methanol Hydrochloride (23). Bromomethyl 2,6-bis(3,4-dichlorophenyl)-4-pyridyl ketone (IIe) (2 g) was suspended in EtOH (40 ml). $NaBH_4$ (250 mg) was added, and the mixt was stirred for 1 hr at room temp. HCl (3 *N*) was added to decompose excess $NaBH_4$, and the mixt was neutralized with Na_2CO_3. Water (50 ml) was added, and the mixt was filtered. The solid was washed with H_2O (x 2, 20 ml) and dried *in vacuo*. There was obtained 1.6 g (95%) of crude epoxide, mp 138–145°, containing *ca.* 5% of an unknown by tlc. The epoxide (1.5 g) and $(n\text{-}C_4H_9)_2NH$ (5 ml) in EtOH (25 ml) were heated at reflux for 3 hr (complete by tlc). Solvent and excess amine were removed *in vacuo*. The residual oil in Et_2O–*i*-PrOH was treated with a satd soln of HCl in *i*-PrOH. The ppt was washed with Et_2O (x 3, 20 ml). Recrystn from EtOH afforded 1.5 g (71%) of **23**, mp 220–222°. The above procedure is typical of the prepn of the 4-pyridinemethanols described in Table III.

Derivatives. The *N*-oxides **4a** and **23a** were prepd by treating the parent compds **4** and **23**, respectively (as the free bases), in Et_2O with 40% AcO_2H in AcOH. The mixt was stirred 2 hr at 25°. For **4a**, the soln was washed with 20% NaOH (x 2) and water (x 2), and dried (Na_2SO_4). The solvent was removed, and the residue was dissolved in Et_2O. Et_2O–HCl was added, and the ppt was recrystd (EtOH) to give **4a** (HCl salt), mp 172–174°. In the case of **23a**, the crude product pptd from the Et_2O reaction mixt as the AcOH salt. This ppt was slurried in MeOH and treated with a little concd HCl. Water was added to the soln to ppt **23a** (HCl salt), mp 174–175° (EtOH).

The *O*-succinoyl derivative **4b** was prepd by heating parent compd **4** (free base) and succinic anhydride in Me_2CO for 1 hr. The solvent was removed. The residue was dissolved in Et_2O and treated with dry HCl. The mixt was stirred at 25° with an equal vol of H_2O for 1 hr. Filtration gave crude **4b**, mp 149–153° (HCl salt), recrystd from CH_3CN.

The *N*-succinoyl derivative **3a** was prepd from parent compd **3** (free base) by treating an Me_2CO soln with succinic anhydride at 25° for 1 hr. The solvent was removed and recrystn from C_6H_6 gave **3a**, mp 104–107°.

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Folate Antagonists. 2. 2,4-Diamino-6-{[aralkyl and (heterocyclic)methyl]amino}quinazolines, a Novel Class of Antimetabolites of Interest in Drug-Resistant Malaria and Chagas' Disease†,‡

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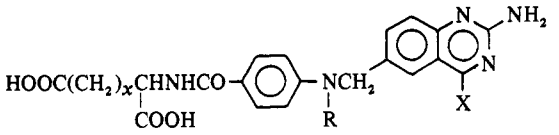
Forty-six 2,4-diamino-6-{[benzyl and (heterocyclic)methyl]amino}quinazolines (VI) were synthesized from the appropriate 2,4-diamino-6-nitroquinazoline (III) by reduction to the corresponding 2,4,6-triaminoquinazoline (IV), condensation of IV with the appropriate benzaldehyde or heterocyclic aldehyde to give the requisite Schiff base V, and reduction of V with H_2 over Raney Ni or with $NaBH_4$. 2,4-Diamino-6-{[(2-chloro-1-naphthyl)methyl]amino}quinazoline (**70**) and 2,4-diamino-6-{[(2-naphthyl)methyl]amino}quinazoline (**71**) were prepared similarly from 2,4,6-triaminoquinazoline and 2-chloro-1-naphthaldehyde and 2-naphthaldehyde, respectively. The condensation of 2 equiv of 2,4,6-triaminoquinazoline with 1 equiv of terephthalaldehyde or 4,4′-(ethylenedioxy)dibenzaldehyde followed by reduction of the Schiff bases afforded 6,6′-[*p*-phenylenebis(methyleneimino)]bis(2,4-diaminoquinazoline) (**76**) and 6,6′-[ethylenebis(oxy-*p*-phenylenemethyleneimino)]bis(2,4-diaminoquinazoline) (**77**). Treatment of 2,4,6-triaminoquinazoline with an acetophenone diethyl ketal in the presence of I_2 gave the corresponding 2,4-diamino-6-[(α-methylbenzylidene)amino]quinazolines, which upon reduction with $NaBH_4$ or H_2/PtO_2 afforded the requisite 2,4-diamino-6-[(α-methylbenzyl)amino]quinazolines (**66, 68, 69**). Forty-four compds were active against *Plasmodium berghei* in mice and 27 ranged from 7 to 190 times as potent as quinine hydrochloride. 2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (**15**) also exhibited strong effects against cycloguanil-, pyrimethamine-, DDS-, and chloroquine-resistant lines of *P. berghei*. Against *P. cynomolgi* in rhesus monkeys, 19 compds eliminated asexual parasites within 1–8 days, and 7 were curative. Twenty-eight quinazolines were active against *Trypanosoma cruzi* in chick embryo cell culture at 0.39–6.25 μg/ml, and six showed antitrypanosomal effects in mice. Data on the inhibitory effects of the triaminoquinazolines against *Streptococcus faecalis* R (*Strep. faecium* var. *durans*), *Strep. faecalis* A (aminopterin-, methotrexate-resistant), *Lactobacillus plantarum*, and *Pediococcus cerevisiae* are presented, and overall structure–activity relationships are discussed.

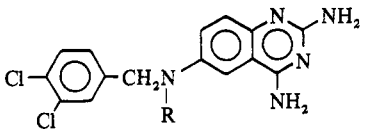
Recent reports from these laboratories have described the synthesis and biological properties of various quinazoline analogs of folic acid.[2-7] Among them, several 2,4-diamino- and 2-amino-4-hydroxyquinazoline Glu and Asp analogs[2] (I, where $x = 1$ or 2; R = H or CH_3; and X = OH or NH_2) exhibit potent inhibitory effects against *Streptococcus faecalis* R (ATCC 8043)[4] [*Strep. faecium* var. *durans* (SF/0)],[8,9]

†This is paper 24 of a series on antimalarial drugs. For paper 23, see ref 1.
‡For the previous paper on folate antagonists, see ref 2.



I

thymidylate synthetase, and dihydrofolic reductase from mammalian and bacterial sources,[4,8] several sublines of the L1210 mouse leukemia,[9,10] and L1210 cells in culture.[9] However, these Glu and Asp analogs lack appreciable antiparasitic activity. In contradistinction, 2,4-diaminoquinazoline antifolics such as 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (IIa) and 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (IIb) display



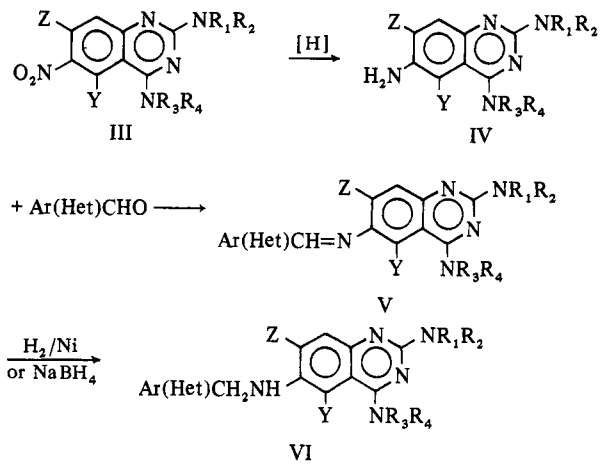
IIa, R = H
b, R = NO

strong antimalarial activity against sensitive and drug-resistant lines of *Plasmodium berghei* in mice, *P. gallinaceum* in chicks, and *P. cynomolgi* and *P. knowlesi* in rhesus monkeys.[5,6] These substances also have an encouraging degree of activity against *Trypanosoma cruzi* in chick embryo cell cultures and in mice.[5,7]

The present communication summarizes in detail the synthesis and biological properties of IIa, other 2,4-diamino-6-{[aralkyl and (heterocyclic)methyl]amino}quinazolines, and related triaminoquinazoline derivs.

Chemistry. The simple 2,4-diamino-6-{[benzyl and (heterocyclic)methyl]amino}quinazolines were synthesized utilizing the general route outlined in Scheme I. Thus the requisite 2,4,6-triaminoquinazoline IV, obtained by redn of the corresponding 2,4-diamino-6-nitroquinazoline III, was condensed with the appropriate benzaldehyde or heterocyclic aldehyde to give the Schiff bases V (methods A-H). The isolation of these intermediate Schiff bases in pure form was usually unnecessary, although several representative compds were purified and characterized (**1-12**, Table I). Condensation of the aldehydes with the 6-amino group of the 2,4,6-triaminoquinazolines was assumed on the basis of

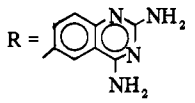
Scheme I



its aromatic character and was confirmed by the failure of 2,4-diaminoquinazoline to react under the conditions employed. Redn of the Schiff bases V with H_2 over Raney Ni (methods L-N) or with $NaBH_4$ (methods O, S, U) afforded the 2,4-diamino-6-[(benzyl)amino]quinazolines (**13-29**, **32-34**, **36-43**, Table II), 2,4-diamino-6-[(benzyl)amino]-(5 or 7)-substituted-quinazolines (**44-48**, Table III), N^2,N^4-(alkyl and aryl)-2,4-diamino-6-[(benzyl)amino]quinazolines (**49-53**, Table IV), and 2,4-diamino-6-{[(heterocyclic)-methyl]amino}quinazolines (**54-58**, Table V) in 15-93% overall yield based on the starting 2,4,6-triaminoquinazoline.

In like manner, treatment of 2,4,6-triaminoquinazoline with 2-chloro-1-naphthaldehyde and 2-naphthaldehyde afforded the intermediate Schiff bases (**63**, Table VI), which upon redn with $NaBH_4$ provided the naphthalene analogs 2,4-diamino-6-{[(2-chloro-1-naphthyl)methyl]amino}quinazoline (**70**) and 2,4-diamino-6-{[(2-naphthyl)methyl]-amino}quinazoline (**71**) in 34 and 59% overall yield, respectively (Table VII). Moreover, the condensation of 2 equiv of 2,4,6-triaminoquinazoline with 1 equiv of terephthalaldehyde or 4,4′-(ethylenedioxy)dibenzaldehyde[11] gave 6,6′-[*p*-phenylenebis(methylidyneimino)] bis(2,4-diaminoquinazoline) (**64**) (80%) and 6,6′-[ethylenebis(oxy-*p*-phenylenemethylidyneimino)] bis(2,4-diaminoquinazoline) (**65**) (79%) (Table VI), which upon hydrogenation over Raney Ni (method M) yielded 6,6′-[*p*-phenylenebis-(methyleneimino)] bis(2,4-diaminoquinazoline) (**76**) (61%)

Table I. 2,4-Diamino-6-[(benzylidene)amino]quinazolines[a] ($XC_6H_4CH{=}NR$)



No.	X	Mp, °C	Yield purified, %	Purification solvent	Method	Formula
1	3,4-Cl_2	258	53	EtOH	C	$C_{15}H_{11}Cl_2N_5$
2	3-Br	244-246	32	$CH_3O(CH_2)_2OH$	A	$C_{15}H_{12}BrN_5$[b]
3	4-Cl	280-282	59	EtOH-H_2O	C	$C_{15}H_{12}ClN_5$
4	4-F	257-259	38	EtOH	A	$C_{15}H_{12}FN_5$
5	2-NO_2	245 dec	78	DMF-EtOH[c]	C	$C_{15}H_{12}N_6O$
6	4-NO_2	314-315 dec	70	DMF	C	$C_{15}H_{12}N_6O$
7	H	222	50	EtOH	E	$C_{15}H_{13}N_5$
8	4-CHO	>360	71	*e*	H	$C_{16}H_{13}N_5O \cdot 0.5H_2O$
9	4-CH_3	273[d]	50	EtOH-H_2O	E	$C_{16}H_{15}N_5$
10	3-OCH_3	223-225	56	EtOH-H_2O	C	$C_{16}H_{15}N_5O$
11	4-OC_2H_5	248-249	39	EtOH-H_2O	A	$C_{17}H_{17}N_5O$
12	4-$N(CH_3)_2$	288-290	74	EtOH	C	$C_{17}H_{18}N_6$

[a] All compds analyzed for C, H, N. [b] Br: calcd, 23.4; found, 23.8. [c] Dissolved in hot DMF and added to EtOH. [d] Also obtained in form of mp 232-235°. [e] Not recrystd.

