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Article

Propyl (E) -3- (furan-2-yl) Acrylate: a synthetic antifungal potential with a regulatory effect on the biosynthesis of ergosterol in *Candida Albicans*

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The genus *Candida* represents the main cause of infections of fungal origin. Some species stand out as disease promoters in humans, such as *C. albicans*, *C. glabrata*, *C. parapsilosis,* and *C. tropicalis*. This study evaluated the antifungal effects of propyl (E) -3- (furan-2-yl) acrylate. The minimum inhibitory concentration of the synthetic compound, amphotericin B and fluconazole alone against four species of *Candida* ranged from 64 to 512 µg/mL, 1 to 2 µg/mL, and 32 to 256 µg/mL, respectively. The synergistic effect of the test substance was observed when associated with fluconazole against *C. glabrata*, there was no antagonism between the substances against any of the tested strains. The potential drug promoted morphological changes in *C. albicans*, decreasing the amount of resistance, virulence, and reproduction structures, such as the formation of pseudohyphae, blastoconidia, and chlamydospores, ensuring the antifungal potential of this substance. It was also possible to identify the fungicidal profile of the test substance through the study of the growth kinetics of *C. albicans*. Finally, it was observed that the test compound inhibited the ergosterol biosynthesis by yeast.

Keywords: Antimicrobial. *Candida*. New drugs. Ergosterol.

INTRODUCTION

Recent works estimated that 1.5 to 2 million people die annually from fungal infections (Denning, Bromley, 2015). The genus *Candida* is one of the principal causes of these infections. There are hundreds of *Candida* species, yet some: *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (Aslani *et al*., 2018) are noted for causing human diseases.

During notable emerging resistance to antibiotics, certain therapeutic options remain limited concerning the treatment of multi-drug resistant microorganisms (Hussain *et al*., 2019). Given this scenario, the search for new antifungal drugs as well as for combined therapies in the context of fighting infections has gained importance. Combination therapies aim to improve host response and reduce mortality. As a consequence of positive interactions between associated drugs, the addition of one compound may allow a reduction in the dose of each other, a very interesting result considering the toxicity of certain antifungal agents, such as amphotericin B (Doern, 2014).

In clinical applications, mechanisms of action are extremely important, and since many antifungals target ergosterol biosynthesis (Prasad, Shah, Rawal, 2016), analysis of ergosterol content in *C. albicans* remains relevant as a way to improve our understanding.

New antifungal drug development is necessary, and furans (being five-membered aromatic compounds present in numerous synthetic and natural products including

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terpenes and alkaloids), are an interesting class of biologically active substances such as Proximicin C, and Tournefolins B and C which present relevant biological activity (Lin *et al*., 1999, Schneider *et al*., 2008).

Furanic compounds are also interesting industrially; 2-furfuraldehyde is a low-cost starting material occurring frequently in nature and is obtained from various biomass sources, such as corncobs, rice husks, and sugarcane bagasse. Yet, given the continuing high mortality rates of patients suffering from *Candida* infection, our study will investigate the antimicrobial potential of furanic compounds against *Candida* spp (Keighley *et al*., 2019).

MATERIAL AND METHODS

Synthesis of the test compound

Propyl (E) -3- (furan-2-yl) acrylate (PFA) was synthesized using a methodology described by Rehan and collaborators (Rehan *et al*., 2017) with adaptations. To a round-bottom flask with a capacity of 100 mL, acrylic (E) -3- (furan-2-yl) acrylic acid (1.38 g, 10 mmol) and propanol (20.0 mL) were added, followed by a slow addition of sulfuric acid (0.4 mL). The flask was coupled to a reflux system and left under stirring and heating (100±5ºC) for 5 hours. Afterward, the flask was cooled to room temperature (28±2ºC) and a saturated sodium bicarbonate solution was added slowly until the solution's pH was neutral (pH = 7). The reaction mixture was transferred to a separator funnel and the product was extracted with ethyl acetate (3 x 50 mL). The organic phases were combined and dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The PFA was purified using liquid column chromatography, with silica gel as the stationary phase and hexane-ethyl acetate systems as the mobile phase starting with only hexane as the eluting solvent and ending with a mixture of 90% hexane and 10% ethyl acetate.

Structural characterization of the PFA was performed using nuclear magnetic hydrogen (NMR ^{1}H) and carbon (NMR ^{13}C) resonance spectroscopic technique with a Varian Unity Plus spectrometer. Deuterated chloroform ($CDCl_3$) was used as a solvent and tetramethylsilane [Si $(CH3)_4$] was used as an internal reference. The chemical shifts were expressed in parts per million (ppm) concerning the central peak of $CDCl_3$ (7.27) for the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, the displacements were obtained about the $CDCl_3$ central peaks (77.0).

Minimum inhibitory concentration

The strains tested were *C. albicans* ATCC 76485, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 90030, and *C. tropicalis* ATCC 13803. After cultivation, the microorganisms were kept on Sabouraud dextrose agar (SDA) under refrigeration (4°C) during the period of the experiments and renewed monthly to maintain the viability of the cells.

The inoculum of each of the tested strains was prepared by adding a few colonies of the microorganism to saline (NaCl 0.9%) until a suspension of approximately 1-5 x 10^6 CFU/mL was formed, adjusted according to the 0.5 McFarland scale. The culture medium used in the tests to assess antifungal activity was Sabouraud dextrose broth (SDB), prepared according to the manufacturer's instructions. Further, the PFA antifungal agent solutions were prepared at the time of testing, initially dissolving them in dimethyl sulfoxide (DMSO), and completing the final volumes with sterile distilled water. Controls were performed with DMSO. Some adaptations were made to the M-27 protocol, such as the use of SDA (CLSI, 2008).

