116953-32-3; **8**, 116953-33-4; **8** (2-ol), 116953-34-5; **8** (2-acetate), 116953-35-6; **9**, 116953-36-7; **10**, 93635-31-5; (*R*)-(+)-**10**, 117020-35-6; (*S*)-(−)-**10**, 117020-38-9; **11**, 116953-37-8; **12a**, 116970-37-7; **12b**, 116953-81-2; **13**, 116953-72-1; **14**, 116953-52-7; **15**, 116953-53-8; **16a**, 116953-39-0; **16b**, 116953-73-2; **16c**, 116953-40-3; **16d**, 116953-74-3; **16e**, 116953-41-4; **16f**, 116953-75-4; **16g**, 116953-43-6; **16h**, 116953-76-5; **17a**, 116970-38-8; **17b**, 116953-54-9; **17c**, 116953-55-0; **17d**, 116953-56-1; **17e**, 116953-57-2; **17f**, 116970-39-9; **17g**, 116953-58-3; **17h**, 116953-59-4; **18a**, 116953-82-3; **18b**, 116953-83-4; **18c**, 116953-84-5; **18d**, 116953-85-6; **18e**, 116953-38-9; (*R*)-(−)-**18e**, 117020-36-7; (*S*)-(+)-**18e**, 117020-37-8; **18f**, 116953-45-8; **18g**, 116953-86-7; **19a**, 116953-77-6; **19b**, 116953-78-7; **19c**, 116953-79-8; **19d**, 116953-80-1; **19e**, 116953-44-7; (*R*)-(−)-**19e**, 117020-39-0; (*S*)-(+)-**19e**, 117020-40-3; **19f**, 116953-46-9; **19g**, 116953-47-0; **20a**, 116953-60-7; **20b**, 116953-61-8; **20c**, 116953-62-9; **20d**, 116953-63-0; **20e**, 116953-64-1; **20f**, 116953-69-6; **20g**, 116953-71-0; **21**, 117064-08-1; (*R*)-(−)-**21**, 116953-65-2; (*S*)-(+)-**21**, 116953-66-3; **22**, 116953-67-4; **23**, 116953-68-5; **24**, 116953-70-9; **25**, 116953-49-2; **25** (iodide salt), 116953-48-1; **27**, 70259-44-8; **28**, 116953-87-8; **28** (2-ol), 83526-68-5; **29**, 116953-50-5; **30**, 116953-88-9; **31**, 70259-28-8; **32**, 116953-89-0; **33**, 116953-51-6; ClCOOPh, 1885-14-9; $H_2NCH_2CH_2NMe_2$, 108-00-9; $H_2NCH_2CH_2OH$, 141-43-5; ClCOOMe, 79-22-1; $C_{18}H_{37}NCO$, 112-96-9; 2-(aminomethyl)pyridine, 3731-51-9; 3-*O*-[(*N*-[2-(dimethylamino)ethyl]-*N*-(phenoxycarbonyl)carbamoyl]-2-*O*-methyl-1-*O*-(octadecylcarbamoyl)glycerol, 116953-42-5; pyrrolidine, 123-75-1.

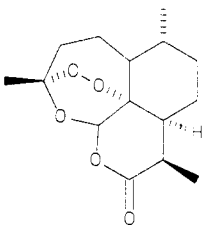
Amine Peroxides as Potential Antimalarials

Jonathan L. Vennerstrom†

Department of Medicinal Chemistry, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C. 20307. *Received April 18, 1988*

Six model amine peroxides (**4**–**9**) were synthesized as targeted antimalarial oxidants. They were approximately 1 order of magnitude more potent than *tert*-butyl hydroperoxide (**3**) in vitro against *Plasmodium falciparum*, but like **3**, they were inactive in vivo against *Plasmodium berghei*.

Several peroxides have shown antimalarial activity. The most notable of these is the complex endoperoxide sesquiterpene lactone artemisinin (**1**),[1-3] a clinically useful antimalarial agent. However, simple peroxides such as H_2O_2 (**2**)[4] and *tert*-butyl hydroperoxide (**3**)[5,6] are also antimalarial albeit much less potent than **1**. The efficacy of **1**–**3** may depend in part on the observation that malaria-infected red cells are selectively damaged by oxidants.



