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Folate Antagonists. 13. 2,4-Diamino-6-[(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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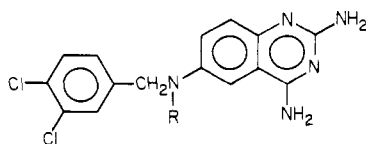
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An array of nonclassical thioquinazoline analogues (VIII) of methotrexate was prepared by cyclization of the requisite 2-amino-5-(arylthio)benzonitrile with chloroformamidine hydrochloride (28-79%). The aminonitrile precursors were obtained by SnCl_2 -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 -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 $<0.25 \mu\text{g/mL}$. 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.

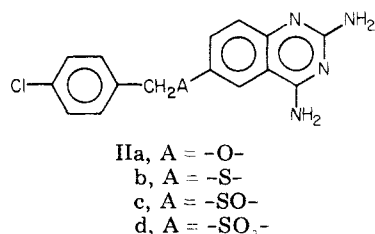
A plethora of 2,4-diamino-6-[(benzyl)amino]quinazoline antifolates, exemplified by 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia),^{4,5} 2,4-diamino-6-[(3,4-di-



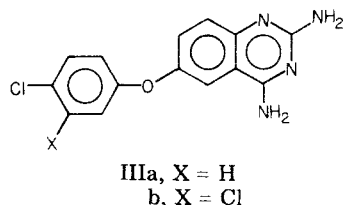
Ia, R = H
b, R = NO
c, R = CH₃

chlorobenzyl)nitrosamino]quinazoline (Ib),^{6,7} and 2,4-di-

amino-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-



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



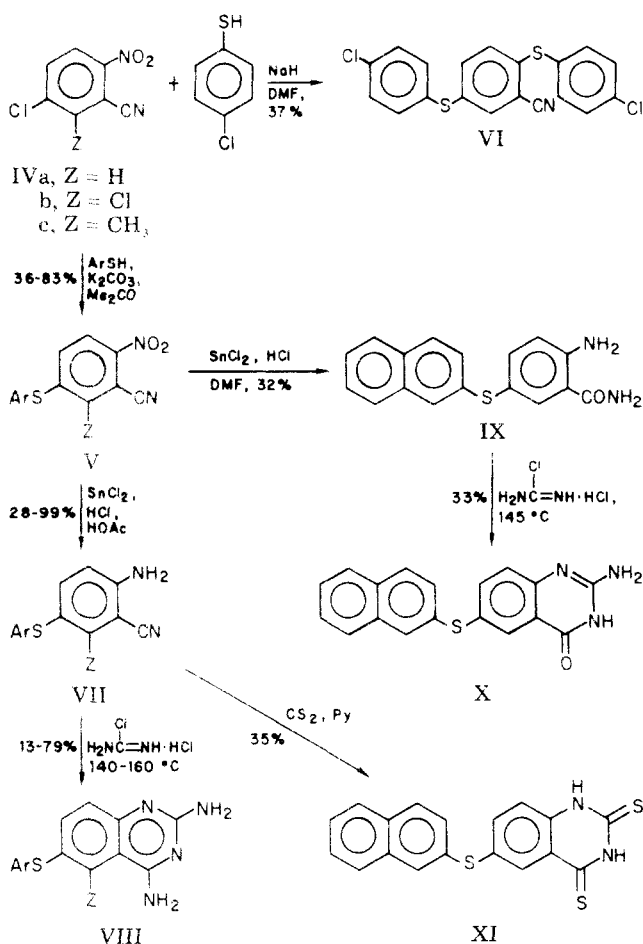
diamino-6-(3,4-dichlorophenoxy)quinazoline (IIIb) displayed oral antimalarial effects against *P. berghei* in mice comparable with or superior to 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia).⁹ We now report the synthesis and biological properties of an array of 2,4-diamino-6-[(phenyl- and 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 K₂CO₃ 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 SnCl₂·2H₂O·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 chloroformamide hydrochloride⁸⁻¹¹ in diglyme or dimethyl sulfone¹² to the desired 2,4-diamino-6-[(phenyl- and naphthyl)thio]quinazolines VIII (64-98, Table III) (13-79%) (procedures VI-VIII).

