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Original article

Synthesis and biological activity of 2-(trifluoromethyl)-1*H*-benzimidazole derivatives against some protozoa and *Trichinella spiralis*

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ABSTRACT

A series of 2-(trifluoromethyl)-1*H*-benzimidazole derivatives (**1a**–**1i**) were synthesized via Phillips cyclocondensation of a substituted 1,2-phenylenediamine and trifluoroacetic acid. The synthesized compounds were evaluated *in vitro* against various protozoan parasites: *Giardia intestinalis*, *Entamoeba histolytica*, *Trichomonas vaginalis* and *Leishmania mexicana*, and they showed nanomolar activities against the first three protozoa tested. The compounds were also tested *in vitro* and *in vivo* against the nematode *Trichinella spiralis*. Compounds **1b**, **1c** and **1e** had the most desirable *in vitro* antiparasitic profile against all parasites studied. In the *in vivo* model against *T. spiralis*, compounds **1b** and **1e** showed good activity against the adult phase at 75 mg/Kg. However, against the muscle larvae stage, only compound **1f** exhibited *in vivo* antiparasitic efficacy.

1. Introduction

Parasitic diseases are still worldwide problems that have a deep impact on public health. Infections caused by protozoa such as *Trypanosoma cruzi*, *Leishmania mexicana*, *Plasmodium falciparum*, *Giardia intestinalis*, *Entamoeba histolytica* or *Trichomonas vaginalis*, and helminths such as *Trichinella spiralis* or *Taenia solium* are worldwide spread diseases that affect mainly underdeveloped countries, where not only tropical or template temperatures prevail, but also poor sanitary and hygiene conditions are common [1–3].

In spite of the progress made during the last fifty years, in the understanding of the biochemistry of the parasites and the development of new antiparasitic agents against these diseases, the availability of adequate chemotherapies remains unsolved. The increasing resistance of *Plasmodium* to antimalarics and the lack of safe drugs against trypanosomiosis and leishmaniosis promote an ongoing drug research effort to identify mechanistically novel, nontoxic, cost effective chemotherapies in the treatment of these sweeping public health problems [4].

The anthelmintic drugs derived from benzimidazole 2-carbamates, such as albendazole (**ABZ**) and mebendazole (**MBZ**), are used mainly to treat endoparasitic diseases in domestic animals and humans. These types of compounds are characterized by a high therapeutic index and low toxicity; however, they find little use in tissue-dwelling parasites mainly due to poor solubility and absorption problems [5].

In order to identify the 2-(trifluoromethyl)-1*H*-benzimidazole system as a promising scaffold for the development of new antiparasitic agents, we have previously synthesized and tested two series of derivatives of this compound [6,7] and a third one that included **ABZ** and **MBZ** analogues [8]. In the last series some derivatives showed *in vitro* activity against *G. intestinalis*, *T. vaginalis* and *T. spiralis*, although less than **ABZ**. In view of the previous results and continuing with our efforts to obtain more information about the antiparasitic potential of the 2-(trifluoromethyl)-1*H*-benzimidazole system we hereby report the synthesis and evaluation of a series of seven new compounds (**1a**–**1g**, Table 1) as direct analogues of two fasciolicide drugs: triclabendazole (**TBZ**) (**1a**–**1d**) and **Alpha** (**1e**–**1g**) [9]. In addition, two analogues (**1h**, **1i**) of an antiprotozoal lead, 5,6-dichloro-2-(methylthio)-1*H*-benzimidazole, reported previously by our group (Fig. 1) [10] were evaluated. Derivatives **1a**–**1i** bear a trifluoromethyl group attached at position 2 of the benzimidazole nucleus instead of a methylthio group.

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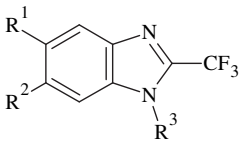
Table 1
Benzimidazole derivatives synthesized.



Compounds	R^1	R^2	R^3	MW	M.p (°C)
1a	2,3-$Cl_2C_6H_3O$[a]	H	H	347.119	172–174
1b	2,3-$Cl_2C_6H_3O$[a]	H	CH_3	361.145	137–139
1c	H	2,3-$Cl_2C_6H_3O$[a]	CH_3	361.145	100–101
1d	2,3-$Cl_2C_6H_3O$[a]	Cl	H	381.564	211–212
1e	$C_{10}H_7O$[b]	Cl	H	362.733	110–112
1f	$C_{10}H_7O$[b]	Cl	CH_3	376.759	98–99
1g	Cl	$C_{10}H_7O$[b]	CH_3	376.759	100–101
1h	Cl	Cl	H	255.024	236–238
1i	Cl	H	CH_3	234.605	108–109

[a] 2,3-dichlorophenoxy.
[b] 1-naphthyloxy.

The introduction of a trifluoromethyl group in bioactive compounds often improves their pharmacodynamic and pharmacokinetic properties. In the former, this group has significant effects on the binding affinity in drug–receptor complexes; meanwhile in the latter it increases membrane permeability and stability against metabolic oxidation [11]. In addition to the 2-trifluoromethyl group, and in order to assess the influence of a hydrogen atom at position 1 of the benzimidazole nucleus on antiparasitic activity, analogues **1b**, **1c** and **1f**, **1g** were designed as 1-methyl regioisomers, respectively.

Furthermore, the antiparasitic activity of compounds **1a**–**1i** was evaluated against protozoa *G. intestinalis*, *E. histolytica*, *T. vaginalis*, *L. mexicana* and against the nematode *T. spiralis*.

