Chem Biol Drug Des 2011; **78:** 810–815

Research Article

doi: 10.1111/j.1747-0285.2011.01185.x

Synthesis of Aryl Aldimines and Their Activity against Fungi of Clinical Interest

Cleiton M. da Silva[1,†], Danielle L. da Silva[1,2,†], Cleide V.B. Martins[2,3], Maria A. de Resende[2], Esther S. Dias[1], Thais F.F. Magalhães[2], Letícia P. Rodrigues[1], Adão A. Sabino[1], Rosemeire B. Alves[1] and Ângelo de Fátima[1,2,*]

[1]*Grupo de Estudos em Química Orgânica e Biológica (GEQOB), Departamento de Química, ICEx, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*
[2]*Departamento de Microbiologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil*
[3]*Centro de Engenharias e Ciências Exatas, UNIOESTE, PR, 85903-000, Brazil*
**Corresponding author: Ângelo de Fátima, adefatima@qui.ufmg.br*
[†]*These authors contributed equally to this work.*

Aldimines are aldehyde-derived compounds that contain a C=N group. Besides its broad industrial applications, this class of non-naturally occurring compounds are found to possess antibacterial, antifungal, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties. Based on this, six aryl aldimines were synthesized from the condensation of aromatic amines with benzaldehydes. The antifungal activities of synthesized compounds were evaluated against nineteen fungal strains that included *Candida* and *Aspergillus* species, *Cryptococcus neoformans*. The aryl aldimines 2-(benzylideneamino)phenol (3) and 4-(benzylideneamino)phenol (8) were the most active compounds against the fungi studied. Compounds 3 and 8 efficiently inhibited the metabolism of *C. neoformans* mature biofilm.

Key words: Antifungal activity, aryl aldimines, *Aspergillus* spp., biofilm, *Candida* spp., *Cryptococcus neoformans*

Received 1 May 2010, revised 3 July 2011 and accepted for publication 3 July 2011

Fungi have remained as some of the neglected pathogens in terms of new drug discovery (1). Consequently, the incidence of invasive fungal infections has steadily increased in the last two decades. Fungal infections are potentially life threatening to patients of compromised immunological system (2,3). Indeed, patients under advanced age, major surgery, immunosuppressive therapy, AIDS and cancer treatment, solid-organ and hematopoietic stem cell transplantation among others are more susceptible to invasive fungal infections (4).

Candida species infections may range from non-life-threatening mucocutaneous illnesses to invasive processes (5). Both mucosal and deep tissue infections are mainly caused by *C. albicans* (5). *Candida* species other than *albicans* have been assumed to be the causal agents of nosocomial infections in immunocompromised patients (6,7). Aspergillosis also offers a risk to human health, being the major cause of mortality in bone marrow-transplanted patients (8). Pulmonary aspergillosis is also a serious complication of patients undergoing lung and heart–lung transplantation (9). The most common *Aspergillus* species recovered from such patients is *A. fumigatus* (10,11). *Cryptococcus* species are also among the life-threatening fungi. Approximately 3–30% of immunocompromised patients because of HIV/AIDS, hematological, or metabolic diseases develop cryptococcosis (12). *Cryptococcus neoformans* is the most common cause of central nervous system's mycosis in HIV-infected patients (12). Recent studies have shown that the species *C. neoformans* is able to form biofilm, a condition that reduces the efficacy of antifungal drugs (13).

The frequent use of antifungal drugs may lead to fungal resistance, affecting the infection treatment's efficiency. The current antifungal drugs available in the market exhibit other drawbacks, such as toxicity, limited efficacy, and high cost (1). This scenario clearly indicates that there is an ongoing requirement for the search of new antifungal agents.

Aldimines, compounds containing the C=N group, are some of the most widely used organic compounds. Besides industrial applications, they show a broad range of biological activities, such as antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties (14,15). A number of studies report the antifungal activity of several aldimines. Some aryl aldimines have been checked for antifungal activity (Figure 1). The compound 4-(4-(3-methoxy-4-hydroxybenzylidene-imino)-phenyl)-morpholine (**1**) was shown to inhibit the growth of *A. niger* and *C. albicans* at minimal inhibitory concentration (MIC) of 23 and 30 μg/mL, respectively (16). The growth of *C. albicans* also was inhibited by 2-((2-hydroxynaphthalen-1-yl)methyleneamino)acetic acid (**2**) that was more active than its corresponding manganese(III) complex (17). The aryl aldimine 4-(benzylideneamino)phenol (**3**) inhibited the budded-to-hyphal form transition in the *C. albicans* (18). Such transition is essential for the establishment of systemic fungal infections. Thus, the ability of compound **3** to inhibit the transition of budded-to-hyphal form brings new perspectives for the development of more effective antifungal therapeutics in the future.