1

This oxidant sensitivity of malaria-infected erythrocytes may arise both from precedent damage by parasite-generated oxidants and from a weakening of oxidant defense mechanisms of the erythrocyte.[7] The inhibition of intraerythrocytic growth of malaria parasites under supraphysiologic concentrations of oxygen[8] and protection against malaria infection by several red blood cell (RBC) disorders that increase the susceptibility of RBCs to oxidative stress exemplify this oxidant sensitivity.[9-16] Numerous articles[7,17-24] have summarized specific mechanisms that may account for the susceptibility of malaria to oxidants.

Structure–activity studies demonstrate that the endoperoxide group in **1** and its analogues is absolutely essential for antimalarial activity,[25] suggestive of an oxidative mode of action. A progressive increase in the potency of **1** with increasing oxygen tensions ranging from 3 to 30% and a significant reduction in the potency of **1** by coadministration of reducing agents[26] support this hypothesis.

Hydrogen peroxide (**2**) has antimalarial properties; micromolar concentrations of **2** kill various murine ma-

†Present address: College of Pharmacy, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, Nebraska 68105.

(1) Qinghaosu Antimalaria Coordinating Research Group *Chin. Med. J.* **1979**, *92*, 811.
(2) Klayman, D. L. *Science (Washington, D.C.)* **1985**, *228*, 1049.
(3) Luo, X.-D.; Shen, C.-C. *Med. Res. Rev.* **1987**, *7*, 29.
(4) Dockrell, H. M.; Playfair, J. H. L. *Infect. Immun.* **1983**, *39*, 456.
(5) Clark, I. A.; Cowden, W. B.; Butcher, G. A. *Lancet* **1983**, i, 234.
(6) Clark, I. A.; Hunt, N. H.; Cowden, W. B.; Maxwell, L. E.; Mackie, E. J. *Clin. Exp. Immunol.* **1984**, *56*, 524.
(7) Vennerstrom, J. L.; Eaton, J. W. *J. Med. Chem.* **1988**, *31*, 1269.
(8) Friedman, M. J. *Nature (London)* **1979**, *280*, 245.
(9) Allison, A. C.; Eugui, E. M. *Lancet* **1982**, 1431.
(10) Friedman, M. J.; Trager, W. *Sci. Am.* **1981**, *244*, 154.
(11) Das, S. K.; Nair, R. C. *Br. J. Hematol.* **1980**, *44*, 87.
(12) Hebbel, R. P.; Eaton, J. W.; Balasingam, M.; Steinberg, M. H. *J. Clin. Invest.* **1982**, *70*, 1253.
(13) Stocks, H.; Kemp, M.; Dormandy, T. L. *Lancet* **1971**, i, 266.
(14) Alving, A. S.; Tarlov, A. R.; Brewer, G.; Carlson, P. E.; Kellermeyer, R. W.; Long, W. K. *Trans. Assoc. Am. Physicians* **1960**, 1xxiii, 80.
(15) Eaton, J. W.; Brewer, G. J. *The Red Blood Cell*; Surgenor, D. M., Ed.; Academic: New York, 1974; pp 435–471.
(16) Brockelman, C. R.; Wongsattayanont, B.; Tan-Ariya, P.; Fucharoen, S. *J. Clin. Microbiol.* **1987**, *25*, 56.
(17) Ames, J. R.; Ryan, M. D.; Klayman, D. L.; Kovacic, P. *J. Free Radicals Biol. Med.* **1985**, *1*, 353.
(18) Clark, I. A.; Cowden, W. B. *Oxidative Stress*; Sies, H., Ed.; Academic: London, 1985; pp 131–149.
(19) Etkin, N. L.; Eaton, J. W. *Erythrocyte Structure and Function*; Brewer, G. J., Ed.; Alan R. Liss: New York, 1975; pp 219–232.
(20) Fairfield, A. S.; Meshnick, S. R.; Eaton, J. W. *Science (Washington, D. C.)* **1983**, *221*, 764.
(21) Nair, C. R.; Gupta, P. H.; Chauhan, D. P.; Vinayak, V. K. *Indian J. Med. Res.* **1984**, *80*, 627.
(22) Stocker, R.; Hunt, N. H.; Buffinton, G. D.; Weidemann, M. J.; Lewis-Hughes, P. H.; Clark, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 548.
(23) Eaton, J. W.; Eckman, J. R.; Berger, E.; Jacob, H. S. *Nature (London)* **1976**, *264*, 758.
(24) Eckman, J. R.; Eaton, J. W. *Nature (London)* **1979**, *278*, 754.
(25) Gu, H. M.; Lu, B. F.; Qu, Z. X. *Acta Pharmacol. Sinica* **1980**, *1*, 48.
(26) Krungkrai, S. R.; Yuthavong, Y. *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 710.

