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## Synthesis and *in vitro* antifungal evaluation of 2-thioalkylaryl-benzimidazoles derivatives against *Candida albicans*

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### ABSTRACT

The aim of this study is to find potent biomolecules against infectious germs. Based on the reactivity of some key positions of the benzimidazole core, the first part of this work consisted of the synthesis of a series of substituted 2-thioalkylaryl-benzimidazoles 3a-d. Then, another series of *N*-alkyl-2-thioalkylaryl-benzimidazoles 5a-d, 7a-c and 9b-c was also prepared from 2-thioalkylaryl-benzimidazoles by substitution on position-1 of benzimidazole core using the corresponding functionalized ethyl. The chemical structures of these compounds are determined by NMR (<sup>1</sup>H, <sup>13</sup>C) and mass spectrometry. The second part concerned the *in vitro* antifungal activity evaluation of some of the synthesized compounds on *Candida albicans*. According to the results of evaluation, four compounds (3b, 3c, 3d and 9c) of the substituted 2-thioalkylaryl-benzimidazoles prove to be potent antifungal agent. Introduction of nitro group (NO<sub>2</sub>) increased significantly the antifungal activity so that their IMQ is ranging between 0.03 and 0.008 µg (or 333 to 1250 times more efficient than the ketoconazole's).

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**Keywords:** synthesis of 2-thioalkylaryl-benzimidazole, antifungal activity, *Candida albicans*.

### INTRODUCTION

Nowadays, *Candida* yeasts are known to cause many infections. These are the most common opportunistic infections (Revankar, 2006; Sanjay et al., 2010) and their frequency doubled between the years 80 and 90 (Zamani et al., 2014). Now, they represent over 80% of yeast infections. Among candidiasis, infection

with *Candida albicans* is the most common in humans (Achkar et al., 2010). This infection, because of its frequency and gravity, is at the forefront of fungal infections. The interest to searching for potent molecules against *Candida albicans* has increased because of its co-infection with HIV (Egusa et al., 2008). Despite the existing therapeutic arsenal, the

efficacy of these molecules is limited by the increase of the microbial resistant strains. These resistant bacteria continue to be a threat to both human and animal health. Therefore, it is crucial to design and synthesize novel anti-infective agents by taking advantage of the flexibility of the benzimidazole core. To achieve our goal, we focused on the structural variations at position-1 and -5 of the 2-thiomethyl-benzimidazole. The above selected molecules were chosen from the chemical class of 5-nitroimidazoles (Figure 1) in which metronidazole (Flagyl®) was the leader and used against anaerobic bacterial infections.

## MATERIALS AND METHODS

### Chemistry

The synthesis of compounds 3a-d (Scheme 1) was performed in ethanolic medium by reacting benzyl chlorides 2a-b with the 5-R<sup>5</sup>-2-thiobenzimidazoles 1a-b. At the end of reaction, the reaction medium was neutralized with a sodium hydrogen-carbonate solution (NaHCO<sub>3</sub>) 5%. For compounds 5a-d (Scheme 2), 7a-c (Scheme 3) and 9b-c (Scheme 4), it was a nucleophilic substitution reaction of the functionalized alkyl at position-1 of compounds 3a-d. The chosen alkyl chain was the ethyl 5-nitroimidazoles of Figure 1. The designed functional groups to be attached to the ethyl moiety were hydroxyl, chloro and morpholino. For fixing the latter, it was necessary to go through the chlorine derivatives 7b-c. These various substitutions at position-1 were synthesized in order to enhance the potential anti-infective activities particularly antifungal activities.

The method (Rajender et al., 1982 ; Caroti et al., 1989 ; Garuti et al., 2000) used for synthesizing compounds 5a-d (scheme 2) and 7a-c (scheme 3) consisted in refluxing the mixture of benzimidazole derivatives 3a-d and alkyl halide in DMF in presence of base (K<sub>2</sub>CO<sub>3</sub>). The reaction generated an amide ion which subsequently reacted with an electrophile to give the *N*-alkylated benzimidazole derivatives.

Molecules 9b-c (Scheme 4) was obtained by exploiting the characteristics of

the nucleofugic chlorine compounds 7b-c and the nucleophilic nitrogen atom of the morpholine 8. Thus, the mixture of two equivalents of 8 and 7b-c in a refluxed THF resulted in derivatives 9b-c. It should be noted that during this reaction, morpholine played both roles, nucleophile and base. When the benzimidazole ring was substituted, the mesomeric electronic circulation between the two nitrogen atoms gave the formation of two compounds. The substitution at position-5 of the benzimidazole ring is made with the nitro group whose impact on the anti-infective activity is recognized (Shinn et al., 1962).

### General procedures for analyses

The spectra of nuclear magnetic resonance (NMR) proton (300-400 MHz) and those of the <sup>13</sup>C atoms (75-100 MHz) were recorded on a Bruker Avance 300 MHz device; Tetramethylsilane (TMS) was used as internal reference chemical shifts, expressed in ppm. Mass spectra were performed on an HP 5889A quadrupole spectrometer in electron impact. Melting points were determined using Koffler bench graduating temperature (40-260 °C). Column chromatography purifications were carried out on type Kieselgel 60 (230-400 mesh, Merck).

