Folate Antagonists. 17. Synthesis and Biological Properties of a 2,4-Diamino-6-thioquinazoline Analog of Aminopterin (1)

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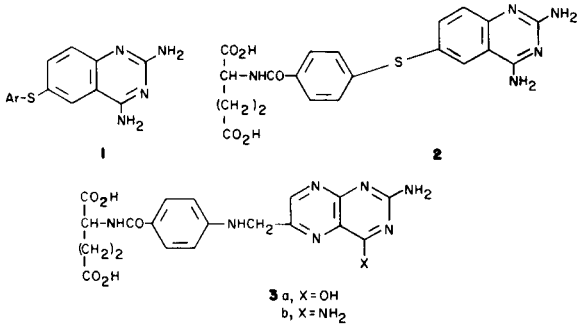
Received October 8, 1979

A multistep route for the synthesis of *N*-[4-[(2,4-diamino-6-quinazolinyl)thio]benzoyl]-L-glutamic acid (2) from 4-mercaptobenzoic acid and 5-chloro-2-nitrobenzonitrile is described. Although this aminopterin analog lacked significant antimalarial activity, it was a potent inhibitor of dihydrofolate reductase from *Trypanosoma cruzi*. The pteroic ester analog **11**, however, was active against *Plasmodium berghei* infections in mice at high doses.

J. Heterocyclic Chem., **17**, 497 (1980).

The potent antimalarial activity of a series of nonclassical folate antagonists, the 2,4-diamino-6-[(aryl)thio]quinazolines (3) **1** prompted the synthesis and biological evaluation of *N*-[4-[(2,4-diamino-6-quinazolinyl)thio]benzoyl]-L-glutamic acid (**2**), a classical folate antagonist related to folic acid **3a** and aminopterin **3b**. This communication describes this work in detail.



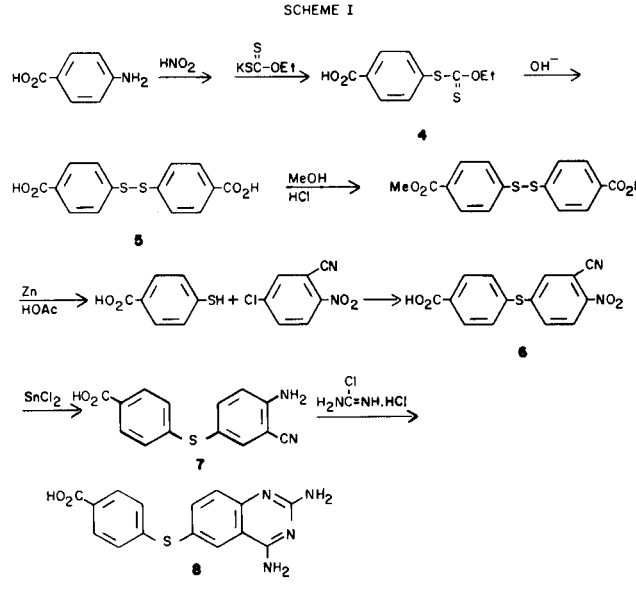
1 **2**

3 a, X = OH
b, X = NH_2

Chemistry.

The route to **2** is shown in Schemes I and II. Diazotization of 4-aminobenzoic acid followed by treatment with carbonodithioic acid, *O*-ethyl ester, potassium salt gave 4-[(ethoxythioxomethyl)thio]benzoic acid (**4**). An attempt to obtain 4-mercaptobenzoic acid by hydrolysis with strong base gave instead the disulfide **5** (4). Initially this was converted to its dimethyl ester by refluxing in methanolic hydrochloric acid, and then treated with zinc and acetic acid (5) to cleave the disulfide and at the same time hydrolyze the ester to provide 4-mercaptobenzoic acid. Later it was found that crude **5** could be treated directly with zinc-acetic acid to provide the desired 4-mercaptobenzoic acid. This material was used directly in the reaction with 5-chloro-2-nitrobenzonitrile to give 4-[(3-cyano-4-nitrophenyl)thio]benzoic acid (**6**). Reduction with stannous chloride afforded the aminonitrile **7** which was cyclized with carbamimidic chloride hydrochloride to give 4-[(2,4-diamino-6-quinazolinyl)thio]benzoic acid (**8**).

