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Antileishmanial Activities of Several Classes of Aromatic Dications

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Aromatic dicationic molecules possess impressive activity against a broad spectrum of microbial pathogens, including *Pneumocystis carinii*, *Cryptosporidium parvum*, and *Candida albicans*. In this work, 58 aromatic cations were examined for inhibitory activity against axenic amastigote-like *Leishmania donovani* parasites. In general, the most potent of the compounds were substituted diphenyl furan and thiophene dications. 2,5-Bis-(4-amidinophenyl)thiophene was the most active compound. This agent displayed a 50% inhibitory concentration (IC_{50}) of 0.42 ± 0.08 μM against *L. donovani* and an in vitro antileishmanial potency 6.2-fold greater than that of the clinical antileishmanial dication pentamidine and was 155-fold more toxic to the parasites than to a mouse macrophage cell line. 2,4-Bis-(4-amidinopheny)furan was twice as active as pentamidine (IC_{50}, 1.30 ± 0.21 μM), while 2,5-bis-(4-amidinopheny)furan and pentamidine were essentially equipotent in our in vitro antileishmanial assay. Carbazoles, dibenzofurans, dibenzothiophenes, and benzimidazoles containing amidine or substituted amidine groups were generally less active than the diphenyl furans and thiophenes. In all cases, aromatic dications possessing strong antileishmanial activity were severalfold more toxic to the parasites than to a cultured mouse macrophage cell line. These structure-activity relationships demonstrate the potent antileishmanial activity of several aromatic dications and provide valuable information for the future design and synthesis of more potent antiparasitic agents.

Protozoal parasitic diseases continue to pose serious public health problems in developing parts of the world. One such disease is leishmaniasis, a spectrum of disease in humans that is caused by several species of the *Leishmania* parasite. These unicellular organisms are related to trypanosomes, the pathogenic parasites responsible for sleeping sickness in Africa and Chagas' disease in South America. Three main clinical variants of leishmaniasis are known: cutaneous, mucocutaneous, and visceral, with symptomatic visceral disease often ending in death if treatment is not provided. In recent years, the coexistence of human immunodeficiency virus and *Leishmania* species causing visceral disease has resulted in several hundred cases of dually infected individuals (4). By current estimates, leishmaniasis affects people in 88 countries, with 350 million at risk of contracting the disease and approximately 2 million new cases each year (www.who.int/emc/diseases/leish/leisdis1.html). The devastating impact of this disease is exemplified by the epidemic of visceral leishmaniasis that occurred in the 1990s in the Sudan (27).

Pentavalent antimonial compounds have been the first-line drugs for leishmaniasis since the 1940s, and two forms of Sb(V) are commonly used. Sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime) are prescribed according to Sb(V) content and are generally considered to be equivalent in terms of efficacy and toxicity. These drugs must be given by injection over a 20- to 28-day course, and common side effects include nausea, hepatotoxicity, and cardiotoxicity (4). Reports of unresponsiveness to antimony treatment have become frequent (24), and a strong correlation between clinical resistance to sodium stibogluconate and decreased in vitro susceptibility to this drug has been reported (20). Amphotericin B is also used as a treatment for visceral leishmaniasis. Past implementation of this drug was limited by toxic side effects, including fever, bone pain, and decreased renal function. New clinical formulations of amphotericin B in lipid complexes have proven to be less toxic than amphotericin B but are more costly, a major problem in treating visceral leishmaniasis in developing countries. Also, amphotericin B and amphotericin B-lipid complexes do not appear to be suitable for treatment of nonvisceral disease (4). Oral miltefosine has shown promise in the treatment of visceral leishmaniasis (18, 31), but no reports of efficacy against cutaneous disease have appeared and the drug has not yet been approved for routine use. Pentamidine is also used frequently for the treatment of leishmaniasis (16, 30). The drawbacks of the clinical use of pentamidine are the route of administration (injection) and the toxicity of the compound. Administration by injection increases the expense of the treatment and makes the use of the drug less practical in developing nations, where cost is a major factor. The clinical side effects of pentamidine include renal and hepatic toxicity, pancreatitis, hypotension, dysglycemia, and cardiac abnormalities (4, 15). There is a clear and urgent need for the development of improved treatments for leishmaniasis that are safe, inexpensive, and orally available.

Dicationic compounds based on pentamidine have shown

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impressive antimicrobial activity against a variety of organisms (2, 13, 25). Dicationic molecules with improved efficacy and reduced toxicity compared to pentamidine have been reported in animal models of pneumocystosis and cryptosporidiosis (5, 6, 26). Many of these compounds also possess excellent activity against *Leishmania* parasites. The antileishmanial activity of several such dicationic molecules was reported in 1990 (3). Since that time, many new aromatic cations have been synthesized and improved assay systems have been developed for the in vitro testing of candidate drugs against amastigote-like parasites (1, 9, 28, 34). We report here the in vitro evaluation of 58 aromatic cations against axenic amastigote-like *Leishmania donovani*. Some of the dicationic agents proved to be more potent than pentamidine in this assay, indicating that the synthesis of new aromatic dications could lead to the development of more effective antileishmanial agents.

MATERIALS AND METHODS

Cationic compounds. All of the cationic agents tested in this work were synthesized in the laboratories of two of the authors (D.W.B. and R.R.T.) as hydrochloride salts. The synthesis and characterization of 49 of the 58 compounds presented here have been described previously; for the original references for these compounds, see Tables 1 to 4. Synthetic details and characterization of compounds 13 to 16, 23 to 25, 36, and **37**, along with the necessary synthetic intermediates, are given below.

2,5-Bis{4-(*N*-isopropylamidino)phenyl}-3,4-dimethyl furan dihydrochloride (compound 13). A suspension of the bis imidate ester hydrochloride (11) (0.9 g, 0.002 mol) with isopropylamine (0.296 g, 0.005 mol) in 30 ml of dry ethanol was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the solid was dissolved in ~50 ml of water and basified to pH 10 with 2 M NaOH (aqueous). The solid was filtered, washed with water, dried, dissolved in warm ethanol, saturated with HCl gas at 0 to 5°C, and stirred at room temperature for 2 h. After the addition of 30 ml of dry ether, the precipitated salt was filtered, washed with ether, and dried under vacuum at 60°C for 24 h to yield 0.69 g (69%) of a yellow crystalline solid with a melting point of 243 to 247°C dec. ^{1}H nuclear magnetic resonance (NMR) (dimethyl sulfoxide [DMSO]-d_6): 9.64 (brd, 2H), 9.54 (br, 2H), 9.26 (br, 2H), 7.90 (brs, 8H), 4.20 (brm, 2H), 2.28 (s, 6H), 1.31 (d, 12H, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6/D_2O): 161.1, 146.3, 134.5, 128.8, 127.2, 124.9, 122.1, 45.0, 21.0, 9.4. Fast atom bombardment mass spectrometry (FABMS): *m/e* 417 (M++1). Analysis calculated for $C_{26}H_{32}N_4O \cdot 2HCl \cdot 0.65H_2O$ (501.23): C, 62.30; H, 7.09; N, 11.17. Found: C, 62.63; N, 7.07; N, 10.72.