Overall, aldimines are interesting prototypes for the design of new antifungal agents (15). Here, we report the synthesis and the antifungal activity of six aryl aldimines (compounds **3**–**8**) against nineteen fungal strains. The effect of the aryl aldimines 4-(benzylideneamino)phenol (**3**) and 2-(benzylideneamino)phenol (**8**) on the metabolism of *C. neoformans* biofilm is also discussed.

Experimental

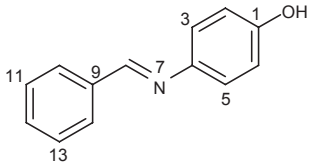
General experimental procedures

All reagents used in the experiments were obtained from commercial sources and used without purification. The determination of compounds melting point (uncorrected) was carried out in an MQAPF-302 apparatus. Infrared spectra were recorded on Perkin–Elmer Spectrum One spectrophotometer. NMR spectra were recorded in a Bruker Avance DRX-200 spectrometer. Chemical shifts are reported in δ units downfield from TMS, and J values are given in Hertz. Elemental analyses were performed by using a CHN Perkin–Elmer 2400 analyzer (Boston, MA, USA).

Synthesis of aldimines

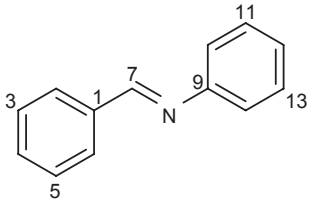
The aldimines **3**–**8** were prepared from the condensation of appropriate amines with benzaldehydes in toluene using a Dean Stark apparatus according to methods previously described (19,20). Solvent was evaporated, and the compounds were purified by crystallization.

4-(benzylideneamino)phenol (3)



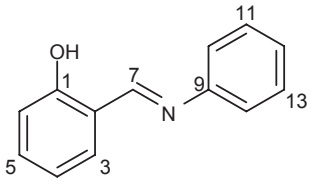
Mp: 184–186 °C [(21): 184–185 °C)]. IR (neat, cm^{-1}) ν: 3062, 3008, 2918, 2789, 2720, 2666, 2578, 2466, 1618, 1604, 1587, 1506, 1450, 1372, 1274, 1235, 1182, 1172, 1106, 978, 835, 823, 753, 717, 685. ^{1}H-NMR [$(CD_3)_2SO$, 200 MHz]: δ 6.84 (d, 2H, $J_{2,3}$ = $J_{6,5}$ = 8.7, H_2, H_6), 7.23 (d, 2H, $J_{3,2}$ = $J_{5,6}$ = 8.7, H_3, H_5), 7.45–7.59 (m, 3H, H_{11}, H_{12}, H_{13}), 7.85–7.99 (m, 2H, H_{10}, H_{14}), 8.62 (s, 1H, H_8), 9.57 (s, 1H, OH). ^{13}C-NMR [$(CD_3)_2SO$, 50 MHz]: 115.8 (C_2, C_6), 122.5 (C_3, C_5), 128.3, 128.8 (C_{10}, C_{11}, C_{13}, C_{14}), 130.9 (C_{12}), 136.5 (C_9), 142.6 (C_4), 156.3 (C_1), 157.2 (C_8). Elemental Analysis for ($C_{13}H_{11}NO$): calculated, C: 79.16, H: 5.62, N: 7.10, O: 8.11; found: C: 79.24, H: 5.46, N: 7.09, O: 8.21.

N-benzylideneaniline (4)



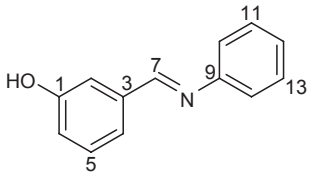
Mp: 50–51 °C [(21): 53–54 °C)]. IR (neat, cm^{-1}) ν: 3061, 3029, 2891, 1625, 1590, 1577, 1483, 1450, 1366, 1192, 1170, 1073, 976, 906, 867, 754, 690. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 7.10–7.61 (m, 8H, H_3, H_4, H_5, H_{10}, H_{11}, H_{12}, H_{13}, H_{14}), 7.81–8.00 (m, 2H, H_2, H_6), 8.44 (s, 1H, H_7). ^{13}C-NMR ($CDCl_3$, 50 MHz): 121.1 (C_{10}, C_{14}), 126.1 (C_{12}), 129.0, 129.3 (C_2, C_3, C_5, C_6, C_{11}, C_{13}), 131.6 (C_4), 136.4 (C_1), 152.3 (C_9), 160.6 (C_7). Elemental Analysis for ($C_{13}H_{11}N$): calculated, C: 86.15, H: 6.12, N: 7.73; found: C: 86.07, H: 6.08, N: 7.74.

