Parallel Solution-Phase Synthesis of Conformationally Restricted Congeners of Pentamidine and Evaluation of Their Antiplasmodial Activities

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Conformationally restricted bisbenzamidines and related congeners have been synthesized and evaluated for activity against two *Plasmodium falciparum* strains. The most active compounds, bisbenzamidines linked by a 1,4-piperazinediyl core, had IC_{50} values between 3 and 18 nM against both chloroquine-susceptible and -resistant parasites and IC_{50} values for cytotoxicity greater than 5 μM, using the A549 human lung epithelial cell line. DNA binding affinity, as estimated by ΔT_m, did not correlate with either antiparasite effects or cytotoxicity. Each of the active bisbenzamidines interfered with the formation of hemozoin in cell-free systems.

Introduction

Malaria is the most common parasitic disease in the world. Each year it affects more than 6% of the global population (300 million cases), primarily in regions between the Tropics of Cancer (23.5° north) and Capricorn (23.5° south). Although most people who become ill survive after an illness of 10−20 days, malaria kills between 1 and 2 million people annually, making it one of the major causes of mortality in developing countries.

Many different drugs have been used to prevent and treat malaria.[1] The best known, safest, and one of the most effective antimalarials is chloroquine (**1**; Figure 1), a drug that can be used for both treatment and chemoprophylaxis. Unfortunately, its intensive use has led to the development of resistant parasites.[2]

As part of our research program[3−6] focused on the development of novel pentamidine (**2**; Figure 1) congeners as potential antimicrobial agents, we decided to examine their antiplasmodial properties because aromatic diamidine compounds have not been studied extensively for their inhibition of malaria parasite growth.[7−10]

In this paper, we describe the synthesis of a series of novel bisbenzamidines, which differ from previously described analogues[9] because the aromatic moieties are linked by a conformationally restricted structure. We also report their activity against two *Plasmodium falciparum* strains: a chloroquine-sensitive strain (Haiti 135) and a chloroquine-resistant strain (Indochina I). The potential toxicity of these compounds against a human lung epithelial cell line was also evaluated. Binding to calf thymus DNA and poly(dA-dT) was measured to determine whether the affinity of these polycationic compounds to DNA (a polyanion) correlated with either their antiplasmodial activity or their cytotoxicity.

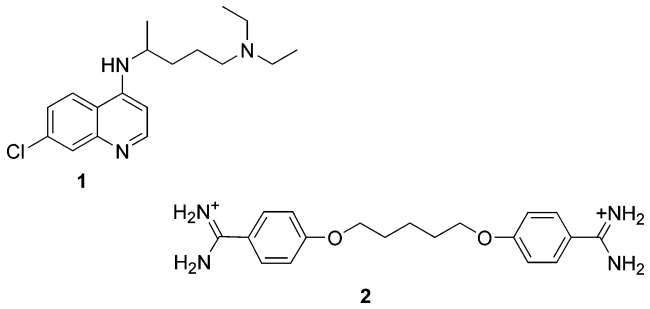

Figure 1. Structures of chloroquine (**1**) and pentamidine (**2**).

Results and Discussion

To prepare the congeners of pentamidine (**2**) examined in this study, we considered **2** as a molecule containing two benzamidine groups bound by a flexible chain. In the first step we decided to modify the nature of the linker by shifting to more rigid moieties containing a saturated ring (cyclopropane), a phenylene system (meta- or para-), or a saturated heterocycle (homopiperazine or piperazine). The parent derivatives **3**−**7** were synthesized as previously reported.[4−6,11−13] Because the piperazine-linked bisbenzamidine **7** was the most potent antiplasmodial agent in the first set of compounds, we decided, in a second step, to consider **7** as a lead molecule and to investigate the effect of modifying the terminal groups of its phenylene rings on antiplasmodial activity. This led us to prepare the highly diverse library of 11 derivatives **8**−**18**.

