Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2221–2224

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Antiparasitic Activity of 1*H*-Benzimidazole Derivatives

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Received 17 May 2001; accepted 16 April 2002

Abstract—Compounds **1**–**18** have been synthesized and tested in vitro against the protozoa *Giardia lamblia*, *Entamoeba histolytica* and the helminth *Trichinella spiralis*. Inhibition of rat brain tubulin polymerization was also measured and compared for each compound. Results indicate that most of the compounds tested were more active as antiprotozoal agents than Metronidazole and Albendazole. None of the compounds was as active as Albendazole against *T. spiralis*. Although only compounds **3**, **9** and **15** (2-methoxycarbonylamino derivatives) inhibited tubulin polymerization, these were not the most potent antiparasitic compounds.

Introduction

Gastrointestinal infections are among the major public health problems in developing countries. Especially amoebiasis (*Entamoeba histolytica*) and giardiasis (*Giardia lamblia*) have high morbidity and mortality indexes due to the effects of severe diarrhea and invasive infections. Although current drug therapy for the treatment of amoebiasis and giardiasis is effective, most available drugs have significant side effects that restrict their use.[1]

Recent studies have established that benzimidazole carbamates (BZC) such as Albendazole, Mebendazole, Flubendazole and Fenbendazole inhibit the in vitro growth of *Trichomonas vaginalis*[2] and *G. lamblia*.[3,4] Clinical reports have shown that Albendazole is as effective as Metronidazole, the choice drug for the treatment of giardiasis, but it is effective neither against *E. histolytica*[5–8] nor *Leishmania donovani*.[9]

BZC, well-known therapeutic agents used mainly as anthelmintics, have a broad antiparasitic spectrum of activity, low toxicity and have been used successfully to treat gastrointestinal helmintic infections. Systemic infections have also been treated with these agents; however, high doses and long treatments are required.[10]

The anthelmintic activity of these compounds is related to their preferential binding to helmintic tubulin over mammalian tubulin.[11–13] For optimal anthelmintic activity, BZC must bear an H at the 1-position of the benzimidazole ring.[14]

At present, the value of both BZC and metronidazole is seriously undermined by the development of resistance in the target parasite species.[15,16] Considering these problems, namely the side effects of the antiprotozoal drugs, and the lack of activity of BZC against *E. histolytica,* there is a pressing need for new therapeutic agents to treat these infections.

As part of our search for basic information about the requirements for antiprotozoal and anthelmintic activity,[17] compounds **1**–**18** (Table 1) were synthesized and tested in vitro against the protozoa *G. lamblia* and *E. histolytica*, and against the helminth *Trichinella spiralis*. The activity of **1**–**18** on rat brain tubulin polymerization was also measured. Although **1**–**18** are known compounds described in the literature, most of them are not commercially available.

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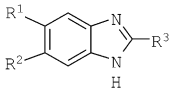
0960-894X/02/$ - see front matter
PII: S0960-894X(02)00346-3

Table 1. Biological activity of compounds **1**–**18** and reference compounds



Compd	R^1	R^2	R^3	IC_{50} (μM)[a]		% of viability reduction of *T. spiralis*[b]
				G. lamblia	*E. histolytica*	
1	H	H	CH_3	0.317	0.007	nr[c]
2	H	H	NH_2	1.902	0.114	9
3	H	H	$NHCO_2CH_3$	0.057	0.204	nr[c]
4	H	H	SH	0.040	0.133	11
5	H	H	SCH_3	0.045	0.393	nr[c]
6	H	H	H	0.008	0.042	nr[c]
7	Cl	H	CH_3	0.156	0.084	14
8	Cl	H	NH_2	0.030	0.125	17
9	Cl	H	$NHCO_2CH_3$	0.066	0.350	19
10	Cl	H	SH	0.081	0.005	13
11	Cl	H	SCH_3	0.005	0.192	15
12	Cl	H	H	0.282	0.039	27
13	Cl	Cl	CH_3	0.065	0.025	8
14	Cl	Cl	NH_2	0.218	0.059	2
15	Cl	Cl	$NHCO_2CH_3$	0.127	0.046	14
16	Cl	Cl	SH	0.078	0.055	23
17	Cl	Cl	SCH_3	0.227	0.356	17
18	Cl	Cl	H	0.358	0.096	18
Albendazole				0.037	56.33	34
Metronidazole				1.22	0.350	nd[d]

[a]IC_{50} The concentration that inhibits the growth of trophozoites at 50%.
[b]Percentage of viability reduction of *T. spiralis* muscle larvae after 3 days of incubation with compounds **1**–**18** and Albendazole at concentration of 3 nM.
[c]nr, no reduction observed.
[d]nd, not determined.

