(10) R. W. Estabrook, G. Martinez-Zedillo, S. Young, J. A. Peterson, and J. McCarthy, *J. Steroid Biochem.*, **6**, 419 (1975).
(11) V. V. Virkar and T. S. Wheeler, *J. Chem. Soc.*, 1679 (1939).
(12) H. S. Mahal and K. Venkataraman, *J. Chem. Soc.*, 1767 (1934).
(13) A. T. M. Dunne, J. E. Gowan, J. Keane, B. M. O'Kelly, D. O'Sullivan, M. M. Roche, P. M. Ryan, and T. S. Wheeler, *J. Chem. Soc.*, 1252 (1950).
(14) N. Amand, D. M. Patel, and K. Venkataraman, *Proc. Indian Acad. Sci., Sect. A*, **28**, 545 (1948).
(15) S. P. Wagh and G. V. Jadhav, *J. Univ. Bombay*, **25**, 23 (1957).
(16) N. V. Nowlan, P. A. Slavin, and T. S. Wheeler, *J. Chem. Soc.*, 340 (1950).
(17) S. A. Kagal, P. Madhavan Nair, and K. Venkataraman, *Tetrahedron Lett.*, 593 (1962).
(18) K. K. Thomas and M. M. Bokadia, *J. Indian Chem. Soc.*, **43**, 713 (1966).
(19) B. J. Ghiya and M. G. Marathey, *J. Indian Chem. Soc.*, **38**, 331 (1961).
(20) T. A. Van der Hoeven, D. A. Haugen, and J. M. Laon, *J. Biol. Chem.*, **249**, 6302 (1974).
(21) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

# Folate Antagonists. 15. 2,4-Diamino-6-(2-naphthylsulfonyl)quinazoline and Related 2,4-Diamino-6-[(phenyl and naphthyl)sulfinyl and sulfonyl]quinazolines, a Potent New Class of Antimetabolites with Phenomenal Antimalarial Activity[1,2]

Edward F. Elslager, Marland P. Hutt, Patricia Jacob, Judith Johnson, Barbara Temporelli, Leslie M. Werbel,* Donald F. Worth,

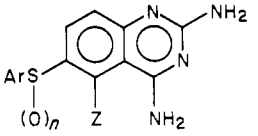
*Department of Chemistry, Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Ann Arbor, Michigan 48106*

and Leo Rane[3]

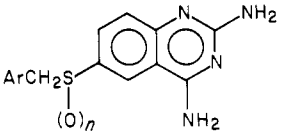
*University of Miami School of Medicine, Medical Research Annex, Miami, Florida 33142.* *Received May 10, 1979*

Oxidation of an array of 2,4-diamino-6-(arylthio)quinazolines provided the corresponding arylsulfinyl and arylsulfonyl analogues. A variety of these nonclassical analogues of methotrexate exhibited suppressive antimalarial activity superior to that of the parent thioquinazolines against drug-sensitive lines of *Plasmodium berghei* in mice and *P. gallinaceum* in chicks, and several displayed potent prophylactic activity against *P. gallinaceum*. The sulfinyl- and sulfonylquinazolines also retained antimalarial effects against chloroquine-, cycloguanil-, and DDS-resistant lines of *P. berghei* in mice and against chloroquine- and pyrimethamine-resistant strains of *P. falciparum* in owl monkeys. Coadministration of one of the most active of these compounds, 2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline (**35**), with sulfadiazine to monkeys infected with *P. falciparum* or *P. vivax* led to greatly enhanced activity and prevented the development of quinazoline resistance.

We have recently described the synthesis and extraordinary antimalarial and antibacterial effects of a group of 2,4-diamino-6-[(phenyl and naphthyl)thio]-quinazolines Ia.[4,5] Oxidation of the related 2,4-diamino-6-[(aralkyl)thio]quinazolines IIa to the corresponding sulfoxides IIb and sulfones IIc resulted in a decrease in antimalarial activity.[6] It was deemed of interest, however, to examine the effect of oxidation on the dramatically more potent 2,4-diamino-6-(arylthio)-quinazolines Ia. The successful outcome of these studies is presented in this article.




Ia, $n = 0$
b, $n = 1$
c, $n = 2$




IIa, $n = 0$
b, $n = 1$
c, $n = 2$

Ar = phenyl or naphthyl

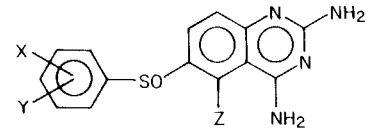
**Chemistry.** The 2,4-diamino-6-[(phenyl and naphthyl)thio]quinazolines Ia, prepared as described previously,[5] when treated either with the bromine complex of 1,4-diazabicyclo[2.2.2]octane[7] in 70% aqueous acetic acid or with a slight excess of 30% hydrogen peroxide in acetic acid afforded the corresponding sulfoxides Ib (Table I; **1–18**).

The sulfones Ic (Table II; **19–37**) were obtained from the parent (arylthio)quinazolines Ia by treatment with a large excess of 30% hydrogen peroxide in glacial acetic acid. Generally, these procedures were straightforward, and the products could be isolated and purified relatively easily. The oxidation of 2,4-diamino-5-chloro-6-[(*o*-chlorophenyl)thio]quinazoline to the corresponding sulfone (**20**; Table II) was more complex, leading to a mixture which included the sulfoxide and the desired sulfone. Fractional crystallization allowed the isolation of the sulfone **20** in only 10% yield, and an attempt to repeat the procedure led only to an inseparable mixture.

**Parenteral Suppressive Antimalarial Screening in Mice.** The sulfoxides Ib (**1–18**; Table I) and sulfones Ic (**19–37**; Table II) described herein were evaluated initially against a normal drug-sensitive strain of *Plasmodium berghei* in mice by the parenteral route.[8,9] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72 h after infection. Compounds are arbitrarily considered to be "active" when they produce at least a 100% increase in the mean survival time of treated mice, and the animals are considered to be "cured" if they survive 60 days postinfection. Results are summarized in Tables III and IV. For comparison, the minimum curative dose (MCD) for the corresponding unoxidized compounds Ia is included, as are data for two reference drugs, cycloguanil hydrochloride and pyrimethamine.

0022-2623/79/1822-1247$01.00/0 © 1979 American Chemical Society

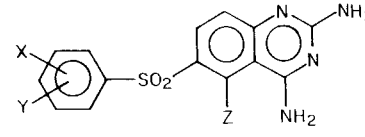
Table I. 2,4-Diamino-6-[(phenyl and naphthyl)sulfinyl]quinazolines



