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Folate Antagonists. 13. 2,4-Diamino-6-[(α,α,α-trifluoro-*m*-tolyl)thio]quinazoline and Related 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines, a Unique Class of Antimetabolites with Extraordinary Antimalarial and Antibacterial Effects[1,2]

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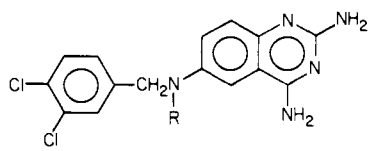
An array of nonclassical thioquinazoline analogues (VIII) of methotrexate was prepared by cyclization of the requisite 2-amino-5-(arylthio)benzonitrile with chloroformamidine hydrochloride (28–79%). The aminonitrile precursors were obtained by $SnCl_2$–HCl reduction (28–99%) of the corresponding 2-nitro-5-(arylthio)benzonitriles, which were synthesized by the condensation of the appropriate 5-chloro-2-nitrobenzonitriles with various arylthiols (36–83%). Many of the thioquinazolines (VIII) showed suppressive antimalarial activity comparable with or superior to chloroquine, cycloguanil, and pyrimethamine against drug-sensitive lines of *Plasmodium berghei* in mice and *Plasmodium gallinaceum* in chicks, and several displayed potent prophylactic activity against *P. gallinaceum*. Moreover, the thioquinazolines retained potent antimalarial effects against chloroquine-, cycloguanil-, pyrimethamine-, and DDS-resistant lines of *P. berghei* in mice and against chloroquine- and pyrimethamine-resistant strains of *Plasmodium falciparum* in owl monkeys. The most active compound, namely, 2,4-diamino-6-[(α,α,α-trifluoro-*m*-tolyl)thio]quinazoline, was designated for preclinical toxicological studies. Numerous substances exhibited in vitro activity against a broad spectrum of pathogenic bacteria at concentrations of <0.25 μg/mL. The thioquinazolines also proved to be potent folate antagonists, causing 50% inhibition of *Streptococcus faecalis* R (ATCC 8043) at drug concentrations ranging from 0.2 to 2.0 ng/mL. Structure–activity relationships are discussed.

A plethora of 2,4-diamino-6-[(benzyl)amino]quinazoline antifolates, exemplified by 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia),[4,5] 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (Ib),[6,7] and 2,4-diamino-6-[(3,4-dichlorobenzyl)methylamino]quinazoline (Ic),[8] exhibit strong antimalarial effects against sensitive and drug-resistant lines of *Plasmodium berghei* in mice, *Plasmodium gallinaceum* in chicks, and *Plasmodium cynomolgi* and *Plasmodium knowlesi* in rhesus monkeys.[4–8] In contradistinction, oxygen and sulfur bioisosteres such as 2,4-diamino-6-[(*p*-chlorobenzyl)oxy]quinazoline (IIa)[9] and 2,4-diamino-6-[(*p*-chlorobenzyl)thio-, sulfinyl-, and sulfonyl]quinazoline (IIb–d)[10] were either much less potent than the triaminoquinazolines Ia–c or lacked appreciable antimalarial activity altogether.[9,10] However, it is noteworthy that potent antimalarial activity was restored when the methylene bridge of IIa was extruded.[9] Thus, 2,4-



Ia, R = H
b, R = NO
c, R = CH_3

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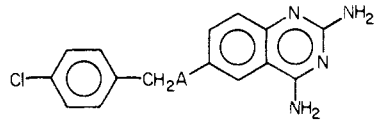
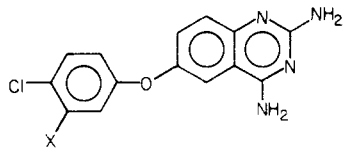


IIa, A = -O-
b, A = -S-
c, A = -SO-
d, A = $-SO_2-$

diamino-6-(*p*-chlorophenoxy)quinazoline (IIIa) and 2,4-



IIIa, X = H
b, X = Cl

diamino-6-(3,4-dichlorophenoxy)quinazoline (IIIb) displayed oral antimalarial effects against *P. berghei* in mice comparable with or superior to 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia).[9] We now report the synthesis and biological properties of an array of 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines (VIII), several of which are among the most potent antimalarial drugs ever reported in experimental animal models.

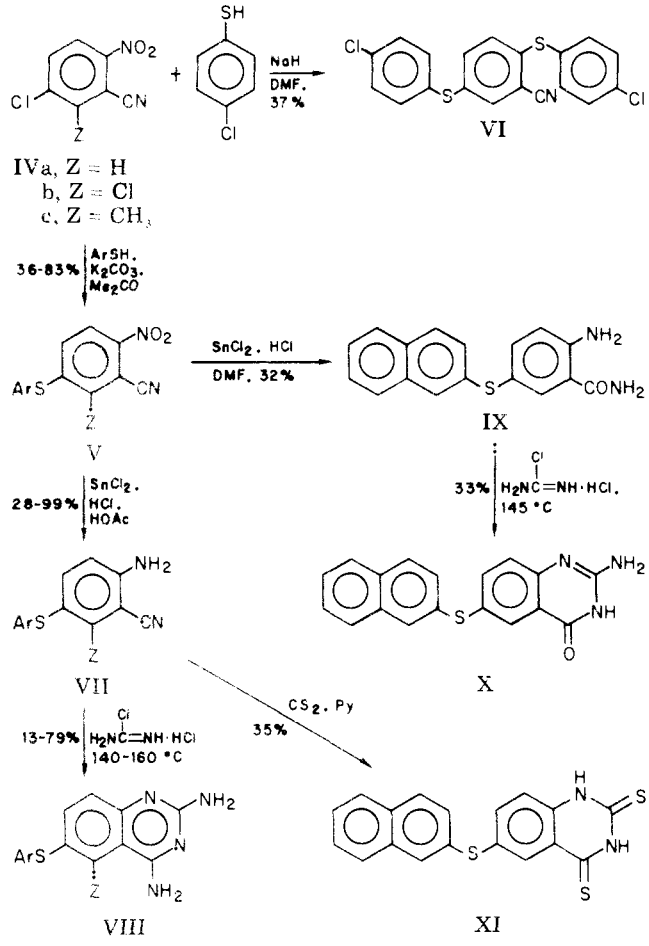
Chemistry. The synthetic approach utilized for the preparation of the 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines (VIII) is outlined in Scheme I. Condensation of the appropriate 5-chloro-2-nitrobenzonitrile (IVa–c) with the requisite arylthiol in acetone or benzene in the presence of K_2CO_3 generated the corresponding 2-nitro-5-[(phenyl- and naphthyl)thio]benzonitriles V (**1–33**, Table I) in 36–83% yield (procedures I–III). An abortive attempt to prepare 2-nitro-5-[(*p*-chlorophenyl)thio]benzonitrile (**10**) from the sodium salt of *p*-chlorobenzenethiol utilizing sodium hydride in dimethylformamide resulted instead in the displacement of the nitro group to give 2,5-bis[(*p*-chlorophenyl)thio]-benzonitrile (VI, 37%). Reduction of V with $SnCl_2 \cdot 2H_2O$–HCl in glacial acetic acid (procedures IV and V) afforded the corresponding 2-amino-5-[(phenyl- and naphthyl)thio]benzonitriles VII (**34–63**, Table II) (28–99%), which were cyclized with chloroformamidine hydrochloride[8-11] in diglyme or dimethyl sulfone[12] to the desired 2,4-diamino-6-[(phenyl- and naphthyl)thio]-quinazolines VIII (**64–98**, Table III) (13–79%) (procedures VI–VIII).

One attempt to effect the reduction of 2-nitro-5-(2-naphthylthio)benzonitrile (**28**) (V, Ar = 2-$C_{10}H_7$; Z = H) with $SnCl_2 \cdot 2H_2O$–HCl in a mixture of glacial acetic acid and *N,N*-dimethylformamide at 60–70 °C gave 2-amino-5-(2-naphthylthio)benzamide (IX, 32%) instead of the expected 2-amino-5-(2-naphthylthio)benzonitrile (**61**). Ring closure of IX utilizing chloroformamidine hydrochloride[11] in diglyme at 145 °C afforded 2-amino-6-(2-naphthylthio)-4(3*H*)-quinazolinone (X, 33%), a thioquinazoline analogue of folic acid.[35] Treatment of a pyridine solution of **61** (VII, Ar = 2-$C_{10}H_7$; Z = H) with carbon disulfide according to the procedure of Taylor et al.[13] gave 6-(2-naphthylthio)-2,4(1*H*,3*H*)-quinazolinedithione (XI) in 35% yield.[35]

Among the requisite 5-chloro-2-nitrobenzonitrile precursors IVa–c, 5-chloro-2-nitrobenzonitrile (IVa) is available commercially.[14] 5,6-Dichloro-2-nitrobenzonitrile (IVb) was obtained in 59% yield by treatment of 1,2,3-trichloro-4-nitrobenzene[14] with cuprous cyanide in 1-methyl-2-pyrrolidone at 145–150 °C. Diazotization of 3-chloro-6-nitro-*o*-toluidine[15] followed by treatment with cuprous cyanide afforded 3-chloro-6-nitro-*o*-tolunitrile (IVc, 71%).

