Folate Antagonists. 14. Synthesis of Pyrazino[2,3-*f*]quinazoline-8,10-diamines and Related Heterocycles as Potential Antimalarial Agents (1,2)

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Amination of 5-chloro-6-nitro-2,4-quinazolinediamine (**2**) afforded 6-nitro-2,4,5-quinazolinetriamine (**3**) which was hydrogenated catalytically to produce 2,4,5,6-quinazolinetetraamine (**5**). Condensation of **5** with the requisite diketones afforded 8,9,10,11-tetrahydropyrimido[5,4-*a*]-phenazine-1,3-diamine (**6**), several pyrazino[2,3-*f*]quinazoline-8,10-diamines (**7-10,13**) as well as two pyrimido[4,5-*f*]phenazinediamines (**11,12**). Amination of **2** in the presence of formamide at 120° led to the formation of 9-nitro-1*H*-pyrimido[4,5,6-*de*]quinazolin-5-amine (**4**). None of these compounds showed suppressive activity when tested parenterally against lethal *Plasmodium berghei* infections in mice. When tested against various bacteria *in vitro*, **7** and **10** exhibited activity against *Streptococcus faecalis* at a concentration below 2.5 μg./ml.

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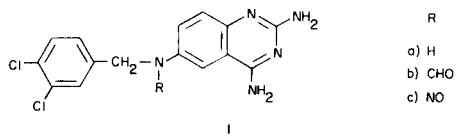
The potent antimalarial effects of various quinazoline-2,4,6-triamines, exemplified by **1a-c**,



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against both sensitive and cycloguanil- and pyrimethamine-resistant plasmodia may be attributed not only to their ability to function as reductase inhibitors, but also to their significant effects on the folate transport mechanism or elsewhere in the folate cycle (2). The anitmalarial potency of this class is generally enhanced by the insertion of a chlorine or methyl substituent at position 5 of the quinazoline ring or by the introduction of formyl, methyl or nitroso groups at N(6) (*i.e.*, **1b,c**). Since this effect may be related to a closer structural resemblance to and concurrent inhibition of tetrahydrofolate coenzymes within the interconversion cycle, it was of interest to devise other related structures which more closely mimicked these enzyme structures. In this paper we report the synthesis and biological evaluation of such a series consisting of the pyrazino[2,3-*f*]quinazolines and related heterocycles.

The synthetic route to the fused pyrazino[2,3-*f*]quinazolines and related compounds is pictured in Scheme I. The key intermediate, 2,4,5,6-quinazolinetetramine **5**, is prepared by amination of 5-chloro-6-nitro-2,4-quinazolinediamine (**2**) (3), followed by catalytic hydrogenation of the resulting 6-nitro-2,4,5-quinazolinetriamine (**3**) over 5% palladium on carbon. When amination of **2** was carried out in the presence of formamide, 9-nitro-1*H*-pyrimido-[4,5,6-*de*]quinazolin-5-amine (**4**) was obtained.

Condensation of the stable dihydrochloride salt of **5** with 1,2-cyclohexanedione in the presence of freshly fused sodium acetate and glacial acetic acid afforded 8,9,10,11-tetrahydropyrimido[5,4-*a*]phenazine-1,3-diamine acetate (1:0.25) (**6**). The other diketones were condensed with 2,4,5,6-quinazolinetetraamine dihydrochloride (**5**) by heating in aqueous acetic acid to give **7-13**. The products are listed in Table I. The isomers are possible from the reaction of the tetraamine with α-oxobenzeneacetaldehyde to give **9**, with 1-phenyl-1,2-propanedione to give **10**, and with 1*H*-indene-1,2,3-trione to give **13**. The presence of such a mixture of isomers in the products was verified by the nmr spectra of **9** and **10** (4). The spectrum of **9** revealed a pair of singlets integrating for 1 proton at δ 9.71 and 9.65, representing the proton attached either to C(2) or C(3). In the case of **10**, the methyl group is represented by a pair of singlets, integrating for three protons, at δ 3.27 and 3.21. For both **9** and **10**, H(5) and H(6) appear as doublets (J = 10) at about δ 8.8 and 8.2. Compound **13** was too insoluble to obtain a spectrum. Dibenzo-[*a,c*]-pyrimido[5,4-*h*]phenazine (**11**), dipyrido[3,2-*a*:2′,3-c]-pyrimido-[5,4-*h*]phenazine (**12**) and 8*H*(and/or 12*H*)-indeno[2′,1′-5,6](and/or [1′,2′:5,6])pyrazino[2,3-*f*]quinazoline (**13**) are novel ring systems.