| no. | X, Y | Z | mp, °C | yield purified, % | purifcn solvent[a] | procedure | reaction time, h | formula | anal. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 2,3,5,6-$F_4$ | H | 301–303 | 56 | EtOH (T) | II | 22 | $C_{14}H_8F_4N_4OS$ | C, H, N |
| 2 | 4-F | Cl | 267–270 | 80 | DMF–$H_2O$ | I | 96 | $C_{14}H_{10}ClFN_4OS$ | C, H, N |
| 3 | 2-Cl | Cl | 311–312 dec | 52 | DMF | II | 168[b] | $C_{14}H_{10}Cl_2N_4OS$ | C, H, N |
| 4 | 3,4-$Cl_2$ | H | 280–282 | 23 | DMF–$H_2O$ | I | 18 | $C_{14}H_{10}Cl_2N_4OS·0.3H_2O$ | C, H, N, $H_2O$ |
| 5 | 4-Br | H | 303–305 | 37 | EtOH | I | 72 | $C_{14}H_{11}BrN_4OS·H_2O$ | C, H, N, $H_2O$ |
| 6 | H | Cl | 284–285 dec | 76 | EtOH | II | 17 | $C_{14}H_{11}ClN_4OS$ | C, H, N |
| 7 | 4-Cl | H | 294–296 | 82 | EtOH (T) | I | 20 | $C_{14}H_{11}ClN_4OS·0.33H_2O$ | C, H, N, $H_2O$ |
| 8 | 4-F | H | 255–257 | 68 | EtOH (T) | I | 24 | $C_{14}H_{11}FN_4OS·0.25C_2H_6O·0.33H_2O$ | C, H, N, $H_2O$ |
| 9 | H | H | 283–285 dec | 78 | MeOH–$H_2O$ | I | 20 | $C_{14}H_{12}N_4OS·0.5H_2O$ | C, H, N, $H_2O$ |
| 10 | 3-$CF_3$ | Cl | 288–291 dec | 53 | DMF–$H_2O$ | II | 20 | $C_{15}H_{10}ClF_3N_4OS$ | C, H, N, S |
| 11 | 3-$CF_3$ | H | 281–283 | 33 | EtOAc | I | 19 | $C_{15}H_{11}F_3N_4OS·H_2O$ | C, H, N, $H_2O$ |
| 12 | 4-$OCH_3$ | H | 259–260.5 | 9 | EtOAc | I | 28 | $C_{15}H_{14}N_4O_2S·0.4H_2O$ | C, H, N, $H_2O$ |
| 13 | 3,4-  | Cl | 312 dec | 79 | DMF | II | 5 | $C_{18}H_{13}ClN_4OS$ | C, H, N |
| 14 | 2,3-  | H | 272–275 | 80 | EtOH (T) | II | 5 | $C_{18}H_{14}N_4OS·0.33C_2H_6O·0.25H_2O$[c] | C, H, N, $H_2O$ |
| 15 | 3,4-  | H | 312–314 | 72 | DMF–$H_2O$ | II | 0.6 | $C_{18}H_{14}N_4OS·0.125H_2O$ | C, H, N, $H_2O$ |
| 16 | 3,4-  | $CH_3$ | >320 | 40 | DMF (T) | II | 2.5 | $C_{19}H_{16}N_4OS·0.1H_2O$ | C, H, N, $H_2O$ |
| 17 | 2-$C_6H_5$ | H | 299–301 | 48 | EtOH (T) | I | 19 | $C_{20}H_{16}N_4OS·0.5C_2H_6O$[c] | C, H, N |
| 18 | 3-$C_6H_5$ | H | 274–275 | 36 | EtOH (T) | I | 28 | $C_{20}H_{16}N_4OS$ | C, H, N |

[a] Most compounds were purified by recrystallization but some were purified by trituration (T) in the hot solvent. [b] Intermittent periods of heating at 45 °C totaling 10 h were also required. [c] The NMR spectrum confirmed the presence of EtOH.

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



| no. | X, Y | Z | mp, °C | yield purified, % | purifcn solvent[a] | reaction time, h[b] | formula | anal. |
|---|---|---|---|---|---|---|---|---|
| 19 | 4-F | Cl | 271 dec | 46 | DMF–$H_2O$ | 96 | $C_{14}H_{10}ClFN_4O_2S$ | C, H, N |
| 20 | 2-Cl | Cl | 299 dec | 10 | DMF–$NH_4OH$ | 168[b] | $C_{14}H_{10}Cl_2N_4O_2S$ | C, H, N |
| 21 | 3,4-$Cl_2$ | H | 308–310 | 70 | DMF–$H_2O$ | 22[d] | $C_{14}H_{10}Cl_2N_4O_2S·0.4H_2O$ | C, H, N, $H_2O$ |
| 22 | 3,5-$Cl_2$ | H | 336–338 | 67 | EtOH (T) | 18 | $C_{14}H_{10}Cl_2N_4O_2S·0.5C_2H_6O·0.4H_2O$[c] | C, H, N, $H_2O$ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 23 | 4-Br | H | 302–304 | 86 | DMF–$H_2O$ | 20 | $C_{14}H_{11}BrN_4O_2S{\cdot}0.75H_2O$ | C, H, N, $H_2O$ |
| 24 | H | Cl | 291–293 dec | 59 | DMF–$H_2O$ | 70 | $C_{14}H_{11}ClN_4O_2S$ | C, H, N |
| 25 | 4-Cl | H | 295.5–297.5 | 47 | EtOH | 20 | $C_{14}H_{11}ClN_4O_2S{\cdot}H_2O$ | C, H, N, $H_2O$ |
| 26 | 4-F | H | 271–273 | 82 | EtOH | 18 | $C_{14}H_{11}FN_4O_2S{\cdot}0.5H_2O$ | C, H, N, $H_2O$[c,d] |
| 27 | H | H | 297–299 | 65 | DMF–$H_2O$ | 20 | $C_{14}H_{12}N_4O_2S{\cdot}0.3H_2O$ | C, H, N, $H_2O$ |
| 28 | 3-$CF_3$ | Cl | 254–256 dec | 20 | EtOH | 96[f] | $C_{15}H_{10}ClF_3N_4O_2S$ | C, H, N |
| 29 | 3-$CF_3$ | H | 308–310 | 76 | EtOH (T) | 21 | $C_{15}H_{11}F_3N_4O_2S$ | C, H, N |
| 30 | 4-Br, 3-$CH_3$ | H | 319–321 | 66 | EtOH | 20 | $C_{15}H_{13}BrN_4O_2S{\cdot}0.33C_2H_6O$[e] | C, H, N |
| 31 | 3-$OCH_3$ | H | 286–288 | 79 | EtOH (T) | 22 | $C_{15}H_{14}N_4O_3S$ | C, H, N |
| 32 | 4-$OCH_3$ | H | 282–283 | 33 | EtOH | 23 | $C_{15}H_{14}N_4O_3S{\cdot}0.8H_2O$ | C, H, N, $H_2O$ |
| 33 | 3,4-  | Cl | 302 dec | 70 | DMF–$H_2O$ | 72 | $C_{18}H_{13}ClN_4O_2S$ | C, H, N |
| 34 | 2,3-  | H | 303–307 | 41 | DMF–$H_2O$ | 18 | $C_{18}H_{14}N_4O_2S$ | C, H, N |
| 35 | 3,4-  | H | >310 | 92 | EtOH (T) | 23 | $C_{18}H_{14}N_4O_2S{\cdot}0.9H_2O$ | C, H, N, $H_2O$ |
| 36 | 2-$C_6H_5$ | H | 278–279.5 | 27 | EtOAc | 42 | $C_{20}H_{16}N_4O_2S$ | C, H, N |
| 37 | 3-$C_6H_5$ | H | 263–265 | 75 | EtOH (T) | 21 | $C_{20}H_{16}N_4O_2S$ | C, H, N |

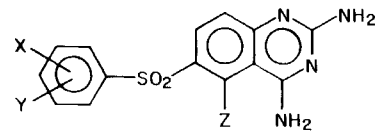
[a] The sulfones were usually purified by recrystallization, but some were purified by trituration (T) in the hot solvent. [b] All sulfones were prepared according to procedure III, except for variations in the temperature and reaction time as indicated. [c] Intermittent periods of heating at 45 °C totaling 10 h were also required. [d] $H_2O$: calcd, 2.75; found, 2.28. [e] The NMR spectrum confirmed the presence of EtOH. [f] Additional 30% aqueous $H_2O_2$ was added after 4, 7, and 22 h. The reaction mixture was also heated at 45 °C for the first 7 h and again between the 22nd and 31st h.

The data does not lend itself to broad generalizations. It does seem clear, however, that oxidation does not produce a generally deleterious effect on antimalarial activity as was previously noted with the (aralkylthio)-quinazolines IIa. Thus, seven sulfoxides (Table IV; **3**, **6**, **7**, **9**, **11**, **15**, and **16**) representing unsubstituted phenyl, chlorophenyl, and naphthyl attached to the sulfur spacer were more active than the parent unoxidized compounds, three others (Table IV; **2**, **17**, and **18**) were of comparable potency, and the remaining eight were less active. Among the sulfones Ic, 11 compounds (Table III; **22**, **24–27**, **29–31**, **34**, **35**, and **37**) representing unsubstituted phenyl, halophenyl, and naphthyl attached to the sulfur spacer were more active than the parent unoxidized compounds, three others (Table III; **19**, **23**, and **32**) were of comparable potency, and the remaining four (Table III; **21**, **28**, **33**, and **36**) were less active. One should consider when examining the data in this fashion that in certain instances (i.e., **36**) we are dealing with a marginally active substituent even in the parent series, so that the term "less active" has little significance. Moreover, in certain cases the parent exhibits such a high degree of potency that a slightly less active derivative (i.e., **23**) is relatively insignificant. Particularly noteworthy is the dramatic increase in potency achieved with the 4-chlorophenyl sulfone (Table III; **25**) and the high potency of both the sulfoxide and sulfone of the β-naphthyl series (Table IV, **15**; and Table III, **35**). In general, it would appear that the overall impact of the oxidation state of the biospacer on potency is $SO_2 > S \geq SO$.

