Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 187–190

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Synthesis and Antiparasitic Activity of 2-(Trifluoromethyl)-benzimidazole Derivatives

Gabriel Navarrete-Vázquez,[a] Roberto Cedillo,[b] Alicia Hernández-Campos,[a] Lilián Yépez,[b] Francisco Hernández-Luis,[a] Juan Valdez,[a] Raúl Morales,[b] Rafael Cortés,[c] Manuel Hernández[c] and Rafael Castillo[a,*]

[a]*Departamento de Farmacia, Facultad de Química, UNAM, CU, Mexico D.F. 04510, Mexico*
[b]*Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, IMSS, Mexico D.F. Mexico*
[c]*Departamento de Biología Celular, CINVESTAV, IPN. Mexico D.F. Mexico*

Received 23 August 2000; revised 1 November 2000

**Abstract**—2-(Trifluoromethyl)benzimidazole derivatives substituted at the 1-, 5-, and 6-positions have been synthesized and in vitro tested against the protozoa *Giardia lamblia, Entamoeba histolytica*, and the helminth *Trichinella spiralis*. Results indicate that all the compounds tested are more active as antiprotozoal agents than Albendazole and Metronidazole. One compound (**20**) was as active as Albendazole against *T. spiralis*. These compounds were also tested for their effect on tubulin polymerization and none inhibited tubulin polymerization. 

## Introduction

Parasitic infections, such as helminthiosis and protozoosis, are still a major problem in developing countries, affecting mainly the infant population. Recent studies have shown that benzimidazole carbamates, Albendazole and Mebendazole, used mainly as anthelmintic agents, tested in vitro are effective against *Giardia lamblia* and *Trichomonas vaginalis,* but not against *Entamoeba histolytic*a.[1–3] Clinical assays have demonstrated that Albendazole is as effective as Metronidazole for giardiosis.[4–6]

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

Since the sites of action of the different benzimidazole carbamates are the same, these compounds have additive action and show crossed resistance, which may undermine their future therapeutic value.[10]

As part of our search for basic information about the structural requirements for antiprotozoal and anthelmintic activity, we have synthesized the 2-(trifluoromethyl)benzimidazole derivatives **16**–**22** (Table 1). The in vitro antiparasitic activity of these compounds on two intestinal protozoa (*Giardia lamblia* and *Entamoeba histolytica*) and one tissue-dwelling helminth (*Trichinella spiralis*) is presented. The activity of **16**–**22** on rat brain tubulin polymerization is also reported.

## Chemistry

The required substituted 1,2-phenylenediamines (**9**–**15**) were prepared by reduction of the corresponding 2-nitroanilines (**1**–**8**) with $H_2$, Raney-nickel. The product of reduction, without isolation, was cyclocondensed with 50% aqueous trifluoroacetic acid to give the corresponding 2-(trifluoromethyl)benzimidazole derivative (**16**–**22**).[11] Compounds **19** and **22** could also be prepared by treatment of the symmetrical precursors **16** and **18** with iodomethane, respectively (Scheme 1).

*Corresponding author. Fax: +1-52-5-6-22-53-29; e-mail: rafaelc@servidor.unam.mx

0960-894X/01/$ - see front matter 
PII: S0960-894X(00)00619-3

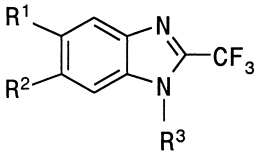
Solid compounds were purified by recrystallization. The structure of the purified compounds was established by spectroscopic and spectrometric data.[19–25]

## Biological Assays

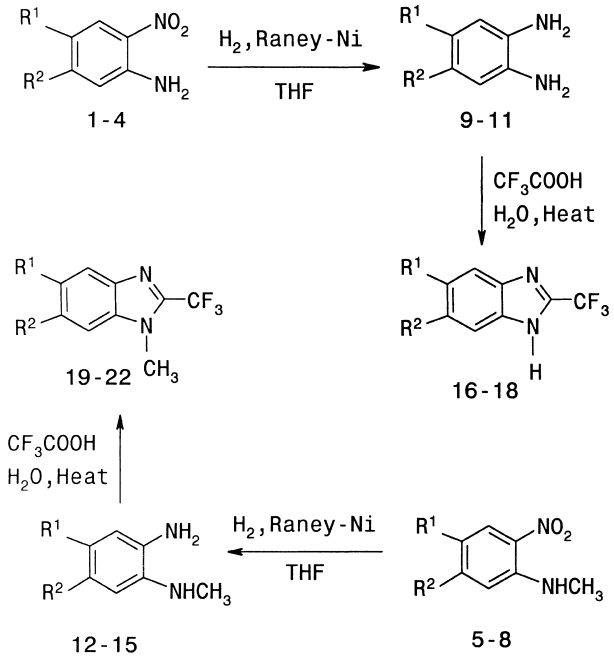
### Culture

*G. lamblia* strain IMSS:0989:1 was cultured in TYI-S-33 modified medium, supplemented with 10% calf serum and bovine bile.[1] *E. histolytica* strain HM1-IMSS was maintained in TYI-S-33 medium supplemented with 10% bovine serum.[12] In vitro susceptibility assays were performed using a method previously described.[1]

