Anticandidosic Activities of New Chalcones Vectorised By Benzimidazole against a Strain of Candida Albicans Pharmacoresistance to Azoles

Coulibaly Songuigama [1], N'guessan D. Jean-Paul[1], Koné W Mamidou[2], Sissouma Drissa[3], Ouattara Mahama[1]

[1]*Département De Chimie Organique Et Chimie Thérapeutique, UFR Sciences Pharmaceutiques, Université FHB, 01 BP V34 Abidjan, Côte d'Ivoire*
[2]*Centre Suisse De Recherches Scientifiques En Côte d'Ivoire, 01 BP 1303 Abidjan*
[3]*Laboratoire De Chimie Organique Structurale, UFR SSMT, Université FHB, 22 BP 582 Abidjan, Côte d'Ivoire*
Corresponding Author: Ouattara Mahama

Abstract: *The objective of this work was to determine the anticandidosic activities of new chalcones against a clinical strain of Candida albicans resistant to antifungal Azole, and then to determine the structural elements conducive to induction of anticandidosic activities. Ten chalcones hybrids, all of which contain phenylpropenone or pyridinylpropenone chains in their respective molecules, have been designed, synthesized and characterized. In order to select the active compounds at the 10 µg threshold, an antifungal screening was performed using the bioautographic method. The Minimum Inhibitory Concentrations (MICs) of the selected compounds were then determined in vitro on the clinical strain of Candida albicans using the microplate dilution method. Among the 10 compounds evaluated, 6 derivatives presented antifungal activity. The reference substances (Fluconazole and Ketoconazole) showed no activity during screening at the 10 µg threshold amount. In addition, the best anticandidosic performance was achieved with C5-halogenated derivatives with MICs of 1.25 µg / ml.*
Our pharmacochemical approach has led to the development of new molecules with benzimidazolyl-arylpropenone structure with anticandidosic activities. This innovative chemical profile opens up new and important avenues of investigation for the development of new antifungal agents.

Keywords: *Benzimidazole. Arylpropenone. Chalcone. anticandidosic. Candida albicans.*

I. Introduction

Candidiasis infections have experienced a resurgence in recent years, with the increase in immunodeficiency situations [1-3]. This resurgence of candidiasis, especially in immunocompromised subjects, is a public health problem [4. In addition, the drug management of candidiasis, which was once very effective with the use of Azoles antifungal agents, is currently confronted with strong drug resistance in some *Candida* strains [5-6]. In this context, it is necessary to develop new molecules that are not resistant to these drugs. This is why we have taken an interest in the benzimidazole heterocycle, which carries in its position 2 the functional arylpropenone chain of chalcones as a potential new anticandiosis. This interest in the benzimidazole nucleus is explained by the fact that it constitutes the heterocyclic support of Chlormidazole, the first synthetic antifungal agent[7-8]. In addition, benzimidazole is primarily a nitrogenous nucleus resulting from the binding of benzene and imidazole. This heterocycle could have anticandidotic properties, such as antifungal azoles. As for the arylpropenone functional sequencing of chalcones, its ability to bind certain thiol-functional infectious enzymes (glutathione S-transferase, cysteine, keratin, etc.) would currently explain the use of its acceptors for the development of new anti-infectious drugs [9-11]. For all these reasons, our research team has conceptualized, synthesized and characterized chalcone hybrids for anti-infectious purposes. After evaluating the anthelmintic and antiplasmodic activities of the said chalcones [12-13], it seemed logical to evaluate their antifungal activities *against* a clinical strain of *Candida albicans* pharmacoresistant to Azoles. These benzimidazole supported chalcone hybrids could lead to drug candidates that are effective against *Candida albicans*. The objective of this work is to determine the Minimum Inhibitory Concentrations of these chalcones in relation to *C. albicans* strain and then to establish the structural elements favourable to the desired Anticandidosic activities.

II. Experimental

1.Reagents, Instrumentation and Measurements

For all compounds, nuclear magnetic resonance spectra (1H, 300 MHz and 13C, 75 MHz) were recorded on a Brucker advance 300 instrument. Mass spectra (MS) were recorded on an HP 5889 quadrupole spectrometer using electronic impact (EI). TLC were carried out using Merck aluminum backed sheets coated with 60 F_{254} silica gel. The melting points (PF) were determined using a Kofler bench and are not corrected. Solvents and reagents come from Sigma Aldrich (France) or Acros Organics (France). Antifungal drugs (Ketoconazole and Fluconazole) in the form of pure powders come from Sigma Chemical Co (USA).