2,5-Bis{4-(*N*-cyclopropylamidino)phenyl}-3,4-dimethyl furan dihydrochloride (compound 14). A suspension of the bis imidate ester dihydrochloride (11) (0.673 g, 0.0015 mol) with cyclopropylamine (0.214 g, 0.00375 mol) in 20 ml of ethanol was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, the solid was suspended in ~50 ml of water, and the pH was adjusted to 10 with 2 M NaOH (aqueous). The solid was filtered, washed with water, dried, dissolved in hot ethanol, saturated with HCl gas at 0°C, and stirred at room temperature for 2 h. After the addition of 30 ml of dry ether, the precipitated salt was filtered, washed with ether, and dried under vacuum at 60°C for 24 h, 0.54 g (72%) to give a yellow crystalline solid with a melting point of 245 to 248°C dec. ^{1}H NMR (DMSO-d_6): 10.17 (br, 2H), 9.8 (br, 2H), 9.22 (br, 2H), 7.96 (d, 4H, J = 8 Hz), 7.89 (d, 4H, J = 8 Hz), 2.88 (brs, 2H), 2.28 (s, 6H), 0.97–0.87 (m, 8H). ^{13}C NMR (DMSO-d_6): 164.0, 146.3, 134.7, 128.8, 126.1, 124.8, 122.3, 24.6, 9.4, 6.4. FABMS: *m/e* 413 (M++1). Analysis calculated for $C_{26}H_{28}N_4O \cdot 2HCl \cdot H_2O$ (503.48): C, 62.25; H, 6.40; N, 11.12. Found: C, 62.11; N, 6.45; N, 11.03.

2,5-Bis{4-(*N*-cyclopentylamidino)-phenyl}-3,4-dimethyl furan dihydrochloride (compound 15). Cyclopentylamine (0.425 g, 0.005 mol) was added to a suspension of the bis imidate ester dihydrochloride (11) (0.9 g, 0.002 mol) in 35 ml of dry ethanol, and this mixture was stirred at room temperature for 12 h. The solvent was distilled under reduced pressure, and the solid was suspended in 50 ml of water and basified to pH 10 with 2 M NaOH (aqueous). The precipitated solid was filtered, washed with water, dried, dissolved in ethanol, saturated with HCl gas at 0 to 5°C, and stirred at room temperature for 2 h. After the addition of 30 ml of dry ether, the precipitated salt was filtered, washed with ether, and dried under vacuum at 50°C for 12 h to yield 0.77 g (70%) of a yellow crystalline solid with a melting point of 280°C dec.; ^{1}H NMR (DMSO-d_6/D_2O): 7.88 (d, 4H, J = 8.4 Hz), 7.83 (d, 4 H, J = 8.4 Hz), 4.19 (m, 2H), 2.26 (s, 6H), 2.08–2.04 (m, 4H), 1.74–1.55 (m, 12H). ^{13}C NMR (DMSO-d_6): 161.9, 146.3, 134.5, 128.9, 127.0, 124.8, 122.1, 54.2, 31.2, 23.5, 9.4. FABMS: *m/e* 468 (M++1). Analysis calculated for $C_{30}H_{36}N_4O \cdot 2HCl \cdot 0.5H_2O$ (550.58): C, 65.44; H, 7.13; N, 10.17. Found: C, 65.02; N, 7.15; N, 9.90.

2,5-Bis{4-(2-tetrahydropyrimidyl)phenyl}-3,4-dimethyl furan dihydrochloride (compound 16). A suspension of the corresponding imidate ester dihydrochloride (11) (0.898 g, 0.002 mol) with 1,3-diaminopropane (0.296 g, 0.004 mol) in 30 ml of dry ethanol was heated at reflux for 12 h. The solvent was distilled under reduced pressure. The solid was suspended in ~50 ml of water, and the pH was adjusted to 10 with 2 M NaOH (aqueous). The solid was filtered, washed with water, and dried to yield a pale yellow crystalline free base (72%) with a melting point of 159 to 160°C. The solid was dissolved in 15 ml of hot ethanol, saturated with HCl gas at 0°C, and stirred at 50°C for 2 h. After the addition of 30 ml of dry ether, the precipitated salt was filtered, washed with ether, and dried under vacuum at 70°C for 24 h to yield 0.77 g (77%) of a yellow crystalline solid with a melting point of 275 to 278°C dec. ^{1}H NMR (DMSO-d_6/D_2O) 7.91 (d, 4H, J = 8.8 Hz), 7.84 (d, 4H, J = 8.8 Hz), 3.05 (t, 8H, J = 6 Hz), 2.26 (s, 6H), 1.99 (q, 4H, J = 6 Hz). ^{13}C NMR (DMSO-d_6/D_2O) 158.8, 146.6, 134.9, 128.3, 126.6, 125.5, 122.8, 38.9, 17.8, 8.8. FABMS: *m/e* 413 (M++1). Analysis calculated for $C_{26}H_{28}N_4O \cdot 2HCl \cdot H_2O$ (503.47): C, 62.07; H, 6.40; N, 11.12. Found: C, 62.09; N, 6.52; N, 10.84.

2,5-Bis{4-(*N*-isopropylamidino)phenyl}-thiophene dihydrochloride (compound 24). 2,5-Bis(4-cyanophenyl)thiophene was prepared by Stille coupling of 4-bromobenzonitrile and 2,5-bis(tributylstannyl)thiophene (19). The bis-nitrile was converted into the corresponding bis imidate ester as previously described (12). Isopropylamine (0.295 g, 0.005 mol) was added to the bis imidate ester dihydrochloride (0.9 g, 0.002 mol) in 30 ml of ethanol and stirred at room temperature for 24 h. The solvent was distilled in a vacuum, the solid was suspended in 50 ml of water and basified to pH 9 with 2 M NaOH (aqueous), and the precipitated free base was filtered, washed with water, and dried in a vacuum at 500°C. The free base was converted into its crystalline yellow dihydrochloride with saturated ethanolic HCl with a melting point of 358 to 600°C dec. ^{1}H NMR (DMSO-d_6/D_2O): 7.84 (d, 4H, J = 8.2 Hz), 7.72 (d, 4H, J = 8.2 Hz), 7.66 (d, 2H), 3.98 (septet, 2H, J = 6.4 Hz), 1.27 (d, 12H, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6/D_2O): 162.1, 143.1, 138.2, 129.5, 128.4, 127.7, 126.1, 45.8, 21.4. FABMS: *m/e* 404 (M + 1). Analysis calculated for $C_{24}H_{28}N_4S \cdot 2HCl$ (427.51): C, 60.37; H, 6.53; N, 11.73. Found: C, 60.62; N, 6.26; N, 11.48.

2(4-Cyanophenyl)thiophene (Fig. 1, c). To a mixture of 2-tributylstannylthiophene (Fig. 1, a) (17.0 g, 0.05 mol) and 4-bromobenzonitrile (Fig. 1b) (9.1 g, 0.05 mol) in 45 ml of dry dioxane (under nitrogen) was added $Pd(PPh_3)_4$ (1.15 g, 2 mol%). The mixture was heated at reflux for 8 h (monitored by thin-layer chromatography [TLC]), the solvent was distilled under a vacuum, and the oily residue was diluted with 100 ml of dichloromethane and 100 ml of 10% KF (aqueous). The mixture was stirred for 1 h, filtered through Celite, and washed with water. The organic layer was dried over Na_2SO_4, filtered, and concentrated under a vacuum, and the resulting oily residue was chromatographed over silica gel (elution with hexane [3:7 hexane-benzene ratio]) to give 7.7 g (83%) of a white solid with a melting point of 85 to 86°C. ^{1}H NMR (DMSO-d_6): 7.81 (brs, 4H), 7.67 (brm, 2H), 7.18 (brm, 1H). ^{13}C NMR (DMSO-d_6): 141.2, 138.0, 132.9, 128.8, 127.9, 126.0, 125.8, 118.6, 109.5. MS: *m/e* 185 (M+). Analysis calculated for $C_{11}H_7NS$ (185.25): C, 71.32; H, 3.81; N, 7.56. Found: C, 71.38; N, 3.87; N, 7.59.

