SYNTHESIS AND ANTIFUNGAL ACTIVITIES OF BENZIMIDAZOLYL-ARYLPROPENONE SCAFFOLDS AS PROMISING INHIBITORS OF AZOLE-RESISTANT CANDIDA STRAINS

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ABSTRACT
In the general context of the emergence of strains resistant to currently available antifungal drugs, we herein report the synthesis and the antifungal activities of some benzimidazoles-arylpropenones against three Azole-resistant Candida strains. These compounds were afforded by a Claisen-Schmidt type condensation reaction between 2-acetylbenzimidazole and the aromatic aldehydes. The chemical structures of the compounds were determined by the usual spectroscopic methods (¹H and ¹³C NMR, MS). The in vitro antifungal activities were assessed on clinical strains of Candida albicans, C. glabrata and C. tropicalis by the microplate dilution method. The results showed that of the 10 compounds tested, 7 possessed antifungal activities unlike the reference drugs (Fluconazole and Ketoconazole) which showed no activity at our limit threshold of 10 μg/mL. Furthermore, two compounds are particularly illustrated by their anti-Candida effectiveness on all three strains with MIC between 1.25 and 5μg / mL.


I-INTRODUCTION
A significant increase in fungal infections was observed in the last three decades. Numerous reports of superficial and invasive systemic infections caused by opportunistic fungal pathogen Candida, are associated with the use of broad spectrum antibiotics, immunosuppressive agents, anti-cancer drugs and anti-HIV.¹ Also, if Candida albicans is by far the most virulent and the main offending agent,² the incidence of non-albicans species such as Candida glabrata, Candida tropicalis, increases alarmingly.³ These non-albicans species are often refractory to conventional treatments than C. albicans.⁴ The five main classes of antifungal drugs are azoles, polyenes, allylamines, fluoropyrimidines and echinocandins.⁵ Azoles are the most commonly used class in particular for the treatment of candidiasis.⁶,⁷ One of the major problems in the treatment of Candida infections is the spread of resistance to antifungal agents, particularly in those individuals undergoing chronic antifungal treatments such as patients with AIDS.⁸ To counteract this resistance, intensive searches for new compounds having antifungal activities have developed around the world. In previous work,⁹ it was reported the antifungal activities of some hybrids of chalcones benzimidazole-based toward a clinical isolate of Candida albicans resistant to azole antifungals. Prompted by these preliminaries results we proposed to extend the screening of these benzimidazole-arylpropenones, against other Candida species (C. glabrata, C. tropicalis) Azole-resistant strains. This study allowed us to select after a structure activity relationship study, the best molecule on the three species of Candida.

II-MATERIALS AND METHODS
II.1 Chemistry
For all the compounds, nuclear magnetic resonance spectra (¹H, ¹³C and 300 MHz, 75 MHz) were registered on a Brucker instrument advance 300. The mass spectra (MS) were recorded on a HP 5889 spectrometer quadrupole in electron impact (EI). Melting points (mp) were determined using a Köfler bench and are uncorrected. The solvents and reagents are from Sigma Aldrich (France) or Acros Organics (France). Antifungal drugs (Ketoconazole and Fluconazole) as pure powders from, is from Sigma Chemical Co (USA).
General method for synthesis of 2-acetylbenzimidazole

The access to benzimidazolyl-2-arylprenones required the prior synthesis of the substituted 2-acetylbenzimidazole or not in position 5. This raw material was obtained by condensation method of Phillips[10] between various orthophenylenediamines properly chosen and lactic acid. To the solution of orthophenylenediamine suitably chosen (4.8 g; 42 mmol) in 50 mL of 4N hydrochloric acid, is added lactic acid (4.5 mL; 60 mmol). The mixture was heated under reflux for 45 min. The cooled reaction mixture is then neutralized with ammonia. The precipitate formed is filtered, washed with water and recrystallized with the same solvent to give 5.94 g of 2-hydroxyethylbenzimidazole. The 2-hydroxyethylbenzimidazole (3.5 g; 22 mmol) in solution in 30 mL acetic acid with 10 mL of an aqueous solution of potassium dichromate (3 g, 11 mmol) was heated under reflux for 45 minutes. The cooled reaction mixture is then neutralized with ammonia to give a precipitate. The precipitate is filtered off and then taken up in hydroxide (75 mmol of sodium hydroxide in 40 mL of water). The mixture is then neutralized with ammonia to give a precipitate. The precipitate is filtered, dried and then recrystallized in a water/Ethanol mixture (1: 1).

