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Synthesis and Antiparasitic Activity of Albendazole and Mebendazole Analogues☆

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Abstract—Albendazole (Abz) and Mebendazole (Mbz) analogues have been synthesized and in vitro tested against the protozoa *Giardia lamblia*, *Trichomonas vaginalis* and the helminths *Trichinella spiralis* and *Caenorhabditis elegans*. Results indicate that compounds **4a**, **4b** (Abz analogues), **12b** and **20** (Mbz analogues) are as active as antiprotozoal agents as Metronidazole against *G. lamblia*. Compound **9** was 58 times more active than Abz against *T. vaginalis*. Compounds **8** and **4a** also shown high activity against this protozoan. Compounds **4b** and **5a** were as active as Abz. None of the Mbz analogues showed activity against *T. vaginalis*. The anthelmintic activity presented by these compounds was poor.

Introduction

Parasitic infections are still a major health problem in developing countries, affecting mainly the infantile population. It has been reported that benzimidazole 2-carbamates (BZC), such as Albendazole (Abz) and Mebendazole (Mbz), used mainly as anthelmintic agents (Fig. 1), inhibit the in vitro growth of protozoa *Giardia lamblia* and *Trichomonas vaginalis*.[1–3] Clinical assays have shown that Abz is as effective as Metronidazole, the choice drug for the treatment of giardiasis.[4–6]

The anthelmintic activity of benzimidazole 2-carbamates has been related to their selective antimitotic activity due to the preferential binding of these agents to helmintic tubulin over mammalian tubulin.[7] Similarly, the action of Abz against *Giardia lamblia* also involves the interaction with tubulin of the *Giardia* cytoskeleton.[2,8] It is suggested that one of the requirements for this action is that the substituted benzimidazole bear a hydrogen atom at the 1-position and a methylcarbamate group at the 2-position.[9,10]

Since the site of action of the different BZC is the same, these compounds have additive action and show crossed resistance, which may undermine their future therapeutic value.[11] Due to their poor solubility and absorption in vivo, BZC have successfully been used to treat gastrointestinal helmintic diseases.

Although systemic infections have also been treated with these agents, high doses and long treatments are required.[12]

As part of our search for basic information about the structural requirements for antiprotozoal and anthelmintic activity,[10,13,14] we have synthesized a series of novel 2-(trifluoromethyl)benzimidazole derivatives, analogues of Abz (**4a–b**, **5a–b**, **8**, and **9**) and Mbz (**12a–b**, and **20**), reported in Table 1. The in vitro antiparasitic activity of these compounds on an intestinal protozoan (*G. lamblia*) and urogenital tract parasite (*T. vaginalis*); and helminths *Trichinella spiralis* and *Caenorhabditis elegans,* is also reported.

Chemistry

Compounds **4a–b**, **5a–b**, **8** and **9** were prepared from the adequate substituted 2-nitroaniline **1a**, **1b**, or **1c**,

☆Taken in part from the PhD thesis of Gabriel Navarrete-Vázquez

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respectively. The sequence shown in Scheme 1 was followed. Aromatic nucleophilic substitution in **1a–b** with 1-propanothiol afforded **2a–b**,[15,17] which upon reduction with H_2, Pd/C gave 1,2-phenylenediamine **3a–b**. Reaction of these with CF_3COOH yielded **4a–b**. The sulfoxides **5a–b** were obtained by treatment of **4a–b** with *m*-CPBA. Reaction of **1c** with NH_4SCN and Br_2 gave **6**,[16,17] which was converted to **7** with a mixture of 1-PrOH, 1-PrBr, KCN, H_2O and then reduced and cyclocondensed with CF_3COOH to give **8**.[13] The same procedure employed above was used to prepare sulfoxide **9**.

Starting with 4-benzoyl-2-nitroanilines **10a–b**, the reduction with $SnCl_2{\cdot}2H_2O$ and cyclocondensation of the *o*-phenylenediamine intermediate with CF_3COOH afforded the corresponding benzimidazole derivatives **12a–b**, respectively (Scheme 2). For the synthesis of the regioisomeric derivative **20**, *o*-phenylenediamine **19** was first prepared through the series of reactions shown in Scheme 3. Thus, 3-chlorotoluene **13**, through nitration and oxidation of the nitration products **14** with $K_2Cr_2O_7$ and H_2SO_4, preferably gave 3-chloro-4-nitrobenzoic acid **15**. The activation of **15** with $SOCl_2$, and Friedel–Crafts acylation of benzene with chloride **16** led to benzophenone **17**, which upon nucleophilic substitution with CH_3NH_2 followed by reduction of **18** with $SnCl_2{\cdot}2H_2O$ afforded the required compound **19**. Cyclocondensation of **19** with CF_3COOH, as shown before, gave **20**. Solid compounds were purified by recrystallization. The structure of the purified compounds was established by spectroscopic and spectrometric data.

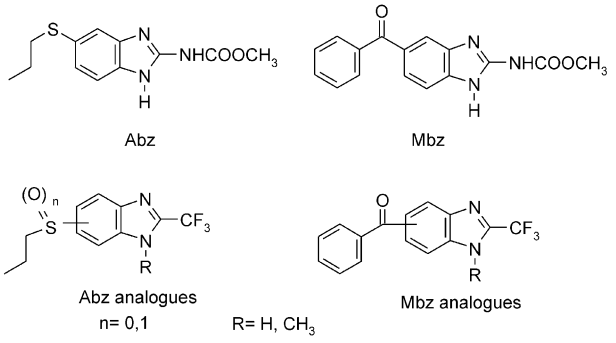
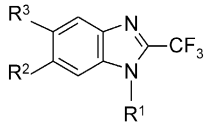


Figure 1. Benzimidazole anthelmintics albendazole and mebendazole and their analogues prepared in this work.