2-Bromo-5-(4-cyanophenyl)thiophene (Fig. 1, d). Bromine (1.76 g, 0.011 mol) in 15 ml of dichloroethane was added in 15 min to a well-stirred solution of 2-(4-cyanophenyl) thiophene (Fig. 1, c) (1.85 g, 0.01 mol) in 50 ml of dichloroethane at 5 to 10°C. The mixture was stirred at room temperature for 2.5 h and then diluted with 50 ml of water and 50 ml of dichloroethane. The organic layer was washed with 5% sodium thiosulfate–5% sodium bicarbonate–water, dried over sodium sulfate, and filtered. The solvent was removed under reduced pressure, and the residual solid was triturated with ether-hexane (1:4) to yield 2.14 g (81%) of an off-white crystalline solid with a melting point of 98 to 99°C. ^{1}H NMR (DMSO-d_6): 7.83 (d, 2H, J = 8.4 Hz), 7.78 (d, 2H, J = 8.4 Hz),7.53 (d, 1H, J = 4 Hz), 7.30 (d, 1H, J = 4 Hz). ^{13}C NMR (DMSO-d_6): 142.7, 136.8, 132.8, 131.9, 126.6, 118.3, 112.9, 109.9. MS: *m/e* 264 (M+). Analysis calculated for $C_{11}H_6BrNS$ (264.14): C, 50.01; H, 2.29; N, 5.30. Found: C, 49.92; N, 2.31; N, 5.16.

2-(3-Cyanophenyl)-5-(4-cyanophenyl)thiophene (Fig. 1, e). Potassium carbonate (3.03 g, 0.022 mol) in 15 ml of water was added to a well-stirred solution of 2-(4-cyanophenyl)-5-bromo-thiophene (Fig. 1, d) (2.64 g, 0.0 1 mol) and 3-cyanophenylboronic acid (1.62 g, 0.011 mol) in 30 ml of *n*-propanol, followed by addition of tetrakis(triphenylphosphine)palladium (0.23 g, 2 mol%). The reaction mixture was heated at reflux for 12 h (under nitrogen), and then the solvent

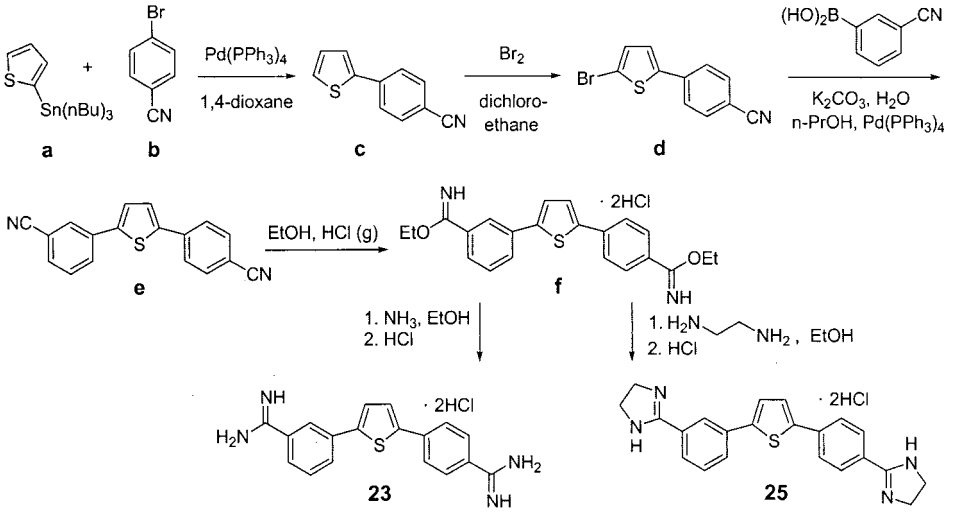


FIG. 1. Synthesis of thiophene dications 23 and 25.

was removed under reduced pressure. The residue was diluted with 50 ml of water, filtered, and dried. The resulting solid was dissolved in 100 ml of chloroform and filtered through Celite, the solvent was removed, and the solid was crystallized from ether-benzene to yield 2.1 g (73%) of a bright yellow crystalline solid with a melting point of 169 to 170°C. ^{1}H NMR (DMSO-d_6): 8.13 (dd, 1H, J = 1.6 Hz), 7.96 (ddd, 1H, J = 0.8 Hz, J = 1.6 Hz, J = 8 Hz), 7.83 (brs, 4H) 7.74 (md, 1H, J = 8Hz), 7.71 (d, 1H, J = 4 Hz), 7.62 (d, 1H, J = 4 Hz), 7.62 (dd, 1H, J = 8 Hz). ^{13}C NMR (DMSO-d_6/D_2O): 142.0, 141.5, 137.2, 134.1, 132.7, 131.1, 129.6, 128.3, 127.2,126.7, 125.5, 118.2, 118.0,112.2, 109.8. MS: *m/e* 286 (M+). Analysis calculated for $C_{18}H_{10}N_2S$ (286.36): C, 75.5; H, 3.52; N, 9.78. Found: C, 75.57; N, 3.62; N, 9.88.

2-{3-(Amidino)phenyl}-5-{4-(amidino)phenyl}-thiophene dihydrochloride (23). The above-described bis-nitrile (Fig. 1, e) (2.86 g, 0.01 mol) in 70 ml of ethanol was saturated with dry HCl gas at 0 to 5°C and then stirred at room temperature for 7 days (monitored by infrared spectroscopy and TLC). Ether was added to the mixture, and the yellow imidate ester dihydrochloride (Fig. 1, f) was filtered and washed with ether. The solid was dried at 40°C for 5 h to yield 4.3 g (95%). The hygroscopic solid was used in the next step without further purification. A suspension of imidate ester dihydrochloride (0.9 g, 0.002 mol) in 35 ml of ethanol was saturated with ammonia gas at 0 to 5°C and stirred at room temperature for 2 days, and the solvent was removed under reduced pressure. The solid was suspended in water, the pH was adjusted to 9, and the pale solid was filtered, washed with water, and dried. The solid was stirred in saturated ethanolic HCl (20 ml) at 50°C for 1 h, diluted with ether, filtered, and dried in a vacuum oven at 75°C for 24 h to yield 0.61 g (78%) of a pale yellow solid with a melting point of 320 to 321°C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.12 (dd, 1H, J = 1.6, J = 8 Hz), 8.01 (brd, 1H, J = 8 Hz), 7.91 (d, 4H, J = 8.8 Hz), 7.88 (d, 4H, J = 8.8 Hz), 7.76 (d, 1H, J = 4 Hz), 7.75 (brd, 1H, J = 8 Hz), 7.72 (d, 1H, J = 4 Hz), 7.69 (dd, 1H, J = 8 Hz). ^{13}C NMR (DMSO-d_6/D_2O): 165.4, 165.0, 143.0, 141.9, 138.5, 134.2, 130.6, 130.3, 129.3, 128.9, 127.5, 127.0, 126.6, 125.6, 125.0. FABMS: *m/e* 321 (M + 1). Analysis calculated for $C_{18}H_{16}N_4S \cdot 2HCl$ (396.35): C, 54.96; H, 4.61, N, 14.24. Found: C, 54.69; H, 4.74; N, 14.02.