2.General procedure for compounds (3a-j)

Access to chalconic derivatives required the prior synthesis of a raw material, namely 2-acetylbenzimidazole, which may or may not be substituted in position 5. This raw material has been obtained by condensation according to the Phillips method [14] (Phillips M, 1928). Between various suitably selected orthophenylenediamines and lactic acid. All the different reaction stages of access to 2-acetylbenzimidazole have been described in a previous work [15]. Subsequently, the 2-acetylbenzimidazoles (1) prepared were engaged in a Claisen-Schmidt type basic condensation reaction with benzaldehyde or with its pyridine analogue (nicotinaldehyde) (2) to lead to the expected new chalcones (3a-3j) (Figure 1). The general method of access to chalcones consisted in adding to an ethanolic solution of sodium hydroxide (75 mmol of sodium hydroxide in 40 ml of ethanol) 2-acetylbenzimidazole (1.5 g, 10 mmol) and arylaldehyde (10, 1 mmol) corresponding. The reaction medium is stirred for 3 to 5 hours at ambient temperatures. After neutralization of the medium with a solution of 30% acetic acid, the precipitate formed was filtered, dried and then recrystallized in a suitably chosen solvent. By proceeding, in this way we isolated 10 chalcones (Table I) all possessing in their respective structures the phenylpropenone (3a, 3c, 3e, 3g, 3i) or pyridinylpropenone (3b, 3d, 3f, 3h, 3j) chain.
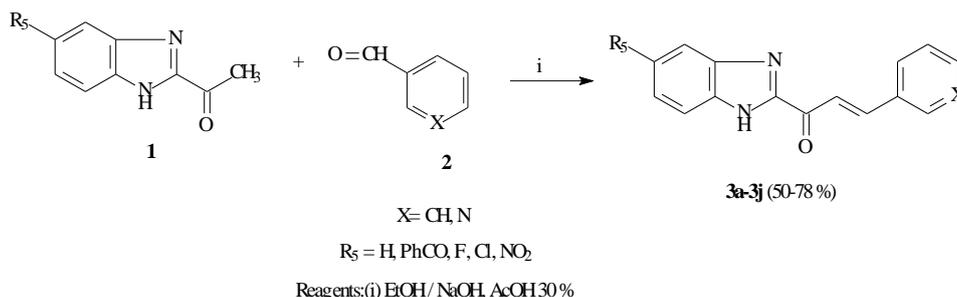

X= CH, N

R_5 = H, PhCO, F, Cl, NO_2

Reagents:(i) EtOH / NaOH, AcOH 30 %

Figure 1 : Synthesis of benzimidazolyl-chalcone.

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

Yield = 78 %; Yellow solid; mp = 216 °C. ^{1}H NMR (DMSO-*d6*, δ ppm): 14 (1H, s, N-H) ; 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); 7,2 (2H, m, Har) ; 7,09 (2H, m, Har); 7,06 (1H, m, Har). ^{13}C NMR (DMSO-*d6*, δ ppm): 181,13 (C=O), 149,49 (C=N) ; 143,97 (CH=CH) ; 139,87 (Car) ; 134,75 (2Car) ; 128,77 (Car) ; 124,22 (2Car) ; 122,51 (2Car); 122,70 (2Car); 121,69 (CH=CH); 117,03 (2Car). ES[+] SM: 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-*d6*, δ ppm): 13,60 (1H,s, N-H); 9,0 (1H, d, *J* = 2 Hz, H$_{ar}$); 8,80 (1H, d, *J* = 2 Hz, H$_{ar}$); 8,65 (1H, m, H$_{ar}$); 8,35 (1H, m, H$_{ar}$); 8,25 (1H, d, *J* = 16,2 Hz, CH=CH); 8,05 (1H, d, *J* = 16,2 Hz, CH=CH) ; 7,60 (2H, m, H$_{ar}$); 7,35 (2H, m, H$_{ar}$). ^{13}C NMR (DMSO-*d6*, δ ppm): 182,13 (C=O) ; 154,02 (Car); 150,77 (Car) ; 150,09 (C=N) ; 144,27 (CH=CH) ; 139,87 (Car) ; 134,75 (2Car) ; 124,22 (Car), 122,51 (2Car) ; 121,69 (CH=CH); 120,89 (Car); 117,03 (2Car). ES[+] SM : 250 [M+H^{+}]**.**