2. Chemistry

The synthetic procedures for the preparation of target compounds (**1a**–**1g**) are outlined in Schemes 1 and 2 and were carried out by well-known methods reported before [6,8,9]. As shown in Scheme 1, commercial 1-fluoro-4-nitrobenzene (**2**) reacted with 2,3-dichlorophenol to give 1,2-dichloro-3-(4-nitrophenoxy)benzene (**3**); this was reduced with H_2 and Raney-Nickel, treated with Ac_2O and then subjected to nitration to give *N*-[4-(2,3-dichlorophenoxy)-2-nitrophenyl]acetamide (**4**). N-methylation of **4** with dimethyl sulfate and KOH afforded *N*-[4-(2,3-dichlorophenoxy)-2-nitrophenyl]-*N*-methylacetamide (**6**). The hydrolysis of **4** and **6**, followed by reduction with H_2 and Raney-Nickel in ethanol, gave the corresponding 1,2-phenylenediamine (**5**, **7**). These intermediates were treated with CF_3CO_2H and HCl as a catalyst to furnish **1a** and **1b**, respectively. For the synthesis of compounds **1c**–**1g** the reactions shown in Scheme 2 were followed. The nucleophilic substitution of the chlorine atom in **8a**, **8b** and **11** by 2,3-dichlorophenol or 1-naphtol followed by catalytic reduction led to **10a**, **10b** and **13a**, **13b**, respectively. The cyclocondensation of these intermediates with CF_3CO_2H, as described above, gave title compounds **1c**–**1e**, and **1g**. Compound **1e** was treated with CH_3I and KOH to furnish a mixture of regioisomers from which **1f** was isolated by silica gel column chromatography. Compounds **1h**, **1i**, **8a**, **8b**, **9b**, **11**, **12b**, **TBZ** and **Alpha** were prepared in our laboratory by known procedures [6,9]. The structure of the new compounds was established from the spectroscopic and spectrometric data. **ABZ**, pentamidine isethionate salt and metronidazole (**MTZ**) were purchased from Sigma–Aldrich.

3. Antiprotozoal activity

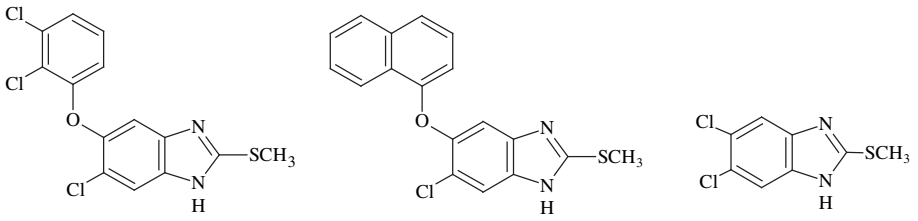
Compounds **1a**–**1i** were evaluated *in vitro* against *G. intestinalis*, *E. histolytica*, *T. vaginalis* and *L. mexicana*.

E. histolytica strain HM1-IMSS and *T. vaginalis* strain GT3 were cultured in a TYI-S-33 modified medium supplemented with 10% calf serum. *G. intestinalis* strain IMSS:0989:1 was maintained in a TYI-S-33 medium supplemented with 10% calf serum and bovine bile. *In vitro* susceptibility assays were performed using a method previously described [12]. Briefly: 5×10^4 trophozoites of *G. intestinalis* or *T. vaginalis* or 6×10^3 trophozoites of *E. histolytica* were incubated for 48 h at 37 °C with increasing concentrations of test compounds. **ABZ** and **MTZ** were used as standard drugs, and dimethyl sulfoxide (DMSO) was used as a suitable solvent and negative control. After incubation, the trophozoites were washed and subcultured for another 48 h in a fresh medium alone. At the end of this period, the 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.

The leishmanicidal activity was performed against *L. mexicana* promastigotes strain MHOM/MX92/UAY68. Parasites were cultured at 26 °C in an RPMI 1640 medium supplemented with 10% fetal bovine serum, 293 mg/L glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin. Promastigotes (2×10^4 parasites/mL) were subcultured every 72 h. To test the effect of the compounds on *Leishmania* growth, 2×10^4 promastigotes/mL were cultured in media containing final concentrations of 0.1, 0.5, 1.5, 10 and 20 μg/mL of each compound for 12, 24 and 48 h. DMSO was used as a negative control and pentamidine was used as a standard drug. The growth inhibition assays were performed in triplicate for each individual concentration. Parasites were fixed in 0.14 M NaCl containing 3.7% formaldehyde and counted under a light microscope in a Neubauer hematocytometer. At 48 h, the leishmanicidal effect of compounds **1a**–**1i** was expressed as IC_{50}.

4. Antitrichinellosis activity

Compounds **1a**–**1i** were evaluated *in vitro* against nematode *T. spiralis* muscle larvae (ML) (systemic phase). **ABZ** was used as a standard drug and DMSO as a suitable solvent and negative

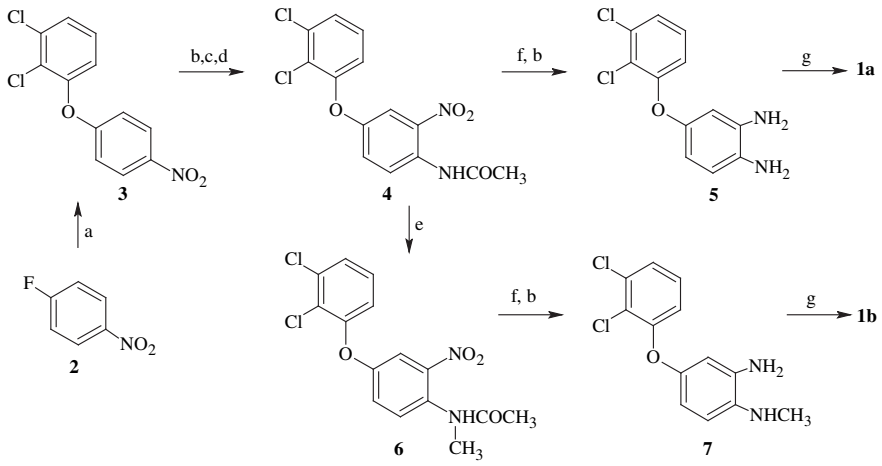


triclabendazole (**TBZ**)

6-chloro-2-(methylthio)-5-(1-naphthyloxy)-1*H*-benzimidazole (**Alpha**)

5,6-dichloro-2-(methylthio)-1*H*-benzimidazole

Fig. 1. Structures of triclabendazole, **Alpha** and 5,6-dichloro-2-(methylthio)-1*H*-benzimidazole.



Scheme 1. Reagents and conditions: (a) K_2CO_3, 2,3-$Cl_2C_6H_3OH$, DMF/H_2O; (b) H_2, Raney-Nickel; (c) Ac_2O; (d) HNO_3/AcO_2; (e) KOH, dimethyl sulfate, dimethoxyethane; (f) KOH, EtOH, heat; (g) CF_3CO_2H, HCl (cat), heat.

