through Celite. The residue after concentration of the filtrate was purified by flash chromatography (hexane–EtOAc = 4:1) to give 7.2 mg (95%) of the 17β-fluoro ketone 11 as a white solid. It was further purified by recrystallization from hexane to give white cottonlike crystals for identification and biological tests: mp 130–131 °C (lit.[39] mp 129–131 °C); ^{1}H NMR (300 MHz, $CDCl_3$) 0.84 (d, 3 H, J = 2.4 Hz, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 4.49 (ddd, 1 H, J_{HF} = 56.1 Hz, J_{HH} = 9.0 Hz, 7.2 Hz, 17α-H); ^{19}F NMR (282.3 MHz, $CDCl_3$) −195.10 (dd, J_{HF} = 57.3 Hz, 26.3 Hz), agreed with the ^{1}H NMR reported in literature;[39] MS (70 eV) m/z (rel intensity) 292 (M^+, 32), 220 (100), 201 (13), 121 (20), 107 (36), 93 (39), 81 (51), 67 (47), 55 (50), 41 (58); HRMS calcd for $C_{19}H_{29}OF$ 292.2202, found 292.2203.

3,3-Difluoroandrostan-17β-ol (12). DHT **53** (0.1 g, 0.34 mmol) was dissolved in CH_2Cl_2 (2 mL) and treated with pyridine (31 μL, 0.38 mmol) as well as CH_3COCl (0.1 mL, 1.4 mmol) at RT for 2 h. The isolated crude product was purified by recrystallization from EtOH and water, giving 0.11 g (98.3%) of the pure DHT acetate as a white needlelike crystalline solid: mp 155–156 °C (Steraloids catalog reported 156–157 °C).

The acetate (50 mg, 0.15 mmol) was treated with DAST (~150 μL) by the same procedure as that described in the synthesis of the compound **51**. Flash chromatography (hexane–EtOAc = 4:1) purification of the isolated product gave 48.5 mg (91%) of the pure geminal difluoro intermediate. It was recrystallized from EtOH and water to give white crystals: mp 126–128 °C; ^{1}H NMR (200 MHz, $CDCl_3$) 0.77 (s, 3 H, 18-CH_3), 0.82 (s, 3 H, 19-CH_3), 2.02 (s, 3 H, acetyl CH_3), 2.89–3.00 (m, 2 H, 2 or 4-H), 4.57 (dd, 1 H, J = 9.6 Hz, 7.6 Hz, 17α-H), agreed with the ^{1}H NMR reported in literature;[37] MS (10 eV) m/z (rel intensity) 354 (M^+, 13), 294 (100), 279 (60), 149 (81), 94 (45); HRMS calcd for $C_{21}H_{32}O_2F_2$ 354.2370, found 354.2381.

The intermediate (20.9 mg, 0.59 mmol) was treated with NaOH–MeOH solution (5 mL, 0.5 N) at RT for 1.5 h. The isolated crude material was purified by flash chromatography (hexane–EtOAc = 2:1) and 18 mg (97.8%) of the pure **12** was obtained as a white solid, which was further purified by flash chromatography twice more to give white crystals for identification and biological tests: mp 149–151 °C (lit.[55] mp 154–156 °C); ^{1}H NMR (200 MHz, $CDCl_3$) 0.72 (s, 3 H, 18-CH_3), 0.82 (s, 3 H, 19-CH_3), 3.63 (br t, 1 H, J = 8.0 Hz, 17α-H), 2.98–3.08 (br s, <1 H, 17β-O*H*); ^{19}F NMR (338.8 MHz, $CDCl_3$) −89.59 (dbr d, J_{FF} = 233.4 Hz, J_{HF} = 2.8 Hz, one of the 3-F), −99.41 (dtt, J_{FF} = 233.7 Hz, J_{HF} = 34.1 Hz, 13.6 Hz, another 3-F); MS (70 eV) m/z (rel intensity) 312 (M^+, 51), 268 (16), 253 (100), 185 (24), 145 (11), 123 (33), 107 (22), 95 (29), 81 (35), 67 (40), 55 (42), 41 (41); HRMS calcd for $C_{19}H_{30}OF_2$ 312.2265, found 312.2270.

Biological Methods. Relative Binding Affinity (RBA). Relative binding affinities of androgens were determined in several receptor and binding protein systems as described in previous publications: androgen receptor (AR),[20,56] progesterone receptor (PgR),[20,27] mineralocorticoid receptor (MR),[43] and sex steroid binding protein (SBP).[57] The standard of the RBA measurement was tritium-labeled R1881 (K_d = 0.6 nM), R5020 (K_d = 0.4 nM), aldosterone (K_d = 3.9 nM), and estradiol (K_d = 1.6 nM) for AR, PgR, MR, and SBP, respectively. RBA values of the standards are 100 by definition.