### General procedure for synthesizing compounds 3

5-R<sup>5</sup>-2-mercaptobenzimidazole **1** (3.30 mmol) was dissolved in 10 mL of anhydrous ethanol and then, 1.2 equivalents of 3-R-benzylchloride **2** was added (Scheme 1). The mixture was refluxed for two hours. Then the reaction medium was neutralized with a 5% solution of potassium bicarbonate. The obtained precipitate **3** (Table 1) was purified by column chromatography on silica gel using ethyl acetate/hexane v/v : 30/70 as an eluent.

#### 2-(thiobenzyl)-1H-benzimidazole **3a**

From 2-mercaptobenzimidazole (0.50 g, 3.30 mmol) and (chloromethyl)benzene (0.50 g, 3.96 mmol), **3a** was obtained (0.70 g, 88%) as white powder ; MP = 122-124 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 4.57 (2H, s, S-CH<sub>2</sub>); 7.10-7.16 (2H, m, H<sub>ar</sub>); 7.22-7.34 (2H, m, H<sub>ar</sub>); 7.44-7.47 (3H, m, H<sub>ar</sub>).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 35.12 (S-CH<sub>2</sub>); 114.08 (2 C<sub>ar</sub>); 121.39 (2 C<sub>ar</sub>); 127.27 (C<sub>ar</sub>); 128.44 (2 C<sub>ar</sub>); 128.80 (2 C<sub>ar</sub>); 137.64 (C<sub>ar</sub>-CH<sub>2</sub>); 149.66 (C=N).  
Mass (m/z) = 240. M<sup>+</sup> = 240.00 (5); M+1 = 241.10 (100); M+2 = 242.10 (17); m/z (%): 243.10 (8).

### 2-(3-nitrobenzylthio)-1H-benzimidazole 3b

From 2-mercaptobenzimidazole (0.50 g, 3.30 mmol) and 1-(chloromethyl)-3-nitrobenzene (0.68 g, 3.96 mmol), **3b** was obtained (0.80 g, 85%) as brown powder; MP = 216-218 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 4.70 (2H, s, S-CH<sub>2</sub>); 7.10-7.15 (2H, m, H<sub>ar</sub>); 7.44-7.62 (3H, m, H<sub>ar</sub>); 7.91-8.38 (2H, m, H<sub>ar</sub>).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 33.94 (S-CH<sub>2</sub>); 113.65 (2 C<sub>ar</sub>); 121.47 (C<sub>ar</sub>); 122.14 (2 C<sub>ar</sub>); 123.50 (C<sub>ar</sub>); 129.85 (C<sub>ar</sub>); 135.53 (C<sub>ar</sub>); 139.63 (2 C<sub>ar</sub>); 140.68 (C<sub>ar</sub>-CH<sub>2</sub>); 147.62 (C=N); 149.06 (C<sub>ar</sub>-NO<sub>2</sub>).

Mass (m/z) = 285. M<sup>+</sup> = 285.1 (5); M+1 = 286.1 (100); M+2 = 287.1 (20).

### 2-(benzylthio)-5-nitro-1H-benzimidazole 3c

From 5-nitro-2-mercaptobenzimidazole (0.64 g, 3.3 mmol) and (chloromethyl)benzene (0.50 g, 3.96 mmol) **3c** was obtained (0.76 g, 81%) as yellow powder; MP = 162-163 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 4.63 (2H, s, S-CH<sub>2</sub>); 7.24-7.35 (3H, m, H<sub>ar</sub>); 7.47-7.49 (2H, m, H<sub>ar</sub>); 7.59-7.62 (1H, m, H<sub>ar</sub>); 8.04-8.08 (1H, m, H<sub>ar</sub>); 8.32- 8.33 (1H, m, H<sub>ar</sub>).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 34.96 (S-CH<sub>2</sub>); 110.34 (C<sub>ar</sub>); 113.25 (C<sub>ar</sub>); 117.46 (C<sub>ar</sub>); 127.45 (C<sub>ar</sub>); 128.50 (2 C<sub>ar</sub>); 128.91 (2 C<sub>ar</sub>); 137.09 (2 C<sub>ar</sub>); 142.13 (C<sub>ar</sub>); 155.9 (N=C-S).

Mass (m/z) = 285. M<sup>+</sup> = 285.82 (100); M+1 = 286.8 (13); m/z (%): 284.9 (35); 283 (20); 281.3 (10).

### 5-nitro-2-(3-nitrobenzylthio)-1H-benzimidazole 3d

From 5-nitro-2-mercaptobenzimidazole (0.64 g, 3.30 mmol) and 1-(chloromethyl)-3-

nitrobenzene (0.68 g, 3.96 mmol) **3d** was obtained (0.82 g, 75%) as yellow powder; MP = 210-211 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 4.76 (2H, s, S-CH<sub>2</sub>); 7.58-7.64 (2H, m, H<sub>ar</sub>); 7.95-7.98 (1H, m, H<sub>ar</sub>); 8.04-8.12 (2H, m, H<sub>ar</sub>); 8.32 (1H, m, H<sub>ar</sub>); 8.41 (1H, m, H<sub>ar</sub>).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 34.06 (S-CH<sub>2</sub>); 117.53 (2 C<sub>ar</sub>); 122.29 (C<sub>ar</sub>); 123.62 (C<sub>ar</sub>); 129.91 (C<sub>ar</sub>); 135.64 (C<sub>ar</sub>); 140.13 (2 C<sub>ar</sub>); 142.20 (C<sub>ar</sub>); 147.65 (N=C-S); 155.37 (C<sub>ar</sub>).