Table II. 2,4-Diamino-6-[(benzyl)amino]quinazolines ($XC_6H_4CH_2NHR$)[l]

No.	X	Mp, °C	Yield purified,[a] %	Purification solvent	Method[b]	Formula	Analyses[c]	Suppressive effects against *P. berghei* in mice			
								Route[d]	Days	SD_{90}, mg/kg per day[e]	Q^f
13	2,4-Cl_2	243–247	44	EtOH–H_2O	AL	$C_{15}H_{13}Cl_2N_5$	C, H, N	G	4	32	2.3
								SC	4	9.7	5.1
14	2,4-Cl_2	230–233	73	H_2O		$C_{15}H_{13}Cl_2N_5 \cdot C_2H_4O_2 \cdot 2H_2O$	C, H, N	G	4	<10	>7.4
								SC	4	<10	>5.0
15	3,4-Cl_2	240–243	40; 73	EtOH	AL; BO	$C_{15}H_{13}Cl_2N_5$	C, H, N	D	6	8.5	8.8
								G	4	18	4.2
								SC	4	4.5	11
16	3,4-Cl_2	220	93	H_2O		$C_{15}H_{13}Cl_2N_5 \cdot C_2H_4O_2$	C, H, N, Cl	D	6	9.5	7.9
17	3-Br	207–210	44	MeCN	AO	$C_{15}H_{14}BrN_5$	C, H, N	D	6	0.4	190
18	4-Br	197–203	44	EtOH–H_2O	AL	$C_{15}H_{14}BrN_5$	C, H, N	G	4	<10	>7.4
19	2-Cl	217–219	50	EtOH–H_2O	BN	$C_{15}H_{14}ClN_5 \cdot 0.33H_2O$	C, H, N	G	4	<10	>7.4
20	3-Cl	176–178	31	EtOH–H_2O	AL	$C_{15}H_{14}ClN_5$	C, H, N	G	4	<20	>3.7
								SC	4	<10	>5.0
21	4-Cl	205–207	40	EtOH–H_2O	CL	$C_{15}H_{14}ClN_5$	C, H, N	G	4	16	4.5
								SC	4	4.6	11
22	4-F	210–214	52	EtOH–H_2O	AL	$C_{15}H_{14}FN_5$	C, H, N	G	4	<10	>7.4
23	2-NO_2	205–206 dec	74		CN	$C_{15}H_{14}N_6O_2$	C, H, N	D	6	170	0.4
24	H	217–219	71; 67	EtOH–H_2O	BN; BO	$C_{15}H_{15}N_5$	C, H, N	G	4	<10	>7.4
								SC	4	8.7	5.7
25	H	298–300	93	H_2O		$C_{15}H_{15}N_5 \cdot HCl \cdot 0.25H_2O$	C, H, N	G	4	<40	>1.8
								SC	4	6.5	7.7
26	H	208–210	86	H_2O		$C_{15}H_{15}N_5 \cdot CH_2O_2 \cdot H_2O$	C, H, N	G	4	8.4	8.8
								SC	4	8.6	5.8
27	2-OH	190–200	75	EtOH–H_2O	BN	$C_{15}H_{15}N_5O$	C; H, N[g,h]	G	4	10	7.4
28	3-OH	135–140	24	EtOH–H_2O	BN	$C_{15}H_{15}N_5O$	C, H, N	G	4	12	6.4
29	4-OH	198–200	18	EtOH–H_2O	AL	$C_{15}H_{15}N_5O \cdot 0.25H_2O$	C, H, N	G	4	64	1.2
								SC	4	>10	<5.0
30	2-NH_2	217–219	62	EtOH–H_2O	P	$C_{15}H_{16}N_6$	C, H, N	D	6	240	0.3
31	4-COOH	>360	83		Q	$C_{16}H_{15}N_5O_2 \cdot 0.67H_2O$	C, H, N	G	4	>40	<1.8
								SC	4	>10	<5.0
32	2-CH_3	217	52	EtOH–H_2O	AL	$C_{16}H_{17}N_5$	H, N; C[i]	G	4	29	2.5
								SC	4	4.3	12
33	3-CH_3	193	49	EtOH–H_2O	AL	$C_{16}H_{17}N_5$	C, H, N	G	4	<10	>7.4
34	4-CH_3	200–201	40	EtOH–H_2O	DN	$C_{16}H_{17}N_5$	C, N; H[j]	G	4	27	2.7
								SC	4	4.5	11
35	4-CH_2OH	117–127	28	EtOH–H_2O	R	$C_{16}H_{17}N_5O \cdot H_2O$	C, H, N	D	6	>105	<0.7
36	3-OCH_3	186–189	59	EtOH–H_2O	CL	$C_{16}H_{17}N_5O$	C, H, N	D	6	21	3.5
								G	4	<10	>7.4
37	4-OCH_3	215–216	20	EtOH–H_2O	AL	$C_{16}H_{17}N_5O$	C, H, N	G	4	25	3.0
								SC	4	4.5	11
38	4-$NHCOCH_3$	256	17	EtOH	AL	$C_{17}H_{18}N_6O$	C, H, N	G	4	>40	<i.8
								SC	4	14	3.6
39	4-OC_2H_5	242–244	24	EtOH–H_2O	AL	$C_{17}H_{19}N_5O$	C, H, N	G	4	18	4.1
								SC	4	4.5	11
40	4-$N(CH_3)_2$	223–225	23	EtOH	CL	$C_{17}H_{20}N_6$	C, H, N	G	4	>40	<1.8
								SC	4	>10	<5.0
41	4-$COOC_2H_5$	229–230	36	EtOH	EL	$C_{18}H_{19}N_5O_2$	C, H, N	SC	4	>10	<5.0

42	2,4,6-$(CH_3)_3$	248–249[k]	43	EtOH–H_2O	AL	$C_{18}H_{21}N_5$	C, H, N	G	4	12	6.2
								SC	4	7.6	6.6
43	3,4,5-$(OCH_3)_3$ Quinine hydrochloride	201–203	39	EtOH–H_2O	AL	$C_{18}H_{21}N_5O_3 \cdot 0.5H_2O$	C, H, N	G	4	9.7	7.6
								D	6	74.5	
	Cycloguanil hydrochloride							G	4	74.0	
								SC	4	50.0	
	Pyrimethamine							D	6	2.1	35
								SC	4	4.5	11
	Trimethoprim							D	6	0.28	270
								SC	4	1.0	50
								D	6	120	0.6

[a]Based on 2,4,6-triaminoquinazoline. [b]Method for Schiff base prepn followed by method for redn. [c]See footnote #. [d]D, compds administered continuously in the diet of mice for 6 consecutive days; G, drugs given by gavage twice daily for 4 days as solns of suspensions in H_2O or 1% aqueous (hydroxyethyl) cellulose; SC, substances administered sc twice daily for 4 days as solns or suspensions in 1% aqueous (hydroxyethyl)cellulose. [e]All doses calcd as free base equiv. SD_{90} represents the daily dose (mg/kg) required for 90% suppression of the parasitemia in treated mice relative to control mice. The SD_{90} was estd graphically using semilog paper. [f]The quinine equiv Q is the ratio of the SD_{90} of quinine hydrochloride to the SD_{90} of the test substance under comparable exptl conditions. [g]H: calcd, 5.4; found, 6.0. [h]N: calcd, 24.9; found, 24.2. [i]C: calcd, 68.8; found, 68.3. [j]H: calcd, 6.1; found, 6.8. [k]Also obtained in a form mp 216–218°. [l]R is 2,4-diamino-6-quinazolinyl (Table I).

Table III. 2,4-Diamino-6-[(benzyl)amino]-(5 or 7)-substituted-quinazolines ($XC_6H_4CH_2NH(5\text{-}Y, 7\text{-}Z\text{-}R)$)[h]

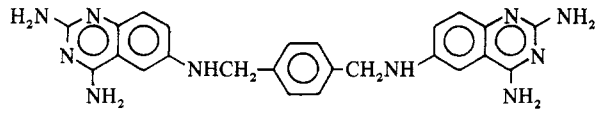
									Suppressive effects against *P. berghei* in mice			
No.	X	Y	Z	Mp, °C	Yield purified,[a] %	Purification solvent	Method[b]	Formula[c,i]	Route[d]	Days	SD_{90}, mg/kg per day[e]	Q^f
44	3,4-Cl_2	Cl	H	218–220	56	DMF	FO	$C_{15}H_{12}Cl_3N_5$	D	6	0.7	110
45	H	Cl	H	187–189	23	EtOH–H_2O	GO	$C_{15}H_{14}ClN_5$	D	6	<14	>5.3
46	3,4-Cl_2	CH_3	H	287–290	28	EtOH–H_2O[g]	BN	$C_{16}H_{15}Cl_2N_5 \cdot HCl \cdot H_2O$	D	6	5.1	15
47	H	CH_3	H	191–195	52	EtOH–H_2O	BN	$C_{16}H_{17}N_5$	D	6	1.3	57
48	H	H	CH_3	213–214	58	EtOH–H_2O	AL	$C_{16}H_{17}N_5$	G	4	>40	<1.8
									SC	4	>10	<5.0

[a–f]See footnotes Table II. [g]From amorphous base in EtOH treated with dil HCl. [h]R is 2,4-diamino-6-quinazolinyl (Table I). [i]All compds analyzed for C, H, N.

Table IV. N^2,N^4-(Alkyl and aryl)-2,4-diamino-6-[(benzyl)amino]quinazolines ($XC_6H_4CH_2NH(2\text{-}R_1R_2, 4\text{-}R_3R_4\text{-}R)$)[k]

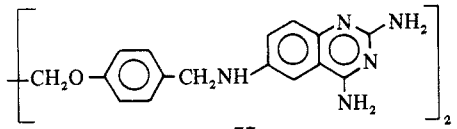
										Suppressive effects against *P. berghei* in mice			
No.	X	NR_1R_2	NR_3R_4	Mp, °C	Yield purified, %	Purification solvent	Method[b]	Formula	Analyses[c]	Route[d]	Days	SD_{90}, mg/kg per day[e]	Q^f
49	H	$N(CH_3)_2$	NH_2	154–155	57[a]	EtOH–H_2O	BO	$C_{17}H_{19}H_5$	C, H, N	D	6	52	1.4
50	3,4-Cl_2	$N(CH_3)_2$	$N(CH_3)_2$	170–172	18[g]	EtOH–H_2O	DN	$C_{19}H_{21}Cl_2N_5$	C, H, N	D	6	24	3.1
51	3,4-Cl_2	$NH(CH_2)_3CH_3$	$NH(CH_2)_3CH_3$	188–192	39[g]	EtOH–H_2O	DN	$C_{23}H_{29}Cl_2N_5$	N; C, H[h,i]	D	6	150	0.5
52	3,4-Cl_2	$N(C_2H_5)_2$	$N(C_2H_5)_2$	146–150	49[g]	C_6H_6–petr ether	DN	$C_{23}H_{29}Cl_2N_5$	C, H, Cl; N[j]	D	6	220	0.3
53	3,4-Cl_2	NHC_6H_5	NHC_6H_5	114–116	37[g]	Me_2CO–MeOH–H_2O	DN	$C_{27}H_{21}Cl_2N_5 \cdot H_2O$	C, H, N	D	6	>63	<1.2

[a–f]See footnotes *a–f*, Table II. [g]Based on the corresponding 2,4-diamino-6-nitroquinazoline. [h]C: calcd, 61.9; found, 61.3. [i]H: calcd, 6.5; found, 7.1. [j]N: calcd, 15.7; found, 14.9. [k]R is 2,4-diamino-6-quinazolinyl (Table I).