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Bisquinolines. 1. *N,N*-Bis(7-chloroquinolin-4-yl)alkanediamines with Potential against Chloroquine-Resistant Malaria

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On the basis of observations that several bisquinolines such as piperaquine possess notable activity against chloroquine-resistant malaria, 13 *N,N*-bis-(7-chloroquinolin-4-yl)alkanediamines were synthesized and screened against *Plasmodium falciparum* in vitro and *Plasmodium berghei* in vivo. Twelve of the thirteen bisquinolines had a significantly lower resistance index than did chloroquine; the resistance index was apparently unrelated to either in vitro or in vivo activity. Except for two compounds, there was a reasonable correlation between in vitro and in vivo activities. Seven of the thirteen bisquinolines had IC_{50}'s of less than 6 nM against both chloroquine-sensitive (D-6) and -resistant (W-2) clones of *P. falciparum* and were curative against *P. berghei* at doses of 640 mg/kg. In contrast to chloroquine, these bisquinolines did not show any toxic deaths at curative dose levels. Four bisquinolines, however, caused skin lesions at the site of injection. Maximum activity was seen in bisquinolines with a connecting bridge of two carbon atoms where decreased conformational mobility seemed to increase activity. Bisquinoline **3** ((±)-*trans*-N^1,N^2-bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine was not only the most potent bisquinoline in vitro, but was clearly unique in its in vivo activity—80% and 100% cure rates were achieved at doses of 160 and 320 mg/kg, respectively. In summary, these preliminary results support the premise that bisquinolines may be useful agents against chloroquine-resistant malaria.

By a large margin, malaria is the most prevalent disease in the world. It is estimated for the year 1986 that some 489 million people contracted malaria, 2.3 million of whom died from the disease.[1] Whereas effective antimalarial drugs exist, drug resistance, particularly resistance to

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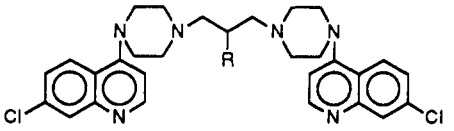
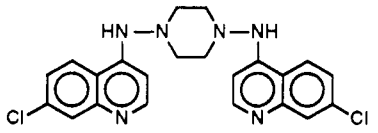
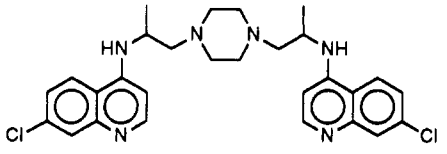


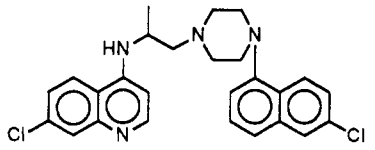
R = H piperaquine
R = OH hydroxypiperaquine



1,4-bis(7-chloro-4-quinolylamino)piperazine



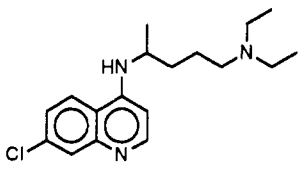
dichloroquinazine



12,494RP

Figure 1. Antimalarial bisquinolines.

chloroquine (CQ), the most useful antimalarial drug, has become an enormous problem.[2] Although the precise



chloroquine

mechanism(s) of action of CQ and mechanism(s) of resistance to chloroquine are incompletely understood,[3] work by Martin et al.[4] demonstrates that verapamil effectively reverses resistance to CQ. Verapamil and other "resistance modulator" drugs which reverse CQ resistance are believed to act by a blockade or inhibition of a putative drug-transporter membrane protein (P-glycoprotein); this protein normally allows the CQ-resistant parasites to pump CQ out of the cell.[5] The effect of these "resistance-modulators" on multidrug transporter proteins found in normal cells[6] remains to be elucidated; however, toxicity is noted with many of these drugs when used in combination with CQ.[7] This toxicity may prove to be a significant obstacle in their therapeutic application.

An alternate strategy which addresses the problem of CQ resistance is drug design based on chemical entities known to be active against CQ-resistant malaria. 4-Quinolinemethanols such as quinine and mefloquine, and the 4-aminoquinoline, amodiaquine, are active to various extents against CQ-resistant malaria.[3c,8] Although some evidence suggests[3c,8e] that CQ resistance may dispose the parasite to resistance to other quinolines, a significant lack of cross resistance among quinoline-containing antimalarials is often observed.[8a,9] In this light, a particularly promising lead may be reports that several bisquinolines are active against CQ-resistant malaria. A systematic study of this ostensibly promising class of compounds, however, has not been reported.[10]