The bisbenzamidines **8**−**13** were synthesized as described for the parent derivative **7** but using the appropriate amines (R = butyl, cyclopentyl, cyclohexyl, isopropyl, benzyl in Scheme 1) or a diamine (1,3-diaminopropane) instead of ammonia. Compounds **14**−**16** were obtained (Scheme 2) from the dialdehyde **21**

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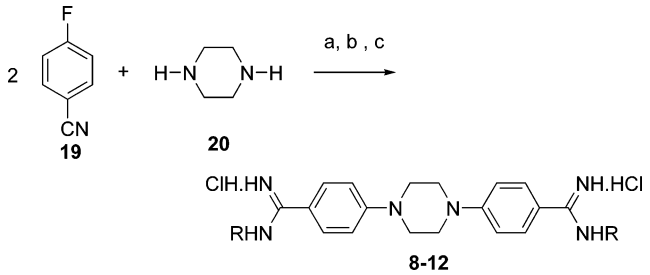
Scheme 1. Synthesis of Compound **8**−**12**[a]



[a] Reagents: (a) K_2CO_3, DMF, reflux; (b) HCl (g), MeOH, CH_2Cl_2; (c) amine, EtOH, reflux.

(prepared from piperazine **20** and 4-fluorobenzaldehyde **22**) and *N*,*N*′-dimethylethanediamine, 1,2-dianilinoethane, or ethylene glycol. The diester **17** was synthesized (Scheme 3) from piperazine (**20**) and ethyl 4-fluorobenzoate (**23**). It was then converted to **18** by reaction with hydrazine.

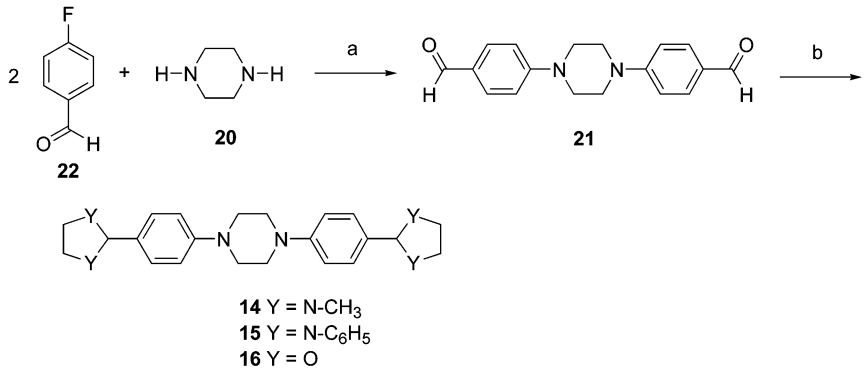
Biological Studies. The antiplasmodial activities of the compounds synthesized were determined by their ability to inhibit the incorporation of ^{3}H-hypoxanthine into nucleic acid via the parasite purine salvage pathway, as reported previously.[14] Each compound was evaluated for activity against two *Plasmodium falciparum* strains: a cloned chloroquine-susceptible strain from Haiti (Haiti 135) and a cloned chloroquine-resistant strain from Indochina (Indochina I). In each case, chloroquine was used as the control. The results are reported as the concentrations of the test compounds necessary to inhibit the incorporation of ^{3}H-hypoxanthine by 50% (IC_{50}, Tables 1 and 2).

Examination of the IC_{50} values for the first set of compounds (Table 1) demonstrates the dramatic influence of the linker on the antiparasite activity of the bisbenzamidines. Indeed, modification of the linker permitted the synthesis of a series of bisbenzamidines ranging in activity from 2000 to 3 nM against the chloroquine-susceptible strain and from 500 to 4 nM against the chloroquine-resistant strain. Interestingly, these compounds had similar activities against both chloroquine-susceptible (Haiti 135) and chloroquine-resistant (Indochina I) strains. In contrast, chloroquine was 33-fold less active against the chloroquine-resistant Indochina I strain than against the chloroquine-susceptible Haiti strain. The piperazine-linked bisbenzamidine **7** emerged as a highly potent lead (IC_{50} = 3−4 nM) with an activity profile superior to pentamidine **2** and chloroquine **1** against both chloroquine-susceptible and chloroquine-resistant parasites.

