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SYNTHESIS AND ANTIFUNGAL ACTIVITY OF NEW O-ALKYLAMIDOXIMES

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Abstract

The continuous prospection for molecules that may be useful in the development of new therapeutic agents is a highly relevant issue, mainly because the launch of new drugs on the market does not accompany the emergence of new resistant microorganisms. In this context, this work describes the synthesis of new O-alkylamidoximes and the evaluation of its antifungal activity. The new O-alkylamidoximes were prepared using easy synthetic protocols and tested against three Candida species using the broth microdilution method. The synthesized compounds were obtained in moderate to good yields in high purity and without any observable decomposition. All tested compounds shown moderate antifungal activity against at least one strain of Candida. Despite the moderate activity of the new compounds, this was the first report involving the antifungal activity of O-alkylamidoximes. In view of the low chemotherapy arsenal and the development of fungal strains resistant to traditional antifungal agents, the present study opens new possibilities for the preparation of a new class of more active antifungal agents.

Keywords: Antifungal. *Candida*. *O*-alkylation. *O*-alkylamidoximes.

1. Introduction

Fungi are widespread in the environment and are found in a variety of habitats (Prasad and Kapoor 2004). These microorganisms make up the normal human microbiota and are commonly found in the skin, gastrointestinal, respiratory, and genitourinary tracts (Kapitan et al. 2019). However, in immunodeficient individuals such as transplanted (Eades and Armstrong-James 2019) and HIV-infected (Wang et al. 2017), the contact with pathogenic fungi (Capote et al. 2016) can lead to severe infections. Thus, fungal infections have been a major concern for health regulators, special due to the considerable morbidity and mortality rates, contributing factors to the increased health costs (Silva et al. 2012).

Among the different etiological agents involved in this type of pathology, *Candida* spp. are the most prevalent, representing approximately 19% of all infections worldwide in intensive care units (Irfan et al. 2017). It is also estimated that around 25 to 75% of healthy individuals to have *Candida* spp colonies in their normal microbiota (Costello et al. 2009). However, different species of this genus are often identified as the cause of invasive infections in humans, with special attention to *C. albicans* due to its prevalence in both

healthy patients and morbid individuals. In addition, *C. albicans* is present in approximately 70% of clinical isolates, being the number of cases generated by other *Candida* species (examples: *C. glabrata*, *C. tropicalis,* and *C. parapsilosis*) continuously growing (Kung et al. 2016).

Associated with these problems are the events of resistance of various microorganisms to antifungal agents (Berman and Krysan 2020). Currently, there is a major concern about the growing cases of microbial resistance worldwide, since, in the absence of efficient antimicrobial agents, it is estimated that incurable diseases will be responsible for about 10 million casualties by 2050 (De Kraker et al. 2016). Thus, the continuous prospection for molecules that may be useful in the development of new therapeutic agents is a highly relevant issue and has been encouraged, mainly due to the appearance of new drugs on the market not to follow the appearance of new resistant microorganisms (Pfaller 2012; Lee and Lee 2018).

In addition to the events of antimicrobial resistance, the limited therapeutic arsenal, the concern about toxicity, drug interactions, and low bioavailability presented by current antifungal agents are issues that deserve attention (Cavaleiro et al. 2006; Spitzer et al. 2017; Wiederhold 2017; Costa-de-Oliveira and Rodrigues 2020). The scenario stimulates the development of new molecules to address these problems. However, the development of new antifungals is challenging, since fungi are eukaryotic organisms which, when compared to human host cells, present only a few different targets (Mccarthy et al. 2017). In this work, *O*-alkylamidoximes, structurally simpler molecules when compared to other antifungal agents have virtually untapped biological potential. Reports of the biological activities for this class of molecules are rare, however, they can act as antipneumocystic (Boykin et al. 1996), antiplatelet (Rehse and Brehme 1998), antithrombotic (Rehse and Brehme 1998), antibacterial (Zhan-Tao et al. 2005), and as poly (ADP-ribose) polymerase-1 (PARP-1) enzyme inhibitors (Szabados et al. 2000). In this context, in this work is described the synthesis of new *O*-alkylamidoximes, and the evaluation of its antifungal activity against three different *Candida* species.