(E)-1-(5-benzoyl-1H-benzimidazol-2-yl)-3-phenylprop-2-en-1-one(3c)

Yield = 75 %; Yellow solid; mp = 210 °C. ^{1}H NMR (DMSO-*d6*, δ ppm): 14,0 (1H, s, N-H); 8,11 (1H, d, *J* = 18 Hz, H$_{ar}$); 8,04 (1H, s, H$_{ar}$); 7,86 (1H, d, *J* = 18 Hz, H$_{ar}$) ; 7,73-7,79 (5H, m, H$_{ar}$); 7,70 (1H, s, H$_{ar}$); 7,64 (1H, d, H$_{ar}$); 7,58 (2H, m, H$_{ar}$) ; 7,54 (2H, m, H$_{ar}$); 7,51 (1H, m, H$_{ar}$). ^{13}C NMR (DMSO-*d6*, δ ppm): 195,42 (C$_{16}$), 180,83 (C$_1$), 151,03 (C$_{10}$), 144,90 (C$_3$), 137,60 (C$_4$), 134,15 (C$_{15a}$), 133,0 (C$_{11a}$), 132,38 (C$_{17}$), 131,25 (C$_{13}$), 129,53 (C$_{20}$), 129,14 (C$_{18}$ et C$_{22}$), 129,0 (C$_{19}$ et C$_{21}$), 128,48 (C$_7$), 127,03 (C$_{12}$), 126,18 (C$_{14}$), 124,22 (C$_5$ et C$_9$), 122,70 (C$_6$ et C$_8$), 122,51 (C$_{15}$), 121,69 (C$_2$), 120,89 (C$_8$). ES[+] SM: 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-*d6*, δ ppm): 14,0 (1H, s, N-H); 9,0 (1H, s, H$_5$); 8,90 (1H, d, *J* = 2 Hz, 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$_2$) ; 7,73-7,79 (5H, m, H$_{18}$ à H$_{22}$); 7,70 (1H, s, H$_{15}$); 7,64 (1H, d, H$_{14}$). ^{13}C NMR (DMSO-*d6*, δ ppm): 195,41 (C$_{16}$), 180,75 (C$_1$), 154,02 (C$_5$), 151,12 (C$_{10}$), 150,77 (C$_7$), 144,95 (C$_3$), 137,61 (C$_4$), 135,41 (C$_{15a}$), 133,0 (C$_{11a}$), 132,38 (C$_{17}$), 131,41 (C$_7$), 131,22 (C$_{13}$), 129,53 (C$_{20}$), 129,14 (C$_{18}$ et C$_{22}$), 129,0 (C$_{19}$ et C$_{21}$), 127,03 (C$_{12}$), 126,18 (C$_{14}$), 124,22 (C$_9$), 122,51 (C$_{15}$), 121,69 (C$_2$), 120,89 (C$_8$). ES$^+$ SM : 354 [M+H$^+$]**.**

(E)-1-(5-chloro-1H-benzimidazol-2-yl)-3-phénylprop-2-en-1-one (3e)
Yield = 78 %; Yellow solid; mp = 228 °C . ^{1}H NMR (DMSO-*d6*, δ ppm): 13,50 (1H, s, N-H); 8,10 (1H, d, *J* = 16 Hz,, 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$_9$ et H$_{14}$) ; 7,25 (1H, m, H$_{14}$).
^{13}C NMR (DMSO-*d6*, δ ppm): 181,19 (C$_1$), 153,72 (C$_{10}$), 143,97 (C$_3$), 143,82 (C$_{15a}$), 142,55 (C$_{11a}$), 141,29 (C$_{13}$), 139,90 (C$_4$), 128,77 (C$_7$), 124,61 (C$_{12}$), 124,22 (C$_5$ et C$_9$), 122,51 (C$_{14}$), 122,70 (C$_6$ et C$_8$), 121,69 (C$_2$), 117,03 (C$_{15}$). ES$^+$ SM : 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-*d6*, δ ppm): 14,0 (1H, s, N-H); 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-*d6*, δ ppm): 181,02 (C$_1$), 154,52 (C$_5$), 151,07 (C$_7$), 150,19 (C$_{10}$), 145,01 (C$_{13}$), 143,86 (C$_3$), 141,76 (C$_{15a}$), 139,20 (C$_4$), 133,0 (C$_{11a}$), 127,63 (C$_{12}$), 122,59 (C$_{14}$), 124,22 (C$_9$), 121,69 (C$_2$), 120,80 (C$_8$), 117,53 (C$_{15}$).