**Table 1.** Synthesized 2-(trifluoromethyl)benzimidazoles (**16–22**)



| Compound | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **16** | H | H | H |
| **17** | Cl | H | H |
| **18** | Cl | Cl | H |
| **19** | H | H | $CH_3$ |
| **20** | Cl | H | $CH_3$ |
| **21** | H | Cl | $CH_3$ |
| **22** | Cl | Cl | $CH_3$ |




**Scheme 1.** Synthesis of 2-(trifluoromethyl)benzimidazole derivatives (**16–22**).

Briefly: $5\times10^4$ trophozoites of *G. lamblia* or $6\times10^3$ trophozoites of *E. histolytica* were incubated for 48 h at 37 °C with increasing concentrations of compounds **16–22**, Albendazole and Metronidazole. As the negative control, trophozoites were incubated with solvent (dimethylsulphoxide) 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.

*T. spiralis* muscle larvae (parenteral phase) were obtained according to the procedure of Dennis et al.[13] For the assay, 1000 larvae were placed in culture plates of 24 wells (Nunclon), which contained RPMI 1640 medium, with 0.0001, 0.001, 0.01, 0.1, and 1 μg/mL 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. Albendazole 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.,[14] with some modifications.[15]

### Inhibition of tubulin polymerization assay

Sixty Winstar rats were sacrificed with an intra-peritoneal overdose of sodium pentobarbital (200 mg/kg body weight) and their brains were quickly removed and homogenized in RB buffer (MES 0.1 M, pH 6.8, EGTA 1 mM, $MgCl_2$ 1 mM). Tubulin was purified according to the methods reported by Shelanski.[16] Tubulin polymerization was carry out by low shear falling ball viscosimetry, calibrated previously with glycerol solutions of known viscosity in vitrex capillary tubes (100 μL, 1.3 mm diameter). In this assay, the polymerization of tubulin is indirectly calculated by relative viscosity.[17] The inhibition of polymerization was assessed after the incubation of tubulin with different concentrations of compounds **16–22** and Albendazole. Dimethylformamide (DMF) was used as solvent. Data calculation and analysis were performed as described.[18]

## Results and Discussion

Compounds **16–22** could be obtained in good yields as white solids with sharp melting points. The spectroscopic and spectrometric data are consistent with the expected structures.

Biological assay results shown in Table 2, indicate that, with exception of **17**, all compounds were more active against *G. lamblia* than Metronidazole (drug of choice in the treatment of giardiosis and amoebiasis); compound **20** was as active as Albendazole. In relation to the activity against *E. histolytica*, all compounds were more active than Metronidazole and even Albendazole. It is interesting to mention that compound **21** (6-chloro-1-methyl-2-(trifluoromethyl) benzimidazole) was 43 and

7000 times more active than Metronidazole and Albendazole, respectively (it is known that Albendazole is not active against *E. histolytica*). On the other hand, compounds **19**–**22**, with a methyl group at the 1-position, were as active against *E. histolytica* as **16**–**22** with an H at that position, suggesting that the H at the 1-position is not necessary for antiprotozoal activity. The fact that **19**–**22** had very good activity against *E. histolytica*, an protozoan in which the major cytoskeleton component is actin, instead of tubulin, suggests a different mechanism of action from that of 2-benzimidazole carbamate compounds.

