



Antifungal activity of aminoalcohols and diamines against dermatophytes and yeast

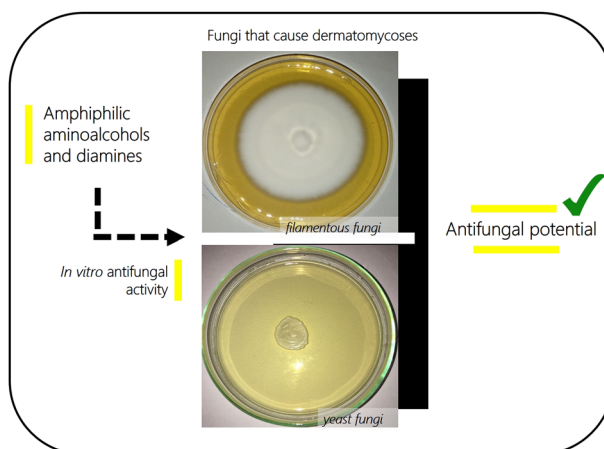
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Abstract

Dermatophytes are infections caused by fungi and yeasts and the drug treatment is considered expensive and extensive. Researchers are synthesizing new organic compounds in order to obtain more effective molecules that provide reduced adverse effects. Our research group has synthesized and evaluated the biological activities of aminoalcohol and diamine derivatives, which were considered active against human pathogenic fungi. Therefore, the objective of this study was to evaluate the *in vitro* antifungal activity of aminoalcohols and diamine derivatives against fungi and yeasts that cause dermatophytes. The minimum inhibitory concentrations (MICs) and the minimum fungicidal concentration (MFC) of aminoalcohol (1–4) and diamine (5–13) derivatives was determined against *Trichophyton mentagrophytes*, *T. rubrum*, *Epidermophyton floccosum*, and *Candida albicans* according to protocols from the Clinical and Laboratory Standards Institute. All molecules exhibited fungicidal activity against the evaluated fungal strains, with the MIC and MFC ranging between 0.12 and 1000 µg/mL for filamentous fungi and 0.6 and 1250 µg/mL for yeasts. The best activity was attributed to diamines compared to aminoalcohols, with an emphasis on molecules 6 and 7. These results demonstrate the antifungal potential of the evaluated aminoalcohols and diamines against the four primary fungal species that cause dermatophytes.

Graphical Abstract



Keywords Aminoalcohols · Diamines · Antifungal activity · Dermatophytes

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Introduction

Dermatophytes are superficial infections that can trigger inflammatory conditions and ulcerations [1–5]. This type of mycosis is associated with several predisposing factors, such as climatic conditions, sports activities, prolonged contact with water, lifestyle, immunological status, chronic diseases, and older age [6].

The main etiological agents of these infections are the microorganisms of the genera *Trichophyton*, *Microsporium*, and *Epidermophyton*, which include the species *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, and *M. canis* [1, 4–7]. In contrast, the genus *Candida* comprises opportunistic yeast fungi whose representatives are responsible for causing candidiasis, but it is also directly associated with dermatomycosis, with *C. albicans* being its primary representative [8]. This species is also responsible for causing several hospital infections from catheters and drains and complications of bacterial sepsis and the urinary system [9].

This type of mycosis is widely distributed among humans and it is considered a serious health problem due to the difficulty of treatment that is attributed to the reduced efficacy of available drugs, prolonged treatment, and high cost, in addition to causing hepatotoxicity. These factors result in low adherence to the drug therapy and consequently the emergence of relapse cases and the selection of strains more resistant to the antifungals [3, 10–12].

The search for compounds with biological activities from natural sources, biomolecules, metals, etc. has been going on for a long time [12]. In this context, organic synthesis plays a fundamental role in the development of new compounds such as aminoalcohols and diamines with potential antibacterial as was verified by [13–20]. In this way new aminoalcohols and diamines were synthesized and described by [15] and [21]. These compounds are the target of this study, have already been evaluated against *Mycobacterium tuberculosis* and *Leishmania* species [14, 15, 19], *Escherichia coli*, and *Pseudomonas aeruginosa*; *Trichomonas vaginalis* [22], *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* [23], and antifungal activity against fungi of the genera *Trichophyton*, *Epidermophyton*, and *Candida* [24].

The several studies were carried out and promising results for the mentioned molecules and their derivatives arouse greater interest in the evaluation of their antifungal activity in vitro against the primary species of dermatophytic and yeast fungi responsible for causing dermatomycoses.