An alternate approach to the synthesis of the 2,4-diamino-6-[(aryl)thio]quinazolines, depicted in Scheme II,

Scheme I



Scheme II

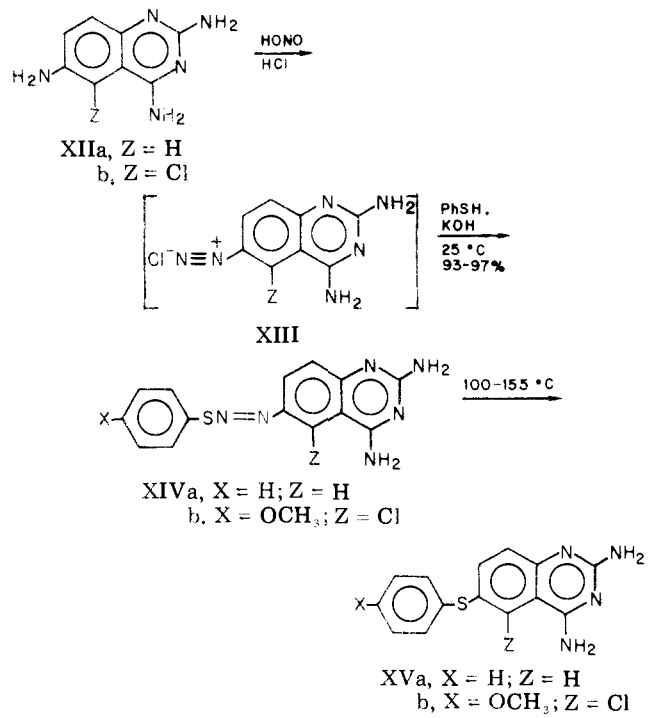
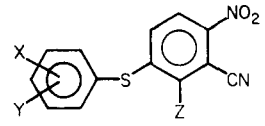


Table I. 2-Nitro-5-[(phenyl- and naphthyl)thio]benzonitriles



no.	X,Y	Z	mp, °C	yield purified, %	purifcn solvent	procedure	formula	analyses
1	2,3,5,6-F_4	H	173-175.5	69	EtOH-MeCN	I	$C_{13}H_4F_4N_2O_2S$	C, H, N
2	2,5-Cl_2	H	106-109	38	EtOAc-MeOH	II	$C_{13}H_6Cl_2N_2O_2S$	C, H, N
3	3,5-Cl_2	H	138-140	54	EtOAc-isooctane	I	$C_{13}H_6Cl_2N_2O_2S$	C, H; N[a]
4	2-Cl	Cl	122-125	36	EtOH	I	$C_{13}H_6Cl_2N_2O_2S$	C, H, N
5	4-F	Cl	175-178	74	MeCN	II	$C_{13}H_6ClFN_2O_2S$	C, H, N
6	H	Cl	126-127.5	66	EtOH	II	$C_{13}H_7ClN_2O_2S$	C, H, N
7	4-Br	H	121-125	58	EtOAc	II	$C_{13}H_7BrN_2O_2S$	C, H, N
8	2-Cl	H	107-110	48	EtOH	II	$C_{13}H_7ClN_2O_2S$	C, H, N
9	3-Cl	H	124-126	56	EtOAc-isooctane	II	$C_{13}H_7ClN_2O_2S$	C, H, N
10	4-Cl	H	107-109	49	MeCN	II	$C_{13}H_7ClN_2O_2S$	C, H, N
11	4-F	H	116.5-119.5	66	EtOH	II	$C_{13}H_7FN_2O_2S$	C, H, N
12	H	H	122-124	51	EtOAc-isooctane	I	$C_{13}H_8N_2O_2S$	C, H, N
13	4-OH	H	161.5-163.5	45	EtOH	I	$C_{13}H_8N_2O_3S$	C, H, N
14	3-CF_3	Cl	117-121	62	2-PrOH	II	$C_{14}H_6ClF_3N_2O_2S$	C, H, N, S
15	3-CF_3	H	96-98.5	73	EtOH	II	$C_{14}H_7F_3N_2O_2S$	C, H, N
16	3,4-Cl_2	CH_3	160-162	44	2-PrOH	II	$C_{14}H_8Cl_2N_2O_2S$	C, H, N
17	4-Br,3-CH_3	H	119-121	59	MeCN	I	$C_{14}H_9BrN_2O_2S$	C, H, N
18	5-Cl,2-CH_3	H	101-105	38	EtOH	II	$C_{14}H_{10}ClN_2O_2S$	H, N; C[b]
19	2-CH_3	H	104-105.5	58	EtOH	I	$C_{14}H_{10}N_2O_2S$	C, H, N
20	3-CH_3	H	110-112	38	C_6H_6-cyclohexane	II	$C_{14}H_{10}N_2O_2S$	C, H, N
21	4-CH_3	H	120-121.5	64	EtOH	I	$C_{14}H_{10}N_2O_2S$	C, H, N
22	3-OCH_3	H	120-122	72	MeCN-H_2O	I	$C_{14}H_{10}N_2O_3S$	C, H, N
23	4-OCH_3	H	86-88	36	$C_6H_5CH_3$-isooctane	II	$C_{14}H_{10}N_2O_3S$	C, H, N
24	4-$NHCOCH_3$	H	241-243.5	67	MeCN	II	$C_{15}H_{11}N_3O_3S$	C, H, N
25	4-$N(CH_3)_2$	H	168.5-170.5	48	MeCN	I	$C_{15}H_{13}N_3O_2S$	C, H, N
26	3,4- 	Cl	145-148	68	2-PrOH-MeCN	II	$C_{17}H_9ClN_2O_2S$	C, H, N
27	2,3- 	H	153.5-155.5	56	MeCN	II	$C_{17}H_{10}N_2O_2S$	C, H, N
28	3,4- 	H	139-141	46	MeCN	III	$C_{17}H_{10}N_2O_2S$	C, H, N
29	4-$C(CH_3)_3$	H	93-95	83	2-PrOH	II	$C_{17}H_{16}N_2O_2S$	C, H, N
30	3,4- 	CH_3	147-149	71	2-PrOH	II	$C_{18}H_{12}N_2O_2S$	C, H, N
31	2-C_6H_5	H	117-118.5	83	EtOAc-isooctane	I	$C_{19}H_{12}N_2O_2S$	C, H, N
32	3-C_6H_5	H	97.5-99	83	EtOAc-isooctane	I	$C_{19}H_{12}N_2O_2S$	C, H, N
33	4-C_6H_5	H	143-145	37	EtOH	I	$C_{19}H_{12}N_2O_2S$	C, H, N

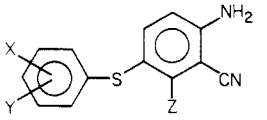
[a] N: calcd, 8.61; found, 9.09. [b] C: calcd, 55.00; found, 55.41.

was attempted predicated on a report by Baker et al.[16] that treatment of the diazonium salt of 5-amino-3,4-dihydroquinazolin-4-one with sodium thiophenoxide on one occasion afforded 5-(phenylthio)-3,4-dihydroquinazolin-4-one (28%), although these authors were unable to duplicate their results.[16] Diazotization of 2,4,6-triaminoquinazoline (XIIa)[17] and of 2,4,6-triamino-5-chloroquinazoline (XIIb)[17] afforded the respective diazonium salts XIII in situ, which upon treatment with a solution of the requisite benzenethiol in aqueous KOH gave the corresponding (2,4-diamino-6-quinazolinyl)(phenylthio)diimides XIVa,b (93–98%). Heating these diimides at 100–155 °C neat or in diglyme or Dowtherm led to the formation of complex mixtures of products from which the desired 2,4-diamino-6-[(phenyl)thio]quinazolines XVa,b could not be isolated in pure form, although TLC indicated their presence based on comparative studies with authentic samples prepared by Scheme I.

Suppressive Antimalarial Screening in Mice. The 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines VIII (**64–98**, Table III) and the related thioquinazoline derivatives X, XI, and XIVa described in the present communication were tested initially against a normal drug-sensitive strain of *P. berghei* in mice by the parenteral route.[18,19] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72 h postinfection. Extension of the mean survival time of the treated mice is interpreted as evidence of antimalarial activity.[20] Compounds are arbitrarily considered to be "active" when they produce at least a 100% increase in the mean survival time of treated mice. Animals that survive to 60 days are considered "cured". The mean survival time of infected control mice in the present study ranged from 6.1 to 6.3 days. X, XI, and XIVa were inactive at 640 mg/kg. Other results are summarized in Table IV.

