(4) P. E. Thompson and L. M. Werbel in "Medicinal Chemistry", Vol. 12, G. D. Stevens, Ed., Academic Press, New York, N.Y., 1972.
(5) J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 812 (1972).
(6) E. F. Elslager, O. D. Bird, J. Clarke, S. C. Perricone, and D. F. Worth, *J. Med. Chem.*, **15**, 1138 (1972).
(7) E. F. Elslager and J. Davoll, "Lectures in Heterocyclic Chemistry", Vol. II, R. N. Castle and L. B. Townsend, Ed., Heterocorporation, Orem, Utah, 1974, pp S97–133.
(8) Private communication from the Walter Reed Army Institute for Research.
(9) R. L. Kisliuk, M. Friedkin, L. H. Schmidt, and R. N. Rossan, *Science*, **156**, 1616 (1967).
(10) E. C. Taylor, K. L. Perlman, I. P. Sword, M. Sequin-Frey, and P. A. Jacobi, *J. Am. Chem. Soc.*, **95**, 6407 (1973); E. C. Taylor, K. L. Perlman, Y. H. Kim, I. P. Sword, and P. A. Jacobi, *ibid.*, **95**, 6413 (1973); E. C. Taylor and T. Kobayashi, *J. Org. Chem.*, **38**, 2817 (1973).
(11) M. Chaykovsky, A. Rosowsky, and E. J. Modest, *J. Heterocycl. Chem.*, **10**, 425 (1973); A. Rosowsky and K. K. N. Chen, Abstracts of Papers, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., March 31–April 5, 1974, MEDI 63.
(12) E. C. Taylor, R. C. Portnoy, D. C. Hochsteller, and T. Kobayashi, *J. Org. Chem.*, **40**, 2347 (1975).
(13) Obtained from Princeton Bio-Medix, Inc., Princeton, N.J. 08540.
(14) The parenteral antimalarial screening in mice and chicks was carried out in the laboratory of Dr. Leo Rane of the University of Miami. Test results were provided through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(15) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(16) M. Chaykovsky, A. Rosowsky, N. Papathanasopoulos, K. K. N. Chen, E. J. Modest, R. L. Kisliuk, and Y. Gaumont, *J. Med. Chem.*, **17**, 1212 (1974).
(17) For a description of the test method, see L. Rane and D. S. Rane, *Proc. Helminthol. Soc. Wash.*, **39**, 283 (1972).
(18) Primate test data were provided by Col. D. Davidson of the Walter Reed Army Institute of Research.
(19) W. Szybalski, *Microb. Genet. Bull.*, **5**, 16 (1951).
(20) A. H. Webb and L. Washington, *Bacteriol. Proc.*, **52**, (1966).
(21) E. F. Elslager, J. Clarke, L. M. Werbel, and D. F. Worth, *J. Med. Chem.*, **15**, 827 (1972).
(22) E. F. Elslager, J. Clarke, J. Johnson, L. M. Werbel, and J. Davoll, *J. Heterocycl. Chem.*, **9**, 759 (1972).
(23) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

Folate Antagonists. 11. Synthesis and Antimalarial Effects of 6-[(Aryloxy- and arylthio-)methyl]-2,4-pteridinediamines and -pteridinediamine 8-Oxides[1-3]

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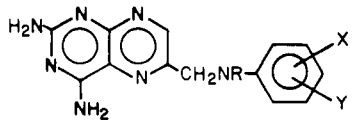
Condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide with 4-chlorophenol gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-oxide (1), which was deoxygenated to obtain the de-*N*-oxide 4. Cyclization of 4 and 1 produced 6-[(4-chlorophenoxy)methyl]-2,4-pteridinediamine and the 8-oxide, respectively. 6-[(Arylthio)methyl]-2,4-pteridinediamines and their 8-oxides were produced analogously. Controlled oxidation of the former gave the anticipated sulfoxide **12** and sulfone **13**. None of these compounds showed significant activity when tested against lethal *Plasmodium berghei* infections in mice or a select list of bacteria in vitro.

One cannot generally predict the effect that replacement of nitrogen by sulfur or oxygen will have on biological activity. The success achieved with certain of the 6-substituted 2,4-quinazolinediamines,[3] however, warranted preparation of the aryloxy and arylthio analogues of the 6-[(arylamino)methyl]-2,4-pteridinediamines I which had



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been shown to have particularly potent prophylactic effects against *Plasmodium gallinaceum* infections.[1]

Chemistry. As shown in Scheme I, condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide[4,5] with 4-chlorophenol in acetone gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-oxide (1) (25% yield). Deoxygenation of 1 with triethyl phosphite gave 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile (4) (80% yield) (see Table I). Cyclization of 4 and 1 with guanidine then produced 6-[(4-chlorophenoxy)methyl]-2,4-pteridinediamine (**10**) (40% yield) and the corresponding 8-oxide **7** (52% yield), respectively.