The minimum inhibitory concentrations of PFA, amphotericin B, and fluconazole against *Candida* spp., were determined using the microdilution method in 96-well microtiter plates (CLSI, 2008). In a 96-well plate, the furanic compound and antifungal agents were tested at concentrations of 1 to 512 μg/mL in serial dilutions of 1:2. Plates with *Candida* spp. (10^5 CFU/well), culture medium and the antifungal agents were incubated at 35°C for 24-48 hours. After which, fungal growth was observed.

MIC was considered the lowest concentration of the substance capable of inhibiting the growth of microorganisms, considering the agent and the microorganism (Wiederhold, 2021). Tests were performed in triplicate.

Morphological transition

Analysis of the furan derivative's interference in *C. albicans* morphological transitions was performed using the microculture technique in a wet chamber (methodology proposed by Dalmau) (Walsh, Hayden, Larone, 2018). We used a cornmeal agar culture medium with Tween 80 at 1%, fractionated in sterile tubes. After homogenization, each culture medium was placed on a glass slide. Immediately after the medium had solidified, the microorganism was seeded on the agar, forming two parallel streaks, and a coverslip was inserted to cover the microculture, and the plates were incubated at 35 ± 2°C for 48-72 hours, after which the plates Petri dishes were incubated at 25 ± 2°C for an additional 48-72 hours. In this way, we obtained the negative control and positive controls (PFA, amphotericin B, and fluconazole) in concentrations corresponding to the MIC and MIC/2. The slides were analyzed daily for 5 days using optical microscopy at 400x magnification, to observe the absence or presence of the characteristic structures.

Time-kill assay

When studying effects on *C. albicans* ATCC 76485 growth, the minimum inhibitory concentrations (and multiples) of PFA, amphotericin B, and fluconazole were tested, and the yeast growth behavior (curve) was observed (Klepser *et al*., 1998).

Initially, at a 1:10 ratio, a suspension of the yeast was inoculated in SDB containing: the test compound, amphotericin B, fluconazole, sterility, and fungal viability controls. After incubation, at 0 h, 4 h, 8 h, and 24 h intervals, a 10 µL aliquot of the solution were seeded evenly on SDA plates. The inoculated plates were incubated at 35 ± 2°C for 24-48 hours. Then Colony Forming Units (CFU) in the Petri dishes were counted and the number of CFU/mL of solution; considering each time, for each substance, and at each concentration was determined. The experiment was carried out in triplicate.

Analysis of ergosterol

To analyze the activity of the furan derivative on *C. albicans* ergosterol biosynthesis the microorganism was inoculated in tubes containing SDB, and the respective molecules to be tested (in their concentrations of MICx2, MIC, and MIC/2) were added. The cultures were incubated at 35°C for 24 hours. The tubes were then centrifuged at 3000 rpm for 15 minutes, the supernatant was discarded, and the wet weight of the sediments was determined. Subsequently, 3 mL of a 25% alcoholic potassium hydroxide solution (KOH 25 g, sterile distilled water 36 mL, completed to 100 mL with 100% ethanol), was added to each test tube. The suspensions were then incubated in a water bath at 80°C for 1 hour. A mixture of 1 mL of water and 3 mL of heptane was then added, followed by a vigorous vortex mix for 5 minutes. Finally, the heptane layer was filtered with a 0.45 µm syringe filter and transferred to properly identified vials for later ergosterol quantification using high-performance liquid chromatography (HPLC) (Arthington-Skaggs, Warnock, Morrison, 2000).

The ergosterol quantification was performed on HPLC equipment (Shimadzu) with a UV detector and a diode array being scanned in the UV-visible region, monitoring at the 282 nm wavelength. The analyses were performed in isocratic mode, with methanol as the mobile phase at a flow rate of 1.4 mL/min. For the stationary phase, an analytical column C18 Shim-pack CLC-ODS (250 x 4.6 mm DI) was used, with a particle size of 5 µm, at a temperature of 30ºC.

The chromatographic analysis was developed and validated using the standard addition method; with the parameters of specificity, linearity, precision, and accuracy, using inter and intraday analyses (in triplicate). Ergosterol (Sigma Chemical) was used as the analytical standard (Cabral, Figueroa, Fariña, 2013). The ergosterol concentration/wet weight (mg) of the microorganism was determined, and the test groups were compared with the control (microorganism). The results were considered significant when $p < 0.05$. The ANOVA - Dunnett's test was used.

Checkerboard

Associations of PFA with amphotericin B and fluconazole against microorganisms were determined using the Fractional Inhibitory Concentration Index

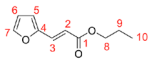
(FICI) and Checkerboard microdilution method (Cuenca-Estrella, 2004). Each substance in combination was varied between MIC/8 and MICx8 and diluted in a 1:2 ratio. Initially, 100 µL of culture medium were added to the microdilution plate wells, and subsequently, 50 µL of each tested substance (in its different concentrations) were inserted in vertical (amphotericin B or fluconazole) and horizontal (PFA) directions on the microplates (Correa-Royero *et al*., 2010). Tests were performed in triplicate.

