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Synthesis and antiprotozoal activity of novel 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1*H*-benzimidazole derivatives

Jaime Pérez-Villanueva [a], Alicia Hernández-Campos [a], Lilián Yépez-Mulia [b], Carlos Méndez-Cuesta [a], Oscar Méndez-Lucio [a], Francisco Hernández-Luis [a], Rafael Castillo [a,*]

[a] Facultad de Química, Departamento de Farmacia, UNAM, DF 04510, Mexico
[b] Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, IMSS, DF 06720, Mexico

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ABSTRACT

A series of 19 new 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1*H*-benzimidazole derivatives was synthesized starting from the properly substituted 1,2-phenylendiamine. These compounds have hydrogen or methyl at position 1; while hydrogen, chlorine, ethoxy or methoxycarbonyl group is at position 5 and/or 6. The novel compounds were tested against protozoa *Trichomonas vaginalis*, *Giardia intestinalis* and *Entamoeba histolytica*. Experimental evaluations revealed strong activity for all tested compounds, having IC_{50} values in the nanomolar range, which were even better than metronidazole, the drug of choice for these parasites.

Parasitic infections caused by protozoa still represent a major public health problem in developing countries. Some important intestinal protozoa include *Giardia intestinalis* and *Entamoeba histolytica*, causal agents of giardiosis and amebiosis, respectively. According to the World Health Organization (WHO), there are estimated 280 million giardiosis cases each year. Interestingly, *G. intestinalis* has been classified as the most common diagnosed flagellate in the intestinal tract.[1,2] Infection by this protozoan usually produces diarrhea and associated symptoms. Furthermore, it is worth mentioning that child incidence is very high and is associated with malnutrition, linear growth retardation and poor cognitive function.[3,4] Regarding *E. histolytica*, WHO estimated that this protozoan causes severe disease in 50 million people each year and has been classified as one of the most common causes of death from parasitic disease.[2,5] In addition to the common symptoms such as diarrhea and dysentery, this protozoan can penetrate the intestinal mucosa and migrate to other organs causing severe damage.[1,2,5] In addition to intestinal infections, the genitourinary infection caused by *T. vaginalis* (trichomonosis) is estimated to be more than 180 million new cases annually.[1] Although trichomonosis in men is generally asymptomatic or mild, in women it causes severe symptoms and consequences. The infection produces deep inflammation of the genital tract and has been associated with preterm labor, low-birth weight, sterility, cervical cancer and a predisposition to HIV infection.[6,7] For these three diseases, metronidazole (MTZ) has been successfully used as the drug of choice for more than 40 years; however, its side effects and the development of resistant strains limit its use.[1] Although some additional chemotherapeutic agents are available (e.g. tinidazole and nitazoxanide used in the treatment of giardiosis), it is still important to have more options of treatment, because of different individual response to drugs. During the last years, an important number of benzimidazole derivatives have been synthesized and evaluated as antiprotozoals by our group and other external research groups.[8–15] Moreover, studies based on the emerging concept of the activity landscape were undertaken to find out the structure–activity relationships (SAR) of benzimidazole derivatives as trichomonicidal and giardicidal agents.[16,17] Besides, quantitative structure–activity relationships (QSAR) studies based on comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were carried out for amebicidal and trichomonicidal benzimidazole derivatives.[18,19] Although some important conclusions have been highlighted with our previous synthetic and SAR studies, the information accumulated is still limited. Therefore, increasing the database of benzimidazole derivatives remains of paramount importance to have access to new SAR features that can lead to the optimization of benzimidazole derivatives as antiprotozoals. Expanding our previous work, herein we report the synthesis of 19 new 2-{[2-(1*H*-imidazol-1-yl)ethyl]sulfanyl}-1*H*-benzimidazole derivatives (**35**–**53**), Fig. 1.

The design of the new benzimidazole derivatives was based on previous SAR conclusions derived from our former studies (Fig. 1).[16–19] It was previously observed that substituted alkylthio

* Corresponding author. Tel.: +52 556 22 52 87; fax: +52 556 22 53 29.
E-mail address: rafaelc@unam.mx (R. Castillo).