Results and discussion

All aminoalcohols and diamines exhibited fungicidal activity against the five fungal strains evaluated in this study, with

the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values ranging between 0.12 and 1000 µg/mL for filamentous fungi and 0.6 and 1250 µg/mL for yeasts (Table 1), which corroborates data from the literature attributing the antimicrobial action to these classes of compounds [15, 17, 19, 25]. The lowest MIC and MFC values were provided by diamine **6**, whose antifungal action could be attributed to its amphiphilic property, since it favors its interaction with the cells, as described in previous studies [21–23, 26, 27]. Among the molecules evaluated, diamine **6** with a lateral aliphatic chain bearing 12 carbon atoms exhibited better antifungal action against the tested microbial strains, with the MIC value in the range of 0.12–4.8 µg/mL.

Diamine **6** also presented the lowest MIC value (0.12 µg/mL) against *T. rubrum* CCT 5507 URM 1666 strain and this MIC value was less than the value obtained with the reference drugs (terbinafine, ketoconazole, itraconazole, and amorolfine). This result deserves attention since Lima et al. [28] have considered this species as the main responsible for dermatomycoses in humans. In addition, the existence of antifungals-resistant clinical isolates of this species is common, which may be related to the presence of transporters associated with cellular efflux identified as TruMDR1 and TruMDR2 [29].

As shown in Table 1, the diamines **5–13** exhibited the best activity (MICs: 0.97–3.9 µg/mL) compared with the aminoalcohols **1–4** (MICs: 0.462–500 µg/mL). There was a reduction in the antifungal activity of the diamines with the increase in the number of carbon atoms present in the spacers between the functional groups. The presence of two- and four-carbon spacers provided MICs of 0.24–15.62 and 0.6–312 µg/mL, respectively. These results suggest a decrease in the biological response due to the increased lipophilicity (longer than 12 carbon atoms in the lateral alkyl chain). Krauss et al. [30] evaluated the antimicrobial activity of *N*-alkyl-trans-decahydroisoquinoline molecules and observed that the biological action was related to the length of the alkyl chain, with the best activity being attributed to the presence of chains composed of 10–12 carbon atoms. Tang et al. [31] evaluated the antifungal potential of quinazolinones, quinoxalines, and benzopyrans and found that the size of the lipophilic chain presents a strong correlation with the biological response.

The highest MIC and MFC values (250 and 2500 µg/mL) were provided by compound **1**. As this aminoalcohol has the shortest carbon chain (eight carbons) among the molecules described in this study, it could be suggested that the increase in the lipophilic characteristic can hamper the described pharmacological action, as demonstrated by [23]. In contrast, compound **3** exhibited the best activity among the described aminoalcohols (MICs: 0.462–500 µg/mL) and has 12 carbon atoms in its side chain; the result may be

Table 1 In vitro antifungal activity of amphiphilic aminoalcohols and diamines

Compounds	Fungal strains									
	<i>Trichophyton mentagrophytes</i> ATCC 11481		<i>Trichophyton rubrum</i> CCT 5507 URM 1666		<i>Epidermophyton floccosum</i> -CCF-IOF-3757		<i>Candida albicans</i> ATCC 10231		<i>Candida albicans</i> Clinical isolated	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	500	1000	250	500	1000	1000	1250	2500	312.5	625
2	1.85	7.4	125	250	500	1000	78	78	39	78
3	0.462	7.4	7.81	7.81	500	1000	19	19	4.8	19
4	250	250	3.9	3.9	15.62	31.25	4.8	4.8	4.8	4.8
5	3.9	7.8	3.9	7.8	15.62	15.62	9.7	9.7	9.7	19
6	0.24	0.48	0.12	0.97	1.95	1.95	4.8	4.8	4.8	4.8
7	0.97	0.97	0.487	0.97	1.95	1.95	1.2	1.2	2.4	2.4
8	1.95	3.90	3.9	7.81	31.25	250	19	78	19	78
9	500	500	125	125	125	250	19	19	19	1250
10	3.9	3.9	0.97	1.95	3.9	3.9	2.4	2.4	2.4	2.4
11	3.9	250	62.5	125	125	125	78	78	78	156
12	31.25	125	31.25	31.25	125	500	39	156.2	19	312
13	1.95	1.95	3.9	7.81	15.62	125	9	19	0.6	78
Terbinafine	0.03	0.03	0.19	0.19	>480	ND	NE	NE	NE	NE
Ketoconazole	0.25	0.25	1	4	>640	ND	NE	NE	NE	NE
Itraconazole	8	64	2	64	>64	ND	NE	NE	NE	NE
Amorolfine	2	4	0,5	1	>16	ND	NE	NE	NE	NE
Amphotericin B	NE	NE	NE	NE	NE	NE	125	500	78	312.5
Nystatin	NE	NE	NE	NE	NE	NE	0.33	3.33	0.08	0.8

Results are expressed as $\mu\text{g mL}^{-1}$

ND not determined ($\text{MIC} > 1000 \mu\text{g mL}^{-1}$ or $\text{MIC} > 5000 \mu\text{g mL}^{-1}$), NE not evaluated, MIC minimum inhibitory concentration, MFC minimum fungicidal concentration

associated with the synergism between the lipophilic chain and the aminoalcohol functional group [21].