2-[(phenylimino)methyl]phenol (5)



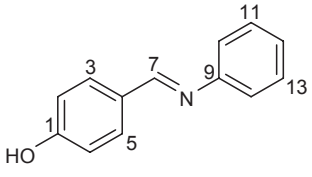
Mp: 50–51 °C [(21): 51–52 °C)]. IR (neat, cm^{-1}) ν: 3058, 2991, 1614, 1589, 1567, 1484, 1458, 1403, 1361, 1280, 1182, 1171, 1150, 908, 834, 757, 687. ^{1}H-NMR ($CDCl_3$, 200 MHz): δ 6.85–7.08 (m, 2H, H_4, H_6), 7.17–7.54 (m, 7H, H_3, H_5, H_{10}, H_{11}, H_{12}, H_{13}, H_{14}), 8.59 (s, 1H, H_7), 13.28 (s, 1H, OH). ^{13}C-NMR ($CDCl_3$, 50 MHz): 117.4 (C_6), 119.3 (C_4), 119.4 (C_2), 121.4 (C_{10}, C_{14}), 127.1 (C_{12}), 129.6 (C_{11}, C_{13}), 132.5 (C_3), 133.3 (C_5), 148.7 (C_9), 161.3 (C_1), 162.9 (C_7). Elemental Analysis for ($C_{13}H_{11}NO$): calculated, C: 79.16, H: 5.62, N: 7.10, O: 8.11; found: C: 79.43, H: 5.52, N: 7.15, O: 7.90.

3-[(phenylimino)methyl]phenol (6)



Mp: 90–91 °C [(22): 90 °C)]. IR (neat, cm^{-1}) ν: 3051, 2915, 2691 2557, 2444, 1621, 1596, 1581, 1447, 1389, 1274, 1221, 1207, 1166, 1156, 1080, 857, 782, 756, 691, 681. ^{1}H-NMR [$(CD_3)_2SO$, 200 MHz]: δ 6.89–7.01 (m, 1H, H_6), 7.19–7.47 (m, 8H, H_2, H_4, H_5, H_{10}, H_{11}, H_{12}, H_{13}, H_{14}), 8.52 (s, 1H, H_7), 9.73 (s, 1H, OH). ^{13}C-NMR [$(CD_3)_2SO$, 50 MHz]: 114.3 (C_2), 118.8 (C_6), 120.4 (C_4), 121.0 (C_{10}, C_{14}), 125.9 (C_{12}), 129.2 (C_{11}, C_{13}), 129.9 (C_5), 137.4 (C_3), 151.5 (C_9), 157.7 (C_1), 160.7 (C_7). Elemental Analysis for ($C_{13}H_{11}NO$): calculated, C: 79.16, H: 5.62, N: 7.10, O: 8.11; found: C: 79.50, H: 5.14, N: 7.10, O: 8.27.

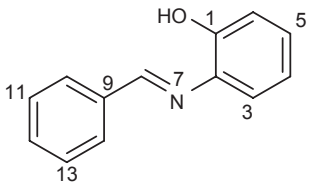
4-[(phenylimino)methyl]phenol (7)



Mp: 194–195 °C [(21): 195–196 °C)]. IR (neat, cm^{-1}) ν: 3047, 2867, 2795, 2733, 2673, 2568, 2478, 1602, 1574, 1515, 1485,

1443, 1387, 1284, 1241, 1189, 1164, 978, 936, 840, 758, 723, 689. ^{1}H-NMR [$(CD_3)_2SO$, 200 MHz]: δ 6.90 (d, 2H, $J_{2,3}$ = $J_{6,5}$ = 8.6, H_2, H_6), 7.13–7.45 (m, 5H, H_{10}, H_{11}, H_{12}, H_{13}, H_{14}), 7.78 (d, 2H, $J_{3,2}$ = $J_{5,6}$ = 8.6, H_3, H_5), 8.45 (s, 1H, H_7), 10.15 (s, 1H, OH). ^{13}C-NMR [$(CD_3)_2SO$, 50 MHz]: 115.7 (C_2, C_6), 120.9 (C_{10}, C_{14}), 125.3 (C_{12}), 127.5 (C_4), 129.2, 130.7 (C_3, C_5, C_{11}, C_{13}), 152.0 (C_9), 160.0 (C_7), 160.7 (C_1). Elemental Analysis for ($C_{13}H_{11}NO$): calculated, C: 79.16, H: 5.62, N: 7.10, O: 8.11; found: C: 78.45, H: 5.29, N: 6.66, O: 7.45.

