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Synthesis of 2-(benzylthio)benzimidazole, 2-[(benzimidazol-2-yl)methylthio]benzimidazole and structural analogues against *Haemoncus contortus*

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The coupling of the derivatives of the 2-mercaptobenzimidazole 1 with the derivatives of the (chloromethyl)benzene 2 gives 2-(benzylthio)benzimidazole 4a-k on the one hand, and with the 2-(chlorométhyl)benzimidazole 3 on the other the 2-(benzimidazolyl methylthio) benzimidazole and analogues 5a-k. We determined the structures of all synthesized compounds by Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). The evaluation of the anthelmintic activities of these molecules on *Haemonchus contortus* showed that the introduction of the nitro group (NO_2) in the structure causes a significant increase of the activity. Among the molecules evaluated *in vitro* for their anti-infectious activity, the compounds 4b, 5d, 5e, 5f and 5h revealed an activity which is comparable to that of the reference molecules (ivermectin and fenbendazole).

Key words: 2-mercaptobenzimidazole, (chloromethyl)benzene, 2-(chloromethyl)benzimidazole, 2-(methylthio)benzimidazole, 2-(benzylthio)benzimidazole,2-(benzimidazolyl methylthio) benzimidazole, anthelmintic, *Haemonchus contortus*.

INTRODUCTION

Intestinal parasitic are very spread infections throughout the world and most of them are rampant in tropical areas mainly in developing countries where all favorable factors for their hatching are gathered: Hot and humid climate, lack or inadequacy of hygiene and sanitization measures and poverty. Although the consequences of the different pathologies are minor in developed countries, they are cruelly dramatic in poor or developing countries. These parasitic ailments are public health issues and are responsible for high rates of morbidity and mortality.

Gastrointestinal nematode infections are major pathologies in both human beings and animals. In sheep farming, this parasitism may be a limiting factor in the production because controlling it requires implementing medicinal measures as well as the implementation of sanitary measures. This curse causes huge economic losses in food-processing (Hussain and Dawson, 2013; Roeber et al., 2013). Several studies worldwide permitted to identify different species of nematodes and it is to be noticed that the most common nematode veterinarian

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and the most dangerous is the species *Haemonchus contortus* (Achi and Zinsstag, 2003; Tehrani et al., 2012). Now, the fight against parasitic diseases rests on the use of molecules containing in their skeleton the benzimidazole. This nucleus is the pharmacophore of many drugs used in therapeutics, namely in the treatment of infectious diseases. In the chemotherapy of intestinal helminthiasis, the most determining discovery is with no doubt the one relating to the biologically active chemical class of compounds, which the most contain the benzimidazole ring in the skeleton such as the thiabendazole, the albendazole , the mebendazole and the flubendazole (Figure 1) which are common use drugs against intestinal worms. However, in veterinary medicine, the most effective means of fight remains the use of anthelmintic drugs whose representative is currently the triclabendazole (Figure 1) which is the most used (Fairweather, 2009). According to its uniqueness, several pharmacochemical investigations about its chemical profile were carried out to extend its spectrum of activity (Mahiuddin et al., 2007; Anelia and al., 2006). But, this chemotherapy shows its limits with the emergence of various strains resisting to many of these drugs (Kaplan, 2004; Fairweather, 2009; Olaechea et al., 2011; Winkelhagen et al., 2012; Van den Brom et al., 2013; Saunders and al, 2013; Ortiz and al., 2013; Brockwell et al., 2014).

In this context, it seems important for us to synthesize new agents with nematicide aims, particularly active on *H. contortus*. To reach our objectives, we carried out an arranged structural variation thanks to the substitute flexibility of positions -2 and -5 of the 2-méthylthiobenzimidazole, basic structure of the triclabendazole. Through a rational analysis of the correlations structure-activity and the perspectives of the requirements of pharmacochemistry, we applied a technique of pharmaco-modulation on the pseudo typical molecule from the basic benzimidazole skeleton by replacing the methylthio group by the methylthioaryl or methylthioheteroaryl group. This replication by molecular rearrangement allows understanding that the chemical arrangement related to the specific structural modification is able to allow improving the molecular environment in order to optimize the pharmacological action or a modulation of the biological activity.

We tested on *H. contortus* these new anthelmintic candidates drugs, stemming from these molecular reorganizations, structural analogues of the triclabendazole, in order to assess their nematocide activities.

MATERIALS AND METHODS

Chemistry

General

Melting points were determined using a Kofler benchto graduating temperature (40-206°C). Purifications by column chromatography were carried out on Kieselgel 60 (230-400 mesh, Merck).^{1}H and ^{13}C measured on a 300 MHz Bruker Avanced apparatus with tetramethylsilane (TMS) served as internal standard: The NMR spectra (^{1}H and ^{13}C) were performed in DMSO. Mass spectra were conducted on a HP5889A spectrometer. All spectrometers analysis was realized in the CEISAM laboratory of the University of Nantes.

General procedure for synthesis of compounds 4a-k: Initially, we prepared 2-mercaptobenzimidazole derivatives **1** (Van Allan and Deacon, 1963) from reaction of *o*-phenylene diamine with carbon disulfide in DMF. Then, to 1 g of compound **1** dissolved in 10 mL of anhydrous ethanol added 1.2 equivalent of (chloromethyl) benzene(derivatives **2** (Scheme 1). The mixture was refluxed for 2 h. The reaction medium was then neutralized with a solution of potassium bicarbonate (5%). The resulting precipitate **4** was filtered off, washed up with cold ethanol and then purified by column chromatography on silica gel. Eluent: ethyl acetate/hexane: v/v : 30/70. Table 1 shows the physicochemical characteristics of compounds **4a-k**.

Synthesis of 2-(benzylthio)-1H-benzimidazole 4a: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and chloromethyl) benzene (1.01 g, 7.99 mmol) **4a** was obtained (1.41 g, 88%) as crystals; MP = 122-124°C.
^{1}H NMR (DMSO, 300 MHz) δ :4.57 (2H, s, S-C$\underline{H}_2$); 7.10-7.16 (2H, m, H_{ar}); 7.22-734 (2H, m, H_{ar}); 7.44-747 (3H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 35.12 (S-$\underline{C}H_2$); 114.08 (2 C_{ar}); 121.39 (2 C_{ar}); 127.27 (C_{ar}); 128.44 (2 C_{ar}); 128.80 (2 C_{ar}); 137.64 (C_{ar}); 149.66 (N=$\underline{C}$-S).
Mass (m/z) = 240. M^+ = 240.1 (5); M+1 = 241.1 (100); M+2 = 242.1 (17); m/z (%): 243.1 (8).

Synthesis of 2-(3-nitrobenzylthio)-1H-benzimidazole4b: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and 1-(chloromethyl)-3-nitrobenzene (1.37 g, 7.99 mmol), **4b** was obtained (1.61 g, 85%) as crystals; MP = 216-218°C.
^{1}H NMR (DMSO, 300 MHz) δ : 4.70 (2H, s, S-C$\underline{H}_2$); 7.10-7.15 (2H, m, H_{ar}); 7.44-7.62 (3H, m, H_{ar}); 7.91-7.94 (2H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ :33.94 (S-$\underline{C}H_2$); 113.65 (2 C_{ar}); 121.47 (C_{ar}); 122.14 (2 C_{ar}); 123.50 (Car); 129.85 (C_{ar}); 135.53 (C_{ar}); 139.63 (2 C_{ar}); 140.68 (C_{ar}); 147.62 (N=$\underline{C}$-S); 149.06 ($\underline{C}$-NO_2).
Mass (m/z) = 285. M^+ = 285.10 (5); M+1 = 286.1 (100); M+2 = 287.1 (20); m/z (%): 288.1 (8).

Synthesis of 2-(4-chlorobenzylthio)-1H-benzimidazole4c: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and 1-chloro-4-(chloromethyl)benzene (1.29 g, 7.99 mmol), **4c** was obtained (1.13 g, 62%) as crystals); MP = 181-182°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.60 (2H, s, S-C$\underline{H}_2$); 7.14-7.18 (2H, m, H_{ar}); 7.37-7.40 (2H, m, H_{ar}); 7.49-7.52 (4H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ : 35.20 (S-$\underline{C}H_2$); 114.91 (2 C_{ar}); 122.46 (2 C_{ar}); 129.30-129.62 (2 C_{ar}); 131.65-132.44 (2 C_{ar}); 132.89 (C_{ar}); 138.03 (C_{ar}); 140.34 (2 C_{ar}); 150.41 (N=$\underline{C}$-S).
Mass (m/z) = 274. M^+ = 274.94 (31.60); M+1 = 275,94 (31,60); m/z (%): 273.98 (77.84); 241.07 (24.85); 148.93 (17.16); 126.90 (30.98); 124.87 (100); 121.90 (21.31); 88.92 (23.59); 85.84 (12.52); 83.82 (15.63); 48.86 (19.66).