Table I: Antifungal susceptibility of Candida clinical isolates of theazole antifungals.

<table>
<thead>
<tr>
<th>Azole antifungals</th>
<th>Candida clinical isolates</th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida tropicalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>KETOCONAZOLE</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>FLUCONAZOLE</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

R: Resistant
S: Sensitive
I: Intermediate

The Antifungal susceptibility shows that clinical strains of Candida are all resistant KETOCONAZOLE and FLUCONAZOLE.

II.3 Antifungal assay

Antifungal screening by bioautography technique

Products in powder form were first solubilized in methanol for the preparation of stock solutions titrating to 1 mg/mL. From each of these stock solutions, a range of 10 dilutions of reason 2 was prepared. Then, 10 μL of each solution, were deposited on glass plates in Silicagel 60 F254. The chromatograms were previously developed in saturated tanks of a mobile chloroform-methanol-water phase in a ratio (65:35:5) and then dried. In addition, Candida albicans fungal inoculum containing approximately 105 cells/mL, was obtained by seeding three colonies of a pure strain for 24 to 48 hours in Tryptone Soya broth. This inoculum was spread on each chromatogram. The plates were incubated at 30°C after solidification of the agar for 24 hours. The plates, then obtained, were impregnated with an aqueous solution of methylthiazolyl chloride Tetrazolium and incubated for 2 to 4 hours. Areas of growth inhibition subsequently
appear as white spots on a purple background. Only those products that have shown a inhibitory zone at the 10 μg threshold have been selected for the determination of Minimum Inhibitory Concentrations (MICs).

Determination of Minimum Inhibitory Concentrations (MIC) by microplate dilution technique
The evaluation of antifungal efficacy by determining the Minimum Inhibitory Concentrations (MICs) was made using the microplate dilution technique. This technique consists of putting in contact a Candida inoculum with an increasing dilution of selected products in 96 well microplates. The preparation of the fungal inoculum is done as previously described in the bioautography technique. The stock solutions of benzimidazolylarylpropenones were prepared with Dimethylsulfoxide (DMSO) at a concentration of 1 mg/ml and then diluted with broth to obtain concentrated solutions at 100μg/mL. Subsequently, 100 μL of this dilution was deposited in the wells in the first column and 50 μL of broth was distributed to the following wells. Subsequently, 50 μL were taken from the first 100 μL of the first well to achieve a range of dilutions increasing for reason 2. Finally, 50 μL of inoculum was distributed to the wells except for the last one, which serves as a control to verify that there is no contamination. The plates were incubated at 30°C for 48 hours. For the revelation of the prepared microplates, 40 μL of aqueous solution of Methyl Thiazolyl Chloride Tetrazolium (MTT) at a concentration of 2.5 mg/mL was distributed to the wells and incubated for a further 30 minutes at room temperature. Wells containing still living cells turn yellow to purple as a result of mitochondrial dehydrogenase activity. The MIC is given by the lowest concentration at which MTT does not turn purple.

III RESULTS AND DISCUSSION
III.1 Chemical results
In total we synthesized and characterized five derivatives of 2-acetyl benzimidazole and ten benzimidazolylarylpropenones. The spectroscopic characteristics, the physicochemical properties and the yields of its compounds are described below.

2-acetyl benzimidazole (1a)
Yield = 72%; Yellow solid. 1H NMR (DMSO-d6, δ ppm): 13.31 (s, 1H, NH); 7.81 (1H, d, = 7.8 Hz, Har); 7.54 (1H, d, J = 7.8 Hz, Har); 7.30-7.38 (2H, m, Har); 2.70 (3H, s, CH3). 13C NMR: (DMSO-d6, δ ppm) 191.5 (C=O), 148.1 (C=N), 142.7 (Car), 134.6 (Car), 125.4 (Car), 122.9 (Car), 121.0 (Car), 112.8 (Car), 26.0 (CH3)

5-Benzoyl-2-acetyl benzimidazole (1b)
Yield = 68%; Yellow solid. 1H NMR (DMSO-d6, δ ppm): 13.50 (s, 1H, NH); 8.04 (1H, s, Ar); 7.68 - 7.77 (5H, m, Har); 7.59 (1H, d, Har); 7.55 (1H, d, Har); 2.72 (3H, s, CH3). 13C NMR (DMSO-d6, δ ppm): 195.5 (C=O), 191.4 (C=O), 151.1 (C=N), 134.2 (Car), 132.4 (Car), 131.3 (Car), 129.5 (Car), 129.1 (Car), 129.1 (2Car), 128.6 (2Car), 124.9 (Car), 122.6 (Car), 113.8 (Car), 26.2 (CH3).