76

and 6,6′-[ethylenebis(oxy-*p*-phenylenemethyleneimino)]-bis(2,4-diaminoquinazoline) (**77**) (76%), respectively (Table VIII).



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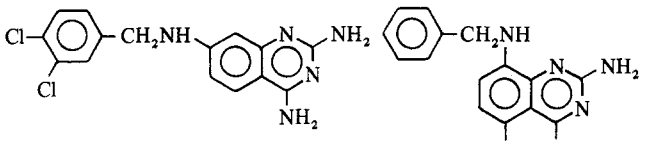
Two representative position isomers of the 2,4-diamino-6-[(benzyl)amino]quinazolines, namely 2,4-diamino-7-[(3,4-dichlorobenzyl)amino]quinazoline (**72**) (59%) and 2,4-diamino-8-[(benzyl)amino]-5-methylquinazoline (**75**) (46%)

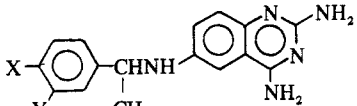


72

75

(Table VIII), were obtained *via* 2,4,7-triaminoquinazoline and 2,4,8-triamino-5-methylquinazoline[2] utilizing the route outlined in Scheme I. 4-Amino-6-[(benzyl)amino]-2-methylquinazoline (**74**) was prepd similarly from 4,6-diamino-2-methylquinazoline.[12]

Initial efforts to prep various 2,4-diamino-6-[(α-methylbenzyl)amino]quinazolines directly from acetophenones by a similar procedure were not encouraging because the condensation of acetophenone with 2,4,6-triaminoquinazoline gave only a trace of 2,4-diamino-6-[(α-methylbenzylidene)amino]quinazoline (**62**). However, the condensation of the requisite acetophenone diethyl ketal with 2,4,6-triaminoquinazoline in $(CH_3OCH_2CH_2)_2O$ in the presence of I_2 afforded moderate yields of the corresponding 2,4-diamino-6-[(α-methylbenzylidene)amino]quinazolines (**61**, **62**, Table VI), which upon redn with $NaBH_4$ or H_2/PtO_2 provided the 2,4-diamino-6-[(α-methylbenzyl)amino]quinazolines (**66**, **68**, **69**) (5-68%, Table VII).



66, X = Y = Cl
68, X = Cl; Y = H
69, X = Y = H

Alternatively, 2,4-diamino-6-[(*p*-chlorophenethyl)amino]-quinazoline (**67**) was prepd utilizing the reaction sequence outlined in Scheme II. The condensation of *p*-chlorophenethylamine with 5-chloro-2-nitrobenzonitrile afforded 5-[(*p*-chlorophenethyl)amino]-2-nitrobenzonitrile (**78**) (54%), which upon redn with $SnCl_2 \cdot 2H_2O$ and HCl gave 2-amino-5-[(*p*-chlorophenethyl)amino]benzonitrile (**79**) (71%). Fusion of the HCl salt of **79** with cyanoguanidine at 160° afforded **67** (5%) (Table VII).

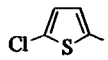
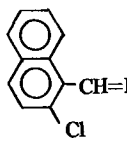
Several other aminoquinazolines were derived from certain 2,4-diamino-6-[(benzyl)amino]quinazolines listed in Table II. Thus hydrogenation of 2,4-diamino-6-[(*o*-nitrobenzyl)amino]quinazoline (**23**) at atmospheric pressure over Pt/C afforded 2,4-diamino-6-[(*o*-aminobenzyl)amino]quin-

Table V. 2,4-Diamino-6-{[(heterocyclic)methyl]amino}quinazolines (Het-CH_2NHR)[h]

No.	Het	Mp, °C	Yield purified,[a] %	Purification solvent	Method[b]	Formula	Analyses[c]	Suppressive effects against *P. berghei* in mice: Route[d]	Days	SD_{50}, mg/kg per day[e]	Q^f
54		186-189	17	MeOH-H_2O	AO	$C_{13}H_{12}ClN_5S \cdot 0.5CH_3OH$	C, H, N; VL[g]	D	6	10	7.1
55		215-216	57	EtOH-H_2O	EL	$C_{13}H_{13}N_5O$	C, H, N	SC	4	16	3.0
56		227-229	41	EtOH-H_2O	BO	$C_{13}H_{13}N_5S$	C, H, N	D	6	41	1.8
57		256	35	EtOH-H_2O	EL	$C_{14}H_{14}N_6$	C, H, N	G SC	4 4	>40 >10	<1.8 <5.0
58		284-288	15	EtOH-H_2O	EL	$C_{14}H_{14}N_6$	C, H, N	SC	4	>10	<5.0

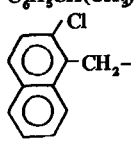
[a-f] See footnotes *a-f*, Table II. [g] Volatile loss at 100°: calcd, 5.0; found, 4.4. [h] R is 2,4-diamino-6-quinazolinyl (Table I).

Table VI. Other Schiff Bases Derived from 2,4,6(or 7)-Triaminoquinazoline (BR)[f]

No.	B	Mp, °C	Yield purified, %	Purification solvent	Method	Formula	Analyses[b]
59		264–266 dec	54	MeOH	A	$C_{13}H_{10}ClN_5S$	C, H, N
60	3′,4′-$Cl_2C_6H_3CH$=N-(7)	265–267	65	EtOH	E	$C_{15}H_{11}Cl_2N_5$	C, H, N
61	4′-$ClC_6H_4C(CH_3)$=N-(6)	259–260	16	EtOH	K	$C_{16}H_{14}ClN_5$	C, H, N
62	$C_6H_5C(CH_3)$ =N-(6)	219–221	48[a]	EtOH	K	$C_{16}H_{15}N_5$	C, H, N
63		238–240	38	$CH_3O(CH_2)_2OH$	J	$C_{19}H_{14}ClN_5 \cdot 1.5C_2H_4O_2 \cdot 0.75H_2O$	C, H, N, H_2O
64	(6)-N=CH-1′-C_6H_4-4′-CH=N-(6)	>360	80	*e*	I	$C_{24}H_{20}N_{10}$	H; C, N[c,d]
65	[-4′-$CH_2OC_6H_4CH$=N-(6)]$_2$	324–326 dec	79	DMF	I	$C_{32}H_{28}N_{10}O_2 \cdot H_2O$	C, H, N

[a]Variable yield. [b]See footnote #. [c]C: calcd, 64.3; found, 63.8. [d]N: calcd, 31.2; found, 30.7. [e]Not recrystd. [f]R is 2,4-diamino-6(or 7)-quinazolinyl (Table I).

Table VII. Other 2,4-Diamino-6-[(aralkyl)amino]quinazolines (Ar-A-NHR)[i]

No.	Ar-A-	Mp, °C	Yield purified, %	Purification solvent	Method	Formula	Analyses[c]	Suppressive effects against *P. berghei* in mice: Route[d]	Days	SD_{90}, mg/kg per day[e]	Q^f
66	3′,4′-$Cl_2C_6H_3CH(CH_3)$-	230–321	5[a]	MeCN	KS	$C_{16}H_{15}Cl_2N_5$	C, H, N	D	6	8.7	8.6
67	4′-$ClC_6H_4(CH_2)_2$-	335–338	5	EtOH		$C_{16}H_{16}ClN_5 \cdot C_7H_8O_3S$[g]	C, H, N	D	6	18	4.0
68	4′-$ClC_6H_4CH(CH_3)$-	231–232	10	EtOH	KS	$C_{16}H_{16}ClN_5 \cdot 0.25C_2H_6O$	C, H, N	D	6	<20, >0.4	>3.7, <190
69	$C_6H_5CH(CH_3)$-	200–202	68	EtOH–H_2O	KT	$C_{16}H_{17}N_5 \cdot H_2O$	H, N; C[h]	D	6	<1.5	>50
70		268–269	34	*i*-PrOH	JU	$C_{19}H_{16}ClN_5$	C, H, N	D	6	9.0	8.3
71	β-Naphthyl-CH_2-	242	59[a]	EtOH–H_2O	CO[b]	$C_{19}H_{17}N_5$	C, H, N	G	4	16	4.8
								SC	4	4.3	12

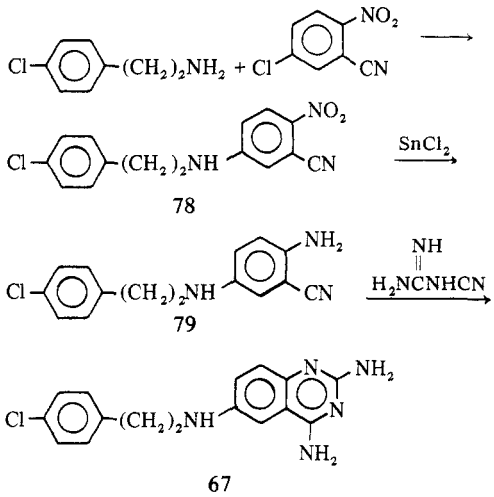
[a-f]See footnotes *a-f*, Table II. [g]$C_7H_8O_3S$ represents *p*-toluenesulfonic acid. [h]C: calcd, 64.6; found, 65.1. [i]R is 2,4-diamino-6-quinazolinyl (Table I).

Table VIII. Miscellaneous 2- and 4-Amino-(6, 7, or 8)-[(benzyl)amino]quinazolines (Ar-A-NHR)[i]

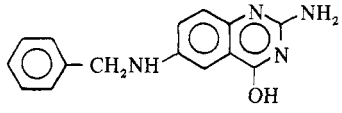
No.	Ar-A-NH	Mp, °C	Yield purified, %	Purification solvent	Method[b]	Formula	Analyses[c]	Suppressive effects against *P. berghei* in mice: Route[d]	Days	SD_{90}, mg/kg per day[e]	Q^f
72	3′,4′-$Cl_2C_6H_3CH_2NH$-(7)	213–216	59	H_2O	CS	$C_{15}H_{13}Cl_2N_5$	C, H, N	*h*			
73	$C_6H_5CH_2NH$-(6)[j]	270–271	74	EtOH–H_2O		$C_{15}H_{14}N_4O$	C, H, N	G	4	>40	<1.8
								SC	4	>10	<5.0
74	$C_6H_5CH_2NH$-(6)[k]	170–171	60[a]	EtOAc	DN	$C_{16}H_{16}N_4$	C, H, N	G	4	>10	<7.4
75	$C_6H_5CH_2NH$-(8)[l]	151–157	46[a]	EtOH–H_2O	JL	$C_{16}H_{17}N_5 \cdot C_2H_4O_2 \cdot 1.5H_2O$	C, H, N	D	6	>150	<0.5
76	(6)-$NHCH_2$-1′-C_6H_4-4′-CH_2NH-(6)	335–336 dec	61	DMF–H_2O	IM	$C_{24}H_{24}N_{10} \cdot 1.5H_2O$	C, H, N	D	6	>350	<0.2
77	[-4′-$CH_2OC_6H_4CH_2NH$-(6)]$_2$	274	76	DMF	IM	$C_{32}H_{32}N_{10}O_2$	C, H; N[g]	D	6	>350	<0.2

[a-f]See footnotes *a-f*, Table II. [g]N: calcd, 23.8; found, 24.3. [h]Inactive against *P. berghei* in mice when administered in a single sc dose of 160 mg/kg. [i]R is 2- or 4-amino-(6, 7, or 8)-quinazolinyl (Table I). [j]4-Hydroxyl. [k]2-Methyl. [l]5-Methyl.