Synthesis of 2-(2,4-dichlorobenzylthio)-1H-benzimidazole4d: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and 2,4-dicholoro-1-(chloromethyl)benzene (1.56 g, 7.99 mmol), **4d** was obtained (1.56 g, 76%) as crystals; MP = 157-158°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.68 (2H, s, SCH_2); 7.16-7.18 (2H, m, H_{ar}); 7.37-7.39 (3H, m, H_{ar}); 7.50-7.52 (2H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 33.63 (S-$\underline{C}H_2$); 114.85 (C_{ar}); 122.43

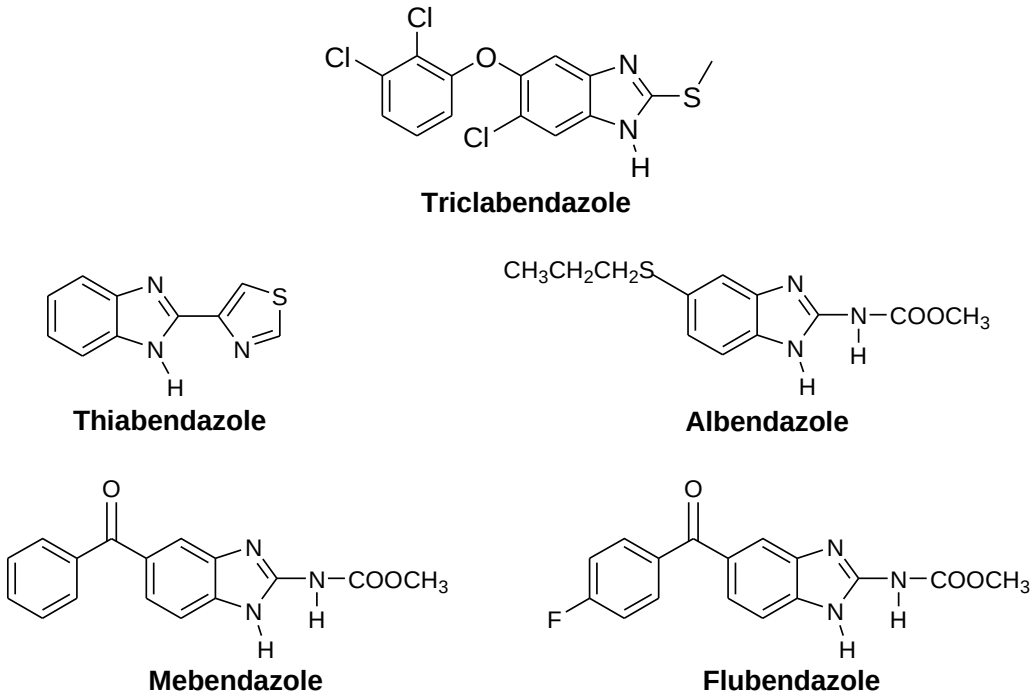


Figure 1. Structure of triclabendazole, thiabendazole, albendazole and flubendazole.

(C_{ar}); 128.33 (C_{ar}); 129.50-129,80 (C_{ar}); 133.22 (C_{ar}); 133.91 (C_{ar}); 140.36 (C_{ar}); 149.75 (N=C-S).
Mass (m/z) = 309. M^+ = 309.90 (27.24); m/z (%): 307.01 (41.82); 274.96 (38.40); 273 (15.67); 272.93 (100); 239.99 (14.68); 162.92 (12.04); 160.88 (41.75); 158.84 (67.05); 148.87 (15.77%); 122.93 (17.02); 121.91 (27.75); 88.93 (15.55).

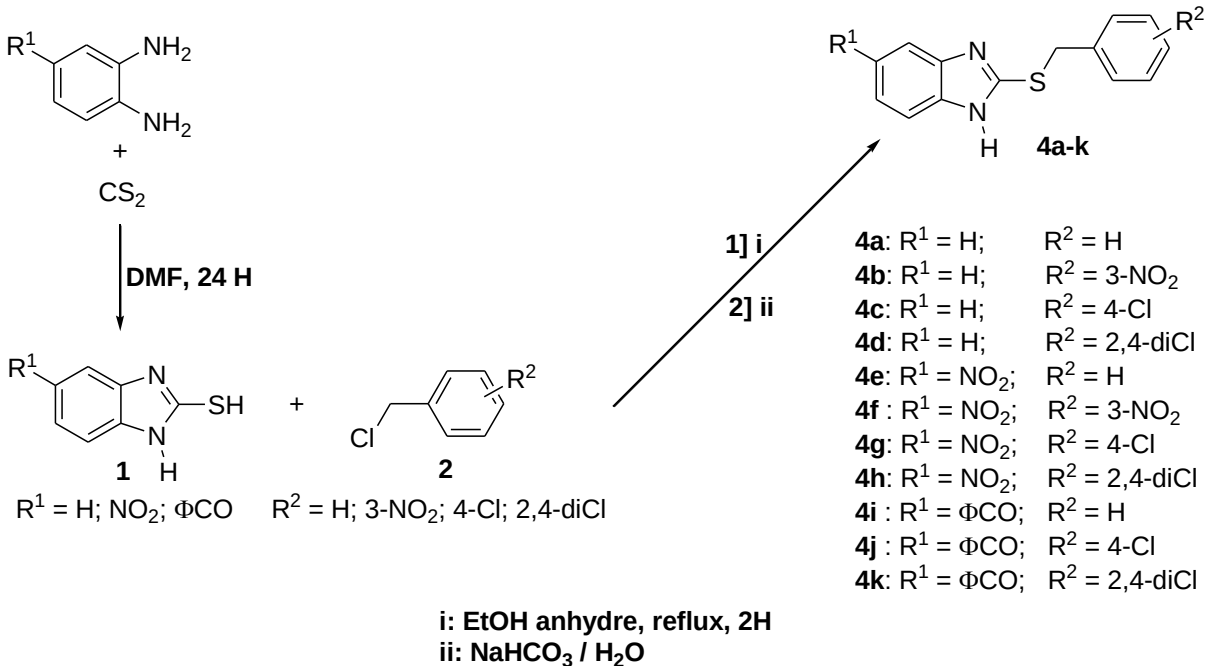
Synthesis of 2-(benzylthio)-5-nitro-1H-benzimidazole 4e: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and (chloromethyl) benzene (0.78 g, 6.15 mmol), **4e** was obtained (1.18 g, 81%) as crystals, MP = 162-163°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.63 (2H, s, S-CH_2); 7.24-7.35 (3H, m , H_{ar}); 7.47-7.49 (2H, m, H_{ar}); 7.59-7.62 (1H, m, H_{ar}); 8.04-8.08 (1H, m, Har); 8.32- 8.33 (1H, m, H_{ar});
^{13}C NMR (DMSO, 75 MHz) δ: 34.96 (S-CH_2); 110.34 (C_{ar}); 113.25 (C_{ar}); 117.46 (C_{ar}); 127.45 (C_{ar}); 128.50 (2 C_{ar}); 128.91 (2 C_{ar}); 137.09 (2 C_{ar}); 142.13 (C_{ar}); 155.9 (N=C-S);
Mass (m/z) = 285. M^+ = 285.32 (100); M+1 = 286.8 (13%); m/z (%): 284.9 (35%); 283 (20%); 281.3 (10%).

Synthesis of 5-nitro-2-(3-nitrobenzylthio)-1H-benzimidazole 4f: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and 1-(chloromethyl)-3-nitrobenzene (1.05 g, 6.15 mmol), **4f** was obtained (1.27 g, 75%) as a crystals; MP = 210-211°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.76 (2H, s, S-CH_2); 7.58-7.64 (2H, m, H_{ar}); 7.95-7.98 (1H, m, H_{ar}); 8.04-8.12 (2H, m, H_{ar}); 8.32 (1H, m, H_{ar}); 8.41 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 34.06 (S-CH_2); 117.53 (2 C_{ar}); 122.29 (C_{ar}); 123.62 (C_{ar}); 129.91 (C_{ar}); 135.64 (C_{ar}); 140.13 (2 C_{ar}); 142.20 (C_{ar}); 147.65 (N=C-S); 155,37 (C_{ar}).
Mass (m/z) = 330. M^+ = 330.32 (4); M+1 = 331 (100); M+2 = 332 (15).

Synthesis of 2-(4-chlorobenzylthio)-5-nitro-1H-benzimidale 4g: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and 1-chloro-4-(chloromethyl)benzene (1.00 g, 6.15 mmol), **4 g** was obtained (1.39 g, 85%) as crystals; MP = 155-156°C.
^{1}H NMR (DMSO, 300 MHz) δ:4.61 (2H, s, S-CH_2); 7.31-7.37 (2H, d, H_{ar}); 7.49-7.31 (3H, 3m, H_{ar}); 8.03-8.06 (1H, dd, H_{ar}); 8.31 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 33.60 (S-CH_2); 114.4 (C_{ar}); 116.1 (C_{ar}); 118.6 (C_{ar}); 128.8-129.1 (4 C_{ar}); 137.7 (C_{ar}); 141.7 (C_{ar}); 147,1 (N=C-S).
Mass (m/z) = 319. M^+ = 319 (5); M+1 = 320.96 (11.17); m/z (%) : 322.02 (2.08); 318.94 (28.89); 126.91 (32.10); 124.87 (100); 88.95 (16.91).

Synthesis of 2-(2,4-dichlorobenzylthio)-5-nitro-1H-benzimidale 4h: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and 2,4-dicholoro-1-(chloromethyl)benzene (1.20 g, 6.15 mmol), **4h** was obtained (1.45 g, 80%) as crystals; MP = 98-100°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.72 (2H, s, S-CH_2); 7.38-7.40 (2H, m, H_{ar}); 7.63-7.64 (1H, m, H_{ar}); 7.70-7.72 (1H, m, H_{ar}); 8.07-8.10 (1H, m, H_{ar}); 8.36 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 32.59 (S-CH_2); 113.42 (C_{ar}); 117.51 (C_{ar}); 127.47 (C_{ar});128.58-128.91 (2 C_{ar}); 131.37 (C_{ar});133.51-134.25 (2 C_{ar}); 139.47 (C_{ar}); 140.32 (C_{ar}); 143.40 (C_{ar}); 155.07 (N=C-S).
Mass (m/z) = 354. M^+ = 354.93 (21.66); m/z (%): 352.89 (29.52); 319.95 (31.20); 317.88 (84.95); 160.86 (65.31); 158.84 (100); 122.84 (16.13); 88.93 (11.21).

