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Activity of diimidazoline amides against African trypanosomiasis

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Abstract

We identified several diimidazoline mono- and diamides that were as potent as pentamidine against *T. brucei rhodesiense* in vitro. All of these were also less cytotoxic than pentamidine, but none was as effective as the latter in a *T. brucei rhodesiense*-infected mouse model. A single imidazoline may be sufficient for high antitrypanosomal activity provided that a second weak base functional group is present.

Keywords

diimidazoline; African trypanosomiasis; *Trypanosoma brucei rhodesiense*

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a neglected vector borne protozoal disease. HAT exists as a chronic infection with *Trypanosoma brucei gambiense* or as an acute infection with *T. brucei rhodesiense*. In the first stage of HAT, parasites multiply within the hemolymphatic system. In the second encephalitic stage, parasites infect the central nervous system and the cerebrospinal fluid. Stage 1 disease can be treated with the diamidine pentamidine, whereas melarsoprol, eflornithine, and their combinations with nifurtimox are the only drugs effective against stage 2 disease, and all of these are poorly tolerated and require parenteral administration.[1] For example, due to its toxicity and lack of oral bioavailability, pentamidine is usually administered only in hospital settings.[2,3] Numerous analogs of pentamidine have been synthesized in order to increase the therapeutic index and provide the option of oral dosing.[1–3] This work culminated in the identification of furamidine, a conformationally restricted analogue of pentamidine, and pafuramidine, the orally active dimethoxyamidine prodrug of furamidine (Figure 1). Pafuramidine advanced to phase III clinical trials, but these were suspended due to nephro- and hepatotoxicity.[1] Despite this setback, efforts continue to identify a next-generation diamidine.[1]

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Our interest in diamidines arose from the potential of diimidazolines as inhibitors of botulinum neurotoxin.[4] In this work, we prepared several diimidazoline terephthanilides as control compounds of our active botulinum neurotoxin inhibitors, and recognized the structural similarity of some of these (e.g. **1**) (Figure 2) to that of pentamidine and other antitrypansomal diamidines (Figure 1). In this respect, the carboxamide isostere of pentamidine (Figure 1) is only slightly less potent and nearly two orders of magnitude less cytotoxic than pentamidine,[5] suggesting that the carboxamide functional group may increase antitrypanosomal selectivity of diamidines and related compounds. Not surprisingly, **1** has been previously investigated for its antitrypanosomal effects[6] where it was shown that this diimidazoline terephthanilide cured *T. brucei brucei*-infected mice at low doses. Thus, we set out to reinvestigate the activity of **1** against HAT and to begin to define its SAR.

As shown in Schemes 1 and 2, **3, 4, 9**, and **10** were obtained by the synthesis of their dinitrile precursors followed by treatment with ethylene diamine and sodium hydrosulfide[7] in dimethylacetamide (DMA) to form the diimidazolines which were isolated as their dimesylate salts by treatment with methanesulfonic acid (MSA) (70–80% overall reaction yields).

The synthesis of indole diimidazolines **11**-**13** and indole monoimidazoline **14** began with formation of nitrostilbene acid **26** from the commercially available precursors **24** and **25** (Scheme 3). The key step was reductive cyclization of **26** to indole nitrile ester **27** in hot triethylphosphite with a $MoO_2(acac)_2$ catalyst in a neat reaction. These reaction conditions advantageously combine features (refluxing triethylphosphite)[8] and (triphenylphosphine and $MoO_2Cl_2(dmf)_2$ in refluxing toluene)[9] of similar reductive cyclization reactions. Nitrile ester **27** was directly converted[10] into dinitrile amide **28**. Hydrolysis of **27** afforded indole nitrile acid **29** that was readily converted into **30**-**32**, the nitrile and dinitrile amide precursors of **12**-**14**. Nitrile to imidazoline formation proceeded as already described. The remaining known diimidazolines **1, 2**, and **5**-**8**[11–14] (Table 1) were obtained by reaction sequences similar to those described in Schemes 1 and 2.[15]

In vitro and in vivo assays with *T. brucei rhodesiense* STIB900 and in vitro cytotoxicity with the rat myoblast L6 cell line were performed as previously described.[16,17] Target compound HAT activity data against *T. brucei rhodesiense* are shown in Tables 1 and 2. Diimidazoline prototype **1** was only slightly less potent against the STIB900 strain of *T. brucei rhodesiense* than were the control drugs malarsoprol and pentamidine. Diimidazoline **2**, the meta analog of **1**, was order of magnitude less potent than the latter, but was similarly cytotoxic. Compounds **3** and **4** demonstrate that insertion of a methylene between the aniline nitrogen atoms and distal phenyl rings of **1** and **2** decreases activity by two to three orders of magnitude. The IC_{50} values for **5**-**7**, the three reversed amides of **1** and **2**, show that at least one aniline nitrogen atom para to an 2-imidazoline substituent is required for high activity. Compound **8**, the biphenyl analog of **1** was only slightly less potent than the prototype, but the resulting increase in molecular weight and aromatic ring count[18] suggests that **8** offers no significant advantage over **1**. Diimidazoline **10** illustrates that removing the central phenyl ring of **1** decreased activity by an order of magnitude. Interestingly, previous work[19] demonstrated that the diamidine analog of **10** had no in vivo activity against HAT species. Comparing **7** to **9** indicates that replacing the central benzene ring with a cyclohexane decreased activity 6-fold and cytotoxicity 1.4-fold; thus there appears to be no benefit in increasing sp^3 carbon count[20] in this series of diimidazolines.