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Examples of such agents (Figure 1) include several bis(quinolyl)piperazines such as piperaquine (13,228RP), hydroxypiperaquine, dichloroquinazine (12,278RP), 12,494RP, and 1,4-bis(7-chloro-4-quinolylamino)-piperazine.[9b,11] In general, these bisquinolines are more potent than CQ, and are active against CQ-resistant malaria. Both piperaquine (PQ) and hydroxypiperaquine are claimed to be effective against CQ-resistant malaria in China,[11f-h,j] although some resistance has been noted for PQ. These bisquinolines have a longer duration of action, and are less toxic than is CQ.[11e,k] Dichloroquinazine is active against CQ-resistant falciparum malaria,[9b] and a mixture of 12,494RP and dichloroquinazine is clinically effective against falciparum malaria and exerts a suppressive effect lasting for 3 weeks.[11a,c] Resistance to dichloroquinazine, however, is noted for CQ-resistant strains of *P. berghei*.[11l,m] Although 1,4-bis(7-chloro-4-quinolylamino)piperazine exhibits some cross-resistance with CQ,[11n] it is significantly more effective than is CQ against *P. berghei* in mice.[11d]

These results suggest that bisquinolines possess both notable activity against CQ-resistant malaria and a longer duration of action than is observed with CQ. However, bis-7-chloro-4-aminoquinolines with simple alkyl bridges have not been described apart from compound **1**[12] shown below. We decided to prepare bisquinolines (*N*,*N*-bis(7-chloroquinolin-4-yl)alkanediamines) **1–13** as antimalarials with potential utility against CQ-resistant malaria and now report our results.

Chemistry

Bisquinolines **1–13** were best obtained via a displacement reaction with 4,7-dichloroquinoline, alkanediamine, and triethylamine in a 2:1:2 ratio using *N*-methylpyrrolidinone as solvent. We had no success with the method of Singh et al.[11d] with K_2CO_3 as base and 2-ethoxyethanol as solvent. Substitution of triethylamine for K_2CO_3, however, gave good results. We later found *N*-methylpyrrolidinone as reported by Tyman et al.[13] to be a better solvent than 2-ethoxyethanol for this reaction. For example, yields for compounds **4** and **7** more than doubled when *N*-methylpyrrolidinone rather than 2-ethoxyethanol was used. Yields for reactions in 2-ethoxyethanol and *N*-methylpyrrolidinone ranged from 23–85% and 49–87%, respectively. Compounds **1–13** were isolated by adding water and ethyl ether or ethyl acetate to the cooled reaction mixtures which initiated product precipitation and dissolved any unreacted starting materials.

Antimalarial Activity

Twelve of the thirteen bisquinolines had a significantly lower resistance index than did CQ, and compared favorably with PQ in this regard (Table I). The resistance index was apparently unrelated to in vitro or in vivo activity. Eight bisquinolines were more potent than was either CQ and PQ against both clones of *P. falciparum*. Except for compounds **8** and **12**, there was a reasonable correlation between in vitro and in vivo antimalarial activities. For example, compounds **2**, **3**, **6**, **7**, and **9–11** which had IC_{50}'s less than 6 nM against *P. falciparum* were either active or curative against *P. berghei* in vivo. Conversely, compounds **1**, **4**, **5**, and **13** which were approximately 1 order of magnitude less potent in vitro, were also without activity in vivo. Skin lesions at the site of injection were observed for compounds **6**, **7**, **9**, and **11** at the 160- and 640-mg/kg doses. Compound **3**, the most potent bisquinoline in vitro, was clearly unique in its in vivo activity; 4/5 and 5/5 mice were cured at 160 and 320 mg/kg, respectively. No other compound was curative at the 160-mg/kg dose.

Compounds **4**, **5**, and **13** with bridges of three, four, or twelve carbon atoms were inactive in both screens; compounds **6–11** with bridges of between five and nine carbon atoms, however, were active. Methyl substitution in the bridge improved activity, e.g. **2** vs **1** and **7** vs **6**. In the three compounds (**1–3**) with a two-carbon bridge, decreased conformational mobility seemed to increase activity. This result suggests that the relative orientation of the two

(10) Examples of bisquinolines inactive against malaria include the succinic acid diester of amodiaquine as a potential repository form (Elslager, E. F.; Tendick, F. H.; Werbel, L. M. Repository Drugs. VIII. Ester and Amide Congeners of Amodiaquine, Hydroxychloroquine, Oxychloroquine, Primaquine, Quinacrine, and Related Substances as Potential Long-Acting Antimalarial Agents. *J. Med. Chem.* **1969**, *12*, 600–607.), and bisquinolines lacking a 4-amino function or with a bridge at the 3 rather than the 4-position (Nasr, M.; Burckhalter, J. H. Bis-Aminoquinolines as Potential Antimalarial Agents and a Novel Fused Ring System, Pyrano[3,2-c:5,6-c']diquinoline. *J. Heterocycl. Chem.* **1979**, *16*, 497–500.; Nasr, M.; Nabih, I.; Burckhalter, J. H. Synthesis of Pyrimido[5,4-c]quinolines and Related Quinolines as Potential Antimalarials. *J. Med. Chem.* **1978**, *21*, 295–298.).