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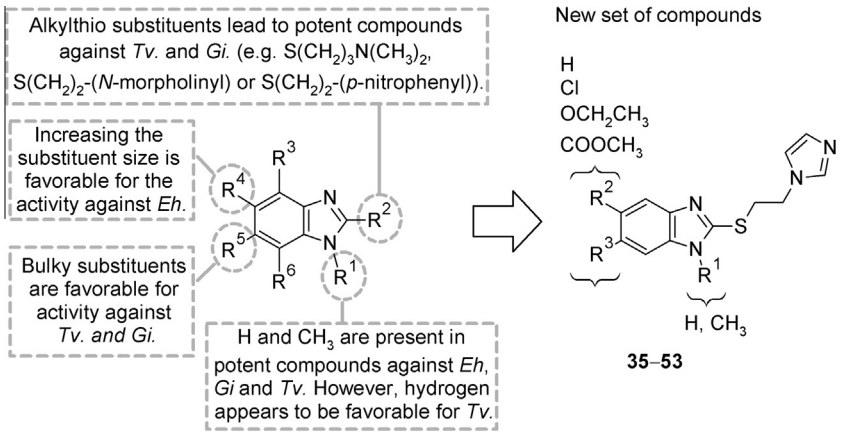
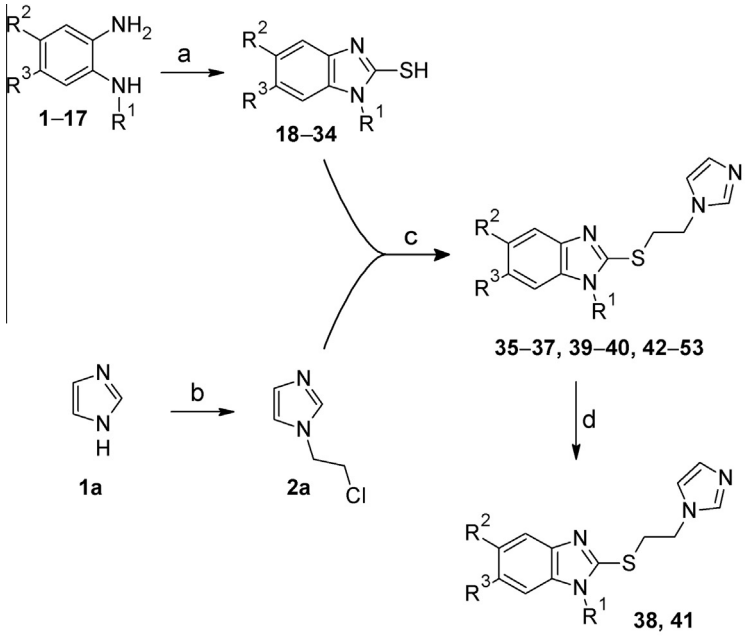


Figure 1. Some SAR conclusions for benzimidazole derivatives tested against *G. intestinalis* (*Gi*), *E. histolytica* (*Eh*), and *T. vaginalis* (*Tv*) which lead to the structure of the 19 new derivatives (**35–53**) synthesized in this work.

groups at position 2 of the benzimidazole nucleus lead to potent compounds against *T. vaginalis* and *G. intestinalis*.[12,17,19] Some of the most active compounds were those with hydrogen acceptors or an aromatic ring attached to the alkyl chain. The new derivatives synthesized in this work contain an alkylthio imidazole group at position 2, a substituent which combines both features. In addition, hydrogen or methyl substituents were present at position 1 of the new derivatives. It is worth noting that both, hydrogen and methyl derivatives, have been reported as strong trichomonicidal and giardicidal agents.[9,14] Also, hydrogen, chlorine, carbomethoxy and ethoxy substituents were considered at position 5 and/or 6 for the new derivatives. As previous QSAR studies of benzimidazole derivatives suggest, the substituents at position 6 are favorable for trichomonicidal and giardicidal activity,[16,17,19] whereas substituents at position 5 play an important role for amebicidal activity.[18] It is important to emphasize that SAR conclusions previously reported are highly dependent on the database analyzed.

Compounds **35–53** were prepared according to the sequence of reactions shown in Scheme 1. The first step was the cyclocondensation of the properly substituted 1,2-phenylenediamine (**1–17**) with carbon disulfide in the presence of potassium hydroxide and ethanol to give the respective 2-mercaptobenzimidazole (**18–34**).[20,21] These were then alkylated with 1-(2-chloroethyl)-1*H*-imidazole to afford title compounds **35–37**, **39**, **40**, and **42–53**.[22] Finally, compounds **38** and **41** were obtained by *N*-methylation of compounds **35** and **37**, respectively.[23] The intermediate 1-(2-chloroethyl)-1*H*-imidazole was prepared under solid–liquid phase transfer-catalysis conditions as described previously by Bogdal et al.,[24] in which commercial imidazole was alkylated with 1,2-dichloroetane in the presence of sodium hydroxide, potassium carbonate and tetrabutylammonium chloride. Compounds **35–53** were obtained as solids with fair global yields and purity. The compounds were characterized using ^{1}H and ^{13}C nuclear magnetic resonance,[25] mass spectrometry and high resolution mass spectrometry (See supplementary data for structure characterization, yields and melting points).