The remaining four compounds (**11**-**14**) are diimidazoline indoles, in which one of the anilide functional groups of **1** was replaced with a pyrrole substructure. Compounds **11**-**14** share some structural similarity with a previously reported[21] set of biphenylbenzimidazole diamidines. Like **1** and pentamidine, diimidazoline indoles **11** and **12** had single digit nM

IC_{50} values, but they were also the most cytotoxic target compounds. Target compound **12** reveals that insertion of a methylene between the aniline nitrogen atom and distal phenyl ring of **11** did not decrease activity; this contrasts to what was observed for **1** vs. **3** (*vide supra*). We also found that the nitrile and dinitrile precursors of target compounds **1**-**14** had *T. brucei rhodesiense* STIB900 IC_{50} values in the range of 10,000 to >150,000 nM demonstrating the importance of the 2-imidazoline substructure for HAT activity. However, comparing the relative activities of monoimidazolines **13** and **14** to their diimidazoline counterpart **12** reveals that only a single imidazoline is required for high activity provided that a second weak base functional group is present. With the exception of **11, 1**-**14** were significantly less cytotoxic than either melarsoprol or pentamidine, consistent with previous data demonstrating lower cytotoxicity for carboxamide analogs of pentamidine.[5] Finally, there was no correlation between *T. brucei rhodesiense* STIB900 and L6 cytotoxicity IC_{50} values for **1**-**14**, similar to what was previously observed for a series of adamantyl monoimidazolines.[22]

The ten target compounds with in vitro IC_{50} values < 150 nM against *T. brucei rhodesiense* STIB900 were administered as three consecutive 40 mg/kg ip doses to *T. brucei rhodesiense*-infected mice on days 1–3 post-infection. In this primary rodent model, all ten compounds were 100% curative. Next, eight of these[23] were tested in a more stringent rodent model with a well-established infection. In this experiment, the compounds were administered as four consecutive 50 mg/kg ip doses to *T. brucei rhodesiense*-infected mice on days 3–6 post-infection (Table 2). Target compounds **2** and **5**-**7** were completely curative and **1** and **10** cured 2/4 infected animals. The partial curative efficacy of **1** contrasts with data from earlier experiments with this diimidazoline where it completely cured[24] *T. brucei brucei*-infected mice at ip doses as low as 3 × 1 mg/kg.[6] Only **8** and **12** were ineffective, and the latter was lethal; all four treated mice died after the first injection of this indole diimidazoline. In this respect, in vitro cytotoxicity seems to have been an inadequate predictor of in vivo toxicity, as **5** and **10** were only slightly less cytotoxic than **12**, but they showed no in vivo toxicity. For the eight compounds tested in the 4 × 50 mg/kg experiment, there was no correlation between in vitro potency and in vivo efficacy. For comparison, in this same experimental format, administration of four 20 mg/kg doses of pentamidine and furamidine cured 2/4 and 3/4 of infected mice.[16]