RESULTS

Esterification of (E) -3- (furan-2-yl) acrylic acid (using propanol) led to propyl (E) -3- (furan-2-yl) acrylate. Propyl (E) -3- (furan-2-yl) acrylate, which was obtained as a yellowish oil in a 92% yield.

The PFA was analyzed using the nuclear magnetic resonance spectroscopy technique - hydrogen (^{1}H NMR) and carbon-13 (^{13}C NMR); the data obtained are described in Table I.

TABLE I - ^{1}H NMR and ^{13}C NMR spectroscopic data for propyl (E) -3- (furan-2-yl) acrylate

Compound Structure	NMR 1H (CDCl3, 400 MHz) ppm	NMR 13C (CDCl3, 100 MHz) ppm
		167.1 (C-1)
	7.47 (d. *J* = 2.4 Hz. H-7)	151.0 (C-4)
	7.42 (d. *J* = 20.3 Hz. H-3)	144.6 (C-7)
	6.59 (d. *J* = 3.9 Hz. H-5)	130.9 (C-3)
	6.45 (dd. *J* = 3.9;	116.0 (C-5)
	2.4 Hz. H-6)	114.5 (C-6)
	6.31 (d. *J* = 21.1 Hz. H-2)	112.2 (C-2)
	4.14 (t. *J* = 8.6 Hz. H-8)	66.1 (C-8)
	1.71 (s. *J* = 9.4 Hz. H-9)	22.1 (C-9)
	0.97 (t. *J* = 10.2 Hz. H-10)	10.4 (C-10)

The MICs of PFA, amphotericin B, and fluconazole against *Candida* spp. were respectively varied: from 64 to 512 µg/mL, from 1 to 2 µg/mL, and from 32 to 256 µg/mL (Table II).

TABLE II - Antifungal MICs for *Candida* spp

ANTIFUNGALS	*C. albicans* ATCC 76485	*C. glabrata* ATCC 90030	*C. parapsilosis* ATCC 22019	*C. tropicalis* ATCC 13803
Propyl (E) -3- (furan-2-yl) acrylate	256 µg/mL	512 µg/mL	128 µg/mL	64 µg/mL
Amphotericin B	1 µg/mL	2 µg/mL	1 µg/mL	1 µg/mL
Fluconazole	256 µg/mL	256 µg/mL	64 µg/mL	32 µg/mL

C. albicans ATCC 76485 microculture was performed in the presence and absence of propyl (E) -3- (furan-2-yl) acrylate (PFA), amphotericin B (ANFB), and fluconazole (FLU) (Figure 1) to verify inhibition of the yeast to hypha transition. It was observed the test compound was effective in reducing the development of virulence structures such as pseudohyphae, and reproduction structures, such as blastoconidia and chlamydospores, compared to the control (absent any test compound). When the microorganism was grown on cornmeal agar in the absence of any drug, pseudohyphae with blastoconidia, with terminal chlamydospores with thick walls were observed. Amphotericin B and fluconazole were more effective than the test drug in inhibiting the formation of virulence and reproduction structures.

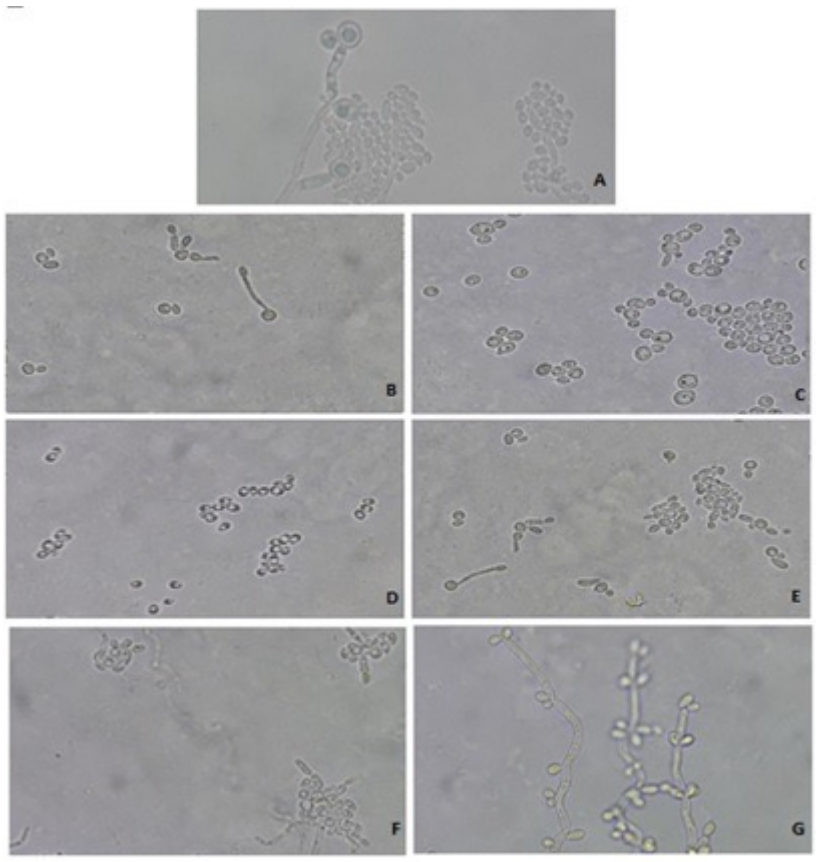


FIGURE 1 - Effect of propyl (E) -3- (furan-2-yl) acrylate and controls on *candida albicans* ATCC 76485 micromorphology after four days of cultivation, at 400x magnification.