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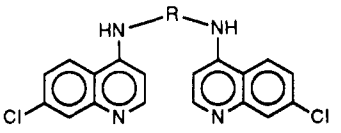
Table I. Antimalarial Activity of 1–13 against *P. falciparum* in Vitro and *P. berghei* in Vivo



compd	R	*P. falciparum* IC_{50} (nM) D-6	*P. falciparum* IC_{50} (nM) W-2	resistance index[a]	*P. berghei* T-C (days)[b] 40 mg/kg	*P. berghei* T-C (days)[b] 160 mg/kg	*P. berghei* T-C (days)[b] 640 mg/kg
1	$(CH_2)_2$	17	27	1.6	0.0	0.4	1.4
2	$CH_2CH(CH_3)$	3.5	3.9	1.1	3.4	5.7	C-3
3	*trans*-1,2-cyclohexyl	1.0	1.4	1.4	8.5	C-4	C-5 (320 mg/kg)
4	$(CH_2)_3$	15	83	5.5	0.2	0.4	1.2
5	$(CH_2)_4$	390	81	0.2	0.5	2.3	5.6
6	$(CH_2)_5$	2.5	3.8	1.5	1.0	5.1[c]	C-1[c]
7	$(CH_2)_3CH(CH_3)CH_2$	2.7	3.0	1.1	1.2	3.6	C-2[c]
8	$(CH_2)_6$	21	23	1.1	0.4	3.9	C-1
9	$(CH_2)_7$	1.9	4.3	2.3	2.6	7.2[c]	C-3[c]
10	$(CH_2)_8$	5.6	3.1	0.6	−0.2	1.0	C-1
11	$(CH_2)_9$	3.0	2.3	0.8	3.1	7.4[c]	15.0A[c]
12	$(CH_2)_{10}$	5.7	3.4	0.6	0.2	0.2	0.6
13	$(CH_2)_{12}$	60	31	0.5	0.0	−0.2	0.0
CQ	–	8.9	100	11	8.7	C-1	C-1,T-3[d]
PQ	–	8.3	16	1.9	–	–	–

[a] IC_{50}(W-2)/IC_{50}(D-6) ratio. [b] T-C is the mean survival time of the treated mice beyond that of the control animals (single dose administered SC 3 days post-infection, $n = 5$). This value must be ≥ twice the mean survival time (6.2 days) of the control animals to be considered active (A). Survival beyond 60 days is considered curative (C), and deaths from 0–2 days post-treatment are attributed to toxicity (T). [c] Skin lesions observed at site of injection. [d] T-C values for CQ represent averages of 10 best data sets from WRAIR.

quinoline heterocycles is important for activity, although ^1H NMR of **3** clearly shows a dynamic equilibrium between its diequatorial and diaxial conformers. This observation is consistent with molecular modeling (PCMODEL) studies which indicate an energy difference of less than 1 kcal/mol between the energy-minimized diaxial and diequatorial conformers of **3**.

Discussion

If one makes an analogy between the length of the connecting bridge between the 4-amino nitrogen atoms in **1–13** and the length of the alkyl fragment between the distal amino and 4-amino nitrogen atoms in CQ, then it is apparent that the number of carbons in each has different effects on antimalarial activity. For CQ and its analogues, a side-chain alkyl fragment of four carbon atoms is optimum; potency drops off rapidly when the number of carbon atoms is decreased or increased.[14] Conversely, in **1–13**, maximum activity is observed with a connecting bridge of two carbons, while minimum activity is seen with a bridge of four carbon atoms suggesting that CQ and **1–13** may act by different mechanisms. However, the geometry of the side chain in CQ analogues is likely more complex than a consideration of the additive bond distances between the two N atoms alone as suggested by Singh et al.[15]

One conceivable pharmacological mechanism for **1–13** is the formation of bis-intercalative complexes with DNA. Such a mechanism has been observed in anticancer bis(9-aminoacridines)[16] where conformation, length, and flexibility of the bridge played an important role in DNA binding. However, Mârquez et al.[17] did not observe such bis-intercalation for two bis(4-amino-7-chloroquinolines) with polyamine bridges. Binding to hemozoin or malaria pigment[18] represents another potential mechanism of action. Such studies are in progress and will be reported elsewhere.