2-(benzylideneamino)phenol (8)



Mp: 89–90 °C [(21): 89–90 °C)]. IR (neat, cm^{-1}) ν_: 3323, 3020, 2902, 1625, 1584, 1574, 1481, 1450, 1380, 1358, 1285, 1249, 1194, 1167, 1147, 1026, 967, 874, 850, 790, 763, 733, 688. ^{1}H-NMR [$(CD_3)_2SO$, 200 MHz]: δ 6.85 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 7.7, H_4), 6.90 (d, 1H, $J_{6,5}$ = 7.7, H_6), 7.11 (t, 1H, $J_{5,4}$ = $J_{5,6}$ = 7.7, H_5), 7.22 (d, 1H, $J_{3,4}$ = 7.7, H_3), 7.46–7.59 (m, 3H, H_{11}, H_{12}, H_{13}), 7.98–8.13 (m, 2H, H_{10}, H_{14}), 8.72 (s, 1H, H_8), 9.04 (s, 1H, OH). ^{13}C-NMR [$(CD_3)_2SO$, 50 MHz]: 116.1 (C_3), 119.2, 119.5 (C_4, C_6), 127.4 (C_5), 128.7, 128.9 (C_{10}, C_{11}, C_{13}, C_{14}), 131.3 (C_{12}), 136.4 (C_9), 138.0 (C_2), 151.2 (C_1), 159.3 (C_8). Elemental Analysis for ($C_{13}H_{11}NO$): calculated, C: 79.16, H: 5.62, N: 7.10, O: 8.11; found: C: 79.19, H: 5.46, N: 7.12, O: 8.25.

Micro-organisms

Candida albicans ATCC 18804, *Candida tropicalis* ATCC 750, *Candida krusei* ATCC 20298, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 2001, *Aspergillus fumigatus* ATCC 16913, *A. nomius* ATCC 15546, and *C. neoformans* ATCC 24067 were obtained from the American Type Culture Collection (Manassas, VA, USA), while *A. flavus* IMI 190443 was acquired from the International Mycological Institute (IMI, England). Clinical isolates of *Candida dubliniensis* (Cd22, Cd23, Cd25, Cd27, Cd28, and Cd29), *A. clavatus*, *A. tamarii*, *A. fumigatus,* and *A. terreus* were also used.

All strains are maintained in the Laboratory of Mycology of the Institute of Biological Sciences at the Federal University of Minas Gerais (Brazil) on slopes of Sabouraud dextrose agar (SDA, Difco, Detroit, MI, USA) or potato dextrose agar (PDA; Difco) and subcultured every 2 months.

Susceptibility assay

The minimum inhibitory concentration (MIC) for each synthesized compound was determined for yeasts and filamentous fungi according to the protocols M27-A2 (NCCLS, 2002) (23) and M38-A (NCCLS, 2002) (24), respectively.

All compounds (**3**–**8**) were primarily dissolved in dimethylsulfoxide (DMSO) and then diluted in $NaHCO_3$-free RPMI-1640 medium (INLAB, Diadema, SP, Brazil) buffered with 165 mM morpholine propanesulfonic acid (MOPS, pH 7.0) and supplemented with 4 mM L-glutamine. The final concentration of DMSO was 5% and did not affect fungal growth as attested for the control treatment. Each compound was tested at concentrations ranging from 0.5 to 256 μg/mL, and fluconazole was used as a positive control.

Yeast species were cultured on SDA for 24 or 48 h, while the filamentous ones were cultured on PDA for 7 days. Fungal inoculum concentrations were adjusted to 1–5 $\times$ 10^3 cells/mL for yeasts and 0.4–5 $\times$ 10^4 cells/mL for filamentous fungi and diluted two-fold in the incubation medium. Three independent experiments were performed each done in duplicate. The MIC end-point for test compounds was defined as the lowest concentration of tested compound necessary to abolish the micro-organism growth. Minimal inhibitory concentration value for fluconazole was defined as the lowest concentration that prevented the fungal growth by 50%.

Biofilm assay

Biofilm assay was performed as described by Ramage and co-workers (2001) (25) with some modifications. Briefly, *C. neoformans* strain was inoculated in yeast peptone dextrose medium (YPD; Difco) and then incubated at 37 °C under stirring at 100 rpm for 24 h. Cells were centrifuged, washed three times in sterile phosphate-buffered saline (PBS) solution, and re-suspended in RPMI-1640 medium. Cells (100 μL of 10^6 units/mL) were transferred to a microplate and incubated at 37 °C for 24 h to allow biofilm formation. Non-adherent cells were carefully removed and the remainder ones washed with sterile PBS solution. An equal volume of compounds **3**, **8,** or fluconazole solutions (at MIC values) was added to yeast cells and the system incubated at 37 °C for 48 h. Formed biofilms were washed three times with sterile PBS solution following the metabolism analysis.