(E)-1-(5-fluoro-1H-benzimidazol-2-yl)-3- phénylprop-2-en-1-one (3g)
Yield = 77 %; Yellow solid; mp = 220°C. ^{1}H NMR (DMSO-*d6*, δ ppm): 13,50 (1H, s, N-H); 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$_8$, et H$_9$) ; 7,23 (1H, m, H$_{14}$).^{13}C NMR (DMSO-*d6*, δ ppm): 181,19 (C$_1$), 158,24 (C$_{13}$), 150,25 (C$_{10}$), 144,50 (C$_3$), 135,45 (C$_{15a}$), 133,20 (C$_{11a}$), 130,90 (C$_4$), 125,70 (C$_{12}$), 124,22 (C$_5$ et C$_9$), 122,70 (C$_6$ et C$_8$), 121,69 (C$_2$), 119,03 (C$_{14}$), 117,03 (C$_{15}$). ES$^+$ SM: 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, N-H); 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* = 16 Hz,, 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-*d6*, δ ppm): 181,0 (C$_1$), 154,50 (C$_5$), 151,40 (C$_7$), 150,19 (C$_{10}$), 145,01 (C$_{13}$), 143,86 (C$_3$), 141,76 (C$_{15a}$), 139,20 (C$_4$), 133,0 (C$_{11a}$), 125,70 (C$_{12}$), 124,22 (C$_9$), 121,69 (C$_2$), 120,80 (C$_8$), 119,90 (C$_6$), 119,61 (C$_{14}$), 117,53 (C$_{15}$). ES$^+$ SM: 284 [M+H$^+$].

(E)-1-(5-nitro-1*H*-benzimidazol-2-yl)-3-phénylprop-2-én-1-one (3i)
Yield = 72 %; Yellow solid; mp = 258 °C. ^{1}H NMR (DMSO-*d6*, δ ppm)**:** 14,0 (1H, s, N-H); 8,54 (2H, m, H$_{12}$); 8,28 (1H, d, *J* = 16 Hz, H$_3$), 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$_5$ et H$_9$) ; 7,09 (2H, m, H$_6$ et H$_8$); 7,06 (1H, m, H$_7$). ^{13}C NMR (DMSO-*d6*, δ ppm): 181,19 (C$_1$), 153,72 (C$_{10}$), 144,89 (C$_{13}$), 143,97 (C$_3$), 143,82 (C$_{15a}$), 142,55 (C$_{11a}$), 139,90 (C$_4$), 131,23 (C$_{12}$), 128,77 (C$_7$), 124,22 (C$_5$ et C$_9$), 122,51 (C$_{14}$), 122,70 (C$_6$ et C$_8$), 121,69 (C$_2$), 120,89 (C$_8$), 117,03 (C$_{15}$). ES$^+$ SM: 294 [M+H$^+$].

(E)-1-(5-nitro-1*H*-benzimidazol-2-yl)-3-(pyridin-3-yl)prop-2-én-1-one (3j)
Yield = 57 %; Yellow solid; mp = 242 °C. ^{1}H NMR (DMSO-*d6*, δ ppm)**:** 13,60 (1H, s, N-H) ; 9,07 (1H, d, *J* = 2 Hz, H$_5$); 8,96 (1H, d, *J* = 2 Hz, 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$_1$), 154,02 (C$_5$), 150,77 (C$_7$), 150,09 (C$_{10}$), 145,31 (C$_{13}$), 144,27 (C$_3$), 139,87 (C$_4$), 135,41 (C$_{15a}$), 133,0 (C$_{11a}$), 127,03 (C$_{12}$), 124,22 (C$_9$), 122,58 (C$_{14}$), 121,69 (C$_2$), 120,89 (C$_8$), 117,53 (C$_{15}$). ES$^+$ SM: 295 [M+H$^+$].

