

Synthesis and Testing of 3-Acetyl-2,5-Disubstituted-2,3-Dihydro-1,3,4-oxadiazole Derivatives for Antifungal Activity Against Selected *Candida* Species

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Uma série de 21 derivados, 1,3,4-oxadiazolinas, foi sintetizada pela ciclização de *N*-acilhidrazonas com anidrido acético e avaliada *in vitro* quanto à sua atividade antifúngica contra seis espécies de *Candida*: *Candida albicans* (ATCC 90028 e LM V-42), *Candida krusei* (ATCC 6258 e LM 12 C) e *Candida tropicalis* (ATCC 13803 e LM 14). As espécies de *Candida* foram consideradas sensíveis a uma série dos compostos, os quais inibiram o crescimento de 50 a 90%, com um intervalo de concentração inibitória mínima (MIC) de 64 a 512 µg mL⁻¹. As estruturas dos compostos foram totalmente confirmadas e caracterizadas pelas técnicas de infravermelho com transformada de Fourier (FTIR), ressonância magnética nuclear (NMR) de ¹H e ¹³C, e espectrometria de massa (MS).

A series of 21 1,3,4-oxadiazoline derivatives was synthesized by cyclization of *N*-acylhydrazones with acetic anhydride and evaluated for their *in vitro* antifungal activity against six *Candida* strains: *Candida albicans* (ATCC 90028 and LM V-42), *C. krusei* (ATCC 6258 and LM 12 C) and *C. tropicalis* (ATCC 13803 and LM 14). The *Candida* strains were found to be sensitive to some of the compounds, which inhibited the growth by 50-90%, with minimum inhibitory concentration (MIC) in the range of 64-512 µg mL⁻¹. The compounds' structures were fully confirmed and characterized by Fourier transform infrared spectroscopy (FTIR), ¹H and ¹³C nuclear magnetic resonance (NMR) and mass spectrometry (MS).

Keywords: 1,3,4-oxadiazoline, antifungal activity, *Candida* species, *N*-acylhydrazones

Introduction

Due to the development of resistant fungous strains and no-longer effective medications, the incidence of systemic fungal infections (with its consequent morbidity and mortality) has been gradually increasing over the last three decades.¹ The resulting requirement for investigation, research and discovery of new antimicrobial agents that are both safer and more effective is of the utmost importance. Derivatives of 1,3,4-oxadiazoline have recently gained importance (for serving as building blocks) for both organic synthesis and medicinal chemistry when developing new therapeutic agents. It has been noted that compounds containing the 1,3,4-oxadiazoline motif generally exhibit excellent antimicrobial activity.²⁻¹²

Therefore, taking into consideration our ongoing research and the importance of the 1,3,4-oxadiazoline scaffold as a good source for the discovery of new and biologically active molecules, our group synthesized new 1,3,4-oxadiazoline heterocyclic compounds, (specifically 3-acetyl-2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoles), and pre-screened these compounds for candidacy as novel antifungal agents.

Results and Discussion

Chemistry

N-acylhydrazones (NAH) belong to the azomethine class of compounds, they are structurally privileged with chemical properties that have proven to be important when designing pharmacologically active prototypes.^{13,14} They are

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Table 1. Physical and chemical properties of novel 1,3,4-oxadiazolines **4b**, **4h**, **4j**, **4l** and **4m-q**

Compound	Molecular formula	Exact mass	mp / °C	Yield / %	GC-MS
					t _r / min
4b	C ₁₈ H ₁₉ N ₃ O ₂	309.15	92-94	66	23.4
4h	C ₁₅ H ₁₂ BrN ₃ O ₂	345.01	120-121	59	24.1
4j	C ₁₇ H ₁₇ N ₃ O ₄	327.12	186-188	57	24.3
4l	C ₁₇ H ₁₅ N ₃ O ₄	325.11	162-164	72	24.5
4m	C ₁₈ H ₁₆ N ₂ O ₄	324.11	140-141	75	24.3
4n	C ₁₉ H ₁₈ N ₂ O ₄	338.13	154-156	70	25.3
4o	C ₁₈ H ₁₅ N ₃ O ₆	369.10	192-194	64	25.8
4p	C ₁₈ H ₁₅ ClN ₂ O ₄	358.07	158-160	71	25.7
4q	C ₁₉ H ₁₈ N ₂ O ₅	354.12	98-100	45	26.5