2. Material and Methods

Reagent materials and equipment

The solvents were distilled before use as reported in the literature (Armarego 2017). Ethanol was dried by distillation from metallic magnesium. Ethyl acetate and hexane were distilled using a vigreux column. All commercially available reagents were used as received. The reactions were monitored by thin-layer chromatography (TLC) using different eluent systems. Compounds were visualized with UV light. Column chromatographic purification was performed using silica gel 60 (70-230 mesh) unless indicated otherwise (Still et al. 1978). All compounds purified by crystallization or chromatography were pure enough for use in other experiments.

The IR spectra were recorded on a Fourier Spectrum 400 FT-IR/FT-NIR Spectrometer Model Perkin Elmer, the samples being prepared as KBr pellet or thin films. The carbon, hydrogen, and nitrogen contents of the compounds were determined by the Dynamic Flash Combustion technique, in a CHNS-O elementary analyzer, CE Instruments, model EA 1110. ^{1}H NMR data were recorded at 400 MHz using a Varian *UNITY PLUS* spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian *Unity Plus*-400 spectrometer using $CDCl_3$ or DMSO-d_6 as the solvents, and calibrated for the solvent signal and tetramethylsilane (TMS) as internal reference. Coupling constants (*J*) were reported in Hertz (Hz).

General procedure for the synthesis of Amidoximes 2a-h

The synthesis of amidoximes 2a-h was based on the methodology previously described in the literature (Barros et al. 2011). To a flask containing hydroxylamine hydrochloride (2.08 g, 30 mmol) and sodium carbonate (1.6 g, 15 mmol) at 25°C was added distilled water (30 mL). The mixture was stirred and then a solution of the appropriate nitrile 1a-h (10 mmol) in ethanol (40 mL) was added dropwise. The reaction mixture was placed in an ultrasonic bath at 55±5 °C and monitored by TLC. After completion, the solvents were removed *in vacuo*. The residual solid was extracted with ethyl acetate (3 x 25 mL) and the combined organic phases dried over anhydrous Na_2SO_4. Filtration, following removal of the solvent under

reduced pressure, provided the corresponding amidoximes 1a-h which were purified by crystallization using hexane/chloroform (10:90) system.

(Z)-N'-hydroxybenzimidamide (3a): white solid: mp 74-76 °C; IR (KBr pellet): ν_{max} 687, 768, 926, 1107, 1384, 1446, 1499, 1590, 1645, 2361, 2893, 3059, 3212, 3356, 3448, cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5,81 (2H, s, NH_2), 7.38-7.36 (3H, m, H_{Aryl}), 7.69-7.67 (2H, m, H_{Aryl}), 9.63 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 125.4, 128.1, 128.9, 133.4, 150.8. Compound data are in accordance with the literature (Ozcan et al. 2013; Andrade et al. 2016).

(Z)-N'-hydroxy-2-methylbenzimidamide (3b): white solid: mp 133-135 °C; IR (KBr pellet): ν_{max} 653, 722, 770, 903, 1107, 1374, 1437, 1580, 1651, 2921, 3152, 3197, 3363, 3479 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.34 (3H, s, CH_{3aryl}), 5.70 (2H, s, NH_2), 7.22-7.16 (2H, m, H_{Aryl}), 7.28-7.25 (2H, m, H_{Aryl}), 9.30 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.7, 125.3, 128.4, 128.8, 130.1, 134.3, 136.2, 152.3. Compound data are in accordance with the literature (Andrade et al. 2016; Tarasenko et al. 2017).

(Z)-N'-hydroxy-3-methylbenzimidamide (3c): white solid: mp 133-135 ºC; IR (KBr pellet): ν_{max} 700, 793, 892, 931, 1085, 1389, 1586, 1647, 2360, 2921, 3039, 3200, 3357, 3454 cm^{-1}; ^1H NMR(400 MHz, DMSO-d_6) δ 2.32 (3H, s, CH_{3aryl}), 5.75 (2H, s, NH_2), 7.21-7.17 (2H, m, H_{Aryl}), 7.28-7.27 (2H, m, H_{Aryl}), 9.57 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 21.1, 122,6, 125.9, 129.7, 129.5, 133.3, 137.1, 150.9. Compound data are in accordance with the literature (Andrade et al. 2016).