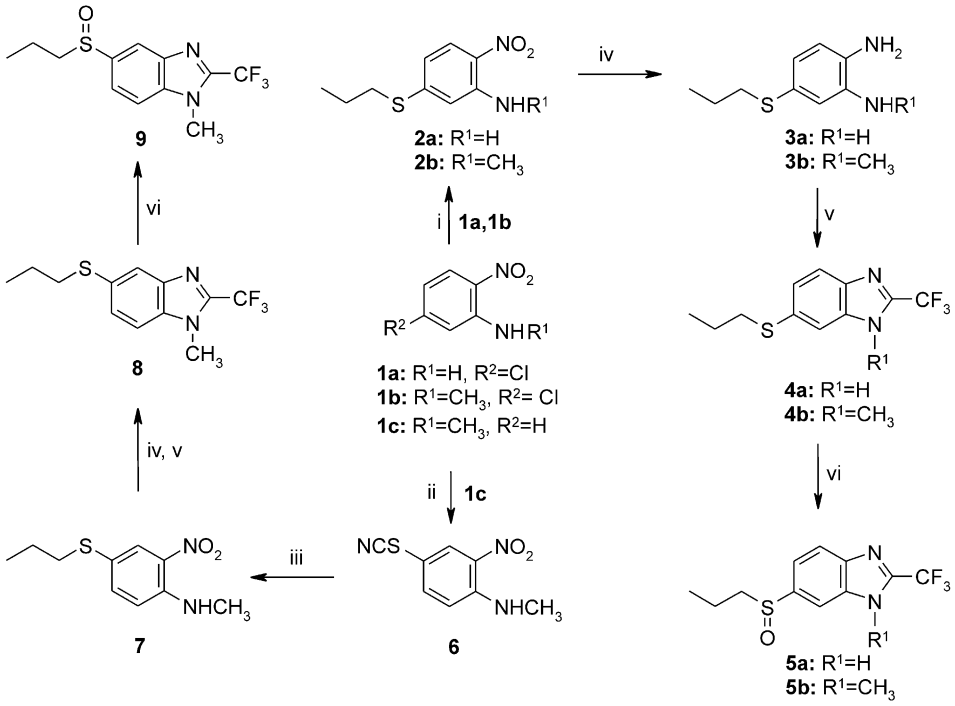
Results and Discussion

In this study, 9 new 2-(trifluoromethyl)benzimidazole derivatives, analogues of Abz and Mbz, have been synthesized

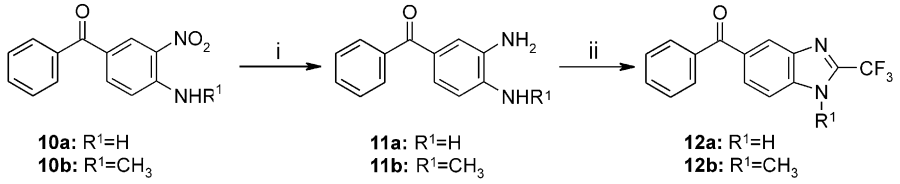
Table 1. Synthesized 2-(trifluoromethyl)benzimidazoles



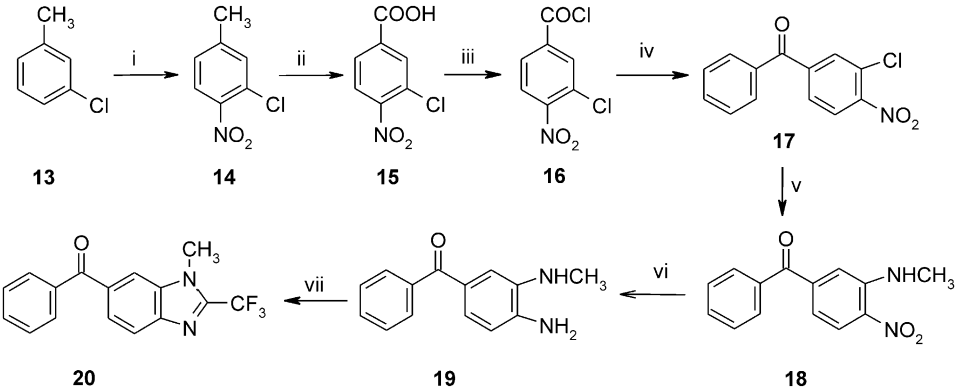
Compd	R^1	R^2	R^3
4a	H	Propylthio	H
4b	CH_3	Propylthio	H
5a	H	Propylsulfinyl	H
5b	CH_3	Propylsulfinyl	H
8	CH_3	H	Propylthio
9	CH_3	H	Propylsulfinyl
12a	H	H	Benzoyl
12b	CH_3	H	Benzoyl
20	CH_3	Benzoyl	H



Scheme 1. Reagents: (i) 1-PrSH, ethylene glycol, 120 °C; (ii) NH_4SCN, Br_2, MeOH; (iii) 1-PrBr, KCN, 1-PrOH, $(Bu)_4NCl$, CH_2Cl_2, reflux; (iv) H_2, Pd/C 10%, MeOH; (v) CF_3COOH, HCl, reflux; (vi) *m*-CPBA, $CHCl_3$, 5 °C.



Scheme 2. Reagents: (i) $SnCl_2{\cdot}2H_2O$, EtOH, reflux; (ii) CF_3COOH, HCl, reflux.



Scheme 3. Reagents: (i) H_2SO_4–HNO_3, 20 °C; (ii) $K_2Cr_2O_7$, H_2SO_4; (iii) $SOCl_2$, benzene; (iv) benzene, $AlCl_3$; (v) $CH_3NH_2{\cdot}HCl$, 1,2-dimethoxyethane, K_2CO_3, 130 °C; (vi) $SnCl_2{\cdot}2H_2O$, EtOH, reflux; (vii) CF_3COOH, HCl, reflux.