All the molecules evaluated in this study exhibited fungicidal action against *E. floccosum* CCF-IOF-3757 ($\text{MFC} \leq 1000 \mu\text{g/mL}$), a fact that indicates their great significance, since this strain was considered resistant to the reference drugs (ketoconazole [$>640 \mu\text{g/mL}$], itraconazole [$>64 \mu\text{g/mL}$], terbinafine [$>480 \mu\text{g/mL}$] and amorolfine [$>16 \mu\text{g/mL}$]) [32] used in the treatment of dermatomycosis. However, this fact is consistent with the studies of [7], which mention that cases of resistance to antifungals are not common.

According to studies conducted by Ouf et al. [33] and Miron et al. [34], the MIC values of terbinafine against the clinical isolates of *T. rubrum* and *E. floccosum* were 4–8 and 0.25–4 $\mu\text{g/mL}$, respectively. In our work, we check that *T. rubrum* CCT 5507 URM 1666 was more susceptible to the same drug described ($\text{MIC} = 0.19 \mu\text{g/mL}$), whereas *E. floccosum* CCF-IOF-3757 was resistant to the above-mentioned allylamine ($\text{MIC} > 480 \mu\text{g/mL}$). The results are not contradictory, since clinical strains were used [33, 34], so it is expected that the MIC values are higher when compared to standard strains. This fact was evidenced for *T.*

rubrum CCT 5507 URM 1666; however, the *E. floccosum* CCF-IOF-3757 strain showed unexpected behavior when presenting $\text{MIC} > 480 \mu\text{g/mL}$.

The antifungal activities of compounds 1–13 against *C. albicans* were analyzed. Results showed that compounds 2–13 presented lower MIC and MFC values than amphotericin B (125 and 500 $\mu\text{g/mL}$, respectively) when tested against *C. albicans* ATCC 10231. Conversely, all compounds (1–13) presented higher MIC and MFC values than nystatin. This experiment was performed in triplicate to confirm the results which repeat the values described in the Table 1.

When tested against the clinical isolate, compounds 2–8, 10, 11, and 13 showed lower MIC and MFC values than amphotericin B. These results are of great significance due the current scenario wherein there is an increase in the number of *C. albicans* isolates resistant to available drug therapy [35]. Compared to nystatin antifungal activity, all the molecules presented higher MIC and MFC values.

Therefore, the results shown in Table 1 are important because these compounds may contribute to the development of a candidate prototype for treating infections caused by resistant fungal strains.

Conclusion

This study demonstrated the fungicidal activity of a series of aminoalcohols **1–4** and diamines **5–13** against *T. mentagrophytes* ATCC 11481, *T. rubrum* CCT 5507 URM 1666, *E. floccosum* CCF-IOF-3757, *C. albicans* ATCC 10231, and a clinical isolate of *C. albicans*. Among the evaluated compounds, diamine **6** and **7** presented the lowest MIC and MFC values against dermatophytes and yeast, respectively. These results can contribute to the synthesis and evaluation of compounds with fungicidal potential against both filamentous fungi and yeasts that cause dermatomycoses.

Material and methods

Chemistry

N-alkylated aminoalcohols **1–4** and diamines **5–13** (Scheme 1) were prepared using a methodology previously described by [15, 25] (Scheme 1).

Fungal strains

Three strains of filamentous fungi, *T. mentagrophytes* ATCC 11481, *T. rubrum* CCT 5507 URM 1666, and *E. floccosum* CCF-IOF-3757, were obtained from the Collection of Tropical Crops (CCT) provided by the André Tosello Foundation (Campinas-SP, Brazil). *C. albicans* ATCC 10231 was provided by the National Institute of Quality Control in the Health-Oswaldo Cruz Foundation (Rio de Janeiro-RJ, Brazil) and a clinical isolate of this species was obtained from a patient from Maurílio Baldi Laboratory at the UFJF University Hospital.