(Z)-N'-hydroxy-4-methylbenzimidamide (3d): white solid: mp 144-146 ºC; IR (KBr pellet): ν_{max} 747, 823, 936, 1099, 1391, 1418, 1588, 1664, 2916, 3049, 3367, 3500 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 2.31 (3H, s, CH_{3aryl}), 5.73 (2H, s, NH_2), 7.17 (2H, d, J = 8.2 Hz, H_{Aryl}), 7.56 (2H, d, J = 8.2 Hz, H_{Aryl}), 9.52 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.8, 125.3, 128.6, 130.5, 138.2, 150.8, Compound data are in accordance with the literature (Andrade et al. 2016; Tarasenko et al. 2017).

(Z)-4-chloro-N'-hydroxybenzimidamide (3e): white solid: mp 128-129 ºC; IR (KBr pellet): ν_{max} 722, 840, 920, 1087, 1380, 1497, 1589, 1655, 1918, 2361, 2893, 3053, 3152, 3346, 3468 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5.86 (2H, s, NH_2), 7.43 (2H, d, J = 8.6 Hz, H_{Aryl}), 7.69 (2H, d, J = 8.6 Hz, H_{Aryl}), 9.73 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 127.1, 128.1, 133.2, 133.4, 149.9. Compound data are in accordance with the literature (Ozcan et al. 2013; Andrade et al. 2016; Tarasenko et al. 2017).

(Z)-4-bromo-N'-hydroxybenzimidamide (3f): white solid: mp 140-141 ºC; IR (KBr pellet): ν_{max} 720, 833, 921, 1010, 1069, 1380, 1492, 1583, 1657, 1914, 2890, 3050, 3108, 3356, 3472 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 5,86 (2H, s, NH_2), 7,57 (2H, d, J = 8,6 Hz, H_{Aryl}), 7,63 (2H, d, J = 8,6 Hz, H_{Aryl}), 9,74 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6 δ 122.1, 127.4, 131.0, 132.5, 149.9. Compound data are in accordance with the literature (Li et al. 2013).

(Z)-N'-hydroxy-4-nitrobenzimidamide (3g): white solid: mp 180-181 ºC; IR (KBr pellet): ν_{max} 700, 810, 860, 922, 1105, 1337, 1512, 1596, 1657, 2844, 3114, 3180, 3351 cm^{-1}; ^1H MNR (400 MHz, DMSO-d_6) δ 6,08 (2H, s, NH_2), 7,97 (2H, d, J = 9,0 Hz, H_{Aryl}), 8,25 (2H, d, J = 9,0 Hz, H_{Aryl}), 10,16 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 123.4, 126.4, 139.5, 147.5, 149.4. Compound data are in accordance with the literature (Li et al. 2013; Ozcan et al. 2013).

(Z)-N'-hydroxyisonicotinimidamide (3h): white solid: mp 178-179 ºC; IR (KBr pellet): ν_{max} 661, 946, 1085, 1218, 1383, 1413, 1539, 1591, 1630, 2750, 2795, 3051, 3156, 3308, 3460 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ6.02 (2H, s, NH_2), 7.67 (2H, d, J = 6.0 Hz, $H_{Heteroaryl}$), 8.59 (2H, d, J = 6.0 Hz, $H_{Heteroaryl}$), 10.07 (1H, s, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 119.6, 140.5, 148.9, 149.7. Compound data are in accordance with the literature (Ozcan et al. 2013).

General procedure for preparing O-alkylamidoximes (3a-h)

To the flask was added the appropriate amidoxime 2a-h (0.5 mmol), sodium hydroxide (32 mg, 0.8 mmol), and DMSO (4 mL). The mixture was stirred for 5 minutes and then bromoacetaldehyde diethyl acetal (118.2 mg, 0.6 mmol) was added. The reaction mixture was stirred at room temperature and monitored by TLC [hexane/ethyl acetate (40:60)]. After completion, the reaction mixture was extracted with ethyl acetate (3 x 20 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude product was purified by a flash column chromatography [hexane/ ethyl acetate (95:5)] to yield compounds 3a-h.