Table 2. In vitro susceptibility of *G. lamblia* and *T. vaginalis* to synthesized compounds, Metronidazole and Abz

Compd	*G. lamblia* IC_{50}, μM	*T. vaginalis* IC_{50}, μM
4a	1.515	0.345
4b	1.403	2.58
5a	6.37	2.78
5b	4.312	4.42
8	10.45	0.2
9	20.89	0.058
12a	2.31	28.44
12b	1.098	29.59
20	1.285	10.64
Metronidazole	1.22	0.2161
Abz	0.037	3.39

Table 3. Percentage of viability reduction of *T. spiralis* muscle larvae after 3 days of incubation with synthesized compounds and Abz[a]

Compd	0.037 μM	0.188 μM	0.37 μM	1.88 μM
Abz	57	58	61	67
4a	nr	18	27	38
4b	11	30	48	56
5a	10	18	30	45
5b	16	24	29	32
8	28	41	43	48
9	8	21	29	42
12a	27	33	36	45
12b	26	32	33	37
20	21	24	28	31

nr = no reduction observed.
[a]Values are means of three experiments.

and tested as antiprotozoal and anthelmintic agents. The main features of these compounds are the substitution of the 2-methylcarbamate group by a 2-trifluoromethyl group in order to enhance solubility and absorption properties and hopefully, antiparasitic activity; and the synthesis of regioisomeric 1-methylbenzimidazole derivatives in order to determine the importance of hydrogen at position 1 on the antiparasitic activity.

Biological assay results shown in Table 2, against *G. lamblia*, indicate that compounds with a hydrogen at position 1 (**4a**, **5a**, **12a**) were less active than Abz and Metronidazole. Compounds with a methyl group at position 1 (**4b**, **5b**, **8**, **9**, **12b**, **20**) were less active than Abz; however, regioisomeric 1-methyl Mbz analogues (**12b**, **20**) were as active as Metronidazole. Taking in consideration the pattern of substitution in Abz analogues, compounds **4b** and **5b**, with the pattern 1,2,6-trisubstituted were more active than **8** and **9**, with the pattern 1,2,5-trisubstituted; also, that those compounds with a propylthio group (**4a**, **4b**, **8**) were more active than their propylsulfinyl analogues (**5a**, **5b**, **9**).

In the other assay, against *T. vaginalis*, Abz analogues (**4a**, **5a**) with a hydrogen at position 1 were more active than Abz, where as Mbz analogue **12a** did not show significant activity. Although these compounds showed good activity, however, none of them were as active as Metronidazole. Another observation is that Abz analogues 1,2,5-trisubstituted (**8**, **9**) were more active than regioisomers 1,2,6-trisubstituted (**4b**, **5b**). Although Abz analogues with a propylthio group (**4a**, **4b**) were more active than those with a propylsulfinyl group (**5a**, **5b**), this fact was not observed with **8** and **9**. Interesting to note is that compound **9** was 4 and 58 times more active than Metronidazole and Abz, respectively.

The in vitro anthelmintic activity results against *T. spiralis* and *C. elegans* are shown in Tables 3 and 4,

Table 4. Percentage of viability reduction of *C. elegans* after 7 days of incubation with synthesized compounds and Mbz[a]

Compd	100 μM	10 μM	1 μM
Mbz	98	99	nr
4a	28	nr	nr
4b	62.4	nr	nr
5a	nr	nr	nr
5b	nr	nr	nr
8	62.2	nr	nr
9	nr	nr	nr
12a	nr	nr	nr
12b	nr	nr	nr
20	nr	nr	nr

[a]nr, no reduction observed.

Table 5. The antiprotozoal benzimidazoles have physical properties compatible with reasonable pharmacokinetics and drug availability

Compd	Mol wt	*C* log *P*	No. of H bond donors	No. of H bond acceptors	No. of criteria met
Rule	< 500	< 5	< 5	< 10	At least 3
4a	260	3.95	1	1	All
4b	274	4.26	0	1	All
5a	276	1.79	1	2	All
5b	290	2.1	0	2	All
8	274	4.26	0	1	All
9	290	2.1	0	2	All
12a	290	3.36	1	2	All
12b	304	3.67	0	2	All
20	304	3.67	0	2	All

respectively. In general, Abz and Mbz analogues showed modest activity, less than that of Abz. However, the pattern of substitution and the electronic properties of the substituent at position 5(6) play an important role. Thus, 1-methyl Abz analogue (**4b**) with an electron donating group at position 6 was more active than **8**, with the same group at position 5. The 1-H Abz analogue (**4a**) with an electron donating group at position 5(6) was the least active of all. On the other hand, compounds **5a**, **12a** with an electron withdrawing group at position 5(6) were more active than their corresponding 1-methyl analogues (**5b**, **9**, **12b**, **20**). None of the compounds showed appreciable activity against *C. elegans* under 100 μM concentrations.

These compounds are fully compatible with Lipiski's rule[18] (Table 5), which should allow for the development of additional antiprotozoal analogues. Their advantages include: (i) physical properties known to be compatible with desirable pharmacokinetic (low molecular weight, favorable *C* log *P*, favorable hydrogen bond donating and accepting capabilities), (ii) potency and efficacy, with IC_{50} values at the low micromolar level, (iii) simple synthetic access and thus low production costs, and (iv) non carbamated groups improving the likelihood of reasonable solubility. Further optimization and pharmacokinetic characterization of this series are ongoing.

Conclusion

The results obtained with the synthesized analogues as antiprotozoal agents are very promising indeed since they broaden the knowledge of the activity of these versatile derivatives of benzimidazole. The fact that some of the 1-methyl analogues were active against *G. lamblia* and *T. vaginalis* confirm what we found in our previous studies, viz. that the H at position 1 and the methylcarbamate group at position 2, of the benzimidazole ring, are not required for antiprotozoal activity; however, they are required for a good anthelmintic activity.[10,13] These findings also imply that the mechanism of action of these novel 2-(trifluoromethyl)benzimidazole derivatives is different from that of the benzimidazole 2-carbamates through inhibition of the polymerization of tubulin.