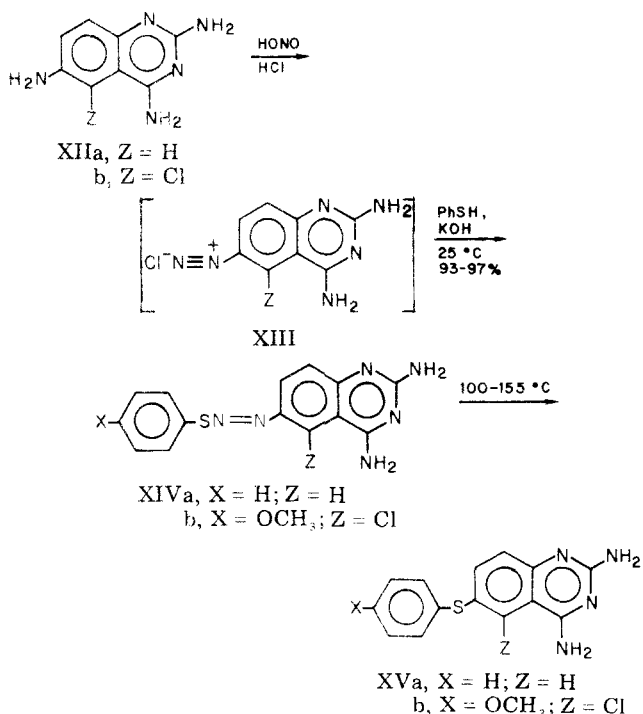
One attempt to effect the reduction of 2-nitro-5-(2-naphthylthio)benzonitrile (28) (V, Ar = 2-C₁₀H₇; Z = H) with SnCl₂·2H₂O·HCl in a mixture of glacial acetic acid and *N,N*-dimethylformamide at 60-70 °C gave 2-amino-5-(2-naphthylthio)benzamide (IX, 32%) instead of the expected 2-amino-5-(2-naphthylthio)benzonitrile (61). Ring closure of IX utilizing chloroformamide hydrochloride¹¹ 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-C₁₀H₇; 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-nitrobenzene¹⁴ with cuprous cyanide in 1-methyl-2-pyrrolidone at 145-150 °C. Diazotization of

Scheme I



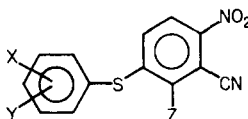




Scheme II



3-chloro-6-nitro-*o*-toluidine¹⁵ 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,

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

|  | | | | | | | | |
|---|--|-----------------|-------------|-------------------|--|-----------|---|----------------------|
| no. | X, Y | Z | mp, °C | yield purified, % | purificn solvent | procedure | formula | analyses |
| 1 | 2,3,5,6-F ₄ | H | 173-175.5 | 69 | EtOH-MeCN | I | C ₁₃ H ₄ F ₄ N ₂ O ₂ S | C, H, N |
| 2 | 2,5-Cl ₂ | H | 106-109 | 38 | EtOAc-MeOH | II | C ₁₃ H ₆ Cl ₂ N ₂ O ₂ S | C, H, N |
| 3 | 3,5-Cl ₂ | H | 138-140 | 54 | EtOAc-isooctane | I | C ₁₃ H ₆ Cl ₂ N ₂ O ₂ S | C, H; N ^a |
| 4 | 2-Cl | Cl | 122-125 | 36 | EtOH | I | C ₁₃ H ₆ Cl ₂ N ₂ O ₂ S | C, H, N |
| 5 | 4-F | Cl | 175-178 | 74 | MeCN | II | C ₁₃ H ₆ ClFN ₂ O ₂ S | C, H, N |
| 6 | H | Cl | 126-127.5 | 66 | EtOH | II | C ₁₃ H ₇ ClN ₂ O ₂ S | C, H, N |
| 7 | 4-Br | H | 121-125 | 58 | EtOAc | II | C ₁₃ H ₇ BrN ₂ O ₂ S | C, H, N |
| 8 | 2-Cl | H | 107-110 | 48 | EtOH | II | C ₁₃ H ₇ ClN ₂ O ₂ S | C, H, N |
| 9 | 3-Cl | H | 124-126 | 56 | EtOAc-isooctane | II | C ₁₃ H ₇ ClN ₂ O ₂ S | C, H, N |
| 10 | 4-Cl | H | 107-109 | 49 | MeCN | II | C ₁₃ H ₇ ClN ₂ O ₂ S | C, H, N |