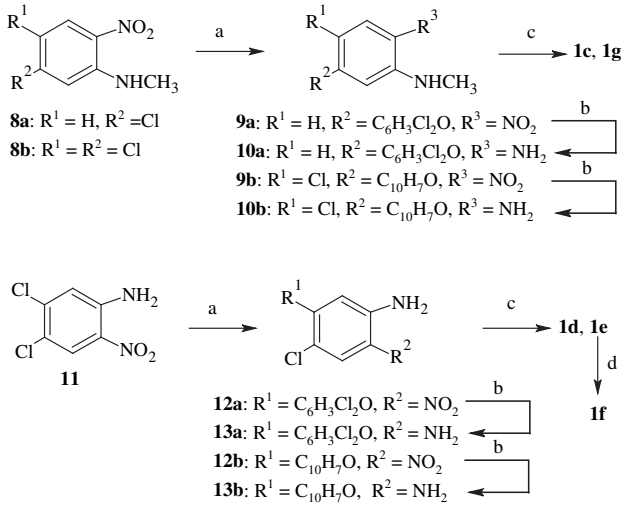
control. *T. spiralis* ML were obtained according to the procedure of Dennis et al. [13]. For the assay, 1000 larvae were placed on culture plates of 24 wells (Nunclon) which contained RPMI 1640 medium with 0.18, 0.37 and 1.80 μM of the test compounds. The parasites were then incubated in a humid 5% CO_2 atmosphere at 37 °C for 3 days, the medium and the compounds were changed each day. After incubation, the viability of the parasites was determined by the MTT colorimetric method described by Comley et al. [14].

Selected compounds were tested *in vivo* against the intestinal (adult worm) and systemic stage (ML) of *T. spiralis* at 75 mg/Kg. At the intestinal level, some compounds were also tested at 50 mg/Kg (Table 3). Biological assays were performed as previously described [15]. **ABZ**, **TBZ** and **Alpha** were used as standard drugs. Animal experiments were performed according to Norma Oficial Mexicana (NOM-062-Z00-1999) published on August 22, 2009.

5. Results and discussion

5.1. Antiprotozoal activity

Derivatives **1a**–**1i** showed amebicidal activity *in vitro* in the nanomolar range (Table 2). These compounds were more active than **ABZ** against *E. histolytica* and *T. vaginalis*. Furthermore, they were more active than **MTZ** against *E. histolytica*; however, only **1b**, **1c** and **1e** were more active than **MTZ** against *T. vaginalis*. In particular, **1b** emerged as the most active derivative against both parasites, being 39 and 13 times more active than **MTZ** with IC_{50} values of 9 nM and 16 nM, respectively. Concerning *G. intestinalis*, all the tested compounds were more active than **MTZ**, among which, compounds **1e**–**1g** showed better activity than **ABZ**. It seems that one important factor contributing to the high giardicidal activity shown, is the 1-naphthyloxy group at position 5 or 6 of the benzimidazole nucleus, implying that an increase of the lipophilicity (Clog $P > 6.0$) of the 2-(trifluoromethyl)-1*H*-benzimidazole system leads to a lower order of IC_{50}. Compounds **1b** and **1d** were as active as **ABZ** with IC_{50} values of 30 and 31 nM, respectively.



Scheme 2. Reagents and conditions: (a) K_2CO_3, 2,3-$Cl_2C_6H_3OH$, DMF/H_2O, or K_2CO_3, 1-naphtol, DMF; (b) H_2, Raney-Nickel; (c) CF_3CO_2H, HCl (cat), heat; (d) CH_3I, KOH, acetone.

When derivatives **1a**–**1i** were evaluated against *L. mexicana* promastigotes, compounds **1b**, **1c**, **1e** and **1h** showed IC_{50} values <25 μM, while **1g** showed moderate activity (Table 2). The remainder of the compounds (**1a**, **1d**, **1f**, **1i**) showed no appreciable activity. Compounds **1c** and **1e** were the most effective (IC_{50} = 4.10 μM and 13.78 μM, respectively); nevertheless, they showed lower leishmanicidal activity than pentamidine (CI_{50} = 2.42 μM). Data presented in Fig. 2 show the dose-dependent effect of compounds **1b**, **1c**, **1e** and **1h** on *L. mexicana* promastigotes growth. In general, the compounds significantly affected the viability of the parasite at 10 and 20 μg/mL. At these concentrations, the leishmanicidal activity of compounds **1c**, **1e** and **1h** was similar at 48 h. Among regioisomers **1b** and **1c** the activity profile was similar between 12 and 24 h; however, at 48 h they showed important differences, being **1c** the most active compound. This behavior may derive from the ability of these compounds to interact with its receptor and not due to their ability to enter the parasite since their lipophilicity is the same (Clog P = 5.67) (Table 2). The different activity of compounds **1b** and **1c** is reflected in the IC_{50} value (24 μM vs 4.10 μM, respectively). It is worth noticing that the leishmanicidal activity depended on the presence of the 2,3-diclorophenoxy group at position 6 since compound **1c** showed 6 times higher leishmanicidal activity than compound **1b**. In addition, the leishmanicidal activity of compound **1a**, with no methyl at position 1, compared with that of compounds **1b** and **1c**, both with a methyl group at position 1, indicates that the methyl group is critical for antiprotozoal activity. This finding is similar to results previously obtained against *G. intestinalis*, *E. histolytica* and *T. vaginalis* with other benzimidazole regioisomers derivatives [6,8]. Nonetheless, in this study a different behavior was shown by compounds **1e**–**1g**. The leishmanicidal activity of compound **1e**

Table 2
In vitro antiprotozoal and anthelmintic activity of benzimidazole derivatives.