2,3-bis(2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay

Cell viability of cryptococcal biofilms was determined semi-quantitatively by the XTT reduction assay as reported elsewhere (13). Briefly, newly formed biofilms were incubated with 50 μL XTT solution (1 mg/mL in PBS) and 4 μM menadione (1 mM in acetone) in the dark at 37 °C for 5 h. The supernatant was then transferred to a new microplate and analyzed spectrophotometrically at 492 nm to determine cell viability.

Statistical analysis

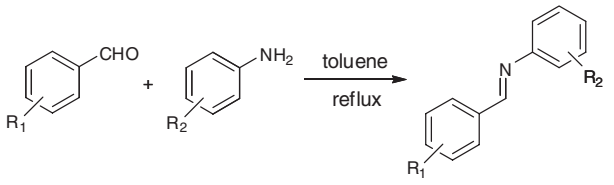
Statistical analysis was carried out by using the SIGMA STAT Software. Experimental data were submitted to one-way analysis of variance (ANOVA). Pairwise comparisons between control and drug-exposed fungi were carried out with Tukey's test. p-value of less than 0.05 was considered statistically significant.

Results and Discussion

All aryl aldimines (compounds **3**–**8**) were obtained in good yields (Table 1). The synthesized compounds were fully characterized by elemental analysis, IR, ^{1}H, and ^{13}C NMR spectroscopy, and the data were consistent with those reported in the literature (21,22).

The minimum inhibitory concentration (MIC) of aryl aldimines **3**–**8** necessary to completely suppress the growth of fungal strains is shown in Table 2. The aryl aldimines **3** and **8** were the most effective compounds inhibiting the growth of all fungi studied, except *A. nomius* (ATCC 15546), *A. flavus* (IMI 190443), and *A. terreus* (clinical isolate).

Table 1: Synthesis of aryl aldimines **3**–**8**



Compound	R^1	R^2	Time (h)	Yields[a] (%)
3	H	4-OH	16	83
4	H	H	36	65
5	2-OH	H	22	83
6	3-OH	H	20	84
7	4-OH	H	16	81
8	H	2-OH	24	63

[a]Isolated yields.

Among the *Candida* species, *C. dubliniensis* Cd22 (clinical isolate) was the most sensitive to compound **3** whose MIC value was 64.0 μg/mL. The growth of *C. glabrata* ATCC 2001 and *C. tropicallis* ATCC 750 was also affected by compound **3** that exhibited MIC values around 99.0 μg/mL.

Eight *Candida* species of eleven strains were inhibited by the aryl aldimine **8** (Table 2). The MIC values for this compound ranged from 124.5 to 176.0 μg/mL. At the studied experimental conditions, no detectable growth-inhibiting activity was observed for aryl aldimines **4**–**7** against *Candida* species.

Aspergillus fumigatus ATCC 16913 and clinical isolate, *A. clavatus* (clinical isolate), and *A. tamarii* (clinical isolate) were the most sensitive to aryl aldimines **3** and **8**, among the *Aspergillus* species. Notably, aryl aldimine **8** (MIC = 10.0 μg/mL) was 6-fold more potent than the reference drug fluconazole in inhibiting the growth of *A. clavatus* (clinical isolate). Also, the MIC value for compound **8** against *A. fumigatus* ATCC 16913 was three-fold lower than that of fluconazole. The aryl aldimine **8** was also more potent than fluconazole in the growth inhibition of the clinical isolates of *A. tamari* and *A. fumigatus* (Table 2). Even though the most expressive results against *Aspergillus* species were obtained from the cell treatment with compound **8**, promising results were achieved from aryl aldimine **3** treatment of *A. fumigatus* ATCC 16913 and clinical isolate, and *A. clavatus* and *A. tamarii* clinical isolates. The aryl aldimines **4**–**7** were also not active against *Aspergillus* species (Table 2). The stability of such compounds was checked by incubating each compound in RPMI medium (pH 7.0) containing DMSO 5% for 3 h at 37 °C in the absence of fungus. The UV–vis spectrum for each compound revealed that only **5** underwent decomposition (data not

Table 2: Minimal inhibitory concentration (MIC[a]; μg/mL) of the aryl aldimines **3**–**8** against fungal strains