3. Anticandidisic assays
Candida albicans strain

The clinical strain of *Candida albicans* "27506 CeDReS strain", was provided by the Center for Diagnosis and Research on AIDS and Opportunistic Diseases (CeDReS) of the University Hospital of Treichville Abidjan (Ivory Coast). 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: Antifungigram of the clinical strain of Candida albicans "27506 CeDReS" against two antifungal azoles

Antifungal azoles	Clinical strain of *Candida albicans* "strain 27506 CeDReS"
Ketoconazole	Resistant
Fluconazole	Resistant

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 [16]. 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).
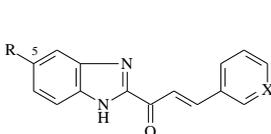
Determination of Minimum Inhibitory Concentrations (MIC) by microplate dilution technique

The evaluation of antifungal efficacy by determining the Minimum Inhibitory Concentrations (MICs) was made using the microplate dilution technique. This technique consists of contacting 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 100 μg/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 active cells turn yellow to violet 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

The results of the antifungal screening showed that of the ten derivatives evaluated, only six derivatives exhibited anticandidosic activity at the threshold quantity of 10 μg (**Table II**). In fact, the Minimum Inhibitory Concentrations (MIC) determined by microplate dilutions showed that, in series, phenylpropenone derivatives, only the compounds (3a, 3e, 3g) were active on the *Candida* strain with concentrations ranging from 5 to 1,25μg / ml. On the other hand in series of pyridinylpropenone derivatives, it is the compounds (3d, 3f, 3h) that have been active on the *Candida* strain for concentrations of between 5 and 1.25 μg / ml. In addition, the reference drugs (Fluconazole and Ketoconazole) proved to be inactive on the *C. albicans* strain at the limit of our experiment (QMI = 10 μg).

Table II: In vitro antifungal activities of 3a-3j compounds and reference substances with respect to *Candida albicans.*

General structure	Compounds	R	X	Candida albicans	
				Anticandidosic screening(MIQ)	MIC (µg/ml)
	3a	H	CH	+	5
	3b		N	-	Nd
	3c		CH	-	Nd
	3d		N	+	5
	3e	Cl	CH	+	1,25
	3f		N	+	1,25
	3g	F	CH	+	5
	3h		N	+	2,5
	3i	NO$_2$	CH	-	Nd
	3j		N	-	Nd

Kétoconazole	-	Nd
Fluconazole	-	Nd

(+): Active (QMI = 10µg)

(-) : Inactive (QMI >10µg)

nd: not determined as inactive for antifungal screening at the 10 µg threshold quantity.