In summary, we identified several diimidazoline mono- and diamides that were as potent as pentamidine against *T. brucei rhodesiense* in vitro, but none of these was as effective as pentamidine in a *T. brucei rhodesiense*-infected mouse model. Second, our data suggest that a single imidazoline may be sufficient for high antitrypanosomal activity provided that a second weak base functional group is present. Third, in vitro cytotoxicity assessment did not seem to be an inadequate predictor of in vivo toxicity for this series of compounds. Although mechanistic studies suggest that diamidines selectively accumulate in HAT species by way of the P2 nucleoside transporter and subsequently concentrate in the mitochondrion where they bind avidly to kinetoplast DNA,[3] the promiscuity of dications such as diamidines is problematic.[25,26] For example, diamidines are active against a wide range of pathogenic microbes and have been investigated as potential anticancer agents.[2] Indeed, we note that **1, 2**, and **5**-**8** had earlier been synthesized and tested for antitumor and antibacterial activities.[12,13,27,28] Moreover, **1** is mutagenic at micromolar concentrations.[29] Accordingly, future work will address the SAR of the imidazoline substructure[6,17] with a goal to increase efficacy and selectivity against HAT species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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15. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a 500 MHz spectrometer. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ (0 ppm) for ^{1}H and 39.5 ppm for ^{13}C NMR.*N*,*N*′-Bis[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]terephthalamide dimesylate (**1**). mp 179–181 °C. ^{1}H NMR (60 °C) δ 2.34 (s, 6H), 4.01 (s, 8H), 7.97 (d, *J* = 8.3 Hz, 4H), 8.08 (d, *J* = 7.8 Hz, 4H), 8.15 (s, 4H), 10.30 (s, 4H), 10.76 (s, 2H); ^{13}C NMR (60 °C) δ 44.33, 116.74, 120.09, 127.97, 129.43, 137.18, 144.53, 164.59, 165.34. Anal. Calcd for $C_{28}H_{32}N_6O_8S_2$: C, 52.16; H, 5.00; N, 13.04. Found: C, 51.94; H, 5.02; N, 12.89.*N*,*N*′-Bis[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]isophthalamide dimesylate (**2**). mp 339–341 °C. ^{1}H NMR δ 2.34 (s, 6H), 4.01 (s, 8H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 8.3 Hz, 4H), 8.09 (d, *J* = 8.3 Hz, 4H), 8.23 (d, *J* = 7.8 Hz, 2H), 8.56 (s, 1H), 10.41 (s, 4H), 10.93 (s, 2H); ^{13}C NMR δ 39.94, 44.50, 116.88, 120.16, 127.57, 129.12, 129.73, 131.53, 134.82, 144.79, 164.55, 165.82. Anal. Calcd for $C_{28}H_{32}N_6O_8S_2{\cdot}0.5H_2O$: C, 51.44; H, 5.09; N, 12.86. Found: C, 51.43; H, 5.31; N, 12.57.*N*,*N*′-Bis[[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]methyl]terephthalamide dimesylate (**3**). mp 306–308 °C. ^{1}H NMR (60 °C) δ 2.36 (s, 6H), 4.01 (s, 8H), 4.61 (d, *J* = 4.9 Hz, 4H), 7.59 (d, *J* = 7.8 Hz, 4H), 7.91 (d, *J* = 7.8 Hz, 4H), 8.00 (s, 4H), 9.19 (brs, 2H), 10.40 (s, 4H); ^{13}C NMR (60 °C) δ 42.57, 44.39, 120.57, 127.25, 127.87, 128.44, 136.52, 146.75, 165.00, 165.79. Anal. Calcd for $C_{30}H_{36}N_6O_8S_2$: C, 53.56; H, 5.39; N, 12.49. Found: C, 53.49; H, 5.46; N, 12.35.*N*,*N*′-Bis[[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]methyl]isophthalamide dimesylate (**4**). mp 219–221 °C. ^{1}H NMR δ 2.35 (s, 6H), 4.01 (s, 8H), 4.60 (s, 4H), 7.51–7.69 (m, 5H), 7.92 (d, *J* = 7.4 Hz, 4H), 8.08 (d, *J* = 6.8 Hz, 2H), 8.45 (s, 1H), 9.34 (brs, 2H), 10.50 (s, 4H); ^{13}C NMR (60 °C) δ 39.93, 42.74, 44.57, 120.76, 126.63, 128.07, 128.69, 128.81, 130.29, 134.49, 147.02, 164.99, 166.17. Anal. Calcd for $C_{30}H_{36}N_6O_8S_2$: C, 53.56; H, 5.39; N, 12.49. Found: C, 53.12; H, 5.80; N, 12.19.*N*-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-4-[[4-(4,5-dihydro-1*H*-imidazol-2-yl)benzoyl]amino]benzamide dimesylate (**5**). mp 277–279 °C. ^{1}H NMR δ 2.34 (s, 6H), 4.01 (s, 4H), 4.06 (s, 4H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.99 (d, *J* = 8.8 Hz, 2H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.07 (d, *J* = 9.3 Hz, 2H), 8.10 (d, *J* = 8.3 Hz, 2H), 8.24 (d, *J* = 8.3 Hz, 2H), 10.37 (s, 2H), 10.66 (s, 1H), 10.69 (s, 2H), 10.81 (s, 1H); ^{13}C NMR δ 39.95, 44.47, 44.76, 116.48, 119.84, 120.00, 125.04, 128.78, 128.80, 129.03, 129.45, 129.66, 139.74, 142.45, 145.11, 164.54, 164.57, 164.75, 165.66. Anal. Calcd for Anal. Calcd for $C_{28}H_{32}N_6O_8S_2$: C, 52.16; H, 5.00; N, 13.04. Found: C, 52.43; H,