A: *C. albicans* ATCC 76485; B: *C. albicans* ATCC 76485 + ANFB MIC; C: *C. albicans* ATCC 76485 + ANFB MIC/2; D: *C. albicans* ATCC 76485 + FLU MIC; E: *C. albicans* ATCC 76485 + FLU MIC/2; F: *C. albicans* ATCC 76485 + PFA MIC; G: *C. albicans* ATCC 76485 + PFA MIC/2.

Antifungal effects on *C. albicans* growth kinetics were verified to evaluate the viability of the yeast in different (microorganism to antifungal) exposure periods. Viability was determined in the absence and presence of various concentrations of PFA, amphotericin B, and fluconazole. Figure 2 contains the results in log CFU/mL according to the time intervals evaluated at the different concentrations used against *C. albicans*. As compared to the initial inoculum, (PFA), and amphotericin B (presenting fungicidal activity) both decreased colony-forming units by > 3 log CFU/mL. Fluconazole was considered fungistatic because for not reduce the number of microorganisms over time.

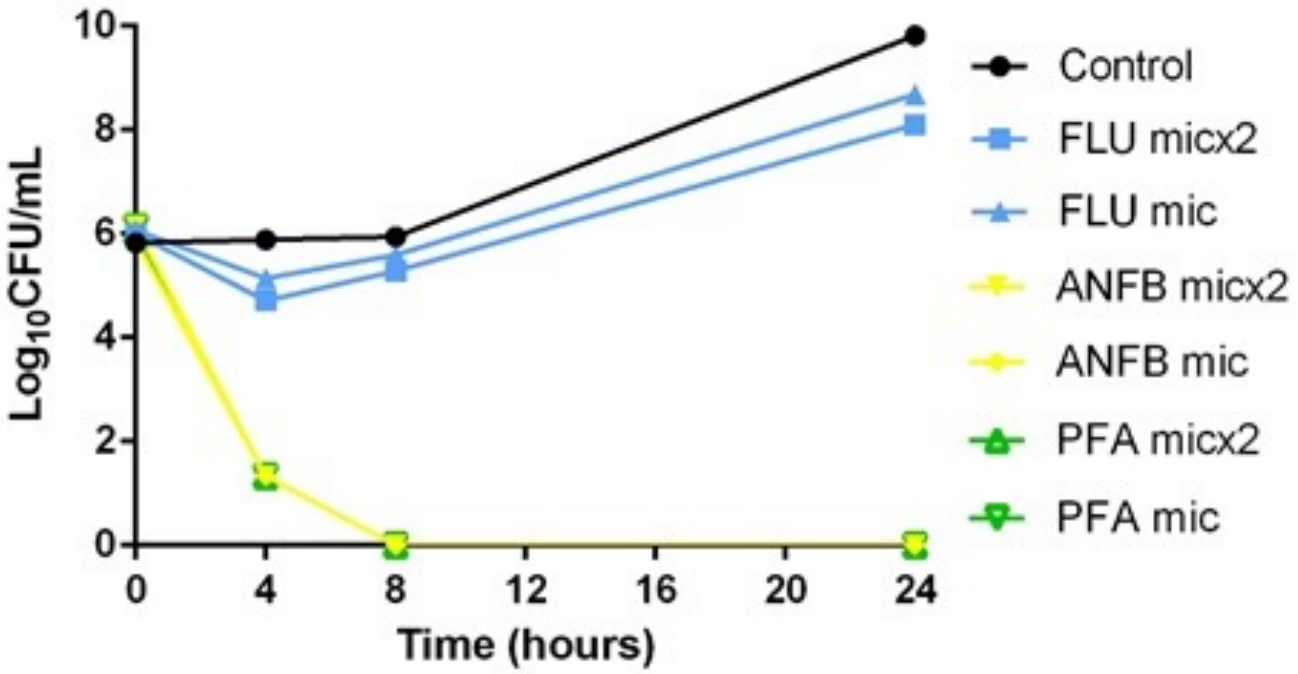


FIGURE 2 - *Viability of Candida albicans ATCC 76485 when exposed to propyl (E) -3- (furan-2-yl) acrylate and controls.*

FLU: fluconazole; ANFB: Amphotericin B; PFA: Propyl (E) -3- (furan-2-yl) acrylate.

To quantify ergosterol, a chromatographic linear calibration model method was established, using the addition of a standard. The method was linear in the range from 0.63 to 10 µg/mL, yielding an R^2 determination coefficient equal to 0.9738. The results found for repeatability and precision revealed concordant results for the method. The variation coefficients (VC%) obtained (given the complexity of the biological matrix) (Chiocchio, Matković, 2011) were mostly below 20%.

The ergosterol retention time was approximately 8.79 minutes (Figure 3). Quantification analyses for ergosterol were performed to verify the effect of the antifungals on sterol biosynthesis in the yeast, which revealing significantly decreased ergosterol content upon exposure to fluconazole at MICx2 and MIC, respectively 69% and 79%. Furthermore, PFA at MICx2, reduced ergosterol biosynthesis by 75%, as compared to the control ($p < 0.05$) (Figure 4).

