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Biphenyl-4-Carboxylic Acid Derived Esters with Antifungal Activity

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ABSTRACT

Candidiasis presents as either a superficial or systemic fungal infection caused by Candida yeast species. Immunocompromised patients such as HIV infected individuals, carriers of chronic disease, diabetics and transplanted patients, as well as the elderly and newborns are the groups with the greatest susceptibility to such infections. Some species of this genus have shown resistance to a wide range of antifungal agents, increasing both infection severity and complexity of treatment. In this work, a collection of biphenyl-4-carboxylic acid ester derivatives was prepared, and the activity of these compounds was investigated against pathogenic species of the Candida genus. Both Fischer esterification and so-called non-Fischer esterification using alkyl halides were employed to prepare the esters. Antifungal assays were performed using broth microdilution methods. The main objective of this work was to trace structure-activity relations for the compounds obtained. Of the eight esters prepared in this study, four presented activity against strains of Candida albicans and Candida tropicalis. The substances yielded good to moderate bioactivities. The bioactivities found amongst the evaluated esters for the tested strains were highest for ethyl 4-biphenyl carboxylate (obtaining MICs ranging from 512 to 1024 µg/mL); and decanoyl 4-biphenyl carboxylate (obtaining an MIC of 512 µg/mL). All of the molecules presenting either a heteroatom or bulky groups close to the ester function demonstrated bioactivity.

Keywords: Esters; Antifungal activity; *Candida* spp

INTRODUCTION

During the last few decades, the frequency of fungal infections has increased significantly. In terms of incidence and mortality these pathogens are becoming more serious, making certain types of fungal infections critical for immunocompromised patients [1,2]. Candidiasis is characterized as the single most common fungal infection, with *C. albicans* being the most frequent etiological agent. However, other Candida species, such as: *C. guilliermondii, C. krusei, C. parapsilosis, C. stellatoidea and C. tropicalis* can also be included in the etiology of this disease. [3]. In general, fungal infections that have Candida species as etiological agents present the clinical manifestation of occurrence through localized mucosal infections and progression towards dissemination, which may involve multiple organs [4]. Azole derivatives (ketoconazole, econozale, sulconazole, miconazole, clotrimazole, fluconazole) are commonly used to treat fungal infections; however, the most commonly used antifungals are the allylamines (naftifine, terbinafine), hydroxypyridone, morpholine, selenium compounds, liposomal amphotericin B, and the echinocandins (caspofungin, micafungin and anidulafungin). The use of these drugs has frequently made the

treatment of such infections more complex, this, due to the fact that certain etiological agents have already acquired resistance to these antifungals [5-7]. Several pharmacological activities (in particular, antimicrobial activity), have been attributed to carboxylic acid derivatives, establishing a relationship between structural characteristic and biological potency. They inhibit the growth of fungi associated with this set of human pathologies [8,9]. Derivatives of benzoic acid are also of great interest for the scientific community, their chemical and biological properties are of great importance to the food and pharmaceutical industries [10,11]. The present study aimed to prepare a collection of eight esters derived from biphenyl-4-carboxylic acid and to evaluate their antifungal potential against strains of the genus *Candida*, tracing their structure-activity relationships.

EXPERIMENTAL SECTION

Chemistry

Purification of the compounds was performed using column chromatography on silica gel 60, ART 7734 MERCK with solvent gradient Hex: EtOAc confirmed by analytical thin layer chromatography on silica gel 60 F254, employing ultraviolet light at two wavelengths (254 and 366 nm), and using a Mineralight apparatus with H2SO4 in 5% ethanol. FTIR spectra were recorded in an FTIR spectrometer IR Prestige-21-Shimadzu model using KBr pellets. 1H and 13C NMR spectra were obtained in Varian MERCURY machines (200 and 50 MHz for 1H and13C, respectively). Deuterated solvent was used ($CDCl_3$). Tetramethylsilane (TMS) was used as the internal standard. Chemical shifts (d) were measured in parts per million (ppm) and coupling constants (J) in Hz.