Scheme II



azoline (**30**) (62%, method P), and $NaBH_4$ redn of α-[(2,4-diamino-6-quinazolinyl)imino]-*p*-tolualdehyde (**8**) gave *p*-{[(2,4-diamino-6-quinazolinyl)amino]methyl}benzyl alcohol (**35**) (28%, method R). α-[(2,4-Diamino-6-quinazolinyl)-amino]-*p*-toluic acid (**31**) was obtained in 83% yield from α-[(2,4-diamino-6-quinazolinyl)amino]-*p*-toluic acid ethyl ester (**41**) by hydrolysis with 0.5 *N* NaOH (method Q), while the hydrolysis of 2,4-diamino-6-[(benzyl)amino]-quinazoline (**24**) with boiling 2 *N* HCl afforded 2-amino-6-[(benzyl)amino]-4-quinazolinol (**73**) in 74% yield.[13]



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Among the aminoquinazolines that were employed as starting materials for the present investigation, 2,4,6-triaminoquinazoline,[2] 2,4,6-triamino-5-chloroquinazoline,[2] 2,4,6-triamino-5-methylquinazoline,[2] 2,4,8-triamino-5-methylquinazoline,[2] and 4,6-diamino-2-methylquinazoline[12] are known and were resynthesized utilizing literature methods. 2,4,6-Triamino-7-methylquinazoline (**82**) was synthesized by a route analogous to that employed previously for the prepn of 2,4,6-triaminoquinazoline,[2] and its structure was confirmed by comparison of its uv spectrum with that of 2,4,6-triaminoquinazoline.[2] Thus the condensation of 2-amino-*p*-tolunitrile with cyanoguanidine gave 2,4-diamino-7-methylquinazoline (**80**) (43%), which was converted to the nitrate salt and treated with fuming HNO_3 and concd H_2SO_4 to give 2,4-diamino-7-methyl-6-nitroquinazoline (**81**) (93%). Redn of **81** with $SnCl_2$ and HCl produced the intermediate triamine **82** (34%). 2,4,7-Triaminoquinazoline (**84**) was obtained by amination of 2,4-dichloro-7-nitroquinazoline[14] with NH_3 in boiling PhOH to give 2,4-diamino-7-nitroquinazoline (**83**) (83%), which upon hydrogenation over Raney Ni afforded **84** (38%).

The intermediate N^2,N^4-(alkyl and aryl)-2,4,6-triaminoquinazolines were synthesized as follows. 2-Chloro-5-nitrobenzonitrile was condensed with 1,1-dimethylguanidine in $C_2H_5O(CH_2)_2OH$ to give 4-amino-2-(dimethylamino)-6-nitroquinazoline (**85**) (69%), which upon hydrogenation over Pd/C in EtOH yielded 4,6-diamino-2-(dimethylamino)-quinazoline (**86**) (69%). The fusion of 2,4-dichloro-6-nitroquinazoline[14] with $NHMe_2 \cdot AcOH$ at 140–150° for 6 hr afforded 2,4-bis(dimethylamino)-6-nitroquinazoline (**87**) (57%), while the condensation of 2,4-dichloro-6-nitroquinazoline with 4 moles of the appropriate amine in EtOH gave 2,4-bis(*n*-butylamino)-6-nitroquinazoline (**88**) (97%), 2,4-bis(diethylamino)-6-nitroquinazoline (**89**) (75%), and 2,4-dianilino-6-nitroquinazoline (**90**) (38%). Hydrogenation of the above 2,4-diamino-6-nitroquinazolines over Pd/C in EtOH at atmospheric pressure afforded EtOH solutions of the corresponding triamines which were not isolated but were employed directly for production of the appropriate Schiff base.

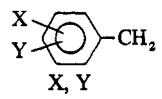
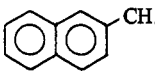
Antimalarial Evaluation. Primary Screening. The 2,4-diamino-6-{[aralkyl and (heterocyclic)methyl]amino}quinazolines described in the present communication were supplied to Dr. Paul E. Thompson and coworkers of these laboratories for antimalarial evaluation. Primary screening was carried out utilizing a normal drug-sensitive strain of *P. berghei* in mice.[5] The drugs were administered by one or more of three regimens: D, continuously in the diet of mice for 6 consecutive days; G, by gavage twice daily for 4 days; or SC, subcutaneously twice daily for 4 days.[5] Results (Tables II–V, VII, VIII) are expressed both in terms of the SD_{90} (daily dose required for 90% suppression of the parasitemia) and quinine equiv *Q* (the ratio of the SD_{90} of quinine hydrochloride to the SD_{90} of the test substance under comparable exptl conditions). Data for the ref drugs quinine hydrochloride, cycloguanil hydrochloride, pyrimethamine, and trimethoprim are included for comparative purposes (Table II).

Secondary Evaluation. *P. cynomolgi* (B strain) infections were induced in rhesus monkeys by giving 0.5×10^6 parasitized erythrocytes iv, and parasite studies were carried out as described previously.[5] Drug treatment was started as soon as daily blood smear examination showed measurable patent infections, usually 5–7 days after inoculation. The quinazolines were administered by stomach tube as suspensions in 0.1% Tween 80 in 1% (hydroxyethyl) cellulose twice daily for 5 or 10 consecutive days. Antimalarial effects, assessed by the examination of thick and thin blood films daily for periods up to 78 days after initiation of treatment, are summarized in Table IX.

Antimalarial Activity in Mice. Structure–Activity Relationships. The effects of chemical modifications at various regions of the basic 2,4-diamino-6-[(benzyl)amino]quinazoline molecule on antimalarial activity in mice are summarized below. These regions include aromatic benzyl substituents (Table II), 5- or 7-quinazoline substitution (Table III), N^2- or N^4-(alkyl or aryl) groups (Table IV), 6-[(heterocyclic)methyl]amino functions (Table V), α-substituted benzyl moieties (Table VII), naphthylmethyl analogs (Table VII), and miscellaneous structural variations (Table VIII).

(1) Aromatic Benzyl Substituents. Twenty-seven compds were active and 19 ranged from 7 to 190 times as potent as quinine hydrochloride (Table II). Consistently strong antimalarial effects were encountered among the halobenzyl derivs **13–22**, and seven compds (**14**, **17–22**) exhibited activity comparable with or superior to 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (IIa) base (**15**) or acetate (**16**). The most active compd, 2,4-diamino-6-[(*m*-bromobenzyl)amino]quinazoline (**17**), was 190 times as potent as quinine hydrochloride and approached pyrimethamine in potency. In constrast with previous studies with antifolates in the cycloguanil[15] and pyrimethamine[16] series, ortho halo substitution (**13**, **14**, **19**) was not deleterious. Moreover, the unsubstituted benzyl deriv **24** and its salts (**25**, **26**) unexpectedly showed activity comparable with IIa, while substitution with one or more OH (**27–29**), Me (**32–34**, **42**), OMe (**36**, **37**, **43**), or OEt (**39**) groups also enabled retention of strong antimalarial effects. However, when the

Table IX. Therapeutic Effects of 2,4-Diamino-6-[(aralkyl)amino]quinazolines (ArCHR'NHR)[i] against *Plasmodium cynomolgi* in Rhesus Monkeys

No.	ArCHR'	Formula	Dose, mg/kg per day[a]	Days	No. of monkeys	Negativity for asexual stages after first dose: Days required	Days remained
	 X, Y						
13	2,4-Cl_2	$C_{15}H_{13}Cl_2N_5$	100	5	1	4	11
14	2,4-Cl_2	$C_{15}H_{13}Cl_2N_5 \cdot C_2H_4O_2 \cdot 2H_2O$	100	5	2	5	>20
15	3,4-Cl_2	$C_{15}H_{13}Cl_2N_5$	100	5	7	2–4	9–17
			50	10	2	1, 2	>40[b]
			25	5	4	3–>23	0–9
18	4-Br	$C_{15}H_{14}BrN_5$	100	5	2	4	12
19	2-Cl	$C_{15}H_{14}ClN_5 \cdot 0.33H_2O$	100	5	2	3, 4	>46, >66[c]
20	3-Cl	$C_{15}H_{14}ClN_5$	100	5	2	3	>52[d]
21	4-Cl	$C_{15}H_{14}ClN_5$	100	5	2	4, 5	>21, 15
22	4-F	$C_{15}H_{14}FN_5$	100	5	2	4	15
24	H	$C_{15}H_{15}N_5$	100	5	4	6–8	3–>78[e]
			50	10	2	5	>44[f]
			25	5	4	4–>37	0–15
25	H	$C_{15}H_{15}N_5 \cdot HCl \cdot 0.25H_2O$	100	5	1	5	>20
26	H	$C_{15}H_{15}N_5 \cdot CH_2O_2 \cdot H_2O$	100	5	2	4	18, >21
32	2-CH_3	$C_{16}H_{17}N_5$	100	5	2	4	>45[g]
33	3-CH_3	$C_{16}H_{17}N_5$	100	5	2	3, 4	>48, 12
34	4-CH_3	$C_{16}H_{17}N_5$	100	5	1	4	>21
36	3-OCH_3	$C_{16}H_{17}N_5O$	100	5	2	4	12, 29
37	4-OCH_3	$C_{16}H_{17}N_5O$	100	5	2	4, 5	>21, >20
39	4-OC_2H_5	$C_{17}H_{19}NO$	100	5	2	3, 4	>46, >45
69	$C_6H_5CH(CH_3)$–	$C_{16}H_{17}N_3 \cdot 0.5H_2O$	100	5	2	4	>45[g]
71		$C_{19}H_{17}N_5$	100	5	2	4	>45, >49[h]
	Quinine hydrochloride		50	5	2	3	10–>27
			25	5	2	5	1–2
	Controls				10	>21	0

[a]Drugs given orally twice daily in 0.1% Tween 80 in 1% (hydroxyethyl)cellulose. [b]Apparently cured: subinoculation on day 68 negative; rechallenge on day 76 produced acute infection. [c]Apparently cured; failed to become positive 14 days after splenectomy on days 34 and 53, respectively. [d]Apparently cured, failed to become positive 14 days after splenectomy on day 42. [e]One monkey apparently cured: subinoculation on day 68 negative; rechallenge on day 81 produced acute infection. [f]Apparently cured: subinoculations on day 68 negative; rechallenge on day 81 produced acute infections. [g]Apparently cured; failed to become positive 14 days after splenectomy on day 34 or 35. [h]Apparently cured; failed to become positive in 14 days after splenectomy on days 34 and 42. [i]R is 2,4-diamino-6-quinazolinyl (Table I).

benzyl function was substituted with NO_2 (**23**), NH_2 (**30**), CO_2H (**31**), CH_2OH (**35**), NHCOMe (**38**), NMe_2 (**40**), or CO_2Et (**41**), activity was reduced or abolished (Table II).

(2) 5- or 7-Quinazoline Substitution. The introduction of Cl or Me functions at position 5 leads to retention or enhancement of antimalarial potency (**44–47**, Table III). 2,4-Diamino-5-chloro-6-[(3,4-dichlorobenzyl)amino]quinazoline (**44**), the most active member of the group, was 110 times as potent (SD_{90} = 0.7 mg/kg per day) as quinine hydrochloride and 12 times as potent as the deschloro analog **15**. This effect is of particular interest, since the corresponding 5 position of tetrahydrofolic acid (FAH_4) is involved in key transformations within the folic acid interconversion cycle.[17] In contradistinction, substitution of the quinazoline ring at position 7 with Me (**48**, Table III) caused a marked diminution of antimalarial effects.

(3) Alkyl or Aryl Substitution at N^2 and N^4. Antimalarial potency was also diminished when the amino groups at positions 2 and 4 were substituted with one or more alkyl or aryl groups (**49–53**, Table IV). This phenomenon is in accord with earlier reports that the replacement of one or both primary amino groups of the 2,4-diaminopyrimidine antimetabolites by secondary or tertiary amino groups leads to a diminution of antimalarial potency,[16,18,19] but is at variance with a more recent report that 4-amino-5-(3,4-dichlorophenyl)-2-(dimethylamino)pyrimidine is more active against *P. berghei* in mice than the corresponding 2,4-diaminopyrimidine.[20]

(4) 6-[(Heterocyclic)methyl]amino Functions. 2,4-Diamino-6-[(furfuryl)amino]quinazoline (**55**) and 2,4-diamino-6-[(2-thenyl)amino]quinazoline (**56**) (Table V) were both less potent than IIa or the unsubstituted benzyl derivs **24–26**. However, 2,4-diamino-6-[(5-chloro-2-thenyl)amino]-quinazoline (**54**) showed activity comparable with IIa. In contradistinction, unsubstituted 2-furyl and 2-thienyl analogs of pyrimethamine lacked appreciable antimalarial effects in mice, although a dibromo-2-thienyl analog was highly active.[16,21] Neither 2,4-diamino-6-[(2-pyridylmethyl)-amino]quinazoline (**57**) or 2,4-diamino-6-[(4-pyridylmethyl)-amino]quinazoline (**58**) exhibited significant antimalarial effects at the dose levels tested.

