Research Article

Synthesis and anthelmintic activity of some hybrid Benzimidazolyl-chalcone derivatives

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Abstract

Purpose: To synthesize hybrid benzimidazolyl-chalcone derivatives, evaluate their anthelmintic activity, and establish some structural elements which could lead to induction and enhancement of this activity.

Methods: A series of 1-(1H-benzimidazol-2-yl)-3-aryl-2-propen-1-one compounds (6a-z) was synthesized by condensation reaction of 2-acetylbenzimidazole with aryl and heteroaryl aldehyde derivatives. The physicochemical characterization of these benzimidazolyl-chalcones was carried out by nuclear magnetic resonance spectroscopy (¹H and ¹³C NMR) and mass spectroscopy (MS). All compounds were screened in vitro for their nematicidal activity against Haemonchus contortus in larval development assay. The anthelmintic activities obtained were compared with those of anthelmintic reference drugs (fenbendazole and ivermectin); 1,3-diphenyl-2-propen-1-one also used as reference for chalcone.

Results: Compounds 6a, 6g, 6w and 6y showed good nematicidal activity (LC₁₀₀) at 0.002 and 0.0092 µg/ml. The activity of these four benzimidazolyl-chalcones is nearly equal to that of fenbendazole. It is also interesting to know that these compounds have anti-haemonchus activity which is equal or more efficient than ivermectin. Four other compounds (6d, 6h, 6o and 6t) possess interesting anthelmintic activities at 0.68 and 0.16 µg/ml.

Conclusion: Preliminary structure-activity relationship studies revealed that arylpropenone group in position 2 of the benzimidazole ring can be considered as new pharmacophore for nematicidal activity.

Keywords: Benzimidazole, Chalcone, Anthelmintic activity, Haemonchus contortus

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INTRODUCTION

Nematodes as well as being the origin of most human parasitic diseases [1,2], remain the major cause of gastrointestinal infections and productivity loss of small ruminant livestock and pets [3,4]. This situation persists for two main reasons. The first is the poor veterinary coverage against *Haemonchus contortus*, the main gastrointestinal parasite of small ruminants [5]. The second reason is related to current misuse of anthelmintic compounds which engenders the occurrence of drug-resistant parasite strains [6-8]. Moreover, the discovery of an anthelmintic vaccine [9] constantly delayed by difficulties in drug development, has made the fight against parasites a major economic and food security issue. In this context, chemotherapy by developing new anthelmintic compounds which can circumvent the problems of drug resistance remains an essential weapon. This is why we have focused our investigations on the chemical series of chalcones and benzimidazoles. Chalcones or 1,3-diphenyl-2-propen-1-ones are known for their multiple anti-infective activities including antimalarial, antileishmanial, antitrypanosomal, antibacterial, anti-tubercular, antifungal and antiviral [10,11]. The diversity of chalcones’ anti-infective activity is due to the presence of enone moiety of which would cause an inhibitory mechanism of some enzymes with thiol function [12,13]. Moreover, appearance, orientation and optimization of biological properties of chalcones depend namely on three structural elements: integrity of enone moiety, nature of aryl rings (A and B) in position 1 and 3 in this functional group, nature and position of modulators R and R’ on the aryl rings (Figure 1). As for the benzimidazole heterocyclic, it is an important vector of many anthelmintic drugs used in both human and veterinary medicine (albendazole, mebendazole, fenbendazole, triclabendazole, thiabendazole, etc) [14]. Despite the great diversity of chalcones’ biological activities, their anthelmintic properties especially their nematicidal activity against *Haemonchus contortus*, has not yet been described or known. Therefore, it seems logical to undertake chemical modulations around chalcones to guide their anti-infective properties toward nematicidal activities. We have, therefore, conceptualized a new hybrid chemical profile of chalcone and benzimidazole anthelmintic by juxtaposition of arylpropenone group and benzimidazole ring (Figure 1). The main objective of this work was to synthesize hybrid benzimidazolyl-chalcone derivatives, and

![Figure 1: Conception and chemical profile of hybrid benzimidazolyl-chalcones (6a-z)](image-url)
then evaluate their nematicidal activities in vitro against *Haemonchus contortus*. Another objective was to establish in this new chemical series, some structural elements which have led to induction of and increasing nematicidal activity.