It is clear that curative activity is retained within a wide variation of substituents on the 6-aryl substituent. Sixteen compounds (**64, 67, 70, 72, 75, 76, 80, 81, 83, 84, 88, 90, 91, 93, 95,** and **97**) encompassing halogen, trifluoromethyl, alkyl, alkoxy, dimethylamino, and phenyl substituents were completely curative at a single sc dose of 80 mg/kg—greater activity than shown by either cycloguanil hydrochloride or pyrimethamine. In addition, nine compounds

Table II. 2-Amino-5-[(phenyl- and naphthyl)thio]benzonitriles



no.	X,Y	Z	mp, °C	yield purified, %	purifcn solvent	procedure	formula	analyses
34	2,3,5,6-F_4	H	108-110	68	EtOH-aq NaOH	IV	$C_{13}H_6F_4N_2S$	C, H, N
35	2,5-Cl_2	H	155-157.5	55	EtOH	IV	$C_{13}H_8Cl_2N_2S$	C, H, N
36	3,4-Cl_2	H	123-125	34	2-PrOH	IV	$C_{13}H_8Cl_2N_2S$	C, H, N
37	2-Cl	Cl	181-184	79	EtOH	IV	$C_{13}H_8Cl_2N_2S$	C, H, N
38	4-F	Cl	156-158	76	EtOH-H_2O	IV	$C_{13}H_8ClFN_2S$	C, H, N
39	H	Cl	171-172.5	66	EtOH	IV	$C_{13}H_9ClN_2S$	C, H, N
40	4-Br	H	133-136.5	63	EtOH	V	$C_{13}H_9BrN_2S$	C, H, N
41	2-Cl	H	104-106.5	57	EtOH	IV	$C_{13}H_9ClN_2S$	H, N; C[a]
42	3-Cl	H	103-105	70	EtOH-H_2O	IV	$C_{13}H_9ClN_2S$	C, H, N
43	4-Cl	H	123-125	67	EtOH-H_2O	V	$C_{13}H_9ClN_2S$	C, H, N
44	4-F	H	92.5-94.5	74	EtOH-H_2O	V	$C_{13}H_9FN_2S$	C, H, N
45	H	H	87-89	81	2-PrOH	IV	$C_{13}H_{10}N_2S$	C, H, N
46	4-OH	H	118.5-122.5	57	2-PrOH	IV	$C_{13}H_{10}N_2OS$	C, H, N
47	3-CF_3	Cl	151-155	76	EtOH-H_2O	IV	$C_{14}H_8ClF_3N_2S$	C, H, N, S
48	3-CF_3	H	114-116	81	EtOH	IV	$C_{14}H_9F_3N_2S$	C, H, N
49	3,4-Cl_2	CH_3	104-106	99	2-PrOH-H_2O	IV	$C_{14}H_{10}Cl_2N_2S \cdot 0.1H_2O$	C, H, N, H_2O
50	4-Br,3-CH_3	H	122-124	64	C_6H_6-isooctane	IV	$C_{14}H_{11}BrN_2S$	C, H, N
51	5-Cl,2-CH_3	H	139-141.5	67	EtOH	IV	$C_{14}H_{11}ClN_2S$	H, N; C[b]
52	2-CH_3	H	96-98	76	EtOH-H_2O	IV	$C_{14}H_{12}N_2S$	C, H, N
53	3-CH_3	H	121-122.5	85	EtOH-H_2O	IV	$C_{14}H_{12}N_2S$	C, H, N
54	4-CH_3	H	114-115	64	2-PrOH-H_2O	V	$C_{14}H_{12}N_2S$	H, N; C[c]
55	3-OCH_3	H	72-74	63	2-PrOH	IV	$C_{14}H_{12}N_2OS$	C, H, N
56	4-OCH_3	H	101-103	71	EtOH	IV	$C_{14}H_{12}N_2OS$	C, H, N
57	4-$NHCOCH_3$	H	158-160	71	EtOAc	IV	$C_{15}H_{13}N_3OS$	C, H, N
58	4-$N(CH_3)_2$	H	135-138	66	EtOH	IV	$C_{15}H_{15}N_3S$	C, H, N
59	3,4- 	Cl	176-179	72	EtOH	IV	$C_{17}H_{11}ClN_2S$	C, H; N[d]
60	2,3- 	H	132-134	62	EtOH-H_2O	IV	$C_{17}H_{12}N_2S$	C, H, N
61	3,4- 	H	140.5-142.5	59	EtOH	IV	$C_{17}H_{12}N_2S$	C, H, N
62	2-C_6H_5	H	156-158	45	EtOH	V	$C_{19}H_{14}N_2S$	C, H, N
63	3-C_6H_5	H	116-119	84	EtOH	IV	$C_{19}H_{14}N_2S$	C, H, N

[a] C: calcd, 59.88; found, 59.46. [b] C: calcd, 61.19; found, 60.46. [c] C: calcd, 69.96; found, 69.36. [d] N: calcd, 9.01; found, 8.57.

were equiactive or more active than the highly potent 2,4-diamino-6-[(3,4-dichlorobenzyl)methylamino]quinazoline (Ic) (**67**, **72**, **76**, **83**, **88**, **90**, **91**, **93**, and **95**), exhibiting curative activity at 20 mg/kg. Once again these included halogen, alkoxy, amino, and aryl substituents. Substitution on the 5 position of the quinazoline ring was examined in seven cases (compounds **69–71**, **79**, **81**, **91**, **95**). The presence of a methyl group or a chlorine in this position was not deleterious to activity but did not seem to offer any advantage.

Twelve of the thioquinazolines (**66**, **72**, **75–77**, **83–86**, **88**, **93**, and **94**, Table III) were also evaluated orally against another normal drug-sensitive strain of *P. berghei* in mice.[21,22] The drugs were given continuously in the diet of mice for 6 consecutive days, and all drug doses were calculated as free base equivalent. Results (Table V) are expressed both in terms of the SD_{90} and the quinine equivalent Q.

Of these, six compounds (**72**, **75**, **76**, **83**, **88**, and **93**) produced a 90% suppression of the parasitemia at daily oral doses of 0.08–1.2 mg/kg and thus ranged from 62 to 880 times as potent as quinine hydrochloride. Once again halogen substitution led to high activity but was not limited to this substituent. The most potent compound, in fact, was the 2-naphthyl analogue (**93**) with a Q of 880.

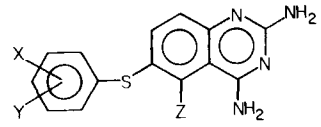
Oral and parenteral base line data for the reference drugs cycloguanil hydrochloride, pyrimethamine, trimethoprim, and the 2,4,6-triaminoquinazolines Ia–c are included for comparative purposes (Tables IV and V).

Suppressive Antimalarial Effects in Chicks. A representative sampling of the 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines (**67**, **72**, **73**, **75–77**, **80**, **83–86**, **90**, **92**, **93**, and **97**, Table III) was also tested for suppressive antimalarial effects against *P. gallinaceum* infections in white Leghorn cockerels (Table VI).[23,24] The drugs were administered to infected chicks in a single subcutaneous dose in peanut oil. In this test, as in the parenteral mouse assay, the antimalarial activity of candidate compounds was assessed by comparing the maximum survival times of treated malaria-infected chicks with the survival times of untreated malaria-infected chicks. A compound was arbitrarily considered to be active against malaria if it produced survival times among treated chicks that were at least 100% greater than the survival times of untreated control animals. Results are summarized in Table VI.

Once again good potency was exhibited by a variety of ring substituents, with extraordinary activity being shown by the 4-dimethylamino analogue (**90**) which was completely curative at 10 mg/kg and highly active as low as 2.5 mg/kg.

Prophylactic Antimalarial Effects in Chicks. Seventeen thioquinazoline derivatives (**65**, **72**, **73**, **76–80**,

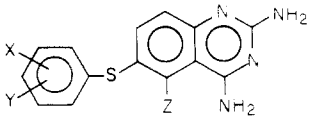
Table III. 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines



no.	X,Y	Z	mp, °C	yield purified, %	purifcn solvent	procedure	formula	analyses
64	2,3,4,5,6-Cl_5	H	283–286	17	EtOAc-$C_6H_5CH_3$	VI	$C_{14}H_7Cl_5N_4S \cdot C_4H_8O_2$[a]	H, N; C[b]
65	2,3,5,6-F_4	H	250–252	47	EtOH-NH_4OH	VII	$C_{14}H_8F_4N_4S \cdot H_2O$	C, H, N, H_2O
66	2,5-Cl_2	H	250–252	24	EtOAc	VI	$C_{14}H_{10}Cl_2N_4S$	C, H, N
67	3,4-Cl_2	H	236–238	44	EtOH	VII	$C_{14}H_{10}Cl_2N_4S \cdot C_2H_6O$[c]	C, H, N
68	3,5-Cl_2	H	223–225	22	EtOAc-isooctane	VII	$C_{14}H_{10}Cl_2N_4S \cdot 0.75C_4H_8O_2$[a]	C, H, N
69	2-Cl	Cl	258–262	79	DMF-NH_4OH	VIII	$C_{14}H_{10}Cl_2N_4S$	C, H, N
70	4-F	Cl	253–255	66	DMF	VIII	$C_{14}H_{10}ClFN_4S$	C, H, N
71	H	Cl	221–222	75	DMF-aq NaOH	VII	$C_{14}H_{11}ClN_4S$	C, H, N
72	4-Br	H	231–234	38	DMF-EtOAc	VII	$C_{14}H_{11}BrN_4S \cdot 0.67H_2O$	C, H, N, H_2O
73	2-Cl	H	246.5–248.5	26	EtOH-NH_4OH	VII	$C_{14}H_{11}ClN_4S$	C, H, N
74	3-Cl	H	209–211	62	DMF-NH_4OH	VII	$C_{14}H_{11}ClN_4S$	C, H, N
75	4-Cl	H	240–242.5	58	DMF-H_2O	VII	$C_{14}H_{11}ClN_4S \cdot 0.33H_2O$	C, H, N, H_2O
76	4-F	H	247–249	16	EtOH-H_2O	VII	$C_{14}H_{11}FN_4S$	C, H, N
77	H	H	189–191	47	EtOH	VII	$C_{14}H_{12}N_4S$	C, H, N
78	4-OH	H	300–302	72	DMF-NH_4OH	VII	$C_{14}H_{12}N_4OS$	C, H, N; H_2O[d]
79	3-CF_3	Cl	180–184	75	DMF-H_2O	VIII	$C_{15}H_{10}ClF_3N_4S$	C, H, N, S
80	3-CF_3	H	229–230.5	63	EtOH-NH_4OH	VII	$C_{15}H_{11}F_3N_4S$	C, H, N
81	3,4-Cl_2	CH_3	247–249	37	DMF-H_2O	VII	$C_{15}H_{12}Cl_2N_4S$	C, H, N
82	4-Br,3-CH_3	H	230–232	41	EtOH	VII	$C_{15}H_{13}BrN_4S \cdot 0.25C_2H_6O$[c]	C, H, N
83	5-Cl,2-CH_3	H	229–231.5	48	EtOH-H_2O	VII	$C_{15}H_{13}ClN_4S \cdot 0.25C_2H_6O$[c]	C, H, N
84	2-CH_3	H	220–222	20	EtOH	VII	$C_{15}H_{14}N_4S$	C, H, N
85	3-CH_3	H	217–219	30	EtOH-H_2O	VII	$C_{15}H_{14}N_4S$	C, H, N
86	4-CH_3	H	250–252	30	EtOH	VII	$C_{15}H_{14}N_4S$	C, H, N
87	3-OCH_3	H	207–209.5	55	EtOH	VII	$C_{15}H_{14}N_4OS$	C, H, N
88	4-OCH_3	H	229–230	25	EtOH	VII	$C_{15}H_{14}N_4OS$	C, H, N
89	4-$NHCOCH_3$	H	>310	23	EtOH	VII	$C_{16}H_{15}N_5OS \cdot 0.75HCl \cdot 0.33H_2O$	C, H, N, H_2O; Cl^-[e]
90	4-$N(CH_3)_2$	H	267–269	45	DMF-H_2O	VII	$C_{16}H_{17}N_5S \cdot C_3H_7NO$[f]	C, H, N
91	3,4- 	Cl	239–242	73	DMF-H_2O	VIII	$C_{18}H_{13}ClN_4S$	C, H, N
92	2,3- 	H	244–246	68	DMF-H_2O	VII	$C_{18}H_{14}N_4S$	C, H, N
93	3,4- 	H	226–228	38	EtOH	VII	$C_{18}H_{14}N_4S$	C, H, N
94	4-$C(CH_3)_3$	H	221–223	21	EtOH	VI	$C_{18}H_{20}N_4 \cdot C_2H_6O$[c]	C, H, N
95	3,4- 	CH_3	277–279	30	DMF-H_2O	VII	$C_{19}H_{16}N_4S \cdot 0.1H_2O$	C, H, N, H_2O
96	2-C_6H_5	H	266–268	62	DMF-NH_4OH	VII	$C_{20}H_{16}N_4S$	C, H, N
97	3-C_6H_5	H	181–183	54	EtOH	VII	$C_{20}H_{16}N_4S \cdot 0.25C_2H_6O \cdot 0.25H_2O$[c]	H, N, H_2O; C[g]
98	4-C_6H_5	H	231–233	13	EtOH-NH_4OH	VII	$C_{20}H_{16}N_4S \cdot H_2O$	C, H, N, H_2O

[a] The NMR spectrum confirmed the presence of EtOAc. [b] C: calcd, 40.89; found, 41.34. [c] The NMR spectrum confirmed the presence of EtOH. [d] H_2O: calcd, 8.67; found, 8.05. [e] Cl^-: calcd, 7.44; found, 7.93. [f] The NMR spectrum confirmed the presence of DMF. [g] C: calcd, 68.32; found, 68.76.

Table IV. Parenteral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines against Trophozoite-Induced *P. berghei* in Mice



			ΔMST; C or T[a] after single sc dose, mg/kg									
no.	X,Y	Z	640	320	160	80	40	20	10	5	2.5	1.25
64	2,3,4,5,6-Cl_5	H		C5	C5	C5	18.1; C2	10.8	6.6	2.7	2.0	
					C5	C5	19.2; C2	10.7	7.1	2.8		
					C5	C5	18.8; C2	11.2	6.8	3.0		
						C5	19.5; C2	11.0	6.8			
65	2,3,5,6-F_4	H	C5	C5	14.9; C3	12.1	8.1	4.9	0.9	0.3	0.3	
				C5	21.9; C2	12.3	8.5	5.1	0.6	0.3		
					15.9; C4	8.9; C2	6.1	2.7	0.5			
						15.4; C1	6.3	3.1				
66	2,5-Cl_2	H	C5	C5	8.8; C2	9.8	8.2	5.8	2.9	2.3		
				C5	9.9; C2	10.1	8.7	5.9	2.9			
					9.7; C3	10.3	8.7	5.9				
67	3,4-Cl_2	H	C5	C5	C5	C5	C5	17.6; C2	12.7; C1	11.5	5.9	0.9
				C5	C5	C5	C5	17.9; C3	12.2; C1	8.5	6.1	1.1
				C5	C5	C5	C5	17.9; C2	11.7; C1	8.7		
					C5	C5	C5	15.9; C4	12.9; C1			
							C5	18.4; C3	13.4; C1			
								17.9; C3				
68	3,5-Cl_2	H	C5	C5	17.9; C4	12.7	6.9	4.1	1.1			
				C5	20.9; C4	12.7	7.1	4.1				
69	2-Cl	Cl	24.9; C4	24.4; C3	15.6; C2	11.7; C1	10.7; C1	4.7				
			27.9; C3		14.9; C2		10.2; C1					
70	4-F	Cl	C1; T4	C5	C5	C5	15.6; C2	6.9				
71	H	Cl	9.9; C3		13.9		4.7	3.9				
72	4-Br	H	C5	C5	C5	C5	C5	14.4; C3	19.9; C1	7.8	3.6	2.2
				C5	C5	C5	C5	20.9; C2	19.6; C1	6.7		
					C5	C5	C5	16.8; C3				
73	2-Cl	H		18.9; C3	11.2; C2	8.3	0.7	0.5	0.5	0.3		
					10.9; C2	8.1	0.5	0.5	0.3			
74	3-Cl	H	C5	20.9; C4	4.9	4.3	3.5	0.5	0.5			
				22.9; C4	5.1	4.5	3.7	0.7				
75	4-Cl	H	C5	C5	C5	25.8; C4	13.1; C2	9.2	9.5	6.0	3.6	1.7
			C5	C5	C5	C5	19.8; C2	11.6	9.4	6.8	2.5	1.7
				C5	C5	C5	20.2; C2	11.9	9.2	6.6		
				C5	C5	C5	19.3; C3	12.0	10.1			
					C5	C5	20.5; C2	11.8	10.1			
							14.1; C4	11.1				
							15.8; C4	11.1				
76	4-F	H	C5	C5	C5	C5	C5	18.0; C1	11.0	7.1	6.1	5.3
				C5	C5	C5	C5	13.5; C2	11.1			
							C5	13.7; C2				
77	H	H		14.7; C3	14.0; C2	9.7	6.3	4.7	3.3	1.1		
					13.9; C2	10.3	6.5	5.1	3.3			
78	4-OH	H	C5	C5	13.6; C2	10.9; C1	11.9	6.3	2.9			
				C5	13.9; C3	11.9; C1	12.1	6.5				
79	3-CF_3	Cl	C5	C5	25.4; C3	19.9; C2	15.2; C1	5.9				
			C5		24.9; C3		14.7; C1					
80	3-CF_3	H	C5	C5	C5	C5	14.9; C3	12.7	9.1	8.7	2.7	0.5
				C5	C5	C5	15.9; C3	13.1	9.3			
							16.4; C3	13.3				
81	3,4-Cl_2	CH_3		C5	C5	C5	C5	15.9; C1	7.9	7.3	2.5	1.3
					C5	C5	C5	15.4; C1	8.1	7.5		
								15.7; C1	8.1			
82	4-Br,3-CH_3	H	C5	C5	C5	14.4; C1	7.7	3.5	1.9			
				C5	C5	11.9; C2	7.9	3.9				
83	5-Cl,2-CH_3	H	C5	C5	C5	C5	25.7; C4	19.4; C2	8.8; C1	9.5	4.1	2.7
				C5	C5	C5	28.8; C3	17.8; C3	10.0; C1			
							27.7; C4	21.7; C2				
84	2-CH_3	H	C2; T3	C5	C5	C5	17.0; C2	10.5	9.8	7.3	5.5	1.1
				C5	C5	C5	17.8; C2	10.8	9.9			
							17.7; C2	10.9				
85	3-CH_3	H	C2; T3	C5	12.7; C3	7.7	6.7	2.3	2.4			
				C5	13.3; C3	8.0	6.6	2.8				
86	4-CH_3	H	C5	C5	C5	9.7; C3	7.1	3.9	2.5			
				C5	C5	16.6; C2	7.5	4.3				
87	3-OCH_3	H	C5	12.2; C2	11.7	8.3	5.7	1.5	0.3			
				13.9; C2	12.1	8.3	5.9	1.7				
88	4-OCH_3	H	C5	C5	C5	C5	C5	25.8; C4	9.8	4.8	3.8	2.2
				C5	C5	C5	C5	C5	9.6			
							C5	C5				