SCHEME I



Since the yield in this step was unsatisfactory for the preparation of any quantity of material, an alternative route (Scheme II) was developed. Thus **6** was methylated in 82% yield with dimethyl sulfate in the presence of 1,1',1"-nitrilotris[2-propanol] (6) to form **9**. Reduction with stannous chloride provided **10** in 70% yield, and this underwent cyclization with carbamimidic chloride hydrochloride to **11** in quantitative yield. Hydrolysis with base then provided **8** in 90% yield. Condensation of the acid **8** with diethyl glutamate in the presence of dicyclohexylcarbodiimide (7) gave *N*-[4-[(2,4-diamino-6-quinazolinyl)thio]benzoyl]-L-glutamic acid diethyl ester (**12**) in low yield, and hydrolysis with dilute base gave the desired product **2**.

Biology.

The quinazoline **2** and its ethyl ester **12**, together with the pteroic acid analogs **8** and **11** were tested against a normal drug-sensitive strain of *Plasmodium berghei* in mice by the parenteral route (8,9). The compounds were dissolved or suspended in peanut oil and were admini-

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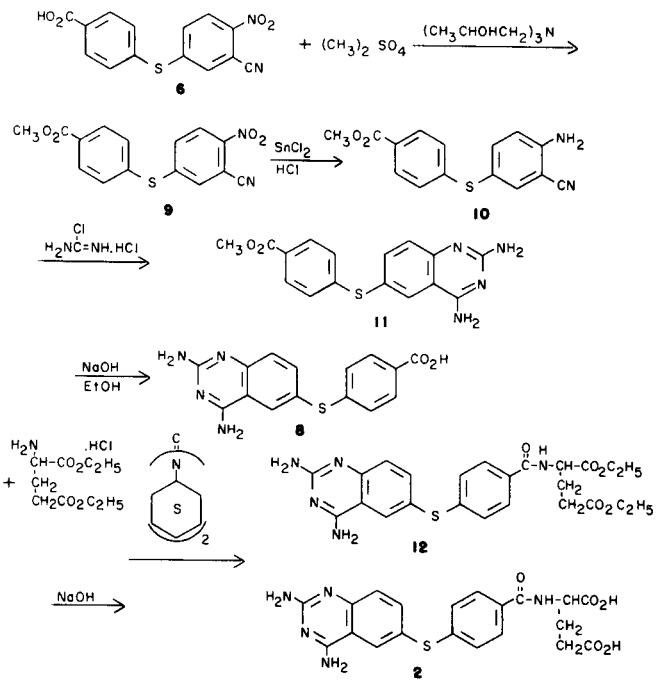
SCHEME II



stered to mice in a single subcutaneous dose 72-hour post infection. Extension of the mean survival time of the treated mice is interpreted as evidence of antimalarial activity (9). Compounds are designated "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 injected control mice ranged from 6.1 to 6.3 days. In this system neither the aminopterin analogs **2** and **12** nor the pteroic acid analog **8** exhibited significant antimalarial activity. Only the ester **11** extended the survival time of treated mice beyond that of the control animals, providing an extension of 7 and 9 days at 320 and 640 mg./kg. respectively.

The thioquinazoline **2** proved to be a very potent and selective inhibitor of dihydrofolate reductase from *Trypanosoma cruzi* (10). The ID_{50} against the enzyme derived from rat liver and *T. cruzi* was 2×10^{-7} *M* respectively, and the relative potency ($1 \times 10^{-5}/ID_{50}$) thus 50 *vs* 20,000. These data provide further evidence that it is possible to achieve selective inhibition of parasite *vs* mammalian reductases, and suggest that **2** is worthy of evaluation against *T. cruzi* infections in animals. In this regard, it is noteworthy that a related compound, 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline, has been shown to possess strong suppressive activity against *T. cruzi* infections in mice (11).

