



Synthesis and SAR of alkanediamide-linked bisbenzamidines with anti-trypanosomal and anti-pneumocystis activity

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ABSTRACT

A series of alkanediamide-linked bisbenzamidines was synthesized and tested in vitro against a drug-sensitive strain of *Trypanosoma brucei brucei*, a drug-resistant strain of *Trypanosoma brucei rhodesiense* and *Pneumocystis carinii*. Bisbenzamidines linked with longer alkanediamide chains were potent inhibitors of both strains of *T. brucei*. However, bisbenzamidines linked with shorter alkanediamide chains were the most potent compounds against *P. carinii*. *N,N'*-Bis[4-(aminoiminomethyl)phenyl] hexanediamide, **4** displayed potent inhibition ($IC_{50} = 2-3$ nM) against *T. brucei* and *P. carinii*, and was non-cytotoxic in the A549 human lung carcinoma cell line. The inhibitory bioactivity was significantly reduced when the amide groups in **4** were moved from the *para* to the *meta* positions or replaced with amides.

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The benzamidine scaffold is an important structural motif found in many bioactive compounds exhibiting antifungal,^{1,2} antiparasitic,^{3,4} anticoagulant,⁵ antitumor⁶ or antiviral⁷ properties. The two benzamidine units in the antimicrobial drug, pentamidine, are attached with a flexible pentyldioxy chain (Fig. 1). Pentamidine is clinically used in the treatment of pneumonia caused by the opportunistic fungus, *Pneumocystis jirovecii*, early stage human African trypanosomiasis (HAT) and antimony-resistant Leishmaniasis. However, its clinical use is hampered by lack of oral bioavailability, and serious adverse effects including hypotension, nephrotoxicity, abdominal pain, pancreatic complications, cardiotoxicity, and hypoglycemia which may progress to insulin-dependent diabetes mellitus.^{4,8} In recent years, a large number of pentamidine analogs have been synthesized with the goals of enhancing its useful therapeutic properties, improving its oral bioavailability, and reducing the undesirable adverse effects.^{1,3,4,9–13} The design strategy in many of these studies has focused on modification of the central pentyldioxy chain, the two aromatic moieties, or the terminal amide groups.

There is clearly an urgent need to develop more effective and safer drugs for the treatment of *P. jirovecii* pneumonia, HAT and Leishmaniasis. The current chemotherapeutic agents suffer from drawbacks such as severe toxicities, limited efficacy, poor oral bioavailability or emergence of microbial resistance. These infectious diseases threaten millions of people worldwide, and are the major cause of human suffering and economic burden, especially in developing countries where these diseases are more prevalent. In

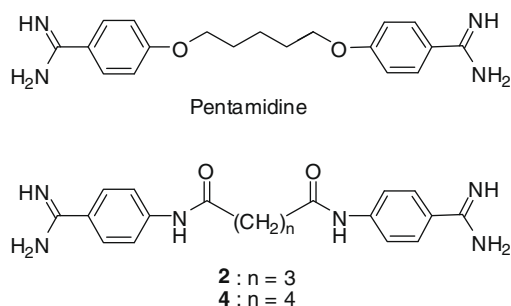


Figure 1. Structures of pentamidine and lead compounds **2** and **4**.

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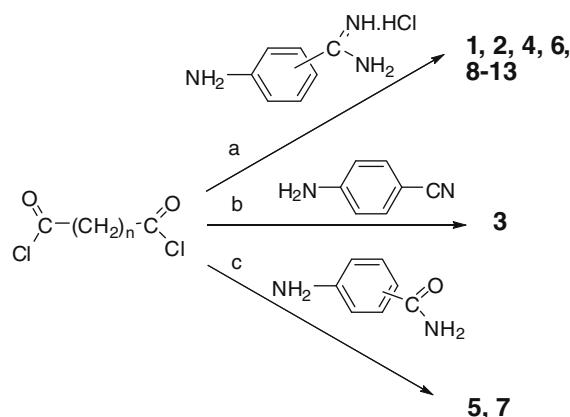
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recent years, the cases of patients dually infected with HIV and parasitic infections, such as Leishmaniasis, have been growing worldwide.¹⁴ This is of concern since coinfections of HIV with *Leishmania* exert a synergistic detrimental effect on the host cellular immune response, reduce the effectiveness of therapies, greatly increases the risk of relapse and mortality rate.¹⁴