5-Nitro-2-acetyl benzimidazole (1c)
Yield = 65%; Yellow solid. 1H NMR (DMSO-d6, δ ppm): 13.31 (s, 1H, NH); 8.54 (1H, d, Har); 8.18 (1H, m, Har); 7.78 (1H, d, Har); 2.70 (3H, s, CH3). 13C NMR (DMSO-d6, δ ppm): 191.5 (C=O), 151.6 (C=N), 146.7 (Car), 143.8 (Car), 142.6 (Car), 131.4 (Car), 119.6 (Car), 117.5 (Car), 26.1 (CH3).

5-Fluoro-2-acetyl benzimidazole (1d)
Yield = 73%; Yellow solid. 1H NMR (DMSO-d6, δ ppm): 13.40 (s, 1H, NH); 7.71 (1H, s, Ar); 7.43 (1H, d, Har); 7.23 (1H, m, Har); 2.68 (3H, s, CH3). 13C NMR (DMSO-d6, δ ppm): 191.1 (C=O), 161.3 (C-F), 149.3 (C=N), 145.2 (Car), 138.3 (Car), 122.4 (Car), 117.4 (Car), 116.9 (Car), 25.9 (CH3).

5-Chloro-2-acetyl benzimidazole (1e)
Yield = 75%; Yellow solid. 1H NMR (DMSO-d6, δ ppm): 13.40 (s, 1H, NH); 7.72 (1H, s, Ar); 7.43 (1H, d, Har); 7.21 (1H, m, Har); 2.68 (3H, s, CH3). 13C NMR (DMSO-d6, δ ppm): 191.2 (C=O), 161.2 (C-Cl), 149.3 (C=N), 145.0 (Car), 138.8 (Car), 122.4 (Car), 117.1 (Car), 116.0 (Car), 25.9 (CH3).

(E) -1- (1H-benzimidazol-2-yl) -3-phenylprop-2-en-1-one (3a)
Yield = 78%; Yellow solid; mp = 216 °C. 1H NMR (DMSO-d6, δ ppm): 14.00 (1H, s, NH); 8.28 (1H, d, J = 16 Hz, CH=CH); 8.15 (1H, d, J = 16 Hz, CH = CH); 8.02 (2H, m, Har); 7.90 (2H, m, Har); 7.20 (2H, m, Har); 7.09 (2H, m, Har); 7.06 (1H, m, Har). 13C NMR (DMSO-d6, δ ppm): 181.1 (C=O), 149.5 (C=N); 144.0 (CH=CH); 139.9 (Car); 134.8 (2Car); 128.8 (Car); 124.2 (2Car); 122.5 (2Car); 122.7 (2Car); 121.7 (CH=CH); 117.0 (2Car). ES + MS: 249 [M + H +].

(E) -1- (1H-benzimidazol-2-yl) -3- (pyridin-3-yl) prop-2-en-1-one (3b)
Yield = 50%; Yellow solid; mp = 244°C. 1H NMR (DMSO-d6, δ ppm): 13.60 (1H, s, NH); 9.0 (1H, d, J = 2 Hz, Har); 8.80 (1H, d, J = 2 Hz, Har); 8.65 (1H, m, Har); 8.35 (1H, m, Har); 8.25 (1H, d, J = 16.2 Hz, CH=CH); 8.05 (1H, d, J = 16.2 Hz, CH=CH); 7.60 (2H, m, Har); 7.35 (2H, m, Har). 13C NMR (DMSO-d6, δ ppm): 182.1 (C=O); 154.0 (Car); 150.8 (Car); 150.1 (C=N); 144.3 (CH=CH); 139.8 (Car); 134.7 (2Car); 124.2 (Car); 122.5 (2 Car); 121.7 (CH=CH); 120.9 (Car); 117.0 (2Car). ES + MS: 250 [M + H +].

