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HEALTH SCIENCES

Antifungal activity of a novel 3-Alkylpyridine analog derived from Marine sponge alkaloids

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Abstract: *Candida* spp. is considered an important cause of healthcare-associated infections worldwide. Currently, the emergence and spread of resistant *Candida* isolates are being increasingly reported, making the development of new agents urgently needed. In this study, we showed the *in vitro* anti-*Candida* activity of seven synthetic 3-alkylpyridine alkaloid analogs. Among them, alkaloid 1 presented a potent antifungal effect, which was independent of its capacity of binding with the fungal membrane ergosterol or cell wall. Analog 1 showed fungistatic and fungicidal effects against *C. albicans* (MIC 7.8 µg/mL and MFC 62.5 µg/mL), *C. glabrata*, *C. krusei* (MIC and MFC 31.2 µg/mL), and *C. tropicalis* (MIC 31.2 µg/mL and MFC 125 µg/mL). The time kill-curve study showed that compound 1 has a potent fungicidal effect *in vitro*, eliminating *C. albicans* cells. Furthermore, an *in vitro* synergistic effect with ketoconazole was observed for compound 1. This compound also eliminated the yeast-to-hypha transition. However, it showed high cytotoxicity against mammalian cells. Taken together, these findings support the use of compound 1 as a prototype to develop new anti-*Candida* agents, but molecular modifications should be done to minimize the high cytotoxicity obtained.

Key words: Antifungal activity, *Candida albicans,* marine sponge alkaloids, synergistic effect, time-kill assay, 3-Alkylpyridine alkaloid analogs.

INTRODUCTION

Candida albicans is the most prevalent fungal pathogen in humans; it is associated with severe fungal infections, accounting for more than 90% of cases of invasive fungal infection (Kauffman 2006, Zida et al. 2016). In addition, this fungus can assume several morphologies, including yeast, hypha, and pseudohypha (Kornitzer 2019). In this context, fungal infections represent a global public health concern. For instance, in the United States, 9% of healthcare-associated bloodstream infections are caused by *Candida* spp. (Pfaller & Diekema 2007, 2010), of which 40–70% are caused by *C. albicans* (Falagas et al. 2010, Pfaller et al. 2012, Kornitzer 2019). Furthermore, candidiasis is currently responsible for approximately 700,000 annual deaths worldwide (Bongomin et al. 2017).

Only a few drugs are available to treat fungal infections compared to those available to treat bacterial infections (Carvalho et al. 2018a). To make this scenario even more adverse, the efficacy of the treatment of fungal infections has been compromised by the acquisition of resistance by these pathogens (Pappas et al. 2016). The increase of opportunistic fungal infections raises the need for the design and synthesis of new antifungal agents. In this context, alkaloid derivatives are an important source of novel compounds with pharmaceutical potentials, as they have been described to

possess various biological activities, such as antiviral (Wang 2006), antitumor (Frenz et al. 2004), antibacterial, antifungal (Wang 2006, Dai et al. 2011, Fyhrquist et al. 2017), and antiparasitic (Hilário et al. 2011, Viana et al. 2016).

Thus, this study aimed to evaluate the anti-*Candida* spectrum of seven synthetic 3-alkylpyridine alkaloid (3-APA) analogs, which presented antibacterial and antibiofilm activities against relevant bacterial pathogens, such as *Klebsiella pneumoniae* and *Staphylococcus aureus* in a previous study (Herrera et al. 2020). Moreover, for the most promising alkaloid, we determined its effect on the fungal membrane ergosterol and cell wall, the anti-virulence activity (*i.e.*, potential to inhibit the yeast-to-hypha transition), and the toxicity against mammalian cells using renal lineages (Vero and BHK-21). Additionally, the synergistic effect of this most active compound was studied after its combination with commercial antifungals (ketoconazole and nystatin) against a fluconazole-resistant *C. albicans* strain.

MATERIALS AND METHODS

Synthesis

The synthesis of 3-APA analogs of theonelladin C (compounds 1, 2a-d, 3, and 4) (Figure 1) was performed as previously described by Hilário et al. (2011), Gonçalves et al. (2014), and Barbosa et al. (2018).

