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# Structure-Activity Relationships of Analogs of Pentamidine against *Plasmodium falciparum* and *Leishmania mexicana amazonensis*

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**The antiprotozoal compound 1,5-di(4-amidinophenoxy)pentane (pentamidine) and 36 of its analogs were screened for in vitro activity against *Leishmania mexicana amazonensis* clone 669 C4S (MHOM/BR/73/M2269) and *Plasmodium falciparum* clones W2 (Indochina III/CDC) and D6 (Sierra Leone I/CDC). Pentamidine and each of the analogs tested exhibited activity in vitro against *L. m. amazonensis* and *P. falciparum*. The pentamidine analogs were more effective against the *P. falciparum* clones than against *L. m. amazonensis*. *P. falciparum* was extremely susceptible to these compounds, with 50% inhibitory concentrations as low as 0.03 μM. While none of the analogs exhibited marked improvement in antileishmanial activity compared with pentamidine, 12 of the pentamidine analogs showed activity approximately equal to or greater than that of the parent compound. From the promising activity exhibited by the pentamidine analogs in this in vitro study and their potential for reduced toxicity relative to the parent drug, pentamidine-related compounds hold promise as new agents for the treatment of protozoal infections.**

The efficacy of aromatic diamidines in the treatment of protozoal diseases was first recognized in the 1930s by investigators searching for agents with therapeutic activity against African trypanosomiasis (14). Early clinical trials examining the activities of pentamidine, propamidine, and stilbamidine revealed that these and other aromatic diamidines are effective against the early stages of African trypanosomiasis (6, 7, 13, 14) and against leishmaniasis (11, 15, 22). Although they are not clinically used in the treatment of malaria, the antiplasmodial activity of aromatic diamidines in monkeys infected with *Plasmodium knowlesi* was demonstrated during the 1940s (1, 4).

Aromatic diamidines not only have antiprotozoal activity but also exhibit activity against bacteria (3), fungi (3), viruses (21), and tumors (12). In the past, their use has mainly been confined to the treatment of protozoal diseases, for which they were first developed. Pentamidine continues to be used in the treatment of the Gambian form of African trypanosomiasis and against antimony-resistant leishmaniasis (17). Pentamidine was first shown to be active against the opportunistic pathogen *Pneumocystis carinii* in 1958 (10), and in the United States, this compound is primarily used to treat *P. carinii* pneumonia in patients with the acquired immune deficiency syndrome. The toxicity and side effects associated with the use of pentamidine in the treatment of *P. carinii* pneumonia in acquired immune deficiency syndrome patients have led to extensive investigations to identify a derivative of pentamidine which is more active against *P. carinii* pneumonia and less toxic than the parent drug.

To this end, over 50 analogs of pentamidine have been synthesized in our laboratory and have been examined for in vivo efficacy against *P. carinii* in the rat model of disease (10a, 18, 19). The design of more-potent analogs of pentamidine against *P. carinii* pneumonia has been hampered by the lack of a reliable in vitro culture system for this organism. In an effort to better understand the mechanism of antiparasitic activity of pentamidine analogs and to determine the range of their antiprotozoal effect, we have examined the structure-activity relationships of pentamidine analogs in vitro against *Leishmania mexicana amazonensis* clone 669 C4S (MHOM/BR/73/M2269) and two clones of *Plasmodium falciparum*, W2 (Indochina III/CDC), which is resistant to chloroquine and susceptible to mefloquine, and D6 (Sierra Leone I/CDC), which is susceptible to chloroquine and resistant to mefloquine.

## MATERIALS AND METHODS

**Chemotherapeutic agents.** Pentamidine and the analogs of pentamidine used in this study were synthesized as mono- or dihydrochloride salts in the laboratories of Richard R. Tidwell, Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill. The syntheses of these compounds were carried out by methods which have been previously described (18, 19). High-performance liquid chromatography, elemental analyses, and proton magnetic resonance were used to determine the purity of the compounds.