Plausible mechanisms of action of bioactive bisbenzamidines include initial binding to AT-rich sites in the minor groove of DNA[15] followed by inhibition of one or more of several DNA-dependent enzymes such as topoisomerases or direct inhibition of the transcription process.[16,17] However, other modes of action such as disruption of polyamine metabolism cannot be ruled out.[18,19] In this work we examined the binding affinity of compounds **2**−**7** for calf thymus DNA and poly(dA-dT) by measuring the change in midpoint of the thermal denaturation curves (ΔT_m) for a 1:5 compound to DNA base pair ratio. On the basis of the ΔT_m data in Table 1, the most promising antiplasmodial agent **7** has a strong binding affinity for DNA and a higher binding affinity for AT-rich DNA (as indicated by the higher ΔT_m values obtained for the poly(dA-dT) sample). Despite this observation, DNA binding is not likely to be a crucial step in the inhibition of parasite growth. This is because the DNA binding (ΔT_m values) of these compounds did not correlate with their antiplasmodial activities (IC_{50} values). For example, although **4** is more potent than **3**, it had a weaker binding affinity for poly-(dA-dT).

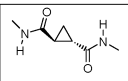
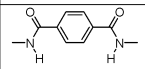
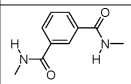
To assess the possibility that pentamidine and other diamidines might act against malaria parasites by binding and inhibiting hemozoin formation, as recently proposed by Stead et al.,[10] we used infrared spectroscopy[20,21] and colorimetry[10] to examine the interactions between ferriprotoporphyrin(IX) (FPIX) and these compounds in cell-free systems. On the basis of infrared spectroscopy (monitoring the concentration of hemozoin by observing the intensity of the band around 1710 cm^{-1}, which represents the carbonyl groups on the porphyrin skeleton), all the benzamidines tested inhibited the formation of hemozoin in acidic acetate solutions mimicking the pH of the parasitic food vacuole, while **14**−**18**, which lack the amidine functionality, did not inhibit hemozoin formation. Colorimetric measurements (monitoring the concentration of FPIX in solution by measuring its absorbance at 400 nm) also demonstrated that benzamidines formed complexes with ferriprotoporphyrin(IX). These results suggest that the mechanism of diamidine action against *Plasmodium falciparum* parasite may be different from their mech-

Scheme 2. Synthesis of Compounds **14**−**16**[a]



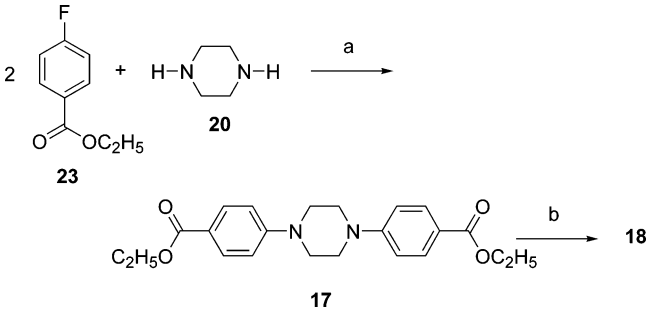
[a] Reagents: (a) K_2CO_3, DMF, reflux; (b) for **14** *N*,*N*′-dimethylethanediamine, PTSA, benzene, Dean−Stark apparatus; for **15** 1,2-dianilinoethane, AcOH, EtOH reflux; for **16** ethylene glycol, PTSA, benzene, Dean−Stark apparatus.