Results of the antifungal screening and antifungigram revealed that *Candida* strain showed decreased susceptibility to antifungal azoles (Fluconazole and Ketoconazole). This clinical strain of *Candida* being isolated in AIDS patients, the resistance observed could be explained by the systematic use of antifungal Azoles in prophylactic or curative treatment of fungal infections in his patients. Indeed, cases of resistance have been reported in people living with HIV/AIDS who have received long-term treatment with Fluconazole [17-18]. Thus, the current medical consensus, which advocates the prevention of candidiasis prophylaxis in immunocompromised subjects, would favour the selection of resistant isolates and cross-resistance to antifungal Azoles [19-20]. A structure-activity relationship analysis of said results shows that the adhesion of benzimidazole to phenylpropenone chain reaction in **Compound 3a** induced anti-*Candida albicans* activity with an MIF of 5 µg / ml. Such efficacy on the Azolee-resistant clinical strain corroborates the intrinsic anti-infectious potential of the phenylpropenone functional grouping of chalcones and the benzimidazole heterocycle [21-23]. The replacement of the benzene nucleus of phenylpropenone of **Compound 3a** with its pyridine-type nitrogen isoster led to pyridinylpropenone in **Compound 3b**. However, such chemical modulation has led to annihilation of the anticandidate activities. This result shows that the concept of ring isostheria alone is not sufficient to induce an anticandidotic serial activity of pyridinylpropenone. Thus, to induce the biological activities of **Compound** 3b, we have introduced various modulators (benzoyl, halogen and nitro) in position 5 of benzimidazole, such as benzimidazole anthelmintics [24]. For example, the introduction of a benzimidazole benzyl group in position 5 of benzimidazole led to activity against the serial *Candida albicans* strain of pyridinylpropenones at the MIC of 5 µg/ml (**Compound 3d**). On the other hand, the C5-benzoyle derivative (**Compound 3c**) did not exhibit any activity at the 10 µg threshold amount during antifungal screening as a series of phenylpropenone derivatives. Thus, the presence of the benzyl grouping led to an inversion of the anticandidotic efficacy. Furthermore, the presence of a halogen atom (chlorine or fluorine) in the C5 position of benzimidazole has led to the maintenance or even improvement of anticandidate activities. Indeed, with a MIC of 1.25 µg/ml, the 5-chlorinated phenylpropenone derivative **(Compound 3)** showed a similar efficacy to the 5-chlorinated pyridinylpropenone derivative **(Compound 3f)**. Such an anti-*Candida* performance is 4 times higher than that of **Compound 3a** on the same strain (MIC = 5 µg/ml). Replacing the chlorine atom with another fluorine-type halogen led to the 5-fluorinated phenylpropenone derivative (**compound 3g**) with a 5 µg/ml MIC and the 5-fluorinated pyridinylpropenone derivative (**compound 3h**) with a MIC of 2.5 µg/ml. These fluorinated derivatives have proven to be less effective than chlorinated derivatives but this activity remains higher than that of **Compound 2a**. In the end, chlorinated and fluorinated derivatives showed the best anticandidositic performances compared to the C. *albicans* strain. Such a performance of halogenated derivatives would undoubtedly be due to their greater lipophilicity. Indeed, this additional degree of lipophilia may be necessary to cross the lipidic fungal membrane and interact with a possible biological target such as ergosterol. C5-nitration, on the other hand, led to a loss of antifungal activity of C5-nitrate derivatives (**composed of 3i and 3j**) at the threshold quantity 10 µg, whatever the series considered. Thus, unlike other drug series (5-nitro imidazolated, 5-nitro furans) where the presence of the nitro group in position 5 is essential to induce anti-infectious properties, it is not very conducive to induction of antifungal activities in the benzimidazolyl-arylpropenone series.

IV. Conclusion

The purpose of this pharmacochemical work was to evaluate the antifungal activities of new benzimidazolyl-chalcones against a clinical strain of pharmacochemo-chemoresistant *Candida albicans*. This study showed that these chalcones are molecules with high antifungal potential with MICs between 5 and 1.25 µg/ml. The structure-activity relationship type discussion established that in the benzimidazolyl-phenylpropenone series, the best performance on this clinical strain is obtained with C5-chlorinated derivatives with MICs of 1.25 µg/ml. In addition, as a series of benzimidazolyl-pyridinylpropenones, the induction of antifungal activities is subject to the introduction of a halogen atom or a C5 benzoyl group of the benzimidazole ring. These notable antifungal activities in relation to this strain of *Candida albicans* resistant to Azoles, open up significant avenues of investigation for the development of new antifungal agents capable in series production of arylpropenones with benzimidazolic support.