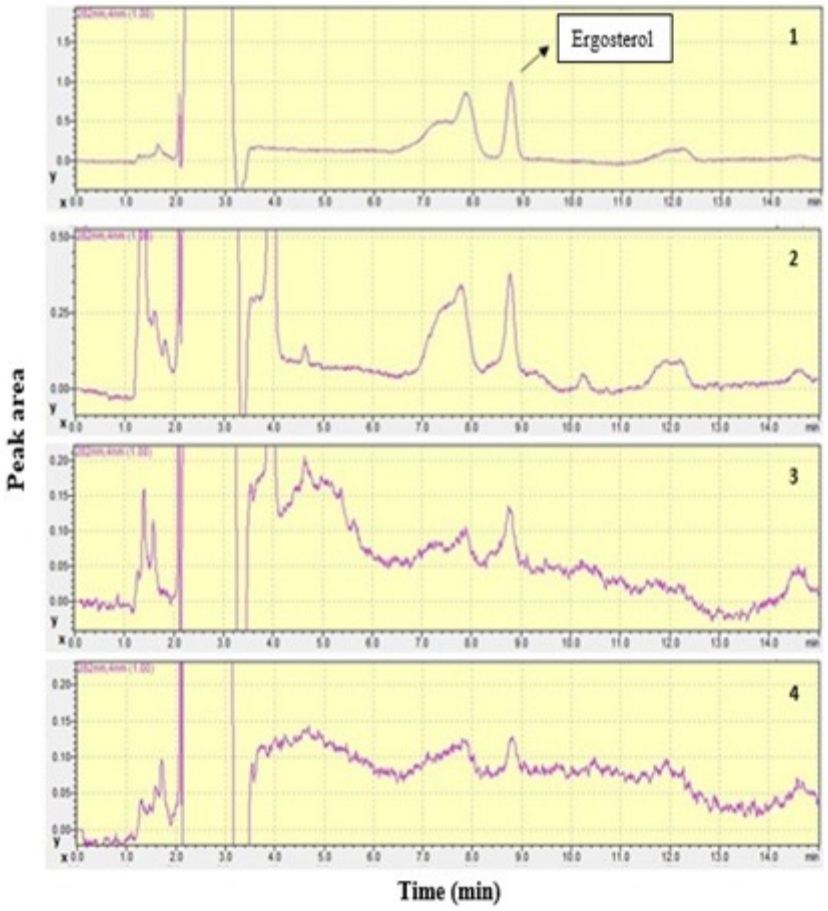


FIGURE 3 - Example chromatograms - microorganism ergosterol pattern when exposed to the tested substances.

Chromatogram 1: Ergosterol standard; Chromatogram 2: Unexposed microorganism; Chromatogram 3: Microorganism exposed to fluconazole at MICx2; Chromatogram 4: Microorganism exposed to propyl (E) -3- (furan-2-yl) acrylate at MICx2.

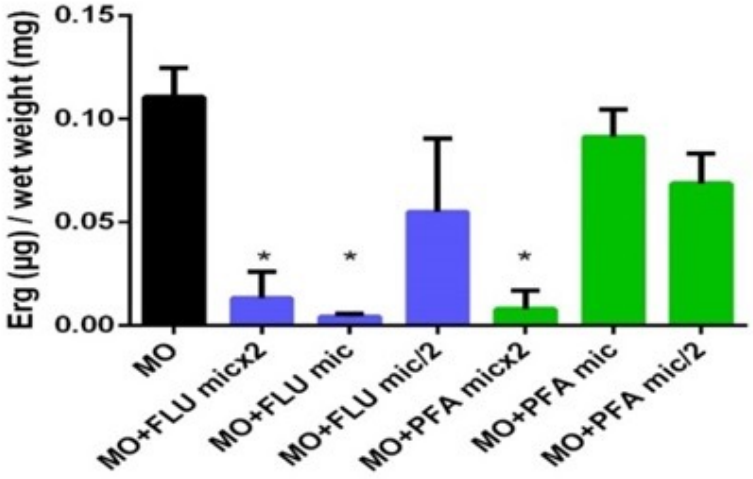


FIGURE 4 - Quantitative analysis of ergosterol content in Candida albicans after treatment with differing concentrations of fluconazole, and propyl (E) -3- (furan-2-yl) acrylate.

MO: microorganism; FLU: fluconazole; PFA: propyl (E) -3- (furan-2-yl) acrylate. * $p < 0.05$ compared to the control (ANOVA - Dunnett's).

When evaluating the effects of PFA in association with amphotericin B or fluconazole against *Candida* spp., the results extended from indifferent to synergistic, there were no antagonistic interactions (Table III).

TABLE III - Associations between propyl (E) -3- (furan-2-yl) acrylate and commercial antifungals for *Candida* spp. - Type of interaction

Microorganism	Antifungals	FIC A	FIC B	FICI	Type of Interaction
C. albicans	PFA + ANFB	1.0	0.5	1.5	Indifference
ATCC 76485	PFA + FLU	0.5	0.125	0.625	Addition
C. glabrata	PFA + ANFB	0.125	1.0	1.125	Indifference
ATCC 90030	PFA + FLU	0.125	0.25	0.375	Synergism
C. parapsilosis	PFA + ANFB	0.125	1.0	1.125	Indifference
ATCC 22019	PFA + FLU	0.125	1.0	1.125	Indifference
C. tropicalis	PFA + ANFB	0.5	0.125	0.625	Addition
ATCC 13803	PFA + FLU	1.0	1.0	2.0	Indifference

PFA: Propyl (*E*)-3-(furan-2-il)acrylate; ANFB: Amphotericin B; FLU: Fluconazole.