(E) -1- (5-benzoyl-1H-benzimidazol-2-yl)-3-phenylprop-2-en-1-one (3c)
Yield = 75%; Yellow solid; mp = 250°C. 1H NMR (DMSO-d6, δ ppm): 14.0 (1H, s, NH); 8.11 (1H, d, J = 18 Hz, Har); 8.04 (1H, s, Ar); 7.86 (1H, d, J = 18 Hz, Har); 7.73 to 7.79 (5H, m, Har); 7.70 (1H, s, Ar); 7.64
(1H, d, Har); 7.58 (2H, m, Har); 7.54 (2H, m, Har); 7.51 (1H, m, Har). 13C NMR (DMSO-d6, δ ppm): 195.4 (C10), 180.8 (C1), 151.0 (C9), 144.9 (C8), 137.6 (C7), 134.2 (C6a), 133.0 (C6), 132.4 (C5), 131.2 (C4), 129.5 (C30), 129.1 (C19 and C25), 129.0 (C18 and C26), 128.5 (C27), 127.0 (C12), 126.2 (C13), 124.2 (C3 and C5), 122.7 (C7 and C8), 122.5 (C15), 121.7 (C2), 120.9 (C6), ES + MS: 353 [M + H+].

(E) -1- (5-benzoyl-1H-benimidazol-2-yl) -3- (pyridin-3-yl)prop-2-en-1-one (3d)
Yield = 55%; Yellow solid; mp = 186°C. 1H NMR (DMSO-d6, δ ppm): 14.0 (1H, s, NH); 9.0 (1H, s, H3); 8.90 (1H, d, J = 2Hz, H2); 8.35 (1H, m, H8); 8.02 (1H, s, H7); 7.97 (1H, d, J = 18 Hz, H1); 7.90 (1H, m, H6); 7.86 (1H, d, J = 18 Hz, H3); 7.73 to 7.79 (5H, m, H13-H22); 7.70 (1H, s, H8); 7.64 (1H, d, H4). 13C NMR (DMSO-d6, δ ppm): 195.4 (C10), 180.7 (C1), 154.0 (C30), 151.1 (C9), 150.8 (C8), 144.9 (C7), 137.6 (C6), 135.4 (C6a), 133.0 (C11a), 132.4 (C11), 131.4 (C11), 129.5 (C29), 129.1 (C20 and C25), 129.0 (C18 and C26), 127.0 (C12), 126.2 (C13), 124.2 (C3 and C5), 122.5 (C15), 121.7 (C2), 120.9 (C6), ES + MS: 354 [M + H+].

(E) -1- (5-chloro-1H-benimidazol-2-yl) -3-phenylprop-2-en-1-one (3e)
Yield = 78%; Yellow solid; mp = 228°C. 1H NMR (DMSO-d6, δ ppm): 13.50 (1H, s, NH); 8.10 (1H, d, J = 16Hz , H3); 7.97 (1H, d, J = 16 Hz, H2); 7.85 (1H, m, H8); 7.76 (1H, m, H7); 7.46-7.51 (5H, m, H6, Hc, Hb, H9, and H14); 7.25 (1H, m, H14). 13C NMR (DMSO-d6, δ ppm): 181.2 (C1), 153.7 (C10), 144.0 (C7), 143.8 (C14a), 142.6 (C11a), 141.3 (C11), 139.9 (C9), 128.8 (C8), 124.6 (C12), 124.2 (C3 and C5), 122.5 (C15), 122.7 (C2), 121.7 (C6 and C8), 121.7 (C7), 117.0 (C13). ES + MS: 283 [M + H+].

(E) -1- (5-fluoro-1H-benimidazol-2-yl) -3-phenylprop-2-en-1-one (3g)
Yield = 77%; Yellow solid; mp = 220°C. 1H NMR (DMSO-d6, δ ppm): 13.50 (1H, s, NH); 8.10 (1H, d, J = 16 Hz, H3); 7.96 (1H, d, J = 16 Hz, H2); 7.86 (1H, m, H8); 7.76 (1H, m, H7); 7.47-7.52 (4H, m, H6, Hc, Hb, and H9); 7.23 (1H, m, H14). 13C NMR (DMSO-d6, δ ppm): 181.2 (C1), 158.2 (C13), 150.3 (C18), 144.5 (C7), 135.4 (C14a), 133.2 (C11a), 130.9 (C8), 125.7 (C2), 124.2 (C3 and C5), 122.70 (C6 and C8), 121.7 (C7), 119.0 (C14), 117.0 (C13). ES + MS: 267 [M + H+].