Mass (m/z) = 330. M<sup>+</sup> = 330.32 (5); M+1 = 331 (100); M+2 = 332 (15).

## General procedure for synthesizing compounds 5

A flask was charged with 2 mmol of 5-R<sup>5</sup>-2-(3-R-benzylthio)-1H-benzimidazole **3**, 10 mL of DMF and 7 g of K<sub>2</sub>CO<sub>3</sub>. The mixture was stirred for 30 minutes and then 6 equivalents of 2-chloroethanol **4** were added (Scheme 2). The reaction mixture was refluxed for 5 hours. Water (25 mL) was added to the reaction medium and the organic layer was extracted 3 times with ethyl acetate and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the crude product **5** (Table 1) was purified by column chromatography on silica gel using ethyl acetate/Hexane v/v: 50/50 as an eluent.

### 2-(benzylthio)-1-(2-hydroxyethyl)-1H-benzimidazole 5a

From 2-(benzylthio)-1H-benzimidazole **3a** (0.48 g, 2 mmol) and 2-chloroethanol (0.97 g, 12 mmol) **5a** was obtained (0.31 g, 55%) as white powder; MP = 126-127 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 3.64-3.69 (2H, m, N-CH<sub>2</sub>); 4.15 (2H, t, J = 6 Hz, CH<sub>2</sub>-OH); 4.60 (2H, s, S-CH<sub>2</sub>); 4.96 (1H, t, J = 6 Hz, CH<sub>2</sub>-OH); 7.14-7.25 (5H, m, H<sub>ar</sub>); 7.26-7.29 (3H, m, H<sub>ar</sub>); 7.32-7.35 (1H, m, H<sub>ar</sub>).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 35.89 (S-CH<sub>2</sub>); 46.32 (N-CH<sub>2</sub>); 59.22 (CH<sub>2</sub>-OH); 117.53 (C<sub>ar</sub>); 121.32-121.44 (C<sub>ar</sub>); 127.33 (C<sub>ar</sub>); 128.43 (C<sub>ar</sub>); 128.88 (2 C<sub>ar</sub>); 136.51

(C<sub>ar</sub>); 137.42 (C<sub>ar</sub>); 142.87 (C<sub>ar</sub>); 151.17 (N=C-S).

Mass (m/z) = 284. M<sup>+</sup> = 284.1 (5); M+1 = 285.1 (100); M+2 = 286.1 (22).

**1-(2-hydroxyéthyl)-2-(3-nitrobenzylthio)-1H-benzimidazole 5b**

From 2-(3-nitrobenzylthio)-1H-benzimidazole **3b** (0.57 g, 2 mmol) and 2-chloroethanol (0.97 g, 12 mmol) **5b** was obtained (0.22 g, 33%) as beige powder; MP = 149-150 °C.

<sup>1</sup>H NMR (DMSO, 300 MHz), δ : 3.65 (2H, t, J = 6 Hz, N-CH<sub>2</sub>); 4.15 (2H, t, J = 6 Hz, CH<sub>2</sub>-OH); 4.73 (2H, s, S-CH<sub>2</sub>); 4.98 (2H, t, J = 6 Hz, OH-CH<sub>2</sub>); 7.15-7.18 (2H, m, H<sub>ar</sub>); 7.47-7.62 (3H, m, H<sub>ar</sub>); 7.93-7.95 (1H, d, H<sub>ar</sub>); 8.08-8.12 (1H, d, H<sub>ar</sub>); 8.39-8.41 (1H, m, H<sub>ar</sub>).

<sup>13</sup>C NMR (DMSO, 75 MHz), δ : 34.56 (S-CH<sub>2</sub>); 46.38 (N-CH<sub>2</sub>); 59.17 (CH<sub>2</sub>-OH); 117.56 (C<sub>ar</sub>); 121.41 (C<sub>ar</sub>); 121.53 (C<sub>ar</sub>); 123.62 (C<sub>ar</sub>); 129.83 (C<sub>ar</sub>); 135.65 (C<sub>ar</sub>); 136.54 (C<sub>ar</sub>); 140.50 (C<sub>ar</sub>); 142.78 (CH<sub>2</sub>-C=N); 147.5 (C<sub>ar</sub>); 150.65 (N=C-S).

**2-benzylthio-1-(2-hydroxyéthyl)-5-nitro-1H-benzimidazole 5c**

From 2-(benzylthio)-5-nitro-1H-benzimidazole **3c** (0.57 g, 2 mmol) and 2-chloroethanol (0.97 g, 12 mmol) **5c** was obtained (0.30 g, 45%) as yellow powder; MP = 150-151 °C.

<sup>1</sup>H NMR (DMSO, 300 MHz), δ : 3.93 (2H, t, J = 3 Hz, N-CH<sub>2</sub>); 4.19 (2H, t, J = 3 Hz, CH<sub>2</sub>-OH); 4.75 (2H, s, S-CH<sub>2</sub>); 5.24 (1H, t, J = 6 Hz, OH); 7.19-7.28 (3H, m, H<sub>ar</sub>); 7.32-7.35 (2H, m, H<sub>ar</sub>); 7.53-7.56 (1H, d, H<sub>ar</sub>); 8.03-8.06 (1H, d, H<sub>ar</sub>); 8.19-8.20 (1H, m, H<sub>ar</sub>).