**Cultivation of parasites.** Two clones of *P. falciparum* were used in these studies, W2 (Indochina III/CDC) and D6 (Sierra Leone I/CDC) (16). These parasite clones were maintained in vitro by modifications of previously described methods (8, 16, 20). Briefly, the clones were maintained in 5-ml suspensions of human type A-positive (A+) erythrocytes at a parasitemia of 0.2 to 0.4% and a hematocrit of 6% in RPMI 1640 culture medium with 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 25 mM $NaHCO_3$, and 10% human plasma. The cultures were grown in sealed 50-ml flasks under an atmosphere of 5% $O_2$, 5% $CO_2$, and 90% $N_2$ and incubated at 37°C. The medium was changed daily, and the cultures were diluted every 2 to 4 days with uninfected erythrocytes in culture medium.

Promastigotes of *L. m. amazonensis* (MHOM/BR/73/M2269) clone 669 C4S were grown at 25°C to early log phase in Schneider drosophila medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% heat-inacti-

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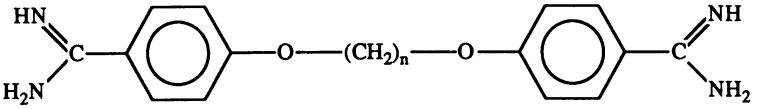
TABLE 1. Antiplasmodial and antileishmanial activities of α,ω-di(4-amidinophenoxy)alkanes



| Compound | n | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|
| | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 1 | 2 | 0.878 ± 0.160 | 0.294 ± 0.079 | 15.822 ± 1.777 |
| 2 | 3 | 0.064 ± 0.006 | 0.038 ± 0.009 | 0.852 ± 0.095 |
| 3 | 4 | 0.260 ± 0.005 | 0.118 ± 0.006 | 1.589 ± 0.272 |
| Pentamidine | 5 | 0.129 ± 0.029 | 0.051 ± 0.015 | 0.820 ± 0.019 |
| 4 | 6 | 0.256 ± 0.072 | 0.123 ± 0.021 | 0.396 ± 0.071 |

[a] Values are means for two or three separate determinations for each compound.

vated fetal bovine serum (GIBCO) and 100 μg of gentamicin sulfate per ml (9). Logarithmically growing promastigotes were maintained by transferring $10^6$ organisms per ml into fresh medium when the organisms approached a density of 4 × $10^7$/ml (5).

**Susceptibility to aromatic diamidines.** Susceptibilities of *L. m. amazonensis* clone 669 C4S and *P. falciparum* clones W2 and D6 were determined by assessing the uptake of [*methyl*-$^3$H]thymidine and [G-$^3$H]hypoxanthine (Du Pont, NEN Research Products, Boston, Mass.), respectively, as a measure of parasite growth. The inhibition of this uptake was used to determine the 50% inhibitory concentrations ($IC_{50}$s) of the compounds tested. The assay for in vitro antimalarial activity was performed as previously described (2, 16). Serial dilutions of diamidines suspended in RPMI 1640 culture medium were prepared in duplicate rows of a 96-well microtiter plate, and 200 μl of parasite suspension at 0.2% parasitemia and 1.5% hematocrit was added to each well. Each plate was incubated in an anaerobic chamber at 37°C under an atmosphere of 5% $O_2$, 5% $CO_2$, and 90% $N_2$ for 24 h, at which time [G-$^3$H]hypoxanthine (20 Ci/mmol) was added to yield 1 to 2 μCi per well. After an additional 18 h of incubation, the cells were harvested onto glass microfiber filters with a Skatron cell harvester. Washed and dried filter disks were counted with a Beckman LS3801 scintillation counter.

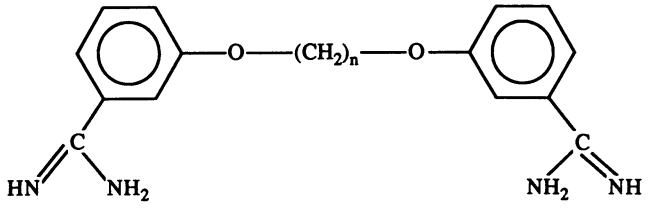
The in vitro assay for antileishmanial activity was performed by established procedures (5). The assay medium consisted of Schneider drosophila medium plus 10% heat-inactivated fetal bovine serum. Serial dilutions of diamidines suspended in assay medium were prepared in duplicate rows of a 96-well microtiter plate, and 200 μl of parasite suspension at 2.5 × $10^6$ cells per ml was added to each well. Each plate was sealed and incubated in air at 25°C for 24 h, at which time [*methyl*-$^3$H]thymidine (20 Ci/mmol) was added to yield 1 to 2 μCi per well. After an additional 18 h of incubation, the cells were harvested and counted as described above.