In reference to our data, it is of interest to compare the antimalarial properties of *N,N'*-bis[3-[(phenylmethyl)amino]propyl]-1,7-diaminoheptane (MDL 27695), the most promising member of a number of bis(benzyl)polyamine analogues designed to interfere with polyamine biosynthesis and function.[19] MDL 27695 has an IC_{50} of 3.0 μM against the D-6 clone of *P. falciparum* in vitro, and, in combination with α-(difluoromethyl)ornithine, cures *P. berghei*-infected mice. Interestingly, although MDL 27695 is 3 orders of magnitude less potent than our more active bisquinolines, it has a similar resistance index.[19a] We suggest that MDL 27695 and bisquinolines **1–13** possess sufficient steric bulk to prevent their efflux by the P-glycoprotein membrane pump.[5]

In summary, our data is consistent with promising results observed with other bisquinolines (Figure 1) against

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CQ-resistant falciparum malaria. From this data, we also observe that, like PQ, bisquinolines 1–13 have much lower resistance indices than does CQ against CQ-resistant *P. falciparum* in vitro. Furthermore, six of the thirteen bisquinolines show superior antimalarial activity (both in vitro and in vivo) to CQ. These preliminary results support the premise that bisquinolines may be useful agents against CQ-resistant malaria. Future work will address the effects of heteroatom and alkyl substitution in the bridge, alternate cyclic bridge systems, and quinoline substitution patterns on antimalarial activity.

Experimental Section

Molecular modeling experiments were performed with PCMODEL 4.0 (Serena Software) on a Macintosh IIcx computer. Melting points were taken with a Mel-Temp capillary apparatus. IR spectra were run as KBr discs on a Perkin-Elmer 1420 spectrophotometer. NMR spectra were obtained with either Varian XL-300 or Bruker AC-200 spectrometers using deuteriated dimethyl sulfoxide with TMS as an internal standard. It was not possible to obtain ^{13}C NMR spectra for 1, 4, and 5 due to their low solubilities in DMSO. Microanalyses were performed by M-H-W Laboratories, Phoenix, AZ. The purity of 1–13 was confirmed with silica gel or alumina TLC. 4,7-Dichloroquinoline and the required diamines are commercially available from Aldrich Chemical Co., with the exception of 2-methylpentamethylenediamine and 1,12-dodecanediamine which were gifts from the Du Pont Co., Petrochemicals Dept. All reactions were conducted under a positive pressure of N_2 subsequent to 10 purge-cycles using a Firestone valve.

Chemistry. Synthesis of 1–13. A solution of 4,7-dichloroquinoline (10 mmol, 1.98 g), triethylamine (10 mmol, 1.01 g), and diamine (5 mmol) in either 2-ethoxyethanol or *N*-methylpyrrolidinone (10 mL) was heated to reflux for 6–24 h under a slight positive N_2 pressure. After the reaction mixture cooled to room temperature, ether or ethyl acetate (15 mL) and water (15 mL) were added with stirring, and the resulting solid was filtered and washed with water and ethyl acetate or ether to provide 1–13. In some cases, cooling of this two-phase mixture was required to induce precipitation of product. When required, crystallization of 1–13 was best accomplished from aqueous EtOH.

N^1,N^2-Bis(7-chloroquinolin-4-yl)ethane-1,2-diamine (1): (1.63 g, 85%); mp 342–345 °C dec (lit.[12] mp 334.5–337 °C); IR 3460, 3230, 3065, 3020, 2970, 2890, 1610, 1580, 1535 cm^{-1}; ^1H NMR δ 3.62 (m, 4 H), 6.58 (d, J = 5.4 Hz, 2 H), 7.47 (dd, J = 9.0, 2.4 Hz, 2 H), 7.48 (t, J = 4.2 Hz, 2 H), 7.79 (d, J = 2.4 Hz, 2 H), 8.23 (d, J = 9.0 Hz, 2 H), 8.41 (d, J = 5.4 Hz, 2 H). Anal. ($C_{20}H_{16}Cl_2N_4 \cdot 0.5H_2O$) C, H, N.

N^1,N^2-Bis(7-chloroquinolin-4-yl)propane-1,2-diamine (2): (1.43 g, 72%); mp 294–296 °C dec; IR 3440, 3070, 2980, 2930, 1610, 1575, 1535 cm^{-1}; ^1H NMR δ 1.35 (d, J = 6.3 Hz, 3 H), 3.49–3.59 (m, 2 H), 4.11–4.20 (m, 1 H), 6.55 (d, J = 5.7 Hz, 1 H), 6.61 (d, J = 5.7 Hz, 1 H), 7.05 (d, J = 8.1 Hz, 1 H), 7.44 (dd, J = 9.0, 2.4 Hz, 1 H), 7.46 (t, J = 2.4 Hz, 1 H), 7.47 (dd, J = 9.0, 2.4 Hz, 1 H), 7.778 (d, J = 1.8 Hz, 1 H), 7.784 (d, J = 1.8 Hz, 1 H), 8.22 (d, J = 9.0 Hz, 1 H), 8.34 (d, J = 9.0 Hz, 1 H), 8.35 (d, J = 5.7 Hz, 1 H), 8.40 (d, J = 5.7 Hz, 1 H); ^{13}C NMR δ 17.88, 46.86, 46.99, 98.76, 98.93, 117.39, 117.47, 123.86, 124.03, 124.21, 127.43, 127.47, 133.34, 149.05, 149.17, 149.43, 150.10, 151.79, 151.83. Anal. ($C_{21}H_{18}Cl_2N_4$) C, H, N.