Acknowledgement

Bibliographic References

[1].   Develoux M and Bretagne S, Candidoses et levuroses diverses, *EMC-Maladies Infectieuses, 2(3)*, 2005, 119–139.
[2].   Yapar N, Epidemiology and risk factors for invasive candidiasis, *Therapeutics and Clinical Risk Management*, *10*, 2014, 95-105.
[3].   Cassone A and Cauda R, *Candida* and candidiasis in HIV-infected patients: where commensalism, opportunistic behavior and frank pathogenicity lose their borders, *Journal of the International AIDS Society*, *26(12)*, 2012, 1457-1472.
[4].   Pfaller M and Diekema D, Epidemiology of invasive candidiasis: a persistent public health problem, *Clinical Microbiology Reviews, 20(1),* 2007, 133-163.
[5].   Kamiar Z, Mohammad JR, Kayvan P, Marjan M, Moosa RG and Hasanein R, Determination of antifungal susceptibility Patterns among the clinical isolates of *Candida Spp, Journal of global infectious diseases*, *3(4),* 2011, 357-360.
[6].   Enwuru CA, Ogunledun A, Idika N, Enwuru NV, Ogbonna F, Aniedobe M and Adeiga A, Fluconazole resistant opportunistic oro-pharyngeal *candida* and non-*candida* yeast-like isolates from HIV infected patients attending ARV clinics in Lagos, Nigeria, *African Health Science, 8(3)*, 2008, 142-148.
[7].   Maertens J, History of the development of azole derivatives, *Clinical Microbiology and Infection*, *10(1)*, 2004, 1-10.
[8].   André B, Antibiotiques, *Agents antibactériens et antifongiques* (Editions Ellipses, 1999,1216p).
[9].   Gupta D, Jain D, Trivedi P, Recent advances in chalcones as anti-infective agents, *International Journal of Chemical Sciences*, *8(1)*, 2010, 649-654.
[10].   Li R, Kenyon GL, Cohen FE, Chen X, Gong B, Dominguez JN, Davidson E, Kurzban G, Miller RE and Nuzum EO, In vitro antimalarial activity of chalcones and their derivatives, *Journal of Medicinal Chemistry*, *38(26),* 1995: 5031-5037.
[11].   Awasthi S, Mishra N, Dixit SK, Singh A, Yadav M, Yadav SS and Rathaur S, Antifilarial activity of 1, 3-diarylpropen-1-one: effect on glutathione-S-transferase, a phase II détoxification enzyme, *American Journal of Tropical Medicine and Hygiene, 80(5),2009*, 764-768.
[12].   Ouattara M, Sissouma D, Koné MW, Menan HE, Touré SA and Ouattara L, Synthesis and anthelmintic activity of some hybrid Benzimidazolyl-chalcone derivatives, *Tropical Journal of Pharmaceutical Research,10 (6)*, 2011, 767-775.
[13].   Ouattara M, Sissouma D, Yavo W and Koné MW, Synthèse et criblage antiplasmodial de quelques benzimidazolyl-chalcones, *International Journal of Biological and Chemical Sciences*, *9(3),* 2015, 1697-1710.
[14].   Phillips MA, The formation of 2-substituted benzimidazoles, *Journal of the Chemical Society, 13*, 1928, 2393-2399.
[15].   Rahalison L, Hamburger MO, Hostettmann K, Monod M and Frenk E, A bioautographic agar overlay method for the detection of antifungal compounds from higher plants, *Phytochemical Analysis*, *2(5)*, 1991, 199-203.
[16].   Andargachew M, Afework K, Belay A, Beyene M, Aschalew G, Martha A, Yeshambel B, Fantahun B, Zewdu H, Feleke M and Emiko I, Frequent detection of 'azole' resistant *Candida* species among late presenting AIDS patients in northwest Ethiopia, *BMC Infectious Diseases, 13(1),* 2013, 82.
[17].   Pfaller M, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W and Ling TA, Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion, *Journal of Clinical Microbiology,48(4)*, 2010, 1366-1377
[18].   Magill S, Shields C, Sears C L, Choti M and Merz W G, Resistance croisée aux derives triazoles chez *Candida sp*: Observation, fréquence dans les isolats sanguins et implications pour les traitements antifongiques, *Journal de Mycologie Médicale*, *17(1),* 2007, S1 -S10.
[19].   Patrick V, Selene F and Alix TC, Antifungal resistance and new strategies to control fungal infections, *International Journal of Microbiology*, *2012(1)*, 2011, 1-26.
[20].   Sissouma D, Ouattara M, Koné MW, Menan HE, Adjou A and Ouattara L, Synthesis and in vitro nematicidal activity of new chalcones vectorised by imidazopyridine, *African journal of pharmacy and pharmacology*, *5(18),* 2011, 2086-2093.
[21].   Bansal Y and Silakari O, The therapeutic journey of benzimidazoles: a review, *Bioorganic & Medicinal Chemistry, 20(21),* 2012, 6208-6236.
[22].   Singh P, Anand A and Kumar V, Recent developments in biological activities of chalcones: A mini review, *European Journal of Medicinal Chemistry,85,*2014, 758-777.
[23].   Velik J, Baliharova V, Fink-Gremmels J, Bull S, Lamka J and Skalova L, Benzimidazole drugs and modulation of biotransformation enzymes. *Research in Veterinary Science, 76(2),* 2004, 95-108.