**EXPERIMENTAL**

**Chemistry**

The synthesis of benzimidazolyl-chalcones (6a-z) was made from 2-acetylbenzimidazole 4 [15] (figure 2). This compound was obtained in two steps from o-phenylenediamine 1. Indeed, condensation of 1 with lactic acid 2 using Phillips method [16] has lead to 2-hydroxyethylbenzimidazole 3. Chromic oxidation of the latter followed by neutralization with ammonia led to 2-acetylbenzimidazole in an overall yield of 72%. This ketone was then condensed with aryl or heteroaryl aldehyde derivatives (5) using the Claisen-Schmidt reaction. The neutralization of the reaction mixture by dilute acetic acid followed by recrystallization gave, compounds 6a-z with yields ranging between 50-86% (Figure 2). The general procedure for synthesis benzimidazolyl-chalcones is reported below. The chosen arylaldehyde (10 mmol) was added to 2-acetylbenzimidazole (1.5 g, 10 mmol) in ethanol solution of sodium hydroxide (75 mmol sodium hydroxide in 40 ml of ethanol). The reaction mixture was subsequently stirred at room temperature for 5 h and neutralized with a solution of 30% acetic acid leading to a precipitate. It was filtered, dried and recrystallized in toluene or toluene/EtOH (4:1) to give compounds 6a-z.

For all compounds 6a-z, melting points were determined on a Köpfier bench and are uncorrected. $^1$H and $^{13}$C NMR spectra were measured on a Brucker Avance 300 spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane (TMS, δ = 0) used as internal reference in DMSO-d6; $J$ values are given in hertz. Splitting patterns have been designated as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; qt, quintuplet; m, multiplet. Mass spectra (MS) were recorded on a JEOL JMS DX300 spectrometer in electrospray ionization mode (ESI mass). The thin layer chromatography (TLC) was performed on silica plates Macherey-Nagel Sil or alumina G/UV254 Macherey-Nagel ALOX N/UV254. Solvents and reagents including arylaldehydes used were from Aldrich (France).

![Figure 2](attachment:figure2.png)

**Figure 2**: General procedure for preparing benzimidazolyl-chalcones 6a-z
Anthelmintic activity

The method for determining anthelmintic activity or larval development assay required *Haemonchus contortus* eggs. Their preparation consists in an experimental infection of farmed sheep from 3000 to 6000 infective larvae of *Haemonchus contortus* L3. After a period of 21 days, the faeces of sheep are infested with parasite eggs. A number of 3000 eggs per gram of faeces has been considered reasonable to perform the anthelmintic tests. The eggs were extracted after grinding, filtration and centrifugation of faeces collected. The suspension was then adjusted with distilled water to a concentration of 80 eggs per 20 ml. The standard anthelmintic drugs (fenbendazole and ivermectin from Sigma Chemical Co, USA) and compounds 6a-z were provided as pure powder. The reference chalcone or 1,3-diphenylpropenone was synthesized in our laboratory according to the classical Claisen-Schmidt using acetophenone and benzaldehyde. All test compounds (6a-z) including anthelmintic standards drugs (7.5 mg) were respectively dissolved in 1ml DMSO and diluted with distilled water to obtain a dilution series in 96 wells microtitration plates. Agar (140 µL) at 45-50 °C containing 2% amphotericin B was added to each well. In the prepared well, were added 80 eggs freshly harvested *Haemonchus contortus*. The microplates were kept at a humid atmosphere (90%) for 6 days at 27° C. The normal larval development in the absence of test products was also performed in wells containing distilled water to serve as an experiment control. The number of hatched eggs and the number of larvae were counted; stages of development and mobility of larvae were recorded. For a development rate between 0 and 5%, the test product was considered active. The tests were repeated three times with all compounds that showed nematocidal activity. The larvicidal concentration (LC100) determined, was the lowest concentration for which the normal larval development was completely blocked (no hatching eggs, paralysis or death of the larvae).