because of its very characteristic signals.^{2,17} For example, in the ¹H NMR spectra of compounds of the series **1** (**4a-l**), two typical signals were observed, one assigned to the methyl protons of the acetyl group linked to the nitrogen atom N-3 in the aliphatic region of 2.27 to 2.30 ppm, and another assigned to methinic protons H-2 in the aromatic region 7.17 to 7.63 ppm. In the ¹³C NMR spectra, signals characteristic of C=O around 167 ppm, and methyl carbon atom CH₃ in the range of 20.1-22.7 ppm, as well as the oxadiazole ring signal C-2 around 88.7 to 92.9 ppm, and C-5 around 152.5 to 163.4 ppm were observed, thus confirming its formation.

In the 1-(2-(4-fluorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl)ethanone, (**4f**) spectrum, it was observed doublets corresponding to couplings ¹³C-¹⁹F, whose coupling constants for one bond ¹J_{CF} 245.0 Hz, two bonds ²J_{CF} 32.0 Hz and three bonds ³J_{CF} 9.0 Hz were assigned respectively to couplings C-F in δ 162.4 (C-14), δ 115.4 (C-11,15) and δ 128.8 (C-12,16) ppm. In the ¹H NMR spectra for series **2** (**4m-q**) compounds, it was observed the characteristic signals: a singlet for hydrogen 3 (acetyl group linked to nitrogen (N-3)) in the aliphatic region of 2.25 to 2.27 ppm, a singlet for hydrogen 3 (acetoxy group linked to the benzene ring) in the aliphatic region of (2.26 to 2.30 ppm), and singlet for hydrogen 1 (H-2) in the aromatic region of 7.17 to 7.26 ppm. In the ¹³C NMR spectra, the characteristic signals observed were: two carbon atoms of the 1,3,4-oxadiazoline core (C-2 and C-5) in the regions of 91.0 to 92.3 ppm and 153.2 to 154.7 ppm, respectively. The chemical shifts of C=O of the acetyl group linked to nitrogen atom (N-3), and the C=O of the acetoxy group were observed in the range of 166.6 to 167.2 ppm and 168.8 to 169.2 ppm, respectively. The characterization of series **3** (**4r-v**) compounds was recently reported by our group.²

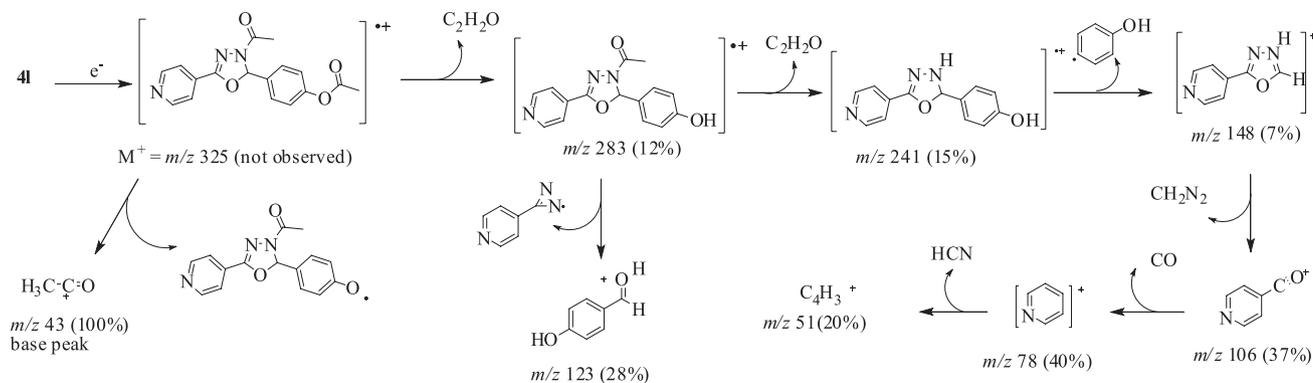
In the infrared spectrum, compounds **4a-q** showed amide C=O absorption bands from 1662 to 1674 cm⁻¹,

C–O–C absorption stretches (oxadiazole ring) from 1095 to 1240 cm⁻¹, and C=N absorptions (oxadiazole ring) from 1604 to 1635 cm⁻¹. The compound 4-(3-acetyl-5-(pyridin-4-yl)-2,3-dihydro-1,3,4-oxadiazol-2-yl)phenyl acetate **4l** and all compounds of series **2** (**4m-q**) also showed absorption bands in the 1755 to 1759 cm⁻¹ range for C=O (acetoxy group).