Replacement of the hydrogen at C-5 of the quinazoline ring with a chlorine or a methyl group effects activity in a more capricious manner. Thus, the antimalarial activity of 5-chloro-2,4-diamino-6-(2-naphthylthio)quinazoline and of 5-chloro-6-[[3-(trifluoromethyl)phenyl]thio]quinazoline is markedly decreased as the oxidation state is increased (SO, **13** and **10**; $SO_2$, **33** and **28**) possibly due to excessive steric constraint at or near a critical enzyme binding site. However, 5-chloro-6-[(2-chlorophenyl)sulfinyl]-2,4-diaminoquinazoline (**3**) and 2,4-diamino-5-methyl-6-(2-naphthylsulfinyl)quinazoline (**16**) are more potent than their unoxidized parents (the corresponding sulfones were not available for testing), while the MCD of 5-chloro-2,4-diamino[(4-fluorophenyl)thio]quinazoline remains unchanged as the oxidation state is increased [although a reduction in toxicity, and thus improvement in therapeutic index, is noted (SO, **2**; $SO_2$, **19**)]. Finally, in the case of 2,4-diamino-5-chloro-6-(phenylthio)quinazoline not only is the toxicity reduced as the oxidation state is increased but the potency is increased. Thus, the MCD is decreased from 640 mg/kg for the parent to 40 mg/kg for the sulfoxide **6** and to 20 mg/kg for the sulfone **24**. One may presume that several interactive effects, including steric and electronic, are involved in this situation.

**Parenteral Suppressive Antimalarial Effects in Chicks.** Seven of the 2,4-diamino-6-[(aryl)sulfinyl and sulfonyl]quinazolines (**1**, **7**, **12**, **25**, **31**, **32**, and **35**) were also tested for suppressive antimalarial effects against *P. gallinaceum* infections in white Leghorn cockerels (Table V).[9,10] The drugs were administered to infected chicks in a single subcutaneous dose in peanut oil. 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 V. Clearly impressive antimalarial activity is also demonstrated in this test system, the 4-chlorophenyl sulfone (**25**) being the most potent compound among the limited number examined.

Table III. Parenteral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(phenyl and naphthylEsulfonyl]quinazolines against Trophozoite-Induced *Plasmodium berghei* in Mice




| 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 | Ia:MCD,[b] cures/dose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | 4-F | Cl | C5 | C5 | C5 | 26.9; C3 | 19.9; C2 | 9.7 | 9.3 | 4.1 | 0.5 | 0.5 | C2/40 |
| | | | C5 | | C5 | | 13.4; C3 | 10.1 | | | | | |
| | | | | | | | 13.9; C3 | | | | | | |
| 20[c] | 2-Cl | Cl | | | | | | | | | | | |
| 21 | 3,4-$Cl_2$ | H | C5 | C5 | C5 | C5 | C5 | 11.2; C2 | 10.3 | 5.9 | 3.5 | 1.1 | C1/10 |
| | | | | C5 | C5 | C5 | C5 | 11.9; C2 | 10.5 | | | | |
| | | | | | | | C5 | 12.2; C2 | | | | | |
| 22 | 3,5-$Cl_2$ | H | C5 | C5 | C5 | C5 | 11.3 | 9.7 | 6.9 | 5.9 | 0.7 | 0.3 | C4/160 |
| | | | | C5 | C5 | C5 | 11.7 | 9.9 | 7.3 | | | | |
| | | | | | | | 11.9 | 9.7 | | | | | |
| 23 | 4-Br | H | | | C5 | C5 | C5 | 9.9; C3 | 13.1 | 10.3 | 6.5 | | C1/10 |
| | | | | | | C5 | C5 | 10.9; C3 | 13.3 | 10.7 | | | |
| 24 | H | Cl | C2, T3 | C5 | C5 | C5 | 22.9; C3 | 11.9; C1 | | | | | C3/640 |
| | | | C2, T3 | | C5 | | 22.4; C3 | | | | | | |
| 25 | 4-Cl | H | | | C5 | C5 | C5 | C5 | C5 | C5 | 12.2 | 7.7 | C4/40 |
| | | | | | | C5 | C5 | C5 | C5 | C5 | 12.5 | | |
| | | | | | | | | C5 | C5 | C5 | | | |
| 26 | 4-F | H | T5 | T5 | C5 | C5 | C5 | C5 | 12.9; C2 | 13.6; C2 | 6.9 | 4.1 | C2/20 |
| | | | | T5 | C5 | C5 | C5 | C5 | 13.9; C3 | | | | |
| | | | | | | | C5 | C5 | | | | | |
| 27 | H | H | C1, T4 | C3, T2 | C5 | 19.4; C3 | 12.3 | 10.7 | 5.1 | 0.5 | 0.3 | | C2/160 |
| | | | | C3, T2 | C5 | 18.9; C3 | 12.1 | 10.7 | 5.3 | 0.3 | | | |
| | | | | | C5 | 12.9; C2 | 12.9 | 7.9 | | | | | |
| | | | | | | 12.6; C2 | 13.1 | 7.7 | | | | | |
| 28 | 3-$CF_3$ | Cl | | 4.9 | 3.7 | 1.1 | 0.7 | 0.7 | 0.5 | | | | C1/40 |
| | | | | 4.7 | | 1.3 | | 0.7 | | | | | |
| 29 | 3-$CF_3$ | H | C5 | C5 | C5 | C5 | C5 | 13.9; C3 | 14.9; C1 | 7.7 | 1.5 | 0.5 | C3/40 |
| | | | | C5 | C5 | C5 | C5 | 15.4; C3 | 11.6; C2 | | | | |
| | | | | | | | C5 | 15.9; C3 | | | | | |
| 30 | 4-Br, 3-$CH_3$ | H | C5 | 18.9; C3 | 17.4; C3 | 10.5 | 5.9 | 1.5 | 0.5 | | | | C2/80 |
| | | | | 19.4; C3 | 22.6; C2 | 10.7 | 6.1 | 1.9 | | | | | |
| 31 | 3-$OCH_3$ | H | C5 | 28.9; C3 | 13.6; C2 | 12.5 | 8.9 | 3.9 | 1.1 | | | | C2/320 |
| | | | | 23.9; C4 | 13.2; C2 | 12.7 | 9.1 | 4.1 | | | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 4-$OCH_3$ | H | C5 | C5 | C5 | C5 | 15.9; C2 | 11.4; C1 | 4.9 | | | | | C5/20 |
| | | | | C5 | C5 | C5 | 20.9; C1 | 12.2; C1 | | | | | | |
| 33 | 3,4- | Cl | C5 | 13.3 | 11.5 | 7.1 | 4.7 | 0.7 | | | | | | C1/5 |
| | | | C5 | | 11.7 | | 4.9 | | | | | | | |
| 34 | 2,3- | H | C5 | 20.9; C2 | 14.9 | 5.5 | 3.5 | 2.9 | 0.7 | | | | | C3/80 |
| | | | | 14.4; C3 | 14.3 | 5.9 | 3.7 | 3.1 | | | | | | |
| 35 | 3,4- | H | C5 | C5 | C5 | C5 | C5 | C5 | C5 | 15.6; C2 | 12.1 | 10.9 | C5/20 | |
| | | | | C5 | C5 | C5 | C5 | C5 | C5 | | | | | |
| | | | | | | | C5 | C5 | | | | | | |
| 36 | 2-$C_6H_5$ | H | 1.1 | 0.7 | 0.7 | 0.5 | 0.3 | 0.3 | | | | | | C1/640 |
| 37 | 3-$C_6H_5$ | H | C5 | C5 | C5 | C5 | 16.9; C4 | 21.9; C4 | 13.9; C2 | 13.6; C2 | 9.3 | 4.1 | C3/40 | |
| | | | | C5 | C5 | C5 | C5 | C5 | 14.2; C2 | | | | | |

[a,b] See footnotes a and b, Table IV. [c] Sufficient material for testing was not available.

