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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-Schimdt type condensation reaction between 2-acetylbenzimidazole and the aromatic aldehydes. The chemical structures of the compounds were determined by the usual spectroscopic methods (^{1}H and ^{13}C 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. Furthermore, two compounds are particularly illustrated by their anti-*Candida* effectiveness on all three strains with MIC between 1.25 and 5µg / mL.

KEYWORDS: Benzimidazole. Arylpropenone. Azoles. *Candida albicans*. *Candida glabrata*. *Candida tropicalis*.

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*.[3] The five main classes of antifungal drugs are azoles, polyenes, allylamines, fluoropyrimidines and echinocandins.[4] Azoles are the most commonly used class in particular for the treatment of candidiasis.[5,6] 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.[7,8] 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 (^{1}H, ^{13}C 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-arylpropenones 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 chloroform. The organic layer is then washed with water, dried over sodium sulphate and then evaporated under reduced pressure. The residue is finally purified by chromatography on silica gel (elution: CH_2Cl_2/AcOEt: 95: 5) or recrystallized in a water/Ethanol mixture (1: 1).

General method of preparation of the benzimidazolyl-arylpropenones

The previously prepared 2-acetylbenzimidazoles have been engaged in a condensation of Claisen-Schmidt in basic medium,[10] with arylaldehydes to drive to the benzimidazolyl-2-arylpropenones expected according to the Protocol below. To an ethanolic solution of sodium hydroxide (75 mmol of sodium hydroxide in 40 mL of ethanol) containing 2-acetylbenzimidazoles 2a-2e (1.5 g, 10 mmol), is added the arylaldehyde (10.1 mmol) properly chosen. The reaction mixture is stirred at room temperature for 3 to 5 hours. The neutralization of the middle with a solution of acetic acid to 30% gave a precipitate which is filtered, dried and then recrystallized in a water/Ethanol mixture (1: 1).

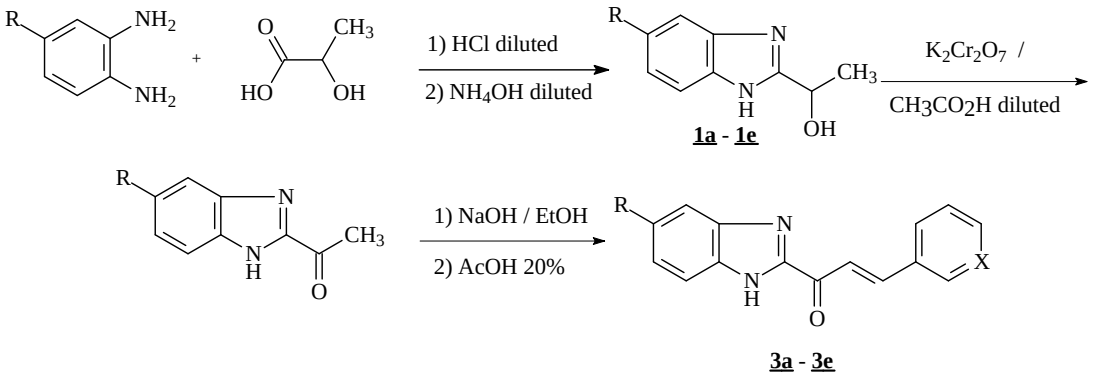


Figure 1: Synthesis of the benzimidazolyl-arylpropenones.

II.2-Clinical Strains of *Candida*

The clinical strains of *Candida* (*C .albicans*, *C. glabrata* and *C. tropicalis*) were provided by the Center for Diagnosis and Research on AIDS and Opportunistic Diseases (CeDReS) of the University Hospital of Treichville Abidjan (Côte d'Ivoire). An antifongigram using the agar diffusion method was previously performed to determine the susceptibility of these strains to antifungal azoles (Ketoconazole, Fluconazole). Results from the antifungigram show resistance to Ketoconazole and Fluconazole (**Table I**).

Table I: Antifungal susceptibility of *Candida* clinical isolates of the azole antifungals.

Azole antifungals	Candida clinical isolates		
	Candida albicans	Candida glabrata	Candida tropicalis
Ketoconazole	**R**	**R**	**I**
Fluconazole	**R**	**R**	**R**

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.[12] Only those products that have shown a inhibitory zone at the 10 µg threshold amount 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-acetylbenzimidazole and ten benzimidazolyl-arylpropenones. The spectroscopic characteristics, the physicochemical properties and the yields of its compounds are described below.