(5) α-Substituted Benzyl Moieties. α-Methyl substitution on benzyl maintained or enhanced antimalarial effects in mice (**66, 68, 69**) (Table VII). Once again it is noteworthy that the α-methyl group occupies a position in the quinazoline molecule which corresponds to key substituents in various FAH_4 coenzymes operating within the folic acid interconversion cycle.[17]

(6) Naphthylmethyl Analogs. Both of the naphthylmethyl analogs studied, namely 2,4-diamino-6-{[(2-chloro-

1-naphthyl)methyl]amino}quinazoline (**70**) and 2,4-diamino-6-{[(2-naphthyl)methyl]amino}quinazoline (**71**) (Table VII), displayed antimalarial activity comparable with IIa, indicating that a naphthylmethyl moiety can substitute effectively for benzyl. By contrast, replacement of phenyl by 1-naphthyl in the pyrimethamine series abolished antimalarial effects.[16]

(7) Miscellaneous Structural Variations. 4-Amino-6-[(benzyl)amino]-2-methylquinazoline (**74**) and 2-amino-6-[(benzyl)amino]-4-quinazolinol (**73**) were devoid of antimalarial effects (Table VIII). These observations parallel results in the cycloguanil and pyrimethamine series,[19] and lend further support to earlier conclusions that a six-membered ring incorporating the sequence $N=C(NH_2)N=C(NH_2)$ plays a key role in conferring optimal antiplasmodial effects among folic acid antagonists.[16,20] Antimalarial activity was also abolished when the benzylamino function was moved to positions 7 or 8 of the quinazoline ring (**72, 75**, Table VIII), or when two quinazoline moieties were joined together at position 6 as in 6,6′-[*p*-phenylenebis(methyleneimino)]bis(2,4-diaminoquinazoline) (**76**) or 6,6′-[ethylenebis(oxy-*p*-phenylenemethyleneimino)]bis(2,4-diaminoquinazoline) (**77**) (Table VIII).

Antimalarial Activity in the Monkey. Nineteen 2,4-diamino-6-[(aralkyl)amino]quinazolines (**13–15, 18–22, 24–26, 32–34, 36, 37, 39, 69**, and **71**) were evaluated against *P. cynomolgi* in rhesus monkeys[5] and the results are summarized in Table IX. The drugs were administered orally to infected monkeys twice daily for 5 or 10 days at doses ranging from 25 to 100 mg/kg per day. At daily doses of 50 or 100 mg/kg for 5 or 10 days, each of the compds eliminated asexual parasites within 1–8 days, and seven compds (**15, 19, 20, 24, 32, 69, 71**) were apparently curative as indicated by subinoculation or splenectomy.

Suppressive Effects against Drug-Resistant Lines of *P. berghei*. In the early stages of this investigation, 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (IIa, **15**) was selected for evaluation against representative drug-resistant lines of *P. berghei* in the mouse to determine whether or not the 2,4,6-triaminoquinazolines represented a unique chemical class with respect to apparent mode of action. In the initial studies **15** was administered sc twice daily for 4 days to mice infected with the parent (P) line or with the T, S, and C lines when they were approximately 30-fold resistant, respectively, to cycloguanil hydrochloride (T), 4,4′-sulfonyldianiline (DDS) (S), and chloroquine (C).[5] The SD_{70} (daily dose required for 70% suppression of the parasitemia) for each line was as follows: line P, 6.0 mg/kg; line T, 4.0 mg/kg; line S, 17.8 mg/kg; line C, 3.2 mg/kg. These results indicated that the T and C lines were fully susceptible and possibly hypersensitive to **15**, but that the S line was approximately 3-fold cross-resistant to it. A subsequent experiment was done utilizing the T and PYR lines when they were >300-fold resistant, respectively, to cycloguanil hydrochloride and pyrimethamine.[5] In this study, compd **15** was given orally to mice by drug-diet for 6 days. The SD_{90} was estimated to be 23.5 mg/kg per day for line T, 8.0 mg/kg per day for line PYR, and 11.5 mg/kg per day for line P. These results indicate that the T line had become approximately 2-fold cross-resistant to **15**, while the PYR line was possibly hypersensitive to it.

The remarkable effects of **15** against these four drug-resistant lines of *P. berghei* suggest that the principal mode of action of **15** and the other triaminoquinazolines may be different from that of cycloguanil hydrochloride, pyrimethamine, DDS, or chloroquine, and stimulated extensive investigations concerning the synthesis and biological properties of related antimetabolites. Accounts of these studies will be set forth in subsequent communications.

Antitrypanosomal Evaluation. Test Procedures. A Brazilian strain of *T. cruzi* was utilized for studies both in culture[5,22] and in mice.[5] The methods of Bayles, *et al.*,[22] were used for studies in chick embryo cell (CEC) cultures. The CEC system provides good conditions for *T. cruzi* to grow extracellularly, invade cells, and multiply intracellularly. Thus the effects of drugs on extracellular growth, cell invasion, intracellular growth, and chick embryo cell toxicity can be assessed concurrently. CEC control data were included to depict the situation prior to and after drug-incubation periods.

Mice were infected by the ip administration of (5–25) × 10^4 *T. cruzi* collected from mice with acute infections.[5] The effects of treatment were assessed from mortality records, the mean survival time, parasitemic counts on day 12 of treatment, and parasite examinations 14 days after the completion of treatment.[5]

Effects against *T. cruzi* in Cultures. Thirty-seven triaminoquinazolines (**13, 14, 18–34, 36–43, 46–48, 55, 57, 58, 69, 71, 74, 76, 77**) were tested in CEC cultures, and antitrypanosomal activity was widespread throughout the series. When incubated for periods of either 48 or 72 hr, compds **13, 14, 18, 19, 21, 22, 24–28, 30, 32–34, 36, 37, 39, 41, 42, 46–48, 55, 69, 71, 74**, and **76** were active against *T. cruzi* at one or more drug concns, ranging from 0.39 to 6.25 μg/ml, that were usually not cytotoxic for chick embryo cells. This activity was reflected by inhibition of extracellular growth, of cell invasion, and of intracellular multiplication. However, none was more potent than IIa reported previously.[5]

Effects against *T. cruzi* in Mice. Thirty-five compds (**13, 14, 18, 19, 21, 22, 24–27, 30, 32–37, 39, 41–53, 55, 56, 69, 74**, and **77**) were administered continuously in the diet of mice infected with *T. cruzi* for 14 days. Only 5 substances (**18, 33, 39, 42, 44**) exhibited significant activity. These drugs increased the mean survival time of treated mice for periods ranging from >5 to >25 days at drug-diet levels of 0.0313–0.125%, but did not effect radical cures. None was more promising in mice than IIa.[5]

Antimetabolite Studies. Background, Materials, and Methods. In the hope that antimetabolite studies utilizing bacterial systems might aid in clarifying the biochemical role of the 2,4,6-triaminoquinazolines and help explain the divergent antiparasitic actions exhibited by these and other small molecular folate antagonists, the inhibitory effects of the quinazolines, pyrimethamine, trimethoprim, cycloguanil hydrochloride, aminopterin, and methotrexate on *Streptococcus faecalis* R, *Strep. faecalis* A, *Lactobacillus plantarum*, and *Pediococcus cerevisiae* were examined.

Strep. faecalis R (*Strep. faecium* var. *durans*, ATCC 8043) is capable of using the fully oxidized form of folate (FA, PGA, pteroylglutamic acid) as well as the various reduced forms. Part of the value of this organism lies in its inability to synthesize folate[23] and its consequent requirement for preformed folate. This allows precise knowledge of the amount of folate available to the organism. Using the minimal medium described in the Experimental Section, the smallest amount of FA allowing complete growth of the organism is 0.4 ng/ml of final strength medium. Inhibition using this substrate reveals the overall strength of the inhibitor without providing information regarding the nature of the inhibition.

N^5-Formyltetrahydrofolic acid (leucovorin, 5-CHO-FAH_4),

a stable, reduced form of folate, provides some information as to the nature of the inhibition. The min concn for good growth using the active L form is also 0.4 ng/ml of final strength medium. Success in overcoming inhibition using this substrate may indicate that the inhibition involves only the reduction of folate, or that the inhibitor blocks the entry of FA into the cell but permits entry of 5-CHO-FAH_4. Failure of the reduced folate to completely reverse the inhibition may mean (a) that the inhibitor is acting on a site other than reductase, which could be further along the folate pathway or unrelated to folate, or (b) that the inhibitor is blocking transport enzymes necessary for the entry of 5-CHO-FAH_4 into the cell. Whichever is true, the half-inhibition figure in the presence of 5-CHO-FAH_4 would indicate the inhibitor concn necessary for this effect to take place.

Finally, a substrate containing 0.4 ng/ml of 5-CHO-FAH_4 together with 10 μg/ml of final strength medium of both adenosine and thymidine was employed. When this concn of both a purine and thymidine are present, *Strep. faecalis* R will grow in the absence of folate. While the requirement for thymidine is specific, we observed that xanthine or guanosine can be used interchangeably with adenosine. Since this combination replaces folate here, inhibition in its presence may indicate that (a) the inhibition lies outside the folic acid cycle or (b) the inhibitor is preventing the uptake or use of the purine or pyrimidine or both.

Strep. faecalis A is a mutant of *Strep. faecalis* R that is resistant to methotrexate and aminopterin.[24] Unfortunately, the FA concn used routinely (500 ng/ml of final strength medium) is considerably higher than the minimal requirement. However, since all of the compds were tested at the same level, it does provide a basis for comparison. With both aminopterin and methotrexate, the *Strep. faecalis* A to *Strep. faecalis* R inhibition ratio is quite high. It seems likely that other inhibitors with a low ratio might be effective against organisms that have become resistant to these two antifolates, while inhibitors with a high ratio might be cross-resistant with them.

Data for *L. plantarum* (ATCC 8014) were included to detect inhibitors acting on the synthesis of folate.

Ped. cerevisiae (ATCC 8081) required 0.4 ng/ml of final strength medium of the L form of 5-CHO-FAH_4.[25] This organism does not do well using an oxidized form of folate.[26] Therefore, it is likely that inhibition of this organism does not involve folate reductase inhibition. It may represent the blockade of a transport mechanism or inhibition at subsequent stages of the folate cycle.

Antimetabolite Effects of Reference Drugs. Pyrimethamine, trimethoprim, cycloguanil hydrochloride, aminopterin, and methotrexate are all highly active against *Strep. faecalis* R utilizing FA as the substrate (Table X). However, the inhibitory effects of pyrimethamine and cycloguanil hydrochloride are strongly reversed by 5-CHO-FAH_4 (Table X), indicating that the major inhibition occurs at the reductase stage. When FAH_2 was used as the substrate, the half-inhibition figures were 8200 and 16,200 ng/ml, re-

Table X. Inhibitory Effects of 2,4-Diamino-6-[(benzyl)amino]quinazolines against *Strep. faecalis* R, *L. plantarum*, and *Strep. faecalis* A ($XC_6H_4CH_2NHR$)[e]

		Concns (ng/ml) causing 50% inhibition				
		Strep. faecalis R			*L. plantarum*	*Strep. faecalis* A
No.	X	FA[a]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[c]	None	FA[d]
13	2,4-Cl_2	10	150		1,400	290
15	3,4-Cl_2	6	112	2,400	550	294
17	3-Br	8	190		770	284
18	4-Br	8	180	5,300	1,300	1,360
19	2-Cl	6	80	5,600	1,340	590
20	3-Cl	24	108		1,140	600
21	4-Cl	10	>400		1,400	580
22	4-F	7	140	13,500	1,540	126
23	2-NO_2	24	114		2,430	600
24	H	13	156	2,900	1,530	530
27	2-OH	96	266		14,000	2,740
28	3-OH	260	680		8,600	1,460
29	4-OH	600	5,000		>40,000	30,200
30	2-NH_2	31	74	>40,000	740	1,320
31	4-COOH	18	3,400	>40,000	25,000	3,600
32	2-CH_3	8	125	13,400	1,800	288
33	3-CH_3	13	222	13,400	2,400	1,300
34	4-CH_3	10	400	5,600	1,400	3,800
35	4-CH_2OH	320	1,680		2,780	5,600
36	3-OCH_3	20	232	27,600	1,900	560
37	4-OCH_3	46	700	24,600	5,000	1,360
38	4-$NHCOCH_3$	260	2,360	>40,000	>40,000	580
39	4-OC_2H_5	46	580	13,600	4,800	1,300
40	4-$N(CH_3)_2$	1,000	19,000		>40,000	37,600
41	4-$COOC_2H_5$	26	>400		34,000	2,560
42	2,4,6-$(CH_3)_3$	70	2,100		800	8,800
43	3,4,5-$(OCH_3)_3$	60	1,500		>40,000	5,100
Pyrimethamine		4	3,100		590	680
Trimethoprim		12	70	>40,000	74	284
Cycloguanil hydrochloride		8	11,400	>400,000	480	560
Aminopterin		2	4	>40,000		>40,000
Methotrexate		0.2	0.6	>40,000	3	3,800

[a] 0.4 ng/ml of FA. [b] 0.4 ng/ml of 5-CHO-FAH_4. [c] 0.4 ng/ml of 5-CHO-FAH_4 + 10 μg/ml of adenosine + 10 μg/ml of thymidine. [d] 500 ng/ml of FA. [e] R is 2,4-diamino-6-quinazolinyl (Table I).