Table 1. In Vitro Antiplasmodial Activity, Cytotoxicity, and DNA Binding Affinity for Compounds **1**−**7**[a]

#	Linker	Antiplasmodial activity IC_{50} (nM)		Cytotoxicity in A549 cells IC_{50} (μM)	DNA binding (ΔT_m, ° C)	
		Haiti 135 (CQ-susceptible)	Indochina I (CQ-resistant)		Calf thymus	Poly(dA-dT)
1 Chloroquine		6	200	40 [b]	-	-
2 Pentamidine		15	45	40	11.1	20.6
3		2000	500	> 526	7.1	11.1
4		100	200	> 228	9.7	7.7
5		40	40	> 122	8.0	9.9
6		150	100	> 204	15.0	23.1
7		3	4	> 413	17.0	23.8

[a] All biological activities resulted from the average of at least two determinations. [b] From the NCI database: http://dtp.nci.nih.gov/webdata.html.

Scheme 3. Synthesis of Compounds **17** and **18**[a]



[a] Reagents: (a) K_2CO_3, DMF, reflux; (b) $NH_2NH_2 \cdot H_2O$, EtOH, H_2O, reflux.

anisms of action against *Trypanosoma brucei*[17,22,23] and *Leishmania donovani*.[19,23,24]

The second set of derivatives is a library of 1,4-diarylpiperazines with alkylated amidines and other substituents in the para position on the phenylene ring. These compounds were prepared on the basis of the promising antiparasite properties of the lead molecule **7**. The results are tabulated in Table 2.

The bis (*N*-alkylbenzenecarboximidamides) **8**−**12** had similar inhibitory activities (IC_{50} values) against both chloroquine-susceptible and chloroquine-resistant parasite strains. Similar findings were obtained for **13**, in which the amidine moieties are within a cyclic structure. These observations indicate that the antiplasmodial activity of the benzamidines is not affected by the pfcrt-based efflux mechanism[25] responsible for chloroquine resistance in *Plasmodium falciparum*.[2] In addition, replacement of the amidine moieties in these compounds by imidazolidine (**14**, **15**), dioxolane (**16**), ester (**17**), or carboxhydrazide (**18**) groups abolished their antiparasite activity. This second result suggests that the terminal groups of biologically active derivatives may need to contain basic, nonsterically hindered amidine moieties.

These bisbenzamidines were also screened for toxicity against mammalian cells in vitro, using the A549 human lung epithelial cell line (Table 2). Each of the active compounds tested had low cytotoxicity for the A549 cell line (IC_{50} values up to 1000-fold higher than the IC_{50} values of these compounds for the two strains of *Plasmodium falciparum*).

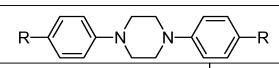
Conclusions

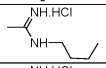
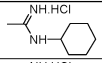
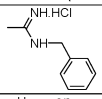
Aromatic diamidines are prescribed widely for the treatment of fungal and protozoal infections, despite their major side effects. However, the diamidines have not been evaluated extensively as potential antimalarials. The results reported here demonstrate that piperazine-linked bisbenzamidines are highly active against both chloroquine-susceptible and chloroquine-resistant *Plasmodium falciparum* and indicate that they inhibit the formation of hemozoin in cell-free systems. The most efficient compounds **7**−**13** were 3- to 11-fold more active than pentamidine (**2**) and were 11- to 50-fold more active than chloroquine (**1**) against the chloroquine-resistant Indochina I strain of *Plasmodium falciparum*. Thus, the 4,4′-(piperazine-1,4-diyl)bisbenzamidines are a promising, novel class of compounds with potential antimalarial activity.

Experimental Section

^{1}H NMR spectra were obtained using a Varian Inova instrument (500 MHz), and chemical shifts (δ) are given in ppm using TMS as the internal reference. IR spectra were

Table 2. In Vitro Antiplasmodial Activity, Cytotoxicity, and DNA Binding Affinity for Compounds **1**, **2**, and **7**−**18**[a]