2-acetyl benzimidazole (1a)

Yield = 72%; Yellow solid. ^{1}H NMR (DMSO-d$_6$, δ ppm): 13.31 (s, 1H, NH); 7.81 (1H, d, *J* = 7.8 Hz, Har); 7.54 (1H, d, *J* = 7.8 Hz, Har); 7.30-7.38 (2H, m, Har); 2.70 (3H, s, CH_3). ^{13}C NMR: (DMSO-d$_6$, 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 (CH_3)

5-Benzoyl-2-acetyl benzimidazole (1b)

Yield = 68%; Yellow solid. ^{1}H NMR (DMSO-d$_6$, 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, CH_3). ^{13}C NMR (DMSO-d$_6$, 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 (CH_3).

5-Nitro-2-acetyl benzimidazole (1c)

Yield = 65%; Yellow solid. ^{1}H 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, CH_3). ^{13}C 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 (CH_3).

5-Fluoro-2-acetyl benzimidazole (1d)

Yield = 73%; Yellow solid. ^{1}H 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, CH_3). ^{13}C 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 (CH_3).

5-Chloro-2-acetyl benzimidazole (1e)

Yield = 75%; Yellow solid. ^{1}H NMR (DMSO-d$_6$, 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, CH_3). ^{13}C NMR (DMSO-d$_6$, 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 (CH_3).

(E) -1- (1H-benzimidazol-2-yl) -3-phenylprop-2-en-1-one (3a)

Yield = 78%; Yellow solid; mp = 216 °C. ^{1}H NMR (DMSO-d$_6$, 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). ^{13}C NMR (DMSO-d$_6$, 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.^{1}H NMR (DMSO-d$_6$, 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). ^{13}C NMR (DMSO-d$_6$, 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. ^{1}H NMR (DMSO-d$_6$, 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). ^{13}C NMR (DMSO-d_6, δ ppm): 195.4 (C_{16}), 180.8 (C_1), 151.0 (C_{10}), 144.9 (C_3), 137.6 (C_4), 134.2 (C_{15a}) , 133.0 (C_{11a}), 132.4 (C_{17}), 131.2 (C_{13}), 129.5 (C_{20}), 129.1 (C_{18} and C_{22}), 129.0 (C_{19} and C_{21}), 128.5 (C_7), 127.0 (C_{12}), 126.2 (C_{14}), 124.2 (C_5 and C_9), 122.7 (C_6 and C_8), 122.5 (C_{15}), 121.7 (C_2), 120.9 (C_8). ES + MS: 353 [M + H +].

(E) -1- (5-benzoyl-1H-benzimidazol-2-yl) -3- (pyridin-3-yl) prop-2-en-1-one (3d)

Yield = 55%; Yellow solid; mp = 186°C. ^{1}H NMR (DMSO-d_6, δ ppm): 14.0 (1H, s, NH); 9.0 (1H, s, H_5); 8.90 (1H, d, *J* = 2Hz, H_7); 8.35 (1H, m, H_8); 8.02 (1H, s, H_{12}); 7.97 (1H, d, *J* = 18 Hz, H_3); 7.90 (1H, m, H_9); 7.86 (1H, d, *J* = 18 Hz, H_2); 7.73 to 7.79 (5H, m, H_{18} - H_{22}); 7.70 (1H, s, H_{15}); 7.64 (1H, d, H_{14}). ^{13}C NMR (DMSO-d_6, δ ppm): 195.4 (C_{16}), 180.7 (C_1), 154.0 (C_5), 151.1 (C_{10}), 150.8 (C_7), 144.9 (C_3) , 137.6 (C_4), 135.4 (C_{15a}), 133.0 (C_{11a}), 132.4 (C_{17}), 131.4 (C_7), 131.2 (C_{13}), 129.5 (C_{20}), 129.1 (C_{18} and C_{22}), 129.0 (C_{19} and C_{21}), 127.0 (C_{12}), 126.2 (C_{14}), 124.2 (C_9), 122.5 (C_{15}), 121.7 (C_2), 120.9 (C_8). ES + MS: 354 [M + H +]

(E) -1- (5-chloro-1H-benzimidazol-2-yl) -3- phenylprop-2-en-1-one (3e)

