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Synthesis and antifungal activities of some benzimidazolyl-chalcones, analogues of chlormidazole

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We report here the synthesis of new benzimidazolyl-chalcones analogues of chlormidazole in order to contribute to the development of new antifungal drugs. Benzimidazolyl-chalcones were obtained by reaction of various aromatic aldehydes (7) with *N*-(4-chlorobenzyl)-2-acetylbenzimidazole (5) and its precursor 2-acetylbenzimidazole (4). After confirming their structure using spectroscopic methods (^{1}H and ^{13}C NMR, MS in EI mode), the compounds were evaluated for their antifungal activities against a clinical strain of *Candida albicans* in order to determine the minimum inhibiting quantity (MIQ). This screening showed that compounds 6e, 6f and 6h had anti-*Candida* efficiencies (MIQ = 5, 1.25 and 0.625 µg) higher than those of chlormidazole (MIQ = 10 µg). Additionally it has shown that improving these activities in benzimidazolyl-chalcones series requires double chemical modulation: Removal of 4-chlorobenzyl on the pyrrole nitrogen of the benzimidazole ring and the introduction or not of modulators such as fluorine (compounds 6f and 6h) on the benzene ring at position 3 of the propenone.

Key words: Benzimidazole, chalcone, chlormidazole, antifungal, *Candida albicans*.

INTRODUCTION

The candidosic infections are diseases due to the development of *Candida* yeasts in human. *Candida albicans* species, the most known and the most dangerous (Develoux and Britain, 2005) can cause serious illnesses that can result in high mortality and increased hospitalization costs especially in immunosuppressed patients (HIV, diabetes, cancers etc.) (Morgan et al, 2005; Lass-Flörl, 2009; Pfaller and Diekema, 2007). Therapeutic support management of these infections constitutes a public health issue with emergence of drug resistant strains to most of the current antifungals [Djohan et al., 2012; Bryskier, 1999; Sanglard and Odds, 2002). This drug resistance is more alarming with azoles antifungals which are the most commonly

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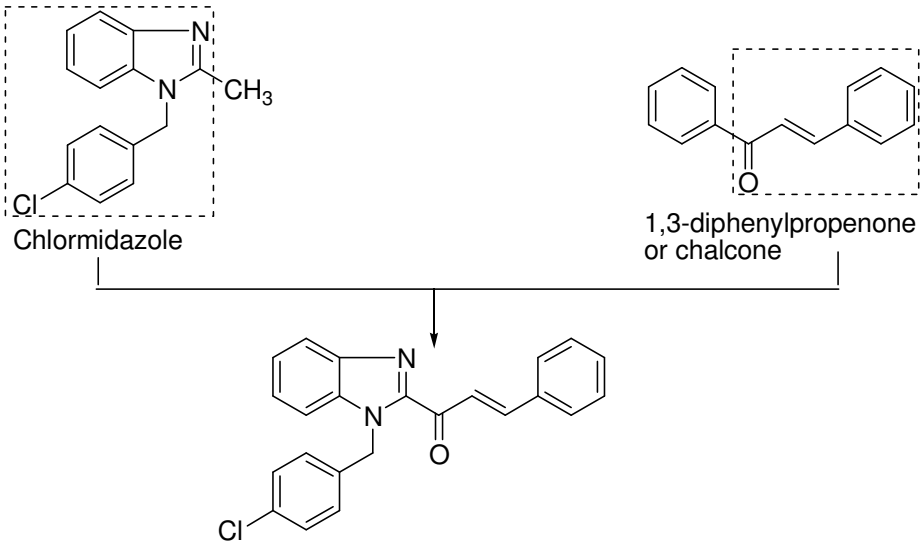


Figure 1. Design of benzimidazolyl-chalcones analogues of chlormidazole.

used drug class (Espinel-Ingroff, 2008; Smagill et al., 2007). Facing this situation, with the development of new drugs, a more effective and able to bypass antifungal drug resistance appears to be a priority need. It is in this perspective that we have proposed to synthesize and evaluate the anti-*Candida* activities of a new series of benzimidazolyl-chalcones, analogues of chlormidazole.