Compounds	Clog *P*[a]	*G. intestinalis* (IC_{50}, μM)	*E. histolytica* (IC_{50}, μM)	*T. vaginalis* (IC_{50}, μM)	*L. mexicana* (IC_{50}, μM)	*T. spiralis* (% reduction, 0.18 μM)	*T. spiralis* (% reduction, 0.37 μM)	*T. spiralis* (% reduction, 1.80 μM)
1a	5.36	0.054	0.018	0.535	NE[b]	20 ± 3	24 ± 4	29 ± 5
1b	5.67	0.030	0.009	0.016	24.00	54 ± 2	62 ± 2	80 ± 3
1c	5.67	0.063	0.019	0.110	4.10	44 ± 2	48 ± 3	67 ± 2
1d	5.88	0.031	0.020	ND[c]	NE[b]	NE[b]	NE[b]	NE[b]
1e	6.14	0.005	0.019	0.086	13.78	43 ± 3	50 ± 2	65 ± 3
1f	6.31	0.023	0.038	0.970	65.23	15 ± 2	29 ± 1	63 ± 3
1g	6.31	0.012	0.017	0.451	50.12	6 ± 2	14 ± 3	18 ± 3
1h	3.75	0.078	0.011	0.235	20.80	NE[b]	NE[b]	19 ± 2
1i	3.30	0.042	0.046	0.238	NE[b]	NE[b]	NE[b]	39 ± 3
ABZ	3.07	0.037	56.600	1.592	NE[b]	58.6 ± 2	61.9 ± 3	67 ± 6
MTZ	−0.02	1.228	0.350	0.216	ND[c]	ND[c]	ND[c]	ND[c]
pentamidine	2.47	ND[c]	ND[c]	ND[c]	2.421	ND[c]	ND[c]	ND[c]

[a] Calculated from ACD/Labs software v.9.0 (Advanced Chemistry Development, Inc.).
[b] NE: no effect.
[c] ND: not determined.

was higher than regioisomers **1f** and **1g**, indicating that when the substituent is 1-naphthyloxy at 5 or 6, the presence of the methyl group at position 1 decreases the antiprotozoal activity.

5.2. Antitrichinellosis activity

The *in vitro* activity of **1a**–**1i** against *T. spiralis* ML is presented in Table 2. Compound **1b** was the most effective nematocidal agent, reducing the parasite metabolic activity by 54% and 62% at the lowest concentrations used (0.18 μM and 0.37 μM), compared with 58% and 61.9% shown by **ABZ** at the same concentrations. Furthermore, **1b** reduced the metabolic activity of *T. spiralis* ML by 80%, when used at 1.80 μM, being more active than **ABZ** (67%). The activity of compounds **1c**, **1e** and **1f** increased with the concentration; in particular, the nematocidal activity of compound **1f** increased from 15% at 0.18 μM–63% at 1.80 μM, while **1a**, **1d**, **1g**, **1h** and **1i** exhibited slight or no activity even at the highest concentration. Interestingly, the gain in potency of **TBZ** analogues **1b** and **1c** versus **1a** is related to the introduction of a methyl group at position 1. The opposite effect, however, was observed in the case of **Alpha** analogues **1f** and **1g** versus **1e**. Regarding regioisomers, **1b**–**1c** or **1f**–**1g**, their antiparasitic profile was related to the relative position of the substituent and the methyl group: the 2,3-dichlorophenoxy group or 1-naphthyloxy group at position 5 led to more active compounds (**1b**, **1f**) than those with the substituent at position 6 (**1c**, **1g**) respectively.

It is well known that the lipophilicity of a drug, determined as Clog *P*, is an important factor involved in parasite penetration [16]. High lipophilicity values are suggestive of good permeability via a passive transcellular uptake mechanism. Nonetheless, our data demonstrate that the increase of Clog *P* of benzimidazole synthesized (Table 2) did not increase antitrichinellosis activity. In fact, although **ABZ** and compound **1b** have a Clog *P* of 3.07 and 5.67 respectively, the percentage of metabolic reduction of *T. spiralis* ML was similar at 0.18 μM and 0.37 μM.

Compounds **1b**, **1e** and **1f** were further tested *in vivo* against the intestinal and systemic stage of *T. spiralis* (Table 3). No dead animals were registered during the assays. Compound **1e** was the most

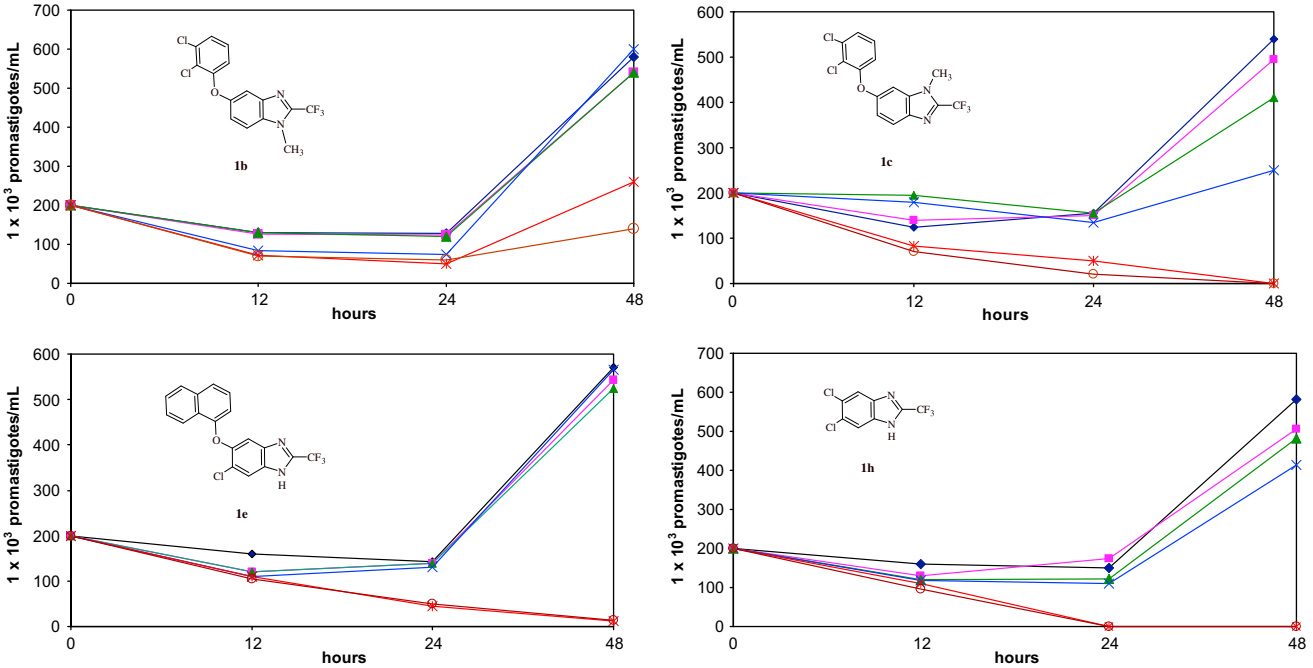


Fig. 2. Effect of compounds **1b**, **1c**, **1e** and **1h** at different concentrations and incubation time on the growth of *L. mexicana* promastigotes. Control (◆), 0.1 μg/mL (■), 0.5 μg/mL (▲), 1.5 μg/mL (×), 10 μg/mL (✳), 20 μg/mL (○).