Yield = 78%; Yellow solid; mp = 228°C.^{1}H NMR (DMSO-d6, δ ppm): 13.50 (1H, s, NH); 8.10 (1H, d, *J* = 16Hz , H_3); 7.97 (1H, d, *J* = 16 Hz, H_2); 7.85 (1H, m, H_{12}); 7.76 (1H, m, H_{15}); 7.46-7.51 (5H, m, H_5, H_6, H_8, H_9 and H_{14}); 7.25 (1H, m, H_{14}).^{13}C NMR (DMSO-d_6, δ ppm): 181.2 (C_1), 153.7 (C_{10}), 144.0 (C_3), 143.8 (C_{15a}), 142.6 (C_{11a}), 141.3 (C_{13}) , 139.9 (C_4), 128.8 (C_7), 124.6 (C_{12}), 124.2 (C_5 and C_9), 122.5 (C_{14}), 122.7 (C_6 and C_8), 121.7 (C_2), 117.0 (C_{15}). ES + MS: 283 [M + H +].

(E) -1- (5-chloro-1H-benzimidazol-2-yl) -3- pyridin-3-ylprop-2-en-1-one (3f)

Yield = 61%; Yellow solid; mp = 188°C. ^{1}H NMR (DMSO-d_6, δ ppm): 14.0 (1H, s, NH); 9.07 (1H, d, H_5); 8.96 (1H, d, H_7); 8.50 (1H, m, H_8); 8.35 (1H, m, H_9); 8.50 (2H, m, H_{12}); 8.30 (1H, d, *J* = 16 Hz, H_3); 8.12 (1H, d, *J* = 16 Hz, H_2); 7.85 (1H, s, H_{15}); 7.70 (1H, m, H_{14}). ^{13}C NMR (DMSO-d_6, δ ppm): 181.0 (C_1), 154.5 (C_5), 151.1 (C_7), 150.2 (C_{10}), 145.0 (C_{13}), 143.8 (C_3), 141.7 (C_{15a}), 139.2 (C_4), 133.0 (C_{11a}), 127.6 (C_{12}), 122.6 (C_{14}), 124.2 (C_9), 121.7 (C_2), 120.8 (C_8), 117.5 (C_{15}).

(E) -1- (5-fluoro-1H-benzimidazol-2-yl) -3- phenylprop-2-en-1-one (3g)

Yield = 77%; Yellow solid; mp = 220°C. ^{1}H NMR (DMSO-d_6, δ ppm): 13.50 (1H, s, NH); 8.10 (1H, d, *J* = 16 Hz, H_3); 7.96 (1H, d, *J* = 16 Hz, H_2); 7.86 (1H, m, H_{12}); 7.76 (1H, m, H_{15}); 7.47-7.52 (4H, m, H_5, H_6, H_8, and H_9); 7.23 (1H, m, H_{14}). ^{13}C NMR (DMSO-d_6, δ ppm): 181.2 (C_1), 158.2 (C_{13}), 150.3 (C_{10}), 144.5 (C_3), 135.4 (C_{15a}), 133.2 (C_{11a}), 130.9 (C_4), 125.7 (C_{12}), 124.2 (C_5 and C_9), 122.70 (C_6 and C_8), 121.7 (C_2), 119.0 (C_{14}), 117.0 (C_{15}). ES + MS: 267 [M + H +].

(E) -1- (5-fluoro-1H-benzimidazol-2-yl) -3- pyridin-3-ylprop-2-en-1-one (3h)

Yield = 55%; Yellow solid; mp = 189°C. ^{1}H NMR (DMSO-d6, δ ppm): 14.0 (1H, s, NH); 9.07 (1H, d, H_5); 8.96 (1H, d, H_7); 8.50 (1H, m, H_8); 8.35 (1H, m, H_9); 8.30 (1H, d, *J* = 16Hz, H_3); 8.12 (1H, d, *J* = 16 Hz, H_2); 7.86 (1H, m, H_{12}); 7.70 (1H, m, H_{15}); 7.25 (1H, m, H_{14}).^{13}C NMR (DMSO-d_6, δ ppm): 181.0 (C_1), 154.5 (C_5), 151.4 (C_7), 150.2 (C_{10}), 145.0 (C_{13}), 143.9 (C_3) , 141.7 (C_{15a}), 139.2 (C_4), 133.0 (C_{11a}), 125.7 (C_{12}), 124.2 (C_9), 121.7 (C_2), 120.8 (C_8), 119.9 (C_6), 119.6 (C_{14}), 117.5 (C_{15}). ES + MS: 284 [M + H +].