**Data analysis.** Data on the uptake of labeled hypoxanthine and thymidine were fitted to a logistic-logarithmic concentration response function by a nonlinear regression method, and drug concentrations required to inhibit 50% incorporation of labeled hypoxanthine and thymidine were determined (2, 16). Statistical analysis was performed on data from two or three separate determinations of each compound by using the StatView 512+ software package (Brainpower, Inc., Calabasas, Calif.) on a Macintosh II microcomputer.

## RESULTS

Pentamidine and 36 analogs of pentamidine were screened for antiplasmodial and antileishmanial activity. The results are summarized in Tables 1 through 6. The parent structure was altered by (i) varying the length of the alkyl bridge connecting the amidinophenoxy moieties from two to six carbons (Table 1), (ii) moving the amidino groups from positions *para* to the ether bridge to *meta* positions (Table 2), (iii) placing substituents on positions *ortho* to the ether bond on both aromatic rings (Table 3), (iv) isosterically replacing the ether oxygens with nitrogens (Table 4), and (v)

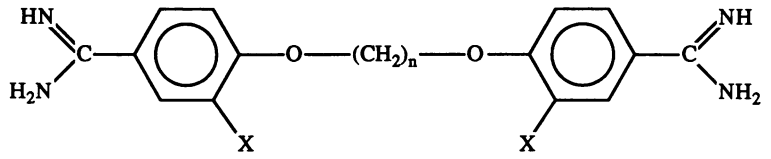
TABLE 2. Antiplasmodial and antileishmanial activities of α,ω-di(3-amidinophenoxy)alkanes



| Compound | n | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|
| | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 5 | 3 | 0.110 ± 0.006 | 0.140 ± 0.035 | 6.100 ± 0.584 |
| 6 | 4 | 0.051 ± 0.006 | 0.110 ± 0.015 | 5.435 ± 0.391 |
| 7 | 5 | 0.085 ± 0.014 | 0.082 ± 0.003 | 2.131 ± 0.203 |
| 8 | 6 | 0.114 ± 0.010 | 0.050 ± 0.015 | 1.034 ± 0.077 |

[a] Values are means for two or three separate determinations for each compound.

TABLE 3. Antiplasmodial and antileishmanial activities of α,ω-di(4-amidino-2-substituted phenoxy)alkanes



| Compound | n | X | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|---|
| | | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 9 | 2 | $NO_2$ | 0.768 ± 0.069 | 1.092 ± 0.179 | >50.0 |
| 10 | 4 | $NO_2$ | 0.112 ± 0.033 | 0.139 ± 0.048 | 5.599 ± 0.399 |
| 11 | 5 | $NO_2$ | 0.114 ± 0.037 | 0.124 ± 0.037 | 1.997 ± 0.471 |
| 12 | 2 | $NH_2$ | 2.629 ± 0.082 | 0.779 ± 0.030 | 22.598 ± 3.444 |
| 13 | 3 | $NH_2$ | 0.175 ± 0.007 | 0.111 ± 0.013 | 3.503 ± 0.426 |
| 14 | 4 | $NH_2$ | 0.339 ± 0.042 | 0.152 ± 0.006 | 7.577 ± 0.372 |
| 15 | 3 | $OCH_3$ | 0.116 ± 0.003 | 0.197 ± 0.010 | 1.785 ± 0.208 |
| 16 | 4 | $OCH_3$ | 0.403 ± 0.028 | 0.389 ± 0.034 | 10.048 ± 1.576 |
| 17 | 5 | $OCH_3$ | 0.101 ± 0.016 | 0.108 ± 0.009 | 3.031 ± 0.515 |
| 18 | 4 | Cl | 0.089 ± 0.029 | 0.144 ± 0.026 | 1.329 ± 0.179 |
| 19 | 5 | Cl | 0.129 ± 0.014 | 0.080 ± 0.009 | 0.703 ± 0.101 |
| 20 | 5 | Br | 0.146 ± 0.029 | 0.181 ± 0.060 | 0.677 ± 0.016 |

[a] Values are means for two or three separate determinations for each compound.

replacing the amidino groups with imidazolino moieties (Table 6). The amidinoanilino and imidazolino compounds were further varied by placing substituents upon their aromatic rings (Tables 5 and 6, respectively).