Table 3
Percentage of adult and muscle larvae load reduction in *T. spiralis* infected mice treated with benzimidazole derivatives.

Compounds	Adult phase[a]		Muscle larvae phase[b]
	50 mg/Kg[a]	75 mg/Kg[a]	75 mg/Kg[a]
1b	ND[c]	58	46
1e	69	80	40
1f	NR[d]	36	64
ABZ	62	73	63
TBZ	41	7	25
Alpha	28	ND[c]	24
Control	0	0	0

[a] Groups of six mice were infected with 800 *T. spiralis* ML and treated at day 3 post-infection (p.i.) with a single dose of the drugs. Animals were sacrificed at day 6 p.i., and adults were obtained as previously described [15].
[b] Groups of 10 mice were infected with 800 *T. spiralis* ML and treated with the drugs at day 28 p.i. for 7 consecutive days. Animals were sacrificed on day 7 after treatment, and ML were obtained as previously described [15].
[c] ND: not determined.
[d] NR: no reduction.

active against *T. spiralis* adult, reducing the parasite burden by 69% and 80% at 50 mg/Kg and 75 mg/Kg, respectively. It was more active than **ABZ**, **TBZ** and **Alpha**. Although compound **1e** reduced just 40% the ML load at the systemic level, it was even more active than **TBZ** and **Alpha**, but not more than **ABZ**. Regarding compound **1b**, at 75 mg/Kg, it reduced the adult and ML burden by 58% and 46%, respectively, being more active than **TBZ** and **Alpha**, and less potent than **ABZ**.

It is worth noting that compounds **1b** and **1e** showed a better antiparasitic activity profile than their respective analogues **TBZ** and **Alpha**. Significantly, the activity of compound **1f** against the adult worm was lower than **1b**, **1e** and **ABZ**; however, it was the most efficient compound against *T. spiralis* ML, reducing the parasite burden by 64%, with activity similar to **ABZ** (63%). The low solubility and hence the low bioavailability of compounds **1b** and **1e** could account for its reduced activity at the systemic level.

It is important to mention that although compound **1f** showed low activity against adult *T. spiralis*, its activity increased against the systemic phase of the parasite; hence, this compound possibly behaved as a prodrug, generating compound **1e** by N-demethylation. A similar process could explain the reduction of the *in vivo* activity of compound **1b**, which could be a prodrug of compound **1a**, that showed scarce *in vitro* activity.

6. Conclusions

The data obtained indicate that the 2-(trifluoromethyl)-1*H*-benzimidazole system is a good scaffold for the development of new antiparasitic agents. Derivatives **1a**–**1i** showed good antiprotozoal activity, most of them higher than choice drugs. The order of parasite susceptibility found was: *E. histolytica* > *G. intestinalis* > *T. vaginalis* > *L. mexicana*. Compounds **1b**, **1c** and **1e** possessed the most desirable *in vitro* antiparasitic profile against all parasites studied. In addition, compound **1f** had the highest activity against the *T. spiralis* systemic stage similar to **ABZ**. These outcomes are very promising and our current efforts will focus on further evaluation of their antiparasitic activity against other tissue-dwelling parasites and to clarify the possible mechanism of action.

7. Experimental

Melting points were determined in open capillary tubes with a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F254 plates (E. Merck) and visualized by irradiation with a UV lamp. Silica gel 60 (70–230 mesh) was used for column chromatography. Infrared (IR) spectra were recorded with a FT Perkin Elmer 16 PC spectrometer on KBr disks, the frequencies (υ) are expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were measured with a Varian EM-390 spectrometer (operating at 300.06 MHz for ^1H and 75.45 MHz for ^{13}C). Chemical shifts are given in parts per million relative to tetramethylsilane (Me_4Si, $\delta = 0$); *J* values are given in Hz. Splitting patterns have been designated as follows: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; bs, broad singlet. Mass spectra (MS) were recorded on a JEOL JMS-SX102A spectrometer by electron impact (EI). The high resolution mass spectra (HRMS) were performed on a Thermo-Electron Trace GC Ultra spectrometer. Elemental analyses (C, H, N) for new compounds were performed on Fisons EA 1108 instrument and agreed with the proposed structures within ±0.4% of the theoretical values. Catalytic hydrogenations were carried out in a Parr shaker hydrogenation apparatus. Starting materials 1-fluoro-4-nitrobenzene (**2**), 2,3-dichlorophenol and 1-naphtol were commercially available (Sigma–Aldrich). The Clog *P* values were obtained using ACD/labs software v. 9.07.

7.1. General procedure for the synthesis of compounds *1a*–*1e*, *1g*

The appropriate 1,2-phenylenediamine (**5**, **7**, **11a**, **11b**, **13a**, **13b**, 0.0313 mol), 1.6 eq of CF_3CO_2H and one drop of concentrated HCl were heated under reflux in a N_2 atmosphere for 3–4 h. After completion of the reaction, the cooled mixture was neutralized with saturated $NaHCO_3$ solution and the crude product was extracted with ethyl acetate. The combined organic extracts were washed with water, dried with Na_2SO_4 and the solvent was removed under vacuum. The residue was dissolved in chloroform, filtered through Al_2O_3 neutral type and concentrated under vacuum to obtain the corresponding benzimidazole.