4.91; N, 12.92.*N*-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-3-[[4-(4,5-dihydro-1*H*-imidazol-2-yl)benzoyl]amino]benzamide dimesylate (**6**). mp 145–147 °C. ^{1}H NMR δ 2.34 (s, 6H), 4.00 (s, 4H), 4.05 (s, 4H), 7.58 (t, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 8.8 Hz, 2H), 8.08 (d, *J* = 8.8 Hz, 3H), 8.17 (d, *J* = 8.3 Hz, 2H), 8.25 (d, *J* = 8.3 Hz, 2H), 8.41 (s, 1H), 10.53 (brs, 2H), 10.80 (s, 1H), 10.82 (s, 1H), 10.85 (brs, 2H); ^{13}C NMR δ 39.96, 44.43, 44.71, 116.68, 120.04, 120.31, 123.48, 124.97, 128.69, 128.90, 129.08, 129.77, 135.10, 139.27, 139.70, 144.94, 164.44, 164.53, 166.29. Anal. Calcd for $C_{28}H_{32}N_6O_8S_2$: C, 52.16; H, 5.00; N, 13.04. Found: C, 51.99; H, 4.85; N, 12.88.*N*,*N*'-1,4-Phenylenebis[4-(4,5-dihydro-1*H*-imidazol-2-yl)benzamide] dimesylate (**7**). mp 196– 198 °C. ^{1}H NMR δ 2.34 (s, 6H), 4.06 (s, 8H), 7.80 (s, 4H), 8.09 (d, *J* = 8.3 Hz, 4H), 8.21 (d, *J* = 8.3 Hz, 4H), 10.54 (s, 2H), 10.68 (s, 4H); ^{13}C NMR δ 39.96, 44.75, 120.98, 128.64, 128.74, 135.09, 140.14, 164.13, 164.59. Anal. Calcd for $C_{28}H_{32}N_6O_8S_2$: C, 52.16; H, 5.00; N, 13.04. Found: C, 51.93; H, 5.25; N, 12.79.*N*,*N*'-Bis[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]biphenyl-4,4'-dicarboxamide dimesylate (**8**). mp 309–310 °C; ^{1}H NMR *δ* 2.33 (s, 6H), 4.02 (s, 8H), 7.99 (t, *J* = 9 Hz, 4H), 8.10 (d, *J* = 9 Hz, 2H), 8.14 (dd, *J* = 9, 3 Hz, 2H), 10.39 (s, 4H), 10.81 (s, 2H); ^{13}CNMR *δ* 39.91, 44.50, 119.17, 120.12, 127.19, 128.44, 128.88, 129.69, 133.88, 144.96, 164.55, 165.91. Anal. Calcd for $C_{34}H_{36}N_6O_8S_2$: C, 56.65; H, 5.03; N, 11.66. Found: C, 56.65; H, 4.90; N, 11.75.*trans*-*N*,*N*'-1,4-Cyclohexanediylbis[4-(4,5-dihydro-1*H*-imidazol-2-yl)benzamide] dimesylate (**9**). mp > 320 °C; ^{1}H NMR *δ* 1.44–1.56 (m, 4H), 1.88–2.00 (m, 4H), 2.32 (s, 6H), 3.76–3.86 (m, 2H), 4.04 (s, 8H), 8.02 (d, *J* = 9Hz, 4H), 8.08 (d, *J* = 9 Hz, 4H), 8.56 (d, *J* = 8 Hz, 2H), 10.62 (s, 4H); ^{13}C NMR *δ* 31.20, 39.91, 44.84, 48.40, 124.50, 128.34, 128.78, 139.98, 164.49, 164.68. Anal. Calcd for $C_{28}H_{38}N_6O_8S_2{\cdot}2H_2O$: C, 48.97; H, 6.16; N, 12.24. Found: C, 49.12; H, 6.04; N, 11.80.*N*-[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]-4-(4,5-dihydro-1*H*-imidazol-2-yl)benzamide dimesylate (**10**). mp 256–258 °C. ^{1}H NMR δ 2.34 (s, 6H), 4.01 (s, 4H), 4.05 (s, 4H), 7.99 (d, *J* = 8.7 Hz, 2H), 8.07 (d, *J* = 8.8 Hz, 2H), 8.10 (d, *J* = 8.3 Hz, 2H), 8.21 (d, *J* = 7.8 Hz, 2H), 10.52 (brs, 4H), 10.97 (s, 1H); ^{13}C NMR δ 39.95, 44.53, 44.97, 117.13, 120.23, 125.57, 128.77, 128.84, 129.72, 139.28, 144.51, 164.43, 164.51, 165.13. Anal. Calcd for $C_{21}H_{27}N_5O_7S_2$: C, 47.99; H, 5.18; N, 13.32. Found: C, 48.10; H, 4.92; N, 13.57.6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-[[[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]amino]carbonyl]phenyl]indole (**11**). mp 185–187 °C. ^{1}H NMR δ 3.71 (s, 4H), 3.84 (s, 4H), 7.20 (s, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 8.5 Hz, 2H), 8.07 (s, 1H), 8.13 (s, 4H), 10.60 (s, 1H), 12.36 (s, 1H); ^{13}C NMR δ 46.65, 48.26, 100.95, 112.11, 119.36, 119.83, 120.54, 123.40, 125.50, 128.35, 128.74, 131.82, 133.82, 134.69, 136.81, 140.34, 142.16, 163.68, 165.38, 165.48. Anal. Calcd for $C_{27}H_{24}N_6O$: C, 72.30; H, 5.39; N, 18.74. Found: C, 71.90; H, 4.98; N, 18.70.6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-[[[[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]methyl]amino]carbonyl]phenyl]indole dimesylate (**12**). mp 177–179 °C. ^{1}H NMR 2.34 (s, 6H), 4.01 (s, 4H), 4.03 (s, 4H), 4.62 (d, *J* = 5.7 Hz, 2H), 7.23 (d, *J* = 1.5 Hz, 1H), 7.57 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.91 (d, *J* = 8.3 Hz, 2H), 8.05 (d, *J* = 8.8 Hz, 2H), 8.06 (s, 1H), 8.10 (d, *J* = 8.8 Hz, 2H), 9.28 (t, *J* = 5.7 Hz, 1H), 10.37 (s, 2H), 10.47 (s, 2H), 12.38 (s, 1H); ^{13}C NMR δ 39.96, 42.69, 44.42, 44.55, 101.04, 112.81, 114.87, 120.74, 121.07, 125.81, 128.04, 128.26, 128.69, 133.14, 133.72, 133.94, 136.52, 141.80, 147.13, 165.00, 165.97, 166.06. Anal. Calcd for $C_{30}H_{34}N_6O_7S_2{\cdot}H_2O$: C, 53.56; H, 5.39; N, 12.49. Found: C, 53.64; H, 4.93; N, 12.70.6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-[[[(4-pyridyl)methyl]amino]carbonyl]phenyl]indole (**13**). mp 257–259 °C. ^{1}H NMR δ 3.63 (s, 4H), 4.54 (d, *J* = 5.8 Hz, 2H), 7.09 (s, 1H), 7.34 (d, *J* = 4.4 Hz, 2H), 7.55 (d, *J* = 9.3 Hz, 1H), 7.57 (d, *J* = 9.3 Hz, 1H), 7.90 (s, 2H), 8.02 (s, 4H), 8.52 (d, *J* = 4.4 Hz, 2H), 9.19 (t, *J* = 5.9 Hz, 1H), 11.87 (s, 1H); ^{13}C NMR δ 41.96, 49.90 (br), 100.51, 110.70, 119.30, 119.88, 122.33, 124.66, 125.11, 128.16, 130.18, 132.86, 134.79, 137.02, 138.67, 148.78, 149.73, 164.82, 166.15. Anal. Calcd for $C_{24}H_{21}N_5O$: C, 72.89; H, 5.35; N, 17.71. Found: C, 72.40; H, 5.40; N, 17.57.6-(4,5-Dihydro-1*H*-imidazol-2-yl)-2-[4-[[(benzyl)amino]carbonyl]phenyl]indole (**14**). mp 224– 226 °C. ^{1}H NMR δ4.52 (d, *J* = 6.3 Hz, 2H), 7.08 (s, 1H), 7.21–7.29 (m, 1H), 7.30–7.40 (m, 4H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.90 (s, 1H), 8.01 (brs, 4H), 9.10 (t, *J* = 6.1 Hz, 1H), 11.88 (s, 1H); ^{13}C NMR δ 42.82, 49.64 (br), 100.43, 110.76, 119.29, 119.88, 124.38, 125.09, 126.93, 127.42, 128.12, 128.47, 130.25, 133.22, 134.59, 136.99, 138.80, 139.87, 164.86, 165.83. Anal. Calcd for $C_{25}H_{22}N_4O{\cdot}H_2O$: C, 72.80; H, 5.86; N, 13.58. Found: C, 73.09; H, 5.51; N, 13.20.