Compounds **7-13** were administered in a single subcutaneous dose to mice infected with a normal drug-sensitive strain of *Plasmodium berghei* (5) and were found devoid of antimalarial activity even at doses up to 640 mg./kg. They were also tested *in vitro* against *Streptococcus faecalis* (MGM-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus, Pseudomonas aeruginosa* (28), *Escherichia coli* (Vogel) and *Shigella sonnei* (C-10), using a modification of the gradient procedure of Szybalski (6) and Webb and Washington (7). Only 2,3-dimethylpyrazino[2,3-*f*]quinazoline-8,10-diamine (**7**) and 2(and 3)-methyl-3(and 2)-phenylpyrazino[2,3-*f*]quin-

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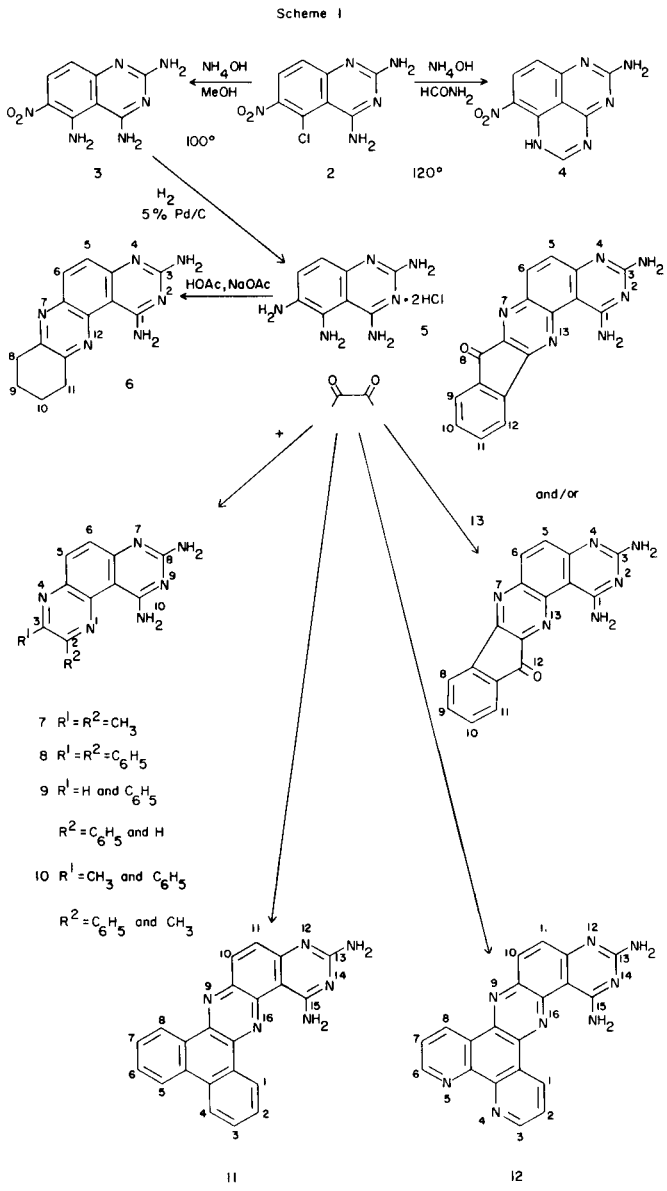
Scheme I



azoline-8,10-diamine (**10**), both of which have a methyl group attached to the pyrazine ring, possessed any antimicrobial activity. That activity was directed only against *S. faecalis* at less than 2.5 μg./ml.

EXPERIMENTAL (8)

6-Nitro-2,4,5-quinazolinetriamine Monoacetate (**3**).

A mixture of 5 g. (0.02 mole) of 5-chloro-6-nitroquinazoline-2,4-diamine (**2**) (3), 20 ml. of 28% ammonium hydroxide, and 20 ml. of methanol was heated in a stainless steel autoclave at 100° for 7 hours, allowed to cool to room temperature, and filtered to collect 4.2 g. of crude product. This was treated with 450 ml. of boiling 20% aqueous acetic acid; the hazy solution was filtered, concentrated to 150 ml., diluted with about 30 ml. of water, and cooled to afford 3.0 g., (51%) of the desired product as the monoacetate salt.

Anal. Calcd. for $C_8H_8N_6O_2 \cdot C_2H_4O_2$: C, 42.85; H, 4.32; N, 29.99. Found: C, 42.62; H, 4.38; N, 29.70.

9-Nitro-1*H*-pyrimido[4,5,6-*de*]quinazoline-5-amine (**4**).

A mixture of 5 g. (0.02 mole) of 5-chloro-6-nitroquinazoline-2,4-diamine (**2**) (3) in 5 ml. of 28% ammonium hydroxide, and 50 ml. of formamide was heated in a stainless steel autoclave at 120° for 13 hours. The autoclave was cooled to room temperature, the contents were removed, and the solid adhering to the sides of the vessel removed by washing with methyl cellosolve. The reaction mixture and wash were combined and filtered to give 3.1 g. (63%) of crude product, m.p. > 300°. An 0.8 g. sample was recrystallized from 150 ml. of dimethylsulfoxide to afford 0.44 g. of pure material, m.p. > 300°.