DISCUSSION

Synthesis of PFA was performed using Fischer esterification (Rehan *et al*., 2017). The formation of the ester was confirmed by ^{1}H NMR and ^{13}C NMR spectra data, described in Table I. Maintenance of double bond stereochemistry was confirmed by the values of the coupling constants of the olefinic protons H-2 (J = 21.1 Hz) and H-3 (J = 20.3 Hz) (Silverstein *et al*., 2005).

A starting point for determining the antifungal activity of a substance is to obtain its MIC. A lower MIC represents greater potency and less likelihood that the microorganism will develop resistance, and indeed, a higher MIC leads to a higher risk of drug resistance (Andrews, 2001).

MIC studies provide input for subsequent in vivo testing of new antifungal agents. Many researchers have investigated the antifungal activity of furanic substances against *Candida* spp. One such study evaluated the inhibitory capacity of two new alkylated furan derivatives and observed potent activity against both fungal and bacterial strains. Other researchers have synthesized furan-derived molecules and observed their (in vitro) inhibitory effects on bacterial and fungal growth (Wu *et al*., 2018, Loğoğlu *et al*., 2010).

Candida albicans is a polymorphic fungus that can grow as yeast or by hyphae. Yeast cells promote the dissemination, and the formation of hyphae and pseudo-hyphae are associated with adhesion, invasion, damage, iron acquisition, and escape from phagocytes and the bloodstream (Vila *et al*., 2017). For virulence, both morphological forms (having distinct functions) are important (Jacobsen *et al*., 2012), thus study of morphological changes promoted by PFA contributed to detailing its antifungal activity.

Compared to the negative control, the synthesized compound promoted an inhibitory effect on the development of virulence structures such as pseudohyphae, blastoconidia, and chlamydospores. This is significant in the sense of decreasing fungal pathogenicity by reducing its virulence. The structures described above are related to microorganism reproduction and virulence and have been the target of many studies aimed at developing antifungals. In addition to secreted aspartic proteases (SAPs), phospholipases, and calcineurin, the morphological transition has also become a therapeutic

target for new antifungals, mainly due to a reduced probability the fungus forming resistance (Calugi, Trabocchi, Guarna, 2011). In the literature, there are many substances capable of inhibiting morphological transition.

Of compounds that inhibit morphological transition (in addition to the compound tested in this work), some substances derived from piperazine, have been demonstrated to reduce the formation of hyphae in *C. albicans* cells by more than 50% (Zhao *et al*., 2018). Inhibition of hyphae formation when *C. albicans* is exposed to substances such as α-longipinene and linalool (and a consequent reduction in microorganism virulence) has also been reported (Manoharan *et al*., 2017). Yet, there are few studies investigating modes of action, though (regardless of the structure of the molecule) most of these studies have demonstrated a common target, components of the Ras1p signaling pathway (Shareck, Belhumeur, 2011). This may further inspire new studies elucidating the mechanisms by which the substances tested in the present study act to inhibit this morphological transition.

Figure 2 reveals reduced *C. albicans* populations during all times for the test compound PFA, which significantly inhibited growth at 4 h; concerning the control. This is characterized by its fungicidal potential to decrease CFU growth by more than 99.9%. Analyzing the effects of the substances in the referred times, we noted significant reductions in fungal growth at 4 h, 8 h, and 24 h (in both concentrations tested); similar to amphotericin. The fungicidal activity of PFA is significant in the scenario of treating immunocompromised individuals, who need fungicidal rather than fungistatic drugs (Klepser *et al*., 1998, Scorneaux, *et al*., 2017).

Similar results have been described by authors analyzing the effects of a benzofuran antifungal agent on the growth curve of *C. albicans*, inhibiting growth in a fungicidal manner (Masubuchi *et al*., 2003). Two novel 1,3,4-oxadiazole class compounds were investigated in vitro, and in contrast to the substance under test, the growth curve kinetics suggested a fungistatic profile that remained for 24 to 36 hours, even better than fluconazole (Capoci *et al*., 2019).

In this study, it was also found PFA decreased ergosterol content in yeast, presenting a fungicidal effect against *C. albicans* by impairing ergosterol biosynthesis and membrane integrity. That the substance acts on ergosterol biosynthesis is of great relevance, since ergosterol is present in fungal cells (and absent in human cells), making it an even more important pharmacological target (Minnebruggen *et al*., 2010).

Other studies have also identified molecules capable of inhibiting ergosterol biosynthesis, the authors note that eucarobustol E (a meroterpenoid of formyl-chloroglucinol) derived from *Eucalipto robusta*, negatively regulates the activity of genes involved in ergosterol biosynthesis, consequently decreasing its production (Liu *et al*., 2017). Another study investigating mechanisms of action for bafilomycin C1 demonstrated that the substance inhibited gene activity related to ergosterol biosynthesis, yet resulted in much smaller ergosterol reductions, (approximately 41% in comparison with the controls) (Su *et al*., 2018) than those encountered in our study. The effects of Combretum zeyheri ethanolic leaf extract on ergosterol biosynthesis in *C. albicans* have also been investigated through ergosterol quantification in the presence and absence of the extract. The authors observed pathway inhibition at both MIC and sub-inhibitory concentrations, with dose-dependent ergosterol reductions (Mutasa, Mangoyi, Mukanganyama, 2015). Further, a new hybrid 1,2,4-triazole-indole molecule has also presented antifungal efficacy, acting to inhibit ergosterol biosynthesis by 82%, a value slightly higher than that found in our study (Pagniez *et al*., 2020).