Conclusion.

The presence of the folic acid side chain as well as related electronegative carboxy and carbethoxy groups on the aryl group of the 2,4-diamino-[(aryl)thio]quinazolines **1** was radically deleterious to antimalarial activity. It is clear that the molecular geometry of the latter which allows them to block the folic acid pathway of the malaria parasite and their transport into the parasitized erythrocyte/parasite in no way depends upon the presence of the terminal glutamic acid residue.

EXPERIMENTAL (12)

4,4'-Dithiobis[benzoic acid] (**5**).

A solution of 13.2 g. (0.109 mole) of 4-aminobenzoic acid, 4.4 g. (0.11 mole) of sodium hydroxide, and 7.5 g. (0.109 mole) of sodium nitrite in 130 ml. of water was added slowly to a cold (0-5°) mixture of 30 ml. of concentrated hydrochloric acid and 40 ml. of ice. The temperature was maintained at 0-5° during the addition. After the addition of the nitrite solution the reaction was stirred 30 minutes and neutralized to Hydrion paper with potassium acetate. The cold diazonium solution was run in a thin stream into a 1 l. beaker containing 50 g. (0.31 mole) of carbonodithioic acid, *O*-ethyl ester, potassium salt in 160 ml. of water at 75-80° with vigorous stirring. The temperature was maintained at 75-80° during the addition and a copious evolution of nitrogen occurred and some oily material appeared at the surface. The reaction mixture was cooled, acidified, and the beige solid that formed was collected by filtration, washed with 100 ml. of water, and dissolved in 80 ml. of 10% sodium hydroxide solution. The solution was heated on a steam bath for 2 hours cooled, and acidified with concentrated hydrochloric acid. The beige solid that resulted was collected by filtration, dried briefly on the filter, and then dried *in vacuo* to give 13.5 g. (44%) of crude disulfide, m.p. 313-325°. Ultraviolet analysis showed this material to contain 34% of 4-mercaptobenzoic acid.

Dimethyl 4,4'-Dithiobis[benzoate].

A mixture of the crude disulfide and 700 ml. of methanol was saturated with dry hydrogen chloride gas and then heated under reflux for 2 hours during which time the reaction mixture became a clear solution. The reaction was allowed to remain at room temperature for 18 hours and the solid that formed was collected to give 3.1 g., m.p. 123-125°. The volume of the filtrate was reduced *in vacuo*, 500 ml. of water was added, and the mixture was extracted with ether. The extract was washed once with water and once with dilute sodium bicarbonate, and dried over magnesium sulfate. The ether was removed *in vacuo* to give 16.7 g. of a soft wax-like solid (this represents more than 100% theoretical yield probably due to some residual solvent). This material was combined with the 3.1 g. obtained above and used as is.

4-Mercaptobenzoic Acid.

A mixture of 19.8 g. (0.059 mole) of crude dimethyl 4,4'-dithiobis[benzoate], 150 ml. of glacial acetic acid, and 15 g. of zinc was heated under reflux for 4 hours. The mixture was cooled and filtered and the solid washed with water. The solid was suspended in 100 ml. of water and made basic with 50% sodium hydroxide solution. This mixture was heated on a steam bath for 5 minutes and then the inorganic solids were collected. The filtrate was acidified with concentrated hydrochloric acid to precipitate the product. This product was dried briefly by suction using a rubber dam, and was then dissolved in 200 ml. of ether. The ether solution was washed with water and then briefly dried over magnesium sulfate. The solvent was removed *in vacuo* at 35°; the resulting solid was triturated with 25 ml. of chloroform and the solid was collected by filtration to give 7.6 g. (42%) of 4-mercaptobenzoic acid, m.p. 193-198°.