	Aryl aldimines						
Micro-organism	**3**	**4**	**5**	**6**	**7**	**8**	Flu[b]
Candida albicans ATCC 18804	157.5	>256	>256	>256	>256	128.0	2.0
Candida tropicalis ATCC 750	99.5	>256	>256	>256	>256	256.0	2.0
Candida krusei ATCC 20298	157.5	>256	>256	>256	>256	124.5	32.0
Candida parapsilosis ATCC 22019	125.0	>256	>256	>256	>256	256.0	1.0
Candida glabrata ATCC 2001	99.0	>256	>256	>256	>256	176.0	1.0
**Candida dubliniensis* Cd 22	64.0	>256	>256	>256	>256	128.0	0.12
**C. dubliniensis* Cd 23	128.0	>256	>256	>256	>256	128.0	0.18
**C. dubliniensis* Cd 25	128.0	>256	>256	>256	>256	128.0	0.18
**C. dubliniensis* Cd 27	128.0	>256	>256	>256	>256	256.0	0.25
**C. dubliniensis* Cd 28	128.0	>256	>256	>256	>256	128.0	0.12
**C. dubliniensis* Cd 29	128.0	>256	>256	>256	>256	128.0	0.25
Aspergillus fumigatus ATCC 16913	128.0	>256	>256	>256	>256	20.0	64.0
Aspergillus nomius ATCC 15546	>256	>256	>256	>256	>256	256.0	>64
Aspergillus flavus IMI 190443	>256	>256	>256	>256	>256	>256	>64
**Aspergillus clavatus*	128.0	>256	>256	>256	>256	10.0	64.0
**Aspergillus tamarii*	128.0	>256	>256	>256	>256	64.0	>64
**Aspergillus fumigatus*	128.0	>256	>256	>256	>256	64.0	>64
**Aspergillus terreus*	>256	>256	>256	>256	>256	>256	>64
Cryptococcus neoformans ATCC 24067	30.0	>256	88.0	>256	256.0	8.0	16.0

[a]Data are geometric means of three independent experiments each carried out in duplicate.
[b]Flu stands for fluconazole (positive control).
Fungal strains marked with an asterisk (*) are clinical isolate.

shown). Indeed, imines derived from salicylidene aldehydes, like **5**, are known to be easily hydrolyzed in aqueous medium (26). A six-membered conformer is established between the *o*-hydroxyl group and the nitrogen atom from C=N bond, which activates the addition of a nucleophile (e.g., H_2O) to the carbon atom at C=N bond (26). Thus, the lack of activity for compounds **4**, **6,** and **7** cannot be attributed to a possible decomposition in the medium.

The growth of *C. neoformans* ATCC 24067 was inhibited by treatment with the aryl aldimines **3**, **5,** and **8** (Table 2). The moderate-to-low activity of **5** is probably derived from its decomposition product(s). Interestingly, compound **8** was twice as potent as fluconazole against *C. neoformans* ATCC 24067. The aryl aldimines **3** and **5** exhibited promising results against *C. neoformans* ATCC 24067 reaching MIC values as low as 30.0 and 88.0 μg/mL, respectively.

Cryptococcus neoformans is opportunistic and accounts for a large number of deaths in Brazil because of systemic mycosis with underlying cause of AIDS (27). The efficacy of antifungal drugs used to treat cryptococcal infections may be compromised by fungal biofilm formation. The promising results obtained with the aryl aldimines **3** and **8** prompted us to further investigate their effect on the metabolism of *C. neoformans* ATCC 24067 mature biofilm. Compounds **3** and **8** at their MIC values (30.0 and 8.0 μg/mL, respectively) impaired the viability of *C. neoformans* ATCC 24067 mature biofilm (Figure 2). Compounds **3** and **8** inhibited the metabolic activity of *C. neoformans* biofilm by 43% and 49%, respectively. Fluconazole at 16.0 μg/mL inhibited the biofilm metabolic activity by 85%. Although compounds **3** and **8** were less effective than fluconazole, the results suggest that these aryl aldimines **3** and **8** are promising prototypes for the design of new *C. neoformans* anti-biofilm agents.

Conclusions

The aryl aldimines **3** and/or **8** showed to have promising antifungal activities against *C. albicans* ATCC 18804, *C. glabrata* ATCC 2001, *C. dubliniensis* Cd 22 CI, *A. fumigatus* ATCC 16913, *A. clavatus* CI, *A. tamarii* CI, *A. fumigatus* CI, and *C. neoformans* ATCC 24067. These compounds were also able to reduce by 40–50% the metabolic activity of *C. neoformans* ATCC 24067 mature biofilm. In conjunction, these results point out aryl aldimine **3** and **8** as lead

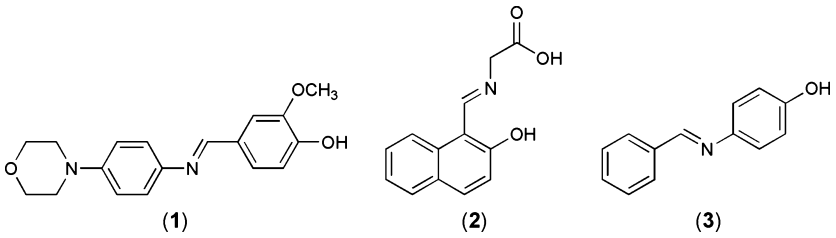


Figure 1: Examples of aryl aldimines of recognized antifungal activities.