Microorganisms

The fungi used were kindly provided by the Reference Microorganisms Laboratory of Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil, and were originated from the American Type Culture Collection (ATCC). The antifungal activity was investigated against *C. albicans* ATCC 10231, *C. albicans* ATCC 18804, *C. glabrata* ATCC 2001, *C. krusei* ATCC 34135, and *C. tropicalis* ATCC 28707.

In addition, eight clinical isolates of *C. albicans* were employed in this study (*i.e.,* five isolates originated from vaginal secretions (Dra. Magna Paiva collection), and three isolates from swabs of the oral cavity (Dra. Susana Johann collection)). The identification of all *C. albicans* clinical isolates was performed using molecular

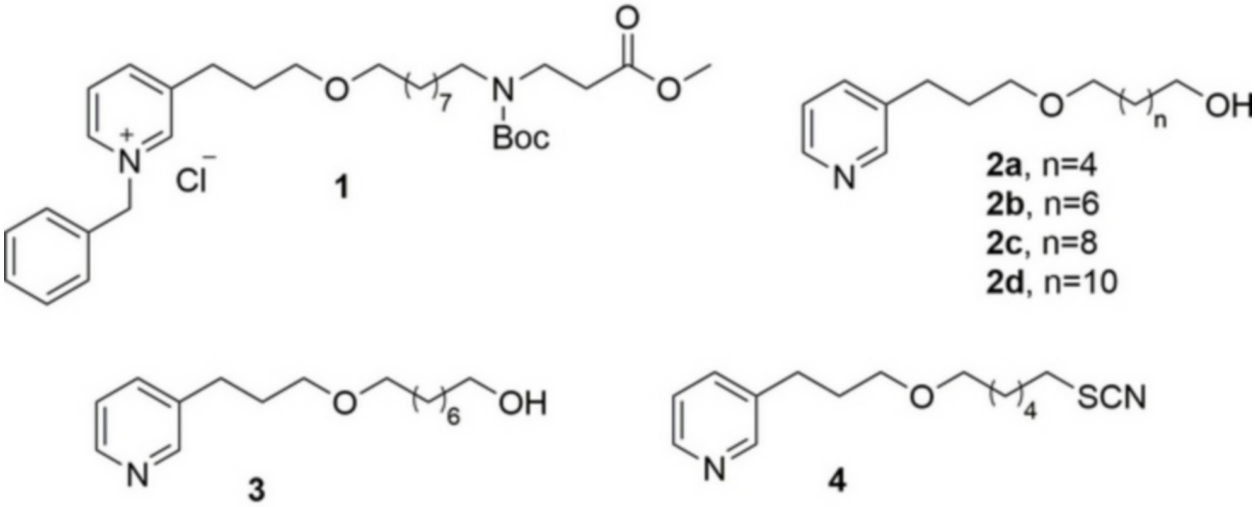

Figure 1. The 3- alkylpyridine alkaloid (3-APA) analogs 1, 2a–d, 3, and 4.

assays. The isolates were kindly provided by the Universidade Federal de Minas Gerais.

Antifungal assay

Inoculum preparation

The fungal inoculum was prepared according to document M27-A3 of the Clinical Laboratory and Standard Institute (CLSI 2008). *Candida* strains were grown in Sabouraud-dextrose (SD) agar (Acumedia, USA) for 48 h, and isolated colonies were suspended in saline solution (0.85 % NaCl; Synth, Brazil). The density of the resultant suspension was adjusted according to the 0.5 McFarland standard corresponding to 10^6 colony-forming units/mL (CFU/mL). Then, the fungal solution was diluted in SD broth (Acumedia, USA) to a concentration of 1.5×10^3 CFU/mL.

Determination of minimum inhibitory concentration (MIC)

The efficacy of seven synthetic alkaloids was examined against *Candida* spp. by the broth microdilution method, following the M27-A3 document of the Clinical and Laboratory Standard Institute (CLSI 2008), with minor modifications (Andrade et al. 2018). Briefly, each alkaloid was dissolved in dimethyl sulfoxide (DMSO; 2 % v/v; Sigma-Aldrich, USA) for stock solution preparation in concentrations ranging from 1.9 to 500 µg/mL. Then, the plates were incubated at 35 ± 2 °C for 48 h, and the MICs were defined as the lowest concentration of compounds that inhibited the visible growth of yeast. DMSO (2% v/v; Sigma-Aldrich, USA) was used as the solvent control. In addition, ketoconazole (Pharmanostra, Brazil) or nystatin (Pharma Nostra, Brazil) were included as the positive control (0.97-500 µg/mL). The assay was performed in triplicate in three independent experiments.