2-{3-(2-Imidazolino)phenyl}-5-{4(2-imidazolino)phenyl}thiophene dihydrochloride (compound 25). A suspension of the imidate ester dihydrochloride (Fig. 1, f) (0.9 g, 0.002 mol) described above with ethylenediamine (0.24 g, 0.004 mol) in 30 ml of dry ethanol was heated at reflux for 12 h. The solvent was removed under reduced pressure, the solid was suspended in ~50 ml of water, and the pH was adjusted to 10 with 2 M NaOH (aqueous). The solid was filtered, washed with water, and dried (free base: white solid with a melting point of 250 to 253°C). This solid was dissolved in hot ethanol, saturated with HCl gas at 0°C, and stirred at 50°C for 2 h. After addition of 40 ml of dry ether, the precipitated salt was filtered, washed with ether, and dried under a vacuum at 70°C for 24 h to yield 0.76 g (83.7%) of a pale yellow solid with a melting point of 360 to 362°C dec. ^{1}H NMR (DMSO-d_6/D_2O): 8.27 (dd, 1H, J = 1.6, J = 8 Hz), 8.02 (md, 1H, J = 8 Hz), 7.96 (d, 4H, J = 8.4 Hz), 7.89 (d, 4 H, J = 8.4 Hz), 7.84 (md, 1H, J = 8 Hz), 7.75 (d, 1H, J = 4 Hz), 7.70 (d, 1H, J = 4 Hz), 7.67 (dd, 1H, J = 8 Hz), 4.01 (s, 4H), 3.98 (s, 4H). ^{13}C NMR (DMSO-d_6/D_2O): 165.1, 164.9, 143.2, 142.0, 139.1, 134.5, 131.3, 130.7, 129.8, 127.9, 127.2, 125.9, 125.4, 123.3, 121.1. FABMS: *m/e* 321 (M + 1). Analysis calculated for $C_{22}H_{20}N_4S \cdot 2HCl \cdot 0.5H_2O$ (454.43): C, 58.14; H, 5.10, N, 12.33. Found: C, 58.00; H, 5.16; N, 12.21.

9-Carboethoxymethyl-3,6-dicyanocarbazole. Sodium hydride (60% dispersion in mineral oil, 0.82 g, 0.021 mol) was washed twice in hexane, dried under reduced pressure, and suspended in dry dimethylformamide (50 ml) under N_2. 3,6-Dicyanocarbazole (25) (3.30 g, 0.015 mol) was added, and the mixture was stirred for 20 min. Ethyl bromoacetate (3.6 ml, 0.0325 mol) was added, and the mixture was stirred overnight. The reaction mixture was poured into water (800 ml). The crude product was filtered off, dried, and then purified by suspension in hot acetone (300 ml) to give a white powder (3.19 g, 69.3%) with a melting point of 275 to 277°C. ^{1}H NMR (DMSO-d_6): 8.66 (s, 2H), 7.95 (dd, 2H, J = 8.6 and 1.5 Hz), 7.88 (d, 2H, J = 8.6 Hz), 5.56 (s, 2H), 4.16 (q, 2H, J = 7.1 Hz), 1.21 (t, 3H, J = 7.1 Hz). Analysis calculated for $C_{18}H_{13}N_3O_2 \cdot 0.2H_2O$: C, 70.44; H, 4.40; N, 13.69. Found: C, 70.31; H, 4.35; N, 13.64.

9-Carboethoxymethyl-3,6-diisopropylamidinocarbazole dihydrochloride (compound 36). 9-Carboethoxymethyl-3,6-dicyanocarbazole (3.04 g, 0.01 mol) and anhydrous ethanol (10 ml, 0.17 mol) were added to 1,4-dioxane (125 ml) saturated with hydrogen chloride at −5°C. The stirred reaction mixture was sealed and allowed to warm to room temperature. After 10 days, the reaction mixture was purged with nitrogen and diluted with dry ether. The crude diimidate intermediate (4.40 g, 93.8%) was filtered off under N_2, suspended in anhydrous ethanol (50 ml), and treated with isopropyl amine (19 ml, 0.22 mol, freshly distilled from KOH). The reaction mixture was sealed and stirred overnight. The precipitated product was filtered off, washed with ether, and dried with ether to give a white powder (2.14 g, 43.2%) with a melting point of 250 to 255°C. ^{1}H NMR (DMSO-d_6): 9.68 (d, 2H, J = 8.0 Hz), 9.50 (s, 2H), 9.21 (s, 2H), 8.76 (s, 2H), 7.91 (dd, 4H, J = 8.9 Hz), 5.61 (s, 2H), 4.20 (m, 2H), 4.15 (q, 2H, J = 7.1 Hz), 1.34 (d, 12H, J = 6.3 Hz), 1.21. (t, 3H, J = 7.1 Hz). FABMS: *m/z* 422 (MH^+ for free base). Analysis calculated for $C_{24}H_{31}N_5O_2 \cdot 0.2HCl \cdot 2H_2O$: C, 56.25; H, 6.88; N, 13.67; Cl, 13.84. Found: C, 56.33; H, 6.90; N, 13.72; Cl, 13.73.

4′,5-Dibromo-2-nitrobiphenyl (Fig. 2, i). Copper powder (14.30 g, 225 mg atoms) was added over 10 min to a mechanically stirred molten mixture of 2,4-dibromonitrobenzene (Fig. 2, g) (21) (21.35 g, 0.076 mol) and 1-bromo-4-iodobenzene (Fig. 2, h) (26.68 g, 0.094 mol). The mixture was maintained at 150 to 175°C (bath temperature) for 4 h. The reaction mixture was extracted with ethyl acetate. The extracts were adsorbed onto silica gel (100 g) and chromatographed on a column of the same (1 kg, 8.5 by 40 cm), eluting with 5% ethyl acetate in hexane. Fractions containing product pure by TLC were evaporated to a yellow solid (16.34 g). Recrystallization from ethanol gave yellow crystals (12.03 g, 44.3%) with a melting point of 105°C. ^{1}H NMR ($CDCl_3$): 7.79 (d, 1H, J = 8.7 Hz), 7.65 (dd, 1H, J = 8.7 and 2.1 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.57 (d, 1H, J = 2.1 Hz), 7.17 (d, 2H, J = 8.5 Hz). Analysis calculated for $C_{12}H_7Br_2NO_2$: C, 40.37; H, 1.98; N, 3.92. Found: C, 40.45; H, 1.99; N, 3.86.

2,6-Dibromocarbazole (Fig. 2, j). A solution of 4′,5-dibromo-2-nitrobiphenyl (Fig. 2, i) (17.85 g, 0.050 mol) in triethylphosphite (20 ml, 0.117 mol) was stirred at 165°C (bath temperature) under N_2 for 20 h. The residue was chromatographed on a column of silica gel (600 g, 7 by 40 cm) eluting with 5% ethyl acetate in hexane. Fractions containing product pure by TLC were evaporated to