| 11 | 4-F | H | 116.5-119.5 | 66 | EtOH | II | C ₁₃ H ₇ FN ₂ O ₂ S | C, H, N |
| 12 | H | H | 122-124 | 51 | EtOAc-isooctane | I | C ₁₃ H ₈ N ₂ O ₂ S | C, H, N |
| 13 | 4-OH | H | 161.5-163.5 | 45 | EtOH | I | C ₁₃ H ₈ N ₂ O ₃ S | C, H, N |
| 14 | 3-CF ₃ | Cl | 117-121 | 62 | 2-PrOH | II | C ₁₄ H ₆ ClF ₃ N ₂ O ₂ S | C, H, N, S |
| 15 | 3-CF ₃ | H | 96-98.5 | 73 | EtOH | II | C ₁₄ H ₇ F ₃ N ₂ O ₂ S | C, H, N |
| 16 | 3,4-Cl ₂ | CH ₃ | 160-162 | 44 | 2-PrOH | II | C ₁₄ H ₈ Cl ₂ N ₂ O ₂ S | C, H, N |
| 17 | 4-Br, 3-CH ₃ | H | 119-121 | 59 | MeCN | I | C ₁₄ H ₉ BrN ₂ O ₂ S | C, H, N |
| 18 | 5-Cl, 2-CH ₃ | H | 101-105 | 38 | EtOH | II | C ₁₄ H ₁₀ ClN ₂ O ₂ S | H, N; C ^b |
| 19 | 2-CH ₃ | H | 104-105.5 | 58 | EtOH | I | C ₁₄ H ₁₀ N ₂ O ₂ S | C, H, N |
| 20 | 3-CH ₃ | H | 110-112 | 38 | C ₆ H ₆ -cyclohexane | II | C ₁₄ H ₁₀ N ₂ O ₂ S | C, H, N |
| 21 | 4-CH ₃ | H | 120-121.5 | 64 | EtOH | I | C ₁₄ H ₁₀ N ₂ O ₂ S | C, H, N |
| 22 | 3-OCH ₃ | H | 120-122 | 72 | MeCN-H ₂ O | I | C ₁₄ H ₁₀ N ₂ O ₃ S | C, H, N |
| 23 | 4-OCH ₃ | H | 86-88 | 36 | C ₆ H ₅ CH ₃ -isooctane | II | C ₁₄ H ₁₀ N ₂ O ₃ S | C, H, N |
| 24 | 4-NHCOCH ₃ | H | 241-243.5 | 67 | MeCN | II | C ₁₅ H ₁₁ N ₃ O ₃ S | C, H, N |
| 25 | 4-N(CH ₃) ₂ | H | 168.5-170.5 | 48 | MeCN | I | C ₁₅ H ₁₃ N ₃ O ₂ S | C, H, N |
| 26 | 3,4-  | Cl | 145-148 | 68 | 2-PrOH-MeCN | II | C ₁₇ H ₉ ClN ₂ O ₂ S | C, H, N |
| 27 | 2,3-  | H | 153.5-155.5 | 56 | MeCN | II | C ₁₇ H ₁₀ N ₂ O ₂ S | C, H, N |
| 28 | 3,4-  | H | 139-141 | 46 | MeCN | III | C ₁₇ H ₁₀ N ₂ O ₂ S | C, H, N |
| 29 | 4-C(CH ₃) ₃ | H | 93-95 | 83 | 2-PrOH | II | C ₁₇ H ₁₆ N ₂ O ₂ S | C, H, N |
| 30 | 3,4-  | CH ₃ | 147-149 | 71 | 2-PrOH | II | C ₁₈ H ₁₂ N ₂ O ₂ S | C, H, N |
| 31 | 2-C ₆ H ₅ | H | 117-118.5 | 83 | EtOAc-isooctane | I | C ₁₉ H ₁₂ N ₂ O ₂ S | C, H, N |
| 32 | 3-C ₆ H ₅ | H | 97.5-99 | 83 | EtOAc-isooctane | I | C ₁₉ H ₁₂ N ₂ O ₂ S | C, H, N |
| 33 | 4-C ₆ H ₅ | H | 143-145 | 37 | EtOH | I | C ₁₉ H ₁₂ N ₂ O ₂ S | C, H, N |