In a similar manner, condensation of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide with arylthiols gave the 3-amino-6-[(arylthio)methyl]-2-pyrazinecarbonitrile 4-oxides **2** and **3** (55 and 31% yields, respectively) which on deoxygenation afforded the corresponding 3-amino-6-[(arylthio)methyl]-2-pyrazinecarbonitriles **5** and **6** (85 and 83% yields, respectively). Cyclization with guanidine then produced the desired 6-[(arylthio)-methyl]-2,4-pteridinediamines **11** and **14** (87 and 54% yields, respectively) and the corresponding 8-oxides **8** and **9** (83 and 47% yields, respectively).

Treatment of 6-[[(4-chlorophenyl)thio]methyl]-2,4-pteridinediamine (**11**) with hydrogen peroxide in glacial acetic acid for 1 h at room temperature gave 6-[[(4-chlorophenyl)sulfinyl]methyl]-2,4-pteridinediamine (**12**) (65% yield). When the oxidation of **11** was allowed to proceed for 17 h, the corresponding 6-[[(4-chlorophenyl)sulfonyl]methyl]-2,4-pteridinediamine (**13**) was obtained in 61% yield.

Oxidation of the sulfur atom was confirmed by the presence of infrared peaks at 1030 cm^{-1} for the sulfoxide and 1150 and 1310 cm^{-1} in the case of the sulfone.

Suppressive Antimalarial Screening in Mice. The 6-[(aryloxy-, arylthio-, arylsulfinyl-, and arylsulfonyl-)-methyl]-2,4-pteridinediamines and *N*-oxides (compounds **7–14**, Table II) were tested against a normal drug-sensitive strain of *P. berghei* in mice by the parenteral route.[6,7] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72 h postinfection. Extension of the

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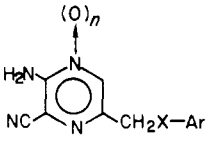
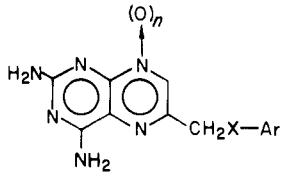
Table I. 3-Amino-6-[(4-aryloxy- and arylthio-)methyl]-2-pyrazinecarbonitriles and 4-Oxides



No.	n	X	Ar	Mp, °C	Yield purified, %	Crystn solvent	Formula	Analyses
1	1	O	4-Cl-C_6H_4	207-208	25	EtOH	$C_{12}H_9ClN_4O_2$	C, H, N
2	1	S	4-Cl-C_6H_4	146-150	55	$C_6H_5CH_3$	$C_{12}H_9ClN_4OS$	C, H, N
3	1	S	2-Naphthyl	157-161	31	EtOH	$C_{16}H_{12}N_4OS$	C, H, N
4	0	O	4-Cl-C_6H_4	191-193	80	EtOH	$C_{12}H_9ClN_4O$	H, N; C[a]
5	0	S	4-Cl-C_6H_4	158-160	85	EtOH	$C_{12}H_9ClN_4S$	C, H, N
6	0	S	2-Naphthyl	125-127	83	2-PrOH	$C_{16}H_{12}N_4S$	C, H, N

[a] C: calcd, 55.29; found, 54.62.

Table II. 6-[(Aryloxy- and arylthio-)methyl]-2,4-pteridinediamines and 8-Oxides



No.	n	X	Ar	Mp, °C	Yield purified, %	Crystn solvent	Formula[a]	Analyses
7	1	O	4-Cl-C_6H_4	311 dec	52	DMF	$C_{13}H_{11}ClN_6O_2$	C, H, N
8	1	S	4-Cl-C_6H_4	269 dec	83	EtOH	$C_{13}H_{11}ClN_6OS$	C, H, N
9	1	S	2-Naphthyl	250-252 dec	47	DMF	$C_{17}H_{14}N_6OS$	C, H, N
10	0	O	4-Cl-C_6H_4	>300	40	DMF	$C_{13}H_{11}ClN_6O \cdot 0.2H_2O$	C, H, N, H_2O
11	0	S	4-Cl-C_6H_4	280-283 dec	87	EtOH	$C_{13}H_{11}ClN_6S$	C, H, N
12	0	SO	4-Cl-C_6H_4	246 dec	65	DMF-H_2O	$C_{13}H_{11}ClN_6OS \cdot {}^1/_3H_2O$	C, H, N, H_2O
13	0	SO_2	4-Cl-C_6H_4	302-305 dec	61	DMF-H_2O	$C_{13}H_{11}ClN_6O_2S \cdot 0.3C_3H_7NO$	C, H, N
14	0	S	2-Naphthyl	260-262 dec	54	DMF	$C_{17}H_{14}N_6S$	C, H, N

[a] C_3H_7NO signifies *N,N*-dimethylformamide. The presence was confirmed by NMR.

mean survival time of the treated mice is interpreted as evidence of antimalarial activity. Compounds are arbitrarily considered to be "active" when they produce at least a 100% increase in the mean survival time of treated mice. All the compounds were devoid of suppressive antimalarial activity at doses up to 640 mg/kg.