Esters Preparation

The esters (from 1-6) were obtained through Fischer esterification reactions in which a carboxylic acid with excess alcohol was used, adding a catalyst (sulfuric acid H_2SO_4) under reflux [12]. Esters 7 - 8 were obtained through esterification reactions using alkyl halides in a basic medium with acetone reflux.

Procedures for Preparation Esters 1-6

To a 250 mL flask, 0.1 or 0.2 g of biphenyl-4-carboxylic acid was added, and dissolved in 20 or 40 mL of the aliphatic alcohol. To this solution was added 0.4 mL of concentrated sulfuric acid (H_2SO4). The flask was coupled to a reflux condenser and placed in an oil bath. The reaction mixture was maintained under reflux with magnetic stirring for 24 hours, and monitored by silica gel analytical thin layer chromatography (ADCC), using as an eluent a hexane and ethyl acetate mixture. After the reaction, the solvent was partially evaporated to about half its volume under reduced pressure. The solution was then transferred to a separating funnel (15 mL of distilled water was added) and extracted with chloroform (3 × 10 mL). The resulting organic phases were combined, neutralized with 5% sodium bicarbonate ($NaHCO_3$), washed with 10 mL of distilled water, and dried with anhydrous sodium sulfate (Na_2SO_4), filtered, and the solvent evaporated with the aid of reduced pressure. For esters 5 and 6, purification was carried out by means of a chromatographic column on silica gel 60, using hexane and ethyl acetate as eluents, in an increasing polarity gradient; this procedure was monitored using CCDA. At the end of each column the purified ester was obtained [13].

Procedures for Preparation of Esters 7-8

In a 100 mL flask, biphenyl-4-carboxylic acid (0.2 g) was dissolved in 13 mL of anhydrous acetone. To this solution the halide (1.04 mmol) was added and 0.6 mL of tri-ethylamine (4.4 mmol). The flask was then coupled to a reflux condenser and placed in an oil bath. The reaction mixture was refluxed with magnetic stirring for 48 hours until consumption of the starting material; the reaction was monitored by CCDA. After the product was formed, the solvent was partially evaporated under reduced pressure to initiate the extraction. The reaction product was then transferred to a separation funnel (15 mL of distilled water was added) and extracted (3 × 10 mL) chloroform. The organics were pooled, treated with 5% sodium bicarbonate ($NaHCO_3$) (3 × 10 mL) to neutralize the solution, which was washed with 10 mL of distilled water, and dried with anhydrous sodium sulfate (Na_2SO_4), after which filtration was carried out and the solvent evaporated with the aid of reduced pressure. The residue was purified by means of a chromatographic column on silica gel 60, eluting with hexane and ethyl acetate, in an increasing polarity gradient, to isolate the desired product [14].

Methyl [1,1'-biphenyl]-4-carboxylate (1)

White amorphous solid; Yield 91.2% (196 mg); IR υmax (KBr, cm^{-1}): 3030, 2987, 1701, 1606, 1502, 1479, 1278, 1004, 752. ^1H NMR ($CDCl_3$, 200 MHz): δ_H8.11 (d, *J* = 8.1 Hz, 2H), 7.64 (dd, *J* = 19.0; 7.6 Hz, 4H), 7.46 (t, *J* = 7.6

Hz, 2H), 7.39 (t, *J* = 6.9 Hz, 1H), 3.94 (s, 3H).^{13}C NMR($CDCl_3$, 50 MHz): δ_C 52,1; 127.0; 127.3; 128.1; 128.9; 129.0; 130.1; 139.9; 145.6; 167.0.

Ethyl [1,1'-biphenyl]-4-carboxylate (2)
Yellow amorphous solid; Yield 92.4% (217 mg); IR υmax (KBr, cm^{-1}): 3028, 2964, 1701, 1606, 1512, 1460, 1276, 1006, 750. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.10 (m, 2H), 7.64 (m, 2H), 7.61 (m, 2H), 7.44 (m, 2H), 7.37 (m, 1H), 4.40 (m, 2H), 1.40 (m, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C14.5; 61.1; 127.1; 127.4; 128.2; 129.0; 129.4; 130.2; 140.2; 145.6; 166.6.