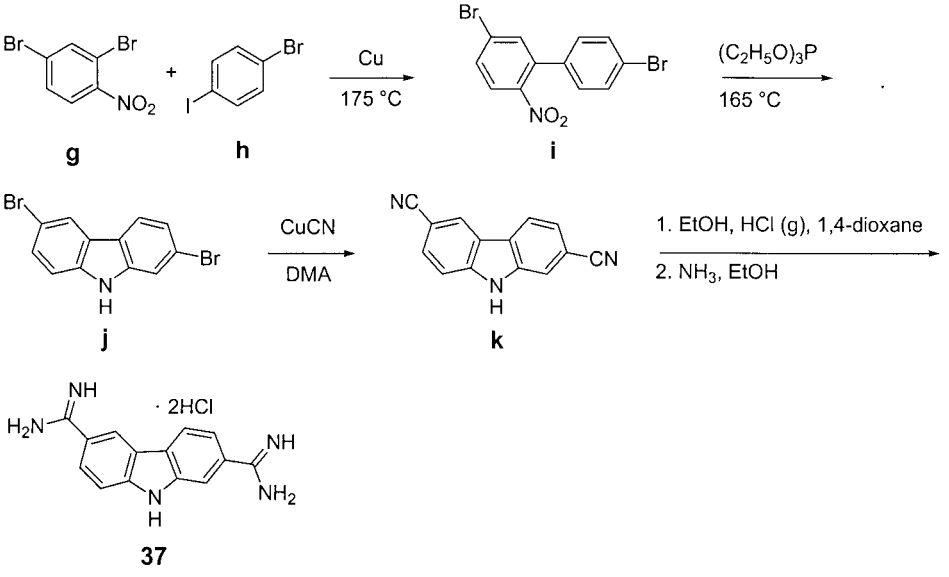


FIG. 2. Synthesis of carbazole diamidine compound 37.

give a white powder (7.88 g, 48.5%) with a melting point of 173 to 174°C. ^{1}H NMR ($CDCl_3$): 8.15 (d, 1H, J = 1.8 Hz), 8.08 (brs, 1 H), 7.86 (d, 1H, J = 8.3 Hz), 7.58 (d, 1H, J = 1.6 Hz), 7.51 (dd, 1H, J = 8.5 and 1.8 Hz), 7.36 (dd, 1H, J = 8.3 and 1.6 Hz), 7.31 (d, 1H, J = 8.5 Hz). EIMS: *m/e* 325 (M^+). Analysis calculated for $C_{12}H_7Br_2N$: C, 44.35; H, 2.17; N, 4.31; Br, 49.17. Found: C, 44.44; H, 2.12; N, 4.25; Br, 49.06.

2,6-Dicyanocarbazole (Fig. 2, k). Copper(I) cyanide (7.54 g, 0.0842 mol) was added to a solution of 2,6-dibromocarbazole (Fig. 2, j) (7.71 g, 0.0237 mol) in *N,N*-dimethylacetamide (100 ml). The mixture was stirred at reflux under N_2 for 18 h. The resulting precipitate was filtered off and stirred for 1.5 h in water containing ethylenediamine (25 ml). The solid was filtered off and stirred for 1 h in water containing NaCN (20 g). The dried crude solid was extracted into $CHCl_3$ in a Soxhlet apparatus. The residue remaining in the thimble (2.4 g) was dissolved in DMSO and filtered through Norit-A. The filtrate was diluted with water to give a precipitate. The solid was filtered off and treated with an aqueous solution containing $FeCl_3$ (10 g) and concentrated HCl (10 ml). The solid was filtered off and dried to give an off-white powder (1.58 g, 30.6%) with a melting point of >300°C. ^{1}H NMR (DMSO-d_6): 12.88 (s, 1H) 8.86 (s, 1H), 8.43 (d, 1H, J = 8.1 Hz), 8.08 (s, 1H), 7.85 (dd, 1H, J = 8.5 and 1.4 Hz), 7.71 (d, 1H, J = 8.5 Hz), 7.65 (dd, 1H, J = 8.1 and 1.2 Hz). Analysis calculated for $C_{14}H_7N_3 \cdot 0.2H_2O$: C, 76.15; H, 3.38; N, 19.03. Found: C, 76.05; H, 3.45; N, 18.98.

2,6-Diamidinocarbazole dihydrochloride (compound 37). 2,6-Dicyanocarbazole (Fig. 2, k) (1.10 g, 0.005 mol) was reacted with anhydrous ethanol (5.0 ml, 0.089 mol) in 1,4-dioxane (75 ml) saturated with hydrogen chloride as described above. After 14 days, the crude diimidate intermediate (2.12 g, 125%) was collected by filtration, suspended in anhydrous ethanol (15 ml), and treated with a solution of ammonia (4.0 g, 0.0023 mol) in ethanol (50 ml). The mixture was sealed and stirred for 62 h. The precipitated product was filtered off and washed with ether to give a tan powder (1.23 g, 75%) with a melting point of >300°C. ^{1}H NMR (DMSO-d_6): 9.39 (brs, 7H), 8.91 (s, 1H), 8.39 (d, 1H, J = 8.1 Hz), 8.08 (s, 1H), 7.96 (d, 1H, J = 8.5 Hz), 7.76 (d, 1H, J = 8.5 Hz), 7.70 (d, 1H, J = 8.1 Hz. FABMS: *m/e* 252 (MH^+ of free base). Analysis calculated for $C_{14}H_{13}N_5 \cdot 2$ HCl $\cdot$ 0.3 H_2O: C, 51.01; H, 4.77; N, 21.25; Cl, 21.51. Found: C, 51.25; H, 4.79; N, 21.08; Cl, 21.42.

Other chemicals. Ethyl bromoacetate and 1-bromo-4-iodobenzene were purchased from Aldrich Chemical Co., Milwaukee, Wis. Other chemicals were obtained from the Sigma Chemical Company unless otherwise indicated. Tissue culture media and supplements were obtained from either the Sigma Chemical Company or GIBCO BRL. Stock solutions were prepared in DMSO and stored at 4°C.

Cell lines. *L. donovani* (World Health Organization designation, MHOM/SD/62/1S-$CL2_D$) promastigotes and axenic amastigote-like parasites were maintained in modified RPMI medium as previously described (34). *L. mexicana* (World Health Organization designation, MNYC/BCZ/62/M379) promastigotes were maintained at 26°C in Schneider's medium (GIBCO BRL) supplemented with 20% fetal bovine serum (Sigma) containing penicillin (50 U/ml) and streptomycin (50 μg/ml). Murine monocyte-like J774.G8 macrophages were maintained by serial passage in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 2.0 mM L-glutamine, penicillin at 50 U/ml, and streptomycin at 50 μg/ml.

Drug susceptibility assays. The susceptibility of the growth of *L. donovani* amastigote-like forms to the compounds was measured in a 3-day assay using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega) as previously described (34). A SpectraMax Plus microplate reader (Molecular Devices, Sunnyvale, Calif.) was used to quantitate reduction of the tetrazolium dye, which is proportional to cell density, by measuring the absorbance in each well at 490 nm. IC_{50}s (the concentrations of the compounds that inhibited parasite growth by 50% compared to that of an untreated control) were determined with the software program SoftMax Pro (Molecular Devices) using the dose-response equation $y = \{(a - d)/[1 + (x/c)^b]\} + d$, where x is the drug concentration, y is the absorbance at 490 nm, a is the upper asymptote, b is the slope, c is the IC_{50}, and d is the lower asymptote.

The toxicity of the compounds to J774.G8 macrophages was also measured with the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay Kit (Promega). A 1-week-old, confluent culture of J774.G8 macrophages was washed three times with Hanks balanced salt solution without calcium and magnesium (Sigma). The cells were then incubated at 37°C in a humidified 5% CO_2 incubator for 15 min with a trypsin-EDTA (0.05% trypsin, 0.53 mM EDTA $\cdot$ 4Na) solution (GIBCO BRL). Cells were subsequently detached with a cell scraper and suspended by vigorous pipetting. Macrophages were then resuspended at a concentration of 5×10^5/ml in DMEM without phenol red indicator supplemented with 10% heat-inactivated fetal calf serum containing penicillin (50 U/ml) and streptomycin (50 μg/ml). Subsequently, 200 μl of this cell solution was added to individual wells of 96-well flat-bottom plates (Costar) and attachment of the macrophages to the bottom of the plate was allowed to occur over a period of 48 h at 37°C in a humidified 5% CO_2 incubator. The medium was then removed, and serial dilutions of drugs in the same DMEM were added to each well. After 72 h of incubation with the test compounds, again at 37°C in a humidified 5% CO_2 incubator, cell viability was determined with the CellTiter 96 Assay Kit by adding 40 μl of assay solution to each well. One to 2 h of incubation was performed at 37°C to allow color development, and then the absorbance of each well at 490 nm was measured in a SpectraMax Pro microplate reader (Molecular Devices). The four-parameter curve given earlier was used to determine the IC_{50}s of the compounds tested.