#	R	Antiplasmodial activity IC_{50} (nM)		Cytotoxicity in A549 cells IC_{50} (μM)	DNA binding (ΔT_m, ° C)	
		Haiti 135 (CQ-susceptible)	Indochina I (CQ-resistant)		Calf thymus	Poly(dA-dT)
1 Chloroquine		6	200	40 [b]	-	-
2 Pentamidine		15	45	40	11.1	20.6
7		3	4	> 413	17.0	23.8
8		3	4	> 9	15.2	23.9
9		3	4	> 43	14.0	23.0
10		3	4	> 21	15.5	23.6
11		4	8	> 125	14.9	22.5
12		6	18	619	18.0	25.1
13		5	5	> 210	12.4	17.9
14		> 500	> 500	-	2.4	1.8
15		> 500	> 500	-	- 1.2	0.0
16		> 500	> 500	-	- 1.2	0.1
17		> 500	> 500	-	0.6	0.0
18		> 500	> 500	-	0.1	0.0

[a] All biological activities resulted from the average of at least two determinations. [b] From the NCI database: http://dtp.nci.nih.gov/webdata.html.

recorded on a Perkin-Elmer Spectrum One instrument operating in the diffuse reflectance mode. Solvents and reagents are commercially available (Aldrich Co., Acros Organics, Fisher Scientific, Sigma Chemical Co.) and were used without further purification.

Compounds **3**,[4,6] **4**,[11] **5**,[4,12,13] **6**,[4,5] **7**,[4,13] and **21**[26] have been described in the literature. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ.

General Procedure for the Preparation of the 4,4′-(1,4-Piperazinediyl)bisbenzenecarboximidamides 8−13. A mixture of 4,4′-(1,4-piperazinediyl)bisbenzonitrile[27] (2 mmol, 0.6 g) in dichloromethane (250 mL) and methanol (10 mL) was saturated with HCl gas, and the reaction medium was left at room temperature for 4 days. The precipitate (crude imidate) was filtered, washed with acetone, and treated with the appropriate (di)amine.

4,4′-(1,4-Piperazinediyl)bis(*N*-butylbenzenecarboximidamide), Dihydrochloride Salt 8. The crude imidate, obtained as above, was treated with butylamine (20 mmol, 2.0 mL) in refluxing ethanol (50 mL) for 30 min. After the mixture was cooled, the solid was filtered and successively washed with water, ethanol, and ether: 25% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.2 (bs, 6 H), 7.7 (d, 4 H, J = 9 Hz), 7.1 (d, 4 H, J = 9 Hz), 3.5 (s, 8 H), 3.4 (t, 4 H, J = 8 Hz), 1.6 (m, 4 H, J = 8 Hz), 1.4 (m, 4 H), 0.9 (t, 6 H, J = 8 Hz); IR 3065, 1671, 1614, 1518, 1383, 1231 cm^{-1}. Anal. ($C_{26}H_{38}N_6$·2HCl) C, H, N.

4,4′-(1,4-Piperazinediyl)bis(*N*-cyclopentylbenzenecarboximidamide), Dihydrochloride Salt 9. The crude imidate, obtained as above, was treated with cyclopentylamine (40 mmol, 4.0 mL) in refluxing ethanol (50 mL) for 90 min. After cooling, the mixture was concentrated under reduced pressure and the residue was successively washed with DMF and ether: 30% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.2−8.7 (bs, 6 H), 7.6 (d, 4 H, J = 9 Hz), 7.1 (d, 4 H, J = 9 Hz), 4.1 (m, 2 H, J = 7 Hz), 3.5 (s, 8 H), 2.0 (m, 4 H, J = 7 Hz), 1.7−1.5 (m, 12 H); IR 3062, 1668, 1601, 1515, 1395, 1232 cm^{-1}. Anal. ($C_{28}H_{38}N_6$·2HCl·H_2O) C, H, N.