(±)-*trans*-N^1,N^2-Bis(7-chloroquinolin-4-yl)cyclohexane-1,2-diamine (3): (1.55 g, 71%); mp 330–333 °C dec; IR 3435, 3250, 3060, 2935, 2860, 1610, 1570, 1535 cm^{-1}; ^1H NMR δ 1.34–1.70 (m, 4 H), 1.72–1.91 (m, 2 H), 2.02–2.21 (m, 2 H), 3.78–3.97 (m, 2 H), 6.74 (d, J = 5.6 Hz, 2 H), 6.94–6.98 (m, 2 H), 7.31 (dd, J = 8.9 Hz, J = 2.0 Hz, 2 H), 7.63 (d, J = 2.0 Hz, 2 H), 8.11 (d, J = 9.1 Hz, 2 H), 8.28 (d, J = 5.5 Hz, 2 H); ^{13}C NMR δ 24.63, 31.55, 55.50, 99.02, 117.30, 123.54, 124.02, 127.25, 133.09, 149.07, 149.78, 151.55. Anal. ($C_{24}H_{22}Cl_2N_4$) C, H, N.

N^1,N^3-Bis(7-chloroquinolin-4-yl)propane-1,3-diamine (4): (0.73 g, 37%); mp 312–314 °C dec; IR 3450, 3240, 3070, 2960, 2880, 1610, 1580, 1535 cm^{-1}; ^1H NMR δ 2.07 (m, 2 H), 3.43 (m, 4 H), 6.51 (d, J = 5.4 Hz, 2 H), 7.40 (t, J = 5.3 Hz, 2 H), 7.45 (dd, J = 9.0 Hz, J = 2.4 Hz, 2 H), 7.78 (d, J = 2.4 Hz, 2 H), 8.29 (d, J = 9.0 Hz, 2 H), 8.37 (d, J = 5.4 Hz, 2 H). Anal. ($C_{21}H_{18}Cl_2N_4$) C, H, N.

N^1,N^4-Bis(7-chloroquinolin-4-yl)butane-1,4-diamine (5): (1.11 g, 54%); mp 339–341 °C dec; IR 3215, 3065, 2960, 1610, 1580, 1550 cm^{-1}; ^1H NMR δ 1.74–1.87 (m, 4 H), 3.21–3.54 (m, 4 H), 6.50 (d, J = 5.3 Hz, 2 H), 7.33 (m, 2 H), 7.40–7.47 (m, 2 H), 7.74–7.77 (m, 2 H), 8.26 (d, J = 9.1 Hz, 2 H), 8.37 (d, J = 5.1 Hz, 2 H). Anal. ($C_{22}H_{20}Cl_2N_4$) C, H, N.

N^1,N^5-Bis(7-chloroquinolin-4-yl)pentane-1,5-diamine (6): (1.07 g, 50%); mp 272–274 °C; IR 3450, 3250, 3070, 2950, 2880, 1610, 1585, 1535 cm^{-1}; ^1H NMR δ 1.49–1.56 (m, 2 H), 1.69–1.78 (m, 4 H), 3.25–3.32 (m, 4 H), 6.46 (d, J = 5.4 Hz, 2 H), 7.32 (t, J = 5.4 Hz, 2 H), 7.44 (dd, J = 9.0, 2.4 Hz, 2 H), 7.78 (d, J = 2.4 Hz, 2 H), 8.28 (d, J = 9.0 Hz, 2 H), 8.38 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 24.25, 27.55, 42.33, 98.56, 117.42, 123.88, 124.04, 127.45, 133.28, 149.08, 150.03, 151.85. Anal. ($C_{23}H_{22}Cl_2N_4$) C, H, N.

N^1,N^5-Bis(7-chloroquinolin-4-yl)-2-methylpentane-1,5-diamine (7): (0.50 g, 23%); mp 228–230 °C; IR 3450, 3065, 2960, 1610, 1580, 1535 cm^{-1}; ^1H NMR δ 0.97 (d, J = 6.6 Hz, 3 H), 1.23–1.36 (m, 1 H), 1.55–2.02 (m, 4 H), 3.03–3.51 (m, 4 H), 6.44 (d, J = 5.4 Hz, 1 H), 6.45 (d, J = 5.4 Hz, 1 H), 7.29 (t, J = 5.1 Hz, 1 H), 7.36 (t, J = 5.7 Hz, 1 H), 7.42 (dd, J = 9.0, 2.1 Hz, 2 H), 7.77 (d, J = 2.4 Hz, 2 H), 8.25 (d, J = 9.0 Hz, 1 H), 8.29 (d, J = 9.0 Hz, 1 H), 8.357 (d, J = 5.4 Hz, 1 H), 8.364 (d, J = 5.4 Hz, 1 H); ^{13}C NMR δ 17.71, 25.18, 31.26, 31.57, 42.64, 48.66, 98.50, 98.58, 117.39, 123.82, 123.90, 123.97, 127.41, 133.26, 149.04, 149.08, 149.99, 150.14, 151.75, 151.79. Anal. ($C_{24}H_{24}Cl_2N_4$) C, H, N.