**Table 2.** In vitro susceptibility of *Giardia lamblia* and *Entamoeba histolytica* to compounds **16**–**22**, Metronidazole and Albendazole

| Compound | *G. lamblia* $IC_{50}$ (μM) | *E. histolytica* $IC_{50}$ (μM) |
|---|---|---|
| **Metronidazole** | 1.220 | 0.350 |
| **Albendazole** | 0.037 | 56.330 |
| **16** | 0.107 | 0.069 |
| **17** | 1.282 | 0.022 |
| **18** | 0.078 | 0.011 |
| **19** | 0.064 | 0.040 |
| **20** | 0.042 | 0.046 |
| **21** | 0.127 | 0.008 |
| **22** | 0.260 | 0.033 |

**Table 3.** Percentage of viability reduction of *T. spiralis* muscle larvae after 3 days of incubation with compounds **16**–**22** and Albendazole[a]

| Compound | $3\times10^{-6}$ μM | $3\times10^{-5}$ μM | $3\times10^{-4}$ μM | $3\times10^{-3}$ μM |
|---|---|---|---|---|
| **Albendazole** | 6 | 13 | 20 | 30 |
| **16** | nr[b] | 1 | 3 | 9 |
| **17** | nr | nr | nr | nr |
| **18** | nr | 1 | 9 | 19 |
| **19** | nr | 1 | 9 | 12 |
| **20** | 2 | 9 | 16 | 39 |
| **21** | nr | nr | nr | nr |
| **22** | 2 | 4 | 9 | 15 |

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

**Table 4.** Effect of compounds **16**–**22** and Albendazole in tubulin polymerization

| Compound | Viscosity[a] | | | |
|---|---|---|---|---|
| | $3\times10^{-5}$ μM[b] | $3\times10^{-3}$ μM[b] | 0.015 μM[b] | 0.024 μM[b] |
| **Albendazole** | 7.04 (±0.7) | 2.07 (±0.1) | nd[c] | nd |
| **16** | 7.55 (±0.3) | 7.56 (±0.1) | 7.34 (±0.2) | 6.92 (±0.1) |
| **17** | 6.59 (±0.6) | 6.70 (±0.3) | 7.14 (±0.4) | 6.47 (±0.1) |
| **18** | 7.00 (±0.2) | 7.03 (±0.1) | 6.68 (±0.7) | 7.22 (±0.1) |
| **19** | 6.68 (±0.9) | 7.41 (±0.3) | 7.31 (±0.3) | 7.16 (±0.1) |
| **20** | 7.30 (±0.2) | 7.58 (±0.9) | 6.94 (±0.2) | 7.54 (±0.3) |
| **21** | 6.44 (±0.8) | 7.39 (±0.1) | 7.07 (±0.7) | 7.26 (±0.7) |
| **22** | 6.93 (±0.6) | 7.63 (±0.9) | 6.63 (±0.3) | 6.94 (±0.7) |

[a]Tubulin viscosity after the polymerization assay, without any compound or Albendazole = 7.66 (it represents 100% polymerization). The incubation of tubulin with the solvent used, DMF 1% = 7.42.
[b]Values are means of three experiments, standard deviation is given in parentheses.
[c]nd, not determined

Regarding the anthelmintic activity of **16**–**22**, it is interesting to note that **20** had similar activity than Albendazole against *T. spiralis* muscle larvae, and that this compound also showed a very good activity against both protozoa tested (Table 3).

The tubulin polymerization data shown in Table 4 indicate that Albendazole inhibited the polymerization of tubulin at low concentrations, whereas **16**–**22** did not upset polymerization of tubulin, even at high concentrations. These results support the idea that one of the requirements for the action on tubulin of the 5(6)-substituted benzimidazole is the presence of an hydrogen atom at the 1-position and a methoxycarbonylamino at the 2-position.[9] Compounds **16**–**18** have an H in the 1-position but do not possess a methoxycarbonylamino at the 2-position, and compounds **19**–**22** do not bear any of the radicals mentioned in these positions. These facts suggest that the mechanism of action of carbamate benzimidazoles is different of that of compounds **16**–**22**. It means that the hydrogens in the 1- and 2-positions are important for the binding of benzimidazoles to tubulin but not for the antiparasitic effect.

The results obtained with compounds **16**–**22** as antiprotozoal agents are very promising, since they broaden the knowledge about the activity of these versatile derivatives of benzimidazole. It is also important to emphasize that the results of inhibition of polymerization of tubulin give us a good idea about the differences in the mode of action of different benzimidazoles. Further studies in this area are in progress in our laboratory.

## Acknowledgements

We thank CONACyT for financing project 25920M, and DGAPA IN204998. We are grateful to Rosa Isela del Villar, Georgina Duarte, Margarita Guzmán, and Marisela Gutiérrez from School of Chemistry, UNAM, for the determination of all spectra. Also, we thank Amparo Tapia for carrying out the biological assays.