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23. Insufficient quantities of **11** and **13** were available for the 4 × 50 mg/kg ip in vivo experiment.
24. The higher in vivo efficacy of TP51 against *T. brucei brucei* reported by Nathan et al. (Ref 6) may be due to infection with a different trypanosome species and that cures were defined as infected animals that survived for more than 30 days beyond controls, whereas in our experiment, cures were defined as survival for more than 60 days post-infection without a parasitemia relapse.
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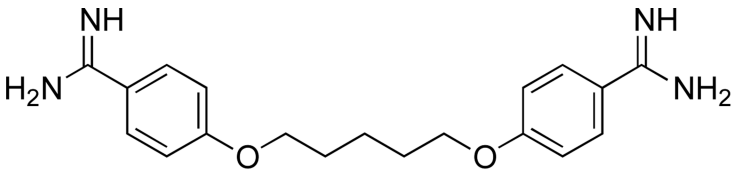
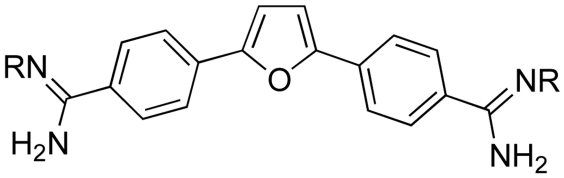
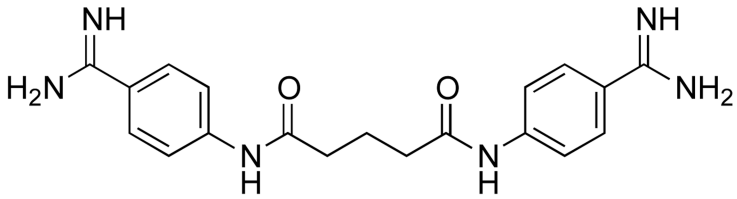