<sup>13</sup>C NMR (DMSO, 75 MHz), δ : 37.25 (S-CH<sub>2</sub>); 46.86 (N-CH<sub>2</sub>); 60.88 (CH<sub>2</sub>-OH); 106.01 (C<sub>ar</sub>); 117.78 (C<sub>ar</sub>); 118.19 (C<sub>ar</sub>); 128.02 (C<sub>ar</sub>); 128.81 (2 C<sub>ar</sub>); 129.13 (2 C<sub>ar</sub>).

Mass (m/z) = 329. M<sup>+</sup> = 329.2 (7); M+1 = 330.2 (100); M+2 = 331.3 (30); m/z (%) ; 206.1 (22); 176.1 (7); 134.1 (6); 90.8 (8).

**1-(2-hydroxyéthyl)-2-(3-nitrobenzylthio)-5-nitro-1H-benzimidazole 5d**

From 5-nitro-2-(3-nitrobenzylthio)-1H-benzimidazole **3d** (0.66 g, 2 mmol) and 2-chloroethanol (0.97 g, 12 mmol) **5d** was obtained (0.34 g, 45%) as yellow oil.

<sup>1</sup>H NMR (DMSO, 300 MHz), δ : 3.7-3.8 (2H, t, J = 6 Hz, N-CH<sub>2</sub>); 4.2-4.3 (2H, t, J = 6 Hz, CH<sub>2</sub>-OH); 4.80 (2H, s, S-CH<sub>2</sub>); 5.01 (1H, t, J = 6 Hz, OH-CH<sub>2</sub>); 7.5-7.7 (2H, m, H<sub>ar</sub>); 7.9-8.2 (3H, m, H<sub>ar</sub>); 8.4-8.45 (2H, m, H<sub>ar</sub>).

Mass (m/z) = 374. M<sup>+</sup> = 374.28 (9); M+1 = 375.2 (100); M+2 = 376.2 (20); m/z (%) : 200.2 (10); 108.2 (13); 106.0 (64); 94 (9).

**General procedure for synthesizing compounds 7**

The mixture of 2 mmol of 5-R<sup>5</sup>-2-(3-R-benzylthio-1H-benzimidazole) **3**, 10 mL of DMF and 7 g of K<sub>2</sub>CO<sub>3</sub> was stirred for 30 minutes in the flask and then 6 equivalents of 1,2-dichloroethane **6** were added (Scheme 3). The reaction mixture was refluxed for 5 hours. Water was added to the reaction medium and the organic layer was extracted 3 times with ethyl acetate and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the crude product **7** (Table 1) was purified by column chromatography on silica gel using ethyl acetate/hexane v/v : 50/50 as an eluent.

**1-(2-chloroéthyl)-2-(benzylthio)-1H-benzimidazole 7a**

From 2-(benzylthio)-1H-benzimidazole **3a** (0.48 g, 2 mmol) and 1,2-dichloroethane (1.19 g, 12 mmol) **7a** was obtained (0.35 g, 57%) white powder; MP = 50-51 °C.

<sup>1</sup>H NMR (DMSO, 300 MHz), δ : 3.91-3.95 (2H, t, J = 6 Hz, CH<sub>2</sub>-Cl); 4.46-4.50 (2H, t, J = 6 Hz, N-CH<sub>2</sub>); 4.61 (2H, s, S-CH<sub>2</sub>); 7.19-7.33 (5H, m, H<sub>ar</sub>); 7.43-7.45 (2H, m, H<sub>ar</sub>); 7.55-7.62 (2H, m, H<sub>ar</sub>).

<sup>13</sup>C NMR (DMSO, 75 MHz), δ : 36.20 (S-CH<sub>2</sub>); 42.72 (CH<sub>2</sub>-Cl); 45.09 (N-CH<sub>2</sub>); 117.77 (2 C<sub>ar</sub>); 121.76-121.82 (2 C<sub>ar</sub>); 127.41 (C<sub>ar</sub>); 128.46 (2 C<sub>ar</sub>); 128.88 (2 C<sub>ar</sub>); 135.91 (C<sub>ar</sub>); 137.25 (C<sub>ar</sub>); 142.84 (CH<sub>2</sub>-C=N); 151.18 (N=C-S).

Mass (m/z) = 302.  $M^+$  = 302 (20);  $M+1$  = 303.1 (100);  $M+2$  = 304 (24); m/z (%): 305.1 (39); 267.1 (14); 91.0 (9).

**1-(2-chloroéthyl)-2-(3-nitrobenzylthio)-1H-benzimidazole 7b**

From 2-(3-nitrobenzylthio)-1H-benzimidazole **3b** (0.57 g, 2 mmol) and 1,2-dichloroethane (1.19 g, 12 mmol) **7b** was obtained (0.44 g, 63%) as beige powder; MP = 87-88 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 3.93-3.97 (2H, t, J = 6 Hz,  $\text{CH}_2\text{-Cl}$ ); 4.45-4.95 (2H, t, J = 6 Hz,  $\text{N-CH}_2$ ); 4.75 (2H, s,  $\text{S-CH}_2$ ); 7.17-7.23 (2H, m,  $\text{H}_{\text{ar}}$ ); 7.55-7.62 (3H, m,  $\text{H}_{\text{ar}}$ ); 7.91-7.94 (1H, d,  $\text{H}_{\text{ar}}$ ); 8.08-8.12 (1H, d,  $\text{H}_{\text{ar}}$ ); 8.38 (1H, m,  $\text{H}_{\text{ar}}$ ).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 34.86 ( $\text{S-CH}_2$ ); 42.73 ( $\text{CH}_2\text{-Cl}$ ); 45.15 ( $\text{N-CH}_2$ ); 117.80 ( $\text{C}_{\text{ar}}$ ); 121.84 ( $\text{C}_{\text{ar}}$ ); 122.23 ( $\text{C}_{\text{ar}}$ ); 123.61 ( $\text{C}_{\text{ar}}$ ); 129.84 ( $\text{C}_{\text{ar}}$ ); 135.63 ( $\text{C}_{\text{ar}}$ ); 135.95 ( $\text{C}_{\text{ar}}$ ); 140.28 ( $\text{C}_{\text{ar}}$ ); 142.77 ( $\text{CH}_2\text{-C=N}$ ); 147.59 ( $\text{C}_{\text{ar}}$ ); 150.66 ( $\text{N=C-S}$ ).