Determination of minimum fungicidal concentration (MFC)

The MFC is considered the lowest concentration of antifungal agent that reduces the viability of the initial fungal inoculum by 99.9% (Lyu et al. 2016, Carvalho et al. 2018b). Briefly, 10 µL of samples from each well that did not show any visible growth in the MIC assay was plated on SD agar (Acumedia, USA) and incubated at 35 ± 2 °C. The solvent (DMSO < 2% v/v) and positive controls (nystatin and ketoconazole) were also tested. The assay was performed in triplicate in three independent experiments.

Time–kill curve

The time-kill curve assay was performed for the most active alkaloid, as previously described (Zore et al. 2011). Briefly, tubes with 10 mL SD broth containing *C. albicans* ATCC 10231 (10^6 CFU/mL) and different concentrations of the most active alkaloid were incubated at 35±2 ºC. Then, 0.1 mL from these tubes were taken at different time intervals (0, 4, 8, 12, 24, 36, and 48 h), inoculated on SD agar, and incubated at 35±2°C for 48 h for posterior colonies count (*i.e.*, determination of CFU/mL). Ketoconazole (125 µg/mL) and DMSO (< 2% v/v) were used as positive and solvent controls, respectively.

Checkerboard assay

The checkerboard microtiter plate assay was used to determine the effect of the combination of the most active alkaloid derivative with commercially available antifungals (ketoconazole and nystatin) (Carvalho et al. 2018b, Lima et al. 2019). Briefly, the alkaloid that showed the most promising activity in the MIC assay was tested at concentrations ranging

from 1.9 to 125 µg/mL. Solutions of ketoconazole and nystatin at the same concentrations were combined in a 1:1 ratio to evaluate the antifungal effect resulting from their interaction with the alkaloid. The fractional inhibitory concentration index (FICI) was determined by the sum of the ratios of the MIC of the sample alone and the MIC of the combinations. For the interpretation of the results, the effect was considered synergistic (FICI≤0.5), additive (0.5≤FICI ≤ 1.0), indifferent (1.0≤FICI ≤4.0), or antagonism (FICI≥4.0) (Carvalho et al. 2018b, Lima et al. 2019).

Phenotypic effects of the alkaloid on fungal cells

Exogenous ergosterol binding assay

The MIC of the most active alkaloid derivative against *C. albicans*, *C. krusei*, *C. glabrata,* and *C. tropicalis* was determined in the absence and presence of exogenous ergosterol (200 µg/mL; Sigma, USA) (Escalante et al. 2008). Nystatin (Phamanostra, Brazil; 2.0 to 500 µg/mL), an antifungal that acts on the ergosterol of the fungal membrane, was used as the positive control.

Sorbitol assay

The effect of the most active alkaloid derivative on the cell wall of *C. albicans*, *C. krusei*, *C. glabrata,* and *C. tropicalis* was evaluated by the determination of the MIC in the presence of sorbitol (0.8 M; Synth, Brazil), which is a known osmotic protector of the fungal wall cell (Escalante et al. 2008). Caspofungin (Sigma, USA; 0.0625 to 62.5 µg/mL), an antifungal that acts on the fungal wall, was used as the positive control.

Inhibition of *Candida* yeast-to-hyphae (y-h) transition

The ability of the most active alkaloid (in MIC and MFC assays) to inhibit the Y-H transition was evaluated according to Andrade et al. (2018). Briefly, hyphae induction was conducted by incubating *C. albicans* ATCC 10231 (10^3 CFU/mL) in a microplate containing fetal bovine serum (FBS) and different alkaloid concentrations. The microplates were incubated for 24, 48, and 72 h at 35±2 °C, and the morphology of the *Candida* cells was visualized using a Nikon TE 2000-U Eclipse microscope equipped with a DC300 F digital imaging system (Leica Microsystems, Germany) with a magnification of 400x. Positive (ketoconazole) and solvent (DMSO 2% v/v) controls were included (Araújo et al. 2013, Andrade et al. 2018).