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

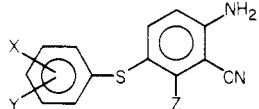
was attempted predicated on a report by Baker et al.¹⁶ that treatment of the diazonium salt of 5-amino-3,4-dihydroquinazolin-4-one with sodium thiophenoxide on one occasion afforded 5-(phenylthio)-3,4-dihydroquinazolin-4-one (28%), although these authors were unable to duplicate their results.¹⁶ Diazotization 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, % | purificn solvent | procedure | formula | analyses |
|-----|--|-----------------|-------------|-------------------|--|-----------|--|---------------------------|
| 34 | 2,3,5,6-F ₄ | H | 108-110 | 68 | EtOH-aq NaOH | IV | C ₁₃ H ₆ F ₄ N ₂ S | C, H, N |
| 35 | 2,5-Cl ₂ | H | 155-157.5 | 55 | EtOH | IV | C ₁₃ H ₈ Cl ₂ N ₂ S | C, H, N |
| 36 | 3,4-Cl ₂ | H | 123-125 | 34 | 2-PrOH | IV | C ₁₃ H ₈ Cl ₂ N ₂ S | C, H, N |
| 37 | 2-Cl | Cl | 181-184 | 79 | EtOH | IV | C ₁₃ H ₈ Cl ₂ N ₂ S | C, H, N |
| 38 | 4-F | Cl | 156-158 | 76 | EtOH-H ₂ O | IV | C ₁₃ H ₈ ClFN ₂ S | C, H, N |
| 39 | H | Cl | 171-172.5 | 66 | EtOH | IV | C ₁₃ H ₉ ClN ₂ S | C, H, N |
| 40 | 4-Br | H | 133-136.5 | 63 | EtOH | V | C ₁₃ H ₉ BrN ₂ S | C, H, N |
| 41 | 2-Cl | H | 104-106.5 | 57 | EtOH | IV | C ₁₃ H ₉ ClN ₂ S | H, N; C ^a |
| 42 | 3-Cl | H | 103-105 | 70 | EtOH-H ₂ O | IV | C ₁₃ H ₉ ClN ₂ S | C, H, N |
| 43 | 4-Cl | H | 123-125 | 67 | EtOH-H ₂ O | V | C ₁₃ H ₉ ClN ₂ S | C, H, N |
| 44 | 4-F | H | 92.5-94.5 | 74 | EtOH-H ₂ O | V | C ₁₃ H ₉ FN ₂ S | C, H, N |
| 45 | H | H | 87-89 | 81 | 2-PrOH | IV | C ₁₃ H ₁₀ N ₂ S | C, H, N |
| 46 | 4-OH | H | 118.5-122.5 | 57 | 2-PrOH | IV | C ₁₃ H ₁₀ N ₂ OS | C, H, N |
| 47 | 3-CF ₃ | Cl | 151-155 | 76 | EtOH-H ₂ O | IV | C ₁₄ H ₈ ClF ₃ N ₂ S | C, H, N, S |
| 48 | 3-CF ₃ | H | 114-116 | 81 | EtOH | IV | C ₁₄ H ₉ F ₃ N ₂ S | C, H, N |
| 49 | 3,4-Cl ₂ | CH ₃ | 104-106 | 99 | 2-PrOH-H ₂ O | IV | C ₁₄ H ₁₀ Cl ₂ N ₂ S·0.1H ₂ O | C, H, N, H ₂ O |
| 50 | 4-Br, 3-CH ₃ | H | 122-124 | 64 | C ₆ H ₅ -isooctane | IV | C ₁₄ H ₁₁ BrN ₂ S | C, H, N |
| 51 | 5-Cl, 2-CH ₃ | H | 139-141.5 | 67 | EtOH | IV | C ₁₄ H ₁₁ ClN ₂ S | H, N; C ^b |
| 52 | 2-CH ₃ | H | 96-98 | 76 | EtOH-H ₂ O | IV | C ₁₄ H ₁₂ N ₂ S | C, H, N |
| 53 | 3-CH ₃ | H | 121-122.5 | 85 | EtOH-H ₂ O | IV | C ₁₄ H ₁₂ N ₂ S | C, H, N |
| 54 | 4-CH ₃ | H | 114-115 | 64 | 2-PrOH-H ₂ O | V | C ₁₄ H ₁₂ N ₂ S | H, N; C ^c |
| 55 | 3-OCH ₃ | H | 72-74 | 63 | 2-PrOH | IV | C ₁₄ H ₁₂ N ₂ OS | C, H, N |
| 56 | 4-OCH ₃ | H | 101-103 | 71 | EtOH | IV | C ₁₄ H ₁₂ N ₂ OS | C, H, N |
| 57 | 4-NHCOCH ₃ | H | 158-160 | 71 | EtOAc | IV | C ₁₅ H ₁₃ N ₂ OS | C, H, N |
| 58 | 4-N(CH ₃) ₂ | H | 135-138 | 66 | EtOH | IV | C ₁₅ H ₁₅ N ₃ S | C, H, N |
| 59 | 3,4-  | Cl | 176-179 | 72 | EtOH | IV | C ₁₇ H ₁₁ ClN ₂ S | C, H; N ^d |
| 60 | 2,3-  | H | 132-134 | 62 | EtOH-H ₂ O | IV | C ₁₇ H ₁₂ N ₂ S | C, H, N |
| 61 | 3,4-  | H | 140.5-142.5 | 59 | EtOH | IV | C ₁₇ H ₁₂ N ₂ S | C, H, N |
| 62 | 2-C ₆ H ₅ | H | 156-158 | 45 | EtOH | V | C ₁₉ H ₁₄ N ₂ S | C, H, N |
| 63 | 3-C ₆ H ₅ | H | 116-119 | 84 | EtOH | IV | C ₁₉ H ₁₄ N ₂ S | 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 SD₉₀ 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, % | purification solvent | procedure | formula | analyses |
|-----|--|-----------------|-------------|-------------------|---|-----------|--|---|