7.1.1. 5(6)-(2,3-Dichlorophenoxy)-2-(trifluoromethyl)-1H-benzimidazole (*1a*)

Recrystallized from cyclohexane–CH_2Cl_2 to yield a white crystalline solid (80% yield). M.p. 172–174 °C; $R_f = 0.26$ ($CHCl_3$/MeOH 99.5:0.5, UV); IR (υ): 2849, 1633, 1577, 1553, 1494, 1448, 1259, 1180, 1141, 962, 906, 767, 714; ^1H NMR (DMSO-d_6) δ: 6.95 (dd, 1H, $J = 1.5$, 8.1, H-6′), 7.11 (dd, 1H, $J = 2.4$, 9.0, H-6), 7.26 (d, 1H, $J = 2.4$, H-4), 7.31 (t, 1H, $J = 8.1$, 8,4, H-5′), 7.40 (dd, 1H, $J_1 = 1.5$, $J_2 = 8.1$, H-4′), 7.76 (d, 1H, $J = 9.0$, H-7), 13.90 (br,1H, exchangeable with D_2O, NH); ^{13}C NMR (DMSO-d_6) δ: 104.43, 117.22, 118.32, 118.53 (q, $J = 269.30$, CF_3), 118.87, 124.75, 125.56, 127.61, 134.42, 134.66, 137.20, 141.42 (q, $J = 41$, C-2), 154.14, 154.36; MS, *m*/*z* (abundance,%): 346 (M^+, 78), 311 ($M^+ - 35$, 100), 348 ($M^+ + 2$, 51), 350 ($M^+ + 4$, 8). Anal. Calcd for $C_{14}H_7Cl_2F_3N_2O$: C, 48.44; H, 2.03; N, 8.07. Found: C, 48.75; H, 2.09; N, 8.09.

7.1.2. 5-(2,3-Dichlorophenoxy)-1-methyl-2-(trifluoromethyl)-1H-benzimidazole (*1b*)

White solid (80% yield). M.p. 137–139 °C; $R_f = 0.71$ ($CHCl_3$/MeOH 99.5:0.5, UV); IR (υ): 1871, 1575, 1491, 1448, 1250, 1234, 1176, 1143, 963, 771, 702; ^1H NMR ($CDCl_3$) δ: 3.96 (s, 3H, NCH_3), 6.82 (dd, 1H, $J_1 = 1.5$, $J_2 = 8.1$, H-6′), 7.12 (t, 1H, $J_1 = 8.1$, $J_2 = 8.4$, H-5′), 7.21 (dd, 1H, $J_1 = 2.4$, $J_2 = 9.9$, H-6), 7.25 (dd, 1H, $J_1 = 1.2$, $J_2 = 8.1$, H-4′), 7.40 (dd, 1H, $J_1 = 0.3$, $J_2 = 2.1$, H-4), 7.44 (dd, 1H, $J_1 = 0.3$, $J_2 = 9.0$, H-7); ^{13}C NMR ($CDCl_3$) δ: 31. 02, 110.09, 111.20, 117.17, 118.17, 118.77 (q, $J = 203.92$, CF_3), 124.47, 125.27, 127.52, 132.67, 134.36, 141.19, 141.59 (q, $J = 38$, C-2), 153.27, 154.49; MS, *m*/*z* (abundance,%): 360 (M^+, 100), 325 ($M^+ - 35$, 98), 362 ($M^+ + 2$, 63), 350 ($M^+ + 4$, 8). Anal. Calcd for $C_{15}H_9Cl_2F_3N_2O$: C, 49.89; H, 2.51; N, 7.76. Found: C, 49.75; H, 2.58; N, 7.79.

*7.1.3. 6-(2,3-Dichlorophenoxy)-1-methyl-2-(trifluoromethyl)-1H-benzimidazole (**1c**)*

Recrystallized from ethanol to yield a white crystalline solid (80% yield). M.p. 100–101 °C; R_f = 0.73 ($CHCl_3$/MeOH 99.5:0.5, UV); IR (υ): 3062, 1889, 1622, 1579, 1522, 1483, 1451, 1413, 1259, 1185, 1131, 1095, 966, 905, ^{1}H NMR ($CDCl_3$) δ: 3.98 (s, 3H, CH_3), 6.89 (dd, 1H, J_1 = 1.5, J_2 = 8.1, H-6′), 6.98 (d, 1H, J = 2.1, H-7), 7.09 (dd, 1H, J_1 = 2.4, J_2 = 9.0, H-5), 7.17 (t, 1H, J_1 = 2.4, J_2 = 9.0, H-5′), 7.30 (dd, 1H, J_1 = 1.5, J_2 = 8.1, H-4′), 7.86 (d, 1H, J = 9.0, H-4); ^{13}C NMR ($CDCl_3$) δ: 30. 98, 99.28, 115.99, 118.27, 118.77 (q, J = 271.10, CF_3), 122.76, 124.82, 125.63, 127.69, 134.48, 136.61, 137.21, 141.10 (q, J = 38.40, C-2), 154.18, 154.82; MS, *m*/*z* (abundance, %): 360 (M^+, 100), 325 ($M^+ - 35$, 97), 362 ($M^+ + 2$, 65), 350 ($M^+ + 4$, 9). Anal. Calcd for $C_{15}H_9Cl_2F_3N_2O$: C, 49.89; H, 2.51; N, 7.76. Found: C, 49.75; H, 2.93; N, 7.77.

*7.1.4. 5(6)-Chloro-6(5)-(2,3-dichlorophenoxy)-2-(trifluoromethyl)-1H-benzimidazole (**1d**)*

White solid (50% yield). M.p. 211–212 °C; R_f = 0.37 ($CHCl_3$/MeOH 90:10, UV); IR (υ): 3398, 3039, 1576, 1550, 1447, 1274, 1158; ^{1}H NMR ($CDCl_3$) δ: 6.74 (dd, 1H, J_1 = 1.5, J_2 = 8.1, H-6′), 7.13 (t, J_1 = 8.4, J_2 = 8.1, 1H, H-5′), 7.27 (dd, J_1 = 1.5, J_2 = 8.1, 1H, H-4′), 7.36 (s, 1H, H-4), 7.86 (s, 1H, H-7); ^{13}C NMR (DMSO-d_6) δ: 115.97, 116.95, 117.96 (q, J = 269.30, CF_3), 120.53, 121.43, 121.70, 124.88, 128.72, 132.97, 147.07, 154.14; MS, *m*/*z* (abundance, %): 380 (M^+, 48), 345 ($M^+ - 35$, 100), 382 ($M^+ + 2$, 47), 384 ($M^+ + 4$, 16). Anal. Calcd for $C_{14}H_6Cl_3F_3N_2O$: C, 44.07; H, 1.58; N, 7.34. Found: C, 45.75; H, 1.63; N, 7.77.