Experimental

Melting points were determined on a Büchi B-450 melting point apparatus and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F_{254} plates (E. Merck). ^{1}H NMR and ^{13}C NMR spectra were measured with a Varian EM-390 (300 and 75.5 MHz) spectrometer. Chemical shifts are given in ppm relative to tetramethylsilane (Me_4Si, $\delta = 0$) in $CDCl_3$; *J* values are given in Hz. The following abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; bs, broad signal. MS were recorded on a JEOL JMS-SX102A spectrometer by electron impact (EI). Catalytic hydrogenations were carried out in a Parr hydrogenation apparatus. Starting materials **1a–c** and **10b** were synthesized in our laboratory from the commercially available 3-chloroaniline, and 4-aminobenzophenone (Aldrich), respectively, via acetylation, nitration, methylation and hydrolysis of the corresponding *N*-Methyl-2-nitroacetanilide. *m*-Chloroperbenzoic acid (*m*-CPBA) 57–86% was from Aldrich. The *C* log *P* values were obtained using ACD/labs software v.4.5.

General method of synthesis of 2-(trifluoromethyl)-1*H*-benzimidazoles 4a, 4b, 8, 12a, 12b, 20

The appropriate 1,2-phenylenediamine (0.0313 mol), 1.6 equivalents of CF_3COOH and one drop of concentrated HCl were heated under reflux in a N_2 atmosphere for 3–4 h. TLC was used to monitor the reaction. The cooled mixture was neutralized with saturated $NaHCO_3$ solution, and the crude benzimidazole was extracted with AcOEt. The solvent was removed under high vacuum (1 mm Hg), and the resulting solid was isolated by filtration through a fritted 60-mL glass funnel packed with Al_2O_3, acid type.

5(6)-(Propylthio)-2-(trifluoromethyl)-1*H*-benzimidazole (4a). Eluted with $CHCl_3$ and recrystallized from cyclohexane-$CHCl_3$. Yield 6.92 g (85%) of white solid. Mp 102–105 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0 (t, 3H, $C\underline{H}_3CH_2$), 1.68 (m, 2H, $CH_3C\underline{H}_2CH_2$), 2.90 (t, 2H, $CH_2C\underline{H}_2S$), 7.42 (dd, 1H, H-6, $J = 8.55$, $J = 1.50$ Hz),

7.65 (d, 1H, H-7, $J=8.85$ Hz), 7.69 (d, 1H, H-4, $J=1.65$ Hz), 10.86 (bs, 1H, N-H) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.27 ($C\underline{H_3}CH_2$), 22.38 ($CH_3C\underline{H_2}CH_2$), 36.68 ($CH_2C\underline{H_2}S$), 116.53 (C-4), 116.92 (C-7), 118.72 (q, CF_3, $J=270.96$ Hz), 127.34 (C-6), 133.91 (C-5), 136.29 (C-7a), 137.58 (C-3a), 141.16 (q, C-2, $J=40.77$ Hz) ppm; MS: *m*/*z* (% rel. int.) 260 (M^+, 100), 231 (78), 218 (95), 187 (50); HRMS: calcd for $C_{11}H_{11}F_3N_2S$: 260.0595, found: 260.0595.

1-Methyl-6-(propylthio)-2-(trifluoromethyl)-1*H*-benzimidazole (4b). Eluted with $CHCl_3$ and recrystallized from cyclohexane. Yield 7.46 g (87%) of white solid. Mp 55–56.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, 3H, $C\underline{H_3}CH_2$), 1.71 (m, 2H, $CH_3C\underline{H_2}CH_2$), 2.97 (t, 2H, $CH_2C\underline{H_2}S$), 3.93 (s, 3H, *N*-CH_3), 7.39 (dd, 1H, H-5, $J=8.40$, $J=1.80$ Hz), 7.43 (d, 1H, H-7, $J=1.50$ Hz), 7.78 (d, 1H, H-4, $J=8.10$ Hz,) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.33 ($C\underline{H_3}CH_2$), 22.46 ($CH_3C\underline{H_2}CH_2$), 30.76 (*N*-CH_3), 36.78 ($CH_2C\underline{H_2}S$), 110.91 (C-7), 118.97 (q, CF_3, $J=271.49$ Hz), 121.63 (C-5), 125.88 (C-6), 134.34 (C-4), 136.47 (C-7a), 139.54 (C-3a), 140.75 (q, C-2, $J=38.70$ Hz) ppm; MS: *m*/*z* (% rel. int.) 274 (M^+, 100), 245 (20), 232 (75); HRMS: calcd for $C_{12}H_{13}F_3N_2S$: 274.0751, found: 274.0767.

1-Methyl-5-(propylthio)-2-(trifluoromethyl)-1*H*- benzimidazole (8). Eluted with hexane and recrystallized from cyclohexane-CH_2Cl_2. Yield 7.98 g (93%) of white solid. Mp 52.4–54.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (t, 3H, $C\underline{H_3}CH_2$), 1.65 (m, 2H, $CH_3C\underline{H_2}CH_2$), 2.92 (t, 2H, $CH_2C\underline{H_2}S$), 3.94 (s, H, *N*-CH_3), 7.36 (d, 1H, H-7, $J=8.7$ Hz), 7.47 (dd, 1H, H-6, $J=8.55$, $J=1.50$ Hz), 7.87 (d, 1H, H-4, $J=1.50$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.30 ($C\underline{H_3}CH_2$), 22.41 ($CH_3C\underline{H_2}CH_2$), 30.94 (*N*-CH_3), 36.93 ($CH_2C\underline{H_2}S$), 110.35 (C-7), 118.87 (q, CF_3, $J=271.42$ Hz), 122.26 (C-4), 128.31 (C-6), 132.09 (C-5), 134.60 (C-7a), 140.96 (q, C-2, $J=39.10$ Hz), 141.21 (C-3a) ppm; MS: *m*/*z* (% rel. int.) 274 (M^+, 98), 245 (30), 232 (100); HRMS: calcd for $C_{12}H_{13}F_3N_2S$: 274.0751, found: 274.0752.