Pentamidine



Furamidine, R = H
Pafuramidine, R = OMe



Pentamidine carboxamide isostere

Figure 1.
Antitrypanosomal diamidines

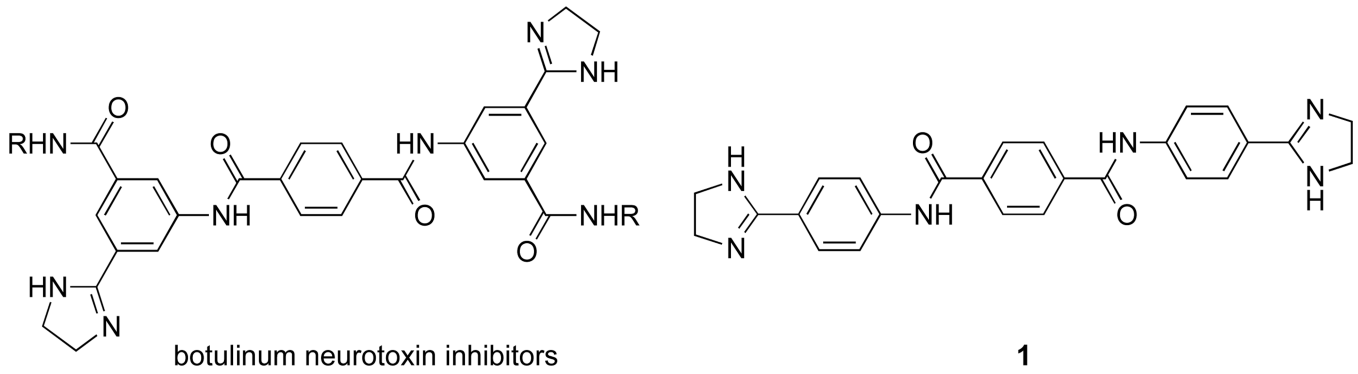
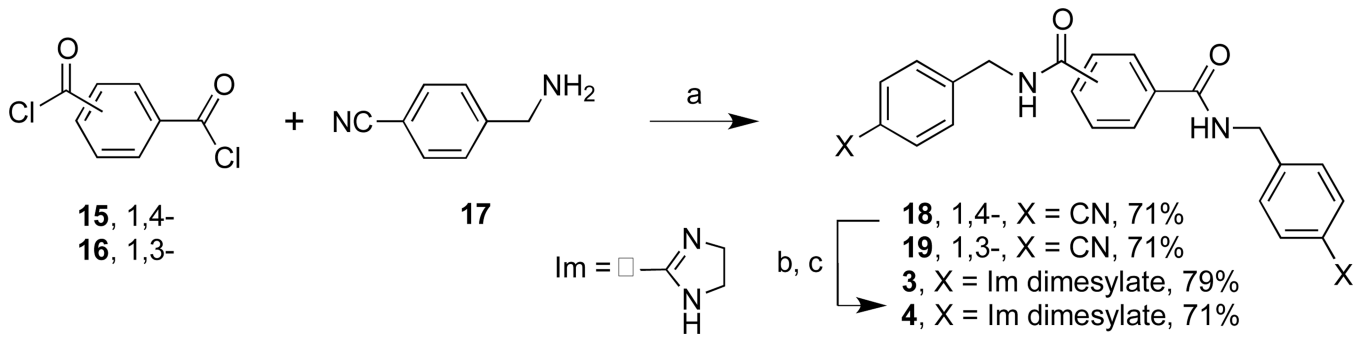
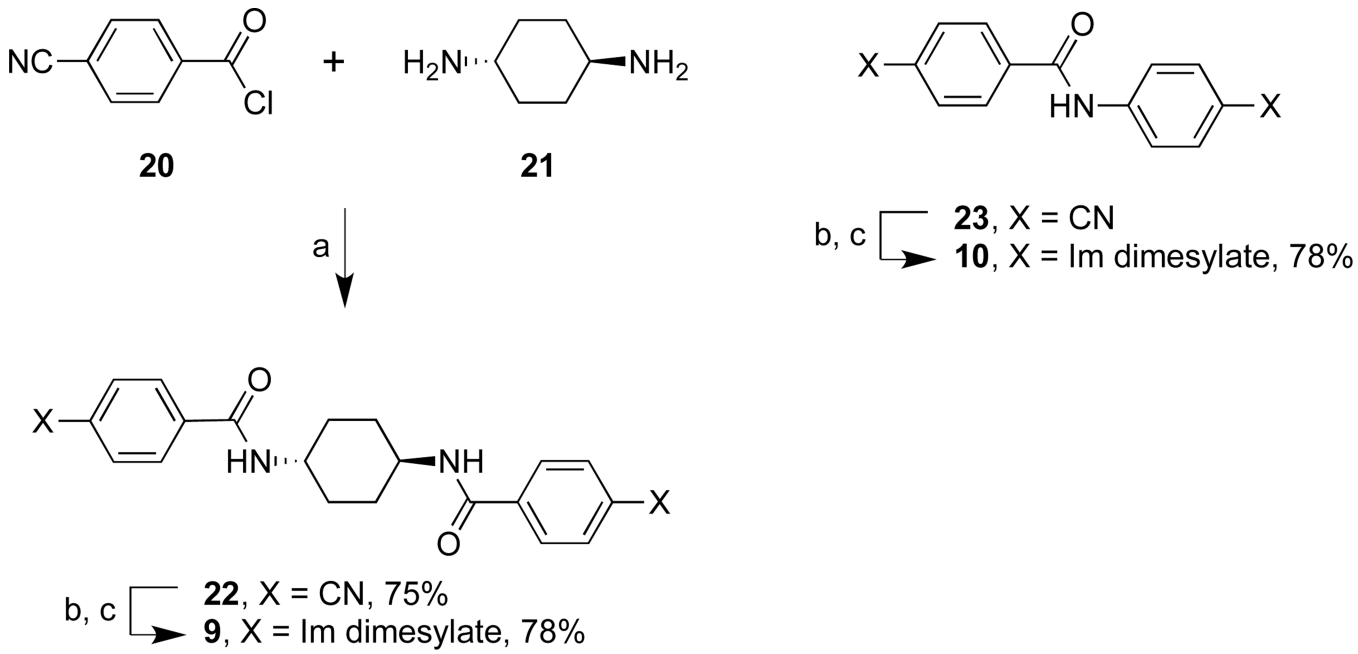