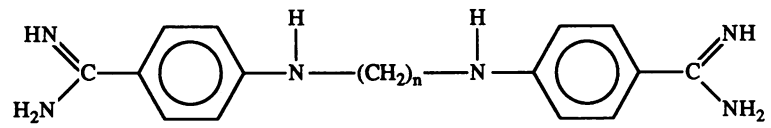
**Antileishmanial activity.** Table 1 contains antileishmanial data from compounds in which the length of the alkyl bridge was varied from two to six carbons. Each of these derivatives exhibited activity against *L. m. amazonensis*, with pentamidine having an $IC_{50}$ of 0.820 ± 0.019 μM (mean ± standard error). The six-carbon analog, hexamidine (compound 4), was approximately twofold more active than pentamidine, and the three-carbon compound, propamidine (compound 2), exhibited activity approximately equal to that of pentamidine. The two- and four-carbon analogs (compounds 1 and 3) showed decreased activity compared with that of pentamidine.

Moving the amidino groups from *para* to *meta* positions resulted in decreased antileishmanial activity (Table 2) compared with that observed for the corresponding *para*-amidinophenoxy compounds (Table 1). Increasing the chain length of the alkyl bridge of the *meta*-amidinophenoxy compounds increased their activity against clone 669 C4S, although the $IC_{50}$ of the most active *meta*-amidinophenoxy compound, the six-carbon analog (compound 8), was more than twice as high as that of the corresponding six-carbon *para*-amidinophenoxy compound (compound 4).

The effect of placing various substituents *ortho* to the ether bridge on both aromatic rings of the *para*-amidinophenoxy compounds is shown in Table 3. Substitution of nitro (compounds 9 to 11), amino (compounds 12 to 14) or methoxy (compounds 15 to 17) groups on the aromatic rings resulted in all cases in decreased antileishmanial activity relative to the unsubstituted 4-amidinophenoxy compounds (Table 1). Chloro (compounds 18 and 19) and bromo (compound 20) substitution, however, slightly increased or did not greatly alter antileishmanial activity, with $IC_{50}$s of 0.703 ± 0.101 μM and 0.677 ± 0.016 μM, respectively, for the chloro-substituted (compound 19) and bromo-substituted (compound 20) derivatives of pentamidine.

Isosteric replacement of ether oxygens with nitrogens (Table 4) produced analogs (compounds 21 to 24) with equal or improved activity against *L. m. amazonensis* in comparison with the corresponding 4-amidinophenoxy compounds (Table 1). As was the case with the *meta*-amidinophenoxy

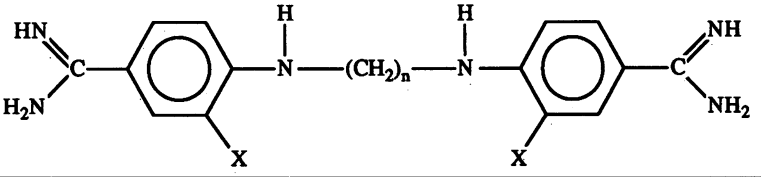
TABLE 4. Antiplasmodial and antileishmanial activities of α,ω-di(4-amidinoanilino)alkanes



| Compound | n | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|
| | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 21 | 3 | 0.076 ± 0.019 | 0.040 ± 0.010 | 0.687 ± 0.060 |
| 22 | 4 | 0.135 ± 0.007 | 0.053 ± 0.007 | 0.671 ± 0.135 |
| 23 | 5 | 0.045 ± 0.010 | 0.030 ± 0.004 | 0.558 ± 0.003 |
| 24 | 6 | 0.053 ± 0.018 | 0.036 ± 0.008 | 0.289 ± 0.055 |

[a] Values are means for two or three separate determinations for each compound.