**Parenteral Prophylactic Antimalarial Effects in Chicks.** Eight sulfinylquinazolines (**1**, **8**, **9**, **11**, **14**, and **16–18**; Table I) and eight sulfonylquinazolines (**21–23**, **27**, **31**, **32**, **35**, and **37**; Table II) were evaluated for prophylactic action in chicks.[10,11] White Leghorn cockerels were parasitized by the intrajugular infection of *P. gallinaceum* sporozoites. The drugs were suspended in peanut oil and were administered subcutaneously in a single dose on the day of infection. The results are summarized in Table VI.

Once again substantial potency was evident among representative examples of both sulfinyl- and sulfonylquinazolines. Curative activity was demonstrated at levels as low as 7.5 mg/kg.

**Expanded Antimalarial Studies.** Several 2,4-diamino-6-[(aryl)sulfinyl and sulfonyl]quinazolines also displayed potent activity when given orally by gavage once daily for 3 days or continuously by drug diet for 6 days to mice infected with a normal drug-sensitive strain of *P. berghei*.[12] The oral antimalarial potency of the sulfoxide **15** and the three sulfones **23**, **26**, and **35** relative to five representative thioquinazolines[4,5] is summarized in Table VII. The quinazoline sulfoxide and sulfones produced a 90% suppression of the parasitemia at daily oral doses of 0.2 to 2.2 mg/kg and ranged from 60 to 830 times as potent as quinine hydrochloride.

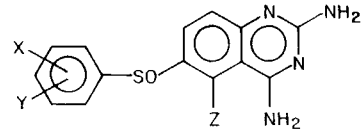
The above 2,4-diamino-6-[(aryl)thio, 4,5-sulfinyl, and sulfonyl]quinazolines were subsequently evaluated orally against chloroquine (C), cycloguanil (T), and dapsone (DDS; S) resistant of *P. berghei*.[14,15] As shown by the data summarized in Table VII, the sulfinyl- and sulfonylquinazolines **15**, **23**, **26**, and **35** displayed little or no cross-resistance with chloroquine, negligible cross-resistance with cycloguanil, and less than two- to sixfold cross-resistance with dapsone.

The demonstration that various 2,4-diamino-6-[(aryl)-thio, sulfinyl, and sulfonyl]quinazolines lacked appreciable cross-resistance to reference drugs in mice prompted the evaluation of representative compounds in monkeys to enable the selection of optimal candidates for preclinical toxicological studies and clinical trial. Seven compounds were evaluated against the chloroquine-susceptible, pyrimethamine-resistant Malayan Camp-CH/Q and the chloroquine-resistant, pyrimethamine-susceptible Vietnam Oak Knoll strains of *P. falciparum* in the *Aotus trivergatus* owl monkey model.[13] All of these compounds exhibited a high order of activity, with remarkably favorable therapeutic indices, against infections with both strains (Table VIII).

The most active compound, 2,4-diamino-6-(2-naphthylsulfonyl)quinazoline (WR-158,122; **35**), was compared with quinine, chloroquine, and pyrimethamine in owl monkeys infected with three strains of *P. falciparum* and two strains of *P. vivax* (Table IX).[13] Against *P. falciparum* infections, the $CD_{90}$ of **35** for each of the three strains was: Vietnam Oak Knoll (pyrimethamine sensitive, chloroquine resistant), 0.098 (mg/kg)/day; Malayan Camp-CH/Q (chloroquine sensitive, pyrimethamine resistant), 0.39 (mg/kg)/day; and Vietnam Smith (quinine, chloroquine, and pyrimethamine resistant), >6.25 (mg/kg)/day (Table IX). Against *P. vivax*, **35** cured the pyrimethamine-susceptible New Guinea Chesson strain at 0.39 (mg/kg)/day, and the pyrimethamine-resistant Vietnam Palo Alto strain at 6.25 (mg/kg)/day (Table IX).

As with other folate antagonists, including cycloguanil and pyrimethamine, the induction of resistance to **35** is facile. Thus, a rapid evolution of resistance to **35** occurred when infections with the Malayan Camp and Oak Knoll strains of *P. falciparum* and the Chesson and Palo Alto strains of *P. vivax* were exposed to subcurative doses.

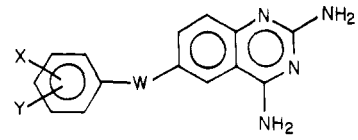
Table IV. Parenteral Suppressive Antimalarial Effects of 2,4-Diamino-6-[(phenyl and naphthyl)sulfinyl]quinazolines against Trophozoite-Induced *Plasmodium berghei* in Mice




| 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 | Ia:MCD,[b] cures/dose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2,3,5,6-$F_4$ | H | C5 | C5 | 16.7 | 14.1 | 10.7 | 1.1 | 0.5 | | | | C2/80 |
| | | | | C5 | 16.9 | 13.9 | 11.1 | 0.9 | | | | | |
| 2 | 4-F | Cl | T5 | C1, T4 | C5 | 27.9; C3 | 20.9; C2 | 4.1 | | | | | C2/40 |
| | | | T5 | | C5 | | 21.2; C2 | | | | | | |
| 3 | 2-Cl | Cl | C2, T3 | C3, T2 | C5 | C5 | C5 | 8.6; C2 | | | | | C1/40 |
| | | | C2, T3 | | C5 | | C5 | | | | | | |
| 4 | 3,4-$Cl_2$ | H | C2, T3 | C4, T1 | C5 | C5 | 30.9; C3 | 15.9; C2 | 8.7 | 7.9 | 4.1 | 1.1 | C1/10 |
| | | | | C4, T1 | C5 | C5 | 21.9; C4 | 15.9; C2 | 9.1 | | | | |
| | | | | | | | 23.9; C4 | 16.9; C2 | | | | | |
| 5 | 4-Br | H | | | C5 | C5 | 25.9; C3 | 13.6; C2 | 10.3 | 6.7 | 3.3 | | C1/10 |
| | | | | | | C5 | 20.9; C4 | 13.6; C2 | 10.1 | 6.9 | | | |
| 6 | H | Cl | T5 | T5 | T5 | 17.9; C4 | 13.4; C3 | 8.5 | | | | | C3/640 |
| | | | T5 | | T5 | | 12.9; C3 | | | | | | |
| 7 | 4-Cl | H | C5 | C5 | C5 | C5 | C5 | C5 | 7.4; C1 | 4.8; C1 | 6.0 | 4.2 | C4/40 |
| | | | | C5 | C5 | C5 | C5 | C5 | 7.3; C1 | | | | |
| | | | | | | | C5 | C5 | | | | | |
| 8 | 4-F | H | C5 | C5 | C5 | 16.9; C3 | 11.5 | 8.9 | 5.5 | | | | C2/20 |
| | | | | C5 | C5 | 18.9; C3 | 11.9 | 9.1 | | | | | |
| 9 | H | H | C5 | C5 | C5 | 15.9; C2 | 11.3 | 4.9 | 1.1 | | | | C2/160 |
| | | | | C5 | C5 | 16.2; C2 | 11.7 | 5.1 | | | | | |
| 10 | 3-$CF_3$ | Cl | | 1.5 | | 1.1 | | 0.5 | | | | | C1/40 |
| 11 | 3-$CF_3$ | H | C5 | C5 | C5 | C5 | C5 | 28.2; C2 | 12.7; C1 | 9.1 | 4.1 | 1.1 | C3/40 |
| | | | | C5 | C5 | C5 | C5 | 21.9; C3 | 13.2; C1 | | | | |
| | | | | | | | C5 | 21.4; C3 | | | | | |
| 12 | 4-$OCH_3$ | H | C5 | C5 | 25.9; C4 | 13.4; C1 | 10.5 | 2.7 | 0.7 | | | | C5/20 |
| | | | | C5 | C5 | 13.2; C1 | 10.7 | 2.9 | | | | | |
| 13 | 3,4-  | Cl | C5 | C5 | C5 | C5 | 23.9; C4 | 9.3 | | | | | C1/5 |
| | | | C5 | | C5 | | 25.9; C3 | | | | | | |
| 14 | 2,3-  | H | C5 | 20.9; C2 | 15.2; C1 | 13.9 | 7.9 | 1.9 | 1.1 | | | | C3/80 |
| | | | | 16.4; C3 | 16.9; C1 | 13.7 | 8.1 | 2.1 | | | | | |
| 15 | 3,4-  | H | C5 | C5 | C5 | C5 | C5 | C5 | C5 | 14.2; C2 | 10.9 | 6.7 | C5/20 |
| | | | | C5 | C5 | C5 | C5 | C5 | 31.9; C4 | | | | |
| | | | | | | | C5 | C5 | | | | | |
| 16 | 3,4-  | $CH_3$ | C5 | C5 | C5 | C5 | C5 | C5 | C5 | 22.9; C3 | 10.7; C1 | 9.7 | C4/20 |
| | | | | C5 | C5 | C5 | C5 | C5 | C5 | | | | |
| | | | | | | | C5 | C5 | | | | | |
| 17 | 2-$C_6H_5$ | H | 12.7; C1 | 5.9 | 3.3 | 0.5 | 0.5 | 0.3 | 0.3 | | | | C1/640 |
| | | | | 5.7 | 3.7 | 0.7 | 0.3 | 0.3 | | | | | |
| 18 | 3-$C_6H_5$ | H | C5 | C5 | C5 | 22.9; C4 | 18.9; C3 | 11.1 | 6.7 | 4.1 | 1.1 | 0.5 | C3/40 |
| | | | | C5 | C5 | 28.9; C3 | 19.9; C3 | 11.3 | 6.9 | | | | |
| | | | | | | | 24.9; C2 | 11.7 | | | | | |
| cycloguanil hydrochloride | | | T5 | C3; T2 | C5 | 21.6; C2 | 13.4; C1 | 7.9 | 4.9 | | | | |
| 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 6.2 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 entry at each dose level represents results with a five-animal group. [b] The minimum curative dose (MCD) for the corresponding unoxidized compounds (Ia)[5] is included for comparative purposes.