0022-2623/89/1832-0064$01.50/0

Table I. Antimalarial Activity of Amine Peroxides against *P. falciparum* in Vitro and *P. berghei* in Vivo

no.	compound	yield, %	*P. falciparum* D-6 clone: IC_{50}, μM	*P. falciparum* W-2 clone: IC_{50}, μM	*P. berghei*, 40, 160, 640 mg/kg: *T* − *C*, days[a]
3	*tert*-butyl hydroperoxide		203[b]	240[b]	0.1, 0.3, 0.7[b]
4	1-[(*tert*-butyldioxy)methyl]-4-phenylpiperidine	94	27.3	26.9	0.3, 0.5, 0.8
5	1-[(*tert*-butyldioxy)methyl]-4-phenylpiperazine	95	25.7	39.8	0.9, 0.7, 0.7
6	*N*-[(*tert*-butyldioxy)methyl]-*N*-ethylaniline	89	54.0	39.4	0.5, 0.3, 1.3
7	*N*-[(*tert*-butyldioxy)methyl]-*N*-methylcyclohexylamine	92	25.1	26.6	0.5, 1.3, 0.7
8	1-[(*tert*-butyldioxy)methyl]-4-methylpiperidine	98	19.7	25.2	0.3, 0.1, 0.7
9	hexamethylene triperoxide diamine	57	2.7	3.8	N/A

[a] *T* − *C* is the mean survival time of the treated mice beyond that of the control animals. This value must be ≥6.2 days in order for a test compound to be considered active. [b] Reference 31.

larias[4] both in vitro and in vivo and the human malaria *Plasmodium falciparum* in vitro.[27] Hydrogen peroxide (**2**) generated by a glucose/glucose oxidase system is also effective, and a significant reversal of killing is achieved by the addition of catalase.[28,29] The low capacity of glutathione peroxidase from *Plasmodium berghei* to reduce hydrogen peroxide may also contribute to its in vitro activity.[30]

tert-Butyl hydroperoxide (**3**) cures *Plasmodium vinckei*[5,6] but not *P. berghei*[31] infected mice, and IC_{50} values of 41–240 μM are observed in vitro against *P. falciparum*.[5,31] The peroxide bond is essential for activity as *tert*-butyl alcohol is without effect.[6] Moreover, **3** has sufficient steric bulk to prevent its reduction by catalase,[32] but is a substrate for GSH peroxidase.[33] Although parasiticidal concentrations of **3** are hemolytic, parasite death is not strictly dependent upon hemolysis, as degenerated parasites are observed inside intact erythrocytes both in vivo and in vitro.[5,6] In addition, doses of **3** that do not cause hemolysis in normal mice do so in parasitized animals.[6]

Iron may contribute in part to the efficacy of **3**, since coadministration of desferrioxamine, a specific iron chelator, completely prevents its antimalarial activity.[5,6] It was suggested[5] that this requirement for iron indicates that these peroxides may damage parasites by causing the generation of hydroxyl radicals via a Fenton[34,35] reaction.

Clearly, peroxide-mediated oxidant damage to the infected erythrocyte and/or resident plasmodia parasite can lead to an interruption of the parasitic life cycle. Simple peroxides such as **3**, however, produce undesirable side effects including hemolysis of uninfected erythrocytes at parasiticidal concentrations. A restricted delivery of peroxides to infected erythrocytes may provide a solution. Accordingly, a series of model amine peroxides containing the *tert*-butylperoxy function ((*tert*-butyldioxy)methyl dialkyl amines) (**4–8**), and a known amine endoperoxide containing three peroxide functions (hexamethylene triperoxide diamine) (**9**) were synthesized (Figure 1) as potential targeted antimalarial oxidants. The rationale for their intended specificity is the selective concentration of weak bases (e.g. amines) in the acidic (pH 5.2) digestive vacuoles[36,37] of the malarial parasite and the oxidant sensitivity of the malarial parasite/host erythrocyte system.