Table IV (*Continued*)

no.	X,Y	Z	ΔMST; C or T[a] after single sc dose, mg/kg									
			640	320	160	80	40	20	10	5	2.5	1.25
89	4-$NHCOCH_3$	H	C5	C5	C5	27.4; C3	13.1	6.3	3.3			
				C5	C5	24.9; C4	12.9	6.5				
90	4-$N(CH_3)_2$	H	C5	C5	C5	C5	C5	C5	10.3	9.7	5.5	2.5
				C5	C5	C5	C5	C5	10.7			
							C5	C5				
91	3,4- 	Cl	C5	C5	C5	C5	C5	15.9; C4	15.9; C2	12.7; C1	7.9	5.5
			C5		C5		C5	17.9; C4				
							C5					
92	2,3- 	H	C5	C5	C5	18.4; C3	14.7	10.7	7.9	1.9	1.1	0.5
				C5	C5	18.9; C3	14.5	10.9	8.3			
							14.7	11.3				
93	3,4- 	H	C5	C5	C5	C5	C5	C5	11.3	6.5	5.3	2.9
				C5	C5	C5	C5	C5				
							C5	C5				
94	4-$C(CH_3)_3$	H	C5	24.8; C3	7.8	6.0	2.6	1.0	1.1			
				17.9; C4	8.1	6.3	2.5	1.3				
95	3,4- 	CH_3	C5	C5	C5	C5	C5	20.9; C4	12.9	10.9	6.5	1.9
				C5	C5	C5	C5	27.9; C3	13.1			
							C5	30.4; C3				
96	2-C_6H_5	H	16.4; C1	12.5	5.7	3.3	0.7	0.7	0.3			
				12.7	5.9	3.5	0.5	0.5				
97	3-C_6H_5	H	C5	C5	C5	C5	20.6; C2	11.3	8.1	7.9	5.3	4.5
				C5	C5	C5	17.4; C3	11.1	8.3			
							18.9; C3	11.3				
98	4-C_6H_5	H		C5	C5	15.6; C2	12.1	7.9	6.5	1.1		
					C5	15.9; C2	12.3	8.1	6.7			
Ia acetate			C5	C5	9.9; C3	12.9	7.1	2.5	0.7			
				C5	9.9; C3	13.1	7.3	2.7	0.7			
Ib acetate				C5		C5		22.4; C1				
Ic base			C5	C5	C5	C5	C5	14.9; C3	7.5	2.9	1.5	0.3
				C5	C5	C5	C5	20.9; C2	9.9			
							C5	16.4; C3				
cycloguanil hydrochloride			T5	C3; T2	C5	21.6; C2	13.4; C1	7.9	4.9			
				C2; T3	C5	21.9; C2	13.4; C1	8.1				
pyrimethamine			C1; T2	C2; T3	C5	C3	C1	7.7	6.1	5.3	4.7	3.1

[a] ΔMST is the mean survival time (days) of treated mice (MSTT) minus the mean survival time (days) of control mice (MSTC). In the present study the MSTC ranged from 6.1 to 6.3 days. T signifies the number of toxic deaths, occurring on days 2–5 after infection, which are attributed to drug action. C indicates the number of mice surviving at 60 days postinfection and termed "cured"; data to establish parasitological cure based on subinoculation are unavailable. Each compound was administered as a single sc dose. Each entry at each dose level represents results with a five-animal group.

82, **83**, **85**, **86**, **88**, **90**, **93**, **96**, and **97**, Table III) were evaluated for prophylactic action in chicks.[23,25] White Leghorn cockerels were parasitized by the intrajugular injection of *P. gallinaceum* sporozoites. All control chicks died between 6 and 11 days postinfection. In the present study, the mean survival time of control animals was approximately 8.5 days. A drug is considered active if the mean survival time of treated chicks is at least twice as long as that of untreated control chicks or if any of the chicks survive to 30 days.

The above drugs were suspended in peanut oil and were administered subcutaneously in a single dose on the day of infection. Each compound was tested in groups of five chicks at three to seven dose levels ranging from 7.5 to 480 mg/kg. Sixteen of the thioquinazolines tested possessed prophylactic activity based on the above criteria (Table VII).

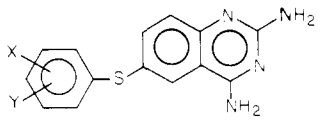
Several compounds possessed outstanding activity, the 4-bromo (**72**) and 4-dimethylamino (**90**) analogues exhibiting a potency comparable with that of pyrimethamine.

Drug Resistance Studies. Expanded chemotherapeutic studies utilizing drug-resistant plasmodia were then undertaken to enable the selection of optimal drug candidates for preclinical toxicological studies and clinical trial. Five of the thioquinazolines (**67**, **72**, **75**, **90**, and **93**) that showed outstanding activity against normal drug-sensitive strains of *P. berghei* in mice were selected for trial against chloroquine-, cycloguanil-, and DDS-resistant lines. These substances displayed no cross resistance with chloroquine, negligible cross resistance with cycloguanil, and less than two- to sixfold cross resistance with DDS.[27,28]

Several of the thioquinazolines (**75**, **80**, **90**, and **93**) were then evaluated against the chloroquine-susceptible, pyrimethamine-resistant Malayan Camp-CH/Q and the chloroquine-resistant, pyrimethamine-susceptible Vietnam Oak Knoll strains of *Plasmodium falciparum* in the *Aotus trivirgatus* owl monkey model.[29,30] These substances cured >50% of the monkeys infected with the pyrimethamine-resistant Malayan Camp strain at daily oral doses ranging from 0.39 to 5 mg/kg per day for 7 days (pyrimethamine = >2.5 mg/kg) and produced >50% cures of the pyrimethamine-sensitive Oak Knoll strain at doses of 0.098–1.56 mg/kg per day for 7 days (pyrimethamine = 0.6 mg/kg).[29–31] The most active compound, namely, 2,4-diamino-6-[(α,α,α-trifluoro-*m*-tolyl)thio]quinazoline (**80**), was designated for preclinical toxicological studies.

Antibacterial Activity. Nearly all of the 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines (**64–66**, **68–79**, and **82–97**, Table III) were tested in vitro against a spectrum of pathogenic bacteria including *Streptococcus*

Table V. Oral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines against Trophozoite-Induced *P. berghei* in Mice[a]



no.	X,Y	form	no. of mice	SD_{90}, mg/kg per day[b]	Q[c]
66	2,5-Cl_2	base	21	4.5	17
72	4-Br	base	21	1.2	62
75	4-Cl	base	35	0.22	340
76	4-F	base	28	0.36	210
77	H	base	21	8.2	9.1
83	5-Cl,2-CH_3	base	28	0.43	170
84	2-CH_3	base	21	4.1	18
85	3-CH_3	base	21	6.2	12
86	4-CH_3	base	28	7.2	10
88	4-OCH_3	base	28	1.2	62
93	3,4- 	base	28	0.08	880
94	4-$C(CH_3)_3$	base	21	18	4.1
Ia		acetate	14	9.5	7.9
Ib		acetate	40	0.27	270
Ic		HCl	63	0.16	470
cycloguanil		HCl	40	2.1	35
pyrimethamine		base	42	0.28	270
trimethoprim		base	21	120	0.6

[a] D, compounds were administered continuously in the diet of mice for 6 consecutive days. [b] All doses were calculated as the free base equivalent. 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 estimated graphically using semilog paper. [c] The quinine equiv Q is the ratio of the SD_{90} of quinine hydrochloride to the SD_{90} of the test substance under comparable experimental conditions.

faecalis (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Pseudomonas aeruginosa* (28), *Escherichia coli*, (Vogel), *Shigella sonnei* (C-10), and *Mycobacterium tuberculosis* $H_{37}Rv$ (Table VIII). A modification of the gradient plate procedure of Szybalski[32] and Webb and Washington[33] was employed throughout.[9] Among them, 26 compounds (**65**, **66**, **68–73**, **75–79**, **83–90**, **93**, **94**, **96**, and **97**) inhibited the growth of *S. faecalis* MGH-2, *S. aureus* UC-76, and *S. aureus* S18713 at drug concentrations of <0.25 μg/mL, and four (**70**, **71**, **76**, and **77**) inhibited the growth of *E. coli* (Vogel) and *S. sonnei* (C-10) at concentrations of <0.25 μg/mL as well (Table VIII). Although end points were not determined, compounds **70**, **71**, **76**, and **77** were equipotent with trimethoprim against all five organisms at the levels tested. In contradistinction with trimethoprim, six thioquinazolines (**68**, **70**, **71**, **79**, **91**, and **96**) were active against *M. tuberculosis* ($H_{37}Rv$) at 5–20 μg/mL, and one compound (**78**) was active against *P. aeruginosa* (28) at 20 μg/mL.

Moreover, the 2,4-diamino-6-(aryl)thioquinazolines proved to be potent folate antagonists. *S. faecalis* R (*Strep. faecium* var. durans, ATCC 8043) is capable of using the fully oxidized form of folate as well as the various reduced forms. Because of this organism's inability to synthesize folate, and its consequent requirement for preformed folate, a precise knowledge of the amount of folate available to the organism is available. Thus, in the presence of 0.4 ng/mL of folic acid, the smallest amount allowing complete growth of the organism, inhibition reveals the overall strength of the inhibitor without providing information as to the nature of the inhibition.[36] A variety of analogues (compounds **67**, **68**, **72**, **74**, **75**, **77**, **81**, **85**, **87**, **90**, **93**, **95**, and **96**) examined in this manner caused 50% inhibition at concentrations of 0.4–2.0 ng/mL, thus exhibiting activity comparable with or superior to pyrimethamine or cycloguanil hydrochloride. Further studies to identify the exact point of inhibition either on the folate transport mechanism or in the folate cycle were not performed.