Mass (m/z) = 347.  $M^+$  = 347.75 (12);  $M+1$  = 348.1 (100); 349 (24); m/z: 350.1 (40); 318.1 (13).

**1-(2-chloroéthyl)-2-benzylthio-5-nitro-1H-benzimidazole 7c**

From 2-(benzylthio)-5-nitro-1H-benzimidazole **3c** (0.57 g, 2 mmol) and 1,2-dichloroethane (1.19 g, 12 mmol) **7c** was obtained (0.32 g, 46%) as yellow powder; MP = 102-103 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 3.96-4.00 (2H, t, J = 6 Hz,  $\text{CH}_2\text{-Cl}$ ); 4.56-4.65 (2H, t, J = 6 Hz,  $\text{N-CH}_2$ ); 4.67 (2H, s,  $\text{S-CH}_2$ ); 7.27-7.35 (3H, m,  $\text{H}_{\text{ar}}$ ); 7.47-7.50 (2H, m,  $\text{H}_{\text{ar}}$ ); 7.79-7.82 (1H, d,  $\text{H}_{\text{ar}}$ ); 8.12-8.15 (1H, d,  $\text{H}_{\text{ar}}$ ); 8.44-8.45 (1H, m,  $\text{H}_{\text{ar}}$ ).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 36.02 ( $\text{S-CH}_2$ ); 42.63 ( $\text{CH}_2\text{-Cl}$ ); 45.58 ( $\text{N-CH}_2$ ); 110.54 ( $\text{C}_{\text{ar}}$ ); 113.39 ( $\text{C}_{\text{ar}}$ ); 117.55 ( $\text{C}_{\text{ar}}$ ); 127.57 ( $\text{C}_{\text{ar}}$ ); 128.51 (2  $\text{C}_{\text{ar}}$ ); 129.02 (2  $\text{C}_{\text{ar}}$ ); 140.70 (2  $\text{C}_{\text{ar}}$ ); 141.97 ( $\text{C}_{\text{ar}}$ ); 142.76 ( $\text{C}_{\text{ar}}$ ); 156.42 ( $\text{N=C-S}$ ).

Mass (m/z) = 347.  $M^+$  = 347.5 (12);  $M+1$  = 348.1 (100);  $M+2$  = 349.0 (25); m/z (%): 350.1 (40); 347.1 (12); 312.1 (10); 108.0 (5); 90.9 (10).

**General procedure for synthesizing compounds 9**

In a round bottle, 5.75 mmol of 1-(2-chloroéthyl)-5-R<sup>5</sup>-2-(3-R-benzylthio)-1H-benzimidazole) was dissolved in 15 mL of THF and then 2 eq. of morpholine was added (Scheme 4). The mixture was refluxed for 48 hours. After cooling, the reaction mixture was filtered off. The filtrate was evaporated under reduced pressure and the crude **9** (Table 1) was purified by column chromatography on silica gel using ethyl acetate/hexane v/v: 50/50 as an eluent.

**1-(2-éthylmorpholine)-2-(3-nitrothiobenzyl)benzimidazole 9b**

From 1-(2-chloroéthyl)-2-(3-nitrobenzylthio)-1H-benzimidazole **7b** (2.00 g, 5.75 mmol) and morpholine (1.00 g, 11.5 mmol) **9b** was obtained (1.00 g, 44%) as yellow oil.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 3.37-3.59 (4H, m,  $\text{CH}_2\text{-N-CH}_2$ ); 4.17-4.19 (2H, t, J = 6 Hz,  $\text{CH}_2\text{-N}$ ); 3.37-3.59 (4H, t, J = 3 Hz,  $\text{CH}_2\text{-O-CH}_2$ ); 4.36-4.39 (2H, t, J = 6 Hz,  $\text{N-CH}_2$ ); 4.74 (2H, s,  $\text{S-CH}_2$ ); 7.16-7.19 (2H, m,  $\text{H}_{\text{ar}}$ ); 7.56-7.59 (3H, m,  $\text{H}_{\text{ar}}$ ); 7.91 (1H, m,  $\text{H}_{\text{ar}}$ ); 8.02 (1H, d,  $\text{H}_{\text{ar}}$ ); 8.38 (1H, m,  $\text{H}_{\text{ar}}$ ).