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



| no. | X, Y | W | single sc dose, mg/kg | MST of chicks, days: treated (MSTT) | MST of chicks, days: controls (MSTC) | MST of chicks, days: ΔMST[a] | no. of chicks: cured[b] | no. of chicks: toxic[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | 2,3,5,6-$F_4$ | SO | 120 | 14.8 | 4.0 | 10.8 | 0 | 0 |
| 7 | 4-Cl | SO | 160 | | 4.0 | | 5 | 0 |
| | | | 80 | | 4.0 | | 5 | 0 |
| | | | 80 | | 4.0 | | 5 | 0 |
| | | | 40 | 27.5 | 4.0 | 23.5 | 3 | 0 |
| | | | 40 | 28.0 | 4.0 | 24.0 | 3 | 0 |
| | | | 20 | 23.0 | 4.0 | 19.0 | 2 | 0 |
| | | | 20 | 23.3 | 4.0 | 19.3 | 2 | 0 |
| | | | 10 | 23.0 | 4.0 | 19.0 | 1 | 0 |
| | | | 10 | 23.0 | 4.0 | 19.0 | 1 | 0 |
| | | | 5 | 15.8 | 4.0 | 11.8 | 0 | 0 |
| | | | 5 | 16.0 | 4.0 | 12.0 | 0 | 0 |
| | | | 2.5 | 4.0 | 4.0 | 0.0 | 0 | 0 |
| 12 | 4-$OCH_3$ | SO | 120 | 24.2 | 4.0 | 20.2 | 0 | 0 |
| 25 | 4-Cl | $SO_2$ | 320 | | 4.0 | | 5 | 0 |
| | | | 160 | | 4.0 | | 5 | 0 |
| | | | 80 | | 4.0 | | 5 | 0 |
| | | | 40 | | 4.0 | | 5 | 0 |
| | | | 20 | | 4.0 | | 5 | 0 |
| | | | 20 | | 4.0 | | 5 | 0 |
| | | | 10 | 20.0 | 4.0 | 16.0 | 3 | 0 |
| | | | 10 | 20.5 | 4.0 | 16.5 | 3 | 0 |
| | | | 5 | 16.0 | 4.0 | 12.0 | 1 | 0 |
| | | | 2.5 | 15.0 | 4.0 | 11.0 | 0 | 0 |
| | | | 1.25 | 7.4 | 4.0 | 3.4 | 0 | 0 |
| | | | 0.63 | 4.0 | 4.0 | 0.0 | 0 | 0 |
| 31 | 3-$OCH_3$ | $SO_2$ | 120 | 13.2 | 4.0 | 9.2 | 0 | 0 |
| 32 | 4-$OCH_3$ | $SO_2$ | 160 | 14.8 | 4.0 | 10.8 | 0 | 0 |
| 35 | 3,4-  | $SO_2$ | 40 | 20.4 | 4.0 | 16.4 | 0 | 0 |
| | | | 20 | 15.0 | 4.0 | 11.0 | 0 | 0 |
| | | | 10 | 13.0 | 4.0 | 9.0 | 0 | 0 |
| | | | 5 | 11.8 | 4.0 | 7.8 | 0 | 0 |
| | | | 2.5 | 8.8 | 4.0 | 4.8 | 0 | 0 |
| | | | 1.25 | 6.0 | 4.0 | 2.0 | 0 | 0 |
| cycloguanil hydrochloride | | | 120 | 22.7 | 3.6 | 19.1 | 1 | 1 |
| | | | 60 | 18.7 | 3.6 | 15.1 | 1 | 1 |
| | | | 30 | 15.3 | 3.6 | 11.7 | 1 | 0 |

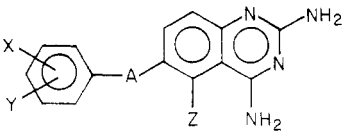
[a] ΔMST is the mean survival time (days) of treated chicks (MSTT) minus the mean survival time (days) of control chicks (MSTC). Each entry at each dose level represents results within a five-animal group. [b] Chicks surviving to 30 days post-infection are termed "cured"; data to establish parasitological 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.

Consequently, it was decided to determine whether or not the liabilities of cross-resistance and developing resistance could be avoided by concomitant delivery of a sulfonamide. Recent observations utilizing the related 2,4,6-triaminoquinazoline antimetabolites have shown that such drugs act synergistically with sulfones and sulfonamides and that the likelihood of acquiring resistance is diminished when combination therapy is employed.[14,15]

The value of adding sulfadiazine to the **35** regimen was examined in monkeys infected with the Malayan Camp, Vietnam Oak Knoll, and Vietnam Smith strains of *P. falciparum* and the Vietnam Palo Alto strain of *P. vivax* (Table X).[13] Three dose levels of sulfadiazine, 1.25, 5, or 20 mg/kg, were used in the combination regimens. Control studies showed that even 80 mg/kg doses of this sulfonamide, administered alone, effected no more than a transitory depression of parasitemia. However, in combination regimens, optimal benefits were attained with 5 mg/kg doses. The therapeutic accomplishments, when this dose of sulfadiazine was combined with, **35**, are summarized in Table X. Comparison of the data in columns 2 and 3 of Table X indicates that the activity of this quinazoline against infections with the various strains is enhanced greatly, from 16- to 64-fold, by concomitant delivery with 5 mg/kg of sulfadiazine. Two equally important negative findings which are not shown must also be stressed—the failure to develop quinazoline resistance when **35** is administered with sulfadiazine and the absence of enhancement of the toxicity of the quinazoline when sulfadiazine is concurrently delivered at doses of 5 or 20 mg/kg. If one assumes that doses of 0.4 mg of **35** per kg of body weight will cure infections even with resistant strains, this gives this quinazoline a therapeutic index in excess of 100.