*7.1.5. 6(5)-Chloro-5(6)-(1-naphthyloxy)-2-(trifluoromethyl)-1H-benzimidazole (**1e**)*

White solid (64% yield). M.p. 110–112 °C; IR (υ): 3053, 2924, 1549, 1441, 1392, 1233, 1191; ^{1}H NMR (DMSO-d_6) δ: 6.75 (d, 1H, J = 7.2, H-2′), 7.40 (dd, 1H, J_1 = 7.2, J_2 = 7.8, H-3′), 7.55–7.63 (m, 2H, H-6′, H-7′), 7.57 (s, 1H, H-4), 7.70 (d, 1H, J = 7.8, H-4′), 7.96–7.99 (m, 1H, H-8′), 8.06 (s,1H, H-7), 8.16–8.19 (m, 1H, H-5′), 14 (br, 1H, exchangeable with D_2O, NH); ^{13}C NMR (DMSO-d_6) δ: 110.40, 110.98, 118.90 (q, J = 320.10, CF_3), 121.28, 123.10, 125.01, 125.98, 126.29, 126.88, 127.85, 134.54, 141.91 (q, J = 41, C-2), 148.65, 152.76; MS, *m*/*z* (abundance, %): 362 (M^+, 100). HRMS Calcd for $C_{18}H_{10}ClF_3N_2O$ 362.733. Found 362.0454.

*7.1.6. 5-Chloro-1-methyl-6-(1-naphthyloxy)-2-(trifluoromethyl)-1H-benzimidazole (**1g**)*

White solid (71% yield). M.p. 98–99 °C; IR (υ): 3053, 1524, 1390, 1239; ^{1}H NMR ($CDCl_3$) δ: 3.93 (s, 3H, NCH_3), 6.84 (dd, 1H, J_1 = 7.4, J_2 = 0.8, H-2′), 6.97 (s, 1H, H-7), 7.38 (dd, 1H, J_1 = 7.8, J_2 = 8.0, H-3′), 7.50–7.59 (m, 2H, H-6′, H-7′), 7.67 (d, J = 8.4, H-4′), 7.88–7.93 (m, 1H, H-8′), 8.02 (s, 1H, H-4), 8.22–8.27 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6) δ: 31.11, 100.94, 112.45, 121.84, 123.88, 125.64, 126.30, 126.87, 127.81, 134.99, 137.32, 151.43, 152.75; MS, *m*/*z* (abundance, %): 376 (M^+, 88), 341 ($M^+ - 35$, 100). Anal. Calc for $C_{19}H_{12}ClF_3N_2O$: C, 60.57; H, 3.21; N, 7.44. Found C, 60.34; H, 3.22; N, 7.21.

*7.2. 6-Chloro-1-methyl-5-(1-naphthyloxy)-2-(trifluoromethyl)-1H-benzimidazole (**1f**)*

Into a stirred mixture of **1e**, methyl iodide and acetone was added a solution of KOH 50% m/v. At the end of the reaction the mixture was worked up by addition of cold water. The precipitate was removed by suction and washed several times with water until neutral pH. The solid residue was a mixture of regioisomers **1f** and **1g**. Title compound **1f** was obtained as a white powder by flash chromatography. White solid (11% yield). M.p. 100–101 °C; IR (υ):1522, 1483, 1393, 1238, 1186; ^{1}H NMR ($CDCl_3$) δ: 3.93 (s, 3H, NCH_3), 6.76 (dd, 1H, J_1 = 7.0, J_2 = 0.8, H-2′), 7.33 (dd, 1H, J_1 = 7.7, J_2 = 8.0, H-3′), 7.47(s, 1H, H-7), 7.48–7.57 (m, 1H, H-6, H-7′), 7.60 (s, 1H, H-4), 7.62 (d, J = 8.3, H-4′), 7.85–7.91 (m, 1H, H-8′), 8.26–8.29 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6) δ: 31.13, 111.55, 111.70, 118.38 (q, J = 299.14, CF_3), 121.86, 123.47, 124.76, 125.56, 126.06, 126.70, 127.76, 134.94, 142.01 (q, J = 39), 149.66, 152.99; MS, *m*/*z* (abundance, %): 376 (M^+, 100). Anal. Calc for $C_{19}H_{12}ClF_3N_2O$: C, 60.57; H, 3.21; N, 7.44. Found C, 60.47; H, 3.24; N, 7.18.

*7.3. General method of synthesis of 1,2-phenylenediamines **5**, **7**, **10a**, **10b**, **13a** and **13b***

A mixture of adequate substituted 2-nitroaniline (0.0282 mol), EtOH (100 mL) and 10% Raney-Nickel was hydrogenated at 25 °C until cessation of H_2 uptake. The catalyst was filtered off on a Whatman paper number 2, washed with EtOH, and the filtrate concentrated to provide a dark purple-colored liquid, which was used immediately in a subsequent step without purification.

*7.4. Synthesis of precursors **3**, **4**, and **6***

*7.4.1. 1,2-Dichloro-3-(4-nitrophenoxy)benzene (**3**)*

A mixture of **2** (30 g, 0.2127 mol, 1 eq), K_2CO_3 (40 g, 0.2894 mol, 1.36 eq), 2,3-dichlorophenol (41.34 g, 0.2551 mol, 1.19 eq), H_2O (20 mL) and DMF (350 mL) was heated at 110 °C for 5 h. The mixture was allowed to cool to room temperature and poured into ice bath. The obtained precipitate was filtered and recrystallized from ethyl acetate to yield a yellow crystalline solid (57 g, 95% yield). M.p. 121–122 °C; IR (υ): 3436, 3077, 1926, 1787, 1612, 1594, 1571, 1510, 1484, 1445, 1339, 1254, 1216, 1166; ^{1}H NMR (299.7, $CDCl_3$) δ: 6.97 (m, 2H, J_1 = 2.4, J_2 = 9.6, H-2′), 7.09 (dd, 1H, J_1 = 1.5, J_2 = 8.4, H-4), 7.29 (t, 1H, J_1 = 8.1, J_2 = 8.1, H-5), 7.42 (dd, 1H, J_1 = 1.5, J_2 = 8.1, H-6), 8.22 (m, 2H, J_1 = 2.4, J_2 = 9.6, H-3′); MS, *m*/*z* (abundance, %): 283 (M^+, 100).