Propyl [1,1'-biphenyl]-4-carboxylate (3)
Yellow amorphous solid; Yield 62.7% (152 mg); IR υmax (KBr, cm^{-1}): 3055, 2953, 1714, 1608, 1502, 1469, 1290, 1004, 740. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.13 (m, 2H), 7.65 (m, 2H), 7.62 (dd, *J* = 2.6, 1.8 Hz, 2H), 7.46 (m, 2H), 7.39 (m, 1H), 4.31 (m, 2H), 1.82 (m, 2H), 1.05 (dd,*J* = 9.5, 5.4 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C10.6; 22.3; 66.7; 127.1; 127.4; 128.2; 129.0; 129.4; 130.2; 140.2; 145.6; 166.7.

Isopropyl [1,1'-biphenyl]-4-carboxylate (4)
Yellow oil; Yield 63.3% (76.8 mg); IR υmax (KBr, cm^{-1}): 3030, 2958, 1718, 1608, 1500, 1450, 1278, 1008, 748. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.10 (d,*J* = 8.7 Hz, 2H), 7.62 (dd, *J* = 16.2, 7.8 Hz, 4H), 7.45 (m, 2H), 7.38 (t, *J* = 7.4 Hz, 1H), 5.28 (hept, *J* = 6.3 Hz, 1H), 1.38 (d, *J* = 6.3 Hz, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C22.1; 68.5; 127.1; 127.4; 128.2; 129.0; 129.8; 130.1; 140.2; 145.5; 166.1.

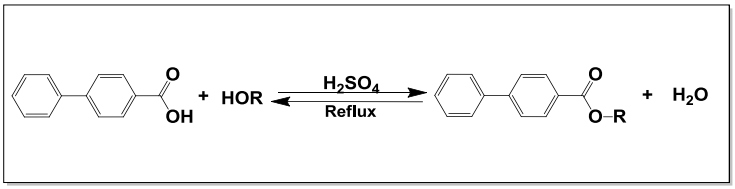
Butyl [1,1'-biphenyl]-4carboxylate (5)
Yellow oil; Yield 93.1% (120.6 mg); IR υmax (KBr, cm^{-1}): 3032, 2927, 1720, 1608, 1512, 1450, 1276, 1006, 748. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.09 (m, 2H),7.76 (m, 2H), 7.48 (m, 4H), 7.42 (m, 1H),4.3 (m, 2H), 1.73 (m, 2H), 1.43 (m, 2H), 0.96 (m, 3H).^{13}C NMR ($CDCl_3$, 50 MHz: δ_C13.4; 18.6; 30.7; 64.8; 126.9; 127.2; 128.1; 128.9; 129.3; 130.0; 140.0; 145.5; 166.5.

2-Methoxyethyl [1,1'-biphenyl]-4-carboxylate (6)
Yellow oil; Yield 65.0% (84 mg); IR υmax (KBr, cm^{-1}): 3030, 2953, 1714, 1608, 1502, 1469, 1292, 1004, 740. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.12 (d, *J* = 8.6 Hz, 2H), 7.62 (m, 4H), 7.41 (m, *J* = 8.8 Hz, 3H), 4.48 (m, 2H), 3.73 (m, 2H), 3.43 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz: δ_C58.9; 63.9; 70.5; 126.9; 127.1; 128.1; 128.7; 128.9; 130.1; 139.9; 145.6; 166.4 (Scheme 1).

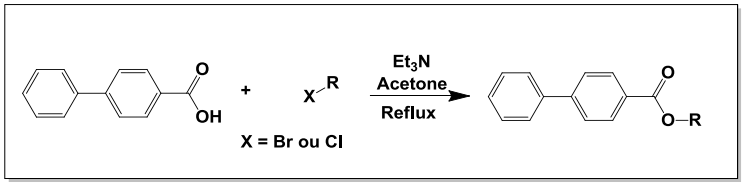
Decyl [1,1'-biphenyl]-4-carboxylate (7)
White crystalline solid; Yield 64.7% (221 mg); IR υmax (KBr, cm^{-1}): 3028, 2970, 1712, 1606, 1514, 1452, 1278, 1006, 742. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.09 (m, 2H), 7.64 (m, 2H), 7.60 (m, 2H), 7.44 (m, 2H), 7.37 (m, 1H), 4.32 (t, *J* = 6.7 Hz, 2H), 1.77 (m, 2H), 1.44 (m, 2H), 1.32 (m, 12H), 0.87 (dd, *J* = 8.3; 5.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz: δ_C14.2; 22.8; 26.2; 28.9; 29.4; 29.7; 32.0; 65.3; 127.1; 127.4; 128.2; 129.0; 129.4; 130.2; 140.2; 145.7; 166.7.