**Antibacterial Activity.** Most of the 2,4-diamino-6-[(aryl)sulfinyl and sulfonyl]quinazolines (**1–5**, **7**, **9**, **11**, and **13–18**, Table I; and **19**, **21–24**, **28**, **29**, and **31–37**, Table II) were tested in vitro against several pathogenic bacteria:

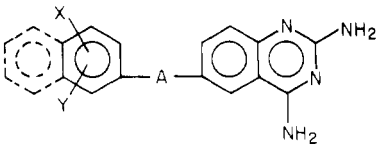
Table VI. Parenteral Prophylactic Antimalarial Effects of 2,4-Diamino-6-[(phenyl and naphthyl)sulfinyl and sulfonyl]quinazolines against Sporozoite-Induced *Plasmodium gallinaceum* in Chicks



| | | | | ΔMST; C or T[a-d] after single sc dose, mg/kg | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| no. | X, Y | Z | A | 480 (320) | 240 (160) | 120 (80) | 60 (40) | 30 (20) | 15 (10) | 7.5 (5) |
| 1 | 2,3,5,6-$F_4$ | H | SO | (C5) | (C5) | (C5) | | | | |
| 8 | 4-F | H | SO | C5 | C5 | C5 | 0.7; C3 | 0.7; C3 | 1.0; C1 | |
| 9 | H | H | SO | | C5 | C5 | C5 | 0.0; C4 | 0.0; C4 | 0.0; C4 |
| 11 | 3-$CF_3$ | H | SO | | C5 | C5 | C5 | C5 | C5 | 6.4; C4 |
| 14 | 2,3- | H | SO | C5 | C5 | C5 | C5 | C5 | C5 | |
| 16 | 3,4- | $CH_3$ | SO | | C5 | C5 | 3.7; C4 | 3.7; C4 | 3.7; C4 | 3.7; C4 |
| 17 | 2-$C_6H_5$ | H | SO | C5 | C5 | C5 | 3.7; C2 | 0.0 | 0.0 | |
| 18 | 3-$C_6H_5$ | H | SO | (C5) | (C5) | (C5) | | | | |
| 21 | 3,4-$Cl_2$ | H | $SO_2$ | C5 | C5 | 0.0; C3 | | | | |
| 22 | 3,5-$Cl_2$ | H | $SO_2$ | C5 | 7.0; C4 | 7.0; C2 | 7.0; C2 | 6.3; C2 | 2.2 | |
| 23 | 4-Br | H | $SO_2$ | C5 | C5 | C5 | C5 | C5 | C5 | |
| 27 | H | H | $SO_2$ | | C5 | C5 | C5 | C5 | C5 | C5 |
| 31 | 3-$OCH_3$ | H | $SO_2$ | C5 | C5 | C5 | 0.0; C4 | 0.0; C4 | 0.0; C3 | |
| 32 | 4-$OCH_3$ | H | $SO_2$ | C5 | C5 | C5 | | | | |
| 35 | 3,4- | H | $SO_2$ | | (C5) | (C5) | (C5) | | | |
| 37 | 3-$C_6H_5$ | H | $SO_2$ | | (C5) | (C5) | (C5) | 0.0; C4 | 0.0; C4 | 2.4; C1 |
| 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 attributable 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.

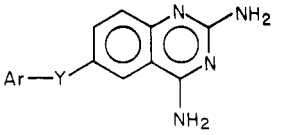
Table VII. Oral Effects of 2,4-Diamino-6-thioquinazolines against Sensitive and Drug-Resistant Lines of *Plasmodium berghei* in Mice

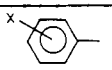
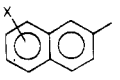


| | | | | | $SD_{90}$, (mg/kg)/day[c] | | | | cross-resistance[d] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | X,Y-Ar | A | regimen[a] | Q[b] ($SD_{75}$) | P | C | T | S | C | T | S |
| | 4-$ClC_6H_4$ | S | D×6 | 493 | 0.4 | 0.3 | | 2.2 | 0 | | 6 |
| | | | D×6 | 150 | 0.6 | | 2.3 | | | 4 | |
| | 3,4-$Cl_2C_6H_3$ | S | G×3 | 482 | 0.2 | <0.4 | <0.4 | 0.8 | 0 | 0 | 4 |
| | 4-$(CH_3)_2NC_6H_4$ | S | G×3 | 188 | 0.8 | <1.6 | | | 0 | | |
| | | | G×3 | 169 | 0.8 | | 0.9 | 1.1 | | 0 | <2 |
| | 4-$BrC_6H_4$ | S | G×3 | >174 | <0.4 | <1.6 | <1.6 | <1.6 | <4 | <4 | <4 |
| 23 | 4-$BrC_6H_4$ | $SO_2$ | G×3 | >256 | <0.4 | <1.6 | <1.6 | <1.6 | <3 | <3 | <3 |
| 26 | 4-$FC_6H_4$ | $SO_2$ | G×3 | 723 | 0.2 | 0.1 | | | 0 | | |
| | | | G×3 | 830 | 0.2 | | 0.6 | 1.0 | | 3 | 6 |
| | 2-$C_{10}H_7$ | S | G×3 | 155 | 1.2 | <1.6 | <1.6 | 2.3 | 0 | 0 | 2 |
| | | | G×3 | 352 | 0.6 | | | 2.8 | | | 5 |
| 15 | 2-$C_{10}H_7$ | SO | G×3 | 152 | 0.8 | 0.2 | 0.1 | | 0 | 0 | |
| | | | G×3 | 214 | 0.7 | | | 2.1 | | | 3 |
| 35 | 2-$C_{10}H_7$ | $SO_2$ | G×3 | 60 | 2.2 | <1.6 | 2.4 | 2.9 | 0 | 0 | <2 |
| | | | G×3 | 250 | 0.8 | <1.6 | | | 0 | | |

[a] Drugs were administered in the diet (D) or by gavage (G) for the number of days indicated. [b] Q is the quinine equivalent of the drug under test calculated at the dose required to suppress the parasitemia by 75%. [c] Dose required for 90% suppression of the parent (P) and chloroquine (C), cycloguanil (T), and DDS (S) resistant lines. [d] Number of fold cross-resistance compared with the parent (P) strain.

Table VIII. Curative Effects of 2,4-Diamino-6-(thio, sulfinyl, and sulfonyl)quinazolines against Resistant *P. falciparum* in the Aotus Monkey



| no. | Ar | X | Y | (mg/kg)/day × 7 required for >50% cures of infections with strains: Malayan Camp CH/Q; C-S, PYR-R[a] | Vietnam Oak Knoll; C-R, PYR-S[b] |
|---|---|---|---|---|---|
| |  | 4-Cl | S | 5.0 | 0.31 |
| | | 4-$N(CH_3)_2$ | S | 2.5 | 1.25 |
| | | 3-$CF_3$ | S | 0.39 | 0.098 |
| 29 |  | 3-$CF_3$ | $SO_2$ | 1.56 | 0.39 |
| | | H | S | 3.125 | 1.56 |
| 15 | | H | SO | 0.78 | 0.39 |
| 35 | | H | $SO_2$ | 0.39 | 0.025 |
| | quinine | | | 80 | 40 |
| | chloroquine | | | 5.0 | >20 |
| | pyrimethamine | | | >2.5 (MTD) | 0.6 |

[a] Chloroquine sensitive, pyrimethamine resistant. [b] Chloroquine resistant, pyrimethamine sensitive.