4,4′-(1,4-Piperazinediyl)bis(*N*-cyclohexylbenzenecarboximidamide), Dihydrochloride Salt 10. The crude imidate, obtained as above, was treated with cyclohexylamine (20 mmol, 2.3 mL) in refluxing ethanol (50 mL) for 30 min. After the mixture was cooled, the precipitate was filtered and successively washed with water, ethanol, and ether: 25% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.2 (bs, 4 H), 9.1 (bs, 2 H), 7.7 (d, 4 H, J = 9 Hz), 7.1 (d, 4 H, J = 9 Hz), 3.5 (m, 2 H), 3.5 (s, 8 H), 1.9 (d, 4 H, J = 8 Hz), 1.8 (d, 4 H, J = 8 Hz), 1.6 (d, 2 H, J = 12 Hz), 1.4 (m, 8 H), 1.1 (m, 2 H); IR 3053, 1673, 1606, 1516, 1234 cm^{-1}. Anal. ($C_{30}H_{42}N_6$·2HCl) C, H, N.

4,4′-(1,4-Piperazinediyl)bis[*N*-(1-methylethyl)benzenecarboximidamide], Dihydrochloride Salt 11. The crude imidate, obtained as above, was treated with isopropylamine (50 mmol, 4.3 mL) in refluxing ethanol (50 mL) for 90 min. After cooling, the mixture was concentrated under reduced pressure and the residue was successively washed with DMF and ether: 40% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.1−8.8 (bs, 6 H), 7.7 (d, 4 H, J = 9 Hz), 7.1 (d, 4 H, J = 9 Hz), 4.0

(m, 2 H), 3.5 (s, 8 H), 1.2 (d, 12 H, $J = 7$ Hz); IR 3077, 1672, 1602, 1516, 1232 cm^{-1}. Anal. ($C_{24}H_{34}N_6 \cdot 2HCl$) C, H, N.

4,4′-(1,4-Piperazinediyl)bis[*N*-(phenylmethyl)benzenecarboximidamide], Dihydrochloride Salt 12. The crude imidate, obtained as above, was treated with benzylamine (20 mmol, 2.2 mL) in refluxing ethanol (50 mL) for 30 min. After the mixture was cooled, the precipitate was filtered and successively washed with water, ethanol, and ether: 50% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 10.0 (s, 2 H), 9.3 (s, 2 H), 8.9 (s, 2 H), 7.8 (d, 4 H, $J = 8$ Hz), 7.4 (m, 8 H), 7.3 (t, 2 H, $J = 7$ Hz), 7.1 (d, 4 H, $J = 8$ Hz), 4.7 (s, 4 H), 3.6 (s, 8 H); IR 3099, 1668, 1607, 1518, 1384, 1235 cm^{-1}. Anal. ($C_{32}H_{34}N_6 \cdot 2HCl$) C, H, N.

1,4-Bis[4-(1,4,5,6-tetrahydropyrimidin-2-yl)phenyl]piperazine, Dihydrochloride Salt 13. The crude imidate, obtained as above, was treated with 1,3-diaminopropane (30 mmol, 2.5 mL) in refluxing ethanol (50 mL) for 90 min. After the mixture was cooled, the precipitate was filtered and successively washed with DMF and ether: 25% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.5 (bs, 4 H), 7.6 (d, 4 H, $J = 9$ Hz), 7.1 (d, 4 H, $J = 9$ Hz), 3.5 (s, 8 H), 3.4 (t, 8 H, $J = 5$ Hz), 1.9 (m, 4 H, $J = 5$ Hz); IR 3271, 1638, 1602, 1516, 1231 cm^{-1}. Anal. ($C_{24}H_{30}N_6 \cdot 2HCl \cdot 2H_2O$) C, H, N.

1,4-Bis(1,3-dimethylimidazolidine-2-yl)piperazine 14. A mixture of 4,4′-(1,4-piperazinediyl)bisbenzaldehyde[26] (**21**, 2.9 g, 10 mmol), *N*,*N*′-dimethylethylenediamine (85%, 2.1 g, 20 mmol), and *p*-toluenesulfonic acid (100 mg) in benzene (100 mL) was heated under reflux in a Dean−Stark apparatus for 5 h. After the mixture was cooled, the solvent was evaporated under reduced pressure to afford a solid, which was recrystallized from acetonitrile: 70% yield; mp 204−206 °C; ^{1}H NMR ($CDCl_3$) δ 7.4 (d, 4 H, $J = 9$ Hz), 7.0 (d, 4 H, $J = 9$ Hz), 3.4 (m, 4 H), 3.3 (s, 8 H), 3.2 (s, 2 H), 2.5 (m, 4 H), 2.2 (s, 6 H); IR 2940, 2826, 1611, 1515, 1449, 1227, 1043, 1032 cm^{-1}. Anal. ($C_{26}H_{38}N_6$) C, H, N.