Table XI. Inhibitory Effects of 2,4-Diamino-6-[(benzyl)amino]-(5 or 7)-substituted-quinazolines against *Strep. faecalis* R, *L. plantarum*, *Strep. faecalis* A, and *Ped. cerevisiae* ($XC_6H_4CH_2NH(5\text{-}Y, 7\text{-}Z\text{-}R)$)[k]

						Concns (ng/ml) causing 50% inhibition							
						Strep. faecalis R			*L. plantarum*			*Strep. faecalis* A	*Ped. cerevisiae*
No.	X	Y	Z	FA[a]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[c]	None	Thiamine[d]	PABA[e]	Adenosine + thymidine[f]	FA[g]	5-CHO-FAH_4 + adenosine + thymidine[h]	5-CHO-FAH_4[i]	5-CHO-FAH_4 + adenosine + thymidine[j]
44	3,4-Cl_2	Cl	H	1	3		14	14	16	71	55	1020		
45	H	Cl	H	2	5		31				66		18	>4,000
46	3,4-Cl_2	CH_3	H	2	3	1,320	44				138			
47	H	CH_3	H	5	17	12,600	82				58			
48	H	H	CH_3	44	>400	29,400	2,400				28,500			

[a-c]See footnotes *a-c*, Table X. [d]1.0 μg/ml of thiamine. [e]1.0 μg/ml of *p*-aminobenzoic acid. [f]1.0 μg/ml each of adenosine and thymidine. [g]500 ng/ml of FA. [h]500 ng/ml of 5-CHO-FAH_4 + 1.0 μg of adenosine + 1.0 μg/ml of thymidine. [i]0.4 ng/ml of 5-CHO-FAH_4. [j]0.4 ng/ml of 5-CHO-FAH_4 + 1.0 μg/ml of adenosine + 1.0 μg/ml of thymidine. [k]R is 2,4-diamino-6-quinazolinyl (Table I).

spectively, for the two inhibitors, suggesting that perhaps major inhibition takes place during the first reduction step and that there is relatively little inhibition in the interconversion cycle.

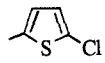
In contradistinction, the inhibitory effects of trimethoprim on *Strep. faecalis* R are only weakly reversed by 5-CHO-FAH_4 (Table X). Aminopterin and methotrexate also show minimal reversal. The former result raises the distinct possibility that trimethoprim acts either on the folate transport mechanism or elsewhere in the folate cycle and suggests that the antimalarials pyrimethamine and cycloguanil hydrochloride on the one hand and trimethoprim on the other may represent different types of folate inhibitors in that the latter appears to produce an inhibitory effect in addition to its activity against folate reductase. The inhibitory effects of cycloguanil hydrochloride, trimethoprim, aminopterin, and methotrexate are totally reversed by the combination of 5-CHO-FAH_4, adenosine, and thymidine (Table X), indicating that they have little if any activity outside of the folate cycle in *Strep. faecalis* R.

Methotrexate is a very potent inhibitor of *L. plantarum*, while pyrimethamine, trimethoprim, and cycloguanil hydrochloride are considerably less active (Table X). Pyrimethamine, trimethoprim, and cycloguanil hydrochloride also possess relatively good activity against the aminopterin- and methotrexate-resistant mutant *Strep. faecalis* A (Table X).

Inhibitory Effects of Triaminoquinazolines against *Strep. faecalis* R. A majority of the 2,4-diamino-6-[(benzyl)amino]-quinazolines and their 5-substituted analogs, 2,4-diamino-6-{[(heterocyclic)methyl]amino}quinazolines, and other 2,4-diamino-6-[(aralkyl)amino]quinazolines exhibit moderate to strong inhibitory effects against *Strep. faecalis* R utilizing FA as the substrate (Tables X-XIII). These substances inhibit one or both reduction stages and are competitive with FA and 5-CHO-FAH_4. Seventeen compds (**13**, **15**, **17-19**, **21**, **22**, **24**, **32-34**, **44-47**, **54**, **69**) caused 50% inhibition at concns of 1-13 ng/ml, and thus exhibited activity comparable with or superior to pyrimethamine, trimethoprim, cycloguanil hydrochloride, and aminopterin (50% inhibition at 2-12 ng/ml). Structural features of this group include halobenzyl (**13**, **15**, **17-19**, **21**, **22**), unsubstituted benzyl (**24**), methylbenzyl (**32-34**), 5-substituted quinazolinyl (**44-47**), 5-chloro-2-thenyl (**54**), and α-methylbenzyl (**69**) functions, each of which also confers potent antimalarial effects (*vide supra*). In general, activity against *Strep. faecalis* R was only partly reversed by 5-CHO-FAH_4 (Tables X-XIII). This suggests that these quinazolines, like trimethoprim, function not only as reductase inhibitors, but also have significant effects either on the folate transport mechanism or elsewhere in the folate cycle. It is interesting to speculate that the known antimalarial effects of the 2,4,6-triaminoquinazolines, trimethoprim, and certain allied pyrimidines and triazines against cycloguanil- and pyrimethamine-resistant plasmodia may be related to this phenomenon. Moreover, in contradistinction with trimethoprim and cycloguanil hydrochloride, compds **15**, **18**, **19**, **22**, **24**, **32-34**, **36**, **37**, **39**, **46**, **47**, **48**, and **71** retain some inhibitory effects against *Strep. faecalis* R even in the presence of 5-CHO-FAH_4, adenosine, and thymidine (Tables X, XI, XIII), which suggests that they may also exert some activity outside the folate cycle.

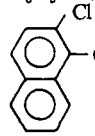
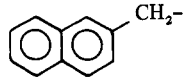
Although there was a good correlation between antimalarial and antibacterial activity among the most potent inhibitors of *Strep. faecalis* R (*vide supra*), correlations were erratic among the less potent inhibitors. For example,

Table XII. Inhibitory Effects of 2,4-Diamino-6-{[(heterocyclic)methyl]amino}quinazolines against *Strep. faecalis* R, *L. plantarum*, and *Strep. faecalis* A, (Het-CH_2NHR)[e]

		Concns (ng/ml) causing 50% inhibition				
		Strep. faecalis R			*L. plantarum*	*Strep. faecalis* A
No.	Het	FA[a]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[c]	None	FA[d]
54		10	124		700	292
55		40	310	>40,000	4000	1200
56		17	94		2400	620
57		33	540	>40,000	3280	1460
58		130	1160	>40,000		6000

[a-e] See footnotes *a-e*, Table X.

Table XIII. Inhibitory Effects of Other 2,4-Diamino-6-[(aralkyl)amino]quinazolines against *Strep. faecalis* R, *L. plantarum*, *Strep. faecalis A*, and *Ped. cerevisiae* (Ar-A-NHR)[f]

		Concns (ng/ml) causing 50% inhibition						
		Strep. faecalis R			*L. plantarum*	*Strep. faecalis* A	*Ped. cerevisiae*	
No.	Ar-A-	FA[a]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[c]	None	FA[d]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[e]
67	Cl-1′-C_6H_4-4′-$(CH_2)_2$-	32	400		1360	1240		
69	$C_6H_5CH(CH_3)$-	6	76		1200	620		
70		<400			760	1480		
71		20	340	2600	2600	1200	540	1310

[a-d] See footnotes *a-d*, Table X. [e] 0.4 ng/ml of 5-CHO-FAH_4 + 1.0 μg/ml of adenosine + 1.0 μg/ml of thymidine. [f] R is 2,4-diamino-6-quinazolinyl (Table I).

compds **23** (2-NO_2), **30** (2-NH_2), **31** (4-COOH), and **41** (4-$COOC_2H_5$) displayed good activity against *Strep. faecalis* R but lacked appreciable antimalarial effects, while compds **27-29** (2-, 3-, and 4-OH) and **38** (4-$NHCOCH_3$) possessed good antimalarial activity but were relatively poor inhibitors of *Strep. faecalis* R (Table X).

Other structural modifications caused a diminution of both antimalarial activity and inhibitory effects against *Strep. faecalis* R. These included substitution of benzyl with 4-CH_2OH (**35**) or 4-NMe_2 (**40**), insertion of Me at position 7 of the quinazoline nucleus (**48**), alkyl or aryl substitution at N^2 or N^4 (**49-53**) (Table XIV), substitution of 2- or 4-pyridylmethyl for benzyl (**57, 58**), replacement of 2-NH_2 by Me (**74**) or of 4-NH_2 by OH (**73**), linking two quinazoline moieties through the 6-amino function (**76, 77**), or moving the benzylamino function to position 8 (**75**) (Table XV).

Although each of the diaminoquinazolines that showed activity against *T. cruzi* in CEC cultures and in mice also produced strong to weak inhibitory effects against *Strep. faecalis* R, no quantitive relationships are apparent. It is concluded that the inhibitory potency of the diaminoquinazolines against *Strep. faecalis* R is not a reliable indicator of relative activity of these compounds against *T. cruzi*.

Inhibitory Effects of Triaminoquinazolines against *L. plantarum*, *Ped. cerevisiae*, and *Strep. faecalis* A. Eight compds (**15, 44-47, 51, 76**, and **77**) produced 50% inhibition of *L. plantarum* at concns ranging from 14 to 550 ng/ml and thus showed activity comparable with pyrimethamine, trimethoprim, and cycloguanil hydrochloride (Tables X, XI, XIV, XV). However, none was as potent as methotrexate (50% inhibition at 3 ng/ml). Attempts to reverse the inhibitory effects of **44** against *L. plantarum* with thiamine, PABA, or adenosine plus thymidine were unsuccessful. *L. plantarum* inhibition does not afford a reliable basis for predicting the relative magnitude of antimalarial or antitrypanosomal effects in this series.

Against the methotrexate-aminopterin-resistant *Strep. faecalis* A, ten 2,4,6-triaminoquinazolines (**13, 15, 17, 22, 32, 44-47, 54**) caused 50% inhibition at concns of 55-294 ng/ml utilizing 500 ng/ml of FA as the substrate (Tables X, XI, XII). These inhibitory concns are equal to or less than that required for trimethoprim (284 ng/ml). The *Strep. faecalis* A to *Strep. faecalis* R inhibition ratios (12-69) for these and many of the other quinazolines are relatively low compared with those observed for aminopterin (>20,000) or methotrexate (19,000). This indicates that there is relatively little cross-resistance between these compds and

Table XIV. Inhibitory Effects of N^2,N^4-(Alkyl and aryl)-2,4-diamino-6-[(benzyl)amino]quinazolines against *Strep. faecalis* R, *L. plantarum*, and *Strep. faecalis* A ($SC_6H_4CH_2NH(2\text{-}R_1R_2, 4\text{-}R_3R_4\text{-}R)$)[e]

				Concns (ng/ml) causing 50% inhibition				
				Strep. faecalis R				
No.	X	NR_1R_2	NR_3R_4	FA[a]	5-CHO FAH_4[b]	5-CHO-FAH_4 + adenosine, thymidine, other substrates[c]	*L. plantarum* None	*Strep. faecalis* A FA[d]
49	H	$N(CH_3)_2$	NH_2	3200				
50	3,4-Cl_2	$N(CH_3)_2$	$N(CH_3)_2$	5200		5800	2700	5600
51	3,4-Cl_2	$NH(CH_2)_3CH_3$	$NH(CH_2)_3CH_3$	1000	1000		280	1400
52	3,4-Cl_2	$N(C_2H_5)_2$	$N(C_2H_5)_2$	2550	1000		1300	2900
53	3,4-Cl_2	NHC_6H_5	NHC_6H_5	3550			2680	700

[a] 0.4 ng/ml of FA. [b] 0.4 ng/ml of 5-CHO-FAH_4. [c] 0.4 ng/ml of 5-CHO-FAH_4 + 1.0 μg/ml of the following: pantothenic acid, nicotinic acid, pyridoxal HCl, biotin, riboflavin, *p*-aminobenzoic acid, adenosine, and thymidine. [d] 500 ng/ml of FA. [e] R is 2,4-diamino-6-quinazolinyl (Table I).