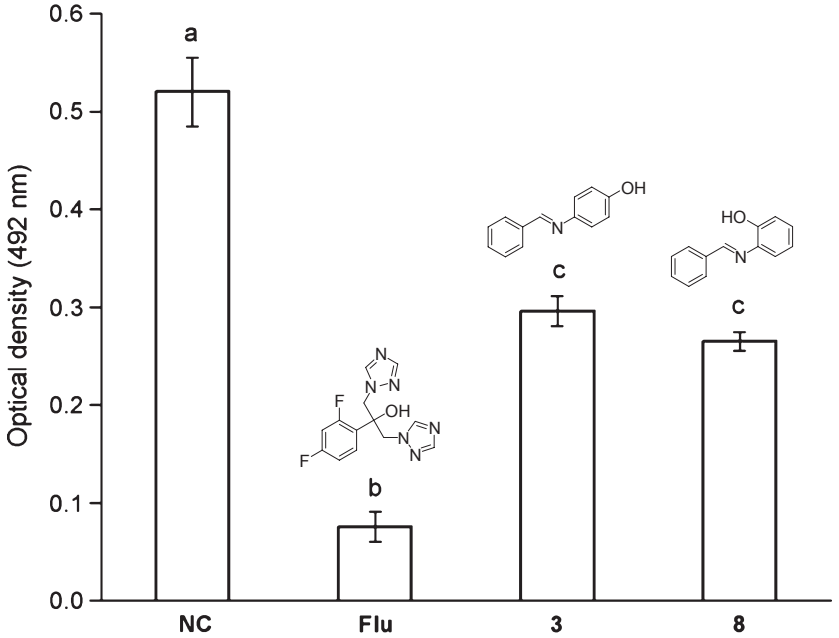


Figure 2: Effect of the aryl aldimines **3** and **8** on the metabolic activity of *Cryptococcus neoformans* biofilms. Biofilms were exposed to the aryl aldimines **3** and **8** at their minimal inhibitory concentration values (30.0 and 8.0 μg/mL, respectively) for 24 h. Metabolic activity was assessed by XTT reduction assay. NC, biofilms incubated with RPMI-1640 medium; Flu, biofilms incubated with fluconazole at MIC value (16.0 μg/mL). Bars represent the means ± SE of three independent experiments carried out in duplicate. Different letters indicate significant difference ($p \leq 0.05$) in relation to results obtained for the negative control.

compounds for the development of new antifungal agents. To the best of our knowledge, this is the first report on the effect of aryl aldimines on the viability of *C. neoformans* ATCC 24067 biofilms.

Acknowledgments

The authors are grateful to Dr Luzia V. Modolo for critical reading of this manuscript. This work was funded by FAPEMIG, CAPES, and CNPq. ADF and MAR are supported by research fellowships from CNPq.