TABLE 5. Antiplasmodial and antileishmanial activities of α,ω-di(4-amidino-2-substituted anilino)alkanes



| Compound | n | X | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|---|
| | | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 25 | 3 | $NO_2$ | 0.162 ± 0.025 | 0.138 ± 0.004 | 4.828 ± 1.213 |
| 26 | 5 | $NO_2$ | 0.248 ± 0.049 | 0.109 ± 0.012 | 1.129 ± 0.223 |
| 27 | 2 | $NH_2$ | >1.000 | >1.000 | 26.243 ± 1.952 |
| 28 | 4 | $NH_2$ | 0.522 ± 0.086 | 0.659 ± 0.012 | 7.878 ± 0.178 |
| 29 | 5 | $NH_2$ | 0.183 ± 0.044 | 0.084 ± 0.022 | 1.160 ± 0.189 |
| 30 | 6 | $NH_2$ | 0.133 ± 0.033 | 0.079 ± 0.008 | 0.991 ± 0.216 |

[a] Values are means for two or three separate determinations for each compound.

compounds, activity in this series increased with increasing alkyl-chain length. The six-carbon 4-amidinoanilino compound (compound 24) was one of the most active antileishmanial compounds tested in this study, with an $IC_{50}$ of 0.289 ± 0.055 μM, slightly lower than that of the corresponding 4-amidinophenoxy compound (compound 4). The three-, four-, and five-carbon 4-amidinoanilino compounds (compounds 21 to 23) also exhibited high antileishmanial activity with $IC_{50}$s slightly lower than or similar to those of the corresponding 4-amidinophenoxy compounds.

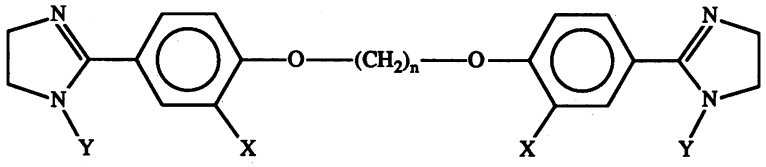
The in vitro activities of nitro- and amino-substituted 4-amidinoanilino derivatives were also evaluated (Table 5). Nitro (compounds 25 and 26) and amino (compounds 27 to 30) substitution resulted in compounds with decreased activity against *L. m. amazonensis* relative to the unsubstituted 4-amidinoanilino compounds (Table 4). The substituted 4-amidinoanilino compounds exhibited activity approximately equal to that of the corresponding substituted 4-amidinophenoxy compounds (Table 3).

Activities of compounds in which the amidino groups were replaced with imidazolino moieties are presented in Table 6. The activities of the unsubstituted 4-imidazolinophenoxy compounds (compounds 31 to 33) against *L. m. amazonensis* were slightly decreased or little changed compared with those of the corresponding unsubstituted 4-amidinophenoxy compounds (Table 1). Methoxy substitution on the aromatic rings *meta* to the imidazolino moiety produced compounds (compounds 34 to 36) with decreased activities relative to those of the unsubstituted 4-imidazolinophenoxy compounds (compounds 31 to 33), and similar or decreased activities relative to those of the methoxy-substituted 4-amidinophenoxy analogs (compounds 15 to 17).

**Antiplasmodial activity.** 4-Amidinophenoxy compounds with alkyl bridges ranging from two to six carbons exhibited relatively high antiplasmodial activities, although the activities against the two clones of *P. falciparum* were different (Table 1). Each of the unsubstituted 4-amidinophenoxy compounds was approximately twice as active against D6 as against W2. Of this series, the three-carbon compound, propamidine (compound 2), had the greatest activity, with $IC_{50}$s of 0.064 ± 0.006 μM and 0.038 ± 0.009 μM against W2 and D6, respectively. Pentamidine was also relatively active, with $IC_{50}$s of 0.129 ± 0.029 μM against W2 and 0.051 ± 0.015 μM against D6.

Moving the amidino groups from *para* to *meta* positions (Table 2) resulted in compounds with similar activities against both *P. falciparum* clones. The variation in activities against W2 and D6 seen in the corresponding *para*-amidinophenoxy compounds (Table 1) was not striking among the compounds with the amidino group in the *meta* position. The $IC_{50}$s for the *meta*-amidinophenoxy compounds against the W2 clone ranged from 0.051 ± 0.006 to 0.114 ± 0.010 μM,