Experimental Section[34]

2-Nitro-5-[(phenyl- and naphthyl)thio]benzonitriles V (1–33, Table I). Procedure I. A mixture of 19.1 g (0.125 mol) of *p*-dimethylaminobenzenethiol, 22.8 g (0.125 mol) of 5-chloro-2-nitrobenzonitrile, and 17.2 g (0.125 mol) of anhydrous K_2CO_3 in 250 mL of Me_2CO was stirred at room temperature for 20 h. The reaction mixture was filtered and the solid was triturated thoroughly with H_2O to give 7.6 g of insoluble material. The Me_2CO filtrate was concentrated to dryness and the residue was recrystallized from MeCN to give 15.6 g of orange crystals. These two fractions were combined and recrystallilzed from 400 mL of MeCN to give 18.0 g (48%) of 2-nitro-5-[[*p*-(dimethylamino)phenyl]thio]benzonitrile (**25**), mp 168.5–170.5 °C.

Procedure II. A solution of 2.0 g (0.01 mol) of 3-chloro-6-nitro-*o*-tolunitrile, 1.6 g (0.01 mol) of 2-naphthalenethiol, 1.5 g (0.011 mol) of anhydrous K_2CO_3, and 50 mL of Me_2CO was heated under reflux for 1.5 h. The warm reaction mixture was filtered and the filtrate was concentrated to dryness. The residue was recrystallized from 2-PrOH to give 2.2 g (71%) of 3-(2-naphthylthio)-6-nitro-*o*-tolunitrile (**30**), mp 147–149 °C.

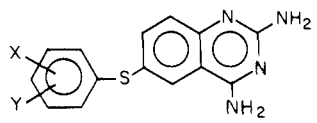
Procedure III. A mixture of 20.5 g (0.128 mol) of 2-naphthalenethiol, 24.5 g (0.134 mol) of 5-chloro-2-nitrobenzonitrile, and 19.5 g (0.141 mol) of anhydrous K_2CO_3 in 300 mL of C_6H_6 was stirred under reflux for 4 h and filtered hot to remove the salt. The C_6H_6 solution was concentrated and cooled. The brittle yellow solid which precipitated was collected and dried. After recrystallization from MeCN, 18.1 g (46%) of 2-nitro-5-(2-naphthylthio)benzonitrile (**28**), mp 139–141 °C, was obtained.

The other requisite 2-nitro-5-[(phenyl- and naphthyl)thio]-benzonitriles not listed in Table I were prepared and purified in a similar manner but were not analyzed and were used directly in the reduction step.

2,5-Bis[(*p*-chlorophenyl)thio]benzonitrile (VI). To a stirred solution of 8.7 g (0.06 mol) of *p*-chlorobenzenethiol in 20 mL of DMF at 5–10 °C was added 2.6 g (0.06 mol) of a 57% dispersion of NaH in mineral oil. The solution was allowed to warm to room temperature, and a solution of 9.1 g (0.05 mol) of 5-chloro-2-nitrobenzonitrile (IVa) in 12 mL of DMF was added. The mixture was stirred overnight at room temperature and then warmed at 50 °C for 0.75 h. The resulting mixture was cooled to room temperature, diluted with 200 mL of $C_6H_5CH_3$, and washed with H_2O. The organic layer was separated and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue, which was washed with isooctane, crystallized. The crude solid was recrystallized twice from MeCN, once from $C_6H_5CH_3$–isooctane, and once from DMF–H_2O to provide 8.6 g (37%) of the title compound, mp 119.5–121.5 °C. Anal. ($C_{19}H_{11}Cl_2NS_2$) C, H, N.

2-Amino-5-[(phenyl- and naphthyl)thio]benzonitriles VII (34–63, Table II). Procedure IV. To a well-stirred solution of 14.8 g (0.066 mol) of $SnCl_2 \cdot 2H_2O$ in a mixture of 40 mL of concentrated HCl and 20 mL of glacial HOAc was added slowly a warm solution of 6.0 g (0.02 mol) of 2-nitro-5-(2-naphthylthio)benzonitrile (**28**) in 60 mL of glacial HOAc. The temperature of the mixture was maintained below 20 °C during the addition by external cooling. The resulting yellow suspension was stirred for 18 h at room temperature during which time a white suspension formed. The product was poured into a stirred ice–water mixture containing 110 mL of 50% aqueous NaOH. The colorless precipitate was collected by filtration, washed with H_2O, and air-dried. This crude product was triturated with boiling 95% EtOH and the insoluble inorganic residue was removed by filtration. The chilled filtrate yielded a colorless crystalline solid which was collected and dried in vacuo at 45 °C for 18 h to give 3.3 g (59%) of 2-amino-5-(2-naphthylthio)benzonitrile (**61**), mp 140.5–142.5 °C.

Table VI. Parenteral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines against Trophozoite-Induced *P. gallinaceum* in Chicks



		ΔMST; C or T[a-c] after single sc dose, mg/kg										
no.	X,Y	320	160	100	80	40	20	10	5	2.5	1.25	0.63
67	3,4-Cl_2			20.8; C1								
72	4-Br					12.8	11.8	8.2	7.2	6.0	3.0	
73	2-Cl	20.8	19.8		10.2	7.6	6.0	0.0				
75	4-Cl	C5	22.2; C2		16.7							
76	4-F	19.3; C1	15.5; C1		13.0	9.2	7.8	7.4	5.8	3.2	0.0	0.0
							8.0	7.2				
77	H	11.4	7.8		6.4	6.2	5.6	0				
80	3-CF_3		15.4									
83	5-Cl,2-CH_3	C5	C5		18.0; C1	7.8	7.4	5.8	3.6	1.4	0.0	0.0
							7.6	5.8				
84	2-CH_3	16.0; C4	17.0; C2		12.0	11.0	7.8	6.6	2.4	0.0	0.0	0.0
							7.6	6.6				
85	3-CH_3	T5	10.7; C2		7.8	6.4	6.2	6.2	0.0	0.0	0.0	0.0
							6.2	6.0				
86	4-CH_3	20.0; C4	22.2		15.2	10.4	7.6	4.6				
90	4-$N(CH_3)_2$						C5	C5	19.8	12.6	10.4	6.6
92	2,3- 						17.4	12.4	11.8	9.0	7.2	2.2
93	3,4- 	16.0; C4	15.0; C4		16.2	12.4	7.8	6.2				
97	3-C_6H_5						0.0	0.0	0.0	0.0	0.0	0.0
Ia base		16.6; C3	15.3; C2		12.9; C2	12.1; C1	7.0	2.2				
Ib acetate		16.9; C4	16.9; C4[d]		16.9; C4							
Ic base		C3; T1	21.0; C3		20.6	15.6	10.6	7.4	6.8	6.0	1.4	
cycloguanil hydrochloride			C1; T1[d]		15.1; C1[e]	11.7; C1[f]						

[a] ΔMST is the mean survival time (days) of treated chicks (MSTT) minus the mean survival time (days) of control chicks (MSTC). [b] Chicks surviving to 30 days postinfection are termed "cured"; data to establish parasitological cure based on subinoculation are unavailable. [c] Deaths occurring within 48 h after infection are attributed to drug action and are counted as toxic deaths. Control birds do not die before 48 h. Each entry at each dose level represents results with a five-animal group. [d] At 120 mg/kg. [e] At 60 mg/kg. [f] At 30 mg/kg.

Procedure V. To a stirred solution of 18.0 g (0.08 mol) of $SnCl_2 \cdot 2H_2O$ in a mixture of 60 mL of concentrated HCl and 10 mL of glacial HOAc was added a warm solution of 7.5 g (0.0258 mol) of 2-nitro-5-[(*p*-chlorophenyl)thio]benzonitrile (**10**) in 50 mL of glacial HOAc. The stirred mixture was heated at 50 °C for 2 h, stirred for 2 h at room temperature, and then poured into 1 L of an ice–H_2O mixture containing 110 mL of 50% aqueous NaOH. The crude product was collected, washed with H_2O, and air-dried. Recrystallization from 70% aqueous EtOH (decolorizing charcoal) afforded 4.5 g (67%) of 2-amino-5-[(*p*-chlorophenyl)thio]benzonitrile (**43**), mp 123–125 °C.

The other intermediate 2-amino-5-[(phenyl- and naphthyl)thio]benzonitriles not listed in Table II were synthesized and purified in an analogous manner but were not analyzed and were used directly in the cyclization reaction.

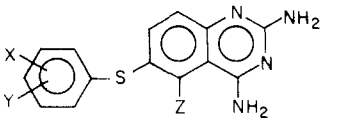
2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines VIII (64–98, Table III). Procedure VI. A mixture of 6.1 g (0.0207 mol) of 2-amino-5-[(2,5-dichlorophenyl)thio]benzonitrile (**35**) and 2.6 g (0.0226 mol) of chloroformamidine hydrochloride[9] in 10 mL of diglyme was stirred and heated in an oil bath to 150 °C (external temperature). As HCl was evolved a clear solution first formed, followed by the separation of a new precipitate. After 1 h at 150 °C, the mixture was cooled and the solid was collected, washed with EtOAc, and air-dried. The crude product was dissolved in hot DMF containing an excess of Et_3N, and the resulting solution was poured into a solution of cold 0.1 N NaOH. The pale yellow solid that separated was collected by filtration and dried. Trituration with boiling EtOH gave an insoluble fraction and a fraction resulting from cooling the EtOH filtrate. Both fractions were recrystallized from EtOAc and dried in vacuo at 50 °C for 24 h to give 1.7 g (24%) of 2,4-diamino-6-[(2,5-dichlorophenyl)thio]quinazoline (**66**), mp 250–252 °C.