$^{13}\text{C}$  NMR (DMSO, 75 MHz),  $\delta$ : 34.65 ( $\text{S-CH}_2$ ); 41.18 ( $\text{N-CH}_2$ ); 53.39 ( $\text{CH}_2\text{-N-CH}_2$ ); 55.39 ( $\text{CH}_2\text{-N}$ ); 66.03 ( $\text{CH}_2\text{-O-CH}_2$ ); 109.76 ( $\text{C}_{\text{ar}}$ ); 117.69 ( $\text{C}_{\text{ar}}$ ); 127.53 ( $\text{C}_{\text{ar}}$ ); 128.51 (2  $\text{C}_{\text{ar}}$ ); 128.94 (2  $\text{C}_{\text{ar}}$ ); 135.74 ( $\text{CH}_2\text{-C=N}$ ); 136.96 ( $\text{C}_{\text{ar}}$ ); 141.59 ( $\text{C}_{\text{ar}}$ ); 150.45 ( $\text{C}_{\text{ar}}$ ); 160.94 ( $\text{N=C-S}$ ).

Mass (m/z) = 398.  $M^+$  = 398.37 (25);  $M+1$  = 399.2 (100);  $M+2$  = 400.2 (35); m/z (%): 370.3 (10); 369.4 (25); 100 (44); 91 (12).

**1-(2-éthylmorpholine)-2-thiobenzyl-5-nitrobenzimidazole 9c**

From 1-(2-chloroéthyl)-2-benzylthio-5-nitro-1H-benzimidazole **7c** (2.00 g, 5.75 mmol) and morpholine (1.00 g, 11.5 mmol) **9c** was obtained (0.80 g, 35%) as yellow powder; MP = 170-171 °C.

$^1\text{H}$  NMR (DMSO, 300 MHz),  $\delta$ : 2.43 (4H, m,  $\text{CH}_2\text{-N-CH}_2$ ); 2.62-2.66 (2H, t, J = 6 Hz,  $\text{CH}_2\text{-N}$ ); 3.49-3.51 (4H, t, 3 Hz,  $\text{CH}_2\text{-O-CH}_2$ ); 4.36-4.40 (2H, t, J = 6 Hz,  $\text{N-CH}_2$ ); 4.73 (2H, s,  $\text{S-CH}_2$ ); 7.31-7.39 (3H, m,  $\text{H}_{\text{ar}}$ );

7.51-7.54 (2H, m, H<sub>ar</sub>); 7.76-7.79 (1H, d, H<sub>ar</sub>); 8.12-8.16 (1H, d, H<sub>ar</sub>); 8.59-8.60 (1H, m, H<sub>ar</sub>).

<sup>13</sup>C NMR (DMSO, 75 MHz), δ : 35.81 (S-CH<sub>2</sub>); 41.81(N-CH<sub>2</sub>); 53.39 (CH<sub>2</sub>-N-CH<sub>2</sub>); 56.81 (CH<sub>2</sub>-N); 66.03 (CH<sub>2</sub>-O-CH<sub>2</sub>); 107.04 (C<sub>ar</sub>); 117.45 (C<sub>ar</sub>); 127.53 (C<sub>ar</sub>); 128.51 (2 C<sub>ar</sub>); 128.94 (C<sub>ar</sub>); 135.74 (C<sub>ar</sub>); 136.96 (C<sub>ar</sub>); 141.87 (C<sub>ar</sub>); 147.29 (C<sub>ar</sub>); 157.93 (N=C-S).

Mass (m/z) = 398. M<sup>+</sup> = 398.37 (25); M+1 = 399.2 (100); M+2 = 400.2 (35); m/z (%): 370.3 (10); 369.4 (25); 100 (44); 91 (12).

#### Antifungal activity

The goal of this work is to synthesize new biomolecules against infectious germs, including *Candida albicans* yeast. The evaluation of antifungal activities, conducted at the Swiss Centre for Scientific Research in Côte d'Ivoire, aims at showing the impact of some substitutions on the antifungal activity in the series of *S*-alkylbenzimidazole or *S*-alkylarylbenzimidazole. Four molecules of the chemical class of 5-R<sup>5</sup>-2-thiobenzylbenzimidazoles **3a-d**, six of the chemical class of *N*-ethyl-2-benzylthiobenzimidazoles **5a-b**, **7a-c** **9c** and a reference drug (ketoconazole) were evaluated on the strain 27506 CeDReS (Centre for Diagnostics and Research on AIDS and other opportunistic diseases). The antifungal activities expressed in Inhibitory Minimum Quantity (IMQ in µg) of the eleven compounds were subsequently determined.

#### Procedure for evaluation of antifungal compounds

The evaluation of *anti-Candida* activity of benzimidazole compounds was performed on a clinical case of *Candida albicans* (strain 27506 CeDReS), provided by the Diagnostic and Research Center on AIDS and Opportunistic Diseases (CeDReS) CHU Treichville Abidjan, Côte d'Ivoire. The screening method used was the "agar overlay" bioautographic technique (Rahalison et al., 1991; Rahalison, 1994; Rahalison et al., 1994). This was a determination method by thin layer chromatography (TLC), minimum

quantity capable of inhibiting the growth of *Candida albicans*. The powder products were first dissolved in methanol to prepare titrating stock solutions: 1 mg/mL. From each of these stock solutions, a range of 10 dilutions of 2 was prepared. Thereafter, 10 µL of each solution was put on the glass plates of Silicagel 60 F254. The chromatograms were previously developed in tanks saturated by a mobile phase CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O : 65/30/5 and dried. Moreover, the fungal inoculum of *Candida albicans* containing approximately 10<sup>5</sup> cells / mL was obtained by inoculation of three colonies of a pure strain from 24 to 48 hours in Tryptone Soya Broth. This inoculum was subsequently spread on each chromatogram. Plates were first incubated at 30 °C after solidification of the agar for 24 hours and then impregnated with an aqueous solution of chloride methylthiazolyl tetrazolium (MTT). Finally after 2 hours incubation, some growth inhibition zones subsequently appeared as white spots having a purple background. Only the products which showed a zone of inhibition were selected for determining their Inhibitory Minimal Quantities (IMQ).