5(6)-Benzoyl-2-(trifluoromethyl)-1*H*-benzimidazole (12a). Eluted with $CHCl_3$ and recrystallized from EtOH. Yield 6.53 g (72%) of white solid. Mp 55–58 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53–7.78 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 7.83 (dd, 1H, H-6, $J=8.40$, $J=1.20$ Hz), 7.85 (d, 1H, H-7, $J=8.40$ Hz), 8.05 (d, 1H, H-4, $J=1.20$ Hz), 10.40 (bs, 1H, N-H) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 113.52 (C-7), 114.66 (q, CF_3, $J=270.96$ Hz), 120.08 (C-4), 121.57 (C-6), 124.46 (C-2′, C-6′), 125.55 (C-3′, C-5′), 128.43 (C-4′), 128.85 (C-5, C-1′), 133.42 (C-3a, C-7a), 138.34 (q, C-2, $J=39.80$ Hz), 196.91 (C=O) ppm; MS: *m*/*z* (% rel. int.) 290 (M^+, 100), 213 (98), 185 (50), 105 (70); HRMS: calcd for $C_{15}H_9F_3N_2O$: 290.0666, found: 290.0690.

5-Benzoyl-1-methyl-2-(trifluoromethyl)-1*H*-benzimidazole (12b). Eluted with $CHCl_3$ and recrystallized from cyclohexane. Yield 7.61 g (80%) of white solid. Mp 103.1–104.2 °C. Mp 55–56.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.01 (s, 3H, *N*-CH_3), 7.49–7.54 (m, 2H, H-3′, H-5′), 7.60–7.66 (m, 3H, H-2′, H-4′, H-6′), 7.81 (dd, 1H, H-6, $J=8.40$, $J=1.50$ Hz), 7.94 (d, 1H, H-7, $J=8.40$ Hz), 8.01 (d, 1H, H-4, $J=0.90$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 31.14 (*N*-CH_3), 112.82 (C-7), 118.74 (q, CF_3, $J=272$ Hz), 121.10 (C-6), 125.79 (C-6), 128.36 (C-3′, C-5′), 130.01 (C-2′, C-6′), 132.53 (C-4), 134.77 (C-4′), 135.80 (C-5), 137.66 (C-1′, C-3a), 143.29 (q, C-2, $J=39.48$ Hz), 143.63 (C-7a), 196.14 (C=O) ppm; MS: *m*/*z* (% rel. int.) 304 (M^+, 90), 227 (100), 199 (20); HRMS: calcd for $C_{16}H_{11}F_3N_2O$: 304.0823, found: 304.0832.

6-Benzoyl-1-methyl-2-(trifluoromethyl)-1*H*-benzimidazole (20). Eluted with $CHCl_3$ and recrystallized from hexane. Yield 7.71 g (81%) of white solid. Mp 156.8–158.1 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.0 (s, 3H, *N*-CH_3), 7.46–7.63 (m, 4H, H-2′,H-3′, H-5′, H-6′), 7.79–7.82 (m, 2H, H-4, H-4′, $J=8.40$ Hz), 8.06 (dd, 1H, H-5, $J=8.70$, $J=1.80$ Hz), 8.29 (d, 1H, H-7, $J=0.90$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 31.15 (*N*-CH_3), 110.36 (C-7), 118.74 (q, CF_3, $J=271.80$ Hz), 125.15 (C-5), 127.02 (C-4), 128.28 (C-3′, C-5′), 129.98 (C-2′, C-6′), 132.37 (C-4′), 133.38 (C-1′), 137.680 (C-6), 138.66 (C-7a), 140.10 (C-3a), 142.60 (q, C-2, $J=39.10$ Hz), 196.01 (C=O) ppm; MS: *m*/*z* (% rel. int.) 304 (M^+, 70), 227 (100), 199 (15); HRMS: calcd for $C_{16}H_{11}F_3N_2O$: 304.0823, found: 304.0844.

General method of synthesis of propylsulfinyl derivatives 5a, 5b, 9

A stirred suspension of **4a**, **4b**, or **8** in $CHCl_3$ was treated, dropwise, with a solution of *m*-CPBA in $CHCl_3$ at 0–5 °C. The mixture was stirred at 5 °C for 30 min, neutralized with 50% $NaHCO_3$ solution, and the organic layer was eliminated in vacuo. The crude product was then purified.

5(6)-(Propylsulfinyl)-2-(trifluoromethyl)-1*H*-benzimidazole (5a). Following the general procedure described above, **4a** (3.5 g, 0.0134 mol) in 30 mL of $CHCl_3$ and *m*-CPBA (3.24 g, 0.0188 mol) in 20 mL of $CHCl_3$ gave **5a** (3.2 g, 0.0116 mol, 86%) as a white solid, after recrystallization from cyclohexane-toluene. Mp 123.2–125.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (t, 3H, $C\underline{H_3}CH_2$), 1.34–1.72 (m, 2H, $CH_3C\underline{H_2}CH_2$), 2.73–2.97 (m, 2H, $CH_2C\underline{H_2}SO$), 7.62 (dd, 1H, H-6, $J=8.40$, $J=1.50$ Hz), 7.89 (d, 1H, H-7, $J=8.40$ Hz), 8.01 (d, 1H, H-4, $J=0.90$ Hz), 13.84 (bs, 1H, N-H) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.88 ($C\underline{H_3}CH_2$), 15.18 ($CH_3C\underline{H_2}CH_2$), 57.71 ($CH_2C\underline{H_2}SO$), 113.43 (C-4), 117.38 (C-7), 118.81 (q, CF_3, $J=271.42$ Hz), 119.53 (C-6), 138.20 (C-3a), 139.12 (C-7a), 140.04 (C-5), 141.76 (q, C-2, $J=39.40$ Hz) ppm; MS: *m*/*z* (% rel. int.) 276 (M^+, 100), 260 (30); HRMS: calcd for $C_{11}H_{11}F_3N_2OS$: 276.0544, found: 276.0563.