Figure 2.
Diimidazolines



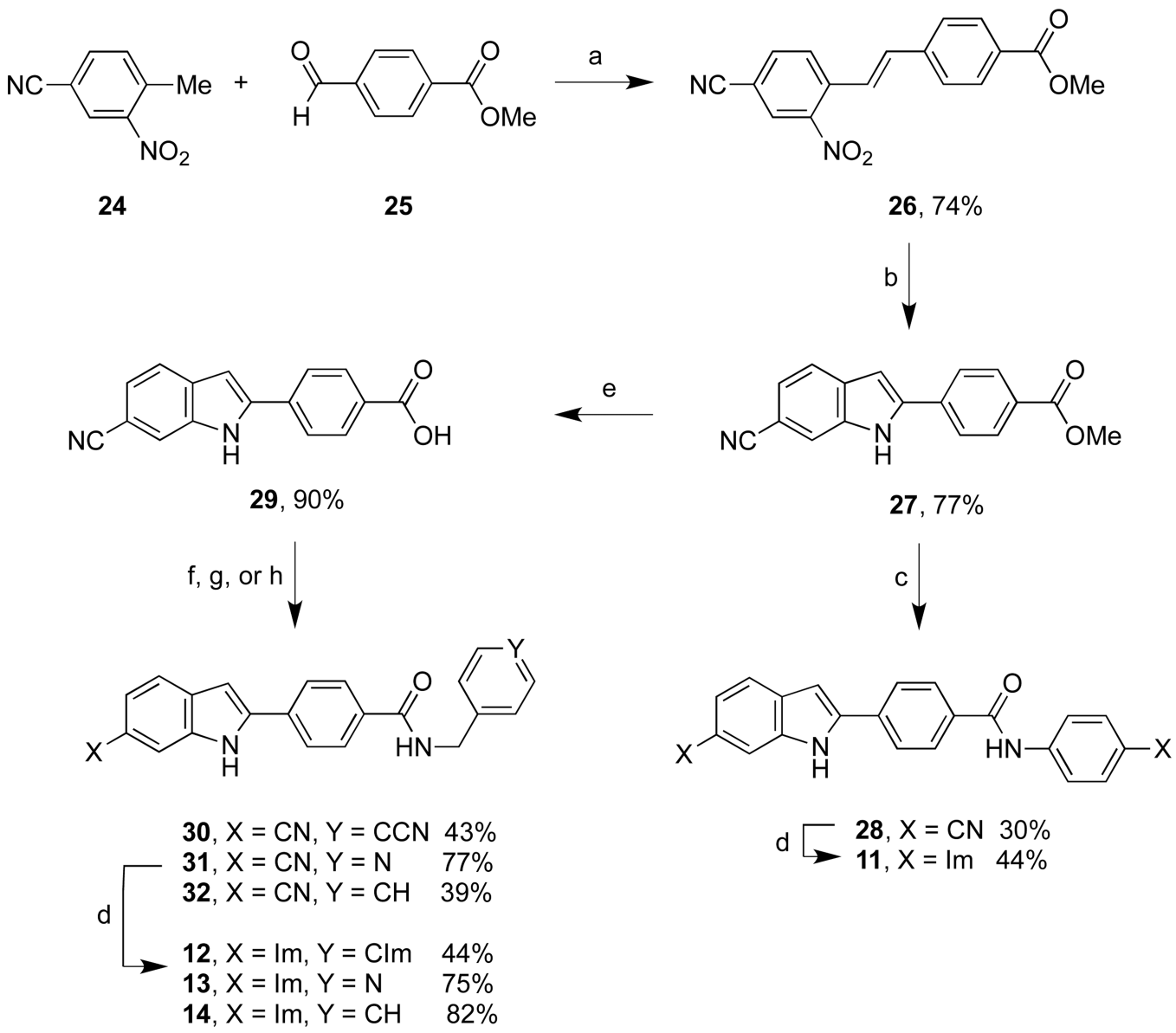
Scheme 1.