The authenticity of the lineages *T. mentagrophytes* ATCC 11481, *T. rubrum* CCT 5507 URM 1666, *C. albicans* ATCC 10231, and the clinical isolate of *C. albicans* was confirmed through molecular analysis as described previously [24, 36, 37]. The strain of *E. floccosum* exhibited

pleomorphism; however, its authenticity was certified through macroscopic and microscopic analyses.

Antifungal activity

The MIC and the MFC values were established as described by Clinical and Laboratory Standards Institute (CLSI) [32, 38, 39].

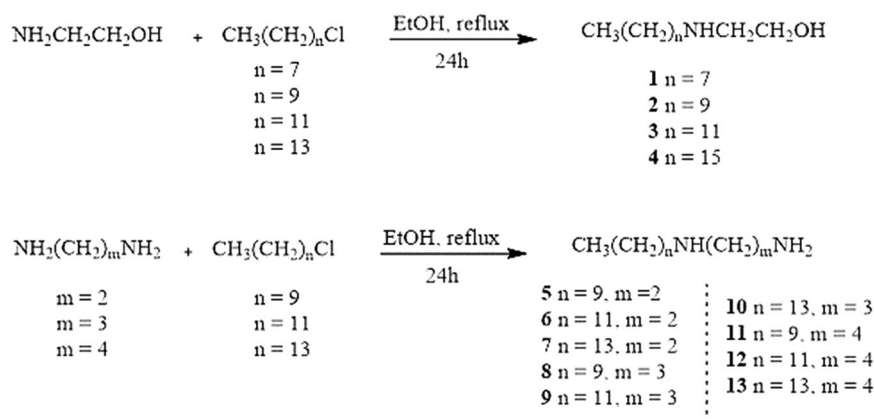
Minimum inhibitory concentration

The MIC value was established according to the CLSI protocols M38-A2, M27-A3, and M27-S4 [32, 38, 39], and the analyses were performed in triplicate. Initially, the filamentous fungi and yeasts were cultivated on Sabouraud dextrose agar for 7 days (filamentous fungi) e 2 days (yeasts). These colonies were used to prepare fungal suspension from successive washes of the surface on the culture medium where the growth microorganism occurred.

The number of viable fungal structures (conidia, blastoconidia, and chlamydoconidia) in the suspensions was analyzed using a spectrophotometer (Libra S12; Biochrom, Cambourne, UK) at a wavelength of 530 nm and a transmittance of 68–70% for filamentous fungi and 89–90% for yeast [32, 38–40]. These transmittance ranges corresponded to $2 \times 10^5 - 2.5 \times 10^6$ CFU/mL for filamentous fungi and $1-5 \times 10^6$ CFU/mL for yeasts. Subsequently, the suspension was diluted in RPMI-1640 culture medium (Sigma, St. Louis, MO, USA) buffered with 3-(*N*-morpholino)propanesulfonic acid (MOPS; JT Baker, Griesheim, Germany), at a ratio of 1:50 (final concentration of $0.4-5.0 \times 10^4$ CFU/mL for filamentous fungi) and 1:2000 (final concentration of $1-5 \times 10^3$ CFU/mL for yeast).

The aminoalcohols **1–4** and the diamines **5–13** were solubilized in RPMI-1640 culture medium, buffered with MOPS, and tested at the final concentrations of 7.8–1000 and 39.06–5000 $\mu\text{g mL}^{-1}$ for filamentous fungi and yeasts, respectively. Fungal growth was evaluated by adding 100 μL RPMI-1640 culture medium buffered with MOPS

Scheme 1 Synthesis of aminoalcohols **1–4** and diamines **5–13**



containing the fungal inoculum. The 96-well sterile plates were incubated at $28 \pm 2^\circ\text{C}$ for 7 days (dermatophytes) or at $37 \pm 2^\circ\text{C}$ for 48 h (yeasts) according to the CLSI. The growth of the fungi was analyzed visually using a SMZ800 microscope (Nikon, Melville, NY). Terbinafine, ketoconazole, itraconazole, amorolfine, amphotericin B, and nystatin were used as reference drugs and assessed according to the M38-A2, M27-A3, and M27-S4 protocols [32, 38, 39].

Minimum fungicidal concentration

The MFC analysis for filamentous fungi was performed by transferring 10 μL volume from the wells where no fungal growth was observed to the wells of another microtiter plate containing 200 μL of Sabouraud dextrose broth (SDB) sterile and without antifungal SDB as described previously [41]. The MFC was determined as the lowest concentration of the molecule that resulted in fungal death. The MFC for *C. albicans* was evaluated using 5 μL from wells without fungal growth, which was transferred to cryotubes containing 1000 μL SDB. The analysis was conducted as described above [42].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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