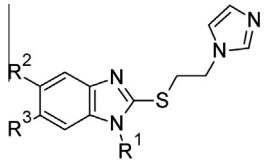
The in vitro antiprotozoal activity of compounds **35–53** was tested following the method previously described for *T. vaginalis*, *G. intestinalis* and *E. histolytica*.[8–15,26] The results obtained from the biological assays are shown in Table 1. All tested compounds



1, **18**, **35**: $R^1=R^2=R^3=H$
2, **19**, **36**: $R^1=H$, $R^2=Cl$, $R^3=H$
3, **20**, **37**: $R^1=H$, $R^2=R^3=Cl$
38: $R^1=CH_3$, $R^2=R^3=H$
4, **21**, **39**: $R^1=CH_3$, $R^2=H$, $R^3=Cl$
5, **22**, **40**: $R^1=CH_3$, $R^2=Cl$, $R^3=H$
41: $R^1=CH_3$, $R^2=R^3=Cl$
6, **23**, **42**: $R^1=H$, $R^2=COOCH_3$, $R^3=H$
7, **24**, **43**: $R^1=H$, $R^2=COOCH_3$, $R^3=Cl$
8, **25**, **44**: $R^1=CH_3$, $R^2=H$, $R^3=COOCH_3$
9, **26**, **45**: $R^1=CH_3$, $R^2=Cl$, $R^3=COOCH_3$
10, **27**, **46**: $R^1=CH_3$, $R^2=COOCH_3$, $R^3=H$
11, **28**, **47**: $R^1=CH_3$, $R^2=COOCH_3$, $R^3=Cl$
12, **29**, **48**: $R^1=H$, $R^2=OCH_2CH_3$, $R^3=H$
13, **30**, **49**: $R^1=H$, $R^2=OCH_2CH_3$, $R^3=Cl$
14, **31**, **50**: $R^1=CH_3$, $R^2=H$, $R^3=OCH_2CH_3$
15, **32**, **51**: $R^1=CH_3$, $R^2=Cl$, $R^3=OCH_2CH_3$
16, **33**, **52**: $R^1=CH_3$, $R^2=OCH_2CH_3$, $R^3=H$
17, **34**, **53**: $R^1=CH_3$, $R^2=OCH_2CH_3$, $R^3=Cl$

Scheme 1. Synthesis of compounds **35–53**: (a) CS_2, KOH, $EtOH/H_2O$, 40–50 °C, 8 h; (b) $ClCH_2CH_2Cl$, Bu_4NCl, NaOH, K_2CO_3, 50 °C, 3 h; (c) CH_3CN, KOH or DBU, 60 °C, 8 h; (d) CH_3I, $(CH_3)_2CO$, K_2CO_3.

Table 1
Chemical structures and biological activity of benzimidazole derivatives **35**–**53**