In the search for new therapeutic alternatives, associating alternative antifungal compounds with conventional antifungals has been much needed (Campitelli *et al*., 2017), since when combined, the drug association often presents reduced toxicity, an increase in the spectrum, a better response rate, reductions in therapy duration, and prevention of resistance; all culminating in greater effectiveness (Kibbler, 2012). In this sense, there is a great need to research such new and effective alternative therapies that involve antifungal agents in the association.

In this study, a synergistic effect resulted when PFA was associated with fluconazole against *C. glabrata* (FICI = 0.375). We note that similar studies have also explored the synergistic potential of antifungal agents associated with both amphotericin B and fluconazole (Lignell *et al*., 2007).

Many combinations are reported in the literature aiming to achieve a synergistic effect between the

different compounds. As an example, synergistic effects of an association of a furan derivative with fluconazole against *C. albicans*, yielding a FICI of 0.27 to 0.50 (Sharafutdinov *et al*., 2010) have been observed. Other researchers have identified improvements in nystatin and fluconazole efficacy when using silver nanoparticles (Hussain *et al*., 2019), and the effectiveness of fluconazole has also been shown to increase when in association with flavonoids (Lu *et al*., 2017).

These findings reinforce the importance of continuing research involving new antifungal agents and associations, which may bring therapeutic success, according to case specificity, a factor that in light of the divergent responses discussed above, should not be neglected.

CONCLUSION

Given this, in vivo tests are needed to further elucidate the promising potential of the PFA in pharmacology. Our study expresses great utility in understanding its antifungal activity against *Candida* spp. Further, the substance may also serve as a starting point for the synthesis of more potent molecules. In addition, we believe that carrying out new experiments in further studies is important to broaden the understanding of the effects of this new compound.

REFERENCES

Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother. 2001;49:1049-1049. https://academic.oup.com/jac/article/48/suppl_1/5/2473513?login=true

Arthington-Skaggs BA, Warnock DW, Morrison CJ. Quantitation of Candida albicans ergosterol content improves the correlation between in vitro antifungal susceptibility test results and in vivo outcome after fluconazole treatment in a murine model of invasive Candidiasis. Antimicrob Agents Chemother. 2000;44(8):2081-2085.

Aslani N, Janbabaei G, Abastabar M, Meis JF, Babaeian M, Khodavaisy S, et al. Identification of uncommon oral yeasts from cancer patients by MALDI-TOF mass spectrometry. BMC Infect Dis. 2018;18(1):24-34.

Cabral ME, Figueroa LIC, Fariña JI. Synergistic antifungal activity of statin–azole associations as witnessed by Saccharomyces cerevisiae- and Candida utilis-bioassays and ergosterol quantification. Rev Iberoam Micol. 2013;30(1):31-38.

Calugi C, Trabocchi A, Guarna A. Novel small molecules for the treatment of infections caused by Candida albicans: a patent review (2002 – 2010). Expert Opin Ther Pat. 2011;21(3):381-397.

Campitelli M, Zeineddine N, Samaha G, Maslak S. Combination antifungal therapy: A review of current data. J Clin Med Res. 2017;9(6):451-456.

Capoci IRG, Sakita KM, Faria DR, Vendramini FAVR, Arita GS, Oliveira AG, et al. Two new 1,3,4-oxadiazoles with effective antifungal activity against Candida albicans. Front Microbiol. 2019;10:2130-2141.

Chiocchio VM, Matković L. Determination of ergosterol in cellular fungi by HPLC. A modified technique. J Arg Chem Soc. 2011;98:10-15.

Clinical Laboratory Standards Institute. "Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard." Third edition. 2008;28.

Correa-Royero J, Tangarife V, Durán C, Stashenko E, Mesa-Arango A. In vitro antifungal activity and cytotoxic effect of essential oils and extracts of medicinal and aromatic plants against Candida krusei and Aspergillus fumigatus. Rev Bras Farmacogn. 2010;20(5):734-741.

Cuenca-Estrella M. Combinations of antifungal agents in therapy–what value are they? J Antimicrob Chemother. 2004;54(5):854-869.

Denning DW, Bromley MJ. How to bolster the antifungal pipeline. Science. 2015;347(6229):1414–1416.

Doern CD. When does 2 plus 2 equal 5? a review of antimicrobial synergy testing. J Clin Microbiol. 2014;52(12):4124-4128.

Hussain MA, Ahmed D, Anwar A, Perveen S, Anis I, Shah MR, et al. Combination therapy of clinically approved antifungal drugs is enhanced by conjugation with silver nanoparticles. Int Microbiol. 2019;22(2):239-246.

Jacobsen ID, Wilson D, Wächtler B, Brunke S, Naglik JR, Hube B. Candida albicans dimorphism as a therapeutic target. Expert Rev Anti Infect Ther. 2012;10(1):85-93.

Keighley C, Chen SCA, Marriott D, Pope A, Chapman B, Kennedy K, et al. Candidaemia and a risk predictive model for overall mortality: a prospective multicentre study. BMC Infect Dis. 2019;19(1):445-455.

Klepser ME, Ernst EJ, Lewis RE, Ernst ME, Pfaller MA. Influence of Test Conditions on antifungal time-kill curve results: Proposal for standardized methods. Antimicrob Agents Chemother. 1998;42(5):1207-1212.

Kibbler CC. The Pro-debate: How can we improve the outcome of invasive fungal infection? The case for combination therapy. Infectio. 2012;16(Suppl 3):3-10.