Reagents and conditions: (a) TEA, DMA, rt, 24 h; (b) ethylene diamine, NaSH, DMA, 120 °C, 2 h; (c) MSA, CH_3CN, 70 °C, 0.5 h.



Scheme 2.

Reagents and conditions: (a) TEA, CH_2Cl_2, rt, 24 h; (b) ethylene diamine, NaSH, DMA, 100 °C, 2–5 h; (c) MSA, CH_3CN, 70 °C, 0.5 h.



Scheme 3.

Reagents and conditions: (a) 4-methylpiperidine, CH_3CN, 90 °C, 48 h; (b) $P(OEt)_3$, $MoO_2(acac)_2$, 130 °C, 2 h; (c) 4-aminobenzonitrile, Me_3Al, PhMe, 75 °C, 17 h; (d) ethylene diamine, NaSH, DMA, 120 °C, 2 h; (e) NaOH, DMA:H_2O (1:1), 70 °C, 4 h; then 1 M aq. HCl; (f-h) 4-(aminomethyl)benzonitrile, 4-(aminomethyl)pyridine, or benzylamine; HOBt, EDCI, TEA, DMA, rt, 24 h.

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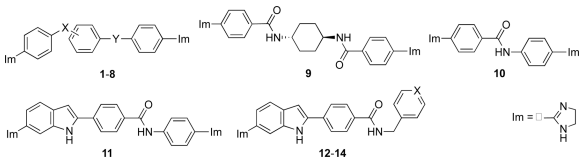
Table 1

In vitro antitrypanosomal activity against the STIB900 strain of *T. brucei rhodesiense* and in vitro cytotoxicity against the L6 cell line.



Compd	Subst. pattern	X	Y	*T. b. rhodesiense* IC_{50} (nM)	L6 IC_{50} (nM)
1[a]	1,4-	NHCO	CONH	4.7	110,000
2[a]	1,3-	NHCO	CONH	51	120,000
3[a]	1,4-	CH_2NHCO	$CONHCH_2$	9,500	92,000
4[a]	1,3-	CH_2NHCO	$CONHCH_2$	64,000	>150,000
5[a]	1,4-	CONH	CONH	11	50,000
6[a]	1,3-	CONH	CONH	120	99,000
7[a]	1,4-	CONH	NHCO	120	92,000
8[a]	1,4-	NHCO	$4\text{-}C_6H_4CONH$	19	>150,000
9[a]	-----	-----	-----	690	130,000
10[a]	-----	-----	-----	86	68,000
11	-----	-----	-----	2.2	19,000
12[a]	-----	CIm	-----	1.5	41,000
13	-----	N	-----	23	89,000
14	-----	CH	-----	2,200	110,000
melarsoprol	-----	-----	-----	3.8	19,000
pentamidine[b]	-----	-----	-----	1.5	5,100

[a] isolated and tested as the dimesylate salt

[b] tested as the diisethionate salt

Table 2

Antitrypanosomal activity of selected compounds in the *T. b. rhodesiense* acute mouse model at doses of 4×50 mg/kg[a].

Compd	Cures[b]	Mean Survival Days[c]
Control	------	6–10
1	2/4	39
2	4/4	>60
5	4/4	>60
6	4/4	>60
7	4/4	>60
8	0/4	11
10	2/4	34
12	0/4	toxic[d]

[a] Administered ip on days 3–6 post-infection.

[b] Cure is defined as survival for more than 60 days after infection without a parasitemia relapse.

[c] Mean survival days is determined for mice with and without parasitemia relapse.

[d] Mice died following first compound dose.