We previously reported on the potent anti-*Pneumocystis carinii* activity of a series of bisbenzamidines linked by various linkers.^{6,15} From these studies, bisbenzamidines linked by a pentanediamide **2** or hexanediamide **4** chain (Fig. 1), emerged as exceptionally potent agents with limited cytotoxicity to mammalian cells. Screening of these compounds against various protozoal parasites showed excellent in vitro activity against *Trypanosoma brucei*. Consequently, compounds **2** and **4** served as lead compounds for the synthesis of the 11 analogs reported in this Letter. These compounds are structurally related to pentamidine, in which the strong electron-donating ether functions of the pentyldioxy chain in pentamidine has been replaced by poor electron-donating amide functions separated by alkanes of increasing chain length (Fig. 1). The effect of moving the terminal bisamidine functions in **4** from the para to the meta positions of the phenyl rings on bioactivity was also investigated.

The synthesized compounds were tested in vitro against a pentamidine-sensitive strain of *Trypanosoma brucei* *brucei* LAB 110 EA-TRO^{16,17}, a drug-resistant strain (melarsoprol, pentamidine, and berenil resistant) of *Trypanosoma brucei* *rhodesiense* KETRI 243^{16,17} and *P. carinii*.¹⁸ The cytotoxicity of the compounds was evaluated with the A549 human lung carcinoma cell line.¹⁸ The biological data is reported in Table 1.

The synthesis of these compounds is accomplished as shown in Scheme 1. Starting with the appropriate diacid chlorides and aminobenzamidine or aminobenzonitrile or aminobenzamide, the resultant final compounds **1–13** were readily obtained in fair yields. The average yields for the bisbenzamidines (**1, 2, 4, 6, 8–13**) and bisbenzamides (**5, 7**) were 40% and 90%, respectively. The chemical structures of the synthesized compounds were confirmed by their spectral data (¹H NMR and FTIR) and their purity



Scheme 1. General procedures for the synthesis of compounds **1–13**. Reagents and conditions: (a) DMF, pyridine, reflux, 30 min to 2 h; (b) and (c) Dioxane, rt, stirred overnight.

was verified by elemental analyses. Representative synthesis and characterization of compounds **2, 3**, and **5** are described.¹⁹

The alkanediamide-linked bisbenzamidines were generally more active against *T. brucei* than *P. carinii* with the exception of compounds **1, 2**, and **4**. Four of the bisbenzamidines, which are linked with a hexanediamide **4**, heptanediamide **9**, octanediamide **10** or decanediamide **12** demonstrated potent activity (IC_{50} = 0.001–0.004 μ M) against both the sensitive and resistant strains of *T. brucei*. The potency of these compounds approached that of pentamidine (IC_{50} = 0.002 μ M). The most active compounds generally showed similar potency against both strains of *T. brucei* with the exception of compound **2** which was about 11-fold less active against the resistant strain. The results indicate that bisbenzamidines linked with longer alkanediamide chains were more active as antitrypanosomal agents. However, those compounds with shorter alkanediamide chains, namely **1, 2**, and **4** were the most active agents against *P. carinii*. The potency of compound **1**

Table 1

Structure, cytotoxicity, and in vitro inhibitory bioactivity of compounds **1–13** against *Trypanosoma brucei* and *Pneumocystis carinii*