#### RESULTS

Thirteen compounds derived from 2-thioalkylarylbenzimidazole **3a-d**, **5a-d**, **7a-c**, and **9b-c** were synthesized and showed in Table 1 including their reaction yields and melting points (MP). All new benzimidazole derivatives contained in position-2 a thioalkylaryl moiety. These heteroaryl compounds, having or not at position-5 a nitro group (NO<sub>2</sub>), were substituted at position-2 by a thiol group which contained either a *meta* nitro on the benzyl group or not. Substitution at position-1 of the benzimidazole ring by various functionalized ethyl groups led to the formation of *N*-alkyl-2-thioalkylarylbenzimidazoles **5a-d**, **7a-c** and **9b-c**. Compounds **3a-d**, **5a-b**, **7a-c**, **9b** and ketoconazole were tested for the antifungal activities. The results are expressed in micrograms (µg) and shown in Table 1. The antifungal results indicated that among the ten tested compounds for *Candida albicans*, only

four (**3b**, **3c**, **3d** and **9c**) showed significant antifungal activity on these fungi. The activities of the other tested molecules were lower than those of the reference product.

## DISCUSSION

The analysis of the antifungal activity results against *Candida albicans* allowed to draw several conclusions. The compound **3a**, having no nitro group, was taken as a basic structure and had no antifungal effectiveness. The appearance and the exaltation of antifungal activity required substitution on the benzimidazole unit as well as on the benzyl moiety. Thus, the introduction of a nitro group led to an exaltation of antifungal properties. Indeed, when this group was placed in *meta* position of the benzyl (compound **3b**), a sharp decrease of the minimum inhibitory amount was observed. This was divided by a factor of more than 40 compared to ketoconazole. When the nitro was placed at position-5 of the benzimidazole (compound **3c**), there was a sharp increase in the activity. Thus, compound **3c** was 31 times more active than **3b**. The

introduction of another nitro group on the benzyl moiety **3c** (compound **3d**) caused a significant decrease of the activity. Indeed, the compound **3d** was 4 times less active than **3c**. However, its activity was still higher than that of **3b** (8 times) and **3a** (more than 333 times). For the *N*-alkyl-2-thioalkylarylbenzimidazole derivatives, the introduction at position-1 of an ethyl chain functionalized by a hydroxyl group (compound **5a**) or chloro (Compound **7a**) did not improve the activity of compound **3a**. In the case of compound **3b**, this substitution resulted in a complete loss of the activity. The introduction of a 3-nitro group on the benzyl moiety of compounds **5a** and **7a** (compound **5b** and **7b**) caused no variation in activity. The introduction of a chloro ethyl chain in compound **3c** (compound **7c**) caused a loss of antifungal activity. The replacement of the chlorine compound **7c** by a morpholino group (compound **9c**) induced antifungal properties (IMQ = 0,5 µg). This was 20 times greater than that of **7c**; but compared to **3c**, it was 62 times less active.

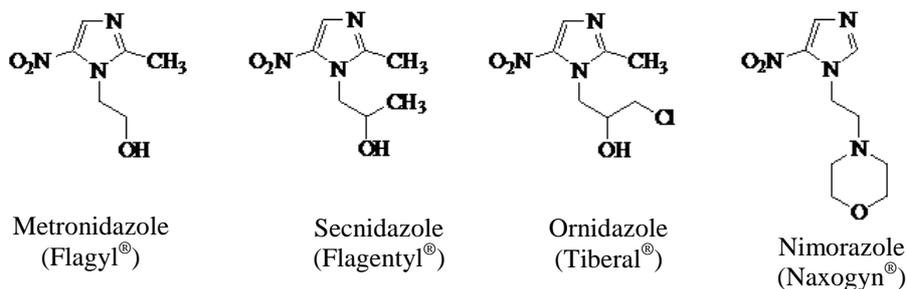
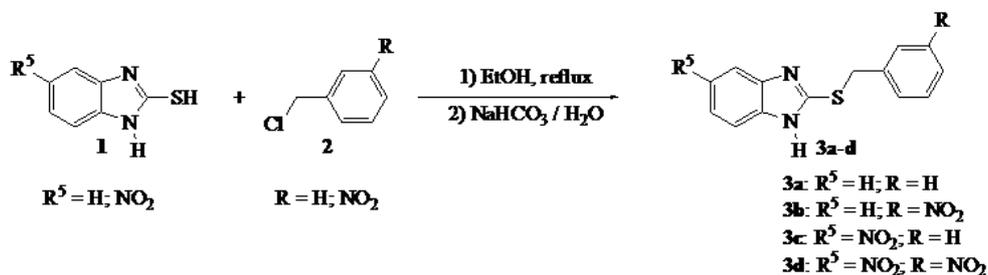
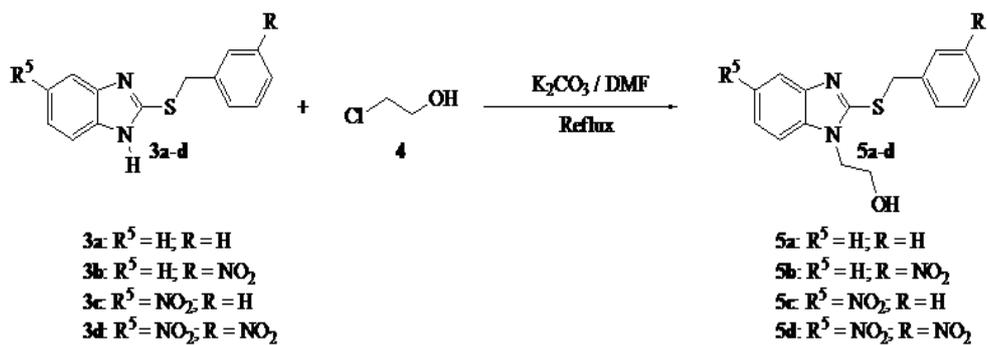