Synthesis of [2-(benzylthio)-1H-benzimidazole-5-yl][phenyl] méthanone 4i: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and (chloromethyl)benzene (0.60 g, 4.72 mmol), **4i** was obtained (1.08 g, 90 %) as crystals; MP = 98-100°C. ^{1}H NMR (DMSO, 300 MHz) δ: 4.66 (2H, s, S-CH_2); 7.28-7.36 (3H, m, H_{ar}); 7.49-7.70 (7H, m, H_{ar}); 7.76-7.81 (2H, m, H_{ar}); 7.87 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 36.00 (S-CH_2); 114.42 (C_{ar}); 124.76



Scheme 1. Synthesis of 2-(benzylthio) benzimidazole 4a-k.

(C_{ar}), 128.31 (C_{ar}); 129.27-129.50 (2 C_{ar}); 129.80-130.27 (2 Car); 131.35-132.33 (2 C_{ar}); 132.62-132.88 (2 C_{ar}); 138.27 (C_{ar}); 139.06 (C_{ar}); 154.86 (C_{ar}); 167.50 (N=C-S); 196.45 (C=O).
Mass (m/z) = 344. M^+ = 344.02 (55.85); M+1 = 345.08 (12.81); m/z (%) : 311.03 (27.23); 104.94 (10.52); 90.93 (100); 76.89 (12.99); 64.92 (10.55).

Synthesis of [2-(4-chlorobenzylthio)-1H-benzimidazol-5-yl][phényl]méthanone 4j: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and 1-chloro-4-(chloromethyl)benzene (0.76 g, 4.72 mmol), **4j** was obtained (1.19 g, 80%) as crystals; MP = 98-100°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.59 (2H, s, S-CH_2); 7.35-7.39 (2H, m, H_{ar}); 7.49-7.58 (5H, m, H_{ar}); 7.63-7.67 (1H, m, H_{ar}); 7.72-7.73 (2H ,m, H_{ar}); 7.74-7.75 (1H, m, H_{ar}); 7.81 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 35.03 (S-CH_2); 114.59 (C_{ar}); 117.95 (C_{ar}); 123.32 (C_{ar}); 129.22-129.29 (2 C_{ar}); 130.25-131.692 (2 C_{ar}); 132.50-132.64 (2 C_{ar}); 138.71 (C_{ar}); 139.87 (C_{ar}); 143.17 (C_{ar}); 147.60 (C_{ar}); 157.88 (N=C-S); 196.73 (C=O).
Mass (m/z) = 378. M^+ = 378 (34.18); M+2 = 380 (12.09); m/z (%): 345.00 (12.52); 126.9 (34.10); 126 (11.12); 124.9 (100); 104.9 (10.16); 88.9 (22.27); 76.9 (19.78).

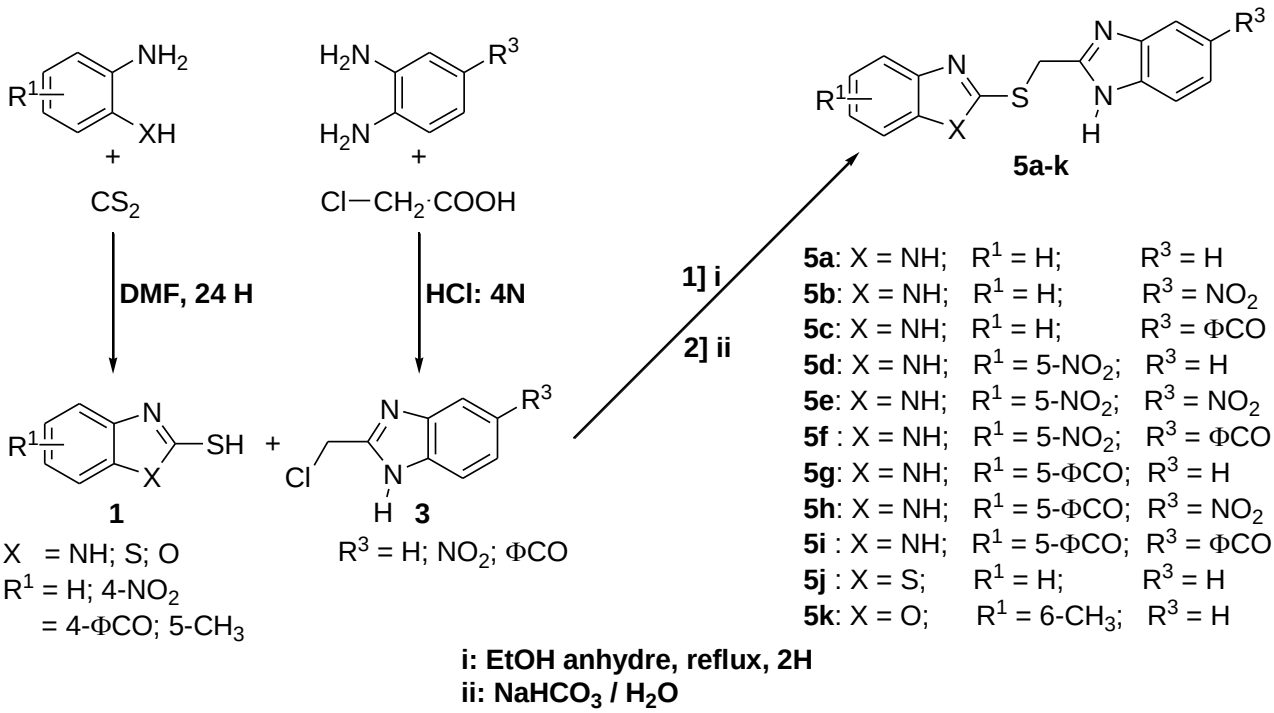
Synthesis of [2-(2,4-dichlorobenzylthio)-1H-benzimidazole-5-yl][phényl]méthanone 4k: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and 2,4-dichloro-1-(chloromethyl)benzene (0.92 g, 4.72 mmol), **4k** was obtained (1.48 g, 91%) as crystals: MP = 110 -111°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.70 (2H, s, H_{ar}); 7.38-7.41 (1H, m, H_{ar}); 7.57-7.70 (5H, m, H_{ar}); 7.73-7.77 (2H, m, H_{ar}); 7. 86 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 33.62 (S-CH_2); 114.67 (C_{ar}); 117.59 (C_{ar}); 125.00 (C_{ar}); 128.54 (C_{ar}); 129.43 (2 C_{ar}); 129.00-130.44 (4 C_{ar}); 131.51 (C_{ar}); 140.11 (C_{ar}); 143.57 (C_{ar}); 153.65 (C_{ar}); 167.95 (N=C-S); 196.58 (C=O).
Mass (m/z) = 413. M^+ = 413 (10); M+1 = 414.17 (11.85); m/z (%) : 411.90 (23.62); 378.97 (37.21); 376158.95 (100); 344.01 (11.42); 197.98 (10.82); 160.14 (43.38); 87 (62.21); 122.88 (10.75); 105.08 (14.74); 85.90 (15.95); 83.80 (20.72); 76.87 (32.84); 48.89 (27.22).

General procedure for synthesis of compounds 5a-k

This preparation involved three steps (Scheme 2): First step include synthesis of 2-mercaptobenzimidazole derivatives and analogues **1** from reaction of *o*-phenylene diamine derivatives or analogues with carbon disulfide in DMF according to Van Allan method (Van Allan and Deacon, 1963). The second relates the formation of 2-(méthylthio)benzimidazole **3** by condensation of *o*-phenylene diamine with chloroacetic acid in hydrochloric acidmedium (Phillips, 1928). In the third step, to 1 g of compound **1** in 10 mL of anhydrous ethanol was added 1.5 equivalent of 2-(chloromethyl)benzimidazole(derivative) **3**. The mixture was refluxed for 2 h. The reaction medium was then neutralized with a solution of sodium hydrogen carbonate (5%). The resulting precipitate **5** was filtered off, washed up with cold ethanol then purified by column chromatography on silica gel. Eluent : ethyl acetate/hexane : v/v : 80/20. Table 2 shows the physicochemical characteristics of compounds **5a-k**.

Synthesis of 2-[(1H-benzimidazol-2-yl)méthylthio]-1H-benzimidazole 5a: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and 2-(chloromethyl)-1*H*-benzimidazole (1.66 g, 10 mmol) **5a** was obtained (1.12 g, 60%) as crystals; MP = 253-256°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.80 (2H, s, S-CH_2); 7.13-7.14 (4H, m, H_{ar}); 7.51 (4H, m, H_{ar}).
^{13}C NMR (DMSO, 75 Hz) δ: 28.86 (S-CH_2); 121.56 (C_{ar}); 149.27 (CH_2-C=N); 150.51 (N=C-S).
Mass (m/z) = 280. M^+ = 280 (7); M+1 = 281 (100); M+2 = 282.1 (20); m/z : 261.1 (25); 132.9 (61); 151 (69).