	R_1	R_2	R_3	IC_{50} (μM) *T. vaginalis*	IC_{50} (μM) *G. Intestinalis*	IC_{50} (μM) *E. histolytica*
35	H	H	H	0.1780 ± 0.0087	0.1167 ± 0.0029	0.1474 ± 0.0058
36	H	Cl	H	0.1704 ± 0.0076	0.0807 ± 0.0025	0.0735 ± 0.0076
37	H	Cl	Cl	0.1405 ± 0.0045	0.0766 ± 0.0090	0.0591 ± 0.0023
38	CH_3	H	H	0.1490 ± 0.0027	0.0871 ± 0.0082	0.0774 ± 0.0055
39	CH_3	H	Cl	0.1281 ± 0.0072	0.0626 ± 0.0039	0.0672 ± 0.0110
40	CH_3	Cl	H	0.1349 ± 0.0024	0.0666 ± 0.0024	0.0660 ± 0.0129
41	CH_3	Cl	Cl	0.0963 ± 0.0022	0.0688 ± 0.0065	0.0642 ± 0.0043
42	H	$COOCH_3$	H	0.1108 ± 0.0023	0.0777 ± 0.0023	0.0612 ± 0.0023
43	H	$COOCH_3$	Cl	0.0727 ± 0.0105	0.0549 ± 0.0021	0.0579 ± 0.0021
44	CH_3	H	$COOCH_3$	0.1138 ± 0.0055	0.0569 ± 0.0045	0.0522 ± 0.0067
45	CH_3	Cl	$COOCH_3$	0.0869 ± 0.0101	0.0556 ± 0.0020	0.0428 ± 0.0040
46	CH_3	$COOCH_3$	H	0.0774 ± 0.0067	0.0616 ± 0.0067	0.0427 ± 0.0112
47	CH_3	$COOCH_3$	Cl	0.0926 ± 0.0020	0.0442 ± 0.0020	0.0513 ± 0.0040
48	H	OCH_2CH_3	H	0.0717 ± 0.0053	0.0566 ± 0.0020	0.0428 ± 0.0044
49	H	OCH_2CH_3	Cl	0.0991 ± 0.0044	0.0465 ± 0.0044	0.0620 ± 0.0044
50	CH_3	H	OCH_2CH_3	0.0717 ± 0.0038	0.0452 ± 0.0083	0.0408 ± 0.0019
51	CH_3	Cl	OCH_2CH_3	0.0698 ± 0.0063	0.0356 ± 0.0084	0.0460 ± 0.0063
52	CH_3	OCH_2CH_3	H	0.0761 ± 0.0094	0.0083 ± 0.0023	0.0298 ± 0.0047
53	CH_3	OCH_2CH_3	Cl	0.0980 ± 0.0042	0.0208 ± 0.0042	0.0148 ± 0.0042
Metronidazole				0.2360 ± 0.0160	1.2260 ± 0.1250	0.3798 ± 0.1461
Albendazole				1.5905 ± 0.0113	0.0370 ± 0.0030	56.5334 ± 18.8445

showed good activity in the nanomolar range. It is worth noting that all compounds were more potent than metronidazole, the drug of choice for these three parasites. The SAR nature of the database presented in this work is essentially continuous. That is, the new set of compounds is in agreement with the similarity principle which establishes that structurally similar compounds have similar biological activities. This conclusion is supported since all molecules in the dataset have less than 15-fold variation in potency. However, these low variations are enough to support some SAR conclusions and are in agreement with most of the observations previously reviewed (Fig. 1).

Results in Table 1, for compounds **35**–**41**, show that dichloro substitution at positions 5 and 6 in compounds **37** and **41** is slightly favorable for antiprotozoal activity, as compared with non-substituted compounds **35** and **38**, respectively. Furthermore, compounds **35** and **38** show a favorable effect of the methyl group at position 1 against the three protozoa tested. This same tendency was observed for compounds **37** and **41** against *T. vaginalis*, but no important changes were observed for *G. intestinalis* and *E. histolytica*. It is interesting to note that all the 1-methyl compounds show comparable or better activity than their respective 1*H* analogue. The only exceptions are compounds **44** and **47**, which have a slightly lower activity against *T. vaginalis* as compared with their 1*H* analogues **42** and **43**, respectively. These results agree with the previous SAR models that suggest that hydrogen at position 1 is favorable for trichomonicidal activity. In general, compounds **43**–**53**, which have a carbomethoxy or an ethoxy substituent at positions 5, 6 present the best activities. In particular, compounds **50**–**53**, with an ethoxy substituent, have similar or higher giardicidal potency than albendazole. It is important to emphasize that compound **52**, which showed a very strong giardicidal activity, was the only one whose activity increased 14-fold against *G. intestinalis*, as compared with unsubstituted analogue **35**, which was the less active compound against all the protozoa tested. Additionally, compounds **50**–**53** were particularly active against *T. vaginalis* and *E. histolytica* as compared with albendazole, which is poorly active. Compounds **50**–**53** can be considered among the most potent benzimidazole derivatives reported against the three protozoa. Noteworthy, antiprotozoal activities are in agreement with the previous SAR studies that indicate the importance of the substituent size at position 5 and 6 of the benzimidazole nucleus for the antiprotozoal activity.[16–19] Also, the strong activity of compounds **35**–**53** agrees with the previous SAR observation that alkylthio groups at position 2, having a hydrogen acceptor or an aromatic ring, lead to potent compounds.