(Z)-N'-(2,2-diethoxyethoxy)benzimidamide (3a): Colorless oil; IR (KBr pellet): ν_{max} 695, 772, 897, 1070, 1265, 1395, 1444, 1502, 1634, 1959, 2882, 2930, 2974, 3058, 3370, 3488 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 3.60 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 3.76 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 4.12 (2H, d, *J* = 5.6 Hz, -CH_2CH-), 4.87 (1H, t, *J* = 5.6 Hz, -CH_2CH-), 4.87 (2H, br s, NH_2), 7.44-7.37 (3H, m, H_{Aryl}), 7.64-7.62 (2H, m, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$), δ 15.4, 62.5, 73.5, 100.4, 125.9, 128.6, 129.9, 132.4,152.3. Compound data are in accordance with the literature (Veerman et al. 2016).

(Z)-N'-(2,2-diethoxyethoxy)-2-methylbenzimidamide (3b): Colorless oil; IR (KBr pellet): ν_{max} 726, 762, 889, 1066, 1387, 1446, 1632, 2880, 2930, 2973, 3354, 3482 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.26 (6H, t, *J* = 6.8 Hz, -CH_2CH_3), 2.44 (3H, s, Aryl-CH_3), 3.61 (2H, dq, *J* = 9.2 and 6.8 Hz, -CH_2CH_3), 3.76 (2H, dq, *J* = 9.2 and 6.8 Hz, CH_2CH_3), 4.07 (2H, d, *J* = 5.2 Hz, -CH_2CH-), 4.83 (2H, br s, NH_2), 4.85 (1H, t, *J* = 5.2 Hz, -CH_2CH), 7.23-7.18 (2H, m, H_{Aryl}), 7.31-7.27 (1H, m, H_{Aryl}), 7.38-7.35 (1H, m, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 19.6, 62.6, 73.3, 100.5, 125.8, 128.8, 129.5, 130.6, 132.5, 136.7, 153.0. Anal. calcd for $C_{14}H_{22}N_2O_3$: C, 63.13%; H, 8.33%; N, 10.52%. Obtained: C, 62.89%; H, 8.21%; N, 10.36%.

(Z)-N'-(2,2-diethoxyethoxy)-3-methylbenzimidamide (4c): Colorless oil; IR (KBr pellet): ν_{max} 703, 789, 896, 1070, 1389, 1588, 1633, 2361, 2878, 2929, 2974, 3366, 3486 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 2.37 (3H, s, CH_3), 3.61 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 3.74 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 4.11 (2H, d, *J* = 5.6 Hz, -CH_2CH-), 4.83 (2H, br s, NH_2), 4.86 (1H, t, *J* = 5.6 Hz, -CH_2CH), 7.29-7.21 (2H, m, H_{Aryl}), 7.46-7.39 (2H, m, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 21.3, 62.4, 73.4, 100.4, 122.9, 126.5, 128.5, 130.7, 132.3, 138.3, 152.6. Anal. calcd for $C_{14}H_{22}N_2O_3$: C, 63.13%; H, 8.33%; N, 10.52%. Obtained: C, 63.01%; H, 8.50%; N, 10.41%.

(Z)-N'-(2,2-diethoxyethoxy)-4-methylbenzimidamide (4d): white solid: mp 50-51 °C; IR (KBr pellet): ν_{max} 822, 930, 1062, 1303, 1396, 1462, 1520, 1622, 2362, 2967, 2930, 2973, 3358, 3471 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 2.37 (3H, s, Aryl-CH_3), 3.61 (2H, dq, *J* = 9.6 and 7.2 Hz, -CH_2CH_3), 3.76 (2H, dq, *J* = 9.6 and 7.2 Hz, -CH_2CH_3), 4.11 (2H, d, *J* = 5.6 Hz, -CH_2CH-), 4.46 (1H, t, *J* = 5.6, Hz, -CH_2CH), 4.85 (2H, br s, NH_2), 7.19 (2H, d, *J* = 8.4 Hz, H_{Aryl}), 7.52 (2H, d, *J* = 8.4 Hz, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 21.3, 62.4, 73.4, 100.4, 125.7, 129.2, 129.5, 139.9, 152.4. Anal. calcd for $C_{14}H_{22}N_2O_3$: C, 63.13%; H, 8.33%; N, 10.52%. Obtained: C, 62.99%; H, 8.54%; N, 10.63%.