Antibacterial Studies. Several of the compounds were tested against a spectrum of pathogenic bacteria including *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Escherichia coli* (Vogel), and *Shigella sonnei* (C-10). A modification of the gradient plate procedure of Szybalski[8] and Webb and Washington[9] was employed throughout. The compounds were either devoid of activity or were marginally active against only *S. faecalis*.

Experimental Section[10,11]

3-Amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-Oxide (1, Table I). A mixture of 8.5 g (0.037 mol) of 3-amino-6-(bromomethyl)-2-pyrazinecarbonitrile 4-oxide, 9.6 g (0.074 mol) of 4-chlorophenol, and 5.4 g (0.039 mol) of powdered K_2CO_3 was heated and stirred under reflux for 1 h. The mixture was filtered and the filtrate was concentrated to an oil which crystallized from ethyl acetate. Recrystallization from EtOH gave 1.0 g of a yellow solid, mp 207–208 °C. An additional 1.6 g was obtained after chromatography of the ethyl acetate filtrate on silica (ethyl acetate–benzene, 3:7), followed by recrystallization from EtOH.

3-Amino-6-[[(4-chlorophenyl)thio]methyl]-2-pyrazinecarbonitrile 4-oxide (2, Table I) and 3-amino-6-[(2-naphthalenylthio)methyl]-2-pyrazinecarbonitrile 4-oxide (3, Table I) were prepared similarly from 4-chlorobenzenethiol and 2-naphthalenethiol. Chromatography over silica with ethyl acetate–benzene (1:1) was required for **3**.

3-Amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile (4, Table I). A solution of 1.6 g (5.8 mmol) of 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile 4-oxide (1, Table I) in 9.9 mL (58 mmol) of triethyl phosphite and 10 mL of DMF was stirred at 120 °C for 1.5 h, allowed to cool, and concentrated in vacuo. Recrystallization of the residue gave 1.1 g, mp 191–193 °C.

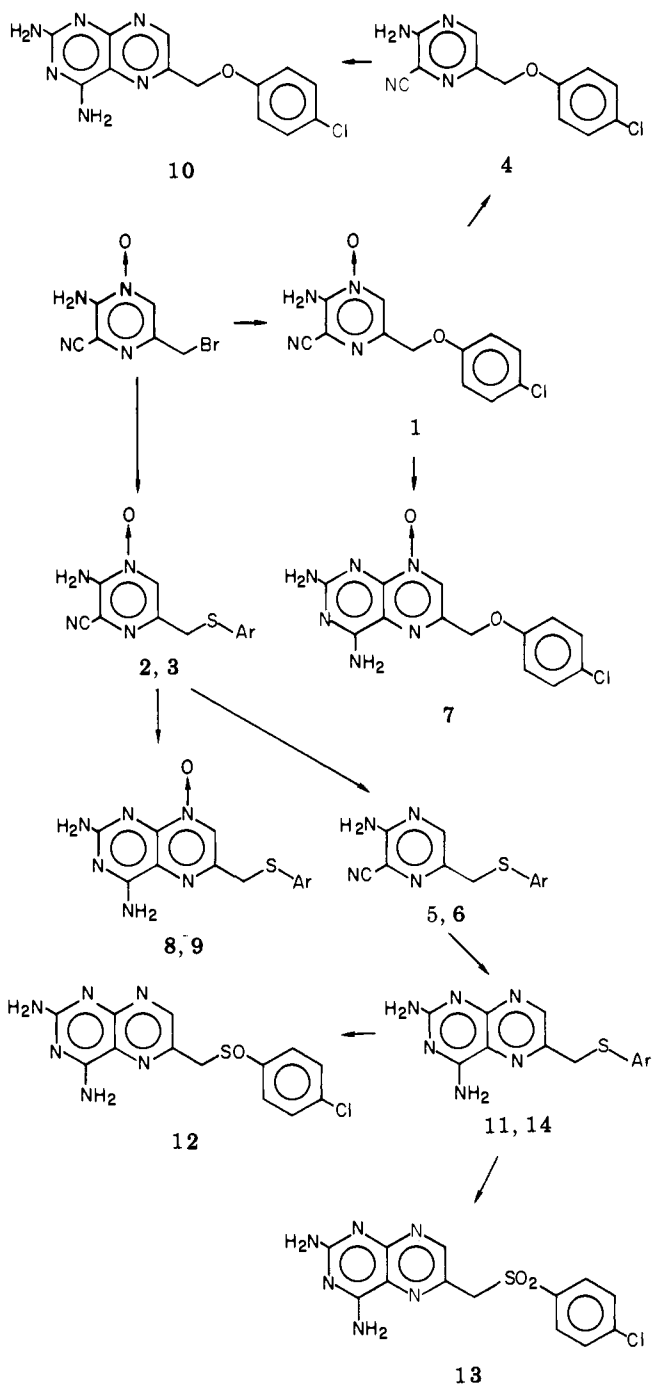
3-Amino-6-[[(4-chlorophenyl)thio]methyl]-2-pyrazinecarbonitrile (5) and 3-Amino-6-[(2-naphthalenylthio)methyl]-2-pyrazinecarbonitrile (6, Table I). These compounds were prepared by deoxygenation of the corresponding 4-oxides (2 and 3) using the procedure given above for 3-amino-6-[(4-chlorophenoxy)methyl]-2-pyrazinecarbonitrile (4).