Cell culture and cytotoxicity assay

The cytotoxicity of the most active alkaloid was evaluated using Baby Hamster Kidney (BHK, ATCC CCL-10) and African green monkey kidney epithelium (Vero, ATCC CCL-81) cells. The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 0.3% solution containing penicillin, streptomycin, and amphotericin B. Cell cultures were maintained at 35±2 ºC in a humidified atmosphere of 5% CO_2. Cells lines were exposed to the alkaloid for 48h, and the cell viability was quantified by the 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) colorimetric assay (Twentyman & Luscombe 1987). Finally, the cytotoxic concentrations for 50% of the cells (CC_{50}) were calculated by linear regression analysis (Souza et al. 2013). In addition, the selectivity index (SI) value was determined by the ratio of the CC_{50} and the MIC (antifungal assay), indicating the specificity of compounds toward pathogens and mammalian cells. Larger

SI values indicate greater cell selectivity (Souza et al. 2013).

RESULTS AND DISCUSSION

The antifungal potential of seven synthetic 3-alkylpyridine alkaloid (3-APA) analogs was evaluated against several *Candida* species frequently involved in invasive candidiasis (Table I). Among them, alkaloid 1 presented fungistatic and fungicidal activities against all *Candida* species evaluated (MIC ranging from 7.8 to 31.25 µg/mL and MFC ranging from 31.25 to 125 µg/mL), revealing an extended spectrum of action that covers the most frequent species involved in candidiasis. This fungicidal activity of compound 1 against all *Candida* species evaluated is relevant because substances that kill pathogens are strong candidates for clinical use (Wong et al. 2014). In addition to the antifungal activity against reference strains of *Candida* spp., this alkaloid also showed a considerable effect on clinical specimens of different origins (Table I). Herein, compound 1 presented fungistatic and fungicidal activity against all oral (MIC 7.8 µg/mL; MFC range of 31.25-125 µg/mL) and vaginal (MIC 7.8 µg/mL; MFC range of 31.25-62.5 µg/mL) isolates of *C. albicans* tested.

In addition, the alkaloid 2d showed activity against all reference strains of *Candida* spp. employed in this study (MIC range of 15.6-62.5 µg/mL; MFC range of 62.5-125 µg/mL), mainly against *C. krusei* (MIC 15.6 µg/mL), an intrinsically azole-resistant species (Carvalho et al. 2018b). This alkaloid also showed antifungal activity against three oral and three vaginal isolates of *C. albicans* (MIC ranging from 31.25 to 62.5 µg/mL and MFC ranging from 62.5 to 250 µg/mL). Similar to these results, Dai et al. (2011) evaluated five synthetic alkaloids that showed fungistatic and fungicidal activity against *C. albicans*, *C. glabrata,* and *C. krusei*, with MIC and MFC ranging from 7.8 to 15 µg/mL. Furthermore, Rane et al. (2012) showed the potential antifungal activity of 20 synthetic compounds derived from marine alkaloids against *C. albicans* (MIC ranging from 1.56 to 12.5 µg/mL). Besides that, another study conducted by Fyhrquist et al. (2017) showed the potential antifungal activity of natural alkaloids against *C. albicans* and *C. glabrata*, with MIC of 5,37 µg/mL. Thus, the results presented in this study corroborate those of previous works that point to alkaloids as a promising class in the development of prototypes for antifungal therapy.

Except for compounds 1 and 2d, all other alkaloids evaluated showed low antifungal activity (MIC of 125-500 µg/mL) (Table I). In contrast to the analogs evaluated, compound 1 has one counterion (Cl^-), which was important for its antifungal activity. As described by Viana (2008), the counterion counterbalances the positive charge of the nitrogen group and plays an essential role in reducing the MIC values. Moreover, the alkaloid 2d was synthesized by the insertion of a long carbon chain into its structure. In this context, studies indicate that long chains appear to be important for the antimicrobial activity of alkaloids (Pernak et al. 2001, Viana 2008).

The time-kill kinetics assay showed that after 8 h of exposure to alkaloid 1 (62.5 µg/mL), a maximum fungicidal effect was observed, resulting in the complete elimination of *C. albicans*. In addition, compound 1 at MIC (31.25 µg/mL) reduced in three logs the fungal load after 48 h of incubation (Figure 2). Ketoconazole did not show a fungicidal effect, which is in accordance with its known fungistatic effect (Hawser & Islam 1999). The growth control continued to grow throughout the experiment (48 h), and no reduction in fungal load was observed with DMSO treatment,

Table I. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of alkaloids against reference strains and clinical isolates of *Candida* spp.