1-Methyl-6-(propylsulfinyl)-2-(trifluoromethyl)-1*H*-benzimidazole (5b). Following the general procedure described above, **4b** (5 g, 0.0182 mol) in 42 mL of $CHCl_3$ and *m*-CPBA (4.1 g, 0.0241 mol) in 30 mL of $CHCl_3$ gave **5b** (4.8 g, 0.0165 mol, 90%) as a white solid, after recrystallization from cyclohexane–petroleum ether. Mp 90.7–91.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0 (t, 3H,

$C\underline{H}_3CH_2$), 1.56–1.77 (m, 2H, $CH_3C\underline{H}_2CH_2$), 2.72–2.97 (m, 2H, $CH_2C\underline{H}_2SO$), 4.14 (s, 3H, *N*-CH_3), 7.59 (dd, 1H, H-5, $J=8.70$, $J=1.50$ Hz), 7.93 (d, 1H, H-4, $J=8.70$ Hz), 8.05 (d, 1H, H-7, $J=1.80$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.34 ($C\underline{H}_3CH_2$), 16.33 ($CH_3C\underline{H}_2CH_2$), 31.74 (*N*-CH_3), 59.64 ($\underline{C}H_2C\underline{H}_2SO$), 108.64 (C-7), 119.44 (C-4), 120.0 (q, CF_3, $J=272.0$ Hz), 122.56 (C-5), 138.20 (C-7a), 141.12 (q, C-2, $J=39.20$ Hz), 143.35 (C-3a) ppm; MS: *m*/*z* (% rel. int.) 290 (M^+, 20), 273 (15), 248 (90), 200 (100); HRMS: calcd for $C_{12}H_{13}F_3N_2OS$: 290.0700, found: 290.0706.

1-Methyl-5-(propylsulfinyl)-2-(trifluoromethyl)-1*H*- benzimidazole (9). Following the general procedure described above, **8** (1.58 g, 0.0057 mol) in 15 mL of $CHCl_3$ and *m*-CPBA (1.37 g, 0.0079 mol) in 15 mL of $CHCl_3$ gave **9** (1.34 g, 0.0045 mol, 80%) as a white solid, after crystallization from cyclohexane–petroleum ether. Mp 130–132 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (t, 3H, $C\underline{H}_3CH_2$), 1.59–1.84 (m, 2H, $CH_3C\underline{H}_2CH_2$), 2.72–2.98 (m, 2H, $CH_2C\underline{H}_2SO$), 4.05 (s, 3H, *N*-CH_3), 7.65 (d, 1H, H-7, $J=8.70$ Hz), 7.82 (dd, 1H, H-6, $J=8.70$, $J=1.80$ Hz), 8.12 (d, 1H, H-4, $J=1.50$ Hz) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.21 ($C\underline{H}_3CH_2$), 15.89 ($CH_3C\underline{H}_2CH_2$), 31.18 (*N*-CH_3), 59.64 ($\underline{C}H_2C\underline{H}_2SO$), 111.49 (C-7), 118.47 (C-4), 119.03 (q, CF_3, $J=272.0$ Hz), 120.65 (C-6), 127.90 (C-3a), 139.40 (C-7a), 140.87 (C-5), 142.01 (q, C-2, $J=39.20$ Hz) ppm; MS: *m*/*z* (% rel. int.) 290 (M^+, 100), 273 (15), 248 (20), 200 (10); HRMS: calcd for $C_{12}H_{13}F_3N_2OS$: 290.0700, found: 290.0701.

2-Nitro-5-(propylthio)aniline (2a). A stirred mixture of **1a** (42.1 g, 0.244 mol), NaOH (12 g, 0.3000 mol), ethylene glycol (11.5 mL), H_2O (587 mL) and 1-PrSH (27.88 g, 0.2527 mol) was heated under reflux for 5 h. The mixture was cooled, filtered by suction and the crude product recrystallized from 2-PrOH. Yield 51 g (98.5%) of a yellow solid. Mp 69–71 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, 3H, $C\underline{H}_3CH_2$), 1.80 (m, 2H, $CH_3C\underline{H}_2CH_2$), 2.90 (t, 2H, $C\underline{H}_2C\underline{H}_2S$), 6.10 (bs, 2H, NH_2), 6.50 (m, 2H, H-4, H-6, $J=9.0$, $J=1.50$, $J=1.70$ Hz), 7.90 (dd, 1H, H-3, $J=9.0$ Hz) ppm; MS: *m*/*z* (% rel. int.) 212 (M^+, 100).

***N*-Methyl-2-nitro-5-(propylthio)aniline (2b).** A stirred mixture of 5-chloro-*N*-methyl-2-nitroaniline (12.1 g (0.0648 mol), KOH (4.55 g, 0.0810 mol), ethylene glycol (33 mL), H_2O (150 mL) and 1-PrSH (5.68 g, 0.0745 mol) was heated at 115 °C for 4 h. The mixture was cooled, filtered by suction and the crude product recrystallized from hexane. Yield 14.2 g (96.8%) of orange crystals. Mp 61–62 °C. ^1H NMR (300 MHz, DMSO-d_6). δ 0.99 (t, 3H, $C\underline{H}_3CH_2$), 1.65 (m, 2H, $CH_3CH_2CH_2$), 2.91 (s, 3H, *N*-CH_3), 3.10 (t, 2H, $CH_2C\underline{H}_2S$), 6.50 (dd, 1H, H-3, $J=9.0$, $J=2.0$ Hz), 6.69 (dd, 1H, H-6, $J=2.0$ Hz), 7.90 (d, 1H, H-4, $J=9.0$ Hz), 8.20 (bs, 1H, N-H) ppm; MS: *m*/*z* (% rel. int.) 226 (M^+, 100).