| 64 | 2,3,4,5,6-Cl ₅ | H | 233-286 | 17 | EtOAc-C ₆ H ₅ CH ₃ | VI | C ₁₄ H ₇ Cl ₅ N ₄ S ₂ C ₈ H ₈ O ₂ ^a | H, N; C ^b |
| 65 | 2,3,5,6-F ₄ | H | 250-252 | 47 | EtOH-NH ₄ OH | VII | C ₁₄ H ₇ F ₄ N ₄ S ₂ H ₂ O | C, H, N, H ₂ O |
| 66 | 2,5-Cl ₂ | H | 250-252 | 24 | EtOAc | VI | C ₁₄ H ₁₀ Cl ₂ N ₄ S ₂ | C, H, N |
| 67 | 3,4-Cl ₂ | H | 236-238 | 44 | EtOH | VII | C ₁₄ H ₁₀ Cl ₂ N ₄ S ₂ C ₆ H ₆ O ^c | C, H, N |
| 68 | 3,5-Cl ₂ | H | 223-225 | 22 | EtOAc-isooctane | VII | C ₁₄ H ₁₀ Cl ₂ N ₄ S ₂ ·0.75C ₈ H ₈ O ₂ ^a | C, H, N |
| 69 | 2-Cl | Cl | 258-262 | 79 | DMF-NH ₄ OH | VIII | C ₁₄ H ₁₀ Cl ₂ N ₄ S ₂ | C, H, N |
| 70 | 4-F | Cl | 253-255 | 66 | DMF | VIII | C ₁₄ H ₁₀ ClFN ₄ S ₂ | C, H, N |
| 71 | H | Cl | 221-222 | 75 | DMF-aq NaOH | VII | C ₁₄ H ₁₁ ClN ₄ S ₂ | C, H, N |
| 72 | 4-Br | H | 231-234 | 38 | DMF-EtOAc | VII | C ₁₄ H ₁₁ BrN ₄ S ₂ ·0.67H ₂ O | C, H, N, H ₂ O |
| 73 | 2-Cl | H | 246.5-248.5 | 26 | EtOH-NH ₄ OH | VII | C ₁₄ H ₁₁ ClN ₄ S ₂ | C, H, N |
| 74 | 3-Cl | H | 209-211 | 62 | DMF-NH ₄ OH | VII | C ₁₄ H ₁₁ ClN ₄ S ₂ | C, H, N |
| 75 | 4-Cl | H | 240-242.5 | 58 | DMF-H ₂ O | VII | C ₁₄ H ₁₁ ClN ₄ S ₂ ·0.33H ₂ O | C, H, N, H ₂ O |
| 76 | 4-F | H | 247-249 | 16 | EtOH-H ₂ O | VII | C ₁₄ H ₁₁ FN ₄ S ₂ | C, H, N |
| 77 | H | H | 189-191 | 47 | EtOH | VII | C ₁₄ H ₁₂ N ₄ S ₂ | C, H, N |
| 78 | 4-OH | H | 300-302 | 72 | DMF-NH ₄ OH | VII | C ₁₄ H ₁₂ N ₄ OS | C, H, N; H ₂ O ^d |
| 79 | 3-CF ₃ | Cl | 180-184 | 75 | DMF-H ₂ O | VIII | C ₁₅ H ₁₀ ClF ₃ N ₄ S ₂ | C, H, N, S |
| 80 | 3-CF ₃ | H | 229-230.5 | 63 | EtOH-NH ₄ OH | VII | C ₁₅ H ₁₀ F ₃ N ₄ S ₂ | C, H, N |
| 81 | 3,4-Cl ₂ | CH ₃ | 247-249 | 37 | DMF-H ₂ O | VII | C ₁₅ H ₁₂ Cl ₂ N ₄ S ₂ | C, H, N |
| 82 | 4-Br, 3-CH ₃ | H | 230-232 | 41 | EtOH | VII | C ₁₅ H ₁₃ BrN ₄ S ₂ ·0.25C ₈ H ₈ O ^c | C, H, N |
| 83 | 5-Cl, 2-CH ₃ | H | 229-231.5 | 48 | EtOH-H ₂ O | VII | C ₁₅ H ₁₃ ClN ₄ S ₂ ·0.25C ₈ H ₈ O ^c | C, H, N |
| 84 | 2-CH ₃ | H | 220-222 | 20 | EtOH | VII | C ₁₅ H ₁₄ N ₄ S ₂ | C, H, N |
| 85 | 3-CH ₃ | H | 217-219 | 30 | EtOH-H ₂ O | VII | C ₁₅ H ₁₄ N ₄ S ₂ | C, H, N |
| 86 | 4-CH ₃ | H | 250-252 | 35 | EtOH | VII | C ₁₅ H ₁₄ N ₄ S ₂ | C, H, N |
| 87 | 3-OCH ₃ | H | 207-209.5 | 55 | EtOH | VII | C ₁₅ H ₁₄ N ₄ OS | C, H, N |
| 88 | 4-OCH ₃ | H | 229-230 | 25 | EtOH | VII | C ₁₅ H ₁₄ N ₄ OS | C, H, N |
| 89 | 4-NHCOCH ₃ | H | >310 | 23 | EtOH | VII | C ₁₆ H ₁₅ N ₅ OS·0.75HCl·0.33H ₂ O | C, H, N, H ₂ O; Cl ^{-e} |
| 90 | 4-N(CH ₃) ₂ | H | 267-269 | 45 | DMF-H ₂ O | VII | C ₁₆ H ₁₇ N ₅ S ₂ C ₆ H ₅ NO ^f | C, H, N |
| 91 | 3,4-  | Cl | 239-242 | 73 | DMF-H ₂ O | VIII | C ₁₈ H ₁₃ ClN ₄ S ₂ | C, H, N |
| 92 | 2,3-  | H | 244-246 | 68 | DMF-H ₂ O | VII | C ₁₈ H ₁₄ N ₄ S ₂ | C, H, N |
| 93 | 3,4-  | H | 226-228 | 38 | EtOH | VII | C ₁₈ H ₁₄ N ₄ S ₂ | C, H, N |
| 94 | 4-C(CH ₃) ₃ | H | 221-223 | 21 | EtOH | VI | C ₁₈ H ₂₀ N ₄ ·C ₆ H ₆ O ^c | C, H, N |
| 95 | 3,4-  | CH ₃ | 277-279 | 30 | DMF-H ₂ O | VII | C ₁₉ H ₁₆ N ₄ S ₂ ·0.1H ₂ O | C, H, N, H ₂ O |
| 96 | 2-C ₆ H ₅ | H | 266-268 | 62 | DMF-NH ₄ OH | VII | C ₂₀ H ₁₆ N ₄ S ₂ | C, H, N |
| 97 | 3-C ₆ H ₅ | H | 181-183 | 54 | EtOH | VII | C ₂₀ H ₁₆ N ₄ S ₂ ·0.25C ₈ H ₈ O·0.25H ₂ O ^c | H, N, H ₂ O; C ^g |
| 98 | 4-C ₆ H ₅ | H | 231-233 | 13 | EtOH-NH ₄ OH | VII | C ₂₀ H ₁₆ N ₄ S ₂ H ₂ O | C, H, N, H ₂ O |