Anal. Calcd. for $C_9H_6N_6O_2$: C, 46.96; H, 2.63; N, 36.51. Found: C, 46.60. H, 2.78; N, 36.21.

2,4,5,6-Quinazolinetetraamine Dihydrochloride (**5**).

A solution of 9.7 g. (0.035 mole) of 6-nitro-2,4,5-quinazolinetriamine monoacetate (**3**) in 10 ml. of acetic acid and 100 ml. of methanol was hydrogenated over 1 g. of 5% palladium on carbon at room temperature and at an initial pressure of 50 psig for 42 hours. The catalyst was removed by filtration and washed with methanol. The combined filtrate-wash was treated with 12 ml. of concentrated hydrochloric acid and chilled to afford 8.0 g. (88%) of the desired product, m.p. decomposes slowly > 280°.

Anal. Calcd. for $C_8H_{10}N_6 \cdot 2HCl$: C, 36.51; H, 4.60; N, 31.94. Found:C, 36.81; H, 4.73; N, 31.67.

8,9,10,11-Tetrahydropyrimido[5,4-*a*]phenazine-1,3-diamine Acetate (1:0.25) (**6**).

A mixtue of 0.71 g. (0.003 mole) of 2,4,5,6-quinazolinetetraamine dihydrochloride (**5**), 0.31 g. (0.003 mole) of 1,2-cyclohexanedione (9a), 0.8 g. (0.01 mole) of freshly fused sodium acetate in 5 ml. of acetic acid was heated under reflux for 2 hours, poured into 25 ml. of water, and filtered. The filtrate was made basic with sodium hydroxide to produce a gummy precipitate. Trituration with ethanol followed by recrystallization from 50% aqueous acetic acid afforded 0.05 g. (7%) of the desired product, containing 0.25 mole of acetic acid, m.p. > 300°. The ir spectrum confirmed the presence of acetic acid (1650 cm^{-1}).

The remaining pyrazinoquinazolines (**7-13**) were prepared by heating at 100°, equal molar amounts of 2,4,5,6-quinazolinetetraamine dihydrochloride (**5**) and the requisite dione in aqeous acetic acid for 0.5-3.5 hours and then cooling. The resulting precipitate was collected and recrystallized, if necessary, from the appropriate solvent. Concentration of aqueous acetic acid, reaction times and recrystallization solvents for each compound appear in Table I. Listed below are the starting diones used.

Dione	Product
2,3-Butanedione (**9a**)	**7**
Diphenylethanedione (**9a**)	**8**
α-Oxobenzeneacetaldehyde monohydrate (**9a**)	**9**
1-Phenyl-1,2-propanedione (**9b**)	**10**
9,10-Phenanthrenedione	**11**
1,10-Phenanthroline-5,6-dione (**9c**)	**12**
1*H*-Indene-1,2,3-trione (**9b**)	**13**

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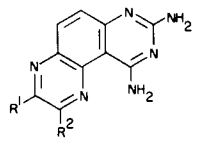
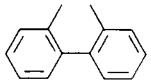
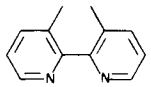
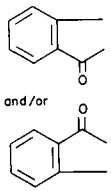
Table I