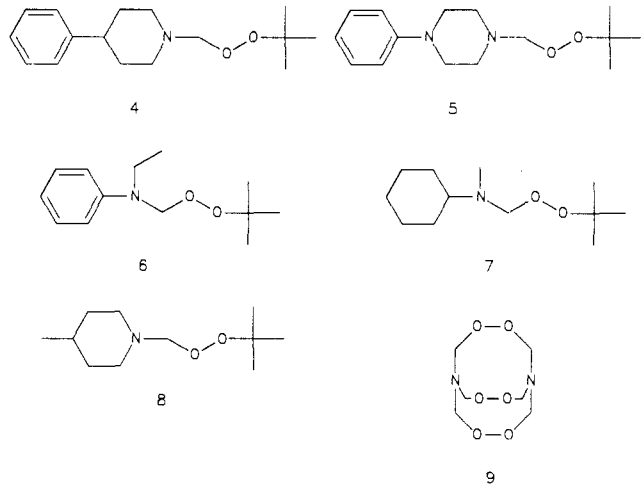


Figure 1. Structures of amine peroxides **4–9**.

Results and Discussion

Chemistry. Amine peroxides **4–9** (Figure 1) were chosen to incorporate the *tert*-butylperoxy or peroxy functional group in structurally diverse Mannich bases containing either exocyclic, endocyclic, and aliphatic or aromatic substituted nitrogen atoms. Synthesis of (*tert*-butyldioxy)methyl dialkyl amines **4–8** is accomplished by using a modified procedure of Rieche et al.;[38] this Mannich reaction, as described in the Experimental Section, affords **4–8** in 89–98% yields. Hexamethylene triperoxide diamine (HMTD) (**9**)[39,40] is synthesized without incident by a literature method[41] in 57% yield. HMTD (**9**) is an explosive peroxide,[39] but relatively insensitive to shock, and in a crystallographic study[41] was found to have an unusual planar geometry about the two bridgehead nitrogen atoms.

Mannich bases of known drugs have been prepared as potential prodrugs,[42–46] but prodrug design was not the

(27) Playfair, J. H. L.; DeSouza, J. B.; Dockrell, H. M.; Lelchuk, R.; Taverne, J. *Molecular Biology of Host-Parasite Interactions*; Agabian, N., Eisen, H., Eds.; Alan R. Liss: New York, 1984; pp 303–309.
(28) Wozencraft, A. O.; Dockrell, H. M.; Taverne, J.; Targett, G. A. T.; Playfair, J. H. L. *Infect. Immun.* **1984**, *43*, 664.
(29) Dockrell, H. M.; Playfair, J. H. L. *Infect. Immun.* **1984**, *43*, 451.
(30) Fritsch, B.; Dieckmann, A.; Menz, B.; Hempelmann, E.; Fritsch, K.-G.; Fritsch, G.; Jung, A. *Parasitol. Res.* **1987**, *73*, 515.
(31) Vennerstrom, J. L.; Acton, N.; Lin, A. J.; Klayman, D. L. *Drug Des. Delivery*, in press.
(32) Sies, H. *Oxidative Stress*; Sies, H., Ed.; Academic: London, 1985; pp 73–90.
(33) Srivastava, S. K.; Awasthi, Y. C.; Beutler, E. *Biochem. J.* **1974**, *139*, 289.
(34) Fenton, H. J. H. *J. Chem. Soc.* **1894**, *65*, 899.
(35) Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125.
(36) Yayon, A.; Cabantchik, Z. I.; Ginsburg, H. *EMBO J.* **1984**, *3*, 2695.
(37) Krogstad, D. J.; Schlesinger, P. H.; Gluzman, I. Y. *J. Cell Biol.* **1985**, *101*, 2302.
(38) Rieche, A.; Schmitz, E.; Beyer, E. *Chem. Ber.* **1959**, *92*, 1206.
(39) Baeyer, A.; Villiger, V. *Ber.* **1900**, *33*, 2479.
(40) Legler, L. *Ber.* **1885**, *18*, 3343.
(41) Schaefer, W. P.; Fourkas, J. T.; Tiemann, G. B. *J. Am. Chem. Soc.* **1985**, *107*, 2461.
(42) Bundgaard, H.; Johansen, M. *J. Pharm. Sci.* **1980**, *69*, 44.