Microorganisms	1		2a		2b		2c		2d		3		4		KET		NYS	
	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]	MIC[a]	MFC[a]
C. albicans ATCC 18804	7.8	62.5	>500	-	250	>500	125	125	62.5	125	125	125	>500	-	31.25	125	4.0	4.0
C. albicans ATCC 10231	7.8	62.5	>500	-	>500	-	125	125	62.5	125	125	125	>500	-	31.25	125	4.0	4.0
C. glabrata ATCC 2001	31.2	31.2	>500	-	>500	-	250	>500	62.5	62.5	125	125	>500	-	125	>500	4.0	4.0
C. krusei ATCC 34135	31.2	31.2	>500	-	>500	-	250	>500	15.6	62.5	62.5	250	>500	-	7.8	7.8	8.0	8.0
C. tropicalis ATCC 28707	31.2	125	250	250	250	250	125	125	31.2	62.5	250	250	>500	-	31.25	62.5	2.0	2.0
CL1	7.8	125	>500	-	250	>500	125	250	62.5	125	125	250	>500	-	0.97	3.9	4.0	4.0
CL2	7.8	31.2	>500	-	>500	-	>500	-	62.5	62.5	125	>500	>500	-	1.95	7.8	4.0	4.0
CL3	7.8	125	>500	-	>500	-	>500	-	>500	-	125	125	>500	-	3.9	7.8	4.0	4.0
CL4	7.8	31.2	>500	-	>500	-	>500	-	>500	-	125	125	>500	-	31.25	250	4.0	4.0
CL5	7.8	62.5	>500	-	125	250	125	250	31.2	125	125	>500	>500	-	7.8	62.5	4.0	4.0
CL6	7.8	31.2	>500	-	250	>500	125	250	31.2	62.5	>500	-	>500	-	31.25	125	4.0	4.0
CL7	7.8	62.5	>500	-	>500	-	62.5	125	62.5	250	>500	-	>500	-	15.6	500	2.0	4.0
CL8	7.8	31.2	>500	-	250	>500	125	125	62.5	125	>500	-	>500	-	15.6	250	2.0	2.0

(-) Not evaluated; KET: Ketoconazole; NYS: Nystatin; [a]MIC and MFC are expressed in µg/mL. CL1-CL-5: Oral isolates; CL6-CL8: Vaginal isolates.

validating our experimental conditions. This fungicidal effect is relevant because the rapid elimination of microorganisms prevents the spread of pathogens and disease progression, guaranteeing the success of the treatment (Nóbrega et al. 2013, Ling et al. 2015, Andrade et al. 2018). Thus, the fungicidal effect of compound 1 is pharmacologically important and desirable in the search for new antifungal drugs.

The use of anti- *C. albicans* agents in combination may be more promising than such compounds used alone (Zida et al. 2016). In this context, the antifungal activity of alkaloid 1 was evaluated after its combination with the imidazole ketoconazole and the polyene nystatin by the checkboard assay. The results showed that compound 1 has a synergistic interaction with ketoconazole (FICI 0.27) and additive with nystatin (FICI 0.7) (Table II). These results suggest that this compound can be used in combination with imidazole antifungals (*e.g.,* ketoconazole) to enhance its therapeutic effect. These findings are significant because drug combinations are often recommended since they may allow a considerable reduction in the concentration of the drug, minimizing the side effects and the final cost of the treatment (Carvalho et al. 2018b).