1,4-Bis(1,3-diphenylimidazolidine-2-yl)piperazine 15. A mixture of 4,4′-(1,4-piperazinediyl)bisbenzaldehyde[26] (**21**, 2.9 g, 10 mmol), dianilinoethane (8.5 g, 40 mmol), and acetic acid (10 mL) in ethanol (100 mL) was heated under reflux for 16 h. After the mixture was cooled, the precipitate was filtered, washed with acetone, and recrystallized from a mixture (1:1) of ethanol and dioxane: 75% yield; mp 250−255 °C (dec); ^{1}H NMR (DMSO-d_6) δ 7.4 (d, 4 H, $J = 8$ Hz), 7.1 (t, 8 H, $J = 8$ Hz), 6.8 (d, 4 H, $J = 8$ Hz), 6.7 (d, 8 H, $J = 8$ Hz), 6.6 (t, 4 H, $J = 8$ Hz), 6.1 (s, 2 H), 3.9 (m, 4 H), 3.7 (m, 4 H), 3.6 (s, protons from the dioxane of recrystallization), 3.1 (s, 8 H); IR 3071, 2963, 2826, 1599, 1482, 1236, 750 cm^{-1}. Anal. ($C_{46}H_{46}N_6 \cdot {}^1/_2C_4H_8O_2$) C, H, N.

1,4-Bis[4-(1,3-dioxolane-2-yl)phenyl]piperazine 16. A mixture of 4,4′-(1,4-piperazinediyl)bisbenzaldehyde[26] (**21**, 2.9 g, 10 mmol), ethylene glycol (10 mL, 180 mmol), and *p*-toluenesulfonic acid (100 mg) in benzene (100 mL) was heated under reflux in a Dean−Stark apparatus for 7 h. After the mixture was cooled, the precipitate was filtered, successively washed with benzene and dichloromethane, and recrystallized from benzene: 55% yield; mp 246−249 °C; ^{1}H NMR (DMSO-d_6) δ 7.3 (d, 4 H, $J = 8$ Hz), 7.0 (d, 4 H, $J = 8$ Hz), 5.6 (s, 2 H), 4.0 (m, 4 H), 3.9 (m, 4 H), 3.3 (s, 8 H); IR 3045, 2960, 2879, 1615, 1523, 1406, 1187, 1038, 830 cm^{-1}. Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

4,4′-(1,4-Piperazinediyl)bis(benzoic acid ethyl ester) 17. A mixture of piperazine (**20**, 0.85 g, 10 mmol), ethyl 4-fluorobenzoate (**23**, 3.36 g, 3.0 mL, 20 mmol), and potassium carbonate (2.8 g, 20 mmol) in DMF (10 mL) was heated under reflux for 8 h. After the mixture was cooled, water (20 mL) was added to precipitate the final product that was filtered, successively washed with water and ethanol, and recrystallized from acetone: 40% yield; mp 199−200 °C; ^{1}H NMR ($CDCl_3$) δ 7.9 (d, 4 H, $J = 8$ Hz), 6.9 (d, 4 H, $J = 8$ Hz), 4.4 (m, 4 H, $J = 9$ Hz), 3.5 (s, 8 H), 1.4 (t, 6 H, $J = 9$ Hz); IR 2981, 2907, 2846, 1710, 1688, 1601, 1519, 1446, 1402 cm^{-1}. Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