intent in this study. It is conceivable, however, that amine peroxides 4–8 may be hydrolyzed preferentially at pH 5.2 of the parasite digestive vacuole vs pH 7.4 of the extracellular fluid. Thus, a potential pH-dependent hydrolysis of 4–8 resulting in a parasite-specific delivery of 3 does formally represent a prodrug strategy. Kinetic NMR experiments, however, showed that with the exception of 6, (*tert*-butyldioxy)methyl dialkyl amines 4–8 are hydrolyzed with half-lives of less than 3 min in both acetate (pH 5.2) and phosphate (pH 7.4) buffers. A pH-dependent hydrolysis is evident only with 6 where a first-order process was observed with a $T_{1/2}$ of 10 and 67 min respectively at pH 5.2 and 7.4. This slower rate of hydrolysis of 6 in contrast to 4, 5, 7, and 8 presumably reflects the diminished basicity of its aniline nitrogen atom.

Biology. Each of the (*tert*-butyldioxy)methyl dialkyl amines 4–8 is approximately 1 order of magnitude more potent than 3 in vitro against the chloroquine-sensitive (D-6) and chloroquine-resistant (W-2) clones of *P. falciparum* (Table I). The (*tert*-butyldioxy)methyl derivative of 4-methylpiperidine (8) is the most potent in vitro against *P. falciparum* of amine peroxides 4–8. These in vitro results are consistent with the basic nitrogen atom in 4–8 contributing to a selective concentration in the acidic food vacuoles of the intraerythrocytic malaria parasites resulting in the observed increase in potency with respect to 3.

Notwithstanding any similar specificity for infected erythrocytes in vivo, 4–8, like 3, are inactive in vivo against *P. berghei*. Therefore, incorporation of the *tert*-butylperoxy function into these amine peroxides did not confer in vivo activity. The precursor secondary amines to 4–8 are likewise inactive in vivo (data not shown). In the in vivo model, other acidic cellular organelles such as chromaffin vesicles[47,48] and lysosomes[49] are also likely to sequester weak bases. More significantly, this lack of in vivo activity may reflect intrinsic differences in susceptibility to oxidants between *P. berghei* and *P. falciparum*.[4,31] Previous results indicate that 3 was ineffective against *P. berghei*,[31] which preferentially invade younger more oxidant resistant reticulocytes, but effective against *P. vinckei*,[5,6] which tend to be confined to oxidant-sensitive mature red cells.[50] Human malaria parasites, not unlike *P. vinckei*, may be more susceptible to oxidants and do not have the same preference for reticulocytes shown by *P. berghei*.[50] Thus, in vivo experiments with the murine malaria parasite *P. vinckei* rather than *P. berghei* may be more valid when screening for antimalarials, particularly oxidant antimalarials.

HMTD (9) is considerably more potent than 4–8 in vitro against *P. falciparum*, but was not tested in vivo for safety considerations. HMTD (9) the only amine endoperoxide tested, has a relative in vitro potency consistent with the observed increased antimalarial potency associated with endoperoxide vs acyclic peroxide functions.[31] There are three peroxide bonds in 9, but only one peroxide bond in 4–8; however, normalization of the inverse of IC_{50} with weight percent peroxide in 4–9 indicates that the increased potency of 9 relative to 4–8 is 1.6–4.5-fold greater than can be accounted for by the presence of additional peroxide functions alone.

Studies in progress include the synthesis of additional amine endoperoxides and peroxide and oxazirane congeners of the antimalarial 4-aminoquinoline drug chloroquine as potential site-specific antimalarial oxidants. The extraordinary specificity of chloroquine for the acidic digestive vacuoles of the malarial parasite is well documented.[51–54] The selective concentration process, however, is only partially explained by the weak-base effect of chloroquine.[55] Thus, oxidant derivatives of chloroquine may be expected to have an added dimension of specificity in the delivery of oxidant functionality to the parasitized erythrocyte.