Pyrazino[2,3-*f*]quinazoline-8,10-diamines and Related Heterocycles



Compound No.	Name	R^1	R^2	M.p., °C.	Reaction (a) Solvent % Aqueous Acetic Acid	Reaction (a) Time (Hours)	Yield Purified %	Purification Solvent	Formula	Analyses Carbon % Calcd.	Carbon % Found	Hydrogen % Calcd.	Hydrogen % Found	Nitrogen % Calcd.	Nitrogen % Found
6	8,9,10,11-Tetrahydropyrimido[5,4-*a*]phenazine-1,3-diamine Acetate (1:0.25)	$-(CH_2)_4-$		>300°	(a)	2.0	7	50% Aqueous Acetic Acid	$C_{14}H_{14}N_6 \cdot 0.25HOAc$	61.90	61.80	5.37	5.47	29.88	29.83
7	2,3-Dimethylpyrazino[2,3-*f*]quinazoline-8,10-diamine	CH_3	CH_3	>300°	10	0.5	64	20% Aqueous Acetic Acid	$C_{12}H_{12}N_6$	59.98	59.66	5.04	5.05	34.98	34.86
8	2,3-Diphenylpyrazino[2,3-*f*]quinazoline-8,10-diamine Hydrate (1:0.3)	C_6H_5	C_6H_5	285-292°	78	1.5	36	90% Aqueous Acetic Acid	$C_{22}H_{16}N_6 \cdot 0.3H_2O$ (b)	71.45	71.49	4.52	4.42	22.73	22.71
9	2(and 3)-phenylpyrazino[2,3-*f*]quinazoline-8,10-diamine Hydrate (1:0.3)	C_6H_5 and H	H and C_6H_5	325° dec	50	2.0	46	DMF/Ammonium Hydroxide	$C_{16}H_{12}N_6 \cdot 0.3H_2O$ (c)	65.43	65.33	4.32	4.41	28.62	28.90
10	2(and 3)-Methyl-3(and 2)-phenylpyrazino[2,3-*f*]-quinazoline-8,10-diamine	C_6H_5 and CH_3	CH_3 and C_6H_5	290-292° dec	85	2.0	58	DMF/Ammonium Hydroxide/Water	$C_{17}H_{14}N_6$	67.53	67.39	4.67	4.90	27.80	28.04
11	Dibenzo[*a*,*c*]pyrimido[5,4-*h*]phenazine-13,15-diamine			>350°	75	2.0	32	DMSO	$C_{22}H_{14}N_6 \cdot 0.15DMSO \cdot 0.7H_2O$ (d)	69.26	69.00	4.25	4.17	21.74	21.60
12	Dipyrido[3,2-*a*:2′,3′-*c*]pyrimido[5,4-*h*]phenazine-13,15-diamine Hydrochloride (1:1.05) Hydrate (1:1.2)			>350°	90	2.5	80	(e)	$C_{20}H_{12}N_6 \cdot 1.05HCl \cdot 1.2H_2O$ (f)	56.62	56.97	3.67	3.72	26.41	26.67
13	1,3-Diamino-8*H*(and/or 12*H*)-indeno[2′,1′:5,6](and/or[1′,2′:5,6])pyrazino[2,3-*f*]quinazolin-8(and/or 12)-one Monoacetate Monohydrochloride	 and/or		>310°	85	2.0	94	(e)	$C_{17}H_{10}N_6O \cdot HOAc \cdot HCl$ (g)	55.55	55.32	3.68	3.60	20.46	20.40

(a) See Experimental for procedure. (b) Calcd. for H_2O: 1.46. Found: 1.28. (c) Calcd. for H_2O: 1.84. Found: 1.38. (d) Calcd. for S: 1.24. Found: 1.07. Calcd. for H_2O: 3.26. Found: 2.90. (e) Product crystallized from reaction mixture. (f) Calcd. for Cl: 8.78. Found: 8.96. Calcd. for H_2O: 5.17. Found: 5.10. (g) Calcd. for Cl: 8.63. Found: 8.85.

REFERENCES AND NOTES

(1) This is communication No. 43 of a series on antimalarial drugs. For paper 42, see: L. M. Werbel, D. McNamara, N. Colbry, J. Johnson, M. Degnan and B. Whitney, *J. Heterocyclic Chem.*, **16**, 881 (1979). For Folate Antagonists 13., see: E. F. Elslager, P. Jacob, J. Johnson, L. M. Werbel, D. F. Worth and L. Rane, *J. Med. Chem.*, **21**, 1059 (1978). This investigation was supported by U. S. Army Medical Research and Development Command Contract DADA-17-72-C-2077. This is contribution No. 1537 to the Army Research Program in Malaria.

(2) A preliminary report of this work was presented by E. F. Elslager and John Davoll, in "Lectures in Heterocyclic Chemistry", Vol. 2, R. N. Castle and L. B. Townsend, Eds., HeteroCorporation, Orem, Utah, 1974, p. S-97.

(3) E. F. Elslager, J. Clarke, L. M. Werbel, D. F. Worth and J. Davoll, *J. Med. Chem.*, **15**, 827 (1972).

(4) A. Bruker WH-90 was used to determine nmr spectra.

(5) The parenteral antimalarial screening was carried out by Dr. Leo Rane and Mrs. D. S. Rane of the University of Miami and test results were supplied through the courtesy of Dr. David P. Jacobus, Dr. T. R. Sweeney, and Dr. E. A. Steck of Walter Reed Army Institute for Research. For a description of the test method, see: T. S. Osdene, P. B. Russell and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(6) W. Szybalski, *Microb. Genet. Bull.*, **5**, 16 (1951).

(7) A. H. Webb and L. Washington, *Bacteriol Proc.*, 52, (1966).

(8) Melting points (corrected) were taken on a Thomas-Hoover capillary melting-point apparatus.

(9) Available from: (a) Aldrich Chemical Co., Milwaukee, Wisconsin; (b) Eastman Organic Chemicals, Rochester, N. Y.; and (c) G. Frederick Smith Chemical Co., Columbus, Ohio.