4,4′-(1,4-Piperazinediyl)bisbenzenecarboxhydrazide 18. A mixture of 4,4′-(1,4-piperazinediyl)bis(benzoic acid ethyl ester) (**17**, 0.4 g, 1 mmol), water (0.5 mL), ethanol (2.5 mL), and hydrazine hydrate (5 mL) was heated under reflux for 8 h. After the mixture was cooled, the precipitate was filtered and re-treated under the same experimental conditions. The precipitate was filtered and washed with water: 80% yield; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (bs, 2H), 7.9 (d, 4 H, $J = 8$ Hz), 7.0 (d, 4 H, $J = 8$ Hz), 4.5 (bs, 4 H), 3.5 (s, 8 H); IR 3307, 2982, 2848, 1690, 1640, 1602, 1278, 940 cm^{-1}. Anal. ($C_{18}H_{22}N_6O_2$) C, H, N.

Antiplasmodial Activity. The parasite strains used for these studies were a cloned chloroquine-susceptible parasite from Haiti (Haiti 135)[28,29] and a cloned chloroquine-resistant parasite from Indochina (Indochina I).[29,30] In vitro antiplasmodial activity was determined by the ability of a compound to inhibit parasite growth, measured by ^{3}H-hypoxanthine incorporation and based on the parasite's requirement for the purine salvage pathway.[31] Synchronous cultures of early ring-stage parasites (600 μL of a 2.5% red blood cell suspension with 0.2% parasitemia in 24-well cell culture plates; Corning Inc., Corning, NY) were grown in an in vitro culture system[32] with varying concentrations of the compounds being tested without supplemental hypoxanthine for 48 h. After a routine medium change using the same drug concentrations at 24 h, the medium change at 48 h included 2 μCi of ^{3}H-hypoxanthine (NEN Perkin-Elmer, Boston, MA). The plates were then incubated for an additional 12−14 h, after which they were harvested (model 1100 Cell Harvester, Skatron, Sterling, VA) on glass microfiber filters (934AH, Whatman, Clifton, NJ). The glass fiber microfilter disks were placed in scintillation vials with 8 mL of scintillation fluid (Cytoscint, ICN, Costa Mesa, CA) and counted in a liquid scintillation counter (Packard TriCarb 2100 TR, Perkin-Elmer, Downers Grove, IL). The 50% inhibitory concentration (IC_{50}) was the concentration that inhibited parasite growth (^{3}H-hypoxanthine accumulation) by 50% in relation to the drug-free control. After a screening to identify the biologically active range, repeat testing was performed in duplicate to determine the actual IC_{50}.

Interaction with Ferriprotoporphyrin(IX) in a Cell-Free System.[10,20,21] The procedures described in the literature were followed by using compounds **1**−**18** and were performed in duplicate. A detailed study of the results will be reported elsewhere.[33]

DNA Binding Affinity Measurements. Thermal denaturation curves were determined by the procedure described in the literature.[34,35] Each ΔT_m value reported in the tables represents the mean of at least two experimental determinations.

Cytotoxicity Evaluation. A bioluminescent ATP assay was used to determine the cytotoxic effects of the compounds on human lung cell monolayers.[36,37] Confluent monolayers of lung cell carcinoma A549 (ATCC 185) were established in 24-well plates containing 1 mL of DMEM plus High Glucose (4.5 g/L) (Fisher Scientific Inc., Cincinnati, OH) with 10% fetal bovine serum (Fisher Scientific), 0.1 mM nonessential amino acids, L-glutamine (0.2 mM), 1X MEM vitamins, and 1.1 μg/mL sodium pyruvate (Fisher Scientific). Media containing varying concentrations of each test compound were added to individual wells in triplicate and were harvested in triplicate at each time point tested. Medium alone was the negative control and antimycin A was the positive control. After incubation and appropriate treatment, 5 μL aliquots were placed directly into the individual wells of a 96-well opaque white plate containing 100 μL of buffer (200 mM Tris, 2.5 mM EDTA, pH 7.75), which were then placed in a FluoSTAR Optima plate reader (BMG Labtechnologies, Inc.). Samples were automatically mixed with the luciferin/luciferase reagent via an injector and immediately measured for light emission at 562 nm.

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