4-(Propylthio)-1,2-phenylenediamine (3a). A mixture of **2a** (6.37 g, 0.0282 mol), EtOH (100 mL) and 10% Pd/C (300 mg) was hydrogenated at 25 °C until cessation of H_2 uptake. The reaction mixture was filtered off on a Whatman paper No. 2, washed with EtOH, and the filtrate concentrated to provide a dark violet-colored liquid, which was used immediately in a subsequent step without purification.

***N*^{2}-Methyl-4-(propylthio)-1,2-phenylenediamine (3b).** A mixture of **2b** (14.2 g, 0.0627 mol), MeOH (120 mL) and 10% Pd/C (700 mg) was hydrogenated at 40 °C until cessation of H_2 uptake. The reaction mixture was filtered off on a Whatman paper No. 2, washed with EtOH, and the filtrate concentrated to provide a dark violet-colored liquid, which was used immediately in a subsequent step without purification.

***N*-Methyl-2-nitro-4-thiocyanoaniline (6).** To a stirred, cooled (3–5 °C) solution of *N*-methyl-2-nitroaniline (12 g, 0.0788 mol) and NH_4SCN (14.9 g, 0.1891 mol) in 80 mL of MeOH was added, droppingly, a solution of Br_2 (13.88 g, 0.0866 mol) in MeOH (16 mL) previously saturated with NaBr. The reaction mixture was stirred for one more h at 5 °C, and then, poured in 200 g of ice-water. Stirring was continued in the hood until the formation of a precipitate. After work up by filtration, the crude product was recrystallized from toluene. Yield 14.81 g (90%) of orange crystals. Mp 109–111 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 3.03 (s, 3H, *N*-CH_3), 6.77 (d, 1H, H-6, $J=6.0$ Hz), 7.48 (dd, 1H, H-5, $J=6.0$ Hz, $J=2.0$ Hz), 8.24 (d, 1H, H-3, $J=2.0$ Hz) ppm; MS: *m*/*z* (% rel. int.) 209 (M^+, 100), 192 (4), 183 (4).

***N*-Methyl-2-nitro-4-(propylthio)aniline (7).** A mixture of **6** (6 g, 0.0287 mol), 1-PrBr (23 g, 0.187 mol), CH_2Cl_2 (60 mL), 1-PrOH (11.16 g), KCN (9 g, 0.1836 mol), $(Bu)_4NCl$ (1 g) and H_2O (27 mL) was heated under reflux for 4 h in a N_2 atmosphere. Workup by extraction with EtOAc and concentration under vacuum left a red oil, which was purified by column chromatography (4×60 cm, 60 g of silica gel, petroleum ether). Yield 6.23g (93%) of red oil. This oil was reduced exactly as **2a**, **2b**. ^1H NMR (300 MHz, DMSO-d_6) δ 0.95 (t, 3H, $C\underline{H}_3CH_2$), 1.53 (m, 2H, $CH_3C\underline{H}_2CH_2$), 2.72 (t, 2H, $C\underline{H}_2C\underline{H}_2S$), 3.03 (s, 3H, *N*-$CH_3$), 6.45 (d, 1H, H-6, $J=6.0$ Hz), 7.30 (dd, 1H, H-5, $J=6.0$, $J=2.0$ Hz), 7.95 (d, 1H, H-3, $J=2.0$ Hz) ppm; MS: *m*/*z* (% rel. int.) 226 (M^+, 100), 197 (10), 183 (33), 151 (12).

3-Amino-4-(methylamino)benzophenone (11b). A mixture of **10b** (9.6 g, 0.0395 mol), $SnCl_2{\cdot}2H_2O$ (53.47 g, 0.2370 mol) and 150 mL of ethanol was stirred at 75° C for 3.5 h under a N_2 atmosphere. After cooling, the mixture was basified (pH = 9–10) with a 50% NaOH solution and then filtered by suction. The solvent was carefully removed in vacuo and the solid residue was extracted with AcOEt (3×50 mL). The combined organic extracts were washed with brine, dried with anhydrous Na_2SO_4 and concentrated in vacuo to give 7.62 g (86%) of **11b** as an orange solid, which was immediately ciclocondensed in the next step. An analytical sample was obtained by recrystallization from EtOH to give orange crystals. Mp 129–131 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 2.85 (s, 3H, *N*-CH_3), 3.40 (s, 3H, N–H, NH_2), 6.50 (m, 1H, H-2), 6.91-7.56 (m, 6H, H-5, H-2′, H-3′, H-4′,

H5′, H-6′), 7.72 (m, 1H, H-6) ppm; MS: *m*/*z* (% rel. int.) 226 (M^+, 100), 211 (27), 149 (58).

3-Chloro-4-nitrobenzoic acid (15). To 3-chlorotoluene (100 g, 0.7900 mol) was added a cold (0–10 °C) mixture of H_2SO_4–HNO_3 (95-79) at 15–22 °C. After the addition, the organic layer was separated, washed with brine and dried with $CaCl_2$ to give a yellow oil (126.48 g) as a mixture of three compounds. To a stirred suspension of this mixture (120 g, 0.699 mol) and $K_2Cr_2O_7$ (236.2 g, 0.9480 mol) was carefully added H_2SO_4 (520 mL). The hot reaction mixture was heated under reflux for 2 h and then cooled with 1000 mL of water. The mixture was filtered and the residue washed with 5% solution of H_2SO_4, water, and filtered to afford 58 g (40%) of yellow-green solid. Mp 181–183.4 °C. An analytical sample was obtained by recrystallization from $CHCl_3$ to give white crystals, Mp 183–184 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, 1H, H-2, J = 1.50 Hz), 8.15 (d, 1H, H-5, J = 8.50 Hz), 8.18 (dd, 1H, H-6, J = 9.0, J = 1.70 Hz), 14.0 (s, 1H, O–H) ppm; MS: *m*/*z* (% rel. int.) 201 (M^+, 100), 171 (38), 143 (24).