The chemical profile of benzimidazolyl-chalcones was conceptualized according to pharmacochemical methodologies of juxtaposition of potential antifungal entities. In this profile, the phenylpropenone of chalcones bears at position 1 a benzimidazole ring. This benzimidazole is substituted on its pyrrole nitrogen by 4-chlorobenzyl to give the *N*-4-chlorobenzylbenzimidazole of chlormidazole (Figure 1). The choice of these two entities (benzimidazole and phenylpropenone) is justified by their high intrinsic ability to induce biological activities of therapeutic interest. Indeed, since the discovery of antifungal properties of benzimidazole by Woolley (1944), this heterocyclic has been used for the development of chlormidazole which is the first azole antifungal with benzimidazole ring used in therapy. As for 1,3-diarylpropenones or chalcones, they hold their multiple biological activities, in particular, anti-infective (antimalarial, antibacterial, antiviral and antifungal) (Nowakowska, 2007) by the presence of propenone group in their structure. Therefore, it seemed appropriate to design a new profile involving chemical entities responsible for antifungal properties of the two compounds namely *N*-4-chlorobenzylbenzimidazole of chlormidazole and 3-phenylpropenone of chalcones (Figure 1).

The objective of this work is to synthesize and select new antifungal drug candidates in benzimidazolyl-chalcones series. Specifically, it is for us to determine in this chemical series, the minimal quantities capable to inhibit the proliferation of *C. albicans* and to establish favorable structural elements for anticandidosic activities.

MATERIALS AND METHODS

Chemistry

Preparation of benzimidazolyl-chalcones derivatives was carried out in two major steps: the first step was to prepare *N*-(4-chlorobenzyl)-2-acetylbenzimidazole (5) and the second consisted of preparing benzimidazolyl-chalcones (6a-d).

Synthesis of *N*-(4-chlorobenzyl)-2-acetylbenzimidazole (5)

Synthesis of compound 5 was carried out using the reaction sequence illustrated in Scheme 1. It started with a condensation according to Phillips method (Phillips, 1928) between orthophenylenediamine (1) and lactic acid (2). The reaction proceeded at reflux in dilute hydrochloric acid. This step led after neutralization with ammonia to 2-hydroxyethyl benzimidazole (3). Compound (3) was then oxidized by potassium dichromate in acetic acid afforded to 2-acetylbenzimidazole (4) after neutralization with ammonia. *N*-chlorobenzylation of compound (4) in presence of sodium hydride gave *N*-(4-chlorobenzyl)-2-acetylbenzimidazole 5.

Synthesis of benzimidazolyl-chalcones 6a to j

N-(4-chlorobenzyl)-2-acetylbenzimidazole 5 previously prepared

and its precursor 4, were engaged in a Claisen-Schmidt condensation with various substituted benzaldehydes 7. This reaction led after neutralization by dilute acetic acid to the expected benzimidazolyl-chalcones 6a to j (Scheme 2). All benzimidazolyl-chalcones derivatives and their precursors were isolated in powder form and characterized by nuclear magnetic resonance (1H and 13C NMR) and mass spectrometry in electron impact mode (EI). Moreover, their melting points were determined on a Kofler bench. As for chlormidazole used in this work as reference antifungal drug and molecular model, it was obtained by total synthesis.

Antifungal activities

To evaluate antifungal activities of all compounds, we used the clinical strain of *C. albicans* 27506 provided by the Centre de Diagnostic et de Recherche sur le SIDA et les Maladies Opportunistes (CeDReS) of CHU Treichville in Abidjan, Cote d'Ivoire. The antimycotic screening method used was the bioautography or "agar overlay" (Homans and Fuchs, 1970; Rahalison, 1994; Rahalison et al., 1994; Rahalison et al., 1991). It is a method to determine *in vitro* the minimum inhibiting quantity (MIQ) fungal growth by thin-layer chromatography (TLC). The products were first solubilized in methanol in order to prepare the stock solutions containing 1 mg/ml. From each of these mother solutions, was prepared a range of 10 dilutions. Thereafter, 10 ml of each solution were deposited on TLC plate. Chromatograms were developed in a saturated mobile phase $CHCl_3$-CH_3OH-H_2O in a ratio (65: 35: 5) and then dried. An inoculums containing approximately 105 cells/ml of *C. albicans* was obtained by inoculation of three colonies of a pure strain for 24 to 48 h in a tryptone soya broth. These inoculums were subsequently spread on each chromatogram. Plates were first incubated at 30°C after solidification of the agar for 24 h and then impregnated with an aqueous solution méthylthiazolyl tetrazolium chloride (MTT). Finally, after incubation for 2 to 4 hours, the growth inhibition zones subsequently appeared as white spots on a purple background. Only products that showed an inhibition zone were selected to determine their Minimum Inhibiting Quantities.