Experimental Section

Melting points were taken with a Thomas-Hoover capillary apparatus. Infrared spectra were run neat on a Perkin-Elmer 283 spectrophotometer. NMR spectra were run on a JEOL FX90Q spectrometer using deuteriated chloroform with TMS as an internal standard. Mass spectra were obtained with a Nermag R10-10 spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI. The secondary amines used as starting materials for the syntheses of 4–8 were purified by distillation.

Chemistry. Synthesis of 4–8. To a solution of secondary amine (10 mmol) in MeOH (1 mL) at 4 °C was added 70% aqueous *tert*-butyl hydroperoxide (12 mmol, 1.08 g) followed by 37% aqueous formaldehyde (12 mmol, 0.36 g). The resulting reaction mixture was stirred for 1 h at 4 °C. Ether (35 mL) and hexane (15 mL) were added, and the solution was dried over K_2CO_3. Removal of solvent in vacuo provided 4–8 as clear oils.

1-[(*tert*-Butyldioxy)methyl]-4-phenylpiperidine (4): IR (neat) 3070, 3040, 2980, 2940, 1605, 1595, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (s, 9 H), 1.73–1.88 (m, 4 H), 2.37–2.53 (m, 1 H), 2.90–3.05 (m, 4 H), 4.65 (s, 2 H), 7.24 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 26.35, 33.77, 42.17, 50.67, 80.09, 92.01, 125.97, 126.73, 128.30, 146.40; MS, *m/e* (relative intensity) 264 (M + 1, 1), 242 (15), 217 (8), 203 (32), 176 (17), 174 (42), 163 (33), 162 (100), 141 (4). Anal. ($C_{16}H_{25}NO_2$) C, H, N.

1-[(*tert*-Butyldioxy)methyl]-4-phenylpiperazine (5): IR (neat) 3070, 3030, 2980, 2940, 2830, 1600, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (s, 9 H), 3.01–3.20 (m, 8 H), 4.64 (s, 2 H), 6.84–6.97 (m, 3 H), 7.17–7.35 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 26.40, 49.59, 49.70, 80.03, 91.19, 116.17, 119.53, 128.95, 151.49; MS, *m/e* (relative intensity) 264 (M, 13), 190 (3), 175 (100), 161 (4), 132 (6), 120 (3). Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

***N*-[(*tert*-Butyldioxy)methyl]-*N*-ethylaniline (6):** IR (neat) 3070, 3020, 2985, 2960, 1605, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (s, 9 H), 1.24 (t, J = 7.2 Hz, 3 H), 3.56 (q, J = 7.2 Hz, 2 H), 5.13 (s, 2 H), 6.77–6.92 (m, 3 H), 7.15–7.32 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.40, 26.40, 45.36, 80.03, 85.34, 113.41 117.90, 128.90, 146.83; MS, *m/e* (relative intensity) 224 (M + 1, 100), 166 (5), 150 (20), 122 (28), 73 (16), 65 (19). Anal. ($C_{13}H_{21}NO_2$) C, H, N.

***N*-[(*tert*-Butyldioxy)methyl]-*N*-methylcyclohexylamine (7):** IR (neat) 2980, 2940, 2860 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15–1.84 (m, 9 H), 1.24 (s, 9 H), 2.56 (s, 3 H), 4.68 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.75, 25.97, 26.35, 31.33, 36.43, 60.64, 79.65, 89.73; MS, *m/e* (relative intensity) 215 (M, 2), 214 (15), 142 (5), 127 (9), 126 (100), 112 (6), 83 (6), 70 (8). Anal. ($C_{12}H_{25}NO_2$) C, H, N.

1-[(*tert*-Butyldioxy)methyl]-4-methylpiperidine (8): IR (neat) 2980, 2955, 2930 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (d, J = 5.4