Table XV. Inhibitory Effects of Miscellaneous 2 and 4-Amino-(6, 7, or 8)-[(benzyl)amino]quinazolines (Ar-A-NHR)[e] against *Strep. faecalis* R, *L. plantarum*, and *Strep. faecalis* A

		Concns (ng/ml) causing 50% inhibition				
		Strep. faecalis R				
No.	Ar-A-NH	FA[a]	5-CHO-FAH_4[b]	5-CHO-FAH_4 + adenosine + thymidine[c]	*L. plantarum* None	*Strep. faecalis* A FA[d]
73	$C_6H_5CH_2NH$-(6)[f]	8000		>40,000		
74	$C_6H_5CH_2NH$-(6)[g]	2920	36,600	>40,000	28,000	>40,000
75	$C_6H_5CH_2NH$-(8)[h]	280	>4,000		3,060	6,000
76	(6)-$NHCH_2$-1′-C_6H_4-4′-CH_2NH-(6)	210	1,080		550	1,280
77	[-4′-$CH_2OC_6H_4CH_2NH$-(6)]$_2$	200	1,510		315	540

[a-d] See footnotes *a-d*, Table X. [e] R is 2- or 4-amino-(6, 7, or 8)-quinazolinyl (Table I). [f] 4-Hydroxy. [g] 2-Methyl. [h] 5-Methyl.

aminopterin or methotrexate utilizing *Strep. faecalis* (Tables X-XV). The inhibitory action of **44** against *Strep. faecalis* A was reversed fairly successfully by 5-CHO-FAH_4 plus adenosine plus thymidine (Table XI).

Two quinazolines (**45**, **71**) were tested against *Ped. cerevisiae* utilizing 5-CHO-FAH_4 as the substrate and each was active (Tables XI, XIII). Compd **45** produced 50% inhibition at 18 ng/ml, and this inhibition was completely reversed by adenosine plus thymidine. Although **71** was a less potent inhibitor of *Ped. cerevisiae* (50% inhibition at 540 ng/ml), the inhibitory effects were only partially reversed by adenosine plus thymidine.

Toxicological Properties. 2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (**15**) suspended in aqueous 1% (hydroxyethyl)cellulose was administered to groups of 5 mice in single oral doses of 25, 50, 100, 200, and 400 mg/kg and sc doses of 100, 200, 400, and 800 mg/kg. The mice were weighed daily for 17 days beginning on the day of dosing. The local reaction at the site of injection was also assessed daily in the mice dosed sc. Oral doses of 400, 200, or 100 mg/kg produced weight loss, with severity and duration proportional to the dose. Oral doses of 50 and 25 mg/kg did not result in weight loss. Subcutaneous doses all produced local irritation at the injection site, with intensity being dose related. Weight loss occurred in all mice dosed sc with degree and duration generally proportional to the dose. No animals died in any of the groups during the 16-day posttreatment observation period.

In a subacute oral rising dose tolerance study in one dog, **15** was administered continuously for 26 days on a schedule of 50 mg/kg for 7 days, 100 mg/kg for 7 days, 200 mg/kg for 7 days, and 400 mg/kg for 5 days. The clinical picture of diarrhea, vomiting, anorexia, and weight loss which occurred at dosage levels of 100 mg/kg and higher was not accompanied by significant biochemical, hematologic, or urinary changes. The lack of striking laboratory changes was paralleled by an absence of significant histological findings. There was no evidence of folic acid antagonism during the course of dosing or terminally.

It should also be noted that none of the infected rhesus monkeys treated therapeutically with **15** at doses of 25, 50, or 100 mg/kg daily for 5 or 10 days (Table IX) exhibited gross evidence of drug intolerance. All animals maintained their weights well, ate well, had formed stools, and appeared normal.

Experimental Section §,#

Antimetabolite Studies. The assay medium representing minimal requirements for good growth of *Strep. faecalis* R (*Strep. faecium* var. *durans*,[27,28] ATCC 8043) and *Strep. faecalis* A contained the following per 100 ml at 2.5 times final strength: Difco casamino acids (treated with 10% Darco G-60 at pH 3.5), 1.25 g; dextrose, 1.9 g; NaOAc, 1.25 g; DL-tryptophan, 0.75 mg; L-cysteine, 25 mg; asparagine, 62.5 mg; KH_2PO_4, 125 mg; K_2HPO_4, 125 mg; $MgSO_4 \cdot 7H_2O$, 50 mg; $FeSO_4 \cdot 7H_2O$, 2.5 mg; $MnSO_4 \cdot H_2O$, 2.25 mg; NaCl, 2.5 mg; pantothenic acid, 5 μg; nicotinic acid, 50 μg; pyridoxal hydrochloride, 1.25 μg; biotin, 25 ng; folate form and concn as indicated in footnotes, Tables X-XV.

L. plantarum (ATCC 8014) assay medium was the same as above with the following exceptions: pantothenic acid, 6.25 μg; nicotinic acid, 20 μg; biotin, 62.5 ng; *p*-aminobenzoic acid, 250 μg; folate, none; pyridoxal hydrochloride, none.

Assay procedures for *Strep. faecalis* R, *Strep. faecalis* A, and *L. plantarum* were the same as those outlined by Capps, *et al.*,[23] except that stock cultures of *L. plantarum* were maintained in Difco Micro Assay Agar stabs.

The assay procedure and medium for *Ped. cerevisiae* (ATCC 8081) were the same as described by Bird, *et al.*[25]

2,4-Diamino-6-[(benzylidene)amino]quinazolines (1-12, Table I) and Other Schiff Bases Derived from 2,4,(6, 7, or 8)-triaminoquin-

§ Melting points (uncorrected) were taken in open capillary tubes in a Townson and Mercer melting point apparatus.

Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

azolines (59–65, Table VI). Isolation of these intermediate Schiff bases in pure form was not usually necessary. However, the properties of some representative compds that were purified and characterized are summarized in Tables I and VI.

Method A. The requisite triaminoquinazoline and aldehyde (0.1 mole of each) in 150 ml of warm DMF were treated with 150 ml of C_6H_6, and the mixt was refluxed under a H_2O separator until no more H_2O was evolved (1–5 hr). The soln was cooled and concd, and the product collected.

Methods B, C. The reactants as in A were boiled under reflux in 200 ml of $C_2H_5O(CH_2)_2OH$ for 3 hr. The soln was used directly (B), or the product was collected after cooling (concg and adding H_2O or EtOH if necessary) (C).

Methods D, E. The reactants as in A were refluxed in 200 ml of EtOH for 3–18 hr, and the soln was used directly (D) or the product was collected after cooling (E).

Method F. 2,4,6-Triamino-5-chloroquinazoline[2] (0.025 mole) and 3,4-dichlorobenzaldehyde (0.05 mole) were stirred in 250 ml of refluxing $C_2H_5O(CH_2)_2OH$ for 20 hr, and the crude product (73%) was collected after cooling.

Method G. 2,4,6-Triamino-5-chloroquinazoline[2] (0.02 mole) and benzaldehyde (0.04 mole) were refluxed in 225 ml of *n*-PrOH for 5 days, and the crude product (41%) was collected after evapg the mixt to 25 ml and cooling to 0°.

Method H. A soln of 3.5 g (0.02 mole) of 2,4,6-triaminoquinazoline[2] in 65 ml of hot $C_2H_5O(CH_2)_2OH$ was added in a thin stream to a stirred soln of 4.0 g (0.03 mole) of terephthalaldehyde in 65 ml of $C_2H_5O(CH_2)_2OH$ at 50°. After standing 3 hr at room temp, the soln was evapd to 25 ml, and the desired α-[(2,4-diamino-6-quinazolinyl)imino]-*p*-tolualdehyde (**8**, Table I) (4.1 g) was collected as an orange powder which could not be recrystd.

Method I. 2,4,6-Triaminoquinazoline[2] (3.5 g, 0.02 mole) and terephthalaldehyde or 4,4¹-(ethylenedioxy)dibenzaldehyde[11] (0.01 mole) were refluxed in 40 ml of $C_2H_5O(CH_2)_2OH$ for 2 hr, and the products (**64**, **65**, Table VI) were collected after cooling.

Method J. 2,4,6-Triaminoquinazoline[2] (8.0 g, 0.046 mole), 2-chloro-1-naphthaldehyde (8.8 g, 0.046 mole), and 120 ml of AcOH were stirred and heated at 110° for 3 hr. Upon cooling, the product was collected, washed with cold AcOH, and slurried in ice and H_2O. Recrystn from $CH_3O(CH_2)_2OH$ gave 6.0 g (38%) of 2,4-diamino-6-{[(2-chloro-1-naphthyl)methylene]amino}quinazoline (**63**), mp 238–240°.

Method K. 2,4,6-Triaminoquinazoline[2] (0.05–0.08 mole), the requisite acetophenone diethyl ketal (0.05–0.08 mole), 0.1–0.3 g of I_2, and 50–150 ml of $(CH_3OCH_2CH_2)_2O$ were refluxed under a short column until no more material boiling below 90° distd (4–7 hr). The hot soln was filtered, and the filtrate was treated with Et_2O (80 ml–1 l.). The product that sepd was crystd from EtOH.

2,4-Diamino-6-[(benzyl)amino]quinazolines (13–43, Table II), 2,4-Diamino-6-[(benzyl)amino]-(5 or 7)-substituted-quinazolines (44–48, Table III), N^2,N^4-(Alkyl and aryl)-2,4-diamino-6-[(benzyl)amino]quinazolines (49–53, Table IV), 2,4-Diamino-6-{[(heterocyclic)methyl]amino}quinazolines (54–58, Table V), Other 2,4-Diamino-6-[(aralkyl)amino]quinazolines (66–71, Table VII), and Miscellaneous 2- and 4-Amino-(6, 7, or 8)-[(benzyl)amino]quinazolines (72–77, Table VIII). Method L, M. The crude benzylidene compd was hydrogenated in EtOH (400 ml for 0.1 mole) (L) or $C_2H_5O(CH_2)_2OH$ (400 ml for 0.01 mole) (M) at 100–110° and 40–70 kg/cm² for 3–5 hr over Raney Ni.[29] The cooled soln was filtered, concd, and dild with H_2O.

Method N. The soln of the benzylidene compd (from B, D) was dild with 200 ml of EtOH, hydrogenated, and worked up as above.

Method O. The requisite benzylidene compd (0.1 mole) in 150 ml of $C_2H_5O(CH_2)_2OH$ at 100° was treated cautiously with a soln of $NaBH_4$ (0.3 mole) in 500 ml of cold MeOH. When the reaction subsided, the mixt was refluxed for 15 min, evapd to 150 ml, and treated with 500 ml of 1 *N* NaOH, and the product was collected or (**49**) isolated with $CHCl_3$.

Method P. 2,4-Diamino-6-[(*o*-nitrobenzyl)amino]quinazoline (**23**) was hydrogenated at atmospheric pressure in AcOH (30 ml/g) over Pt/C, and the filtered, concd soln was dild with H_2O and basified with NH_4OH to give 2,4-diamino-6-[(*o*-aminobenzyl)amino]-quinazoline (**30**).