| Compd | n | R | R' | IC_{50} (μ M) ^a | | | | Cytotox. A549 ^e |
|-------------|----|------------------------------|------------------------------|-----------------------------------|--------------------------------------|--------------------------------|--|----------------------------|
| | | | | <i>T.b. brucei</i> ^b | <i>T.b. rhodesiense</i> ^c | <i>P. carinii</i> ^d | | |
| 1 | 2 | <i>p</i> -Am | <i>p</i> -Am | 9.0 | 2.19 | 0.442 | | >44.2 |
| 2 | 3 | <i>p</i> -Am | <i>p</i> -Am | 0.009 | 0.096 | 0.003 ^f | | 2276 |
| 3 | 3 | <i>p</i> -CN | <i>p</i> -CN | 6.50 | 7.90 | NT ^g | | NT |
| 4 | 4 | <i>p</i> -Am | <i>p</i> -Am | 0.003 | 0.002 | 0.002 ^f | | 1193 |
| 5 | 4 | <i>p</i> -CO-NH ₂ | <i>p</i> -CO-NH ₂ | 2.80 | 1.10 | >100 | | NT |
| 6 | 4 | <i>m</i> -Am | <i>m</i> -Am | 0.041 | 0.021 | 33.7 | | NT |
| 7 | 4 | <i>m</i> -CO-NH ₂ | <i>m</i> -CO-NH ₂ | NT | 1.97 | >100 | | >261 |
| 8 | 4 | <i>p</i> -Am | <i>m</i> -Am | 0.012 | 0.007 | 2.27 | | >227 |
| 9 | 5 | <i>p</i> -Am | <i>p</i> -Am | 0.002 | 0.002 | 2.37 | | >237 |
| 10 | 6 | <i>p</i> -Am | <i>p</i> -Am | 0.003 | 0.001 | 1.95 | | 176 |
| 11 | 7 | <i>p</i> -Am | <i>p</i> -Am | 0.400 | 0.240 | 2.44 | | 2.87 |
| 12 | 8 | <i>p</i> -Am | <i>p</i> -Am | 0.002 | 0.004 | 4.28 | | 249 |
| 13 | 10 | <i>p</i> -Am | <i>p</i> -Am | 0.008 | 0.007 | 3.07 | | 18 |
| Pentamidine | | | | 0.002 | 0.002 | 0.50 ^f | | 24.0 ^f |

^a Each value is the average of at least two determinations.

^{b,c} The *Trypanosoma brucei* *brucei* strain was Lab 110 and the *Trypanosoma brucei* *rhodesiense* strain was KETRI 243 (see Refs. 16 and 17).

^d *Pneumocystis carinii* was obtained from rat lungs (see Ref. 18).

^e Cytotoxicity with A549 epithelial lung cell monolayers from human carcinoma (see Ref. 18).

^f Data obtained from Ref. 15.

^g NT, not tested.

(IC₅₀ = 0.442 μ M) was equivalent to pentamidine (IC₅₀ = 0.50 μ M), whereas, compounds **2** (IC₅₀ = 0.003 μ M) and **4** (IC₅₀ = 0.002 μ M) were 167–250-fold more potent than pentamidine. The remaining compounds were significantly less active than pentamidine. The bioactivity against *T. brucei* or *P. carinii* was reduced by at least 10-fold or greater if the bisamidine groups in **4** were moved from the para to the meta positions as in **6** or replaced with amides as in **5**.

In addition to high potency against *T. brucei* or *P. carinii*, several bisbenzamidines exhibited very low cytotoxicity against the A549 cell line, namely, **2**, **4**, **8**, **9**, **10**, and **12**. The selectivity index of these compounds for *T. brucei* (SI_{T.b}) or *P. carinii* (SI_{P.c}) can be defined as the ratio of the cytotoxic IC₅₀ to the *T. brucei* or *P. carinii* IC₅₀ values. Using this definition, the most selective compounds in this group against *P. carinii* were compounds **2** (SI_{P.c} = 758,667) and **4** (SI_{P.c} = 596,500), respectively. The selectivity of compounds **2**, **4**, **8**, **9**, **10**, and **12** for *T. brucei* ranged from 18,917 to 596,500. The selectivity indexes of pentamidine for *P. carinii* and *T. brucei* were 48 and 12,000, respectively. This indicates that several of the compounds in this series are highly selective for the pathogenic cells (*P. carinii* and *T. brucei*) than for the mammalian cells, and their selectivity profiles are superior to that of pentamidine. Because of their high potency against *P. carinii* and low cytotoxicity, compounds **2** and **4** were further evaluated in an immunosuppressed mouse model of Pneumocystosis.¹ When evaluated at different doses (5, 10, 20, and 40 mg/kg), compound **2** emerged as the most promising anti-Pneumocystis drug. This compound was highly efficacious against the infection at 20 mg/kg and 40 mg/kg doses, with >1000-fold reductions in organism burden, and resulted in improved survival curves versus those for pentamidine-treated mice at the same doses.