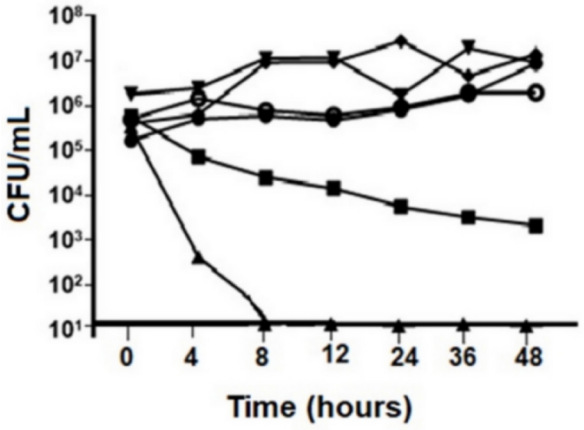

Figure 2. Time-kill curve assay of alkaloid 1 against *Candida albicans* ATCC 10231. Compound 1 was evaluated at 7.8 µg/mL ( ), 31.25 µg/mL ( ), and 62.5 µg/mL ( ). Ketoconazole (125 µg/mL;) was employed as the positive control, DMSO (v/v<2%;) was employed as vehicle control, and untreated fungal cells as growth control ( ).

Next, the effects on the fungal membrane and cell wall were evaluated for alkaloid 1 against *C. albicans*. The damage observed in the fungal cells was not associated with the binding of compound 1 to the membrane ergosterol because the MIC value with exogenous ergosterol remained unchanged for all species evaluated. The antifungal activity of nystatin, which acts on the fungal membrane through ergosterol binding, decreased in the presence of exogenous ergosterol for all species tested, validating our experimental conditions (Table III). Furthermore, no increase in the MIC value was observed for all species evaluated in the presence of sorbitol (Table III), a substance that acts as an osmotic protector of the fungal cell wall (Andrade et al. 2018). Caspofungin, a drug with a mechanism of action associated with structural alterations of the fungal cell wall, showed an increase in the MIC after sorbitol supplementation, validating the experimental conditions employed in this study. Thus, the action of alkaloid 1 on fungal cell components remains to be determined in future studies.

Yeast-to-hyphal transition is a key in the pathophysiology of invasive candidiasis (Pappas et al. 2018, Lima et al. 2019). Thus, the effect of the most promising alkaloid (1) was evaluated against this important virulence factor of *C. albicans*. This compound at 15.6 µg/mL reduced the Y-H transition, and the data were confirmed over 72 h (Figure 3). In the absence of treatment (growth control), the Y-H transition was observed, but it was affected by the treatment with ketoconazole (125 µg/mL), a reference drug used as control positive. In addition, DMSO v/v <2%, used as solvent control, did not inhibit Y-H transition, validating the conditions employed.

To the best of our knowledge, this is the first time that a total reduction in the Y-H transition by the treatment with a synthetic alkaloid is reported. This finding is significant since the yeast-to-hyphal morphogenetic switch plays a vital role in the transition from candidemia to the subsequent tissue invasion (Richardson & Warnock 1997, Kornitzer 2019). Moreover, the establishment of candidemia might also be favored by the enhanced ability of the hyphae to penetrate the mucous membranes and underlying tissues and enter the bloodstream (Koh et al. 2008). In addition, hyphae formation in the phagosome was shown to contribute to the ability of *C. albicans* cells to escape phagocytosis and kill the macrophage (Marcil et al. 2002, Ghosh et al. 2009, McKenzie et al. 2010). In this context, the ability of compound 1 to inhibit Y-H transition in *Candida albicans* may contribute not only to its direct antifungal activity but also to potentiate the host antifungal immune response.

Finally, the safety of alkaloid 1 was assessed through the cytotoxicity assay in mammalian renal cells (BHK-21 and Vero strains). Compound 1 presented a CC_{50} of 36.61±8.30 µg/mL and 7.78±0.1060 µg/mL against BHK-21 and Vero cells, respectively. Ketoconazole was also considerably cytotoxic, showing a CC_{50} of 42.48±1.16 µg/mL and 10.31±0.60 µg/mL for Vero and BHK-21 cells, respectively. The selectivity index (SI) of compound 1 to different *Candida* species regarding BHK-21 and Vero cells is shown in Table IV. The SI of compound 1 ranged from 0.25 to 4.69, in which the higher SI value was observed for *C. albicans* ATCC 10231 (IS 4.69), which is a fluconazole-resistant strain (Lima et al. 2019). However, ketoconazole, a reference antifungal widely used in clinical practice, showed larger SI values (0.25-37.4 for both cell lines).

An important limitation for the use of alkaloids is their high cytotoxicity, which has already been described in another study (Barbosa et al. 2018). Thus, the main challenge for the development of new antifungals is related to the fact that both fungi and human cells are eukaryotic, making it challenging to identify specific metabolite targets and fungal structures, which results in the high toxicity of antifungal agents (Morace et al. 2014, Andrade et al. 2018).