Table IX. Curative Effects of Quinine, Chloroquine, Pyrimethamine, and 35 against Sensitive and Drug-Resistant Strains of *P. falciparum* and *P. vivax* in Owl Monkeys

| strain | curative dose, mg of base/kg of body wt, administered po once daily for 7 days: quinine | chloroquine | pyrimethamine | 35 |
|---|---|---|---|---|
| *P. falciparum* | | | | |
| Malayan Camp-CH/Q | >20; <40 (RI) | >5; <10 (RI) | >2.5[a] (RIII) | 0.39 |
| Vietnam Oak Knoll | 80 (RIII) | >2 × 20 (RIII) | ~0.15 (S) | 0.098 |
| Vietnam Smith | >80 (RIII) | >20 (RIII) | >2.5[a] (RIII) | >6.25 |
| *P. vivax* | | | | |
| New Guinea Chesson | 40 (S) | 2.5 (S) | 0.625 (S) | 0.39 |
| Vietnam Palo Alto | 40 (S) | 2.5 (S) | >2.5[a] (RIII) | 6.25 |

[a] MTD.

Table X. Influence of Sulfadiazine on the Capacity of 35 to Cure Established Infections with Various Strains of *P. falciparum* and *P. vivax* in Owl Monkeys

| strain | $CD_{90}$: 35 daily po dose, mg/kg × 7: alone | with 5 mg/kg sulfadiazine × 7[a] |
|---|---|---|
| *P. falciparum* | | |
| Malayan Camp-CH/Q | 0.39 | 0.025 |
| Vietnam Oak Knoll | 0.098 | 0.00156 |
| Vietnam Smith | >6.25 | 0.39 |
| *P. vivax* | | |
| New Guinea Chesson | 0.39 | |
| Vietnam Palo Alto | 6.25 | 0.098 |

[a] No development of resistance in >200 monkeys.

*Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S 18713) *Staphylococcus aureus*, *Pseudomonas aeruginosa* (28), *Escherichia coli* (Vogel), *Shigella sonnei* (C-10), and *Mycobacterium tuberculosis* $H_{37}Rv$ (Table XI). A modification of the gradient plate procedure of Szybalski[16] and Webb and Washington[17] was employed throughout. Among them, **5**, **7**, **9**, **11**, **15**, **18**, and **23** 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 one compound (**2**) inhibited the growth of *E. coli* (Vogel) and *S. sonnei* (C-10) at concentrations of <0.25 μg/mL. None of the compounds was equipotent with trimethoprim against all five organisms.

Moreover, the 2,4-diamino-6-(arylsulfonyl)quinazolines proved to be potent folate antagonists. *Streptococcus 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 μg/mL 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.[18] Several thioquinazolines (**21**, **23**, **25–28**, **31**, **35**, and **37**) examined in this manner caused 50% inhibition at a concentration of 0.6–1.0 ng/mL, thus exhibiting activity 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.

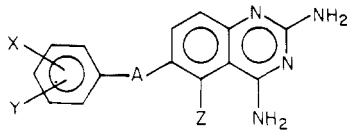
In a specific antifolic bioassay with WR-158,122 the following data for 50% inhibitory concentration (at μg/L) of drug were recorded: *S. faecalis*, 0.007 (reversed by folic acid); *Lactobacillus casei*, 0.140 (reversed by folic acid); *Pediococcus cerevisiae*, 0.160 (reversed by folinic acid).[19] Additionally, WR-158,122 was shown to inhibit *P. berghei* dihydrofolate reductase 50% at a concentration of $2.4 \times 10^{-9}$ M.[20]

**Conclusion.** The studies in the laboratory models reported above augur well for the potential clinical utility of this class of compound in man. It is hoped that implementation of adequate studies to allow such a verdict will be forthcoming in the near future.

## Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are corrected. The progress of the oxi-

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



**1-18**, A = SO
**19-37**, A = $SO_2$

| | | | minimum inhibitory concn, μg/mL[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| no. | X, Y | Z | *S.f.* MGH-2[c] | *S.a.* UC-76[d] | *S.a.* S18713 | *S.a.* 28[e] | *E.c.* vogel[f] | *S.s.* C-10[g] | *M.t.* $H_{37}Rv$[h] |
| 1 | 2,3,5,6-$F_4$ | H | <0.25 | 1.0 | 1.5 | >25 | >25 | >25 | 25 |
| 2 | 4-F | Cl | <0.25 | <0.25 | 1.0 | >25 | <0.25 | <0.25 | 2.5 |
| 3 | 2-Cl | Cl | <0.25 | 2.0 | >25 | >25 | >25 | >25 | >25 |
| 4 | 3,4-$Cl_2$ | H | <0.25 | >25 | >25 | >25 | >25 | >25 | N[b] |
| 5 | 4-Br | H | <0.25 | <0.25 | <0.25 | >25 | 2.5 | >25 | N |
| 7 | 4-Cl | H | <0.25 | <0.25 | <0.25 | >25 | 1.5 | 20 | N |
| 9 | H | H | <0.25 | <0.25 | <0.25 | >25 | 1.5 | 15 | 20 |
| 11 | 3-$CF_3$ | H | <0.25 | <0.25 | <0.25 | >25 | >25 | >25 | N |
| 13 | 3,4-  | Cl | <0.25 | >25 | >25 | >25 | >25 | >25 | >25 |
| 14 | 2,3-  | H | >25 | >25 | >25 | >25 | >25 | >25 | N |
| 15 | 3,4-  | H | <0.25 | <0.25 | <0.25 | >25 | <0.25 | 2.5 | N |
| 16 | 3,4-  | $CH_3$ | <0.25 | >25 | >25 | >25 | >25 | >25 | N |
| 17 | 2-$C_6H_5$ | H | <0.25 | 2.5 | 5.0 | >25 | >25 | >25 | 25 |
| 18 | 3-$C_6H_5$ | H | <0.25 | <0.25 | <0.25 | >25 | >25 | >25 | N |
| 19 | 4-I | Cl | <0.25 | 2.0 | >25 | >25 | 10 | >25 | >25 |
| 21 | 3,4-$Cl_2$ | H | <0.25 | 0.5 | 1.0 | >25 | 2.5 | 20 | 20 |
| 22 | 3,5-$Cl_2$ | H | 1.0 | 2.5 | 10 | >25 | >25 | >25 | >25 |
| 23 | 4-Br | H | <0.25 | <0.25 | <0.25 | >25 | 2.5 | >25 | N |
| 24 | H | Cl | <0.25 | 2.0 | 2.0 | >25 | 2.0 | 2.0 | >25 |
| 28 | 3-$CF_3$ | Cl | <0.25 | >25 | >25 | >25 | >25 | >25 | >25 |
| 29 | 3-$CF_3$ | H | 0.25 | 1.5 | 2.0 | >25 | >25 | >25 | 25 |
| 31 | 3-$OCH_3$ | H | <0.25 | 1.0 | 2.0 | >25 | 2.5 | >25 | 25 |
| 32 | 4-$OCH_3$ | H | <0.25 | 2.5 | 2.5 | >25 | 2.5 | 2.5 | N |
| 33 | 3,4-  | Cl | <0.25 | >25 | >25 | >25 | >25 | >25 | >25 |
| 34 | 2,3-  | H | >25 | >25 | >25 | >25 | >25 | >25 | >25 |
| 35 | 3,4-  | H | >25 | 25 | >25 | >25 | >25 | >25 | >25 |
| 36 | 2-$C_6H_5$ | H | 0.25 | >25 | >25 | >25 | >25 | >25 | N |
| 37 | 3-$C_6H_5$ | H | <0.25 | 1.0 | 1.5 | >25 | >25 | >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.* = *Straphylococcus aureus*. [e] *P.a.* = *Pseudomonas aeruginosa*. [f] *E.c.* = *Escherichia coli*. [g] *S.s.* = *Shigella sonnei*. [h] *M.t.* = *Mycobacterium tuberculosis*.

dations was followed by TLC using silica gel plates (Eastman) and a solvent mixture of MeOH–EtOAc–$Et_3N$, 25:75:1. Satisfactory infrared spectra were obtained for all compounds. The sulfinyl compounds exhibited a strong absorption band at or about 1040 $cm^{-1}$, characteristic of the S=O stretching vibrations, and the sulfonyl compounds exhibited strong absorption bands at or about 1160 and 1310 $cm^{-1}$, characteristic of O=S=O. Nuclear magnetic resonance spectra were obtained as necessary to confirm the structure or the presence of solvate. The preparation of the precursor 2,4-diamino-6-[(phenyl and naphthyl)thio]quinazolines has been described.[5]

**Preparation of 2,4-Diamino-6-[(phenyl and naphthyl)-sulfinyl]quinazolines (Table I; 1–18). Procedure I.** The preparation of 2,4-diamino-5-chloro-6-[(*p*-fluorophenyl)sulfinyl]quinazoline (Table I; **2**) is described as an example. A mixture of 1.0 g (0.003 mol) of 2,4-diamino-5-chloro-6-[(*p*-fluorophenyl)thio]quinazoline and 0.7 g (0.0016 mol) of diazabicyclooctane dibromide in 30 mL of 70% (v/v) aqueous acetic acid was allowed to stand with intermittent agitation for 4 days. The solution was diluted with water and made alkaline with 50% aqueous NaOH. The resulting precipitate was collected, washed with water, air-dried, and recrystallized from DMF–$H_2O$ to afford 0.84 g (80%) of the desired product, mp 267–270 °C.