(43) Deutsch, H. M.; Gelbaum, L. T.; McLaughlin, M.; Fleischmann, T. J.; Earnhart, L. L.; Haugwitz, R. D.; Zalkow, L. H. *J. Med. Chem.* **1986**, *29*, 2164.
(44) Koch, S. A. M.; Sloan, K. B. *Pharm. Res.* **1987**, *4*, 317.
(45) Sieger, G. M.; Barringer, W. C.; Krueger, J. E. *J. Med. Chem.* **1971**, *14*, 458.
(46) Winstead, M. B.; Barr, D. E.; Hamel, C. R.; Renn, J.; Parker, H. I.; Neumann, R. M. *J. Med. Chem.* **1965**, *8*, 117.
(47) Johnson, R. G.; Carlson, N. J.; Scarpa, A. *J. Biol. Chem.* **1978**, *253*, 1512.
(48) Johnson, R. G.; Scarpia, A. *J. Gen. Physiol.* **1976**, *68*, 601.
(49) Dean, R. T.; Jessup, W.; Roberts, C. R. *Biochem. J.* **1984**, *217*, 27.
(50) Cox, F. E. G. *Bull. Soc. Pathol. Exot. Ses. Fil.* **1983**, *76*, 503.
(51) Fitch, C. D. *Proc. Natl. Acad. Sci. U.S.A.* **1969**, *64*, 1181.
(52) Fitch, C. D.; Yunis, N. G.; Chevli, R.; Gonzalez, Y. *J. Clin. Invest.* **1974**, *54*, 24.
(53) Macomber, P. B.; O'Brien, R. L.; Hahn, F. E. *Science (Washington, D.C.)* **1966**, *152*, 1374.
(54) Polet, H.; Barr, C. F. *J. Pharm. Exp. Ther.* **1968**, *168*, 187.
(55) Krogstad, D. J.; Schlesinger, P. H. *Am. J. Trop. Med. Hyg.* **1987**, *36*, 213.

Hz, 3 H), 1.23 (s, 9 H), 1.12–1.68 (m, 5 H), 2.75–2.87 (m, 4 H), 4.59 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 21.96, 26.35, 30.30; 34.64, 50.24, 79.98, 92.11; MS, *m/e* (relative intensity) 302 (M + 1, 24), 200 (45), 186 (14), 128 (27), 112 (100), 73 (14). Anal. ($C_{11}H_{23}NO_2$) C, H, N.

1,6-Diaza-3,4,8,9,12,13-hexaoxabicyclo[4.4.4]tetradecane (HMTD) (9).[41] Hexamethylenetetramine (50 mmol, 7.0 g) was dissolved in 30% aqueous hydrogen peroxide (22.5 g) with stirring at 4 °C. Powdered citric acid hydrate (11.5 g) was added slowly over a period of 10 min, followed by continued stirring for 3 h at 4 °C. The reaction mixture was warmed to room temperature and **9** was obtained as fine white crystals (5.87 g, 57%) after washing with water (250 mL) and MeOH (50 mL) and air-drying: mp 154 °C exploded; ^1H NMR ($CDCl_3$) δ 4.80 (s). Anal. ($C_6H_{12}N_2O_6$) C, H, N.

Biological Methods. The in vitro assays were conducted by using a modification of the semiautomated microdilution technique of Desjardins et al.[56] and Milhous et al.[57] Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were utilized in susceptibility testing. Test compounds were dissolved in DMSO and serially diluted with culture media. The uptake of tritiated hypoxanthine was used as an index of inhibition of parasite growth. The compounds described herein were tested against a drug-sensitive strain of *P. berghei* (strain KBG 173) in mice according to a method previously described.[58]

Kinetic Experiments. The rates of hydrolysis of **4–8** were determined by ^1H NMR. Amine peroxides **4–8** were dissolved (45 mM) in CD_3CN–deuteriated buffer, 2:1, with 1,1,2,2-tetrachloroethane as an internal standard. The acetate (pH 5.2) and phosphate (pH 7.4) buffers (50 mM) were chosen to correspond to the average pH of the parasitic digestive vacuole and the extracellular fluid, respectively. The rates of hydrolysis were obtained by determining peak integration ratios for the methylene protons in **4–8** and the protons in the internal standard and comparison to standard curves.

Acknowledgment. This work was done while J.L.V. held a National Research Council (WRAIR) Research Associateship at the Walter Reed Army Institute of Research. The author is grateful to Dr. Daniel L. Klayman for serving as a sponsor during this associateship period, and thanks Drs. Wilbur K. Milhous (WRAIR) and Arba L. Ager, Jr. (University of Miami) for the in vitro and in vivo antimalarial test results, respectively.