Synthesis of 2-[(1H-benzimidazol-2-ylthio)méthyl]-5-nitro-1H-benzimidazole 5b: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and 2-(chloromethyl)-5-nitro-1*H*-benzimidazole (2.11 g, 10



Scheme 2. Synthesis of 2-(benzimidazolyl methylthio) benzimidazole and analogues 5a-k.

mmol), **5b** was obtained (1.32 g, 61%) as a crystals; MP = 265-266°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.99 (2H, s, S-C$\underline{H}_2$); 7.24-7.27 (2H, m, H_{ar}); 7.53-7.60 (2H, m, H_{ar}); 7.67-7.70 (1H, m, H_{ar}); 8.11-8.13 (1H, m, H_{ar}); 8.47-8.48 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 Hz) δ: 30.04 (S-$\underline{C}H_2$); 113.16 (C_{ar}); 114.89 (2 C_{ar}); 115.64 (C_{ar}); 123.52 (2 C_{ar}); 132.48-132.66 (2 C_{ar}); 143.27-143.62 (2 C_{ar}); 149.92 (CH_2-$\underline{C}$=N); 156.58 (C_{ar}); 167.92 (N=$\underline{C}$-S).
Mass (m/z) = 325. M^+ = 325.35 (42.61); M+1 = 326 (12.60); m/z (%) : 149.9 (100); 148.9 (23.30); 117 (10.39); 106 (11.39); 89.9 (10.72); 62.9 (14.44).

Synthesis of [2-((1H-benzimidazol-2-ylthio)méthyl)-1H-benzimidazol-5-yl][phényl]méthanone 5c: From 2-mercaptobenzimidazole (1.00 g, 6.66 mmol) and [2-(chloromethyl)-1*H*-benzimidazol-5-yl] [phenyl] methanone (2.70 g, 10 mmol), **5c** was obtained (2.00 g, 78%) as crystals; MP = 190-191°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.89 (2H, s, S-C$\underline{H}_2$); 7.17-7.19 (2H, m, H_{ar}); 7.51-7.59 (4H, m, H_{ar}); 7.66-7.75 (5H, m, H_{ar}); 7.94 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 29.88 (S-$\underline{C}H_2$); 115.83 (C_{ar}); 119.06 (C_{ar}); 122.86 (C_{ar}); 125.18 (C_{ar}); 129.49-129.70 (2 C_{ar}); 132.17-132.55 (2 C_{ar}); 132.74 (C_{ar}); 133.21 (C_{ar}); 139.05-142.51 (3 C_{ar}); 150.06 (CH_2-$\underline{C}$=N); 154.87 (C_{ar}); 168.05 (N=$\underline{C}$-S); 196.85 ($\underline{C}$=O).
Mass (m/z) = 384. M^+ = 384.03 (100); M+1 = 385.09 (26.67); m/z (%): 351.08 (35.20); 235.01 (35.74); 149.94 (27.33); 129.90 (20.76); 76.94 (15).

Synthesis of 2-[(1H-benzimidazol-2-yl)méthylthio]-5-nitro-1H-benzimidazole 5d: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and 2-chloromethyl-1*H*-benzimidazole (1.28 g, 7.68 mmol), **5d** was obtained (1.18 g, 71%) as crystals; MP = 247-248°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.88 (2H, s, S-C$\underline{H}_2$); 7.14-7.19 (2H, dd, H_{ar}); 7.50-7.55 (2H, dd, H_{ar}); 7.62-7.65 (1H, d, H_{ar}); 8.06-8.09 (1H, dd, H_{ar}); 8.37-8.38 (1H, m, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 28.76 (S-$\underline{C}H_2$); 113.45 (C_{ar}); 115.03 (C_{ar}); 117.55 (C_{ar}); 121.84 (C_{ar}); 139.02 (C_{ar}); 142.21 ($\underline{C}H_2$-C=N); 150.03 (C_{ar}); 155.47 (N=$\underline{C}$-S).
Mass (m/z) = 325. M^+ = 325 (4); M+1 = 326 (100); M+2 = 327.2 (22); m/e (%) : 196 (10); 166 (11); 133 (37); 132 (11).

Synthesis of 5-nitro-2-[(5-nitro-1H-benzimidazol-2-yl)méthylthio]-1H-benzimidazole 5e: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and 2-chloromethyl-5-nitro-1*H*-benzimidazole (1.63 g, 7.68 mmol), **5e** was obtained (1.00 g, 53%) as crystals; MP = 180-181°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.97 (2H, s, S-C$\underline{H}_2$); 7.64-7.75 (2H, m, H_{ar}); 8.08-8.12 (2H, m, H_{ar}); 8.39-8.47 (2H, m, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 28.64 (S-$\underline{C}H_2$); 117.58 (C_{ar}); 128.59 (C_{ar}); 142.21 (CH_2-$\underline{C}$=N); 142.55 (C_{ar}); 155.37 (N=$\underline{C}$-S).
Mass (m/z) = 370. M^+ = 370 (19.54); m/z (%): 194.9 (100); 164.9 (37.26); 148.9 (57.42); 136.9 (21.72); 121.9 (30.48); 89.9 (46.08); 104.9 (46.15); 62.2 (61.52).

Synthesis of [2-((5-nitro-1H-benzimidazol-2ylthio)méthyl)1H-benzimidazol-5-yl][phényl]méthanone 5f: From 5-nitro-2-mercaptobenzimidazole (1.00 g, 5.12 mmol) and [2-(chloromethyl)-1*H*-benzimidazol-5-yl] [phenyl] methanone (2.08 g, 7.68 mmol), **5f** was obtained (1.58 g, 72%) as crystals; MP = 161-162°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.98 (2H, s, S-C$\underline{H}_2$); 7.55-7.59 (2H, m, H_{ar}); 7.65-7.76 (6H, m, H_{ar}); 7.95-7.96 (1H, m, H_{ar}); 8.08-8.11 (1H, dd, H_{ar}); 8.39 (1H, d, H_{ar}).
^{13}C NMR (DMSO, 75 MHz) δ: 29.59 (S-$\underline{C}H_2$); 111.59 (C_{ar}); 114.41 (C_{ar}); 115.69 (C_{ar}); 118.50 (C_{ar}); 118.92 (C_{ar}); 125.01 (C_{ar}); 129.28-129.53 (2 C_{ar}); 130.32 (2 C_{ar}); 132.62-132.97 (C_{ar}); 138.93 (C_{ar}); 140.50 (CH_2-$\underline{C}$=N); 142.34-144.46 (C_{ar}); 156.12 (C_{ar}); 167.81 (N=$\underline{C}$-S); 196.51 ($\underline{C}$=O).
Mass (m/z) = 429. M^+ = 429.87 (30.59); m/z (%) : 428.97 (100); 396.14 (21.54); 236.06 (51.34); 235.03 (96.31); 148.95 (55.25);

129.99 (44.82); 105.62 (43.28); 76.94 (49.60).

Synthesis of [2-((1H-benzimidazol-2yl)méthylthio)-1H-benzimidazol-5-yl][phényl]méthanone 5g: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and 2-(chloromethyl)-1*H*-benzimidazole (0.98 g, 5.90 mmol), **5g** was obtained (1.30 g, 86%) as crystals; MP = 255-256°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.91 (2H, s, S-C$\underline{H}_2$,); 7.17-7.21 (2H, dd, H_{ar}); 7.54-7.61 (4H, m, H_{ar}); 7.64-7.7 (4H, m, H_{ar}); 7.73-7.78 (1H, m, H_{ar}); 7.91 (1H, m, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 29.77 (S-$\underline{C}H_2$); 116.00 (3 C_{ar}); 122.89 (C_{ar}); 124.91 (C_{ar}); 129.40 (C_{ar}); 129.64 (2 C_{ar}); 130.43 (2 C_{ar}); 131.43 (C_{ar}); 132.48 (C_{ar}); 132.66 (3 C_{ar}); 139.95 (C_{ar}); 151.32 (CH_2-$\underline{C}$=N); 153.97 (C_{ar}); 167.91 (N=$\underline{C}$-S); 196.57 ($\underline{C}$=O).
Mass (m/z) = 384. M^+ = 384.04 (58.75); M+1 = 385.08 (16.21); m/z (%) : 253.99 (71.46); 176.91 (62.42); 131.99 (56.79); 130.07 (100); 76.88 (52.35).

Synthesis of [2-((5-nitro-1H-benzimidazol-2yl)méthylthio)-1H-benzimidazol-5-yl][phényl]méthanone 5h: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and 2-(chloromethyl)-5-nitro-1*H*-benzimidazole (1.25 g, 5.90 mmol), **5h** was obtained (1.42 g, 84%) as crystals; MP = 93-94 °C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.96 (2H, s, S-C$\underline{H}_2$,); 7.60 (2H ,m, H_{ar}); 7.65-7.70 (5H, m, H_{ar}); 7.89 (1H, m, H_{ar}); 8.10-8.13 (1H, dd, H_{ar}); 8.47-8.48 (1H, d, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 29.70 (S-$\underline{C}H_2$); 113.64-118.73 (3 C_{ar}); 124.94 (C_{ar}); 129.63 (C_{ar}); 130.42 (2 C_{ar}); 131.49 (2 C_{ar}); 132.47 (C_{ar}); 132.66 (C_{ar}); 133.06 (C_{ar}); 139.04 (C_{ar}); 143.53 (CH_2-$\underline{C}$=N); 153.59 (C_{ar}); 156.73 (C_{ar}); 167.92 (N=$\underline{C}$-S); 196,55 ($\underline{C}$=O).
Mass (m/z) = 429. M^+ = 429.01 (56.19); m/z (%) : 253.00 (78.44); 176.94 (100); 129.9 (25.66); 104.94 (26.51); 76.92 (31.40).