**Procedure II.** The preparation of 2,4-diamino-5-chloro-6-[(*o*-chlorophenyl)sulfinyl]quinazoline (Table I; **3**) is described as an example. A mixture of 2.5 g (0.0074 mol) of 2,4-diamino-5-chloro-6-[(*o*-chlorophenyl)thio]quinazoline, 15 mL of 30% $H_2O_2$, and 29 mL of HOAc was stirred at room temperature for 28 h and poured into iced 50% NaOH. The resulting precipitate was collected, washed with water, and recrystallized from DMF to give 1.4 g (52%) of the desired product, mp 311–312 °C dec.

**Preparation of 2,4-Diamino-6-[(phenyl and naphthyl)-sulfonyl]quinazolines (Table II; 19–37).** The preparation of 2,4-diamino-6-(phenylsulfonyl)quinazoline hydrate (Table II; **27**) is given as an example. To a stirred slurry of 5.4 g (0.02 mol) of 2,4-diamino-6-(phenylthio)quinazoline in 100 mL of 70% aqueous HOAc was added 40 mL (0.4 mol) of 30% $H_2O_2$. After stirring for 20 h at room temperature, the mixture was poured into ice–water containing excess NaOH. The precipitate was collected, washed thoroughly with acetone, and recrystallized from

DMF–$H_2O$ to give 4.0 g (65%) of the product as an off-white solid, mp 297–299 °C.

## References and Notes


(1) This is paper 44 of a series on antimalarial drugs. For paper 43, see J. Johnson, E. F. Elslager, and L. M. Werbel, *J. Heterocycl. Chem.*, **16**, in press (1979).
(2) This investigation was supported by the U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is contribution No. 1542 to the Army Research Program on Malaria.
(3) Deceased June 21, 1973.
(4) E. F. Elslager, Proceeding of the International Symposium on Medicinal Chemistry, 4th, J. Maas, Ed., Elsevier, Amsterdam, 1974, pp 227–270.
(5) E. F. Elslager, P. Jacob, J. Johnson, L. M. Werbel, D. F. Worth, and L. Rane, *J. Med. Chem.*, **21**, 1059 (1978).
(6) E. F. Elslager, J. Davoll, P. Jacob, A. M. Johnson, J. Johnson, and L. M. Werbel, *J. Med. Chem.*, **21**, 639 (1978).
(7) S. Oae, Y. Ohnishi, S. Kozuka, and W. Tagaki, *Bull. Chem. Soc. Jpn.*, **39**, 364 (1964).
(8) The parenteral antimalarial screening in mice was carried out by Dr. Leo Rane, and test results were provided through the courtesy of Dr. T. R. Sweeney and Dr. E. A. Steck of the Walter Reed Army Institute of Research.
(9) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).
(10) 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.
(11) For a description of the test method, see L. Rane, and D. S. Rane, *Proc. Helminthol. Soc. Wash.*, **39**, 283 (1972).
(12) Oral antimalarial testing was performed by Dr. Paul E. Thompson and colleagues in the laboratories of Parke-Davis and Co.
(13) These tests were performed by Dr. L. H. Schmidt: L. H. Schmidt, *W.H.O., Tech. Rep. Ser.*, **no. 529** (1973); L. H. Schmidt, *Trans. R. Soc. Trop. Med. Hyg.*, **67**, 446 (1973). For a definition of the magnitude of RI, RIII drug resistance, see *W.H.O., Tech. Rep. Ser.*, **no. 375**, (1967); **529** (1973).
(14) P. E. Thompson, A. Bayles, and B. Olszewski, *Exp. Parasitol.*, **25**, 32 (1969).
(15) P. E. Thompson, A. Bayles, and B. Olszewski, *Am. J. Trop. Med. Hyg.*, **19**, 12 (1970).
(16) W. Szybalski, *Microb. Genet. Bull.*, **5**, 16 (1954).
(17) A. H. Webb and L. Washington, *Bacterial. Proc.*, **52** (1966).
(18) For a more complete description of such antimetabolite studies, see: J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 812 (1972).
(19) These studies were carried out by Dr. C. C. Smith and co-workers at the University of Cincinnati.
(20) These data were provided by Col. D. E. Davidson of the Walter Reed Army Institute of Research.


# *Notes*

## Syntheses and Biological Activities of 7β-Methyl Steroids


Wen-Hsiung Chiu* and Manfred E. Wolff

*Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143.*

*Received March 15, 1979*


The preparation of 7β-methyl-5α-dihydrotestosterone acetate and its 2-thia-*A*-nor analogue is described. Biological evaluation shows that a 7β-Me largely decreases myotrophic–androgenic activity in both 5α-dihydrotestosterone and the 2-thia-*A*-nor analogue. Testing for antitumor activity shows that the reduction in breast tumor weight was not significant for either compound, but the final tumor size in the animals treated with 7β-methyl-2-thia-*A*-nor steroid at 10 (mg/kg)/day was significantly reduced. The effects of 7β-Me steroids on the various organ weights are also described. The influence of 7β-Me substituent on the biological activities of androgens may be mediated through direct interaction of the substituent with the receptor surface in contact with the third dimension of the steroid molecule.


Breast cancer is the single most important cause of cancer deaths in women in the U.S.[1] Like cancer of other organs under hormonal control, breast cancer may respond to hormonal therapy;[2] androgen treatment, in the form of testosterone propionate, produces regressions in about 25% of all patients[3] but produces undesirable virilization. By introduction of a 7β-methyl group into methyltestosterone, an equally efficacious antineoplastic drug (calusterone)[4] lacking strong masculinizing properties is produced.

In a previous study,[5] using derivatives of 2-thia-*A*-nor-5α-androstan-17β-ol as probes of steroid–receptor interactions, we showed that enhancing groups, such as 7α-Me, known to be useful in carbocyclic steroids, such as testosterone and 5α-dihydrotestosterone (DHT), could be introduced into heterocyclic steroids, such as 2-thia-*A*-nor steroids, to give similar increases in myotrophic–androgenic activity. Since the introduction of a 7β-Me into methyltestosterone decreased myotrophic–androgenic activity while increasing antitumor activity, one might expect similar effects on introducing such a group into 5α-DHT or into 2-thia-*A*-nor steroid. For this reason, the preparation of 7β-methyl-5α-dihydrotestosterone acetate (**2**) and 7β-methyl-2-thia-*A*-nor-5α-androstan-17β-ol acetate (**6**) was undertaken.

6-Dehydro-7-methyltestosterone acetate (**1**) was prepared in good yield by a reported procedure.[6] Catalytic hydrogenation of **1** in acetic acid gave a product (**2**) which had a positive CD curve and a positive Cotton effect in the ORD. It was assigned the 5α configuration **2** on this basis. The assignment of a 7β-Me configuration to **2** was made on the basis of the catalytic hydrogenation of the dienone system in **2** which would be expected to proceed by cis addition of hydrogen to the α face and the fact that **2** was not identical with the 7α-Me epimer.[5] Under similar conditions, Beyler et al.[7] obtained a 7β-methyl-5α-androstan-3-one derivative in very good yield from the

0022-2623/79/1822-1257$01.00/0 © 1979 American Chemical Society