## References and Notes

1. Cedillo-Rivera, R.; Muñoz, O. *J. Med. Microbiol.* **1992**, *37*, 221.
2. Chavez, B.; Cedillo-Rivera, R.; Martinez-Palomo, A. *J. Protozool.* **1992**, *39*, 510.
3. Fears, S. D.; O'Jare, J. *Antimicrob. Agents Chemother.* **1998**, *32*, 144.
4. Hall, A.; Nahar, Q. *Trans. Roy. Soc. Trop. Med. Hyg.* **1993**, *87*, 84.
5. Romero-Cabello, R.; Robert, L.; Muñoz-Garcia, R.; Tanaka, J. *Rev. Lat.-Amer. Microbiol.* **1996**, *37*, 315.
6. Rodriguez-Garcia, R.; Aburto-Bandala, M.; Sanchez-Maldonado, M. *Bol. Med. Hosp. Infant. Mex.* **1996**, *53*, 173.
7. Friedman, P. A.; Platzer, E. G. *Biochem. Biophys. Acta* **1990**, *630*, 271.
8. Reynoldson, J. A.; Thompson, R. C.; Horton, R. J. *Parasitol. Today* **1992**, *9*, 150.
9. Lacey, E. *Int. J. Parasitol.* **1998**, *18*, 885.

10. Singh, S.; Sharma, S. *Med. Res. Rev.* **1991**, *11*, 581.
11. Holan, G.; Samuel, E. L.; Ennis, B. C.; Hinde, R. W. *J. Chem. Soc.* **1967**, 20.
12. Chavez, B.; Espinoza, M.; Cedillo, R.; Ramirez, A.; Martinez, A. *Arch. Med. Res.* **1992**, *23*, 63.
13. Dennis, D. T.; Despommier, D. D.; Davis, N. *J. Parasitol.* **1970**, *56*, 974.
14. Townson, D. H.; Morris, D. L. *Trans. Roy. Soc. Trop. Med. Hyg.* **1989**, *83*, 664.
15. Cedillo-Rivera, R.; Ramirez, A.; Muñoz, O. *Arch. Med. Res.* **1992**, *23*, 59.
16. Shelanski, M. L.; Gaskin, F.; Cantor, Ch. R. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 765.
17. MacLean-Fletcher, S. D.; Pollard, T. D. *J. Cell. Biol.* **1980**, *85*, 414.
18. Pollard, T. D. *Meth. Cell. Biol.* **1982**, *24*, 301.
19. 2-(Trifluoromethyl)-1*H*-benzimidazole (**16**). Ethanol–water (yield 80.8%); mp 209–211 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.218 (bs, 1H), 7.754 (m, 2H), 7.412 (m, 2H); MS: $m/z$ 186 $M^+$.
20. (6)-Chloro-2-(trifluoromethyl)-1*H*-benzimidazole (**17**). Column chromatography (yield, 68.03%); mp 197–199 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 13.41 (bs, 1H), 7.67 (bs, 2H), 7.292–7.38 (dd, 1H, $J$ = 8.6 Hz); MS: $m/z$ 220 $M^+$.
21. 5,6-Dichloro-2-(trifluoromethyl)-1*H*-benzimidazole (**18**). Toluene (yield 84.5%); mp 236–238 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.692 (s, 1H), 8.004 (s, 1H); MS: $m/z$ 220 $M^+$.
22. 1-Methyl-2-(trifluoromethyl)benzimidazole (**19**). Ethanol–water (yield 85.2%); mp 98–100 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.8 (d, 1H), 7.76 (d, 1H), 7.465 (t, 1H), 7.365 (t, 1H), 3.91 (s, 3H); MS: $m/z$ 200 $M^+$.
23. 5-Chloro-1-methyl-2-(trifluoromethyl)benzimidazole (**20**). Petroleum ether (yield 80.7%); mp 108–109 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.85 (d, 1H, $J$ = 2 Hz), 7.42 (dd, 1H, $J$ = 8 Hz), 7.371 (dd, 1H, $J$ = 8 Hz), 3.945 (s, 3H); MS: $m/z$ 234 $M^+$.
24. 6-Chloro-1-methyl-2-(trifluoromethyl)benzimidazole (**21**). Cyclohexane (yield 81.5%); mp 158–160 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.77 (d, 1H, $J$ = 8 Hz), 7.44 (d, 1H, $J$ = 2 Hz), 7.33 (dd, 1H, $J$ = 8 Hz), 3.915 (s, 3H); MS: $m/z$ 234 $M^+$.
25. 5,6-Dichloro-1-methyl-2-(trifluoromethyl) benzimidazole (**22**). Ethanol (yield 82.1%); mp 164–165 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 7.94 (s, 1H), 7.56 (s, 1H) 3.94 (s, 3H); MS: $m/z$ 268 $M^+$.