(E) -1- (5-fluoro-1H-benimidazol-2-yl) -3- pyridin-3-ylprop-2-en-1-one (3h)
Yield = 55%; Yellow solid; mp = 189°C. 1H NMR (DMSO-d6, δ ppm): 14.0 (1H, s, NH); 9.07 (1H, d, H3); 8.96 (1H, d, H2); 8.50 (1H, m, H8) 8.35 (1H, m, H7); 8.25 (1H, d, J = 16 Hz, H1); 8.05 (1H, d, J = 16 Hz, H2); 7.80 (1H, s, H9); 7.70 (1H, s, H14). 13C NMR (DMSO-d6, δ ppm): 182.0 (C1), 154.0 (C3), 150.8 (C7), 150.1 (C10), 145.3 (C7), 144.3 (C3), 139.8 (C8), 135.4 (C18), 133.0 (C11a), 127.0 (C12), 124.2 (C6 and C8), 122.6 (C14), 121.7 (C2), 120.9 (C6 and C8), 117.5 (C13). ES + MS: 295 [M + H+].

III.2 Biological results

III.2.1-Results of the anticandidiasis screening by bioautography technique
The results of the anticandidiasis screening (Table II) showed that seven of our compounds (3a, 3c, 3d, 3e, 3f, 3g, 3h) are active on at least one strain of Candida. On the other hand, reference drugs (Fluconazole and Ketoconazole) were inactive on the three strains of Candida at the limit threshold of our experiment (QMI = 10 μg).
Table II: Results of the antifungal screening 3a-3j compounds, Ketoconazole and Fluconazole against Candida.

<table>
<thead>
<tr>
<th>general structure</th>
<th>Compounds</th>
<th>R</th>
<th>X</th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida Tropicalis</th>
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<tbody>
<tr>
<td></td>
<td>3a</td>
<td>H</td>
<td>CH</td>
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</table>

(-): not determined as having no activity. At the threshold quantity of 10 µg.

After screening, the Minimum Inhibitory Concentrations (MIC) of derivatives with anticandidosic activities greater than the threshold of 10 µg was determined by dilution method on microplates. The minimum inhibitory concentrations (MIC) against each Candida species are expressed in micrograms per milliliter (µg / mL), and summarized in Table III:

III.2.2-Results of the anticandidosic activities by dilution method on microplates

Table III: Antifungal activities in vitro 3a-3d compounds and reference compounds against strains of Candida.

<table>
<thead>
<tr>
<th>General structure</th>
<th>Compounds</th>
<th>R</th>
<th>X</th>
<th>MIC (µg / mL)</th>
<th>Candida albicans</th>
<th>Candida glabrata</th>
<th>Candida Tropicalis</th>
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<tr>
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</table>

(-): not determined as having no activity anticandidosique antifungal screening the threshold quantity of 10 µg.

These MICs showed that only compounds (3a, 3c, 3d, 3e, 3f, 3g, 3h) are active on Candida strains with concentrations ranging from 1.25 to 5 µg / mL.