The mechanism of action for the antiprotozoal activity of compounds **35**–**53** is still unknown. Interestingly, we found in previous studies that benzimidazole derivatives that are not benzimidazole carbamates (BC) do not inhibit β-tubulin polymerization as antiparasitic BC do.[8,9] Therefore, a different mechanism of action is implied in the antiprotozoal activity of the current derivatives.

In summary, compounds **35**–**53** resulted in new structures which were able to be synthesized with acceptable yields starting from the properly substituted 1,2-phenylendiamine. The novel compounds were characterized by using spectrometric and spectroscopic methods. Biological assays revealed that the novel compounds have strong activity against *T. vaginalis*, *G. intestinalis* and *E. histolytica*, which is better than metronidazole. Particularly, compounds **50**–**53** exhibited the best activities; among these, compound **52** was the most active against *G. intestinalis*. The new compounds expand our database of benzimidazole derivatives and increase our knowledge concerning the structural requirements for antiprotozoal activity. These results are useful for the design of new stronger antiprotozoal compounds.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.05.012.

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21. *General procedure for the synthesis of 2-mercaptobenzimidazole derivatives (**18**–**34**)*: the proper 1,2-phenylenediamine **1**–**17** (16 mmol) was added over a stirred solution of ethanol (50 mL), water (5 mL), potassium hydroxide (19.2 mmol) and carbon disulfide (19.2 mmol). The mixture was heated overnight under a N_2 atmosphere at 50 °C. The cold reaction mixture was then neutralized with acetic acid and poured into 250 mL of cold water for complete precipitation. The solid was separated and dried using vacuum filtration. The crude product was purified by recrystallization, adding norite to the solvent.
22. *General procedure for the synthesis of 2-{[2-(1H-imidazol-1-yl)ethyl]sulfanyl}-1H-benzimidazole derivatives (**35**–**37**, **39**, **40** and **42**–**53**)*: the proper 2-mercaptobenzimidazole **18**–**34** (6 mmol) in 20 mL of acetonitrile was stirred at room temperature and treated with KOH (6.6 mmol, as a 50% aqueous solution) or DBU (6.6 mmol, for ester derivatives **23**–**28**). Then, 1-(2-chloroethyl)-1*H*-imidazole (10.2 mmol) was added and the mixture was heated at 60 °C for 6–8 h. Next, the cold reaction mixture was neutralized with acetic acid and the solvent was removed under vacuum. The evaporation residue was extracted with ethyl acetate (30–50 mL), the organic phase was washed thrice with 20 mL of water, then dried with anhydrous sodium sulfate, and evaporated under vacuum. The residue was purified by column chromatography using ethyl acetate as a mobile phase.
23. *General procedure for the synthesis of 2-{[2-(1H-imidazol-1-yl)ethyl]sulfanyl}-1H-benzimidazole derivatives (**38** and **41**)*: a solution of the proper benzimidazole derivative, **35** or **37** (2 mmol) in 2-propanone (5 mL) was treated with KOH (2.2 mmol, as a 50% aqueous solution). Then, methyl iodide (2.2 mmol) was added and the mixture was stirred for 4 h at room temperature. Finally, the reaction was poured into 50 mL of cold water and the solid product was collected on a Büchner funnel. The crude product was purified by filtration using a column packed with silica gel using ethyl acetate as solvent.
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25. Some signals in ^{13}C NMR for 1*H* substituted benzimidazole derivatives are duplicated due to tautomeric effect. This same effect decreases the intensity of ^{13}C signals; therefore, in some compounds signals are weak or can be missed.
26. *Biological assays*: *Trichomonas vaginalis* strain GT3, *Giardia intestinalis* isolate IMSS:0981:1 and *Entamoeba histolytica* strain HM1-IMSS were used in all the experiments. Trophozoites of *G. intestinalis* were maintained in a TYI-S-33 medium supplemented with 10% calf serum and bovine bile. *E. histolytica* and *T. vaginalis* trophozoites were maintained in TYI-S-33 medium supplemented with 10% bovine serum. 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 different concentrations of the compound to be tested, each added as solutions in DMSO. As a negative control, parasite cultures received an equivalent amount of DMSO only, while ABZ and MTZ were included as positive controls. At the end of the treatment period, the cells were washed and subcultured for another 48 h in a fresh medium to which no drug was added. The trophozoites were then counted with a haemocytometer and the 50% inhibitory concentration (IC_{50}), together with the respective 95% confidence limit was calculated by Probit analysis. Experiments were carried out in triplicate and repeated at least twice.