4-Chlorobenzyl [1,1'-biphenyl]-4-carboxylate (8)
Yellow amorphous solid; Yield 95.5% (311 mg); IR υmax (KBr, cm^{-1}): 3026, 2949, 1730, 1612, 1502, 1450, 1282, 1049, 1008, 746.^{1}H NMR ($CDCl_3$, 200 MHz): δ_H8.12 (d, *J* = 8.3 Hz, 2H), 7.63 (dd, *J* = 22.6; 7.8 Hz, 4H), 7.45 (m,2H), 7.35 (m, 5H), 5.33 (s, 2H).^{13}C NMR ($CDCl_3$, 50 MHz: δ_C 65.9; 127.1; 127.3; 128.2; 128.6; 128.8; 129.6; 130.2; 134.2; 134.6; 139.9; 140.9;145.9; 166.2 (Scheme 2).

Procedure



Scheme 1: Preparation of esters of 1-6



Scheme 2: Preparation of esters 7-8

Antifungal Activity Evaluations of the Prepared Esters

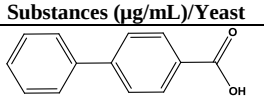
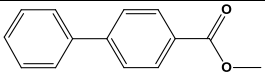
The following *Candida* strains were used: *C. albicans* (ATCC-76485), *C. albicans* (LM-111), *C. tropicalis* (ATCC-13803) and *C. tropicalis* (LM-14). The method used was broth microdilution. The compounds prepared were suitably solubilized in dimethylsulfoxide (DMSO) in proportions of up to 10%, and Tween 80 at 0.02%; filled with sterile distilled water (Q.S.P. 3 ml) to obtain an emulsion at the initial concentration of 1024 μg/ml [15-17].

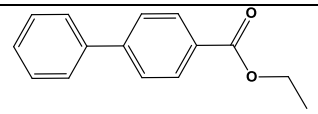
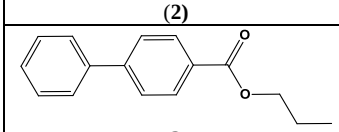
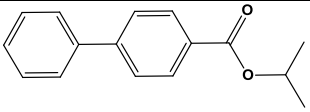
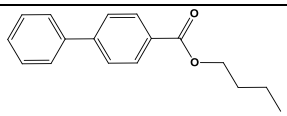
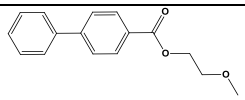
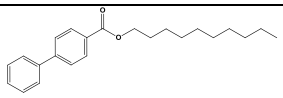
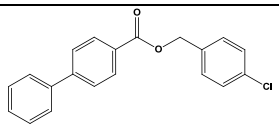
To control the results of the biological assays, nystatin was used as a standard: 100 IU/mL/yeast. The minimum inhibitory concentration (MIC) of the tested products was determined; which were evaluated at concentrations ranging from 1024 to 64 μg/mL. After the MIC determination, the minimum fungicidal concentration (MFC) was calculated. The tests were performed in duplicate and the results were expressed as the geometric mean of the MIC and MFC values.