Procedure VII. A mixture of 3.2 g (0.0116 mol) of 2-amino-5-(2-naphthylthio)benzonitrile (**61**) and 1.5 g (0.013 mol) of chloroformamidine hydrochloride[9] in 7 mL of dry diglyme was stirred and heated at 150 °C (oil bath temperature) for 0.75 h. Upon cooling, the reaction mixture was diluted with 7 mL of diglyme and the solid was collected by filtration, washed with EtOAc, and dried. The product was recrystallized once from aqueous EtOH containing excess NH_4OH and once from anhydrous EtOH. After drying in vacuo at 50 °C for 18 h, 2,4-diamino-6-(2-naphthylthio)quinazoline (**93**) (1.4 g, 38%) was obtained as pale yellow crystals, mp 226–228 °C.

Procedure VIII. A mixture of 2.2 g (0.0067 mol) of 6-amino-2-chloro-3-[(α,α,α-trifluoro-*m*-tolyl)thio]benzonitrile (**47**), 1.5 g (0.0134 mol) of chloroformamidine hydrochloride,[11] and 2.0 g of Me_2SO_2 was ground and heated with stirring to 165 °C (external temperature) in an oil bath. The mixture was maintained at this temperature for 0.6 h. During this time a solution formed at 158 °C and 15 min later a solid precipitated. The reaction mixture was cooled, suspended in 100 mL of hot H_2O, and made basic with 50% aqueous NaOH. A soft sticky precipitation formed which solidified upon cooling. The solid was collected by filtration, washed with H_2O, and crystallized from DMF·H_2O (decolorizing charcoal) to give 1.9 g (75%) of 2,4-diamino-5-chloro-6-[(α,α,α-trifluoro-*m*-tolyl)thio]quinazoline (**79**) as yellow crystals, mp 180–184 °C.

2-Amino-5-(2-naphthylthio)benzamide (IX). To a stirred solution of 19.3 g (0.0875 mol) of $SnCl_2 \cdot 2H_2O$ in a mixture of 50 mL of concentrated HCl and 10 mL of glacial HOAc was added a warm (70 °C) solution of 8.1 g (0.0264 mol) of 2-nitro-5-(2-naphthylthio)benzonitrile (**28**) in 70 mL of glacial HOAc and 30 mL of DMF. The internal temperature was maintained at 60–70 °C during the 0.5-h addition period. The pale yellow suspension was stirred overnight at room temperature and filtered, and the filtrate was poured into cold NaOH. The colorless solid which precipitated was collected, washed with H_2O, and dried. The crude product was triturated with hot EtOH and filtered. Evaporation

Table VII. Parenteral Prophylactic Antimalarial Effects of 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines against Sporozoite-Induced *P. gallinaceum* in Chicks



			ΔMST; C or T[a-d] after single sc dose, mg/kg						
no.	X,Y	Z	480 (320)	240 (160)	120 (80)	60 (40)	30 (20)	15 (10)	7.5 (5)
65	2,3,5,6-F_4	H	C5	C5	C5	C5	2.7; C4	1.7; C4	
72	4-Br	H	C5	C5	C5	C5	C5	C5	C5
				C5	C5	C5	C5	C5	
73	2-Cl	H	(1.9; C4)	(C5)	(17.9; C4)				
76	4-F	H	(C3; T2)	(C4; T1)	(0.5; C4)	(0.5; C4)	(0.5; C4)	(6.5; C1)	
77	H	H	T5	1.6; T4	2.4				
78	4-OH	H	C5	C5	C5	C5	C5	4.7; C3	
79	3-CF_3	Cl	(C5)	(C5)	(C5)				
80	3-CF_3	H	(C5)	(C5)	(C5)	(C5)	(C5)	(7.3; C4)	
82	4-Br,3-CH_3	H	C4; T1	C4; T1	C4; T1	C4; T1	C4; T1	C4; T1	
83	5-Cl,2-CH_3	H	(C5)	(C5)	(C5)	(C5)	(C5)	(7.0; C4)	
				(C5)	(C5)	(C5)			
85	3-CH_3	H	(C2; T2)	(C2; T2)	(4.7; C3)	(1.7; C3)	(4.2; C1)	(1.8)	
				(C2; T2)	(C2; T1)	(3.1; C3)	(3.8; C2)	(2.7)	(0.3)
86	4-CH_3	H	C3; T2	C5	C5	C5			
88	4-OCH_3	H	(C4; T1)	(C5)	(C5)	(C5)	(3.2; C4)	(2.2; C4)	
90	4-$N(CH_3)_2$	H	C5	C5	C5	C5	C5	C5	
				C5	C5	C5			
93	3,4- 	H	(C5)	(C5)	(C5)				
96	2-C_6H_5	H	C5	C5	C5	0.0; C4	0.0; C4	0.0; C4	
97	3-C_6H_5	H	C5	C5	0.3; C4	0.3; C4	1.0; C2	0.7	
Ia acetate			C4, T1	0.0; C4	0.0; C4	0.0; C4	0.0; C4	3.1; C2	2.3
				C5	0.0; C4	0.0; C4	0.0; C4	3.1	C5
				C5	C5	C5	C5	C5	
Ib acetate			C4; T1	C5	C5	C5	0.0; C4	0.0; C3	
			(C5)	(C5)	(C5)				
cycloguanil hydrochloride			T5	T5	T5	1.6	2.3	1.6	
				T5		0.4; T1		0.0	
pyrimethamine			(C3; T2)	(C5)	(C5)	(C5)	(C5)	(C5)	

[a] ΔMST is the mean survival time (days) of treated chicks (MSTT) minus the mean survival time (days) of control chicks (MSTC). [b] All control chicks die between 6 and 11 days, with a MSTC of approximately 8.5 days. Chicks surviving to 30 days postinfection are termed "cured" and are designated C. [c] Deaths occurring before day 6 are usually attributed to drug toxicity and are designated T. Each entry at each dose level represents results with a five-animal group. [d] Results enclosed in parentheses correspond to doses designated by parentheses.

of the filtrate left a solid residue which was recrystallized from $C_6H_5CH_3$. Several small crops were collected giving 2.05 g, mp 200–202 °C. The EtOH insoluble material was triturated with Me_2CO and filtered. The filtrate was evaporated to dryness and the residue was recrystallized from $C_6H_5CH_3$ to give an additional 0.4 g: mp 192–195 °C; total yield, 2.45 g (32%). The IR spectrum contained a carbonyl band at 1658 cm^{-1}. Anal. ($C_{17}H_{14}N_2OS$) C, H, N.

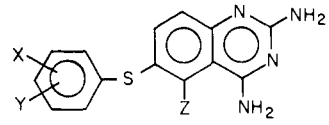
2-Amino-6-(2-naphthylthio)-4(3*H*)-quinazolinone (X). A mixture of 2.2 g (0.0075 mol) of 2-amino-5-(2-naphthylthio)-benzamide (IX) and 0.97 g (0.0084 mol) of chloroformamidine hydrochloride[11] in 5 mL of dry diglyme was heated to 145 °C (bath temperature). A brisk evolution of HCl occurred and a clear solution formed from which a new solid was deposited. After 1.5 h at 145 °C, the mixture was cooled and diluted with Et_2O, and the solid was collected and dried. The crude product was recrystallized from 90% aqueous EtOH containing excess Et_3N. After drying in vacuo for 18 h, 0.4 g (17%) of the title compound, mp 310 °C dec, was obtained. A second crop, 0.4 g, brought the total yield to 33%. The IR spectrum showed carbonyl absorption at 1660 cm^{-1}. Anal. ($C_{18}H_{13}N_3OS$) C, H, N, S.

6-(2-Naphthylthio)-2,4(1*H*,3*H*)-quinazolinedithione (XI). A solution of 4.5 g (0.016 mol) of 2-amino-5-(2-naphthylthio)-benzonitrile (61) in 18 mL of pyridine and 18 mL of CS_2 was heated under reflux for 2 h. The cooled solution was diluted with 270 mL of anhydrous EtOH and the yellow solid that formed was collected, washed with EtOH and Et_2O, and dried to give 2.0 g (35%) of the desired product, mp 308–310 °C. Anal. ($C_{18}H_{12}N_2S_3$) C, H, N.

2,3-Dichloro-6-nitrobenzonitrile (IVb). A mixture of 45.3 g (0.20 mol) of 1,2,3-trichloro-4-nitrobenzene[14] and 17.9 g (0.21 mol) of CuCN in 120 mL of 1-methyl-2-pyrrolidone was stirred and heated in an oil bath at 145–150 °C (external temperature) for 6 h. The reaction mixture was allowed to cool to room temperature and was stirred at room temperature for 18 h. The mixture was poured with stirring into 1.6 L of H_2O. Within 15 min, the precipitate had solidified and the crude brown-black solid was collected by filtration, washed thoroughly with H_2O, and air-dried. A second identical run was made, and the combined crude solids were stirred with 1.2 L of boiling EtOH for 45 min and filtered. The filtrate was treated with decolorizing charcoal, the charcoal was removed by filtration, and the filtrate was concentrated to dryness in vacuo. The residue was crystallized from 400 mL of EtOH to give 51.0 g (59%) of product, mp 75–92 °C, which was of satisfactory purity for use in successive reactions. A sample was recrystallized from EtOH to give an analytical sample, mp 90–93 °C. Anal. ($C_7H_2Cl_2N_2O_2$) C, H, Cl, N.