*7.4.2. N-[4-(2,3-Dichlorophenoxy)-2-nitrophenyl]acetamida (**4**)*

A mixture of **3** (11.5 g, 0.040 mol), MeOH (250 mL) and 10% Raney-Nickel (2 g) 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 4-(2,3-dichlorophenoxy)aniline (10 g, 97% yield), which was used immediately in a subsequent step without purification. A stirred mixture of this compound (10 g, 0.0393 mol), acetic anhydride (4.46 mL, 0.4724 mol, 1.2 eq) and three drops of H_2SO_4 was heated at 80 °C for 3 min. The mixture was cooled, worked up by addition of cold water, filtered by suction and the crude product was recrystallized from EtOH to yield a yellow solid (10.5 g, 90% yield). To a cold mixture of *N*-[4-(2,3-dichlorophenoxy)phenyl]acetamide (8 g, 0.0270 mol) and anhydride acetic (44 mL) was added an HNO_3 (3 mL, 70.4%) at 0–10 °C. After the addition, the organic layer was separated, washed with brine and dried with $CaCl_2$. The obtained precipitate was filtered and recrystallized from EtOH to yield a yellow crystalline solid (8.32 g, 90% yield). M.p. 122–124 °C; R_f = 0.5 ($CHCl_3$/MeOH 99.5:0.5, UV); IR (υ): 3372, 3085, 1693, 1586, 1520, 1445, 1344, 1256, 970; ^{1}H NMR ($CDCl_3$) δ: 2.29 (s, 3H, $COCH_3$), 6.96 (dd, 1H, J_1 = 1.5, J_2 = 8.1, H-6′), 7.23 (t, 1H, J_1 = 8.4, J_2 = 8.4, H-5′), 7.29 (dd, 1H, J_1 = 3.0, J_2 = 9.3, H-5), 7.36 (dd, 1H, J_1 = 1.5, J_2 = 8.4, H-4′), 7.73 (d, 1H, J = 2.7, H-3), 8.75 (d, J = 9.3, H-6), 10.19 (br, 1H, NH); MS, (abundance, %): *m*/*z* 340 (M^+, 30), 298 ($M^+ - 42$, 100).

*7.4.3. N-[4-(2,3-Dichlorophenoxy)-2-nitrophenyl]-N-methylacetamide (**6**)*

Into a stirred mixture of **4** (8.30 g, 0.0243 mol) in dimethyl sulfate (4.60 g, 3.46 mL, 0364 mol, 1.5 eq) and monoglyme (15 mL) was added a solution of KOH 50% m/v (2.04 g, 0.0364 mol, 1.5 eq) at 32–35 °C. The resulting solution was stirred for 3 h at 32 °C. The mixture was cooled, worked up by addition of cold water and extracted with EtOAc. The combined organic extracts were washed

with brine, dried with anhydrous Na_2SO_4 and concentrated in vacuo to give a yellow solid (19 g, 95% yield). M.p. 134–135 °C; R_f = 0.2 ($CHCl_3$/MeOH 99:1, UV); IR (υ): 3072, 3040, 2930, 1957, 1665, 1541, 1451, 1428, 1267; ^{1}H NMR ($CDCl_3$) δ: 1.83 (s, 3H, $COCH_3$), 3.19 (s, 3H, N–CH_3), 7.11 (dd, 1H, $J_1 = 1.5$, $J_2 = 8.1$, H-6′), 7.20 (dd, 1H, $J_1 = 2.7$, $J_2 = 8.7$, H-5), 7.26 (t, 1H, $J_1 = 8.1$, $J_2 = 8.1$, H-5′), 7.33 (d, 1H, $J = 8.7$, H-6), 7.44 (dd, 1H, $J_1 = 1.5$, $J_2 = 8.1$, H-4′), 7.48 (d, $J = 2.3$, H-3); MS, m/z (abundance, %): 354 (M^+, 1), 308 ($M^+ - 46$, 100).

7.5. Synthesis of precursors ***9a*** and ***12a***

A mixture of **8a** or **11** (0.0536 mol, 1 eq), K_2CO_3 (8.14 g, 0.0589 mol, 1.1 eq), 2,3-dichlorophenol (9.61 g, 0.0589 mol, 1.1 eq), H_2O (8 mL) and DMF (60 mL) was heated at 125 °C for 3 h. The mixture was allowed to cool to room temperature and poured into ice bath. The obtained precipitate was filtered.

7.5.1. 5-(2,3-Dichlorophenoxy)-N-methyl-2-nitroaniline (***9a***)

Recrystallized from ethanol to yield a crystalline solid (12.4 g, 81% yield). M.p. 124–126 °C; R_f = 0.27 (hexane/EtOAc 85:15, UV); IR (υ): 3386, 1631, 1578, 1566, 1508, 1448, 1328, 1258, 1218, 842; ^{1}H NMR ($CDCl_3$) δ: 2.94 (s, 3H, N–CH_3), 6.13 (dd, 1H, $J_1 = 2.4$, $J_2 = 9.6$, H-4), 6.27 (d, 1H, $J = 2.7$, H-6), 7.07 (dd, 1H, $J_1 = 1.2$, $J_2 = 8.7$, H-6′), 7.26 (t, 1H, $J_1 = 8.4$, $J_2 = 8.4$, H-5′), 7.39 (dd, 1H, $J_1 = 1.2$, $J_2 = 8.1$, H-4′), 8.16 (d, 1H, $J = 9.1$, H-3), 10.49 (br, 1H, N–H); MS, m/z (abundance, %): 312 (M^+, 100).

7.5.2. 4-Chloro-5-(2,3-dichlorophenoxy)-2-nitroaniline (***12a***)

Recrystallized from benzene–ethanol to yield an orange crystalline solid (12.5, 70% yield). M.p. 149–150 °C; R_f = 0.59 (hexane/$CHCl_3$/EtOAc 50:35:15, UV); IR (υ): 3465, 3344, 3174, 3112, 3039, 1559, 1546, 1224; ^{1}H NMR ($CDCl_3$) δ: 5.93 (s, 1H, H-6), 6.13 (br, 1H, exchangeable with D_2O, N–H), 7.09 (dd, 1H, $J_1 = 1.80$, $J_2 = 1.2$, $J_3 = 8.1$, H-6′), 7.29 (t, 1H, $J_1 = 8.4$, $J_2 = 8.4$, H-5′), 7.42 (dd, 1H, $J_1 = 1.8$, $J_2 = 1.2$, $J_3 = 8.1$, H-4′), 8.26 (s, 1H, C-3); MS, m/z (abundance, %): 332 (M^+, 94), 262 ($M^+ - 70$, 100).

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