Registry No. 4, 116437-26-4; 5, 116437-27-5; 6, 64254-25-7; 7, 116437-28-6; 8, 116466-10-5; 9, 283-66-9; *tert*-butyl hydroperoxide, 75-91-2; 4-phenylpiperidine, 771-99-3; 1-phenylpiperazine, 92-54-6; *N*-ethylaniline, 103-69-5; *N*-methylcyclohexylamine, 100-60-7; 4-methylpiperidine, 626-58-4.

(56) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710.
(57) Milhous, W. K.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. *Antimicrob. Agents Chemother.* **1985**, *27*, 525.
(58) Osdene, T. S.; Russell, P. B.; Rane, L. *J. Med. Chem.* **1967**, *10*, 431.

Conformationally Restricted Analogues of Atriopeptin(103–125)amide

Kerry L. Spear,* Emily J. Reinhard, Ellen G. McMahon, Gillian M. Olins, Maria A. Palomo, and Dennis R. Patton

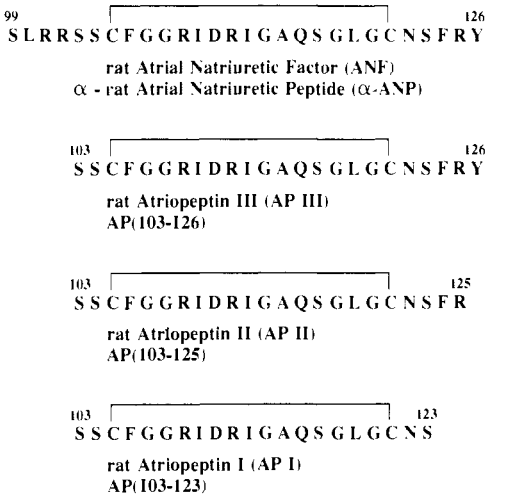
Cardiovascular Diseases Research Department, Searle Research and Development Division, G. D. Searle and Company, c/o Monsanto Company, 700 Chesterfield Village Parkway, Chesterfield, Missouri 63198. *Received February 19, 1988*

Conformationally restricted analogues of atriopeptin(103–125)amide were prepared by synthesizing novel bicyclic peptides in which a second disulfide bridge linking residues 108 and 117 was introduced. These syntheses were shown to proceed with no significant scrambling of the disulfide bonds and demonstrated that structurally defined bicyclic analogues of atrial peptides could be easily prepared. The conformationally restrained analogues described here were found to be biologically active with potencies (EC_{50}s) ranging from 0.05 to 3 μM. In addition, these bicyclic peptides (and many of the monocyclic precursors) were found to bind selectively to a class of specific tissue binding sites that have not been shown to be associated with any known second messenger system (NVR binding sites). Since affinity for the receptor class linked to vasorelaxation was negatively affected by the conformational restrictions described here, binding of atrial peptides to this class of receptors appears to have more specific conformational requirements than does binding to the NVR sites.

A group of cyclic peptides[1] has been isolated from mammalian atria that mediate a variety of in vitro and in vivo responses, including vasorelaxation, diuresis, and natriuresis. This family of compounds varies in length from 21 to 28 amino acids and shares a common cyclic core of 17 residues (see Scheme I). Several different names have been applied to this hormone, including atriopeptin (AP), atrial natriuretic factor (ANF), and atrial natriuretic peptide (ANP).

Because of their unique physiological activity, both the physical and biological properties of AP have been intensively studied. Unfortunately, despite the availability of a variety of spectral information, the solution-phase conformational properties of these peptides remain poorly defined. For example, nuclear magnetic resonance (NMR)

Scheme I. Amino Acid Sequences and Proposed Names of Atrial Peptides



(1) For recent reviews, see: Needleman, P.; Adams, S. P.; Cole, B. R.; Currie, M. G.; Geller, D. M.; Michener, M. L.; Saper, C. B.; Schwartz, D.; Standaert, D. G. *Hypertension* **1985**, 7, 469. Ballerman, B. J.; Brenner, B. M. *J. Clin. Invest.* **1985**, 76, 2041. *Atrial Hormones and Other Natriuretic Factors*; Mulrow, P. J., Schrier, R., Eds.; American Physiological Society: Bethesda, MD, 1987. *Endocrinology and Metabolism Clinics of North America*; Rosenblatt, M., Jacobs, J. W., Eds.; W. B. Saunders Co.: Philadelphia, PA, 1987; Vol. 16.

0022-2623/89/1832-0067$01.50/0