RESULTS

We have synthesized and characterized 26 hybrid benzimidazolyl-chalcone compounds 6a-z. All synthesized compounds have in their respective molecule, the 1H-benzimidazole heterocyclic and the 3-aryl(heteroaryl)-2-propenone group. The aryl represented by phenyl ring was variously substituted by modulators such as alkyl, hydroxy, alkoxy, aminoalkyl halogen and nitro. As for heteroaryl rings, it’s pyridine and furan. The physicochemical results especially chemical groups characteristic (NH, -C=, -CO-CH=CH-) and ESI mass of all compounds 6a-z and their yields are reported below in table 1. Nematicidal activities of synthesized compounds were evaluated on eggs and larvae of *Haemonchus contortus* and then compared with the anthelmintic efficacy of anthelmintic standards drugs (fenbendazole and ivermectin). Also, to determine structural elements of nematocidal activity, we have evaluated the anthelmintic activity of 1,3-diphenylprop-2-en-1-one or standard chalcone. The results of anthelmintic tests (Table 1) showed that four compounds (6a, 6g, 6w and 6y) have good nematicidal activity (LC100) at 0.002 and 0.0092 µg/ml. Four other compounds (6d, 6h, 6o and 6t) possess interesting anthelmintic activities at 0.68 and 0.16 µg/ml. For the other compounds their nematicidal concentrations are lower and range from 424.5 to 2.87 µg/ml.

DISCUSSION

Analysis of our results (Table 1) in a preliminary structure-activity relationship studies, showed that the replacement of phenyl group (A) of standard chalcone or 1,3-diphenylpropenone (Figure 1) by 2-benzimidazolyl group, increased nematicidal activity against *Haemonchus contortus*. Indeed, compound 6a (LC100 = 0.002 µg/ml) was 6000 times more active than the
Table 1: Physicochemical data for characteristic groups and in vitro anthelmintic activity of compounds 6a-z against *Haemonchus contortus*