RESULTS

Chemical results

We synthesized and isolated 10 chalcones derivatives (Table 1) carring a benzimidazole in their phenylpropenone moiety. These compounds were divided in two series: the *N*-4-chlorobenzyles derivatives (compounds 6a to 6e) and the non-*N*-substituted derivatives (compounds 6f to 6j). Moreover, benzene ring in position 3 of the propenone in both series carried various modulators like (Cl, F) and nitro (NO_2) (Figure 2). The spectroscopic NMR proton characterization (Table 1) of benzimidazolyl-chalcones showed three characteristic peaks: from 5.91 to 5.95 ppm for NCH2Ph, from 7.86 to 8.15 ppm for H2 and from 8.01 to 8.40 ppm for H3. As for the 13C spectra, we noted four main peaks: from 47.76 to 48.25 ppm NCH2Ph, from 121.69 to 123.01 ppm for C2, from 136.55 to 144.99 ppm for C3, from 180.00 to 182.72 ppm for C = O. The molecular peaks in Mass Spectrometry Spectrometry of these new chalcones (Table I) varied between 267 and 452 depending on their substituents.

Antifungal activities results

The results of anti-*Candida albicans* screening in Table 2 showed that the strain CeDReS had sensitivity for the various products tested at different concentrations. Thus chlormidazole presented anticandidosic activities of 10 µg at the limit of our experimentation. It is the same for the benzimidazolyl-chalcones 6a, analogue of chormidazole and six derivatives of this compound. As against three other derivatives that is, compounds 6e, 6f and 6h, they were able to induce much better anticandidosic activities, respectively at 5, 1.25 and 0.625 µg.

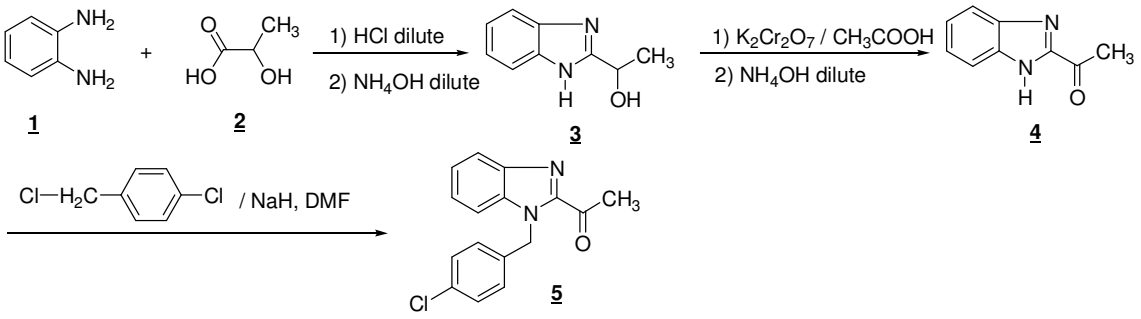
DISCUSSION

Pharmacochemical method of apposition of multiple pharmacophores entities allowed to establish that the replacement of the 2-methyl of chlormidazole by phenylpropenone led to a new chalcone (compound 6a) having anticandidosic activities (MIQ =10 µg). These activities were superimposed to those of chlormidazole. Tests to improve activities of compound 6a by pharmacomodulation (Figure 3) showed that the introduction of modulators like halogen (Cl, F) or nitro on the benzene ring in position 3 (compounds 6b to 6d) did not improve the anti-*Candida* activities which were superposable to those of compound 6a and chlormidazole (MIQ = 10 µg). But the concomitant presence of chlorine and nitro on the benzene ring (compound 6e) was suitable to the improvement of anti-*Candida* activities with a MIQ of 5 µg. This anti-*Candida* efficiency was 2 times higher than those of benzimidazolyl-chalcone 6a and chlormidazole.