The fragmentation most observed in the mass spectra of the new compounds (**4b**, **4g**, **4j**, **4l** and **4m-q**) is exemplified by compound **4l**. In the mass spectrum of compound **4l**, it was not detected a peak related to the molecular ion (*m/z* 325), this due to the presence of an acetyl radical which easily promotes the loss of one ketene molecule, and the transfer of a hydrogen to form a phenol. However, the radical cation (*m/z* 283) originating from the first breaking of the molecular ion, and corresponding to the elimination of a neutral ketene, was not observed. The peak at *m/z* 241 originates from the radical cation (*m/z* 283) through loss of a second neutral ketene molecule. The base peak was assigned to the acylium ion (*m/z* 43). In Scheme 2, it is outlined a fragmentation pattern proposal for that observed in the mass spectrum of compound **4l**.

Antifungal activity

The *in vitro* antifungal activity of compounds **4a-v** was evaluated by the microdilution method against six strains of pathogenic fungi, *Candida albicans* (ATCC 90028 and LM V-42), *Candida krusei* (ATCC 6258 and LM 12 C) and *Candida tropicalis* (ATCC 13803 and LM 14), using nystatin as the drug standard (Table 2). The compounds were tested at concentrations from 32 to 1024 μg mL⁻¹, and solubilized in dimethylsulfoxide, DMSO (Sigma Chemical) to 10% to avoid interferences with the microorganisms. The antifungal activity of the products was interpreted, and considered active or not, according to the following



Scheme 2. Fragmentation pattern proposal for the compound **4l**.

Table 2. Antifungal activity of 1,3,4-oxadiazoline **4m-q** and **4r-v**

Compounds	Minimum inhibitory concentration (MIC) / ($\mu\text{g mL}^{-1}$)					
	<i>Candida</i> Strains					
	<i>C. albicans</i> ATCC 90028	<i>C. albicans</i> LM V-42	<i>C. krusei</i> ATCC 6258	<i>C. krusei</i> LM 12C	<i>C. tropicalis</i> ATCC 13803	<i>C. tropicalis</i> LM 14
4m	R	1024	R	1024	R	R
4n	R	128	128	R	R	R
4o	R	R	R	R	R	R
4p	R	R	R	R	R	R
4q	R	R	R	R	R	R
4r	64	64	256	R	512	512
4s	128	128	128	R	R	128
4t	128	R	256	R	R	256
4u	512	128	128	R	R	64
4v	64	128	64	R	128	128
Control yeast	+	+	+	+	+	+
Nystatin	-	-	-	+	+	-

R: resistant; +: growth of the microorganism; -: no growth of the microorganism.

parameters: 50-100 $\mu\text{g mL}^{-1}$ = good activity; 100-500 $\mu\text{g mL}^{-1}$ = moderate activity; 500-1000 $\mu\text{g mL}^{-1}$ = low activity; greater than 1000 $\mu\text{g mL}^{-1}$ = inactive product.¹⁸

From the 21 compounds tested, compounds **4a-m** and **4o-q** showed no inhibitory activity against the mentioned yeasts in the bioassays. Compound **4n** showed moderate inhibitory effect against *Candida albicans* (LM V-42), and *Candida krusei* (ATCC 6258) at a minimum concentration of 128 $\mu\text{g mL}^{-1}$. The *Candida* species were sensitive to compounds of series **3** (**4r-v**), these compounds inhibited the growth by 50 to 90% (MIC range of 64-512 $\mu\text{g mL}^{-1}$). The best results were against *Candida albicans* (ATCC 90028) exhibited by compounds **4r** and **4v** with an MIC of 64 $\mu\text{g mL}^{-1}$, while compounds **4u** and **4v** exhibited the best results against *Candida krusei*, and *Candida tropicalis* with an MIC of 64 $\mu\text{g mL}^{-1}$, respectively. *Candida krusei* (LM 12C) showed no sensitivity to any of the tested compounds. The

standard drug, nystatin at 100 IU mL^{-1} inhibited the growth of four (67%) of the *Candida* strains. The results therefore were considered between good and moderate in terms of biological activity, and taking into account the parameters established.¹⁸ The results show that compounds **4r-v** may be considered promising for possible development of new antifungal agents, and, that the 5-nitrofuranyl group in these compounds is important for their activity.