N^1,N^6-Bis(7-chloroquinolin-4-yl)hexane-1,6-diamine (8): (1.55 g, 71%); mp 284–286 °C dec; IR 3450, 3300, 3105, 3065, 3010, 2930, 2830, 1610, 1570, 1535 cm^{-1}; ^1H NMR δ 1.42–1.53 (m, 4 H), 1.63–1.74 (m, 4 H), 3.23–3.29 (m, 4 H), 6.45 (d, J = 5.4 Hz, 2 H), 7.31 (t, J = 5.1 Hz, 2 H), 7.43 (dd, J = 9.0, 2.1 Hz, 2 H), 7.77 (d, J = 2.1 Hz, 2 H), 8.27 (d, J = 9.0 Hz, 2 H), 8.37 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 26.37, 27.71, 42.29, 98.52, 117.39, 123.86, 124.02, 127.41, 133.25, 149.06, 150.01, 151.83. Anal. ($C_{24}H_{24}Cl_2N_4$) C, H, N.

N^1,N^7-Bis(7-chloroquinolin-4-yl)heptane-1,7-diamine (9): (1.96 g, 87%); mp 218–220 °C; IR 3450, 3060, 2935, 2860, 1610, 1580, 1535 cm^{-1}; ^1H NMR δ 1.39 (br s, 6 H), 1.53–1.77 (m, 4 H), 3.16–3.32 (m, 4 H), 6.44 (d, J = 5.5 Hz, 2 H), 7.31 (t, J = 5.1 Hz, 2 H), 7.44 (dd, J = 9.0, 2.2 Hz, 2 H), 7.79 (d, J = 2.2 Hz, 2 H), 8.29 (d, J = 9.1 Hz, 2 H), 8.39 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 26.64, 27.73, 28.64, 42.35, 98.54, 117.43, 123.91, 124.08, 127.46, 133.30, 149.09, 150.04, 151.87. Anal. ($C_{25}H_{26}Cl_2N_4$) C, H, N.

N^1,N^8-Bis(7-chloroquinolin-4-yl)octane-1,8-diamine (10): (1.80 g, 77%); mp 216–219 °C; IR 3450, 3350, 3070, 2940, 2865, 1610, 1580, 1540 cm^{-1}; ^1H NMR δ 1.35 (br s, 8 H), 1.57–1.75 (m, 4 H), 3.15–3.31 (m, 4 H), 6.44 (d, J = 5.5 Hz, 2 H), 7.28 (t, J = 5.1 Hz, 2 H), 7.43 (dd, J = 9.0, 2.3 Hz, 2 H), 7.77 (d, J = 2.2 Hz, 2 H), 8.27 (d, J = 9.1 Hz, 2 H), 8.38 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 26.58, 27.73, 28.79, 42.36, 98.55, 117.42, 123.92, 124.08, 127.44, 133.30, 149.08, 150.04, 151.87. Anal. ($C_{26}H_{28}Cl_2N_4$) C, H, N.

N^1,N^9-Bis(7-chloroquinolin-4-yl)nonane-1,9-diamine (11): (1.82 g, 76%); mp 161–164 °C; IR 3455, 3370, 3065, 2930, 2860, 1610, 1575, 1540, 1535 cm^{-1}; ^1H NMR δ 1.14–1.50 (br s, 10 H), 1.54–1.73 (m, 4 H), 3.13–3.32 (m, 4 H), 6.44 (d, J = 5.5 Hz, 2 H), 7.28 (t, J = 5.1 Hz, 2 H), 7.43 (dd, J = 9.0, 2.3 Hz, 2 H), 7.77 (d, J = 2.2 Hz, 2 H), 8.28 (d, J = 9.0 Hz, 2 H), 8.38 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 26.61, 27.74, 28.78, 28.98, 42.37, 98.53, 117.43, 123.90, 124.08, 127.46, 133.30, 149.09, 150.04, 151.87. Anal. ($C_{27}H_{30}Cl_2N_4$) C, H, N.

N^1,N^{10}-Bis(7-chloroquinolin-4-yl)decane-1,10-diamine (12): (2.15 g, 87%); mp 200–204 °C; IR 3445, 3285, 3060, 2930, 2855, 1610, 1580, 1535 cm^{-1}; ^1H NMR δ 1.28 (br s, 12 H), 1.52–1.74 (m, 4 H), 3.15–3.31 (m, 4 H), 6.44 (d, J = 5.4 Hz, 2 H), 7.28 (t, J = 5.1 Hz, 2 H), 7.43 (dd, J = 8.9, 2.3 Hz, 2 H), 7.70 (d, J = 2.2 Hz, 2 H), 8.27 (d, J = 7.0 Hz, 2 H), 8.38 (d, J = 5.4 Hz, 2 H); ^{13}C NMR δ 26.70, 27.72, 28.80, 28.94, 42.37, 98.53, 117.44, 123.89, 124.08, 127.46, 133.29, 149.10, 150.04, 151.86. Anal. ($C_{28}H_{32}Cl_2N_4$) C, H, N.