In the *Leishmania*-infected macrophage assay, initial steps were identical to those used in the macrophage toxicity assay. After washing and trypsinization of the macrophage cell line as described above, J774.G8 cells were mixed with a late-log-phase culture of *L. mexicana* promastigotes to yield a solution containing 5×10^5 macrophages/ml and 25×10^5 promastigotes/ml in the supplemented DMEM medium described previously. The macrophage-parasite mixture was then pipetted into 96-well flat-bottom plates at 200 μl/well.

Infection and attachment of the macrophages were allowed to occur over a period of 48 h at 33°C in a humidified 5% CO_2 incubator. Wells were washed three times with Hanks balanced salt solution to remove extracellular parasites, and then serial dilutions of drugs in supplemented DMEM were added to each well. The plate was returned to the CO_2 incubator for an additional 72 h of incubation at 33°C. Macrophages in each well were subsequently detached by pipetting and scraping with the tip of a microliter pipettor. A 75-μl volume of the cell suspension was transferred to a Cytospin funnel, and the cells were centrifuged onto microscope slides at 800 rpm for 5 min with a Cytospin centrifuge (Shandon). The slides were allowed to air dry and were then fixed in methanol for 5 s. After evaporation of the methanol, the slides were stained with 5% Giemsa stain (Fisher) in phosphate buffer (3.1 mM dibasic potassium phosphate, 8.3 mM monobasic sodium phosphate) for 45 min. After thorough washing in flowing tap water, the slides were allowed to air dry before being viewed by oil immersion microscopy to determine the percentage of infected cells.

RESULTS

Chemistry. The multistep synthesis of novel unsymmetrical thiophene dications 23 and 25 is given in Fig. 1. Stille coupling between 2-tributylstannylthiophene (a) and 4-bromobenzonitrile (b) gave 2-(4-cyanophenyl)thiophene (c), which was brominated to provide 2-bromo-5-(4-cyanophenyl)thiophene (d). This thiophene derivative was coupled with 3-cyanophenylboronic acid to give 2-(3-cyanophenyl)-5-(4-cyanophenyl)-thiophene (e). The unsymmetrical dicyanophenyl thiophene was converted to the corresponding diimidate ester (f) by treatment with ethanolic HCl, and compound f was used to synthesize both compounds 23 and 25. The former was obtained by treatment of the diimidate ester with ethanolic ammonia, while the latter was provided by reaction of the diimidate ester with ethylenediamine in ethanol. Figure 2 outlines the multistep synthesis of unsymmetrical carbazole diamidine (compound 37). A crossed Ullmann reaction of 2,4-dibromonitrobenzene (g) (21) and 1-bromo-4-iodobenzene (h) gave 4′,5-dibromo-2-nitrobiphenyl (i). Ring closure giving 2,6-dibromocarbazole (j) was effected with triethyl phosphite. Cyanodebromination with copper(I) cyanide gave 2,6-dicyanocarbazole (k). Subjection of the latter to standard Pinner reaction conditions (25) gave the asymmetric diamidine (compound 37), isolated as the dihydrochloride salt.

We examined different classes of cationic molecules for activity against *L. donovani* axenic amastigotes and compared their activity to that of pentamidine, which possesses an IC_{50} of 2.59 ± 0.54 μM against these parasites (Table 1). For compounds possessing IC_{50}s against the parasites that are near or less than 10 μM, the toxicity of these agents against a J774.G8 macrophage cell line was also determined.

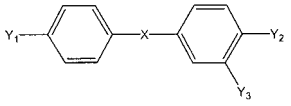
Diphenyl furans and thiophenes. The in vitro antileishmanial activities of a wide range of diphenyl furans and thiophenes are reported in Table 1. Compounds 1 to 16 are 2,5-diphenyl furan dications. When the furan ring possesses hydrogen atoms at positions 3 and 4, as in compounds 1 to 6, 2,5-bis-(4-amidinophenyl)furan (compound 1) is the most active of this series, with antileishmanial activity that is indistinguishable from that of pentamidine. This compound is 9.7-fold less toxic to the macrophage cell line than to *L. donovani*. Substituting a cyclopentyl group for hydrogen at each of the amidine groups, as in compound 4, decreases its antileishmanial activity 5.7-fold, and a further decrease in activity is observed with the isopropyl substitution found in compound 3. Other substitutions to the amidine group in this series essentially inactivate compounds 2, 5, and 6 against *Leishmania*. When the 2,5-disubstituted furan possesses a methyl group at position 3, cyclopropyl or isobutyl substitution of the amidine group, as in compounds 8 and 9, confers greater antileishmanial activity than isopropyl substitution, as in compound 7. Compound 9 was also 6.1-fold more active against *Leishmania* than against the macrophage cell line. Addition of a carboxyethyl group at position 4 of the furan ring deactivates compounds 10 and 11 compared to compounds 7 and 8. In compounds 12 to 16, methyl groups are present at both the 3 and 4 positions of the furan ring. Amidine, isopropyl amidine, cyclopropyl amidine, and cyclopentyl amidine compounds 12 to 15 have similar activities, while ditetrahydropyrimidine compound 16 is less active. It is interesting that while compound 1 is 6.5-fold more active than its dimethyl furan analog compound 12, cyclopentyl diamidines 4 and 15 are equally active, while the isopropyl diamidine-substituted dimethyl furan compound 13 is 2.8-fold more active than corresponding furan compound 3.

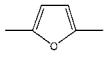
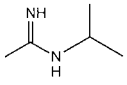
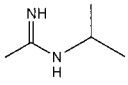
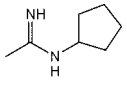
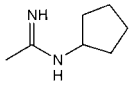
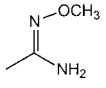
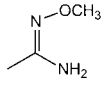
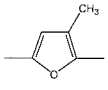
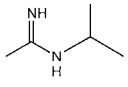
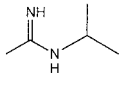
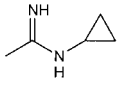
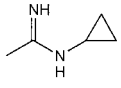
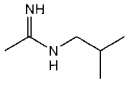
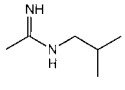
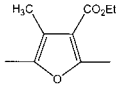
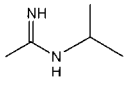
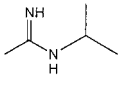
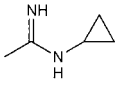
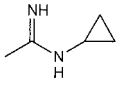
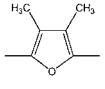
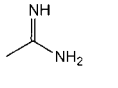
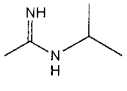
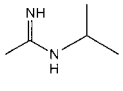
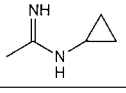
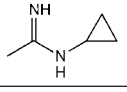
Five 2,4-diphenyl furans (compounds 17 to 21) were also tested. These compounds all possessed activity against *L. donovani*, with 2,4-bis-(4-amidinopheny)furan 17 being about twice as active as pentamidine and corresponding 2,5-diphenyl furan compound 1. Compound 17 was also 19-fold more active against the parasites than against the macrophage cell line. Isopropyl and cyclopentyl diamidines 18 and 21 were also 5.0-fold and 3.4-fold more active than their 2,5-disubstituted counterparts (compounds 3 and 4, respectively). Four 2,5-diphenyl thiophenes (compounds 22 to 25) displayed outstanding in vitro antileishmanial activity. 2,4-Bis-(4-amidinophenyl)furan 22 exhibited the best activity of any of the compounds studied, possessing 6.2-fold greater antiparasitic activity than pentamidine. This compound was 155-fold more potent against *Leishmania* than against the macrophage cell line. Isomeric diamidine compound 23 was 3.2-fold more potent than pentamidine but was less active and more toxic than compound 22. Isopropyl diamidine compound 24 was less active than diamidine compounds 22 and 23 but possessed superior antiparasitic activity than corresponding 2,5- and 2,4-diphenyl furan isopropyl diamidine compounds 3 and 13.