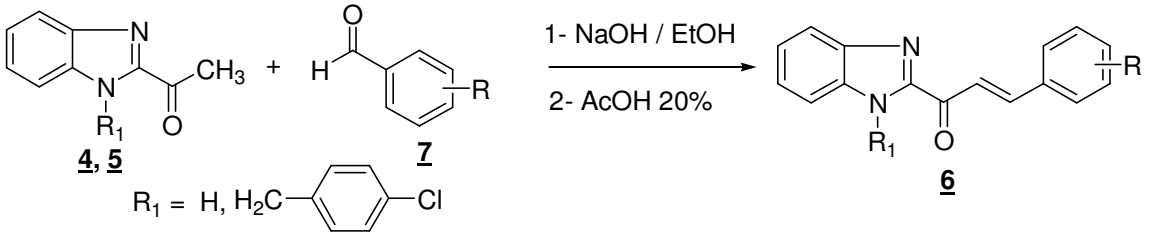
The removal of *N*-4-chlorobenzyl in compound 6a gave compound 6f, surprisingly, this improved anticandidosic activities with a MIQ of 1.25 µg. This antifungal efficacy was 8 times higher than those of its *N*-substituted analogue (compound 6a) and chlormidazole. Taking chalcone derivative 6f as reference, tests to improve anticandidosic activities by the introduction of chlorine and/or nitro group (compounds 6g, 6i and 6j) into its benzene ring was not satisfactory. Indeed the anti-*candida* activities of these compounds remained at 10 µg like those of *N*-4- chlorobenzyl derivatives 6a to d. However, the presence of fluorine doubled the removal of *N*-4-chlorobenzyl (compound 6h) led to the exaltation of anticandidosic efficiency with MIQ of 0.625 µg. In fact, the derivative 6h was, respectively 2 and 16 times more effective than those of 6f and 6a. If the non substitution of pyrrole nitrogen of the benzimidazole showed positive

Table 1. Physicochemical characteristics of 6a to j compounds.