| Compound | Ar | Yield (%) | Mp (°C) | H, 13C NMR of NH, -C=N, -CO-CH=CH- (DMSO-d6, δ ppm) and ESI mass | LC100 (µg/ml) |
|----------|----|-----------|---------|---------------------------------------------------------------|-------------|
| 6a       |    | 78        | 215-217 | 1H: 14 (1H, s, NH); 8.28 (1H, d, J = 16 Hz, CH=CH); 8.15 (1H, d, J = 16 Hz, CH=CH). 13C: 181.13 (C=O); 149.49 (C=N); 143.97 (CH=CH); 121.69 (CH=CH). MS (m/z): 249 (M+1) | 0.002       |
| 6b       | H | 66        | 204-207 | 1H: 13.54 (1H, s, NH); 8.20 (1H, d, J = 15.8 Hz, CH=CH); 8.06 (1H, d, J = 15.8 Hz, CH=CH). 13C: 180.89 (C=O); 149.02 (C=N); 143.30 (CH=CH); 121.69 (CH=CH). MS (m/z): 263 (M+1) | 12.0        |
| 6c       | CH3 | 66 | 201-203 | 1H: 13.20 (1H, s, NH); 8.15 (1H, d, J = 15.6 Hz, CH=CH); 8.03 (1H, d, J = 15.6 Hz, CH=CH). 13C: 181.09 (C=O); 149.02 (C=N); 143.30 (CH=CH); 121.69 (CH=CH). MS (m/z): 263 (M+1) | 2.87        |
| 6d       | CH3 | 64 | 216-219 | 1H: 13.60 (1H, s, NH); 8.20 (1H, d, J = 15.8 Hz, CH=CH); 8.09 (1H, d, J = 15.8 Hz, CH=CH). 13C: 180.79 (C=O); 149.82 (C=N); 145.30 (CH=CH); 121.69 (CH=CH). MS (m/z): 263 (M+1) | 0.16        |
| 6e       | CH3 | 61 | 240-243 | 1H: 12.0 (1H, s, NH); 8.10 (1H, d, J = 16.2 Hz, CH=CH); 7.93 (1H, d, J = 16.2 Hz, CH=CH). 13C: 181.11 (C=O); 151.92 (C=N); 144.01 (CH=CH); 121.69 (CH=CH). MS (m/z): 263 (M+1) | 424.5       |
| 6f       |    | 58        | 203-206 | 1H: 14 (1H, s, NH); 7.99 (1H, d, J = 15.6 Hz, CH=CH); 7.94 (1H, d, J = 15.6 Hz, CH=CH). 13C: 181.13 (C=O); 149.60 (C=N); 145.16 (CH=CH); 121.69 (CH=CH). MS (m/z): 265 (M+1) | 2.87        |
| 6g       |    | 69        | 257-259 | 1H: 14 (1H, s, NH); 8.02 (1H, d, J = 15.6 Hz, CH=CH); 7.98 (1H, d, J = 15.6 Hz, CH=CH). 13C: 181.13 (C=O); 150.10 (C=N); 145.60 (CH=CH); 121.69 (CH=CH). MS (m/z): 265 (M+1) | 0.002       |
| 6h       |    | 55        | >260    | 1H: 14 (1H, s, NH); 8.05 (1H, d, J = 15.6 Hz, CH=CH); 7.96 (1H, d, J = 15.6 Hz, CH=CH). 13C: 181.18 (C=O); 150.60 (C=N); 145.20 (CH=CH); 121.69 (CH=CH). MS (m/z): 265 (M+1) | 212.3       |
| 6i       | HCO | 68 | 198-201 | 1H: 13.54 (1H, s, NH); 8.30 (1H, d, J = 16 Hz, CH=CH); 8.10 (1H, d, J = 16 Hz, CH=CH). 13C: 181.23 (C=O); 149.34 (C=N); 138.76 (CH=CH); 121.69 (CH=CH). MS (m/z): 279 (M+1) | 424.5       |
| 6j       | OCH3 | 69 | 165-167 | 1H: 13.54 (1H, s, NH); 8.30 (1H, d, J = 16 Hz, CH=CH); 8.12 (1H, d, J = 16 Hz, CH=CH). 13C: 181.23 (C=O); 149.34 (C=N); 138.76 (CH=CH); 121.49 (CH=CH). MS (m/z): 279 (M+1) | 424.5       |
Table 1: Physicochemical data for characteristic groups and in vitro anthelmintic activity of compounds 6a-z against Haemonchus contortus (continued)