(Z)-4-chloro-N'-(2,2-diethoxyethoxy)benzimidamide (4e): white solid: mp 43-44 ºC; IR (KBr pellet): ν_{max} 834, 927, 1063, 1301, 1403, 1497, 1620, 2662, 2869, 2935, 2977, 3349, 3462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 3.60 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 3.75 (2H, dq, *J* = 9.2 and 7.2 Hz, -CH_2CH_3), 4.10 (2H, d, *J* = 5.2 Hz, -CH_2CH-), 4.84 (1H, t, *J* = 5.2 Hz, -CH_2CH), 4.84 (2H, br s, NH_2), 7.36 (2H, d, *J* = 8.4 Hz, H_{Aryl}), 7.57 (2H, d, *J* = 8.4 Hz, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 62.4, 73.5, 100.3, 127.1, 128.8, 130.8, 135.9, 151.2. Anal. calcd for $C_{13}H_{19}ClN_2O_3$: C, 54.45%; H, 6.68%; N, 9.77%. Obtained: C, 54.51%; H, 6.77%; N, 9.59%.

(Z)-4-bromo-N'-(2,2-diethoxyethoxy)benzimidamide (4f): white solid: mp 55-57 ºC; IR (KBr pellet): ν_{max} 823, 837, 926, 1009, 1063, 1303, 1401, 1493, 1625, 2361, 2868, 2930, 2972, 3347, 3462 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 3.60 (2H, dq, *J* = 8.8 and 7.2 Hz, -CH_2CH_3), 3.75 (2H, dq, *J* = 8.8 and 7.2 Hz, -CH_2CH_3), 4.10 (2H, d, *J* = 5.6 Hz, -CH_2CH-), 4,84 (1H, t, *J* = 5,2 Hz, -CH_2CH-), 4.85 (2H, br s, NH_2), 7.51 (4H, m, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 62.4, 73.5, 100.3, 124.1, 127.4, 131.3, 131.7, 151.3. Anal. calcd for $C_{13}H_{19}BrN_2O_3$: C, 47.14%; H, 5.78%; N, 8.46%. Obtained: C, 47.22%; H, 5.71%; N, 8.37%.

(Z)-N'-(2,2-diethoxyethoxy)-4-nitrobenzimidamide (4g): Colorless oil; IR (KBr pellet): ν_{max} 698, 758, 858, 907, 1050, 1344, 1517, 1600, 1636, 2882, 2932, 2976, 3368, 3481 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 3.61 (2H, dq, *J* = 9.6 and 7.2 Hz, -CH_2CH_3), 3.76 (2H, dq, *J* = 9.6 and 7.2 Hz, -CH_2CH_3), 4.15 (2H, d, *J* = 5.2 Hz, -CH_2CH-), 4.84 (1H, t, *J* = 5.2 Hz, -CH_2CH-) 4.94 (2H, br s, 2H, NH_2), 7.82 (2H, d, *J* = 8.8 Hz, H_{Aryl}), 8.24 (2H, d, *J* = 8.8 Hz, H_{Aryl}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 62.4, 73.8, 100.2, 123.8, 126.6, 138.3, 148.6, 150.0. Anal. calcd for $C_{13}H_{19}N_3O_5$, C, 52.52%; H, 6.44%; N, 14.13%. Obtained: C, 52.69%; H, 6.36%; N, 14.21%.

(Z)-N'-(2,2-diethoxyethoxy)isonicotinimidamide (4h): Colorless oil; IR (KBr pellet): ν_{max} 835, 928, 1064, 1302, 1404, 1496, 1624, 2870, 2932, 2975, 3349, 3459 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (6H, t, *J* = 7.2 Hz, -CH_2CH_3), 3.59 (2H, dq, *J* = 9.6 and 7.2 Hz, 2H, -CH_2CH_3), 3.73 (2H, dq, *J* = 9.6 and 7.2 Hz, -CH_2CH_3), 4.11 (2H, d, *J* = 5.6 Hz, -CH_2CH), 4.83 (1H, t, *J* = 5.6 Hz, -CH_2CH-), 4.99 (2H, br s, NH_2), 7.51 (2H, dd, *J* = 4.8 and 2.4 Hz, $H_{Heteroaryl}$), 8.61 (2H, *J* = 4.8 and 2.4 Hz, $H_{Heteroaryl}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 62.4, 73.7, 100.2,

119.9, 139.8, 149.7, 150.1. Anal. calcd for $C_{12}H_{19}N_3O_3$, C, 56.90%; H, 7.56%; N, 16.59%. Obtained: C, 56.99%; H, 7.42%; N, 16.70%.