III.3 DISCUSSION

The results of the antifungal screening revealed that the Candida strains have decreased susceptibility to the antifungal Azoles (Fluconazole and Ketoconazole). As these clinical Candida strains are isolated from AIDS patients, the observed resistance could be explained by the systematic use of antifungal Azoles as a prophylactic or curative treatment for mycoses in patients. Indeed, cases of resistance have been reported in people living with HIV / AIDS who have received long-term Fluconazole therapy. Thus, the current medical consensus advocating the prophylaxis of candidiasis in immunocompromised individuals would promote selection of resistant isolates and cross-resistance to antifungal azoles. The structure-activity relationship analysis of the anti-Candida tests showed that benzimidazole covalently linked to the phenylpropenone chain in position 2 (compound 3a) induced an anti-Candida albicans activity with a MIC of 5 µg / mL. Such efficacy on the drug-resistant clinical strain with azoles confirms the intrinsic anti-infectious potential of the arylpropenone functional group of chalcones and benzimidazole heterocycle reported in the literature. However, the compound 3a is inactive on the Candida glabrata and Candida tropicalis species at the threshold amount of 10 µg / mL. In order to improve the anticandidosic activities of compound 3a and expand its antifungal spectrum to the other species of Candida, various chemical modulations have been undertaken around it. These allow establishing that the replacement
of the benzene ring homocycle of compound 3a by pyridinic heterocycle (compound 3b) has led to the annihilation of the antifungal activity, whatever the strain of Candida considered. On the other hand, the introduction of a benzoyl group at the 5-position of the benzimidazole of compound 3a causes a loss of activity on Candida albicans in favor of Candida tropicalis (MIC = 2.5 μg / mL). Indeed the C5-benzoyl derivative or compound 3c, inactive on C. albicans and C glabrata was shown effective on C. tropicalis. The combination of the two preceding modulations, namely the replacement of the benzene homocycle by a pyridine and the C5 benzylation of the benzimidazole (compound 3d), enabled the maintenance of the anti-albicans activity at 5 μg / mL and anti-tropicalis activity at 2.5 μg / mL comparable to the activities of the compounds 3a and 3c. The introduction of a chlorine atom at the position 5 of the benzimidazole of compound 3a has enabled compound 3e to be endowed with potent antifungal activities against Candida albicans (MIC = 1.25 μg / mL). This anti-Candida performance was 4-fold more active than that of compound 3a on the same strain (CMI = 5 μg/mL). Moreover, 3e extended its antifungal efficacy to Candida glabrata and Candida tropicalis with MIC of 5μg/mL. The C5 chloro derivative was found to be effective in inducing antifungal activity against the three resistant strains of Candida tested. The replacement of the benzene ring of 3e by a hexagonal type pyridine heterocycle (compound 3f) led to loss of antifungal activities towards Candida glabrata and Candida tropicalis. Indeed, the chloro pyridinylpropenone (3f) was only active against C. albicans with a CMI of 1.25 μg/mL. The replacement of the chlorine of the compound 3e by the Fluorine atom, led to a decline in effectiveness of the compound 3g against C. albicans by a factor of 4 (MIC = 5 μg / mL). In addition, this C5-fluorinated derivative (compound 3g) is totally inefficiency on C. glabrata and C. tropicalis. On the other hand, the replacement of the benzene ring of the compound 3g by a pyridinic heterocycle led to compound 3h effective against the three strains of Candida. This fluoro pyridinylpropenone derivative is effectively active on C. albicans (MIC = 2.5 μg / mL), in addition to inducing anti-glabrata activities at 5 μg / mL and anti-tropicalis activities at 2.5 μg / mL. The introduction of a nitro group (3i and 3j) is not in favour of the appearance of the anti-Candida activity. These 2 compounds exerted no antifungal activity on all strains at the threshold limit of our experimentation (QMI = 10 μg).

CONCLUSION
This work of pharmacocchemistry reports the synthesis and evaluation of the antifungal activities of some benzimidazolyl-arylprenopones against three azole-resistant Candida strains. This study showed that benzimidazolyl-arylprenopones had a high antifungal potential with MICs ranging from 1.25 to 5μg / mL. The structure-activity relationship studies revealed that the best performance on Candida albicans is obtained with chloro derivative with a MIC of 1.25 μg / mL. The chloro and fluoro compounds remain the most effective on C. glabrata with a MIC of 5 μg / mL. On the other hand, with MICs of 2.5 μg / mL, the benzoyl compounds and the fluorinated derivative turned out to be the most effective against C. tropicalis. Against the three azole-resistant strains of Candida, C5-halogenated compounds showed the best antymycotic profile with MICs ranging from 1.25 to 5 μg / mL. In addition, the chemical modulations undertaken showed that the presence of a nitro group at the 5-position of benzimidazole led to a loss of antifungal activities. The induction of antifungal activities was related to the presence of a halogen atom or a C5 benzoyl group of the benzimidazole ring. These results allow us to validate the benzimidazolyl-arylpropenone chemical scaffold as a promising pharmacophore with high antifungal potential.

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REFERENCES