Chemistry

1*H*-Benzimidazoles: Compounds **1**, **7**, and **13** were prepared by the method of Phillips.[18] The appropriate 1,2-phenylenediamine and excess of acetic acid were boiled for 4 h. The reaction mixture was neutralized with concentrated ammonium solution, and the crude benzimidazole was isolated by filtration. Compounds **6**, **12**, and **18** were prepared in the same way, using formic acid instead of acetic acid.

2-Amino-1*H*-benzimidazoles: The method of Leonard et al.[19] was used for the general synthesis of compounds **2**, **8**, and **14**. A suspension of the appropriate 1,2-phenylenediamine and cyanogen bromide in ethanol–water was warmed to 70 °C for 1 h, the mixture was neutralized with concentrated ammonium hydroxide, and the crude product was isolated by filtration.

(1*H*-Benzimidazol-2-yl)carbamic acid methyl esters: Compounds **3**, **9**, and **15** were synthesized according to the procedure of Raeymakers et al.[20] A 25% aqueous solution of sodium hydroxide was added to an ice-cold stirred mixture of 2-methylthiopseudourea sulfate and methyl chloroformate in water until the pH of the reaction mixture reached 8.0. Care was taken to keep the temperature between 10 and 15 °C. The pH of the reaction mixture was then adjusted to 5.0 with glacial acetic acid. To the above suspension was added the appropriate 1,2-phenylenediamine. The resulting reaction mixture was stirred at 95 °C for 2 h and then cooled at room temperature. A solid precipitated from the reaction mixture and was collected by filtration.

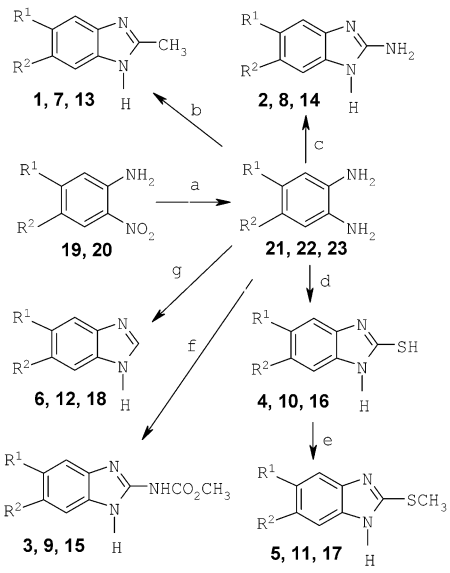
1*H*-Benzimidazole-2(3*H*)-thiones: The method of Van Allan[21] was used for the synthesis of compounds **4**, **10**, and **16**. In this procedure a mixture of the appropriate 1,2-phenylenedimine, ethanol, potassium hydroxide and carbon disulfide was stirred for 2 h at 70 °C. After cooling, the mixture was neutralized with 20% acetic acid solution, and the crude benzimidazole was isolated by filtration.

2-Methylthio-1*H*-benzimidazoles: Compounds **5**, **11**, and **17** were prepared as described by Iddon.[22] A mixture containing ethanol, potassium hydroxide, water and the 1*H*-Benzimidazole-2(3*H*)-thiones (**4**, **10**, and **16**) was cooled to −5 °C. Then methyl iodide was added and the mixture was stirred at room temperature for 1 h, the solid formed was collected by filtration.

Solid compounds were purified by recrystallization. The structure of **1**–**18** was established by spectroscopic and spectrometric data (Scheme 1).

Biological Assays

The biological assays for compounds **1**–**18** were carried out exactly as recently described.[17] These included: subculture methods to determine the susceptibility of *G. lamblia* and *E. histolytica* to these compounds, MTT/PMS assay to calculate the percentage of viability reduction of *T. spiralis* induced by **1**–**18** and measurement of the inhibition of tubulin polymerization after the incubation with **1**–**18**. In this case, *G. lamblia* isolate IMSS:1090:1 was used.



Scheme 1. Synthesis of 1*H*-benzimidazole derivatives (**1**–**18**): (a) H_2, Raney-nickel/THF; (b) CH_3CO_2H; (c) BrCN/EtOH; (d) CS_2/KOH; (e) CH_3I/EtOH; (f) 1-carbomethoxy-2-methyl-2-thiopseudourea; (g) HCO_2H. R^1 and R^2 have the same meaning as in Table 1.