3-Chloro-6-nitro-*o*-tolunitrile (IVc). A mixture of 1.87 g (0.01 mol) of 3-chloro-6-nitro-*o*-toluidine,[15] 1.0 g (0.01 mol) of H_2SO_4, and 20 mL of HOAc was warmed to obtain a solution and then cooled to 15 °C. *n*-Butyl nitrite (1.5 mL, 0.013 mol) was added slowly and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with 100 mL of Et_2O and the solid diazonium compound was collected by filtration. To a freshly prepared solution of CuCN [from 5.3 g (0.021 mol) of $CuSO_4 \cdot 5H_2O$, 5.8 g (0.089 mol) of KCN, and 30 mL of H_2O], the diazonium salt was added portionwise. The foaming reaction mixture was stirred for 1 h at room temperature and then at 75

Table VIII. In Vitro Antibacterial Effects of 2,4-Diamino-6-[(phenyl- and naphthyl)thio]quinazolines



			min inhibitory concn, μg/mL[a]						
no.	X,Y	Z	*S.f.* MGH-2[c]	*S.a.* UC-76[d]	*S.a.* S18713[d]	*P.a.* 28[e]	*E.c.* Vogel[f]	*S.s.* C-10[g]	*M.t.* $H_{37}Rv$[h]
64	2,3,4,5,6-Cl_5	H	1.5	10	20	>25	>25	>25	N[b]
65	2,3,5,6-F_4	H	<0.25	<0.25	<0.25	>25	2.0	15	N
66	2,5-Cl_2	H	<0.25	<0.25	<0.25	>25	1.0	10	N
68	3,5-Cl_2	H	<0.25	<0.25	<0.25	>25	2.0	20	20
69	2-Cl	Cl	<0.25	<0.25	<0.25	>25	<0.25	1.0	>25
70	4-F	Cl	<0.25	<0.25	<0.25	>25	<0.25	<0.25	5.0
71	H	Cl	<0.25	<0.25	<0.25	>25	<0.25	<0.25	10
72	4-Br	H	<0.25	<0.25	<0.25	>25	1.0	2.0	N
73	2-Cl	H	<0.25	<0.25	<0.25	>25	<0.25	2.0	N
74	3-Cl	H	10	2.5	5.0	>25	10	20	>25
75	4-Cl	H	<0.25	<0.25	<0.25	>25	1.0	5.0	N
76	4-F	H	<0.25	<0.25	<0.25	>25	<0.25	<0.25	N
77	H	H	<0.25	<0.25	<0.25	>25	<0.25	<0.25	N
78	4-OH	H	<0.25	<0.25	<0.25	20	2.5	15	>25
79	3-CF_3	Cl	<0.25	<0.25	<0.25	>25	0.5	2.0	10
82	4-Br,3-CH_3	H	10	5	10	>25	20	20	>25
83	5-Cl,2-CH_3	H	<0.25	<0.25	<0.25	>25	<0.25	1.5	N
84	2-CH_3	H	<0.25	<0.25	<0.25	>25	<0.25	1.0	N
85	3-CH_3	H	<0.25	<0.25	<0.25	>25	<0.25	1.5	N
86	4-CH_3	H	<0.25	<0.25	<0.25	>25	<0.25	0.5	N
87	3-OCH_3	H	<0.25	<0.25	<0.25	>25	<0.25	1.0	N
88	4-OCH_3	H	<0.25	<0.25	<0.25	>25	<0.25	1.5	N
89	4-$NHCOCH_3$	H	<0.25	<0.25	<0.25	>25	2.5	>25	>25
90	4-$N(CH_3)_2$	H	<0.25	<0.25	<0.25	>25	1.5	10	N
91	3,4- 	Cl	<0.25	1.5	5.0	>25	10	>25	10
92	2,3- 	H	10	2.5	5.0	>25	15	20	>25
93	3,4- 	H	<0.25	<0.25	<0.25	>25	<0.25	2.5	N
94	4-$C(CH_3)_3$	H	<0.25	<0.25	<0.25	>25	10	15	N
95	3,4- 	CH_3	<2.5	<2.5	<2.5	>25	<2.5	<2.5	N
96	2-C_6H_5	H	<0.25	<0.25	<0.25	>25	2.5	>25	20
97	3-C_6H_5	H	<0.25	<0.25	<0.25	>25	10	>25	>25
trimethoprim			<0.25	<0.25	<0.25	>25	<0.25	<0.25	>25

[a] Gradient plate test. [b] N = not tested. [c] *S.f.* = *Streptococcus faecalis.* [d] *S.a.* = *Staphylococcus aureus.* [e] *P.a.* = *Pseudomonas aeruginosa.* [f] *E.c.* = *Escherichia coli.* [g] *S.s.* = *Shigella sonnei.* [h] *M.t.* = *Mycobacterium tuberculosis.*

°C for 1 h. The reaction mixture was filtered hot and the solid was extracted with 100 mL of boiling EtOH. The EtOH solution was concentrated in vacuo to dryness and the residue was recrystallized from aqueous EtOH to give 1.4 g (71%) of product, mp 91–94 °C. Anal. ($C_8H_5ClN_2O_2$) C, H, N.

(2,4-Diamino-6-quinazolinyl)(phenylthio)diimide (XIVa). 2,4,6-Triaminoquinazoline (XIIa)[17] (1.75 g, 0.010 mol) was dissolved in 25 mL of H_2O and 4.3 mL of concentrated HCl and diazotized at 0–2 °C by the addition of a solution of 0.69 g (0.010 mol) of $NaNO_2$ in 5 mL of H_2O. The mixture was stirred at 0–5 °C for 0.5 h and was added portionwise over 20 min to a solution of 1.21 g (0.011 mol) of benzenethiol in 25 mL of H_2O and 5.6 g of KOH maintained at 40–45 °C. After the addition was complete, the reaction mixture was stirred at 40–45 °C for 1 h, cooled to room temperature, and acidified with HOAc. The resulting precipitate was collected by filtration and the filter cake was slurried in 1 N NaOH. The precipitate was collected, washed with cold H_2O, and dried to give 2.75 g (93%) of product, mp 158 °C dec. For analysis, a sample was recrystallized from $CHCl_3$–MeOH (1:1) to give brilliant yellow crystals, mp 159 °C dec. Anal. ($C_{14}H_{12}N_6S$) C, H, N.

(2,4-Diamino-5-chloro-6-quinazolinyl)[(*p*-methoxyphenyl)thio]diimide (XIVb). To a slurry of 2.28 g (0.01 mol) of 2,4,6-triamino-5-chloroquinazoline hydrate (XIIb)[17] in 50 mL of H_2O was added 4.3 mL of concentrated HCl. A solution rapidly formed, followed by the precipitation of the hydrochloride salt. The mixture was cooled to −5 °C, treated with a solution of 0.69 g (0.010 mol) of $NaNO_2$ in 5 mL of H_2O, and stirred at 0–5 °C for 0.5 h. The cold diazonium salt solution was slowly added to a solution of 1.54 g (0.011 mol) of *p*-methoxybenzenethiol in 5.6 g of KOH and 25 mL of H_2O, and the mixture was stirred at room temperature for 2.5 h. The resulting yellow suspension was neutralized with HOAc, and the precipitate was collected by filtration, slurried in 50 mL of 1 N NaOH, collected, washed with H_2O, and dried. The dark yellow crystals thus obtained weighed 3.52 g (98%), mp 100–105 °C with foaming. Anal. ($C_{15}H_{13}ClN_6OS$) C, H.

References and Notes

(1) This is paper 39 of a series on antimalarial drugs. For paper 38, and the previous communication on folate antagonists, see ref 10.
(2) This investigation was supported by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is Contribution No. 1484 to the Army Research Program on Malaria.
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(19) For a description of the test method, see ref 20.
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(22) For a description of the test method see ref 5 and 6.
(23) Parenteral antimalarial screening against *P. gallinaceum* in chicks was carried out by Dr. Leo Rane at the University of Miami, and test results were supplied through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
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(36) For a more complete description of such antimetabolite studies, see ref 4. These studies were carried out by Dr. C. C. Smith and co-workers at the University of Cincinnati, Cincinnati, Ohio.

Notes

Synthetic Sulfur-Containing Amino Acids. Inhibition of Transport Systems in S37 Ascites Tumor Cells

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The preparation of a series of synthetic sulfur-containing amino acids is described. These compounds included heterocyclic analogues of L-cysteine, DL-norcysteine, and DL-homocysteine. The amino acids were assessed for their ability to inhibit the neutral amino acid transport systems of the Sarcoma 37 ascites tumor cell and their inhibitions were compared with those of L-methionine and L-ethionine. Transport studies indicated that the amino acids synthesized were capable of inhibiting the uptake of [^{3}H]-L-histidine in the S37 cell.

Amino acid uptake in mammalian cells may be by multiple transport systems of overlapping specificities.[1] In 1962 Ahmed and Scholefield[2] proposed the systematic use of competitive inhibitions to determine whether uptake of a given solute was by a single transport system or by several. Oxender and Christensen[3] utilized patterns of competitive inhibition in proposing that uptake of neutral amino acids in Ehrlich ascites tumor cells was mediated by two major transport systems, one termed the L system and one identified as the A system. An alternate approach of good utility in discriminating the amino acid transport systems of S37 ascites tumor cells from each other is based on histidine interactions with two neutral amino acid systems with disparate kinetic parameters such that bi-

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