[2-((5-benzoyl-1H-benzimidazol-2yl)méthylthio)-1H-benzimidazol-5-yl][phényl]méthanone 5i: From (2-mercaptobenzimidazol-5-yl) (phenyl) methanone (1.00 g, 3.93 mmol) and [2-(chloromethyl)-1*H*-benzimidazol-5-yl] [phenyl] methanone (1.60 g, 5.90 mmol), **5i** was obtained (1.00 g, 52%) as crystals; MP = 150-154°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.95 (2H, s, S-C$\underline{H}_2$); 7.55-7.60 (4H, m, H_{ar}); 7.66-7.69 (6H, m, H_{ar}); 7.90 (2H, m, H_{ar}); 7.95 (2H, m, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 29.73 (S-$\underline{C}H_2$); 124.81 (C_{ar}); 129.26 (4 C_{ar});130.30 (4 C_{ar}); 131.45 (C_{ar}); 131.82 (2 C_{ar});132.91 (C_{ar}); 139.01 (C_{ar}); 154.46 (N=$\underline{C}$-S); 196.44-196.55 (2 $\underline{C}$=O).
Mass (m/z) = 488. M^+ = 488.10 (71.89); m/z (%) : 253.91 (53.38); 234.94 (72.20); 176.90 (22.37); 158.90 (33.46); 129.94 (31.15); 104.92 (85.89); 76.86 (100).

Synthesis of 2-[(1H-benzimidazole-2yl)methylthio]benzothiazol 5j: From 2-mercaptobenzothiazole (1.00 g, 5.98 mmol) and 2-(chloromethyl)benzimidazole (1.49 g, 8.97 mmol), **5d** was obtained (0.96 g ,54%) as crystals; MP = 176-177°C.
^{1}H NMR (DMSO, 300 MHz) δ: 4.90 (2H, s, S-C$\underline{H}_2$); 7.15-7.19 (2H, m, H_{ar}); 7.34-7.59 (4H, m, H); 7.59-7.91 (1H, m, H_{ar}); 8.01-8.04 (1H, m, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 30.31 (S-$\underline{C}H_2$); 121.25 (2 C_{ar}); 121.86 (2 C_{ar}); 124.56 (C_{ar}); 126.39 (C_{ar}); 134.79 (2 C_{ar}); 149.53 (CH_2-$\underline{C}$=N); 152.47 (C_{ar}); 165.59 (N=$\underline{C}$-S).

Synthesis of synthesis of 2-[(1H-benzimidazole-2yl)methylthio]-6-methylbenzoxazole 5k: From 5-methyl-2-mercaptobenzoxazole (1.00 g, 6.05 mmol) and 2-(chloromethyl)-1*H*-benzimidazole (1.51 g, 9.08 mmol), **5k** was obtained (0.91 g, 51%) as a crystals; MP = 181-182°C.
^{1}H NMR (DMSO, 300 MHz) δ: 2.42 (3H, s, C$\underline{H}_3$); 4.85 (2H, s, S-C$\underline{H}_2$); 7.15-7.19 (3H, m, H_{ar}); 7.48-7.54 (4H, m, H_{ar}).
^{13}C (DMSO, 75 MHz) δ: 21.07 ($\underline{C}H_3$); 29.40 (S-$\underline{C}H_2$); 162.42 (N=$\underline{C}$-S); 151.65 (C_{ar}); 149.40 (CH_2-$\underline{C}$=N); 139.01 (C_{ar}); 134.36 (C_{ar}); 125.6 (C_{ar}); 121.86 (2 C_{ar}); 117.76 (C_{ar}); 110.33 (C_{ar}).
Mass (m/z) = 295. M^+ = 295 (5); M+1 = 296.1 (100); M+2 = 297.1 (20).

Anthelmintics activities

The nematocide test used is an enhancement of the method initially described by Diehl et al. (2004). This required prior a reasonable number of 3000 eggs of *H. contortus* obtained by experimental infection of breeding sheep. Compounds **4a-k** and **5a-k** and the anthelmintic drugs (fenbendazole and ivermectin) (7.5 mg per sample) were dissolved in 1 mL of DMSO and diluted with distilled water to obtain a dilution series in 96 well of microtiter plates. Some agar (l40 µL, 45-50°C) containing 2% of amphotericin B was added to each well with also 80 of *Haemonchus* eggs. The plates were maintained at 27°C in a humid atmosphere (90%) for 6 days. The normal development of larvae without products on trial was also carried in wells containing distilled water to serve as control to the experiment. The number of hatched eggs and the number of larvae was counted the stages of development and the mobility of larvae was recorded. For a development rate between 0 and 5%, the test molecule was considered as an active one. The tests were carried out repeatedly three times with all compounds showing a nematocide activity.

RESULTS

Chemical results

We synthesized and isolated 22 molecules carrying both benzimidazole and methylthio moiety in their structure. These compounds, structural analogues of triclabendazole was obtained by total synthesis with a yield varying between 51 and 91% and divided in three series (Figure 2)**.**

The 2-(benzylthio) benzimidazole derivatives **4a-k** (Table 1), the 2-(benzimidazolyl methylthio) benzimidazole derivatives **5a-i** (Table 2) and their analogues 2-(benzimidazolyl methylthio) benzothiazole **5j** and 2-(benzimidazolyl methylthio) benzoxazole **5k** (Table 2). Moreover, benzene ring in the first two series carried various modulators such us nitro (NO_2), benzoyl (PhCO) and chloro (Cl).

The spectroscopic proton NMR characterization (Table 1) of all synthesized compounds showed one characteristic peak between 2.42 and 4.99 ppm corresponding to the chemical shift of the proton S-C$\underline{H}_2$-. Concerning ^{13}C spectra, we noted two main peaks: From 28.64 to 36.00 ppm for S-$\underline{C}H_2$- and from 140.50 to 167.95 ppm for N=$\underline{C}$-S. The molecular peaks in mass spectrometry of these methylthio benzimidazoles (Table 1) varied between 240 and 488 depending on their substituents.

Anti-Haemonchus activities

Regarding nematocidal activities of synthesized compounds **4a-k** and **5a-k** (Table 3), the antiparasitic

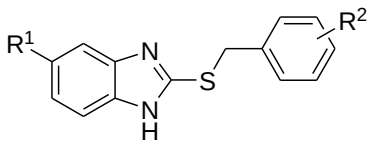
Table 1. Physicochemical characteristics of compounds4a-k.