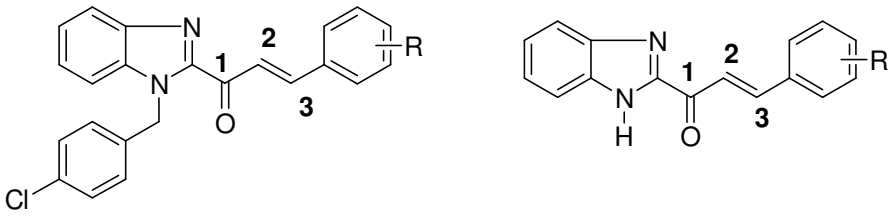
Compounds	Physicochemical characteristics
6a	RMN ^{1}H: 8.29 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 7.92 (1H, d, *J* = 15.8 Hz, C<u>H</u>=CH); 5.94 (2H, s, CH_2). RMN ^{13}C: 182.72 (C=O); 144.99 (CH=<u>C</u>H); 122.84 (<u>C</u>H=CH); 48.20 (CH_2). SM: 373 ($[M]^+$, 100). Purification: column chromatography (eluent: hexane / dichloromethane: 30/70). Yield = 73%. MP = 150°C
6b	RMN ^{1}H: 8.29 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 7.92 (1H, d, *J* = 15.8 Hz, C<u>H</u>=CH); 5.95 (2H, s, CH_2). RMN ^{13}C: 182.72 (C=O); 141.86 (CH=<u>C</u>H); 123.01 (<u>C</u>H=CH); 47.76 (CH_2). SM: 407 ($[M]^+$, 80). Purification: column chromatography (eluent: hexane / dichloromethane: 50/50). Yield = 40%. MP > 260°C
6c	RMN ^{1}H: 8.25 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 7.86 (1H, d, *J* = 15.8 Hz, C<u>H</u>=CH); 5.93 (2H, s, CH_2). RMN ^{13}C: 182.35 (C=O); 136.55 (CH=<u>C</u>H); 122.02 (<u>C</u>H=CH); 48.25 (CH_2). SM: 391 ($[M+H]^+$, 100). Purification: column chromatography (eluent: hexane / dichloromethane: 30/70). Yield = 79%. MP = 168°C
6d	RMN ^{1}H: 8.40 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 8.09-7.95 (4H, m, C<u>H</u>=CH et H_{Ar}); 5.94 (2H, s, CH_2). RMN ^{13}C: 182.38 (C=O); 137.46 (CH=<u>C</u>H); 122.04 (<u>C</u>H=CH); 48.25 (CH_2). SM: 417 ($[M]^+$, 60). Purification: washing in hot hexane; Yield = 62%. MP= 140°C
6e	RMN ^{1}H: 8.39 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 8.10-7.85 (4H, m, C<u>H</u>=CH et H_{Ar}); 5.94 (2H, s, CH_2). RMN ^{13}C: 183.01 (C=O); 136.58 (CH=<u>C</u>H); 122.03 (<u>C</u>H=CH); 48.25 (CH_2). SM: 452 ($[M]^+$, 35). Purification: washing in hot hexane; Yield = 50%. MP= 150°C
6f	RMN ^{1}H: 8.28 (1H, d, *J* = 16 Hz, CH=C<u>H</u>); 8.15 (1H, d, *J* = 16 Hz, C<u>H</u>=CH). RMN ^{13}C: 181.13 (C=O); 143.97 (CH=<u>C</u>H); 121.69 (<u>C</u>H=CH). SM: 249 ($[M+H]^+$, 100). Purification: recrystallization from toluene. Yield = 54%. MP = 216°C
6g	RMN ^{1}H: 8.01 (1H, d, *J* = 15 Hz, CH=C<u>H</u>); 7.96 (1H, d, *J* = 15 Hz, C<u>H</u>=CH). RMN ^{13}C: 180.00 (C=O); 142.25 (CH=<u>C</u>H); 122.01 (<u>C</u>H=CH). SM: 283,72 ($[M+H]^+$, 100). Purification: recrystallization from ethanol/toluene: 1/4. Yield = 47%. MP>260°C.
6h	RMN ^{1}H: 8.01 (1H, d, *J* = 15.8 Hz, CH=C<u>H</u>); 7.91 (1H, d, *J* = 15.8 Hz, C<u>H</u>=CH). RMN ^{13}C: 180.80 (C=O); 142.55 (CH=<u>C</u>H); 122.01 (<u>C</u>H=CH). SM: 267 ($[M+H]^+$, 100). Purification: recrystallization from toluene. Yield = 81%. MP = 222°C
6i	RMN ^{1}H: 8.39 (1H, d, *J* = 15.9 Hz, CH=C<u>H</u>); 8.10-7.85 (2H, m, C<u>H</u>=CH et H_{Ar}). RMN ^{13}C: 182.38 (C=O); 137.40 (CH=<u>C</u>H); 122.04 (<u>C</u>H=CH). SM: 294 ($[M+H]^+$, 100). Purification: recrystallization from ethanol/toluene: 1/1. Yield = 49% MP= 200°C.
6j	RMN ^{1}H: 8.39 (1H, d, *J* = 15.9 Hz, CH=C<u>H</u>); 8.10-7.85 (2H, m, C<u>H</u>=CH et H_{Ar}). RMN ^{13}C: 183.01 (C=O); 136.58 (CH=<u>C</u>H); 122.09 (<u>C</u>H=CH). SM: 328 ($[M+H]^+$, 100). Purification: recrystallization from ethanol/toluene: 1/1. Yield = 45%. MP= 208°C



Scheme 1. Synthesis of *N*-(4-chlorobenzyl)-2-acetylbenzimidazole 5



Scheme 2. Synthesis of benzimidazolyl-chalcones 6a to j.