Conclusions

In this work, it was synthesized a series of twenty-one 1,3,4-oxadiazoline compounds, and their structures were confirmed by the techniques FTIR ^1H and ^{13}C NMR and MS. For *in vitro* antifungal activity against *Candida* strains, compounds **4r-v** showed good to moderate activity, inhibiting the growth of the strains used by

50-90% with an MIC of 64-512 $\mu\text{g mL}^{-1}$. These may be promising compounds for the development of new antifungal agents.

Experimental

Chemistry

All used reagents and solvents were purchased from commercial sources (Sigma-Aldrich, Brazil), and used without a further purification. The purification of the compounds was performed by re-crystallization in ethanol, and confirmed by determining the melting range on an MQAPF-3 hotplate, and by gas chromatography with low resolution mass spectrometer (GC-MS-QP2010) Shimadzu. The FTIR spectra were obtained on an FTIR spectrometer model IRPrestige-21-Shimadzu, using KBr pellets. ^1H and ^{13}C NMR spectra were obtained on two different machines: a Varian 200 NMR (200 and 50 MHz for ^1H and for ^{13}C , respectively), and a Varian 500 NMR (500 and 125 MHz for ^1H and ^{13}C , respectively), deuterated dimethyl sulphoxide ($\text{DMSO-}d_6$) was used as the solvent and tetramethylsilane (TMS) was used for the internal standard. Chemical shifts (δ) were measured in parts *per million* (ppm) and the coupling constants (J) in Hz.

Antifungal activity

Microorganisms

The microbiological assays used: *Candida albicans* (ATCC 90028 and LM V-42), *C. krusei* (ATCC 6258 and LM 12 C) and *C. tropicalis* (ATCC 13803 and LM 14). The strains were acquired from the Institute Adolfo Lutz of São Paulo and Mycology Laboratories of the Departments of Pharmaceutical Sciences at the University of Sao Paulo and the Federal University of Paraíba, respectively. The fungal strains were maintained in appropriate medium, Sabouraud Dextrose Broth-SDB (DIFCO Laboratories, France-USA), and stored at 4 °C and 35 °C. The suspension of microorganisms was prepared according to the 0.5 McFarland scale tube, and adjusted by means of a spectrophotometer (Leitz-Photometer 340-800) to 90% T (530 nm) corresponding to approximately 10^6 UFC mL^{-1} .¹⁹⁻²¹

Culture medium

The antifungal activity assays were performed in Sabouraud Dextrose Broth-SDB (DIFCO Laboratories, France-USA), which was prepared and used according to manufacturer instructions.

Determination of minimum inhibitory concentration (MIC)

The MIC value was determined by the microdilution method, using 96 well microtiter plates with background in a “U” and in duplicate. To each well of the plate, it was added 100 μL of liquid medium SDB doubly concentrated. Then, 100 μL of the product solution (also doubly concentrated) were dispensed into the wells of the first line of the plate. By means of serial dilution (ratio of two), it was obtained concentrations from 2,048 to 64 $\mu\text{g mL}^{-1}$, so that in the first row of the plate, the highest concentration, and in the latter, the lower concentrations. Finally, 10 μL of inoculum were added to the wells where each column of the plate referred specifically to a strain. The same was also done in the culture medium with fungal drug nystatin (100 UI). The plates were incubated at 37 °C for 24-48 h. For each strain, MIC was defined as the lowest concentration able to inhibit fungal growth visually observed in the wells, when compared to the control. All tests were performed in duplicate, and the results were expressed as the geometric mean of the MIC values obtained in the two trials.²¹⁻²³

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Supplementary Information

Supplementary information is available free of charge at <http://jbcbs.sbg.org.br> as PDF file.

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