| Compound | Ar         | Yield (%) | Mp (°C) | H, 13C NMR of NH, -C=N, -CO-CH=CH- (DMSO-d6, δ ppm) and ESI mass | LC100 (µg/ml) |
|----------|------------|-----------|---------|------------------------------------------------------------------|--------------|
| 6k       |            | 70        | 202-205 | H: 13.54 (1H, s, N-H); 8.18 (1H, d, J = 16.4 Hz, CH=CH); 8.08 (1H, d, J = 16.4 Hz, CH=CH).  
13C: 181.23 (C=O); 149.34 (C=N); 138.76 (CH=CH); 121.49 (CH=CH). MS (m/z): 279 (M+1) | 0.68         |
| 6l       |            | 68        | 216-218 | H: 13.48 (1H, s, N-H); 8.25 (1H, d, J = 16.2 Hz, CH=CH); 8.12 (1H, d, J = 16.2 Hz, CH=CH).  
13C: 181.18 (C=O); 149.04 (C=N); 138.84 (CH=CH); 121.49 (CH=CH). MS (m/z): 309 (M+1) | 424.5        |
| 6m       |            | 70        | 204-207 | H: 13.48 (1H, s, N-H); 8.21 (1H, d, J = 16.2 Hz, CH=CH); 8.12 (1H, d, J = 16.2 Hz, CH=CH).  
13C: 181.18 (C=O); 149.04 (C=N); 138.84 (CH=CH); 121.49 (CH=CH). MS (m/z): 309 (M+1) | 424.5        |
| 6n       |            | 52        | >260    | H: 12.0 (1H, s, N-H); 8.10 (1H, d, J = 16.2 Hz, CH=CH); 7.93 (1H, d, J = 16.2 Hz, CH=CH).  
13C: 180.61 (C=O); 149.07 (C=N); 144.51 (CH=CH); 122.61 (CH=CH). MS (m/z): 292 (M+1) | 424.5        |
| 6o       |            | 70        | 245-248 | H: 14 (1H, s, N-H); 7.99 (1H, d, J = 15 Hz, CH=CH); 7.95 (1H, d, J = 15 Hz, CH=CH).  
13C: 180.71 (C=O); 149.95 (C=N); 142.29 (CH=CH); 121.69 (CH=CH). MS (m/z): 284 (M+1) | 0.16         |
| 6p       |            | 85        | >260    | H: 14 (s, 1H, NH); 8.01 (1H, d, J = 15 Hz, CH=CH); 7.96 (1H, d, J = 15 Hz, CH=CH).  
13C: 180.0 (C=O); 149.80 (C=N); 142.25 (CH=CH); 122.01 (CH=CH). MS (m/z): 284 (M+1) | 12.0         |
| 6q       |            | 69        | 237-240 | H: 14 (1H, s, N-H); 8.0 (1H, d, J = 15 Hz, CH=CH); 7.90 (1H, d, J = 15 Hz, CH=CH).  
13C: 180.69 (C=O); 149.0 (C=N); 142.4 (CH=CH); 121.82 (CH=CH). MS (m/z): 284 (M+1) | 424.5        |
| 6r       |            | 74        | 241-244 | H: 13.89 (s, 1H, NH); 8.06 (1H, d, J = 15.2 Hz, CH=CH); 7.91 (1H, d, J = 15.2 Hz, CH=CH).  
13C: 180.0 (C=O); 150.40 (C=N); 148.20 (CH=CH); 121.01 (CH=CH). MS (m/z): 317 (M+1) | 424.5        |
| 6s       |            | 71        | 258-261 | H: 14 (1H, s, N-H); 8.20 (1H, d, J = 16.2 Hz, CH=CH); 7.88 (1H, d, J = 16.2 Hz, CH=CH).  
13C: 180.88 (C=O); 149.21 (C=N); 141.22 (CH=CH); 121.69 (CH=CH). MS (m/z): 328 (M+1) | 424.5        |
Table 1: Physicochemical data for characteristic groups and in vitro anthelmintic activity of compounds 6a-z against Haemonchus contortus (continued)