3-Chloro-4-nitrobenzoyl chloride (16). A mixture of **15** (50 g, 0.248 mol) and thionyl chloride (87.8 g, 0.744 mol) was heated in a dry atmosphere until gas evolution was ceased. The excess of thionyl chloride was distilled from the reaction and the residue was used immediately for the next step.

3-Chloro-4-nitrobenzophenone (17). A stirred mixture of **16** (64 g, 0.248 mol), benzene (130 mL) and $AlCl_3$ (45 g, 0.3383 mol) was heated at 80 °C, under a N_2 atmosphere, for 3 h. The cooled mixture was poured in ice-water (500 g). After work up by filtration and extraction with EtOAc, the crude product was recrystallized from ethanol. Yield 52.3 g (80.7%) of a light brown solid. Mp 97.5–98.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.57 (m, 2H, H-3′, H-5′), 7.64–7.70 (m, 1H, H-4′), 7.77–7.81 (m, 3H, H-2′, H-6′, H-6), 7.94 (d, 1H, H-2, J = 2.10 Hz), 7.95 (d, 1H, H-5, J = 6.30 Hz) ppm; MS: *m*/*z* (% rel. int.) 261 (M^+, 78), 105 (100), 77 (44).

3-(Methylamino)-4-nitrobenzophenone (18). A stirred mixture of **17** (10 g, 0.0388 mol), 1,2-dimethoxyethane (150 mL), CH_3NH_2·HCl (7.74 g, 0.1147 mol) and K_2CO_3 (15.82 g, 0.1147 mol) in H_2O (8 mL) was heated for 6 h at 130° C in a Parr reactor. Then, the cooled reaction mixture was poured over H_2O (500 mL). The orange precipitate was filtered off and recrystallized from ethanol. Yield 8.6 g (88.6%) of an orange solid. Mp 121.2–122.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.0 (s, 3H, *N*-CH_3), 6.92 (dd, 1H, H-6, J = 8.70, J = 1.80 Hz), 7.23 (d, 1H, H-2, J = 1.50 Hz), 7.48–7.53 (m, 3H, H-2′, H-4′, H-6′), 7.82–7.85 (m, 2H, H-3′, H-5′), 8.07 (s, 1H, N-H), 8.25 (d, 1H, H-5, J = 8.70 Hz) ppm; MS: *m*/*z* (% rel. int.) 256 (M^+, 100), 161 (35), 105 (70).

4-Amino-3-(methylamino)benzophenone (19). A mixture of **18** (6 g, 0.0234 mol), $SnCl_2$·$2H_2O$ (31.7 g, 0.1406 mol) and EtOH (120 mL) was stirred at 75° C for 4 h under a N_2 atmosphere. After cooling, the mixture was basified (pH = 9–10) with a 50% NaOH solution and then filtered by suction. The solvent was removed carefully in vacuo, and the solid residue was extracted with AcOEt (3×50 mL). The combined organic extracts were washed with brine, dried with anhydrous Na_2SO_4 and concentrated in vacuo. The yellow residue was recrystallized from benzene. Yield 4.3 g (81.3%) of **19** as yellow crystals, which were immediately ciclocondensed in the next step. Mp 118.7–120.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.71 (s, 3H, *N*-CH_3), 4.92 (s, 1H, N-H), 5.52 (s, 2H, NH_2), 6.55 (d, 1H, H-5, J = 8.70 Hz), 6.88 (m, 2H, H-2, H-6, J = 8.70, J = 1.50, J = 1.80 Hz), 7.43–7.62 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′) ppm; MS: *m*/*z* (% rel. int.) 226 (M^+, 100), 211 (27), 149 (81).

Biological assays

Culture. *G. lamblia* strain IMSS:0989:1 and *Trichomonas vaginalis* strain GT3 were cultured in TYI-S-33 modified medium, supplemented with 10% calf serum and bovine bile.[1] In vitro susceptibility assays were performed using a method previously described.[1] Briefly: 4×10^4 trophozoites of *G. lamblia* or *T. vaginalis* were incubated for 48 h at 37 °C with increasing concentrations of synthesized compounds, Abz, and Metronidazole. As the negative control, trophozoites were incubated with dimethylsulphoxide (DMSO) used in the experiments. After the incubation, the trophozoites were washed and subcultured for another 48 h in fresh medium alone. At the end of this period, trophozoites were counted and the 50% inhibitory concentration (IC_{50}) was calculated by Probit analysis. Experiments were carried out in triplicate and repeated at least twice.

Trichinella spiralis muscle larvae (parenteral phase) were obtained according to the procedure of Dennis et al.[19] For the assay, 1000 larvae were placed in culture plates of 24 wells (Nunclon), which contained RPMI 1640 medium, with 0.037, 0.188, 0.37, 1.88 μM of the compounds tested. The parasites were then incubated in a humid 5% CO_2 atmosphere at 37 °C for 3 days, changing the medium and the compounds each day. Abz was used in this test as a positive control, and the solvent employed, as a negative control. After the incubation, the viability of the parasites was determined by the colorimetric method described by Townson et al.,[20] with some modifications.[21]

C. elegans assay was performed following the method of Simpkin and Coles[22] with slight modifications. Tests were carried out in 24-well plates and four wells were used for each experimental group. To each well, 1.0 mL of culture medium (with 9 mg/mL chloramphenicol instead of ampicillin) was added followed by 7.5 μL of the appropriate compound solution or solvent (DMSO). Finally, 0.5 mL of culture medium containing 10–15 *C. elegans* larvae (L2 or L3 obtained of synchronous cultures) was added to each well. The effect of compounds on the development and reproductive capacity of *C. elegans* was determined by comparing the population levels attained in the control and test wells after an incubation period of 7 days at 20 ± 1 °C. Mebendazole was used in this test as positive control.

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