RESULTS AND DISCUSSION

In the present work, eight biphenyl-4-carboxylic acid esters with different alkyl or benzyl substitutions (alcohol or halide used in the reactions) were prepared and the antifungal capacity of the compounds was investigated using broth microdilution method for the determination of inhibitory concentrations (MIC)s of these bioactive esters; the results are presented in Table 1 with the biphenyl-4-carboxylic acid. The minimum inhibitory concentration (MIC) for antifungal activity, expressed in μg/mL, of the bioactive esters was considered as the lowest concentration capable of visually inhibiting the growth of the microorganism when compared to the controls containing culture medium or inoculum. The MIC activity of the prepared esters was analyzed in accordance with the following criteria: 50-500 μg/mL was considered a compound with excellent activity, 600-1500 μg/mL was characterized as a compound with moderate activity, and valuesabove 1500 μg/mL indicated a weak or inactive compound against *Candida* spp. strains [18,19]. Of the eight esters evaluated, four showed bioactivity against strains of *Candida* spp.; demonstrating good to moderate bioactivity. Decanoyl 4-biphenyl carboxylate (**7**) was the compound with the strongest bioactivity, presenting the lowest MICs against all of the strains tested. In the present study, in addition to investigating the antifungal activity of esters against *Candida* spp., the influence of the structural characteristics of the prepared substances on biological activity, i.e., the structure-activity relationship was also analyzed. First, methyl 4-biphenyl carboxylate (1), the simplest compound among the prepared esters with only one carbon in its alkyl chain, did not show bioactivity; the ester ethyl 4-biphenylcarboxylate (2) presented moderate activity for *C. albicans* strains and good activity for *C. tropicalis* species. Thus it can be suggested that adding a carbon to the alkyl chain gives this ester bioactivity; changing from good to moderate.

Table 1: Results of antifungal activity evaluation against *Candida* spp. in MIC (μg/mL) for the prepared esters

	Candida albicans	***Candida albicans***	***Candida tropicalis***	***Candida tropicalis***
	ATCC-76485	**LM-111**	**ATCC-13803**	**LM-14**
Substances (μg/mL)/Yeast				
 **Biphenyl-4-carboxylic acid**	+	+	+	+
 **(1)**	+	+	+	+

 **(2)**	1024	1024	512	512
 **3**	+	+	+	+
 **4**	1024	1024	1024	1024
 **5**	+	+	+	+
 **6**	1024	1024	1024	1024
 **7**	512	512	512	512
 **8**	+	+	+	+
Culture medium	-	-	-	-
Control: nistatin	-	-	-	-

The compound propyl 4-biphenyl carboxylate (3) was not bioactive; isopropyl 4-biphenyl carboxylate (4) was bioactive for all tested strains, with moderate activity. These two esters are isomers, but the difference in alkyl volume close to the ester function was determinant for antifungal activity. Butyl 4-biphenyl carboxylate (5) had no activity, yet 2-methoxyethyl 4-biphenyl carboxylate (6) was moderately bioactive against all strains, suggesting that the presence of a heteroatom; oxygen in the alkyl chain makes the molecule bioactive.

Decanoyl 4-biphenyl carboxylate (7) obtained the best activity against all strains tested had the longest alkyl chain of the compounds prepared in the present work, this chain is formed by ten carbons, which may suggest that such increase yields higher lipophilicity to the molecule, thus, increasing permeation, and allowing greater activity, (the longer the carbon chain the greater the lipophilicity). The substance may also bind to cell membrane sterols and cause extravasation of cellular constituents and even cell death. However, this assertion would apply only to specific carbon chain lengths, and depend on several factors involving both the tested molecule and the microorganism.

4-chlorobenzyl 4-biphenyl carboxylate (8) did not present bioactivity; comparison with ester (7) suggests that exchanging a carbonic chain with an aromatic ring did not result in activity for the compound. This, since the aromatic ring is a bulky group which may hinder the binding of the molecule to its target, making the molecule less active or even inactive as seen in the results obtained in this study.

CONCLUSION

Eight structurally related esters were obtained using biphenyl-4-carboxylic acid as a starting material. All products were prepared via easy to perform reactions and presented good yields ranging from 62.7 to 95.5%. Regarding antifungal activity for the eight prepared esters, four presented bioactivity against strains of *Candida* spp., inhibiting fungal growth in MICs that varied from moderate to good. Of all the preparations, the ester obtaining the best activity and lowest MIC also had the largest alkyl chain, providing greater bioactivity and higher potency against all

of the tested strains. In accordance with the results, these compounds can be used in product studies involving better biological profiles and contributing to new drug prototype research, and thus to obtain more effective antifungals.

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