^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 H₂O: 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

Δ MST; 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 C5 | C5 C5 C5 | 18.1; C2 19.2; C2 18.8; C2 19.5; C2 | 10.8 10.7 11.2 11.0 | 6.6 7.1 6.8 6.8 | 2.7 2.8 3.0 | 2.0 | |
| 65 | 2,3,5,6-F ₄ | H | C5 | C5 C5 | 14.9; C3 21.9; C2 15.9; C4 | 12.1 12.3 8.9; C2 15.4; C1 | 8.1 8.5 6.1 6.3 | 4.9 5.1 2.7 3.1 | 0.9 0.6 0.5 | 0.3 0.3 | 0.3 | |
| 66 | 2,5-Cl ₂ | H | C5 | C5 C5 | 8.8; C2 9.9; C2 9.7; C3 | 9.8 10.1 10.3 | 8.2 8.7 8.7 | 5.8 5.9 5.9 | 2.9 2.9 | 2.3 | | |
| 67 | 3,4-Cl ₂ | H | C5 | C5 C5 C5 | C5 C5 C5 | C5 C5 C5 | C5 C5 C5 C5 | 17.6; C2 17.9; C3 17.9; C2 15.9; C4 18.4; C3 17.9; C3 | 12.7; C1 12.2; C1 11.7; C1 12.9; C1 13.4; C1 | 11.5 8.5 8.7 | 5.9 6.1 | 0.9 1.1 |
| 68 | 3,5-Cl ₂ | H | C5 | C5 C5 | 17.9; C4 20.9; C4 | 12.7 12.7 | 6.9 7.1 | 4.1 4.1 | 1.1 | | | |
| 69 | 2-Cl | Cl | 24.9; C4 27.9; C3 | 24.4; C3 | 15.6; C2 14.9; C2 | 11.7; C1 | 10.7; C1 10.2; C1 | 4.7 | | | | |
| 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 C5 | C5 C5 C5 | C5 C5 C5 | 14.4; C3 20.9; C2 16.8; C3 | 19.9; C1 19.6; C1 | 7.8 6.7 | 3.6 2.2 | |
| 73 | 2-Cl | H | | 18.9; C3 | 11.2; C2 10.9; C2 | 8.3 8.1 | 0.7 0.5 | 0.5 0.5 | 0.5 0.3 | 0.3 | | |
| 74 | 3-Cl | H | C5 | 20.9; C4 22.9; C4 | 4.9 5.1 | 4.3 4.5 | 3.5 3.7 | 0.5 0.7 | 0.5 | | | |
| 75 | 4-Cl | H | C5 C5 | C5 C5 C5 C5 | C5 C5 C5 C5 | 25.8; C4 C5 C5 C5 | 13.1; C2 19.8; C2 20.2; C2 19.3; C3 20.5; C2 14.1; C4 15.8; C4 | 9.2 11.6 11.9 12.0 11.8 11.1 11.1 | 9.5 9.4 9.2 10.1 10.1 | 6.0 6.8 6.6 | 3.6 2.5 | 1.7 1.7 |
| 76 | 4-F | H | C5 | C5 C5 | C5 C5 | C5 C5 | C5 C5 C5 | 18.0; C1 13.5; C2 13.7; C2 | 11.0 11.1 | 7.1 | 6.1 5.3 | |
| 77 | H | H | | 14.7; C3 | 14.0; C2 13.9; C2 | 9.7 10.3 | 6.3 6.5 | 4.7 5.1 | 3.3 3.3 | 1.1 | | |
| 78 | 4-OH | H | C5 | C5 C5 | 13.6; C2 13.9; C3 | 10.9; C1 11.9; C1 | 11.9 12.1 | 6.3 6.5 | 2.9 | | | |
| 79 | 3-CF ₃ | Cl | C5 C5 | C5 | 25.4; C3 24.9; C3 | 19.9; C2 | 15.2; C1 14.7; C1 | 5.9 | | | | |
| 80 | 3-CF ₃ | H | C5 | C5 C5 | C5 C5 | C5 C5 | 14.9; C3 15.9; C3 16.4; C3 | 12.7 13.1 13.3 | 9.1 9.3 | 8.7 | 2.7 0.5 | |
| 81 | 3,4-Cl ₂ | CH ₃ | | C5 | C5 C5 | C5 C5 | C5 C5 | 15.9; C1 15.4; C1 15.7; C1 | 7.9 8.1 | 7.3 7.5 | 2.5 1.3 | |
| 82 | 4-Br,3-CH ₃ | H | C5 | C5 C5 | C5 C5 | 14.4; C1 11.9; C2 | 7.7 7.9 | 3.5 3.9 | 1.9 | | | |
| 83 | 5-Cl,2-CH ₃ | H | C5 | C5 C5 | C5 C5 | C5 C5 | 25.7; C4 28.8; C3 27.7; C4 21.7; C2 | 19.4; C2 17.8; C3 17.8; C3 10.9 | 8.8; C1 10.0; C1 | 9.5 | 4.1 2.7 | |
| 84 | 2-CH ₃ | H | C2; T3 | C5 C5 | C5 C5 | C5 C5 | 17.0; C2 17.8; C2 17.7; C2 | 10.5 10.8 10.9 | 9.8 9.9 | 7.3 | 5.5 1.1 | |
| 85 | 3-CH ₃ | H | C2; T3 | C5 C5 | 12.7; C3 13.3; C3 | 7.7 8.0 | 6.7 6.6 | 2.3 2.8 | 2.4 | | | |
| 86 | 4-CH ₃ | H | C5 | C5 C5 | C5 C5 | 9.7; C3 16.6; C2 | 7.1 7.5 | 3.9 4.3 | 2.5 | | | |
| 87 | 3-OCH ₃ | H | C5 | 12.2; C2 13.9; C2 | 11.7 12.1 | 8.3 8.3 | 5.7 5.9 | 1.5 1.7 | 0.3 | | | |
| 88 | 4-OCH ₃ | H | C5 | C5 C5 | C5 C5 | C5 C5 | C5 C5 C5 | 25.8; C4 C5 C5 | 9.8 9.6 | 4.8 | 3.8 2.2 | |