Method Q. α-[(2,4-Diamino-6-quinazolinyl)amino]-*p*-toluic acid ethyl ester (**41**) was refluxed for 5 min with 0.5 *N* NaOH, and the soln was cooled and filtered with the addn of decolorizing charcoal, and the filtrate was adjusted to pH 6 with 2 *N* HCl to give α-[(2,4-diamino-6-quinazolinyl)amino]-*p*-toluic acid (**31**) (83%).

Method R. α-[(2,4-Diamino-6-quinazolinyl)imino]-*p*-tolualdehyde (**8**) (10.8 g), suspended in 200 ml of MeOH, was treated with 8.5 g of $NaBH_4$ in 400 ml of MeOH at 0°. When the reaction subsided, the mixt was refluxed for 15 min, cooled, treated with 3 g of $NaBH_4$, again refluxed 15 min, and filtered with charcoal. An addnl 1 g of $NaBH_4$ was added, the solvent was evapd below 50°, and the residue was treated with 500 ml of 1 *N* NaOH to give the crude product *p*-{[(2,4-diamino-6-quinazolinyl)amino]methyl}benzyl alcohol (**35**).

Method S. To a soln of 2.5 g (0.0075 mole) of 2,4-diamino-7-[(3,4-dichlorobenzylidene)amino]quinazoline (**60**) in 25 ml of $CH_3O(CH_2)_2OH$ was added portionwise 0.5 g (0.0132 mole) of $NaBH_4$, and the mixt was stirred overnight at room temp. The mixt was poured into 500 ml of ice and H_2O, and the ppt was collected, washed with H_2O, dried, and recrystd from 20% aqueous AcOH. The partial AcOH salt of 2,4-diamino-7-[(3,4-dichlorobenzyl)amino]-quinazoline weighed 1.9 g (59%), mp 213–216°. *Anal.* ($C_{15}H_{13}Cl_2N_5 \cdot 1.2C_2H_4O_2 \cdot 1.4H_2O$) C, H, N, Cl, H_2O.

A hot soln of 0.2 g of the AcOH salt in EtOH contg 1 ml of dil NH_4OH was poured into ice and H_2O. The ppt was collected, washed with H_2O, and dried *in vacuo* at 70° to give the base (**72**), mp 213–216°.

Method T. A suspension of 2,4-diamino-6-[(α-methylbenzylidene amino]quinazoline (**62**) in EtOH (50 ml/g) was hydrogenated at atmospheric pressure over PtO_2 to give 2,4-diamino-6-[(α-methylbenzyl)amino]quinazoline (**69**), pale yellow crystals from EtOH–H_2O.

Method U. A soln of 2,4-diamino-6-{[(2-chloro-1-naphthyl)-methylene]amino}quinazoline (**63**) (4.2 g, 0.01 mole) in 50 ml of DMF was cooled to 3° and 1.2 g (0.03 mole) of $NaBH_4$ was added portionwise over 15 min with cooling. The mixt was allowed to warm to room temp and was stirred at room temp for 0.5 hr and poured into H_2O. The crude product (2.6 g) was recrystd from *i*-PrOH to give 1.2 g (34%) of 2,4-diamino-6-{[(2-chloro-1-naphthyl)methyl]-amino}quinazoline (**70**) as yellow crystals, mp 268–269°.

2,4-Diamino-6-[(*p*-chlorophenethyl)amino]quinazoline *p*-Toluenesulfonate (67). 2-Amino-5-[(*p*-chlorophenethyl)amino]-benzonitrile·HCl (**79**) (4.9 g, 0.016 mole) and cyanoguanidine (1.4 g, 0.017 mole) were ground together and heated to a melt (160°). After heating at 160° for 15 min, the reaction mixt was cooled and recrystd from *i*-PrOH–Et_2O. The product was extd with a $CHCl_3$–dil NaOH mixt, the $CHCl_3$ layer was sepd, and volatile materials were removed *in vacuo*. The residue was dissolved in EtOH and treated with an excess of *p*-toluenesulfonic acid in EtOH. The product that sepd was crystd from EtOH to give 0.4 g (5%) of product as yellow cryst, mp 335–338°.

2-Amino-6-[(benzyl)amino]-4-quinazolinol (73). 2,4-Diamino-6-[(benzyl)amino]quinazoline (**24**) was heated under reflux for 2 hr with 2 *N* HCl (10 ml/g). The mixt was dild with H_2O, basified with NH_4OH, and the yellow solid was collected and recrystd from EtOH–H_2O.

5-[(*p*-Chlorophenethyl)amino]-2-nitrobenzonitrile (78). 5-Chloro-2-nitrobenzonitrile (Aldrich) (9.1 g, 0.05 mole) and *p*-chlorophenethylamine (Sapon) (15.5 g, 0.10 mole) were heated under reflux in 75 ml of $MeO(CH_2)_2OH$ for 4 hr. Volatile materials were removed *in vacuo* on a rotary evaporator, and the residue was shaken between $CHCl_3$ and 1 *N* HCl. The insoluble material was collected and washed with a $CHCl_3$–1 *N* HCl mixt to give 6.6 g of a yellow solid, mp indef >131°. The combined $CHCl_3$ extracts were washed with 1 *N* HCl and H_2O, and the $CHCl_3$ was removed *in vacuo* to give a waxy orange solid, mp 90–110°. The two fractions were combined and recrystd twice from MeOH to give 8.2 g (54%), mp 140–141°. *Anal.* ($C_{15}H_{12}ClN_3O_2$) C, H, N, Cl.

2-Amino-5-[(*p*-chlorophenethyl)amino]benzonitrile (79). 5-[(*p*-Chlorophenethyl)amino]-2-nitrobenzonitrile (**78**) (7.7 g, 0.025 mole) was added to a soln of 21.0 g (0.09 mole) of $SnCl_2 \cdot 2H_2O$ in 65 ml of concd HCl and 25 ml of AcOH over a period of 15 min while maintaining the temp at 25–30° with external cooling. The mixt kept below 25° for 2 hr and was stirred overnight at room temp. The solid that sepd was collected and washed with H_2O. The stannous chloride complex was added to 300 ml of warm H_2O, 15 g of 50% NaOH was added, the mixt was stirred for 0.5 hr, and the process was repeated. The crude product was collected and crystd from *i*-PrOH to give 4.8 g (71%) of pure product, mp 96–98°. *Anal.* ($C_{15}H_{14}ClN_3$) C, H, N.

The base was dissolved in 300 ml of Et_2O and treated with 25% HCl in *i*-PrOH. The HCl salt thus obtained, mp 217–220°, was used in the reaction with cyanoguanidine.

2,4,6-Triamino-7-methylquinazoline (82). The title compd was prepd utilizing procedures described previously for the synthesis of 2,4,6-triaminoquinazoline.[2] Equimolar amounts of 2-amino-*p*-tolunitrile and cyanoguanidine were boiled under reflux with 2 *N* HCl for 2 hr. H_2O and 2 *N* NaOH were added, and the soln was treated with decolorizing charcoal and cooled to give 2,4-diamino-7-methyl-

quinazoline **(80)** (43%) as colorless needles from H_2O, mp 230–232°. *Anal.* ($C_9H_{10}N_4$) C, H, N.

Nitration of the nitrate salt of **80** with fuming HNO_3 and concd H_2SO_4 below 10° afforded 2,4-diamino-7-methyl-6-nitroquinazoline **(81)** in 93% crude yield. Crystn from AcOH gave an orange powder, mp >360°. *Anal.* ($C_9H_9N_5O_2 \cdot 0.5H_2O$) C, H, N.

Redn of the crude nitro compd **81** with $SnCl_2 \cdot 2H_2O$ and HCl gave 2,4,6-triamino-7-methylquinazoline **(82)** (34%) as beige needles from H_2O, mp 268–270°; λ_{max} (pH 6.8 buffer) 208, 242, 357 mμ (ε 14,900, 42,800, 4660). *Anal.* ($C_9H_{11}N_5$) C, H; N: calcd, 37.0; found, 36.5.

2,4,7-Triaminoquinazoline (84). NH_3 was bubbled through a boiling soln of 30.0 g (0.123 mole) of 2,4-dichloro-7-nitroquinazoline[14] in 280 g of PhOH for 2 hr. The reaction mixt was cooled to 60°, poured into 1.5 l. of 20% NaOH, and chilled. The ppt was collected and recrystd twice from AcOH to give 27.1 g (83%) of 2,4-diamino-7-nitroquinazoline (AcOH salt) **(83)** as orange-yellow cryst, mp 330–332° dec. *Anal.* ($C_8H_7N_5O_2 \cdot C_2H_4O_2$) C, H, N.

A hot soln of 5.0 g (0.018 mole) of **83** in AcOH was poured slowly, with stirring, into cold dil NH_4OH. The ppt was collected, washed with H_2O, and dried to give 3.7 g (0.018 mole) of the base. A mixt of the base and 0.5 g of Raney Ni in 50 ml of $CH_3O(CH_2)_2OH$ was hydrogenated for 17 hr at an initial temp of 25° and 3.17 kg/cm². The mixt was filtered, and the filtrate was evapd to dryness *in vacuo*. The residue was crystd twice from H_2O (decolorizing charcoal) and dried *in vacuo* at 85° for 48 hr to give 1.2 g (38%) of 2,4,7-triaminoquinazoline **(84)** as a tan, hydrated solid, mp 141–143°. *Anal.* ($C_8H_9N_5 \cdot H_2O$) C, H, N; H_2O: calcd, 9.3; found, 8.4.

4,6-Diamino-2-(dimethylamino)quinazoline (86). 1,1-Dimethylguanidine (0.2 mole) was prepd by mixing solns of its nitrate salt and of NaOEt in abs EtOH, filtering, and evapg. 2-Chloro-5-nitrobenzonitrile (0.1 mole) and $C_2H_5O(CH_2)_2OH$ (120 ml) were added, and the mixt was stirred under reflux for 3 hr. The mixt was cooled, 120 ml of Et_2O was added, and the intermediate 4-amino-2-(dimethylamino)-6-nitroquinazoline **(85)** was collected. Crystn from EtOH afforded orange needles, mp 266–268° (69%). *Anal.* ($C_{10}H_{11}N_5O_2$) C, H, N.

Hydrogenation of **85** in EtOH over Pd/C at atmospheric pressure gave 4,6-diamino-2-(dimethylamino)quinazoline **(86)** (69%) as pale yellow prisms from EtOH, mp 188–189°. *Anal.* ($C_{10}H_{13}N_5$) C, H, N.

6-Amino-2,4-bis(substituted-amino)quinazolines. 2,4-Dichloro-6-nitroquinazoline[14] (25.0 g, 0.102 mole) and $HNMe_2 \cdot HOAc$ (130 g) were heated for 6 hr at 140–150°. Addn of the melt to H_2O and basification gave 14.5 g (57%) of 2,4-bis(dimethylamino)-6-nitroquinazoline **(87)** as yellow needles from EtOH, mp 199–203°. *Anal.* ($C_{12}H_{15}N_5O_2$) C, H, N.

The following three compds sepd on cooling after boiling 5.0 g (0.02 mole) of 2,4-dichloro-6-nitroquinazoline[14] with 4 moles of the appropriate amine in 40 ml of EtOH for 4 hr: 2,4-bis(*n*-butylamino)-6-nitroquinazoline **(88)** (6.0 g, 97%), yellow needles from EtOH, mp 173–175° [*Anal.* ($C_{16}H_{23}N_5O_2$) C, H, N]; 2,4-bis(diethylamino)-6-nitroquinazoline **(89)** (4.9 g, 75%), yellow needles from EtOH, mp 91–93° [*Anal.* ($C_{15}H_{23}N_5O_2$) C, H, N]; and 2,4-dianilino-6-nitroquinazoline **(90)** (2.8 g, 38%), dark orange needles from DMF–H_2O, mp 260–262° [*Anal.* ($C_{20}H_{15}N_5O_2$) C, H, N].

The above 2,4-diamino-6-nitroquinazolines were hydrogenated over Pd/C in EtOH at atmospheric pressure, and the filtered solns of the 2,4,6-triaminoquinazolines were used directly in the formation of the corresponding Schiff bases.

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