In succeeding preparations of 4-mercaptobenzoic acid, the use of the dimethyl ester of 4,4'-dithiobis[benzoic acid] was eliminated and the crude 4,4'-dithiobis[benzoic acid] was cleaved with zinc-acetic acid as described above. One run using 11.0 g. of the disulfide gave 4.2 g. (30%) of product, m.p. 203-215°, and another run using 30 g. of disulfide gave

11.3 g. (38%), m.p. 205-215°. Subliming a small sample of this material at 210° and 0.25 mm pressure gave material with melting point of 198-210° (immersed at 210°, m.p. 215-217°). Literature melting point is 216-217°.

4-[(3-Cyano-4-nitrophenyl)thio]benzoic Acid (**6**).

To a solution of 11.3 g. (0.074 mole) of 4-mercaptobenzoic acid, 600 ml. of acetone, and 21.5 ml. (0.148 mole) of triethylamine was added with virorous stirring 13.4 g. (0.074 mole) of 5-chloro-2-nitrobenzonitrile. The reaction mixture became bright red and became warm; it was stirred for 15 minutes. The acetone volume was reduced by half *in vacuo* and the mixture was poured into 1.5 l. of water. This solution was acidified with concentrated hydrochloric acid and the resulting yellow solid was collected by filtration. This solid was dried *in vacuo* at 45° and then recrystallized from 500 ml. of acetonitrile to give 16.2 g. (72 %) of the product, m.p. 211-213°.

Anal. Calcd. for $C_{14}H_8N_2O_4S$: C, 56.00; H, 2.68; N, 9.33. Found: C, 55.80; H, 2.78; N, 9.18.

4-[(4-Amino-3-cyanophenyl)thio]benzoic Acid (**7**).

To a cloudy solution of 30 g. (0.133 mole) of stannous chloride in 80 ml. of concentrated hydrochloric acid and 40 ml. of glacial acetic acid was added a warm solution of 12.0 g. (0.04 mole) of 4-[(3-cyano-4-nitrophenyl)thio]benzoic acid (**6**) in 40 ml. of glacial acetic acid and 40 ml. of *N,N*-dimethylformamide. The temperature rose to 75° and the bright yellow color changed to a cream color within 5 minutes. The reaction mixture was stirred for an additional 10 minutes and poured into 800 ml. of water. The beige solid was collected by filtration and dried *in vacuo* at 45°. The solid was dissolved in 1400 ml. of methanol and dry hydrogen sulfide gas was bubbled through the solution for 8 minutes. The light cream colored solid which appeared was collected and discarded. The solvent was removed *in vacuo*, the residue was triturated with 50 ml. of methanol and the beige solid collected to give 8.0 g. (74%) of the product, m.p. 253-254°.

Anal. Calcd. for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36. Found: C, 62.03; H, 3.74; N, 10.17.

4-[(2,4-Diamino-6-quinazolinyl)thio]benzoic Acid, Monohydrate (**8**).

A mixture of 2.7 g. (0.01 mole) of 4-[(4-amino-3-cyanophenyl)thio]benzoic acid (**7**), 1.1 g. (0.01 mole) of carbamimidic chloride hydrochloride, and 10 ml. of diglyme was heated to 135-140° for 45 minutes. An additional 0.2 g. of carbamimidic chloride hydrochloride was added and the mixture was heated for an additional 30 minutes. The reaction was cooled and filtered, and the solid washed with 5-10 ml. of diglyme and then with 10-15 ml. of water. The crude solid was dried *in vacuo* to give 0.9 g. of material, m.p. 337-341°. This solid was dissolved in a small amount of hot *N,N*-dimethylformamide, treated with charcoal, and filtered through celite. Several drops of water were added and an amorphous dark material was removed by filtration. More water was added to precipitate a dark solid which was filtered and dried *in vacuo* at 45° to give 0.3 g. (10%) of the monohydrate of the desired product, m.p. 335-339°.