Table IV (Continued)

| no. | X,Y | Z | Δ MST; 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 | | | |
| 90 | 4-N(CH ₃) ₂ | H | C5 | C5 | C5 | 24.9; C4 | 12.9 | 6.5 | | | | |
| | | | | C5 | C5 | C5 | C5 | C5 | 10.3 | 9.7 | 5.5 | 2.5 |
| 91 | 3,4-  | Cl | C5 | C5 | C5 | C5 | C5 | C5 | 15.9; C4 | 15.9; C2 | 12.7; C1 | 7.9 |
| | | | | | | | C5 | C5 | 17.9; C4 | | | 5.5 |
| 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 | | | |
| 93 | 3,4-  | H | C5 | C5 | C5 | C5 | C5 | C5 | 11.3 | 6.5 | 5.3 | 2.9 |
| | | | | 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 | | | |
| 96 | 2-C ₆ H ₅ | H | 16.4; C1 | 12.5 | 5.7 | 3.3 | 0.7 | 0.3 | | | | |
| 97 | 3-C ₆ H ₅ | H | C5 | 12.7 | 5.9 | 3.5 | 0.5 | 0.5 | | | | |
| | | | | C5 | C5 | C5 | 20.6; C2 | 11.3 | 8.1 | 7.9 | 5.3 | 4.5 |
| 98 | 4-C ₆ H ₅ | H | C5 | C5 | C5 | 15.6; C2 | 12.1 | 7.9 | 6.5 | 1.1 | | |
| | | | | C5 | 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 | | | |
| Ib acetate | | | | C5 | 9.9; C3 | 13.1 | 7.3 | 2.7 | 0.7 | | | |
| Ic base | | C5 | C5 | C5 | C5 | C5 | C5 | 22.4; C1 | | | | |
| | | | | C5 | C5 | C5 | C5 | 14.9; C3 | 7.5 | 2.9 | 1.5 | 0.3 |
| cycloguanil | | | T5 | C3; T2 | C5 | 21.6; C2 | 13.4; C1 | 7.9 | 4.9 | | | |
| hydrochloride | | | | 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 Δ MST 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 |
| 1a | | acetate | 14 | 9.5 | 7.9 |
| 1b | | acetate | 40 | 0.27 | 270 |
| 1c | | 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. SD₉₀ represents the daily dose (mg/kg) required for 90% suppression of the parasitemia in treated mice relative to control mice. The SD₉₀ was estimated graphically using semilog paper. ^c The quinine equiv Q is the ratio of the SD₉₀ of quinine hydrochloride to the SD₉₀ 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* H₃₇Rv (Table VIII). A modification of the gradient plate procedure of Szybalski³² and Webb and Washington³³ 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* (H₃₇Rv) 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 Section³⁴

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 K₂CO₃ in 250 mL of Me₂CO was stirred at room temperature for 20 h. The reaction mixture was filtered and the solid was triturated thoroughly with H₂O to give 7.6 g of insoluble material. The Me₂CO 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 recrystallized 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 K₂CO₃, and 50 mL of Me₂CO 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.

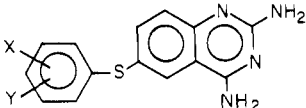


Procedure III. A mixture of 20.5 g (0.128 mol) of 2-naphthalenethiol, 24.5 g (0.134 mol) of 5-chloro-2-nitrobenzonitrile, and 19.5 g (0.141 mol) of anhydrous K₂CO₃ in 300 mL of C₆H₆ was stirred under reflux for 4 h and filtered hot to remove the salt. The C₆H₆ solution was concentrated and cooled. The 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 C₆H₅CH₃, and washed with H₂O. The organic layer was separated and dried over MgSO₄, 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 C₆H₅CH₃-isooctane, and once from DMF-H₂O to provide 8.6 g (37%) of the title compound, mp 119.5–121.5 °C. Anal. (C₁₉H₁₁Cl₂NS₂) 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 SnCl₂·2H₂O 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 H₂O, 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

| <div style="text-align: center;">  </div> | | | | | | | | | | | | |
|--|--|----------|-----------------------|----------|-----------------------|-----------------------|------|------|------|------|------|------|
| 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 | | | | |
| | | | | | | | 5.6 | 0 | | | | |
| 77 | H | 11.4 | 7.8 | | 6.4 | 6.2 | | | | | | |
| 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 ₆ H 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 SnCl₂·2H₂O 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-H₂O mixture containing 110 mL of 50% aqueous NaOH. The crude product was collected, washed with H₂O, 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 hydrochloride⁹ 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 Et₃N, 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.