(E) -1- (5-nitro-1H-benzimidazol-2-yl) -3-phenylprop-2-en-1-one (3i)

Yield = 72%; Yellow solid; mp = 258°C. ^{1}H NMR (DMSO-d_6, δ ppm): 14.00 (1H, s, NH); 8.54 (2H, m, H_{12}); 8.28 (1H, d, *J* = 16 Hz, H_3); 8.20 (1H, m, H_{14}); 8.12 (1H, d, *J* = 16 Hz, H_2); 7.90 (1H, m, H_{15}); 7.20 (2H, m, H_5 and H_9); 7.09 (2H, m, H_6 and H_8); 7.06 (1H, m, H_7). ^{13}C NMR (DMSO-d_6, δ ppm): 181.2 (C_1), 153.7 (C_{10}), 144.9 (C_{13}), 143.9 (C_3), 143.8 (C_{15a}), 142.6 (C_{11a}), 139.9 (C_4), 131.3 (C_{12}), 128.8 (C_7), 124.2 (C_5 and C_9), 122.51 (C_{14}), 122.70 (C_6 and C_8), 121.69 (C_2), 120.89 (C_8), 117.03 (C_{15}). ES + MS: 294 [M + H +].

(E) -1- (5-nitro-1H-benzimidazol-2-yl) -3- (pyridin-3-yl) prop-2-en-1-one (3j)

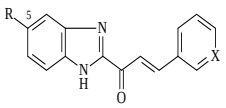
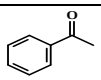
Yield = 57%; Yellow solid; mp = 242°C. ^{1}H NMR (DMSO-d6, δ ppm): 13.60 (1H, s, NH); 9.07 (1H, d, *J* = 2 Hz, H_5); 8.96 (1H, d, *J* = 2Hz, H_7); 8.50 (1H, m, H_8); 8.35 (1H, m, H_9); 8.25 (1H, d, *J* = 16.2 Hz, H_3); 8.05 (1H, d, *J* = 16.2 Hz, H_2); 8.02 (1H, s, H_{12}); 7.70 (1H, s, H_{15}); 7.64 (1H, d, H_{14}). ^{13}C NMR (DMSO-d6, δ ppm): 182.0 (C_1), 154.0 (C_5), 150.8 (C_7), 150.1 (C_{10}), 145.3 (C_{13}), 144.3 (C_3), 139.8 (C_4), 135.4 (C_{15a}), 133.0 (C_{11a}), 127.0 (C_{12}), 124.2 (C_9), 122.6 (C_{14}), 121.7 (C_2), 120.9 (C_8), 117.5 (C_{15}). ES + MS: 295 [M + H +].

III.2 Biological results

III.2.1-Results of the anticandidosic screening by bioautography technique

The results of the anticandidosic 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*.

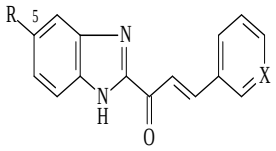
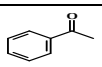
general structure	Compounds	R	X	*Candida albicans*	*Candida glabrata*	*Candida Tropicalis*
	**3a**	H	CH	+	-	-
	3b		N	-	-	-
	3c		CH	-	-	+
	3d		N	+	-	+
	3rd	Cl	CH	+	+	+
	3f		N	+	-	-
	3g	F	CH	+	-	-
	3h		N	+	+	+
	3i	NO_2	CH	-	-	-
	3d		N	-	-	-
Ketoconazole				-	-	-
Fluconazole				-	-	-

(-): 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 10µg 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.

General structure	Compounds	R	X	MIC (µg / mL)		
				Candida albicans	*Candida glabrata*	*Candida Tropicalis*
	**3a**	H	CH	**5**	-	-
	3b		N	-	-	-
	3c		CH	-	-	**2.5**
	3d		N	**5**	-	**2.5**
	3rd	Cl	CH	**1.25**	**5**	**5**
	3f		N	**1.25**	-	-
	3g	F	CH	**5**	-	-
	3h		N	**2.5**	**5**	**2.5**
	3i	NO_2	CH	-	-	-
	3d		N	-	-	-
Ketoconazole				-	-	-
Fluconazole				-	-	-

(-): 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 5µg / 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.[13,14] 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.[15,16] 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.[17-19] 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 benzoylation 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 anti-fungal 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 anticandidosic 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 pharmacochemistry reports the synthesis and evaluation of the anticandidosic activities of some benzimidazolyl-arylpropenones against three **azole-resistant *Candida* strains**. This study showed that benzimidazolyl-arylpropenones 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 antimycotic 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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