TABLE 6. Antiplasmodial and antileishmanial activities of α,ω-di(4-imidazolinophenoxy)alkanes



| Compound | n | X | Y | $IC_{50}$ (μM) ± SE[a] for: | | |
|---|---|---|---|---|---|---|
| | | | | *P. falciparum* W2 | *P. falciparum* D6 | *L. m. amazonensis* |
| 31 | 3 | H | H | 0.124 ± 0.011 | 0.061 ± 0.025 | 1.773 ± 0.149 |
| 32 | 4 | H | H | 0.540 ± 0.072 | 0.164 ± 0.012 | 2.710 ± 0.234 |
| 33 | 5 | H | H | 0.548 ± 0.046 | 0.072 ± 0.012 | 1.719 ± 0.247 |
| 34 | 3 | $OCH_3$ | H | 0.039 ± 0.012 | 0.177 ± 0.049 | 2.258 ± 0.203 |
| 35 | 4 | $OCH_3$ | H | 0.387 ± 0.067 | 0.385 ± 0.010 | 6.415 ± 1.076 |
| 36 | 5 | $OCH_3$ | H | 0.066 ± 0.003 | 0.236 ± 0.035 | 4.041 ± 0.385 |

[a] Values are means for two or three separate determinations for each compound.

while the $IC_{50}$s for these compounds against the D6 clone ranged from 0.050 ± 0.015 to 0.140 ± 0.035 μM.

The antimalarial activities of compounds in which nitro (compounds 9 to 11), amino (compounds 12 to 14), methoxy (compounds 15 to 17), chloro (compounds 18 and 19), and bromo (compound 20) substituents were placed on the aromatic rings of the 4-amidinophenoxy compounds are shown in Table 3. In general, the placement of substitution groups on the aromatic rings resulted in approximately equal or decreased activity against *P. falciparum* relative to that of the unsubstituted 4-amidinophenoxy compounds (Table 1).

Replacement of the ether oxygens with nitrogens produced compounds with approximately equal or increased antiplasmodial activities (Table 4) relative to those of the corresponding 4-amidinophenoxy compounds (Table 1). The D6 clone was very susceptible to the 4-amidinoanilino compounds tested (compounds 21 to 24), with $IC_{50}$s of 0.030 ± 0.004 to 0.053 ± 0.007 μM. Nitro-substituted (compounds 25 and 26) and amino-substituted (compounds 27 to 30) 4-amidinoanilino derivatives (Table 5) were less active against the *P. falciparum* clones than were the corresponding unsubstituted 4-amidinoanilino compounds (Table 4). The substituted 4-amidinoanilino compounds had activities approximately equal to those of the corresponding substituted 4-amidinophenoxy compounds (Table 3).

Compounds in which the amidino groups were replaced with an imidazolino moiety were also examined for in vitro activity against *P. falciparum* (Table 6). Little change in antiplasmodial activity was noted in unsubstituted 4-imidazolinophenoxy compounds (compounds 31 to 33) compared with the corresponding 4-amidinophenoxy derivatives (Table 1). The three compounds in which methoxy groups were placed on the aromatic rings *meta* to the imidazolino moiety (compounds 34 to 36) exhibited a slight increase or little change in antiplasmodial activities compared with the corresponding unsubstituted 4-imidazolinophenoxy compounds (compounds 31 to 33). The methoxy-substituted 4-imidazolinophenoxy compounds (compounds 34 to 36) were slightly more active or showed little change in activity against the W2 clone compared with the corresponding methoxy-substituted 4-amidinophenoxy compounds (compounds 15 to 17). The three-carbon 4-imidazolinophenoxy compound was one of the most active compounds against W2, with an $IC_{50}$ of 0.039 ± 0.012 μM, but exhibited more-moderate activity against D6 ($IC_{50}$ of 0.177 ± 0.049).

## DISCUSSION

The data presented in this study indicate that *Leishmania* and *Plasmodium* spp. are susceptible to strongly dicationic diamidines and di-imidazolines. In general, pentamidine and all the analogs tested exhibited at least minimal activity against the protozoans used in this study. The *P. falciparum* clones W2 and D6 were more susceptible to the pentamidine analogs than was *L. m. amazonensis*. It should be noted that a direct comparison between the efficacies of drugs against various organisms is not always possible. It is also difficult to determine whether in vitro drug activity will correlate with activity in vivo. Many factors such as bioavailability, toxicity, and drug metabolism may influence in vivo activity. Preliminary data concerning the in vivo toxicity of these compounds in rats infected with *P. carinii* pneumonia are available and are useful in evaluating the potential clinical applications of these compounds (10a, 15, 18). In vivo studies with the rat model of *P. carinii* pneumonia suggest that many of the analogs are more potent and less toxic than the parent compound (10a, 15, 18).