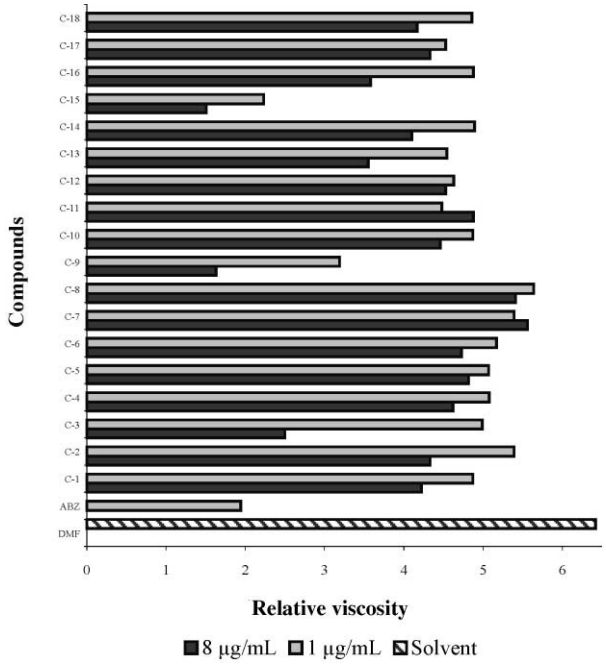


Figure 1. Effect of compounds **1**–**18** and Albendazole on tubulin polymerization.

Results and Discussion

Compounds **1**–**18** were obtained as white solids with sharp melting points. The spectroscopic and spectrometric data were consistent with the expected structure.[25–42] Results of the biological assays against *G. lamblia*, *E. histolytica* and *T. spiralis* are shown in Table 1.

With the exception of **2**, all compounds were more active against *G. lamblia* than Metronidazole, especially compounds **6**, with a hydrogen at 1-position and no substitution in the benzenoid ring and **11**, with a methylthio group at the 2-position and a chlorine at the 5-position. These compounds were 152.5 and 244 times more active than Metronidazole and 4.6 and 7.4 times more active than Albendazole, respectively. Compounds **4**, **5**, and **8**, were as active as Albendazole. It is interesting to note the effect of the 2-mercapto group in **4**, **10**, and **16**, which maintained the same order of magnitude of activity in spite of the pattern of substitution in the 5, 6 positions.

With respect to the activity against *E. histolytica*, most of the compounds were more active than Metronidazole. The most potent compounds tested, **1** and **10**, were 50 and 70 times more potent than Metronidazole, respectively. Compounds **3**, **9**, and **15**, with a 2-methoxycarbonylamino group, such as Albendazole, were more active than this drug, indicating that large groups at the 5(6)-position drastically decrease the activity against this parasite.

In contrast with antiprotozoal action, the anthelmintic activity of some of these compounds was moderate. Although none of them was as active as Albendazole, the 5(6) monosubstitution in the benzenoid ring, as in Albendazole and the 5,6 disubstitution (**15**–**18**) led to active compounds. This moderate activity is observed regardless of the nature of the substituent at the 2-position.

The tubulin polymerization data shown in Figure 1 indicate that only Albendazole and compounds **3**, **9**, and **15** inhibited the polymerization of tubulin. These results confirm that the requirements for the action on tubulin of the benzimidazole ring is the presence of a hydrogen atom at the 1-position and a methoxycarbonylamino group at the 2-position.[11] Compounds **9** and **15** inhibited the polymerization of tubulin more than compound **3** did, suggesting that the substitution at the 5- and/or 6-position with a chlorine atom improves the interaction. The rest of the compounds studied without the requirements mentioned did not inhibit the polymerization of tubulin. These results show that there is no correlation between the activity against the protozoa or *T. spiralis* with the capacity to inhibit the polymerization of tubulin. This suggests that the antiprotozoal nature of the test BZC compounds is distinctly different[17,23,24] from their action against helminths.

The results so far obtained with compounds **1**–**18** as antiparasitic agents are very promising, since they broaden the knowledge of the activity of these versatile derivatives of benzimidazole, especially about the antiprotozoal activity.

Acknowledgements

We thank CONACyT for financial support in project 25920M, and DGAPA in project IN204998. We are grateful to Rosa Isela del Villar, Georgina Duarte, Margarita Guzmán, and Marisela Gutierrez, from the USAI, Facultad de Química, for the determination of all spectra.