Fungal strains

Candida guilliermondii ATCC 6260, *Candida albicans* ATCC 76615 and *Candida albicans* ATCC 76485 were provided by the fungal culture collection from the Laboratório de Micologia da Universidade Federal da Paraíba, Brazil.

Minimum inhibitory concentration (MIC)

The determination of minimum inhibitory concentration (MIC) was performed by broth microdilution according to CLSI (Clinical and Laboratory Standards Institute) document M27-A2 (CLSI 2017). The culture medium used was Sabouraud Dextrose Agar. The inoculum of each of the microorganisms was prepared according to the 0.5 McFarland standard containing approximately 1-5 x 10^6 CFU.mL^{-1} (colony forming units.mL^{-1}). Experiments were conducted at approximately 1-5 x 10^5 CFU.mL^{-1} in each well. The solutions with compounds 3a-h were prepared at the time of the tests. Dimethylsulfoxide (DMSO) was used to solubilize compounds 3a-h (the concentration of DMSO was always less than 0.5%). Compounds 3a-h was tested at concentrations ranging from 512 to 8 µg.mL^{-1} at 1:2 serial dilutions. Initially, 100 µl of the culture medium was added to each well of a plate. Then, a 100 µL of the solution of compounds 3a-h (final concentration 512 µg.mL^{-1}) was added to the first line of the plate and a 1:2 serial dilution to 1 µg.mL^{-1} concentration was performed. Finally, 10 µL of the inoculum was added to each well of the plate. The plate was incubated at 35ºC for 24-48 hours. The minimum inhibitory concentration was considered the lowest concentration capable of inhibiting the visible growth of the microorganism. The experiment was performed in triplicate and controls with DMSO (culture medium + inoculum + DMSO) at the same concentration used to solubilize 3a-h were prepared to demonstrate that the growth of the microorganism is not influenced by DMSO.

MIC was considered the lowest concentration of the substances capable of causing visual inhibition of the growth of the strains used in the microbiologic assays, being confirmed by the addition of 20 µL of 2.0% TTC (2,3,5 triphenyl tetrazolium chloride) in each cavity.

3. Results and Discussion

The general strategy for the synthesis of the new antifungal agents involved two reaction steps, the preparation of the amidoximes 2 from different nitriles followed by the alkylation of the obtained compounds to afford the *O*-alkylamidoximes. The synthesis started from the reaction of commercially available nitriles 1a-h and hydroxylamine hydrochloride under sonication (Barros et al. 2011; Andrade et al. 2016). In all cases, the corresponding amidoximes 2a-h were obtained in short reaction times and moderate to high yields after purification by crystallization (Figure 1). The characterization data of the obtained compounds are in accordance with the literature (Li et al. 2013; Ozcan et al. 2013; Andrade et al. 2016; Tarasenko et al. 2017).