The mechanism of action of bisbenzamidines has been attributed to strong binding to DNA at AT rich sites followed by subsequent inhibition of DNA-dependent enzymes and/or direct inhibition of transcription.^{8,20–22} In this series of compounds, the introduction of poor electron-donating amide groups in the central linker resulted in significant reduction in DNA binding compared to pentamidine. The binding (ΔT_m) of pentamidine to poly (dA-dT) was 21.6 °C whereas the values ranged from 7.5 °C to 14.4 °C for the other bisbenzamidines reported here (data not shown). Although compounds that demonstrated strong potency against *P. carinii* were strong binders to DNA (ΔT_m = 11.4–14.4 for compounds **1**, **2** and **4**), several other poor inhibitors of *P. carinii* (e.g., **6** and **10**) also showed strong binding to DNA (ΔT_m = 13.8 °C and 13.2 °C, respectively). These results are consistent with our previous reports^{3,6,17,18,23,24} which indicated that the anti-*P. carinii* or antitrypanosomal activity of a large number of bisbenzamidines synthesized in our laboratory do not correlate directly with their DNA binding affinity.

In conclusion, we have synthesized and evaluated the structure–activity relationships of a series of alkanediamide-linked bisbenzamidines as potential new agents against *T. brucei* and *P. carinii*. The high selectivity indexes of these compounds against both pathogens warrant further investigations especially in animal models of trypanosomiasis. We will report the findings of such investigations in the near future.

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- The compounds reported in this study were synthesized in the laboratory of Professor Tien Huang at Xavier University of Louisiana (New Orleans, LA). *N,N'*-Bis[4-(aminoiminomethyl)phenyl] pentanediamide, dihydrochloride salt, (**2**) was synthesized by heating under reflux for 30 min a mixture of 4-aminobenzonitrile monohydrochloride (2.08 g, 10 mmol), glutaryl chloride (0.64 mL, 5 mmol), and pyridine (8 mL, 100 mmol) in *N,N*-dimethyl formamide (40 mL). The precipitate was filtered and thoroughly washed successively with water and acetone. Yield: 50%. Mp: >300 °C (decomp.). ¹H NMR (DMSO-*d*₆): 10.5 (s, 2H); 9.1 (br s, 8H); 7.8 (dd, 8H); 2.5 (t, 4H); 1.9 (q, 2H) ppm. IR: 3094; 1923; 1659; 1604; 1351 cm⁻¹. Anal. Calcd for C₁₉H₂₂N₆O₂·2HCl (438.13): C, 51.94; H, 5.51; N, 19.13. Found: C, 51.79; H, 5.31; N, 18.89. (M-H-W Laboratories, Phoenix, AZ). *N,N'*-Bis[4-(cyanophenyl)pentanediamide, (**3**) was synthesized by stirring at room temperature overnight a mixture of 4-aminobenzonitrile (9.25 g, 80 mmol), and glutaryl chloride (3.38 g, 20 mmol) in dioxane (200 mL). The precipitate was filtered and washed successively with water, ethanol and ether. Yield: 68%. Mp: 238–239 °C. ¹H NMR (DMSO-*d*₆): 10.3 (s, 2H); 7.7 (2d, 8H, *J* = 8 Hz); 2.4 (t, 4H); 1.9 (q, 2H) ppm. IR: 3264; 2221; 1667; 1592; 1506; 1442 cm⁻¹. Anal. Calcd for C₁₉H₁₆N₄O₂ (332.36): C, 68.66; H, 4.85; N, 16.86. Found: C, 68.83; H, 5.00; N, 16.69. (M-H-W Laboratories, Phoenix, AZ). *N,N'*-Bis[4-(aminocarbonyl)phenyl]hexanediamide, (**5**) was synthesized by stirring at room temperature overnight a mixture of 4-aminobenzonitrile (5.44 g, 40 mmol), and adipoyl chloride (1.83 g, 10 mmol) in dioxane (100 mL). The precipitate was filtered and washed successively with water and ethanol. Yield: 90%. Mp: >300 °C. ¹H NMR (DMSO-*d*₆): 10.1 (s, 2H); 7.8 (d, 4H, *J* = 7 Hz); 7.8 (s, 2H); 7.6 (d, 4H, *J* = 8 Hz); 7.2 (s, 2H); 2.4 (br m, 4H); 1.6 (m, 4H) ppm. IR: 3371; 3317; 3171; 1672; 1651; 1620; 1512 cm⁻¹. Anal. Calcd for C₂₀H₂₂N₄O₄ (382.41): C, 62.82; H, 5.80; N, 14.65. Found: C, 62.75; H, 5.76; N, 14.53. (M-H-W Laboratories, Phoenix, AZ).
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