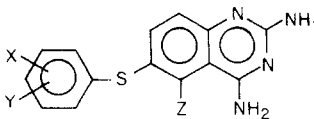

Procedure VII. A mixture of 3.2 g (0.0116 mol) of 2-amino-5-(2-naphthylthio)benzonitrile (61) and 1.5 g (0.013 mol)

of chloroformamidine hydrochloride⁹ 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 NH₄OH 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 Me₂SO₂ 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 H₂O, and made basic with 50% aqueous NaOH. A soft sticky precipitation formed which solidified upon cooling. The solid was collected by filtration, washed with H₂O, and crystallized from DMF·H₂O (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 SnCl₂·2H₂O 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 H₂O, 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

| | | |  | | | | | | |
|---------------------------|--|----|---|-----------|------------|-----------|-----------|-----------|---------|
| | | | Δ MST; 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 |
| 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) | |
| 85 | 3-CH ₃ | H | (C2; T2) | (C2; T2) | (4.7; C3) | (1.7; C3) | (4.2; C1) | (1.8) | |
| 86 | 4-CH ₃ | H | C3; T2 | C5 | C5 | (3.1; C3) | (3.8; C2) | (2.7) | (0.3) |
| 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 | |
| 93 | 3,4-  | H | (C5) | (C5) | (C5) | | | | |
| 96 | 2-C ₆ H ₅ | H | C5 | C5 | C5 | 0.0; C4 | 0.0; C4 | 0.0; C4 | |
| 97 | 3-C ₆ H 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 |
| 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 Δ MST 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 C₆H₅CH₃. Several small crops were collected giving 2.05 g, mp 200–202 °C. The EtOH insoluble material was triturated with Me₂CO and filtered. The filtrate was evaporated to dryness and the residue was recrystallized from C₆H₅CH₃ 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. (C₁₇H₁₄N₂OS) C, H, N.





2-Amino-6-(2-naphthylthio)-4(3H)-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 chloroformamidinium hydrochloride¹¹ 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 Et₂O, and the solid was collected and dried. The crude product was recrystallized from 90% aqueous EtOH containing excess Et₃N. 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. (C₁₈H₁₃N₃OS) C, H, N, S.

6-(2-Naphthylthio)-2,4(1H,3H)-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 CS₂ 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 Et₂O, and dried to give 2.0 g (35%) of the desired product, mp 308–310 °C. Anal. (C₁₈H₁₂N₂S₃) C, H, N.

2,3-Dichloro-6-nitrobenzonitrile (IVb). A mixture of 45.3 g (0.20 mol) of 1,2,3-trichloro-4-nitrobenzene¹⁴ 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 H₂O. Within 15 min, the precipitate had solidified and the crude brown-black solid was collected by filtration, washed thoroughly with H₂O, 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. (C₇H₂Cl₂N₂O₂) 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 H₂SO₄, 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 Et₂O and the solid diazonium compound was collected by filtration. To a freshly prepared solution of CuCN [from 5.3 g (0.021 mol) of CuSO₄·5H₂O, 5.8 g (0.089 mol) of KCN, and 30 mL of H₂O], 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, $\mu\text{g/mL}^a$ | | | | | | |
|-----|--|-----------------|--|----------------------------|-----------------------------|-------------------------|----------------------------|---------------------------|---|
| 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 ₆ H ₅ | H | <0.25 | <0.25 | <0.25 | >25 | 2.5 | >25 | 20 |
| 97 | 3-C ₆ H 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. (C₈H₅ClN₂O₂) 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 H₂O 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 NaNO₂ in 5 mL of H₂O. 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 H₂O 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 H₂O, and dried to give 2.75 g (93%) of product, mp 158 °C dec. For analysis, a sample was recrystallized from CHCl₃-MeOH (1:1) to give brilliant yellow crystals, mp 159 °C dec. Anal. (C₁₄H₁₂N₆S) 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 H₂O 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 NaNO₂ in 5 mL of H₂O, 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 H₂O, 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 H₂O, and dried. The dark yellow crystals thus obtained weighed 3.52 g (98%), mp 100–105 °C with foaming. Anal. (C₁₅H₁₃ClN₆OS) 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.
- (3) Deceased June 21, 1973.

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- (20) T. S. Osden, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
- (21) The oral antimalarial screening against *P. berghei* in mice was carried out by Dr. Paul E. Thompson and co-workers, Department of Pharmacology, Parke, Davis and Co., Ann Arbor, Mich.
- (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 [^3H]-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 Scholefield² 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 Christensen³ 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-