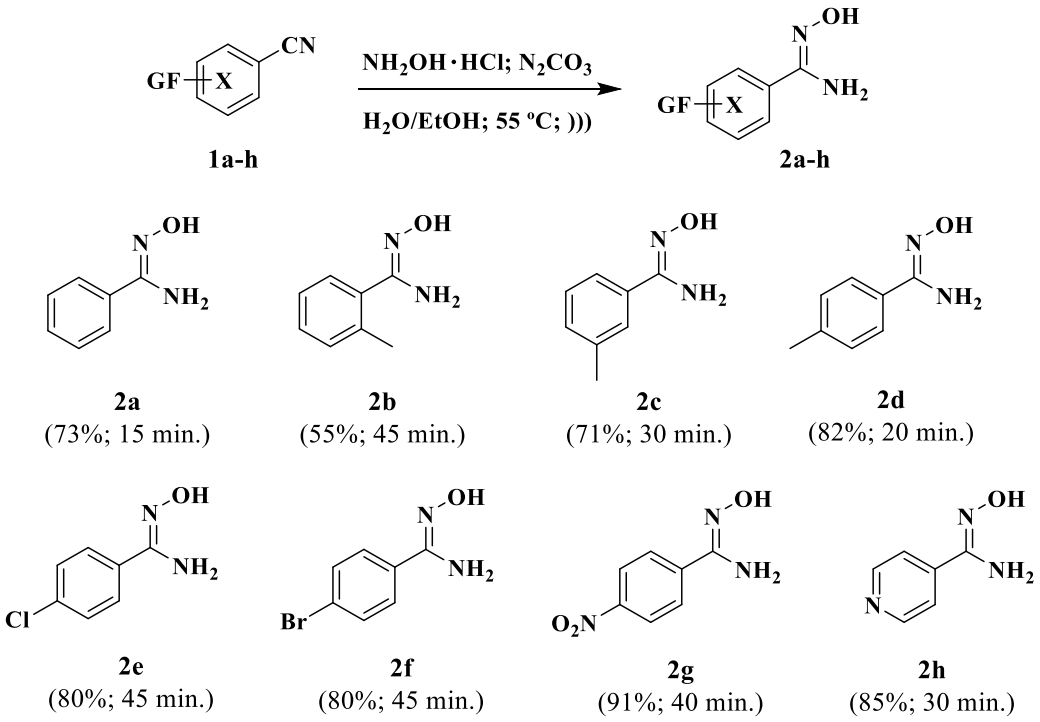

Figure 1. Synthesis of amidoximes.

Thus, compound 2a was prepared at the shortest reaction time probably due to its high solubility in the reaction medium and lower steric hindrance. Steric factors are also important, the *ortho* isomer 2b was obtained in lower yield and longer reaction time when compared to the *meta* isomers and *para* isomers, 2c and 2d, respectively. When halogens were present in the aromatic ring the corresponding compounds 2e and 2f were obtained in higher yields. The best result was observed when the nitro group, a strong electron-withdrawing group was used, where compound 2g was obtained in 91% yield. The method proved also to be efficient for the synthesis of heteroaromatic compounds, where 2h was obtained in good yield after a half-hour.

The *O*-alkylation of amidoximes has few examples described in the literature (Coviello 1964; Veerman et al. 2016). In this way, the synthesis of alkylated amidoximes was revisited to find the best condition for this reaction. The desired compounds, 3a-h were obtained in moderate to good yields after 2 to 7 hours depending on the group present in the aromatic ring (Figure 2).