Pentamidine and other aromatic diamidines have previously been shown to be effective against *Leishmania* spp. (11, 15, 22), and pentamidine is currently used in the treatment of antimony-resistant leishmaniasis (17). Thus, the identification of a more potent and/or less toxic derivative of pentamidine might have clinical applications in the treatment of leishmaniasis. Twelve pentamidine analogs examined in this study exhibited antileishmanial activity approximately equal to or higher than that of pentamidine. These include the unsubstituted 4-amidinophenoxy alkanes, propamidine (compound 2) and hexamidine (compound 4), and the unsubstituted six-carbon-chain 3-amidinophenoxy compound (compound 8). The chloro-substituted (compound 19) and bromo-substituted (compound 20) derivatives of pentamidine were similar in activity to pentamidine or were slightly more potent, as were the 4-amidinoanilino alkanes (compounds 21 through 24). Three of the substituted 4-amidinoanilino compounds (compounds 26, 29, and 30) exhibited antileishmanial activity approximately equal to that of pentamidine.

The *P. falciparum* clones W2 and D6 were in general very susceptible to pentamidine and the derivaties of pentamidine screened in this study. Aromatic diamidines have not been clinically used in the treatment of malaria, but previous in vivo studies (1, 4) and the current in vitro data suggest that further investigation is warranted. Several compounds examined in this study deserve further examination because of their in vitro activity against *P. falciparum* and their low in vivo toxicity, as observed in rat *P. carinii* pneumonia studies (10a, 15, 18). The three-carbon-chain 4-amidinophenoxy (compound 2) and the three- and five-carbon-chain 4-amidinoanilino compounds (compounds 21 and 23) as well as some of the substituted 4-amidinophenoxy (Table 3) and 4-amidinoanilino (Table 5) compounds were quite active against both W2 and D6. Likewise, the three- and five-carbon-chain unsubstituted 4-imidazolinophenoxy compounds (compounds 31 and 33) were also quite potent against both clones. One compound which had excellent activity against *P. falciparum* clone W2, 1,3-di(4-imidazolino-2-methoxyphenoxy)propane (compound 34), had previously demonstrated 10 times the activity of pentamidine against *P. carinii* pneumonia in the rat model of disease (10a). This compound was also found to be active against *P. carinii* pneumonia upon oral administration (10a). The *meta*-amidinophenoxy compounds (compounds 5 to 8) exhibited approximately equal activity against both clones of *P. falciparum*. The similarities in response by both the chloroquine-resistant and mefloquine-susceptible clone W2 and the chloroquine-susceptible and mefloquine-resistant clone D6 to the *meta*-amidinophenoxy compounds warrant further investigation and might also provide a tool with which to examine the mechanism(s) of drug resistance in *P. falciparum*.

This study has yielded no additional data to assist in determining the mechanism(s) by which pentamidine and its analogs exert antimicrobial activity. However, while amidinophenoxy compounds are effective inhibitors of trypsin, imidazolino compounds have been found to be devoid of antitrypsin activity and yet are active against *P. falciparum* and *L. m. amazonensis* in vitro and *P. carinii* pneumonia in vivo (18). Thus, it is unlikely that the antiprotease activity of these compounds is related to their antiprotozoal activity. Previous work has also revealed that all of the molecules bind to calf thymus DNA, as measured by a thermal denaturation assay (18). It has also been possible to demonstrate correlations between antiprotozoal activity and DNA binding (unpublished data).

A better understanding of the mechanism(s) by which these compounds act would greatly aid in the design of more-effective antiprotozoal agents. The continuing emergence of drug-resistant parasites makes the development of additional antiprotozoal drugs of vital importance. The data presented in this study indicate that *L. m. amazonensis* and *P. falciparum* are susceptible to these aromatic diamidines and di-imidazolines. This study has identified several compounds with promising antileishmanial and antiplasmodial activities that are worthy of further in vitro and in vivo studies to assess their therapeutic potential. The antimalarial and antileishmanial activities of aromatic diamidines and di-imidazolines, coupled with their potential for reduced toxicity relative to pentamidine, suggest that they hold promise for the treatment of these and other infections.

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