N^1,N^{12}-Bis(7-chloroquinolin-4-yl)dodecane-1,12-diamine (13): (1.70 g, 65%); mp 188–190 °C; IR 3460, 3070, 2930, 2860, 1610, 1580, 1540 cm^{-1}; ^1H NMR δ 1.24–1.35 (m, 16 H), 1.59–1.69 (m, 4 H), 3.22–3.28 (m, 4 H), 6.46 (d, J = 5.7 Hz, 2 H), 7.57 (t, J = 5.3 Hz, 2 H), 7.44 (dd, J = 9.0, 2.1 Hz, 2 H), 7.78 (d, J = 2.1 Hz, 2 H), 8.29 (d, J = 9.0 Hz, 2 H), 8.39 (d, J = 5.7 Hz, 2 H); ^{13}C NMR δ 18.49, 25.42, 26.55, 27.69, 28.74, 28.91, 42.34, 55.97, 98.52,

117.41, 123.86, 124.05, 127.41, 133.26, 149.07, 150.03, 151.83. Anal. ($C_{30}H_{36}Cl_2N_4 \cdot H_2O$) C, H, N.

Screening Methods. In vitro activity against *P. falciparum* was determined using a modification of the semiautomated microdilution technique of Desjardins et al.[20] and Milhous et al.[21] Two *P. falciparum* malaria parasite clones, designated as Sierra Leone (D-6) and Indochina (W-2), are used in susceptibility testing. The former is resistant to mefloquine, and the latter to CQ, pyrimethamine, sulfadoxine, and quinine. Test compounds are dissolved in dimethyl sulfoxide, and solutions are serially diluted with culture media. Erythrocytes with 0.25 to 0.5% parasitemia are added to each well of a 96-well microdilution plate to give a final hematocrit of 1.5%. Inhibition of uptake of tritiated hypoxanthine is used as an index of antimalarial activity. Results are reported as IC_{50} (ng/mL) values.

In vivo activity against *P. berghei* was obtained against a drug-sensitive strain of *P. berghei* (strain KBG 173).[22] Each test compound is administered sc to five male mice per dilution in a single subcutaneous dose 3 days after infection. Results are expressed in T-C values which indicate the mean survival time of the treated mice beyond that of the control animals; untreated mice survive on average 6.2 days. Compounds are classified as active (A) when the mean survival time of the treated mice is twice that of the controls (>6.2 days), and curative (C) when one or more test animals live 60 days postinfection. Deaths from 0–2 days post-treatment are attributed to toxicity (T).

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Clinical Analysis by ^1H Spin-Echo NMR. 2.† Oxidation of Intracellular Glutathione as a Consequence of Penicillamine Therapy in Rheumatoid Arthritis

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Spin echo NMR analysis is used to monitor the effect of penicillamine on intact erythrocytes obtained from patients suffering from rheumatoid arthritis during a 12-week period of therapy. The results are compared to the previously reported in vitro effects of the compound (McKay, C. N. N.; et al. *Biochim. Biophys. Acta* **1986**, *888*, 30–35). At clinical assessment at week 12, the 20 patients were divided into responder and nonresponder groups. The intracellular glutathione in the responder group is more oxidized ($P < 0.01$) than in the nonresponder group. A retrospective analysis of the two patient groups at the initial assessment following the commencement of therapy indicated that in the nonresponder group intracellular glutathione was significantly more reduced ($P < 0.02$) than in the responder group. It is postulated that penicillamine stimulates cellular defense against the oxidation of the cell membrane at the expense of cytosolic glutathione. This initial study suggests that spin-echo NMR analysis of erythrocyte glutathione can act as an early indicator of a clinical response to penicillamine therapy.

Introduction

Penicillamine is one of a limited number of disease-modifying antirheumatic drugs used in the treatment of rheumatoid arthritis. The chemical processes underlying its action are still unknown. However, it may act by reacting with sulfhydryl sites on plasma proteins,[4] in the cytosol,[5] or at the cell membrane.[3,6] In a previous in vitro study of the action of penicillamine on intact, viable erythrocytes using ^1H spin-echo nuclear magnetic resonance spectroscopy (NMR),[5] we reported that intracellular glutathione became more oxidized. The effect was multiplicative with more cytosolic thiol being affected than penicillamine thiol applied. This clinical study was initiated in an attempt to discover whether the in vitro re-

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