Anal. Calcd. for $C_{15}H_{12}N_4O_2S \cdot H_2O$: C, 54.54; H, 4.27; N, 16.96; H_2O, 5.45. Found: C, 54.33; H, 4.43; N, 16.97; H_2O, 5.66.

4-[(3-Cyano-4-nitrophenyl)thio]benzoic Acid Methyl Ester (**9**).

A solution of 12.0 g. (0.04 mole) of 4-[(3-cyano-4-nitrophenyl)thio]benzoic acid, (**6**), 9.5 g. (0.05 mole) of 1,1′,1″-nitrilotris[2-propanol] and 5.6 g. (0.044 mole) of dimethyl sulfate in 400 ml. of acetone was heated on a steam bath for 1 hour. The acetone was allowed to evaporate during the course of the reaction so that most of it had evaporated by the end of the 1 hour. Water was added and the mixture was heated on the steam bath for 5-10 minutes. The resulting yellow solid was collected and dried *in vacuo* to give 12.0 g. of crude material, m.p. 144-145°. Recrystallization from acetonitrile gave 10.3 g. (82%) of analytical material, m.p. 149-150°.

Anal. Calcd. for $C_{15}H_{10}N_2O_4S$: C, 57.32; H, 3.21; N, 8.91. Found: C, 57.19; H, 3.35; N, 9.01.

4-[(4-Amino-3-cyanophenyl)thio]benzoic Acid, Methyl Ester (**10**).

To a cloudy solution of 22.2 g. (0.10 mole) of stannous chloride dihydrate in 60 ml. of concentrated hydrochloric acid and 30 ml. of acetic acid was added a warm solution of 9.4 g. (0.03 mole) of 4-[(3-cyano-4-nitrophenyl)thio]benzoic acid methyl ester (**9**) in 45 ml. of *N,N*-dimethylformamide. The reaction was strongly exothermic and the flask was swirled in an ice bath. The color changed from bright yellow to light yellow and a heavy precipitate appeared. After 3-4 minutes in the ice bath the reaction mixture was poured into 125 g. of 50% sodium hydroxide solution in 1 l. of water. The solid was collected, washed with water, and dried *in vacuo* to give 7.6 g. of crude product. This material was passed through an alumina column with ethyl acetate. The homogeneous fractions were combined and the solvent removed *in vacuo*. The resulting solid was recrystallized from benzene to give 4.4 g. of material, m.p. 148-150°. The addition of hexane to the benzene filtrate provided another 1.6 g. of material, m.p. 146-149°, making the total yield 6.0 g. (70%).

4-[(2,4-Diamino-6-quinazolinyl)thio]benzoic Acid Methyl Ester, 0.75 Hydrate (**11**).

A mixture of 2.8 g. (0.01 mole) of 4-[(4-amino-3-cyanophenyl)thio]benzoic acid methyl ester, (**10**), 2.2 g. (0.02 mole) of carbamimidic chloride hydrochloride and 15 g. of sulfonylbis[methane] was treated slowly over 15 minutes to 165° and then maintained there for 20 minutes whereupon the reaction mixture became a clear yellow solution. After an additional 20 minutes at 165° a solid began to appear. The reaction was cooled, triturated with 350 ml. of water and then 50 ml. of concentrated ammonium hydroxide was added to give a yellow solid. The solid was collected and dried *in vacuo* to give 3.3 g. of crude material, m.p. 252-275°. The solid was dissolved in hot *N,N*-dimethylformamide, filtered and 10% ammonium hydroxide was added until no further precipitation occurred. The solid was collected and dried *in vacuo* at 45° to give 3.4 g. (99%) of the desired product as the 0.75 hydrate, m.p. 253-256°.

Anal. Calcd. for $C_{16}H_{14}N_4O_2S \cdot 0.75H_2O$: C, 56.54; H, 4.59; N, 16.48; H_2O, 3.97. Found: C, 56.23; H, 4.69; N, 16.71; H_2O, 3.72.

4-[(2,4-Diamino-6-quinazolinyl)thio]benzoic Acid (**8**).