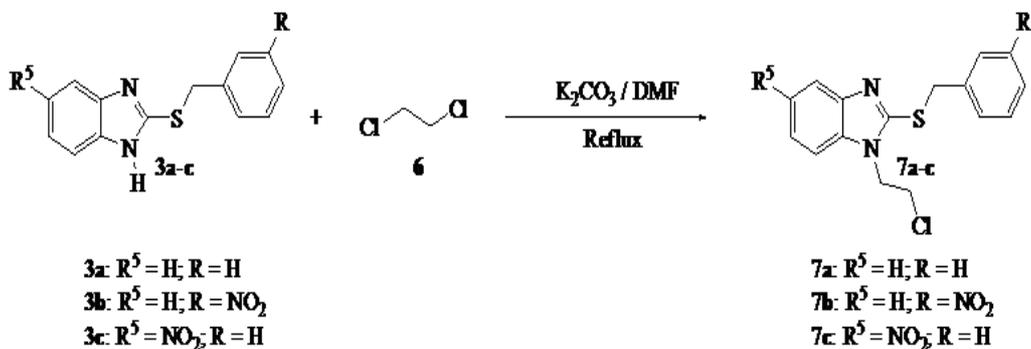
Figure 1 : 5-Nitroimidazole derivatives.



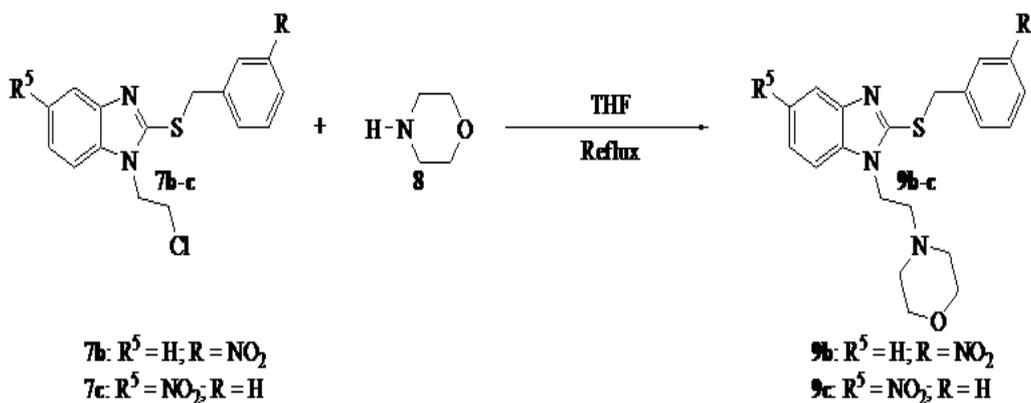
Scheme 1: Synthesis of compounds 3a-d.



Scheme 2: Synthesis of compounds 5a-d.



Scheme 3 : Synthesis of compounds 7a-c.



Scheme 4: Synthesis of compounds 9b-c.

**Table 1:** Physical and chemical characteristics of 2-thioalkylarylbenzimidazoles and their antifungal activity in vitro of *Candida albicans*.

Compounds	R <sup>5</sup>	R	Yield (%)	MP (°C)	IMQ (µg)
3a	H	H	88	122-124	> 10
3b	H	NO <sub>2</sub>	85	216-218	0,25
3c	NO <sub>2</sub>	H	81	162-163	0,008
3d	NO <sub>2</sub>	NO <sub>2</sub>	75	210-211	0,03
5a	H	H	55	126-127	> 10
5b	H	NO <sub>2</sub>	33	149-150	> 10
5c	NO <sub>2</sub>	H	45	150-151	-
5d	NO <sub>2</sub>	NO <sub>2</sub>	45	oil	-
7a	H	H	57	50-51	> 10
7b	H	NO <sub>2</sub>	63	87-88	> 10
7c	NO <sub>2</sub>	H	46	102-103	> 10
9b	H	NO <sub>2</sub>	44	oil	-
9c	NO <sub>2</sub>	H	35	170-171	0,5
<b>Ketoconazole</b>	-	-	-	-	10

### Conclusion

This work enabled new benzimidazole derivatives which contained at position-2 of a thioalkylaryl chain by total chemical synthesis. However, it should be noted that *N*-alkylation was not favorable to the antifungal activity. The antimicrobial activities of these products showed that the induction and the improvement of such activities were mostly related to the presence of a nitro group on the benzimidazole ring as well as on the aryl of the thioalkylaryl sequence. This could open some routes for investigating anti-infective 2-thioalkylaryl-benzimidazoles.

### COMPETING INTERESTS

The authors declare they have no competing interests.

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