Carbazoles. Twelve carbazoles were also tested for in vitro antileishmanial activity (Table 2). These carbazoles were disubstituted at either the 3 and 6 positions (compounds 26 to 28), the 2 and 7 positions (compounds 29 to 36), or the 2 and 6 positions (compound 37). Compounds 26 to 28 and compound 37 were inactive against *L. donovani*. In the 2,7-substituted carbazole series, however, diamidine 29 was only 3.1-fold less active than pentamidine and was 8.6-fold more potent against the parasites than against the macrophage cell line. The isopropyl diamidine in this series, compound 35, was nearly as active as diamidine 29 and was less toxic than compound 29 to the macrophage cell line. Diimidazoline compound 30 was the only other compound in this series that possessed measurable antileishmanial activity. Compound 31, differing from compound 30 only by methylation of the carbazole nitrogen, did not display antiparasitic activity.

Dibenzofurans and dibenzothiophenes. Closely analogous to the carbazole dications are dibenzofuran and dibenzothiophene compounds 38 to 51 (Table 3). In general, the dibenzo-

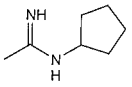
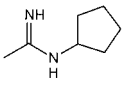
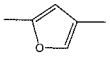
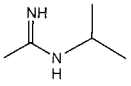
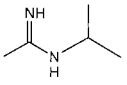
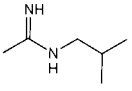
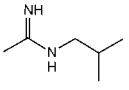
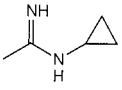
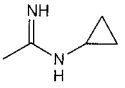
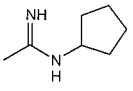
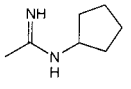
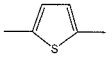
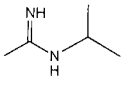
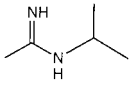
TABLE 1. Structures and in vitro activities of diphenyl furans and thiophenes



Compound (reference)	X	Y_1	Y_2	Y_3	IC_{50}[a] (μM) vs:	
					L. donovani	J774.G8 cell line
Pentamidine	$O(CH_2)_5O$			H	2.59 ± 0.54	>100
1 (11)				H	2.76 ± 0.60	26.84 ± 2.44
2 (11)	"			H	>100	NT[b]
3 (8)	"			H	60.57 ± 17.01	NT
4 (8)	"			H	15.75 ± 5.05	NT
5 (7)	"			H	>100	NT
6 (7)	"			H	>100	NT
7 (6)				H	26.58 ± 7.9	NT
8 (6)	"			H	14.21 ± 0.41	NT
9 (6)	"			H	10.71 ± 0.48	65.12 ± 13.25
10 (6)				H	53.67 ± 9.79	NT
11 (6)	"			H	68.70 ± 9.39	NT
12 (11)				H	17.96 ± 2.11	NT
13	"			H	21.27 ± 3.2	NT
14	"			H	14.20 ± 1.63	NT

Continued on following page

TABLE 1—*Continued*

Compound (reference)	X	Y_1	Y_2	Y_3	IC_{50}[a] (μM) vs:	
					L. donovani	J774.G8 cell line
15	"			H	13.17 ± 0.75	NT
16	"			H	28.38 ± 5.85	NT
17 (14)				H	1.30 ± 0.21	24.71 ± 1.71
18 (14)	"			H	12.07 ± 0.74	NT
19 (14)	"			H	10.39 ± 0.41	77.92 ± 24.3
20 (14)	"			H	7.12 ± 1.90	16.28 ± 5.14
21 (14)	"			H	4.62 ± 1.18	>100
22 (12)				H	0.42 ± 0.08	65.3 ± 4.6
23	"		H		0.82 ± 0.03	9.63 ± 0.43
24	"			H	7.74 ± 0.25	21.26 ± 2.58
25	"		H		12.14 ± 1.81	NT

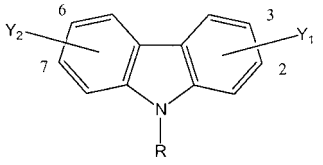
[a] Mean ± standard deviation of at least two independent measurements.
[b] NT, not tested.

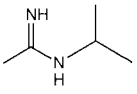
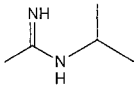
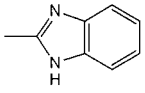
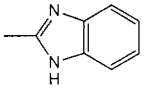
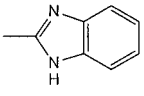
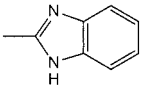
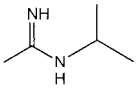
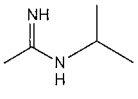
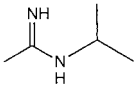
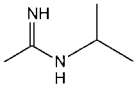
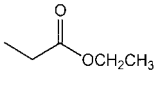
furans were ineffective against *Leishmania* in vitro. Only 3,7-substituted diamidine compound 42 and corresponding isopropyl diamidine compound 44 possessed weak antiparasitic activity. Corresponding dibenzothiophene compounds 48 and 51 were more potent, however. Diamidine compound 48 was only 4.1-fold less potent than pentamidine, and isopropyl diamidine compound 51 possessed 2.5-fold less antileishmanial activity than pentamidine. Isopropyl diamidine compound 51 was also relatively nontoxic to the macrophage cell line.

Benzimidazoles. Seven benzimidazole-containing cations have also been examined for activity against *L. donovani* axenic amastigotes (Table 4). Cyclopentyl diamidine compound 55 displayed moderate activity against the parasites. When phenyl substituents were added as spacers between the central 2,5-disubstituted furan ring and the cyclopentyl amidine-containing benzimidazole ring systems (compound 58), antileishmanial activity decreased 3.9-fold. The only other benzimidazole compound that possessed measurable antiparasitic activity in this series was cyclopropyl diamidine-containing compound 57.

Assays with *Leishmania*-infected macrophages. Agents with activity against axenic amastigotes comparable or superior to that of pentamidine (compounds 1, 17, and 21 to 23) were also tested for activity in J774.G8 cells infected with *L. mexicana*. In this assay, the clinical antileishmanial agent amphotericin B reduced parasite burdens in infected cells with an IC_{50} of 46 ± 6 nM (mean ± standard deviation of four independent experiments), while pentamidine was inactive. We observed no reduction in parasitemia in *L. mexicana*-infected macrophages at concentrations of compounds 1, 17, and 21 to 23 that were nontoxic to the J774.G8 cell line.