Table II. Fractional inhibitory concentration index (FICI) obtained from combinations of alkaloid 1 and ketoconazole or nystatin against *Candida albicans* ATCC 10231.

Antifungal	FICI	Effect
	alkaloid 1	
Ketoconazole	0.27	Synergistic
Nystatin	0.7	Additive

Table III. Action of alkaloid 1 on membrane and cell wall of four *Candida* spp.

	MIC (µg/mL)*											
	***C. albicans* ATCC 10231**			***C. glabrata* ATCC 2001**			***C. krusei* ATCC 34135**			***C. tropicalis* ATCC 28707**		
	MIC	**MIC with Ergosterol**	**MIC with Sorbitol**	**MIC**	**MIC with Ergosterol**	**MIC with Sorbitol**	**MIC**	**MIC with Ergosterol**	**MIC with Sorbitol**	**MIC**	**MIC with Ergosterol**	**MIC with Sorbitol**
1	7.8	7.8	7.8	31.25	31.25	31.25	31.25	31.25	31.25	31.25	31.25	31.25
NIS	4.0	31.25	ND	4.0	31.25	ND	8.0	31.25	ND	2.0	3.9	ND
CSP	4.0	ND	15.6	0.5	ND	1.0	0.0625	ND	0.25	0.0625	ND	0.25

***Values are the average of three readings. NIS: Nistatin; CSP: Caspofungin. ND: Not determined.**

However, the safety assessment of compounds of pharmaceutical interest by the *in vitro* cytotoxicity assay is preliminary, and these results do not eliminate the potential of analog 1 as a prototype for prospecting new antifungal drugs (Mokoka et al. 2013). Importantly, cytotoxicity is not the only factor to be considered in determining the therapeutic potential of a compound. For example, amphotericin B, a widely used polyene in clinical practice, presents high cytotoxic activity against microglia cell line (Klepser 2011) and known nephrotoxic effect in humans (Mayer et al. 2013). Thus, it is noteworthy that complementary assays, such as evaluation of acute toxicity in mice, are necessary to better elucidate the toxicity of alkaloid 1.

CONCLUSIONS

In conclusion, seven 3-alkylpyridine alkaloid analogs were synthesized and described. The results indicate that the alkaloid 1