| Compound | Ar | Yield (%) | Mp (°C) | $^1$H, $^{13}$C NMR of NH, -C=N -CO-CH=CH- (DMSO-d$_6$, δ ppm) and ESI mass | LC$_{100}$ (µg/ml) |
|----------|----|-----------|---------|-----------------------------------------------------------------------------------------------------------------|------------------|
| 6t       |     | 86        | 249-251 | $^1$H: 14 (1H, s, N-H); 8.15 (1H, d, J = 16.2 Hz, CH=CH); 7.88 (1H, d, J = 16.2 Hz, CH=CH). $^{13}$C: 180.0 (C=O); 148.90 (C=N); 142.25 (CH=CH); 121.69 (CH=CH). MS (m/z): 328 (M$^+$1) | 0.16 |
| 6u       |     | 73        | 227-230 | $^1$H: 14 (1H, s, N-H); 8.01 (1H, d, J = 15 Hz, CH=CH); 7.95 (1H, d, J = 15 Hz, CH=CH). $^{13}$C: 179.91 (C=O); 149.40 (C=N); 142.55 (CH=CH); 122.01 (CH=CH). MS (m/z): 328 (M$^+$1) | 424.5 |
| 6v       |     | 76        | 221-224 | $^1$H: 14 (1H, s, N-H); 8.01 (1H, d, J = 15 Hz, CH=CH); 7.91 (1H, d, J = 15 Hz, CH=CH). $^{13}$C: 180.80 (C=O); 148.04 (C=N); 142.55 (CH=CH); 122.01 (CH=CH). MS (m/z): 267 (M$^+$1) | 12.0 |
| 6w       |     | 69        | 199-202 | $^1$H: 13.78 (1H, s, N-H); 8.70 (1H, d, J = 16.3 Hz, CH=CH); 8.10 (1H, d, J = 16.2 Hz, CH=CH). $^{13}$C: 182.86 (C=O); 151.31 (C=N); 145.39 (CH=CH); 121.52 (CH=CH). MS (m/z): 294 (M$^+$1) | 0.0092 |
| 6x       |     | 80        | 229-231 | $^1$H: 13.82 (1H, s, N-H); 8.70 (1H, d, J = 16.3 Hz, CH=CH); 8.10 (1H, d, J = 16.2 Hz, CH=CH). $^{13}$C: 182.86 (C=O); 151.31 (C=N); 145.39 (CH=CH); 121.52 (CH=CH). MS (m/z): 294 (M$^+$1) | 2.87 |
| 6y       |     | 50        | 243-245 | $^1$H: 13.60 (1Hs, N-H); 8.25 (1H, d, J = 16.2 Hz, CH=CH); 8.05 (1H, d, J = 16.2 Hz, CH=CH). $^{13}$C: 182.13 (C=O); 150.09 (C=N); 144.27 (CH=CH); 121.69 (CH=CH). MS (m/z): 250 (M$^+$1) | 0.002 |
| 6z       |     | 78        | 213-216 | $^1$H: 13.92 (1Hs, N-H); 8.71 (1H, d, J = 16.2 Hz, CH=CH); 7.97 (1H, d, J = 16.2 Hz, CH=CH). $^{13}$C: 181.89 (C=O); 149.47 (C=N); 138.18 (CH=CH); 131.18 (CH=CH). MS (m/z): 239 (M$^+$1) | 2.87 |