Compounds	Physicochemical characteristics
4a	NMR^{1}H:4.57 (2H, s, S-C<u>H</u>$_2$); 7.10-7.16 (2H, m, H$_{ar}$); 7.22-734 (2H, m, H$_{ar}$); 7.44-747 (3H, m, H$_{ar}$).NMR ^{13}C :35.12 (S-<u>C</u>H$_2$); 114.08 (2 C$_{ar}$); 121.39 (2 C$_{ar}$); 127.27 (C$_{ar}$); 128.44 (2 C$_{ar}$); 128.80 (2 C$_{ar}$); 137.64 (C$_{ar}$); 149.66 (N=<u>C</u>-S). SM: 240([M+H]$^+$, 100). Yield = 88%. MP = 122-124°C.
4b	NMR ^{1}H: 4.70 (2H, s, S-C<u>H</u>$_2$); 7.10-7.15 (2H, m, H$_{ar}$); 7.44-7.62 (3H, m, H$_{ar}$); 7.91-7.94 (2H, m, H$_{ar}$).NMR ^{13}C: 33.94 (S-<u>C</u>H$_2$); 113.65 (2 C$_{ar}$); 121.47 (C$_{ar}$); 122.14 (2 C$_{ar}$); 123.50 (Car); 129.85 (C$_{ar}$); 135.53 (C$_{ar}$); 139.63 (2 C$_{ar}$); 140.68 (C$_{ar}$); 147.62 (N=<u>C</u>-S); 149.06 (<u>C</u>-NO$_2$).SM : 285 (M+H]$^+$,100).Yield = 85%. MP = 216-218 °C.
4c	NMR ^{1}H:4.60 (2H, s, S-C<u>H</u>$_2$); 7.14-7.18 (2H, m, H$_{ar}$); 7.37-7.40 (2H, m, H$_{ar}$); 7.49-7.52 (4H, m, H$_{ar}$). NMR ^{13}C: 35.20 (S-<u>C</u>H$_2$); 114.91 (2 C$_{ar}$); 122.46 (2 C$_{ar}$); 129.30-129.62 (2 C$_{ar}$); 131.65-132.44 (2 C$_{ar}$); 132.89 (C$_{ar}$); 138.03 (C$_{ar}$); 140.34 (2 C$_{ar}$); 150.41 (N=<u>C</u>-S). SM: 274. ([M]$^+$, 32). Yield = 62%. MP = 181-182°C.
4d	NMR ^{1}H: 4.68 (2H, s, S-C<u>H</u>$_2$); 7.16-7.18 (2H, m, H$_{ar}$); 7.37-7.39 (3H, m, H$_{ar}$); 7.50-7.52 (2H, m, H$_{ar}$).NMR ^{13}C :33.63 (S-<u>C</u>H$_2$); 114.85 (C$_{ar}$); 122.43 (C$_{ar}$); 128.33 (C$_{ar}$); 129.50-129,80 (C$_{ar}$); 133.22 (C$_{ar}$); 133.91 (C$_{ar}$); 140.36 (C$_{ar}$); 149.75 (N=<u>C</u>-S).SM : 309. ([M]$^+$, 28). Yield = 76%. MP = 157-158°C.
4e	NMR ^{1}H: 4.63 (2H, s, S-C<u>H</u>$_2$); 7.24-7.35 (3H, m, H$_{ar}$); 7.47-7.49 (2H, m, H$_{ar}$); 7.59-7.62 (1H, m, H$_{ar}$); 8.04-8.08 (1H, m, Har); 8.32- 8.33 (1H, m, H$_{ar}$). NMR ^{13}C: 34.96 (S-<u>C</u>H$_2$); 110.34 (C$_{ar}$); 113.25 (C$_{ar}$); 117.46 (C$_{ar}$); 127.45 (C$_{ar}$); 128.50 (2 C$_{ar}$); 128.91 (2 C$_{ar}$); 137.09 (2 C$_{ar}$); 142.13 (C$_{ar}$); 155.9 (N=<u>C</u>-S). SM: 285. ([M]$^+$, 100). Yield = 81%. MP = 162-163°C.
4f	NMR ^{1}H: 4.76 (2H, s, S-C<u>H</u>$_2$); 7.58-7.64 (2H, m, H$_{ar}$); 7.95-7.98 (1H, m, H$_{ar}$); 8.04-8.12 (2H, m, H$_{ar}$); 8.32 (1H, m, H$_{ar}$); 8.41 (1H, m, H$_{ar}$). NMR ^{13}C: 34.06 (S-<u>C</u>H$_2$); 117.53 (2 C$_{ar}$); 122.29 (C$_{ar}$); 123.62 (C$_{ar}$); 129.91 (C$_{ar}$); 135.64 (C$_{ar}$); 140.13 (2 C$_{ar}$); 142.20 (C$_{ar}$); 147.65 (N=<u>C</u>-S); 155,37 (C$_{ar}$). SM; 330 ([M+H]$^+$, 100).Yield = 75%. MP = 210-211 °C.
4g	NMR ^{1}H: 4.61 (2H, s, S-C<u>H</u>$_2$); 7.31-7.37 (2H, d, H$_{ar}$); 7.49-7.31 (3H, 3m, H$_{ar}$); 8.03-8.06 (1H, dd, H$_{ar}$); 8.31 (1H, m, H$_{ar}$). NMR ^{13}C: 33.60 (S-<u>C</u>H$_2$); 114.4 (C$_{ar}$); 116.1 (C$_{ar}$); 118.6 (C$_{ar}$); 128.8-129.1 (4 C$_{ar}$); 137.7 (C$_{ar}$); 141.70 (C$_{ar}$); 147,1 (N=<u>C</u>-S). SM: 319. ([M]$^+$, 5).Yield = 85%. MP = 155-156°C.
4h	NMR ^{1}H: 4.72 (2H, s, S-C<u>H</u>$_2$); 7.38-7.40 (2H, m, H$_{ar}$); 7.63-7.64 (1H, m, H$_{ar}$); 7.70-7.72 (1H, m, H$_{ar}$); 8.07-8.10 (1H, m, H$_{ar}$); 8.36 (1H, m, H$_{ar}$). NMR ^{13}C: 32.59 (S-<u>C</u>H$_2$); 113.42 (C$_{ar}$); 117.51 (C$_{ar}$); 127.47 (C$_{ar}$); 128.58-128.91 (2 C$_{ar}$); 131.37 (C$_{ar}$); 133.51-134.25 (2 C$_{ar}$); 139.47 (C$_{ar}$); 140.32 (C$_{ar}$); 143.40 (C$_{ar}$); 155.07 (N=<u>C</u>-S). SM: 354 ([M]$^+$, 22). Yield = 80%. MP = 98-100°C.
4i	NMR ^{1}H: 4.66 (2H, s, S-C<u>H</u>$_2$); 7.28-7.36 (3H, m, H$_{ar}$); 7.49-7.70 (7H, m, H$_{ar}$); 7.76-7.81 (2H, m, H$_{ar}$); 7.87 (1H, m, H$_{ar}$). NMR ^{13}C :36.00 (S-<u>C</u>H$_2$); 114.42 (C$_{ar}$); 124.76 (C$_{ar}$), 128.31 (C$_{ar}$); 129.27-129.50 (2 C$_{ar}$); 129.80-130.27 (2 Car); 131.35-132.33 (2 C$_{ar}$); 132.62-132.88 (2 C$_{ar}$); 138.27 (C$_{ar}$); 139.06 (C$_{ar}$); 154.86 (C$_{ar}$); 167.50 (N=<u>C</u>-S); 196.45 (<u>C</u>=O). SM : 344([M]$^+$, 56). Yield = 90%. MP = 98-100°C.
4j	NMR ^{1}H: 4.59 (2H, s, S-C<u>H</u>$_2$); 7.35-7.39 (2H, m, H$_{ar}$); 7.49-7.58 (5H, m, H$_{ar}$); 7.63-7.67 (1H, m, H$_{ar}$); 7.72-7.73 (2H ,m, H$_{ar}$); 7.74-7.75 (1H, m, H$_{ar}$); 7.81 (1H, m, H$_{ar}$). NMR ^{13}C :35.03 (S-<u>C</u>H$_2$); 114.59 (C$_{ar}$); 117.95 (C$_{ar}$); 123.32 (C$_{ar}$); 129.22-129.29 (2 C$_{ar}$); 130.25-131.692 (2 C$_{ar}$); 132.50-132.64 (2 C$_{ar}$); 138.71 (C$_{ar}$); 139.87 (C$_{ar}$); 143.17 (C$_{ar}$); 147.60 (C$_{ar}$); 157.88 (N=<u>C</u>-S); 196.73 (<u>C</u>=O). SM : 378 ([M]$^+$, 34). Yield = 80%. MP = 98-100°C.
4k	NMR ^{1}H: 4.70 (2H, s, H$_{ar}$); 7.38-7.41 (1H, m, H$_{ar}$); 7.57-7.70 (5H, m, H$_{ar}$); 7.73-7.77 (2H, m, H$_{ar}$); 7. 86 (1H, m, H$_{ar}$). NMR ^{13}C: 33.62 (S-<u>C</u>H$_2$); 114.67 (C$_{ar}$); 117.59 (C$_{ar}$); 125.00 (C$_{ar}$); 128.54 (C$_{ar}$); 129.43 (2 C$_{ar}$); 129.00-130.44 (4 C$_{ar}$); 131.51 (C$_{ar}$); 140.11 (C$_{ar}$); 143.57 (C$_{ar}$); 153.65 (C$_{ar}$); 167.95 (N=<u>C</u>-S); 196.58 (<u>C</u>=O). SM: 413 ([M]$^+$, 10).Yield = 91%. MP = 110-111°C.

gμ / mL) and **5d** (CL_{100} = 0.002 gμ / mL) had respectively a nematicidal activity in the same magnitude order as that of the reference molecules fenbendazole (CL_{100} = 0.0005 μg/mL) and ivermectin (CL_{100} = 0.009 μg/mL). Compounds **5e** (CL_{100} = 0.68 μg/mL), **5f** (CL_{100} = 0.68 μg/mL) and **5h**(CL_{100} = 0.038 μg/mL) had lower activity than that of the reference product. **4a**, **4f**, **4g**, **4h** and **4k** had very high larvicidal concentrations (CL_{100} = 2.86 μg/mL) compared to the reference molecules. Larvicidal concentration (CL_{100}) was the lowest concentration for which the normal larval development was completely blocked (non-hatching eggs, paralysis or death of larvae).

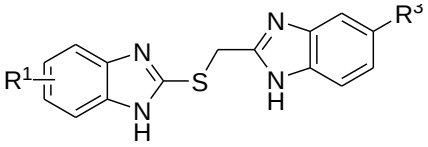
DISCUSSION

Analysis of the results of nematicide activities against *H. contortus* in connection with the structural changes made in the series of 2-(benzylthio) benzimidazole derivatives **4a-k** (Table 1) allowed to make the following observations.