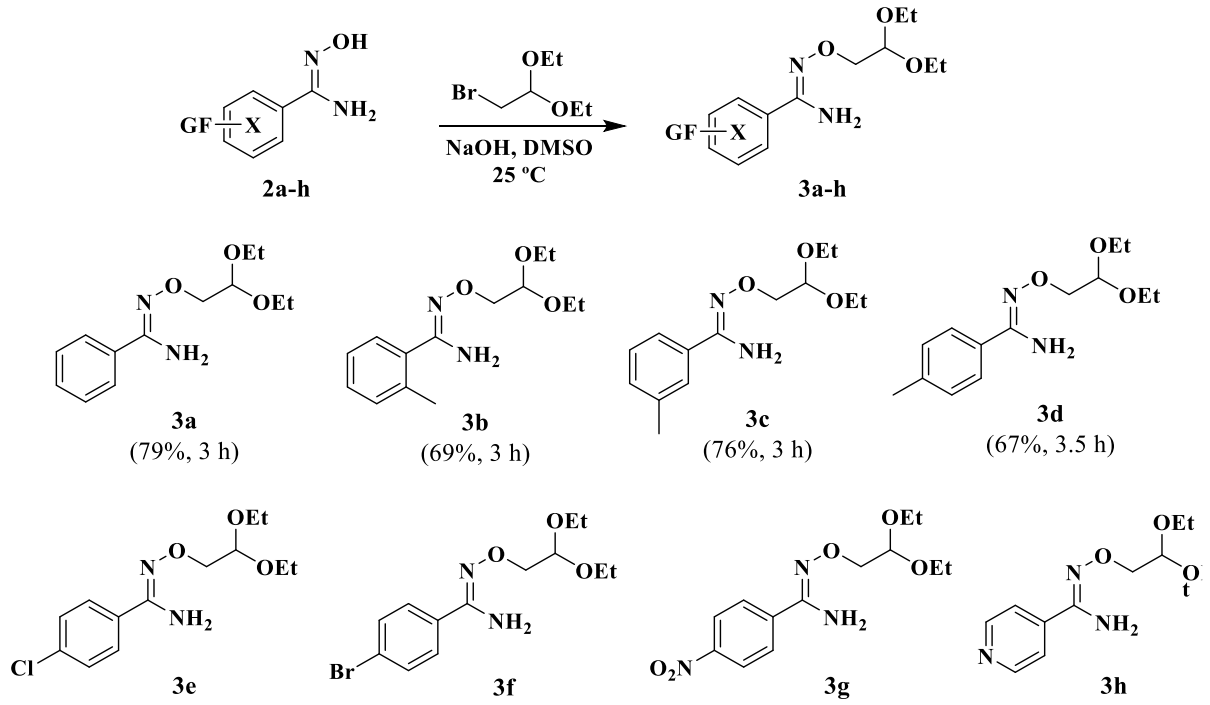

Figure 2. Synthesis of *O*-alkylated-amidoximes.

Compounds 3a-h were then submitted to antifungal activity against three *Candida* species due to their prevalence in the epidemiology of fungal infections (Whaley et al. 2017) using the broth microdilution method (CLSI 2017). Thus, the Minimum Inhibitory Concentration (MIC) (Espinel-Ingroff et al. 2005) capable of visually inhibiting 100% of fungal growth for the synthesized compounds was evaluated. The results are depicted in Table 1.

Table 1. Minimum inhibitory concentrations for compounds 3a-h.

Entry	Compound	MIC, mM (mg.mL^{-1})		
		Candida guilliermondii ATCC 6260	*Candida albicans* ATCC 76615	*Candida albicans* ATCC 76485
1	3a	2.03 (512)	2.03 (512)	>2.03 (>512)
2	3b	1.92 (512)	1.92 (512)	>1.92 (>512)
3	3c	>1.92 (>512)	1.92 (512)	>1.92 (>512)
4	3d	1.92 (512)	>1.92 (>512)	>1.92 (>512)
5	3e	1.79 (512)	1.79 (512)	1.79 (512)
6	3f	1.55 (512)	1.55 (512)	>1.55 (>512)
7	3g	0.86 (256)	0.86 (256)	1.72 (512)
8	3h	>2.02 (>512)	2.02 (512)	>2.02 (>512)
9[a]	-	0.11 (32)	0.11 (32)	0.83 (256)

[a] Fluconazole was used as a positive control.

In general, all compounds shown antifungal activity against at least one strain of *Candida*. As described by Morales et al. (2008) a compound with poor antimicrobial activity shows MIC values above of 1500 µg.mL^{-1}, moderate activity in the range of 500-1500 µg.mL^{-1}, and good activity with MIC values in the range of 50-500 µg.mL^{-1}. It is also interesting to note that the position of the substituents attached to the aromatic ring did not imply significant changes in MIC values. For example, compounds 3b-d which contain a methyl group at the *ortho*, *meta,* or *para* positions, respectively, exhibited similar MIC values (Table 1, entries 2-4). Aromatic rings containing a halogen as substituent 3e and 3f exhibited better activities against all strains tested (Table 1, entries 5 and 7). These findings are in accordance with the commercially available antifungal agents chlorotrimazole and econazole. Structure-activity studies have revealed that the presence of a halogen is critical for drug activity for both compounds (Kasper et al. 2015).

The best result was observed for compound 3g, which contains the strongly electron-withdrawing group nitro group (Table 1, entry 7). This is an interesting result, while a number of antibiotics bearing nitro groups were reported from bacteria, such as chloramphenicol and pyrrolnitrin (Al-Zereini et al. 2007) but not for antifungal agents. Finally, compound 3h exhibits an antifungal activity similar to 3a, which has no substituents at the aromatic ring (Table 1, entries 1 and 8).

One of the most important steps in the process in the development of new drugs is the prospecting of new prototypes that can be optimized based on planned molecular modifications for viable therapeutic options (Pinzi and Rastelli 2019). Further studies in the mode of action of the synthesized compounds are underway in our laboratories.

4. Conclusions

New *O*-alkylamidoximes were obtained in moderate to good yields (68-81%) without any observable decomposition using a simple, reproducible, and non-expensive method. All *O*-alkylamidoximes showed *in vitro* antifungal activity against at least one of the tested strains of *Candida*. These findings are the first step in the development of new drugs, providing important information for future research to obtain new classes of antifungal agents.

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conception and design, acquisition of data, and drafting the article; FREITAS, J.C.R.: conception and design, acquisition of data, and drafting the article. All authors have read and approved the final version of the manuscript.

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