Lignell A, Johansson A, Löwdin E, Cars O, Sjölin J. A new in-vitro kinetic model to study the pharmacodynamics of antifungal agents: inhibition of the fungicidal activity of amphotericin B against Candida albicans by voriconazole. Clin Microbiol Infect. 2007;13(6):613-619.

Lin Y-L, Tsai Y-L, Kuo Y-H, Liu Y-H, Shiao M-S. Phenolic Compounds from Tournefortia sarmentosa. J Nat Prod. 1999;62(11):1500-1503.

Liu R-H, Shang Z-C, Li T-X, Yang M-H, Kong L-Y. In Vitro antibiofilm activity of Eucarobustol E against Candida albicans. Antimicrob Agents Chemother. 2017;61(8):2707-2716.

Loğoğlu E, Yilmaz M, Katircioğlu H, Yakut M, Mercan S. Synthesis and biological activity studies of furan derivatives. Med Chem Res. 2010;19(5):490-497.

Lu M, Li T, Wan J, Li X, Yuan L, Sun S. Antifungal effects of phytocompounds on Candida species alone and in combination with fluconazole. Int J Antimicrob Agents. 2017;49(2):125-136.

Manoharan RK, Lee J-H, Kim Y-G, Kim S-I, Lee J. Inhibitory effects of the essential oils α-longipinene and linalool on biofilm formation and hyphal growth of Candida albicans. Biofouling. 2017;33(2):143-155.

Masubuchi M, Ebiike H, Kawasaki K, Sogabe S, Morikami K, Shiratori Y, et al. Synthesis and biological activities of benzofuran antifungal agents targeting fungal N-myristoyltransferase. Bioorg Med Chem. 2003;11(20):4463-4478.

Minnebruggen GV, François IEJA, Cammue BPA, Vroome V, Borgers M, Shroot B. A general overview on past, present and future antimycotics. Open Mycol J. 2010;4(1):22-32.

Mutasa T, Mangoyi R, Mukanganyama S. The Effects of combretum zeyheri leaf extract on ergosterol synthesis in Candida albicans. J Herbs Spices Med Plants. 2015;21(2):211-217.

Pagniez F, Lebouvier N, Na YM, Garnier IO, Picot C, Le Borgne M, et al. Biological exploration of a novel 1,2,4-triazole-indole hybrid molecule as antifungal agent. J Enzyme Inhib Med Chem. 2020;35(1):398-403.

Prasad R, Shah AH, Rawal, MK. Antifungals: mechanism of action and drug resistance. In: Yeast Membrane Transport. Springer. 2016;892:327-349.

Rehan M, Nallagonda R, Das BG, Meena T, Ghorai P. Synthesis of functionalized benzo[b]furans via oxidative cyclization of o-cinnamyl phenols. J Org Chem. 2017;82(7):3411–3424.

Schneider K, Keller S, Wolter FE, Röglin L, Beil W, Seitz O, et al. Proximicins A, B, and C—Antitumor Furan Analogues of Netropsin from the Marine ActinomyceteVerrucosispora Induce Upregulation of p53 and the Cyclin Kinase Inhibitor p21. Angew Chemie Int Ed. 2008;47(17):3258-3261.

Scorneaux B, Angulo D, Borroto-Esoda K, Ghannoum M, Peel M, Wring S. SCY-078 Is fungicidal against candida species in time-kill studies. Antimicrob Agents Chemother. 2017;61(3):1961-1971.

Sharafutdinov IS, Ozhegov GD, Sabirova AE, Novikova VV, Lsovskaya SA, Khabibrakhmanova AM, et al. Increasing susceptibility of drug-resistant candida albicans to fluconazole and terbinafine by 2 (5h)-furanone derivative. Molecules. 2010;25(3):642-660.

Shareck J, Belhumeur P. Modulation of morphogenesis in candida albicans by various small molecules. Eukaryot Cell. 2011;10(8):1004-1012.

Silverstein RM, Webster FX, Kiemle DJ, Bryce DL. Identification Of Organic Compounds. 7th. New York: College of Environmental Science and Forestry, 2005.

Su H, Han L, Ding N, Guan P, Hu C, Huang X. Bafilomycin C1 exert antifungal effect through disturbing sterol biosynthesis in Candida albicans. J Antibiot. 2018;71(4):467-476.

Vila T, Romo JA, Pierce CG, McHardy SF, Saville SP, Lopez-Ribot JL. Targeting Candida albicans filamentation for antifungal drug development. Virulence. 2017;8(2):150-158.

Walsh TJ, Hayden RT, Larone DH. Larones's Medically Importante Fungi: A Guide To Identification, 6th. Washington: American Society of Microbiology (ASM press), 2018.

Wiederhold NP. Antifungal Susceptibility Testing: A Primer for Clinicians. Open Forum Infect Dis. 2021;8(11):1-13.

Wu X, Pang XJ, Xu LL, Zhao T, Long XY, Zhang QY, et al. Two new alkylated furan derivatives with antifungal and antibacterial activities from the plant endophytic fungus Emericella sp. XL029. Nat Prod Res. 2018;32(22):2625-2631.

Zhao S, Huang J-J, Sun X, Huang X, Fu S, Yang L, et al. (1-aryloxy-2-hydroxypropyl)-phenylpiperazine derivatives suppress Candida albicans virulence by interfering with morphological transition. Microb Biotechnol. 2018;11(6):1080-1089.

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