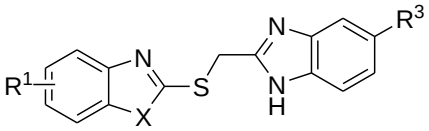
(Methylthio) benzimidazole 4 a-k

4a: R^1 = H; R^2 = H
4b: R^1 = H; R^2 = 3-NO_2
4c: R^1 = H; R^2 = 4-Cl
4d: R^1 = H; R^2 = 2,4-diCl
4e: R^1 = NO_2; R^2 = H
4f : R^1 = NO_2; R^2 = 3-NO_2
4g: R^1 = NO_2; R^2 = 4-Cl
4h: R^1 = NO_2; R^2 = 2,4-diCl
4i : R^1 = ΦCO; R^2 = H
4j : R^1 = ΦCO; R^2 = 4-Cl
4k: R^1 = ΦCO; R^2 = 2,4-diCl



(Methylthio) bisbenzimidazole 5a-i

5a: R^1 = H; R^3 = H
5b: R^1 = H; R^3 = NO_2
5c: R^1 = H; R^3 = ΦCO
5d: R^1 = 5-NO_2; R^3 = H
5e: R^1 = 5-NO_2; R^3 = NO_2
5f : R^1 = 5-NO_2; R^3 = ΦCO
5g: R^1 = 5-ΦCO; R^3 = H
5h: R^1 = 5-ΦCO; R^3 = NO_2
5i : R^1 = 5-ΦCO; R^3 = ΦCO

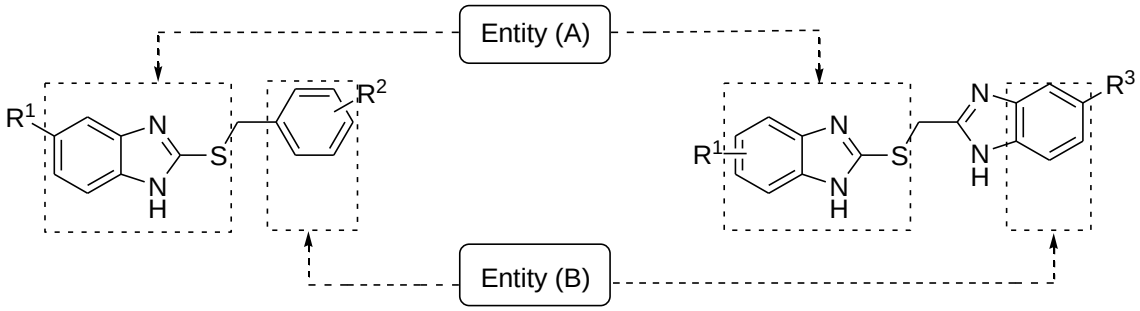


X = S; O

(Methylthiobenzimidazol) benzothiazole 5j et (Methylthiobenzimidazole) benzoxazole 5k

5j : X = S; R^1 = H; R^3 = H
5k: X = O; R^1 = 6-CH_3; R^3 = H

Figure 2. Structure of analogues of triclabendazole synthesized.



(Methylthio) benzimidazole 4a-k **(Methylthio) bisbenzimidazole 5a-i**

Figure 3. Entities (A) and (B) in compounds 4 and 5.

First, presence of benzyl group on the 2-mercapto-benzimidazole (entity (A), (Figure 3) engendered the appearance of nematicide activities. The substitutions on this nucleus caused changes of activity.

Also, when benzyl ring carried a nitro group (NO_2) in the isomeric position-3, an increase of helminthicide activity was observed. Indeed, the product **4b** (CL_{100} = 0.0005 µg/mL) was nearly 6000 times more active than the unsubstituted derivative **4a** (CL_{100} = 2.86 µg/mL). In addition, compared to the reference nematicide molecules, the product **4b** was 18 times more effective than ivermectin (CL_{100} = 0.009 µg/mL) and had an activity equivalent to that of fenbendazole (CL_{100} = 0.0005 µg/mL). The presence of halogenated entities on the benzene ring namely chlorine, induced a decrease of the nematocidal activity. Thus, *para*-chloro derivative **4c** had

Table 2. Physicochemical characteristics of compounds5a-k.

Compounds	Physicochemical characteristics
5a	NMR^{1}H: 4.80 (2H, s, S-C<u>H</u>$_2$); 7.13-7.14 (4H, m, H$_{ar}$); 7.51 (4H, m, H$_{ar}$). NMR ^{13}C : 28.86 (S-<u>C</u>H$_2$); 121.56 (C$_{ar}$); 149.27 (CH$_2$-<u>C</u>=N); 150.51 (N=<u>C</u>-S).SM : 280 ([M+H]$^+$, 100). Yield = 60%. MP = 253-254°C.
5b	NMR ^{1}H: 4.99 (2H, s, S-C<u>H</u>$_2$); 7.24-7.27 (2H, m, H$_{ar}$); 7.53-7.60 (2H, m, H$_{ar}$); 7.67-7.70 (1H, m, H$_{ar}$); 8.11-8.13 (1H, m, H$_{ar}$); 8.47-8.48 (1H, m, H$_{ar}$). NMR ^{13}C : 30.04 (S-<u>C</u>H$_2$); 113.16 (C$_{ar}$); 114.89 (2 C$_{ar}$); 115.64 (C$_{ar}$); 123.52 (2 C$_{ar}$); 132.48-132.66 (2 C$_{ar}$); 143.27-143.62 (2 C$_{ar}$); 149.92 (CH$_2$-<u>C</u>=N); 156.58 (C$_{ar}$); 167.92 (N=<u>C</u>-S). SM: 325 ([M]$^+$, 43). Yield = 61%. MP = 265-266°C.
5c	NMR ^{1}H: 4.89 (2H, s, S-C<u>H</u>$_2$); 7.17-7.19 (2H, m, H$_{ar}$); 7.51-7.59 (4H, m, H$_{ar}$); 7.66-7.75 (5H, m, H$_{ar}$); 7.94 (1H, m, H$_{ar}$).NMR ^{13}C : 29.88 (S-<u>C</u>H$_2$); 115.83 (C$_{ar}$); 119.06 (C$_{ar}$); 122.86 (C$_{ar}$); 125.18 (C$_{ar}$); 129.49-129.70 (2 C$_{ar}$); 132.17-132.55 (2 C$_{ar}$); 132.74 (C$_{ar}$); 133.21 (C$_{ar}$); 139.05-142.51 (3 C$_{ar}$); 150.06 (CH$_2$-<u>C</u>=N); 154.87 (C$_{ar}$); 168.05 (N=<u>C</u>-S); 196.85 (<u>C</u>=O). SM: 384([M]$^+$,100).Yield = 78%. MP = 190-191°C.
5d	NMR ^{1}H: 4.88 (2H, s, S-C<u>H</u>$_2$); 7.14-7.19 (2H, dd, H$_{ar}$); 7.50-7.55 (2H, dd, H$_{ar}$); 7.62-7.65 (1H, d, H$_{ar}$); 8.06-8.09 (1H, dd, H$_{ar}$); 8.37-8.38 (1H, m, H$_{ar}$). NMR ^{13}C : 28.76 (S-<u>C</u>H$_2$); 113.45 (C$_{ar}$); 115.03 (C$_{ar}$); 117.55 (C$_{ar}$); 121.84 (C$_{ar}$); 139.02 (C$_{ar}$); 142.21 (<u>C</u>H$_2$-C=N); 150.03 (C$_{ar}$); 155.47 (N=<u>C</u>-S). SM: 325 ([M+H]$^+$, 100).Yield = 71%. MP = 247-248°C.
5e	NMR ^{1}H: 4.97 (2H, s, S-C<u>H</u>$_2$); 7.64-7.75 (2H, m, H$_{ar}$); 8.08-8.12 (2H, m, H$_{ar}$); 8.39-8.47 (2H, m, H$_{ar}$). NMR ^{13}C: 28.64 (S-<u>C</u>H$_2$); 117.58 (C$_{ar}$); 128.59 (C$_{ar}$); 142.21 (CH$_2$-<u>C</u>=N); 142.55 (C$_{ar}$); 155.37 (N=<u>C</u>-S). SM: 370. ([M]$^+$, 19.54).Yield = 53%. MP = 180-181°C.
5f	NMR ^{1}H: 4.98 (2H, s, S-C<u>H</u>$_2$); 7.55-7.59 (2H, m, H$_{ar}$); 7.65-7.76 (6H, m, H$_{ar}$); 7.95-7.96 (1H, m, H$_{ar}$); 8.08-8.11 (1H, dd, H$_{ar}$); 8.39 (1H, d, H$_{ar}$). NMR ^{13}C : 29.59 (S-<u>C</u>H$_2$); 111.59 (C$_{ar}$); 114.41 (C$_{ar}$); 115.69 (C$_{ar}$); 118.50 (C$_{ar}$); 118.92 (C$_{ar}$); 125.01 (C$_{ar}$); 129.28-129.53 (2 C$_{ar}$); 130.32 (2 C$_{ar}$); 132.62-132.97 (C$_{ar}$); 138.93 (C$_{ar}$); 140.50 (CH$_2$-<u>C</u>=N); 142.34-144.46 (C$_{ar}$); 156.12 (C$_{ar}$); 167.81 (N=<u>C</u>-S); 196.51 (<u>C</u>=O). SM : 429. ([M+H]$^+$, 100). Yield = 72%. MP = 161-162°C.
5g	NMR ^{1}H: 4.91 (2H, s, S-C<u>H</u>$_2$,); 7.17-7.21 (2H, dd, H$_{ar}$); 7.54-7.61 (4H, m, H$_{ar}$); 7.64-7.7 (4H, m, H$_{ar}$); 7.73-7.78 (1H, m, H$_{ar}$); 7.91 (1H, m, H$_{ar}$). NMR ^{13}C: 29.77 (S-<u>C</u>H$_2$); 116.00 (3 C$_{ar}$); 122.89 (C$_{ar}$); 124.91 (C$_{ar}$); 129.40 (C$_{ar}$); 129.64 (2 C$_{ar}$); 130.43 (2 C$_{ar}$); 131.43 (C$_{ar}$); 132.48 (C$_{ar}$); 132.66 (3 C$_{ar}$); 139.95 (C$_{ar}$); 151.32 (CH$_2$-<u>C</u>=N); 153.97 (C$_{ar}$); 167.91 (N=<u>C</u>-S); 196.57 (<u>C</u>=O). SM: 384 ([M]$^+$, 59). Yield = 86%. MP = 255-256°C.
5h	NMR ^{1}H: 4.96 (2H, s, S-C<u>H</u>$_2$,); 7.60 (2H,m, H$_{ar}$); 7.65-7.70 (5H, m, H$_{ar}$); 7.89 (1H, m, H$_{ar}$); 8.10-8.13 (1H, dd, H$_{ar}$); 8.47-8.48 (1H, d, H$_{ar}$). NMR ^{13}C: 29.70 (S-<u>C</u>H$_2$); 113.64-118.73 (3 C$_{ar}$); 124.94 (C$_{ar}$); 129.63 (C$_{ar}$); 130.42 (2 C$_{ar}$); 131.49 (2 C$_{ar}$); 132.47 (C$_{ar}$); 132.66 (C$_{ar}$); 133.06 (C$_{ar}$); 139.04 (C$_{ar}$); 143.53 (CH$_2$-<u>C</u>=N); 153.59 (C$_{ar}$); 156.73 (C$_{ar}$); 167.92 (N=<u>C</u>-S); 196.55 (<u>C</u>=O). SM : 429 ([M]$^+$, 56). Yield = 84%. MP = 93-94°C.
5i	NMR ^{1}H: 4.95 (2H, s, S-C<u>H</u>$_2$); 7.55-7.60 (4H, m, H$_{ar}$); 7.66-7.69 (6H, m, H$_{ar}$); 7.90 (2H, m, H$_{ar}$); 7.95 (2H, m, H$_{ar}$). NMR ^{13}C : 29.73 (S-<u>C</u>H$_2$); 124.81 (C$_{ar}$); 129.26 (4 C$_{ar}$);130.30 (4 C$_{ar}$); 131.45 (C$_{ar}$); 131.82 (2 C$_{ar}$);132.91 (C$_{ar}$); 139.01 (C$_{ar}$); 154.46 (N=<u>C</u>-S); 196.44-196.55 (2 <u>C</u>=O). SM: 488 ([M]$^+$, 72). Yield = 52%. MP = 150-154°C.
5j	NMR ^{1}H: 4.90 (2H, s, S-C<u>H</u>$_2$); 7.15-7.19 (2H, m, H$_{ar}$); 7.34-7.59 (4H, m, H); 7.59-7.91 (1H, m, H$_{ar}$); 8.01-8.04 (1H, m, H$_{ar}$). NMR ^{13}C: 30.31 (S-<u>C</u>H$_2$); 121.25 (2 C$_{ar}$); 121.86 (2 C$_{ar}$); 124.56 (C$_{ar}$); 126.39 (C$_{ar}$); 134.79 (2 C$_{ar}$); 149.53 (CH$_2$-<u>C</u>=N); 152.47 (C$_{ar}$); 165.59 (N=<u>C</u>-S). Yield = 54%. MP = 176-177°C.
5k	NMR ^{1}H: 2.42 (3H, s, C<u>H</u>$_3$); 4.85 (2H, s, S-C<u>H</u>$_2$); 7.15-7.19 (3H, m, H$_{ar}$); 7.48-7.54 (4H, m, H$_{ar}$). NMR ^{13}C: 21.07 (<u>C</u>H$_3$); 29.40 (S-<u>C</u>H$_2$); 162.42 (N=<u>C</u>-S); 151.65 (C$_{ar}$); 149.40 (CH$_2$-<u>C</u>=N); 139.01 (C$_{ar}$); 134.36 (C$_{ar}$); 125.6 (C$_{ar}$); 121.86 (2 C$_{ar}$); 117.76 (C$_{ar}$); 110.33 (C$_{ar}$). SM : 295 ([M+H]$^+$, 100).Yield = 51%. MP = 181-182°C.