1,3-diphenylprop-2-en-1-one or chalcone
ivermectine
fenbendazole

standard chalcone (LC$_{100}$ = 12.0 µg/ml). In addition, this compound had a nematicidal activity about 5 times higher than that of ivermectin (LC$_{100}$ = 0.0092 µg/ml). However, the larvicidal action of 6a was less than fenbendazole (LC$_{100}$ = 0.0005 µg/ml). Maintaining the benzimidazole ring doubled by introduction of group low electron donating such as methyl on the phenyl group (B) of our compound 6a (Figure 1), supports emergence of nematicidal activity. However, it seems to be related to isomeric position of methyl on phenyl group. The isomer $\text{para}$ methyl 6d (LC$_{100}$ = 0.16 µg/ml) had a larvicidal activity 18 times greater than that of isomer $\text{meta}$ methyl 6c (LC$_{100}$ = 2.87 µg/ml). This latter had an activity 4 times greater than $\text{ortho}$ methyl 6b (LC$_{100}$ = 12.0 µg/ml) which was equivalent to that of standard chalcone. However, none of these methylated isomers possessed nematicidal activity comparable to that of fenbendazole and ivermectin. Furthermore, replacement of methyl in $\text{para}$ position by another alkyl group as isopropyl...
(6e) with more lipophilicity, causes nematicidal activity loss \(\text{LC}_{100} = 424.5\ \mu\text{g/ml}\). Introduction on the phenyl group of compound 6a of another electron donating substituent, such as methoxy, has induced anti-haemonchus activity with the single isomer para methoxy 6k \(\text{LC}_{100} = 0.68\ \mu\text{g/ml}\). This compound showed a nematicidal activity 18 times greater than that of reference chalcone. Furthermore, duplication of methoxy group at positions 3 and 4 or 2 and 5 (compounds 6l and 6m) caused the loss of nematicidal activity \(\text{LC}_{100} = 424.5\ \mu\text{g/ml}\). It is the same for compounds mono-methoxylated in position ortho or meta (6i and 6j). Presence of halogen on phenyl group (B) induced anthelmintic properties according to the type of halogen. It was also dependent on the position of halogen on this phenyl group. Thus, the isomers ortho and the para brominated (6s and 6u), the meta chlorinated isomer (6q) and the isomer 2,4-dichloro (6r) have no larvicidal activity. By contrast, para chloro (6p) and para fluoro (6v) derivatives had their activities equivalent to that of reference chalcone \(\text{LC}_{100} = 12.0\ \mu\text{g/ml}\). Only ortho chloro (6o) and meta bromo(6t) compounds \(\text{LC}_{100} = 0.16\ \mu\text{g/ml}\) had nematicidal activities higher than that of the standard chalcone. Both compounds have equivalent activity to that of para methylated compound (6d). However, this action was low compared to that induced by ivermectin and fenbendazole. The modulation of phenyl ring by adding group strongly electron donating such as hydroxy, has induced nematicidal activity which depends on isomeric position of the group. Only the ortho and the meta hydroxylated compounds (6f and 6g) had anti-haemonchus concentrations better than that of standard chalcone. Moreover, this activity was enhanced when the hydroxy was in position meta on phenyl group (6g, \(\text{LC}_{100} = 0.002\ \mu\text{g/ml}\)). Compared with the nematicidal activities of our reference drugs, compound 6g was 5 times more active than ivermectin, but lower than fenbendazole. The anthelmintic efficacy of 6g was however equivalent to the derivative none substituted (compound 6a). The presence of group more electron donating like dimethylamine at position para on the phenyl (6n), caused loss of nematicidal activities \(\text{LC}_{100} = 424.5\ \mu\text{g/ml}\). Such a modulator would be unfavorable to the emergence of nematicidal activity. When we introduced entities that are strong electron repelling such as nitro on phenyl group, only the meta nitro isomer (6w; \(\text{LC}_{100} = 0.0092\ \mu\text{g/ml}\)) has induced anti-haemonchus activity equal to that of ivermectin. As for para nitro derivative (6x), it has not presented a good nematicidal activity \(\text{LC}_{100} = 2.87\ \mu\text{g/ml}\). However, this compound remained more effective than our standard chalcone. The replacement of phenyl group by heteroaryl rings, such as 3-pyridinyl or 2-furyl, induced nematicidal activity. The 3-pyridinyl derivative (6y) exalted anthelmintic properties compared to that of reference chalcone. However, this compound presented the same nematicidal action \(\text{LC}_{100} = 0.002\ \mu\text{g/ml}\) as that of the unsubstituted derivative (6a) and the meta hydroxylated derivative (6g). This substitution of phenyl by a 3-pyridinyl ring did not change the anti-haemonchus activity. In contrary, the 2-furyl derivative (6z, \(\text{LC}_{100} = 2.87\ \mu\text{g/ml}\)) did not increase nematicidal activity compared to that of compounds 6a, 6g, or 6y. It remained 1500 times lower, even if it is more efficient than the standard chalcone. Compared to fenbendazole (benzimidazole anthelmintic drug), our hybrid benzimidazolyl-chalcones compounds (6a-z) were not substituted in their positions 5 and/or 6. However anthelmintic effectiveness of benzimidazole drugs would be based on blocking these sites of metabolism [17]. This structural difference may explain why these compounds were less active against Helamonchus than fenbendazole in vitro. This was not in itself a failure of our 8 active compounds (6a, 6d, 6g, 6h, 6o, 6t, 6w, and 6y). Rather, it was a good prospect for their pharmacology modulation in position 5 (or 6) as in benzimidazole anthelmintic drugs.
CONCLUSION

In this study, we designed, synthesized and evaluated the anthelmintic activity against *Haemonchus contortus* of 26 hybrid benzimidazolyl-chalcone compounds. Preliminary structure-activity relationship studies have helped to highlight structural elements of the nematicidal activities. These show that replacement of phenyl group in position 1 of 1,3-diphenyl-2-propen-1-one by benzimidazole ring induces the occurrence of a potential anthelmintic activity. This activity was also modulated by attaching different substituents on the phenyl group of benzimidazolyl-chalcones. The arylpropenone group, like carbamate or thioalkyl in position 2 of benzimidazole ring, is a true anthelmintic pharmacophore. Our hybrid benzimidazolyl-chalcones are therefore promising candidates for the development of new anthelmintic agents substituted at positions 5 and/or 6 of the benzimidazole ring.

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