TABLE 2. Structures and in vitro activities of carbazoles



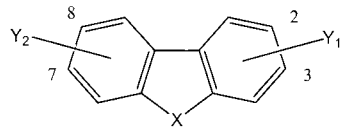
Compound (reference)	Y_1	Y_2	Positions Y_1, Y_2	R	IC_{50}[a] (μM) vs:	
					L. donovani	J774.G8 cell line
26 (25)			3, 6	H	>100	NT[b]
27 (25)			3, 6	H	>100	NT
28 (25)			3, 6	H	>100	NT
29 (25)			2, 7	H	7.92 ± 2.92	67.88 ± 6.37
30 (25)			2, 7	H	26.45 ± 3.99	NT
31 (25)			2, 7	CH_3	>100	NT
32 (25)		OCH_3	2, 7	H	>100	NT
33 (25)		OCH_3	2, 7	CH_3	>100	NT
34 (25)			2, 7	H	>100	NT
35 (5)			2, 7	H	10.19 ± 2.45	>100
36			3, 6		>100	NT
37			2, 6	H	>100	NT

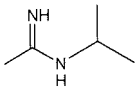
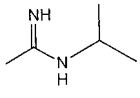
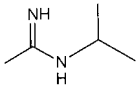
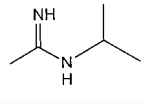
[a] Mean ± standard deviation of at least two independent measurements.
[b] NT, not tested.

DISCUSSION

Data presented in Tables 1 to 4 indicate that several of the dications tested possess in vitro antileishmanial activity comparable or superior to that of the clinical antileishmanial agent pentamidine. Of the compounds examined in this work, the diphenyl furan and thiophene dications described in Table 1 possess the best antileishmanial activity. Three major classes of diphenyl furans and thiophenes were tested: 2,5-diphenyl furans, 2,4-diphenyl furans, and 2,5-diphenyl thiophenes. In each class, the diamidines were the most potent, with 2,5-bis-

TABLE 3. Structures and in vitro activities of dibenzofurans and dibenzothiophenes



Compound (reference)	Y	Positions Y_1, Y_2	X	IC_{50}[a] (μM) vs:	
				L. donovani	J774.G8 cell line
38 (33)		2, 8	O	>100	NT[b]
39 (33)		2, 8	O	>100	NT
40 (33)		2, 8	O	>100	NT
41 (33)		2, 8	O	>100	NT
42 (33)		3, 7	O	49.15 ± 5.6	NT
43 (33)		3, 7	O	>100	NT
44 (33)		3, 7	O	83.96 ± 22.3	NT
45 (26)		2, 8	S	>100	NT
46 (26)		2, 8	SO_2	>100	NT
47 (26)		2, 8	S	>100	NT
48 (26)		3, 7	S	10.60 ± 3.9	NT
49 (26)		2, 8	S	>100	NT
50 (26)		3, 7	S	>100	NT
51 (26)		3, 7	S	6.46 ± 1.04	>100

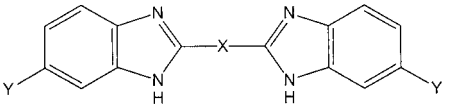
[a] Mean ± standard deviation of at least two independent measurements.
[b] NT, not tested.

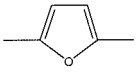
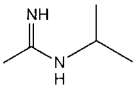
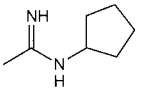
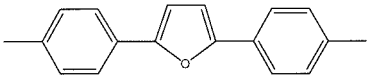
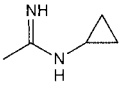
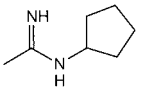
(4-amidinophenyl)thiophene (compound 22) possessing the best activity of all of the agents tested. Since the 2,4-diphenyl furans were more active than their 2,5-diphenyl furan counterparts, it will be of interest to prepare 2,4-diphenyl thiophene-containing dications in the future.

Addition of an alkyl group to one of the nitrogen atoms of the amidine group decreased the antileishmanial potency of the diphenyl furans and thiophenes. In the 2,5-diphenyl furan series, substitution of an alkyl group on the amidino nitrogen generally leads to an increase in DNA binding affinity, and in particular, dialkylamidine compounds 3 and 4 bind more avidly to the duplex oligomer $d(CGCGAATTCGCG)_2$ than does 2,5-bis-(4-amidinopheny)furan compound 1 (8). However, different aromatic dication analogs of pentamidine can also have distinct preferences for specific DNA sequences (32) and it is conceivable that particular DNA sequences could be targets for the dications in *Leishmania*. Although there appears to be a correlation between DNA binding affinity and the antipneumocystis activity of such compounds (8), DNA binding affinity may not be the only factor contributing to the antileishmanial activity of these compounds. Multiple mechanisms of action have been proposed for pentamidine in kinetoplastid parasites, including inhibition of serine proteases (22), inhibition of topoisomerase II (29), and disruption of polyamine metabolism (10). With regard to polyamine metabolism, cationic polyamine analogs themselves have displayed activity against kinetoplastid parasites (35). It is therefore possible that the compounds presented in this work interact at several target sites within the parasite and that addition of an alkyl group to the amidine function in the diphenyl furans and thiophenes leads to unfavorable interactions with an important, as yet unidentified, parasite receptor.

The carbazoles, dibenzofurans, dibenzothiophenes, and benzimidazoles presented in Tables 2 to 4 were less active against *L. donovani* than were the diphenyl furans and thiophenes. Since amidine and alkylamidine substitutions also occurred in the molecules presented in Tables 2 to 4, the geometry of the substituents imposed by the carbazole, dibenzofuran, dibenzothiophene, and benzimidazole linkers must be less favorable for antileishmanial activity compared to the diphenyl furans and thiophenes. In the carbazole series, the 2,7-substituted diamidines and diisopropyl amidines are active while the corresponding 3,6-substituted compounds are not. In the dibenzofuran and dibenzothiophene systems, which are, by convention, numbered differently than the carbazoles, the 3,7-substituted compounds are active while the 2,8-substituted compounds are not. Thus, the positioning of the amidine and isopropyl amidine groups is more favorable for antileishmanial activity in the 2,7-substituted geometry (carbazole numbering system). It is also interesting that in the molecules present in Tables 2 to 4, the amidines are not always the most active. In the 2,7-disubstituted carbazole series, diamidine 29 and diisopropyl amidine 35 are equally potent within experimental error. The same is true of 3,7-disubstituted dibenzothiophene compounds 48 and 51, and in the case of the benzimidazoles, the diamidines are less active than alkyl-substituted amidines. The distinct structure-activity relationships among the different classes of agents are consistent with the hypothesis that the aromatic dications have multiple action mechanisms and also

TABLE 4. Structures and in vitro activities of benzimidazoles



Compound (reference)	X	Y	IC_{50} (μM)[a]
52 (13)			>100
53 (13)	"		>100
54 (13)	"		>100
55 (13)	"		18.24 ± 0.31
56 (17)			>100
57 (17)	"		74.70 ± 16.14
58 (17)	"		71.64 ± 1.89

[a] Versus *L. donovani*.

suggest that particular classes of molecules interact preferentially with different targets within the parasite.

Although pentamidine is used clinically for the treatment of leishmaniasis, this compound has very poor activity in antileishmanial assays measuring the effects of drug on parasite-infected macrophages (23). Agents with potency comparable or superior to that of pentamidine in the axenic amastigote assay were examined in our *Leishmania*-infected macrophage model in an attempt to identify candidate compounds for further study in vivo. Unfortunately, we did not observe activity in our infected macrophage cell line with any of the compounds described in this paper. We are optimistic, however, about the potential of related dicationic molecules as antileishmanial drug candidates. Information obtained from the structure-activity relationships outlined here is currently being used to design new molecules that have displayed superior antileishmanial activity in preliminary experiments, and we look forward to providing future accounts of the antiparasitic potential of novel aromatic dications.

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