6a R = H
6b R = 4-Cl
6c R = 4-F
6d R = 3-NO_2
6e R = 2-Cl, 5-NO_2

6f R = H
6g R = 4-Cl
6h R = 4-F
6i R = 3-NO_2
6j R = 2-Cl, 5-NO_2

Figure 2. Structure of benzimidazolyl chalcones synthesized.

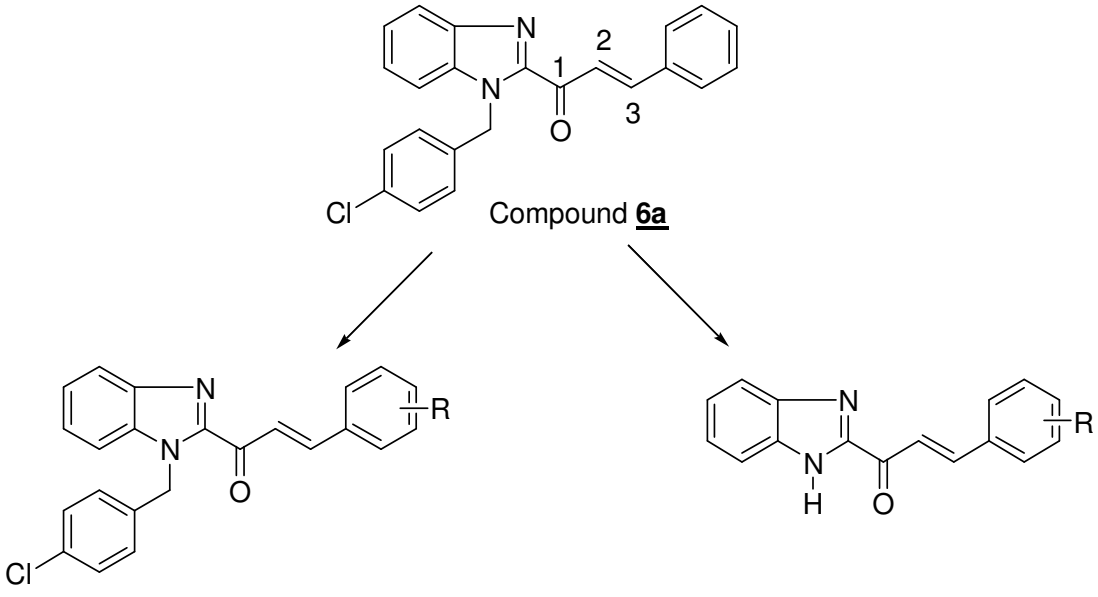


Figure 3. Structural variations of compound 6a.

Table 2. *In vitro* antifungal activities of 6a-6j compounds and reference substance against *Candida albicans.*

Compounds	MQI (µg)
6a	10
6b	10
6c	10
6d	10
6e	5
6f	1.25
6g	10
6h	0.625
6i	10
6j	10
Chlormidazole	10

impact on anthelmintic activities (AFECT, 1998), it also seems to be essential for induction of anticandidosic activities.

Finally, in the series of benzimidazolyl-chalcones, induction and enhancement of anticandidosic activities passed through a double modulation namely the deletion of the 4-chlorobenzyl on the pyrrole nitrogen of the benzimidazole nucleus and the modulation or not of the homocycle benzene at position 3 of the propenone by a fluorine.

Conclusion

Research of new antifungal in helping to fight against mycotic diseases allowed us to conceptualize and synthesize a new series of benzimidazolyl-chalcones. Evaluation of antifungal activities against *C. albicans* of these benzimidazolyl-chalcones showed that the induction and the enhancement of these activities were due to a double chemical modulation. This is the removal of 4-chlorobenzyl on pyrrole nitrogen of the benzimidazole ring and the introduction or not of modulator such as fluorine on the homocycle benzene at position 3 of the propenone. Accordingly, compounds 6f and 6h having, respectively MIQ of 1.25 and 0.625 µg were able to completely inhibit the growth of *C. albicans*. These compounds could be the leaders of a new class of total synthesis antifungal.

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Conflict of interest

Authors have none to declare.

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