a larvicidal activity (CL_{100} = 12.03 µg/mL) less than that of **4a** and introducing two chlorines on the benzene ring (compound **4d**) caused the disappearance of the activity (LC_{100} = 424 µg/mL).

Secondly, the introduction of a substituent such as nitro on the benzimidazole ring in position-5 produced a decrease in the activity. So, compound **4f** had a larvicidal concentration (CL_{100} = 2.86 µg/mL) 5720 higher than that of compound **4b**. The different substitutions made did not cause an improvement of this activity, it is the case of chloro isomer **4g** (CL_{100} = 2.86 µg/mL) and 2,4-dichloro **4h** (CL_{100} = 2.86 µg/mL) which had their activity equal to that of compound **4f**; these had their activity four times greater than that of the non-nitrated derivative **4c** (CL_{100} = 12.03 µg/mL). However, none of these substituted isomers at position-5 of benzimidazole by nitro group had larvicidal activity comparable to those of fenbendazole and ivermectin.

Thirdly, replacing the nitro groupat position-5 by another group such as benzoyl led to the loss of

Table 3. Larvicidalconcentration of 4a-k, 5a-kcompounds and reference molecules.

Compounds	$CL_{100\mu}$g/mL	Compounds	CL_{100}µg/mL
4a	2.86	**5a**	424
4b	0.0005	**5b**	424
4c	12.03	**5c**	424
4d	424	**5d**	0.002
4e	-	**5e**	0.68
4f	2.86	**5f**	0.68
4g	2.86	**5g**	12.03
4h	2.86	**5h**	0.038
4i	424	**5i**	424
4j	424	**5j**	212
4k	2.86	**5k**	12.03
Ivermectin	*0.009*	***Fenbendazole***	*0.0005*

nematicide activity. Nevertheless, only one compound of this series seemed to have a larvicidal activity, the **4k** (CL_{100} = 2.86 µg/mL) compared to **4a**.

As for the obtained results after evaluating the nematicide activity of 2-(benzimidazolyl methylthio) benzimidazole derivatives **5a-k** (Table 2) and their analogues allowed to make some interpretations.

First, replacing benzene moiety by benzimidazolyl inhibited the nematocidal activity. The product **5a** (CL_{100} = 424 µg/mL) had larvicidal concentration 18 times greater than that induced by the benzyl derivative **4b** (CL_{100} = 0.0005 µg/mL). The double substitution on the benzene homocycle (B) using substituents such as nitro (**5b**: CL_{100} = 424 µg/mL) and benzoyl (**5c**: CL_{100} = 424 µg/mL) had no effect on the nematicide activity compared to compound **4a** (CL_{100} = 2.86 µg/mL).

Secondly, introducing a nitro group at position-5 of the 2-methylthiobenzimidazole on the entity (A) of compound **5a** caused an enhancement of the activity. Thus, the larvicide product concentration **5d** (CL_{100} = 0.002 µg/mL) obtained was multiplied by a factor of 212,000. It also showed an anti-*Haemonchus* which was 4 times lower respectively than those of fenbendazole and ivermectin. However, the introduction of substituents on the benzene homocyle (B) such as nitro and benzoyl decreased the ctivities.So, compounds **5e** and **5f** (CL_{100} = 0.68 µg/mL) were 340 times less active than the unsubstituted derivative **5d**. Their activities however were greater than that of the unsubstituted derivatives on the entity (A). These compounds possessed nematocidal activity 623 times greater than that of **5a**.

Thirdly, the replacement of nitro on the compound **5b** of the entity (A) by a benzoyl group **5g** (CL_{100} = 12.03 µg/mL) appeared to inhibit the nematocidal activity and it was 35 times more active than the unsubstituted derivative **5a**. However, this larvicidal concentration remained significantly higher than those of ivermectin (CL_{100} = 0.009 µg/mL) and fenbendazole (CL_{100} = 0.0005 µg/mL). When homocycle entity (B) **5g** was substituted in its position-5 by nitro group **5h** (CL_{100} = 0.038 µg/mL), there was an increase of the larvicidal activity. This was 316 times greater than **5g**. Compound **5h** showed an anti-*Haemonchus* efficiency respectively 76 times and 4 times less than that of fenbendazole and ivermectin. The presence in the same position of the benzoyl **5i** (LC_{100} = 424 µg/mL) caused the greatest loss of larvicidal activity. In this series, a third substitution has been carried out. This was the replacement of the benzimidazole ring of the entity (A) by benzothiazole **5j** and benzoxazole **5k**. This homology replication resulted in slight improvement in the nematicide activity compared to compound **5a**.The larvicide concentration was divided by a factor 2 (**5d**) and 35 (**5k**). These activities remained very low compared to the reference compounds. A comparison of compounds **5j** and **5k** showed that the benzoxazole derivative exhibit anti-*Haemonchus* activity 18 times greater than its analogue benzothiazole.

Conclusion

The syntheses carried out around the chemical series of 2-mercaptobenzimidazole allowed to obtain new compounds, derivatives and structural analogues of 2 (methylthio) benzimidazole which have been characterized by NMR (^{1}H and^{13}C) and mass spectroscopy. *In vitro* nematocide assays against *H. contortus* of the synthesized compounds revealed the anthelmintic activities of compounds **4b**, **5d**, **5e**, **5f** and **5h**. Among them, **4b** and **5d** were active compared to fenbendazole or ivermectin. The structure-activity relationship study showed that coupling a benzene group with the methylthio group of 2-mercaptobenzimidazole was more advantageous to the appearance of nematocidal activities than that of a benzimidazole. In addition, the introduction of nitro (NO_2) group on the benzene ring was favorable to an increase of nematicide activities.

Conflict of Interests

The authors have not declared any conflict of interests.

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