6-[(2-Naphthalenylthio)methyl]-2,4-pteridinediamine 8-Oxide (9, Table II). A mixture of 1.2 g (0.0039 mol) of 3-amino-6-[(2-naphthalenylthio)methyl]-2-pyrazinecarbonitrile 4-oxide (3, Table I) and a freshly prepared solution of 0.0039 mol of guanidine base in 30 mL of EtOH was heated and stirred under reflux for 2 h, allowed to cool, and filtered. The filter cake was washed successively with 2-propanol, H_2O, and acetone. Recrystallization from DMF gave 0.64 g (47%).

Compounds **7**, **8**, **10**, **11**, and **14** (Table II) were prepared in an analogous manner by cyclization of the appropriate pyrazine or pyrazine *N*-oxide (Table I) with guanidine in EtOH.

6-[[(4-Chlorophenyl)sulfinyl]methyl]-2,4-pteridinediamine (12, Table II). A mixture of 0.50 g (0.0016 mol) of 6-[[(4-chlorophenyl)thio]methyl]-2,4-pteridinediamine (11) and 4 mL

Scheme I



2, 5, **8**, 11, Ar = 4-C_6H_4Cl
3, **6**, 9, **14**, Ar = 2-naphthalenyl

of 30% H_2O_2 in 9 mL of glacial CH_3CO_2H was stirred at room temperature for 1 h and poured into iced water containing 9.5 mL of 50% NaOH. The resulting precipitate was collected by filtration and washed with H_2O. Recrystallization from DMF–H_2O gave 0.35 g (64%), mp 246 °C dec.

6-[[(4-Chlorophenyl)sulfonyl]methyl]-2,4-pteridinediamine (13, Table II). A mixture of 0.50 g (0.0016 mol) of 6-[[(4-chlorophenyl)thio]methyl]-2,4-pteridinediamine (11) and 4 mL of 30% H_2O_2 in 9 mL of glacial CH_3CO_2H was stirred at room temperature for 17 h and then poured into a mixture of ice and 11 mL of 50% NaOH. The resulting precipitate was collected by filtration and washed with H_2O. Recrystallization from DMF–H_2O gave 0.36 g (61%), mp 302–305 °C dec.

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References and Notes

(1) This is communication 39 of a series on antimalarial drugs. For paper 38, see D. F. Worth, J. Johnson, E. F. Elslager, and L. M. Werbel, *J. Med. Chem.*, preceding paper in this issue.
(2) This investigation was supported by U.S. Army Medical Research and Development Command Contract DA 17-72-C-2077. This is Contribution No. 1477 to the Army Research Program on Malaria.
(3) A preliminary report of this work appeared in "Medicinal Chemistry IV, Proceedings of the 4th International Symposium on Medicinal Chemistry, Noordwijkerhout, The Netherlands, Sept 9–13, 1974", J. Maas, Ed., Elsevier Scientific, New York, N.Y., 1974.
(4) For recent work of Professor Taylor developing this methodology, see E. C. Taylor, R. C. Portnoy, D. C. Hochsteller, and T. Kobayashi, *J. Org. Chem.*, **40**, 2347 (1975).
(5) Obtained from Princeton Bio-Medix, Inc.
(6) The parenteral antimalarial screening in mice was carried out in the laboratory of Dr. Leo Rane of the University of Miami. Test results were provided through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(7) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(8) W. Szybalski, *Microb. Genet. Bull.*, **5**, 16 (1951).
(9) A. H. Webb and L. Washington, *Bacteriol. Proc.*, 52 (1966).
(10) Melting points (corrected) were taken on a Thomas-Hoover capillary melting point apparatus.
(11) Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.