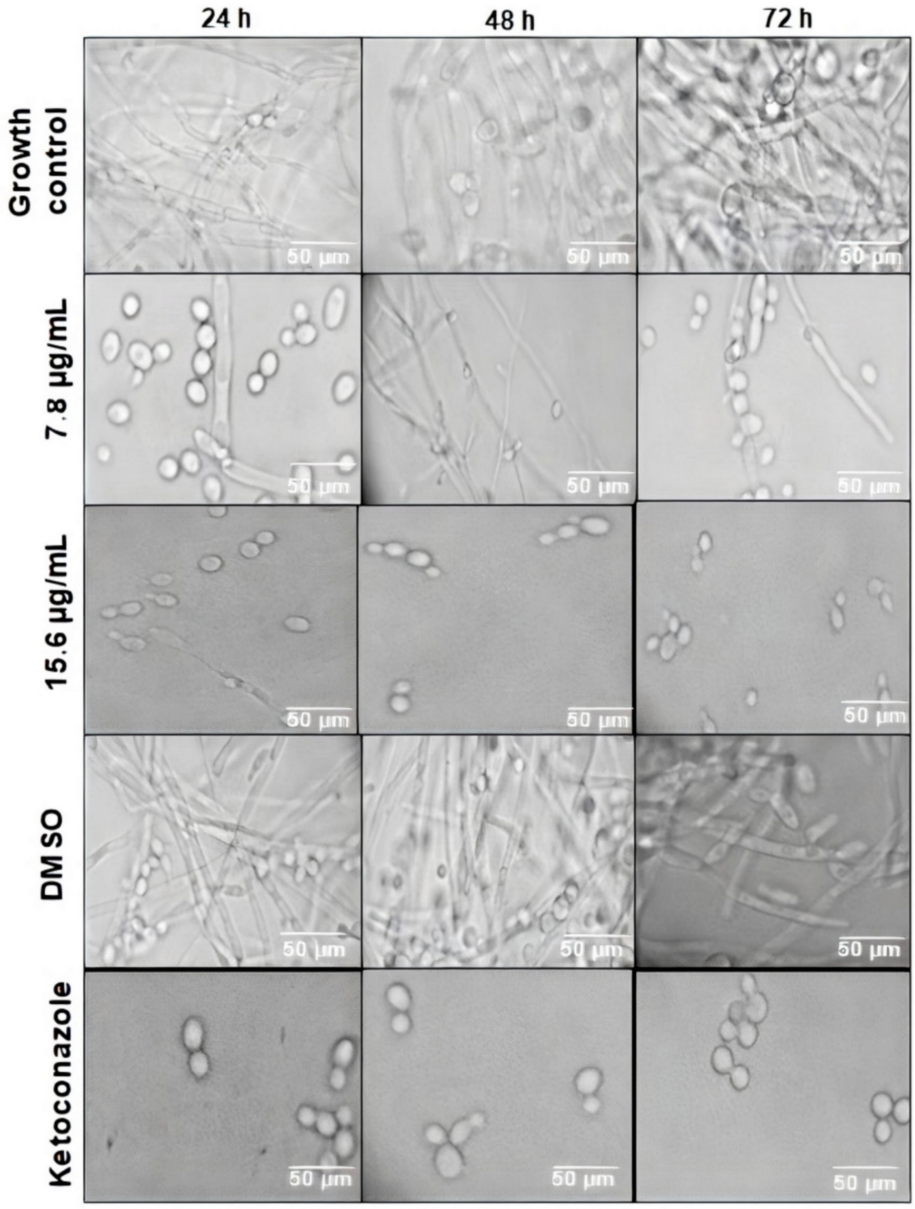

Figure 3. Hyphae formation of *Candida albicans* ATCC 10231. *Candida albicans* cells were cultured with alkaloid 1 (7.8 and 15.6 µg/mL) for 24, 48, and 72 h at 37 °C. Ketoconazole was used as the positive control and DMSO as vehicle control. The experiments were performed in duplicate and repeated three times. Representative microphotographs are shown. The white bar represents a length of 50 µm (magnification of ×400).

showed promising fungistatic and fungicidal activity against *Candida* species, which was independent of its capacity of binding to the membrane ergosterol or fungal cell wall. The fungicidal effect was confirmed by the time-kill curve study, with a maximum fungicidal effect after 8 h. Furthermore, compound 1 interacts synergistically with ketoconazole (FICI 0.27). We also showed that this compound is associated with a total reduction in the Y-H transition of *C. albicans*. However, it presented considerable cytotoxicity *in vitro* against mammalian cells with low SI values. In summary, alkaloid 1 stands out as a promising prototype in the development of new effective drugs against *Candida* spp. However, molecular modifications of this compound are encouraged to reduce its toxicity and potentiate the antifungal effect.

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Table IV. Selective index (SI) of alkaloid 1 and ketoconazole to different *Candida* species regarding BHK-21 and Vero cells.

Microorganisms	1		Ketoconazole	
	Vero	BHK-21	Vero	BHK-21
C. albicans ATCC 18804	1.00	4.69	0.25	1.17
C. albicans ATCC 10231	1.00	4.69	0.25	1.17
C. glabrata ATCC 2001	0.25	1.17	0.06	0.29
C. krusei ATCC 34135	0.25	1.17	1.00	4.69
C. tropicalis ATCC 28707	0.25	1.17	0.25	1.17
CL1	1.00	4.69	8.02	37.74
CL2	1.00	4.69	3.99	18.77
CL3	1.00	4.69	1.99	9.39
CL4	1.00	4.69	0.25	1.17
CL5	1.00	4.69	1.00	4.69
CL6	1.00	4.69	0.25	1.17
CL7	1.00	4.69	0.50	2.35
CL8	1.00	4.69	0.50	2.35
Overall mean	0.82	3.88	1.41	6.63

Selectivity index (SI) is a ration of CC_{50} to MICs values. CL1-CL-5: Oral isolates; CL6-CL8: Vaginal isolates.

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JMSF and GHRV conceived, designed, and coordinated the research. JTA, WGL, CSB, AMMNG, MKPS, FBM, and JMCP conducted the experiments. All authors discussed the results and contributed to the final manuscript.