References

1. Sundriyal S., Sharma R.K., Jain R. (2006) Current advances in antifungal targets and drug development. Curr Med Chem;13: 1321–1335.
2. Andriole V.T. (1999) Current and future antifungal therapy: new targets for antifungal agents. J Antimicrob Chemother;44:151–162.
3. Ascioglu S., Rex J.H., de Pauw B., Bennett J.E., Bille J., Crokaert F., Denning D.W. *et al.* (2002) Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis;34:7–14.
4. Nucci M., Marr K.A. (2005) Emerging fungal diseases. Clin Infect Dis;41:521–526.
5. Pappas P.G., Rex J.H., Sobel J.D., Filler S.G., Dismukes W.E., Walsh T.J., Edwards J.E. (2004) Guidelines for treatment of candidiasis. Clin Infect Dis;38:161–189.
6. Walsh T.J., Gonzalez C., Roilides E., Mueller B.U., Ali N., Lewis L.L., Whitcomb T.O., Marshall D.J., Pizzo P.A. (1995) Fungemia in children infected with the human immunodeficiency virus: new epidemiologic patterns, emerging pathogens, and improved outcome with antifungal therapy. Clin Infect Dis;20:900–906.
7. Girmenia C., Martino P., de Bernardis F., Gentile G., Boccanera M., Monaco M., Antonucci G., Cassone A. (1996) Rising incidence of *Candida parapsilosis* fungemia in patients with hematologic malignancies: clinical aspects, predisposing factors, and differential pathogenicity on the causative strains. Clin Infect Dis;23:506–514.
8. Pannuti C., Gingrich R., Pfaller M.A., Kao C., Wenzel R.P. (1992) Nosocomial pneumonia in patients having bone marrow transplant attributable mortality and risk factors. Cancer;69:2653–2662.
9. Kanj S.S., Welty-Wolf K., Madden J., Tapson V., Baz M.A., Davis R.D., Perfect J.R. (1996) Fungal infections in lung and heart-lung transplant recipients. Report of 9 cases and review of the literature. Medicine;75:142–156.
10. Denning D.W. (1998) Invasive aspergillosis. Clin Infect Dis;26:781–805.
11. Walsh T.J., Pizzo P.A. (1988) Nosocomial fungal infections: a classification for hospital-acquired fungal infections and mycoses arising from endogenous flora or reactivation. Annu Rev Microbiol;42:517–545.
12. Lin X. (2009) *Cryptococcus neoformans*: morphogenesis, infection, and evolution. Infect Genet Evol;9:401–416.
13. Martinez L.R., Casadevall A. (2006) Susceptibility of *Cryptococcus neoformans* biofilms to antifungal agents *in vitro*. Antimicrob Agents Chemother;50:1021–1033.
14. Przybylski P., Huczynski A., Pyta K., Brzezinski B., Bartl F. (2009) Biological properties of Schiff bases and azo derivatives of phenols. Curr Org Chem;13:124–148.
15. da Silva C.M., da Silva D.L., Modolo L.V., Alves R.B., Martins C.V.B., de Resende M.A., de Fátima A. (2011) Schiff bases: a short review of their biological activities. J Adv Res;2:1–8.
16. Panneerselvam P., Nair R.R., Vijayalakshmi G., Subramanian E.H., Sridhar S.K. (2005) Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. Eur J Med Chem;40:225–229.
17. Sakiyan I., Loğoğlu E., Arslan S., Sari N., Şakiyan N. (2004) Antimicrobial activities of *N*-(2-hydroxy-1-naphthalidene)-amino acid (glycine, alanine, phenylalanine, histidine, tryptophane) Schiff bases and their manganese(III) complexes. Biometals;17:115–120.
18. Toenjes K.A., Munsee S.M., Ibrahim A.S., Jeffrey R., Edwards J.E. Jr, Johnson I. (2005) Small-molecule inhibitors of the budded-to-hyphal-form transition in the pathogenic yeast *Candida albicans*. Antimicrob Agents Chemother;49:963–972.
19. Han S.Y., Inoue H., Terada T., Kamoda S., Saburi Y., Sekimata K., Saito T., Kobayashi M., Shinozaki K., Yoshida S., Asami T. (2003) *N*-benzylideneaniline and *N*-benzylaniline are potent inhibitors of lignostilbene-α,β-dioxygenase, a key enzyme in oxidative cleavage of the central double bond of lignostilbene. J Enzyme Inhib Med Chem;18:279–283.
20. Seomoon D., Jaemyung A., Lee P.H. (2009) Synthetic method for the preparation of 2-aminomethyl-1,3-diene derivatives through indium-mediated 1,3-butadien-2-ylation of imines. Org Lett;11:2401–2404.
21. Huneck S., Schreiber K., Grimmecke H.D. (1984) Schiff's bases and derivated secondary amines as plant growth inhibitors. J Plant Growth Regul;3:75–84.
22. Joergensen K.A., Shabana R., Scheibye S., Lawesson S.O. (1980) Studies on organophosphorus compounds. XXXII. Reactions of 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide with compounds containing the N $\rightarrow$ O function. Bull Soc Chem Belg;89:247–253.
23. Clinical and Laboratory Standards Institute. (2002) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved standard, 2nd edn. NCCLS document M27-A2. Villanova: Clinical and Laboratory Standards Institute.
24. Clinical and Laboratory Standards Institute. (2002) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. Approved standard, 2nd edn. NCCLS document M27-A2. Villanova: Clinical and Laboratory Standards Institute.
25. Ramage G., Walle K.V., Wickes B.L., López-Ribot J.L. (2001) Biofilm formation by *Candida dubliniensis*. J Clin Microbiol;39: 3234–3240.
26. Reeves R.L. (1965) On the mechanism, substituent effects, and intramolecular catalysis in Schiff base hydrolysis. J Org Chem; 30:3129–3135.
27. Prado M., da Silva M.B., Laurenti R., Travassos L.R., Taborda C.P. (2009) Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006. Mem Inst Oswaldo Cruz;104:513–521.