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25. 2-Methyl-1*H*-benzimidazole (**1**). Water (83%); mp 176–178 °C.
26. 2-Amino-1*H*-benzimidazole (**2**). Water (90%); mp 228–229 °C.
27. (1*H*-Benzimidazol-2-yl)carbamic acid methyl ester (**3**). Ethanol (85%); mp 305–307 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.6 (bs, 2H, NH), 7.3 (m, 2H, H-4, H-7), 7.0 (m, 2H, H-5, H-6), 3.7 (s, 3H, CH_3). MS: m/z 191 M^+.
28. *H*-Benzimidazole-2(3*H*)-thione (**4**). DMF (84%); mp 301–303 °C.
29. 2-Methylthio-1*H*-benzimidazole (**5**). Ethanol–water (91%); mp 201–203 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.2 (bs, 1H, NH), 7.4 (m, 2H, H-4, H-7), 7.1 (m, 2H, H-5, H-6), 2.7 (s, 3H, CH_3). MS: m/z 164 M^+.
30. *H*-Benzimidazole (**6**). Water (80%); mp 172–173 °C.
31. 5(6)-Chloro-2-methyl-1*H*-benzimidazole (**7**). Acetone–water (80%); mp 209–211 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.3 (bs, 1H, NH), 7.5 (d, J = 1.8 Hz, 1H, H-4), 7.4 (d, J = 8.7 Hz, 1H, H-7), 7.1 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H, H-6), 2.4 (s, 3H, CH_3). MS: m/z 166 M^+.
32. 2-Amino-5(6)-chloro-1*H*-benzimidazole (**8**). Water (87%); mp 168–170 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.8 (bs, 3H, NH), 7.7 (d, J = 1.8 Hz, 1H, H-4), 7.2 (d, J = 8.2 Hz, 1H, H-7), 7.2 (dd, J = 8.2 Hz, J = 1.8 Hz, 1H, H-6). MS: m/z 167 M^+.
33. [5(6)-Chloro-1*H*-benzimidazol-2-yl] carbamic acid methyl ester (**9**). DMF (54%); mp 296–297 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.7 (bs, 2H, NH), 7.4 (d, J = 1.8 Hz, 1H, H-4), 7.4 (d, J = 8.4 Hz, 1H, H-7), 7.1 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H, H-6), 3.7 (s, 3H, CH_3). MS: m/z 225 M^+.
34. 5(6)-Chloro-1*H*-benzimidazole-2-(3*H*)-thione (**10**). Ethanol–water (53%); mp 327–329 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.7 (bs, 2H, NH), 7.1 (m, 3H, H-4, H-6, H-7). MS: m/z 184 M^+.
35. 5(6)-Chloro-2-methylthio-1*H*-benzimidazole (**11**). Toluene (79%); mp 242–243 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.2 (bs, 1H, NH), 7.4 (d, J = 1.8 Hz, 1H, H-4), 7.3 (d, J = 8.7 Hz, 1H, H-7), 7.0 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H, H-6), 2.74 (s, 3H, CH_3). MS: m/z 198 M^+.
36. 5(6)-Chloro-1*H*-benzimidazole (**12**). Water (81%); mp 125–126 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.7 (bs, 1H, NH), 8.2 (s, 1H, H-2), 7.6 (d, J = 1.8 Hz, 1H, H-4), 7.5 (d, J = 8.7 Hz, 1H, H-7), 7.1 (dd, J = 8.7 Hz, J = 1.8 Hz, 1H, H-6). MS: m/z 152 M^+.
37. 5,6-Dichloro-2-methyl-1*H*-benzimidazole (**13**). Methanol (76%); mp 260–262 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 12 (bs, 1H, NH), 7.7 (s, 2H, H-4, H-7), 2.6 (s, 3H, CH_3). MS: m/z 200 M^+.
38. 2-Amino-5,6-dichloro-1*H*-benzimidazole (**14**). Ethanol–water (86%); mp 260–262 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.9 (bs, 1H, NH), 7.3 (s, 2H, H-4, H-7), 6.5 (sb, 2H, NH_2). MS: m/z 201 M^+.
39. (5,6-Dichloro-1*H*-benzimidazol-2-yl) carbamic acid methyl ester (**15**). Ethanol (80%), mp 250–253 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 11.8 (bs, 2H, NH), 7.6 (s, 2H, H-4, H-7), 3.3 (s, 3H, CH_3). MS: m/z 259 M^+.
40. 5,6-Dichloro-1*H*-benzimidazole-2(3*H*)-thione (**16**). Acetic acid (75%); mp 344–346 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.7 (bs, 2H, NH), 7.3 (d, J = 0.6, 2H, H-4, H-7). MS: m/z 218 M^+.
41. 5,6-Dichloro-2-methylthio-1*H*-benzimidazole (**17**). Ethanol–water (89%); mp 234–235 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.8 (bs, 1H, NH), 7.6 (s, 2H, H-4, H-7), 2.6 (s, 3H, CH_3). MS: m/z 232 M^+.
42. 5,6-Dichloro-1*H*-benzimidazole (**18**). Ethanol (76%), mp 208–209 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 11.2 (bs, 1H, NH), 7.9 (s, 1H, H-2), 7.7 (s, 2H, H-4, H-7). MS: m/z 186 M^+.