A solution of 0.56 g. (0.017 mole) of 4-[(2,4-diamino-6-quinazolinyl)thio]benzoic acid methyl ester (**11**) in 3.0 ml. of 1*N* sodium hydroxide and 100 ml. of absolute ethanol was heated under reflux until thin layer chromatography indicated that no further starting material was present (1.5 hour). After 50 ml. of water and 3.0 ml. of 1*N* hydrochloric acid was added, the ethanol was removed *in vacuo* to give a light yellow solid which was collected by filtration and washed successively with water, diethyl ether, methanol and diethyl ether. The solid was dried *in vacuo* at 40° to give 0.50 g. (90%), m.p. 348-350° of the product as the 1.25 hydrate.

Anal. Calcd. for $C_{18}H_{12}N_4O_2S \cdot 1.25H_2O$: C, 53.79; H, 4.36; N, 16.73; H_2O, 6.72. Found: C, 53.60; H, 4.55; N, 16.60; H_2O, 6.61.

N-[4-(2,4-Diamino-6-quinazolinyl)thio]benzoyl-L-glutamic Acid, Diethyl Ester (**12**).

To a slurry of 1.86 g. (5.0 mmoles) of 4-[2,4-diamino-6-quinazolinyl)thio]benzoic acid (**8**) in 40 ml. of *N,N*-dimethylformamide was added 1.36 g. (5.0 mmoles) of pentachlorophenol and 1.03 g. (5.0 mmoles) of dicyclohexylcarbodiimide. The reaction was allowed to remain at room temperature for 3 hours, filtered to remove unchanged starting material and *N,N*-dicyclohexylurea, and cooled in an ice bath. In another flask, 1.15 g. (5.0 mmoles) of diethyl-L-glutamate hydrochloride was dissolved in 2.5 ml. of cold *N,N*-dimethylformamide; 0.7 ml. (5.0 mmoles) of triethylamine was added and the triethylamine hydrochloride which precipitated was removed by filtration and washed with several drops of *N,N*-dimethylformamide. The filtrate and washings were combined and added to the cooled *N,N*-dimethylformamide solution of activated ester prepared above and a trace of 1*H*-benzotriazol-1-ol was added as a catalyst. The reaction was kept at 40° for 24 hours and at room temperature for 24 hours. After diluting with 200 ml. of ethyl acetate, the reaction was washed successively twice with water, twice with 1*N* sodium hydroxide and once more with water. The ethyl acetate layer was dried over magnesium sulfate and concentrated *in vacuo* to give a soft solid

which when triturated with diethyl ether gave 0.61 g. of an off white solid. The solid was dissolved in 3 ml. of *N,N*-dimethylformamide, filtered to remove any residual *N,N*-dicyclohexylurea and the product was precipitated with water. The resulting solid was reprecipitated from ethyl acetate with hexane in two crops to give 0.33 g. of material. This material was combined with material from another 2.0 mmoles run to give 0.43 g. (13%) of product, m.p. 133-138°.

Anal. Calcd. for $C_{24}H_{27}N_5O_3S$: C, 57.92; H, 5.47; N, 14.08. Found: C, 57.86; H, 5.78; N, 13.83.

N-[4-[(2,4-Diamino-6-quinazolinyl)thio]benzoyl-L-glutamic Acid, 0.25 Formula Weight Hydrochloride, 1.3 Hydrate. (**2**).

N-[4-[(2,4-Diamino-6-quinazolinyl)thio]benzoyl-L-glutamic acid, diethyl ester (**12**) (1.21 g., 0.024 mole) was dissolved in 120 ml. of absolute ethanol, and a small amount of undissolved solid was removed by filtration through a fine sintered glass funnel. To the filtrate was added 53.5 ml. of 1.0 *N* sodium hydroxide and this solution was stirred at room temperature for 20 minutes. Fifty ml. of water was added and the ethanol was removed *in vacuo*. A light yellow solid impurity was removed by filtering the aqueous solution through a fine sintered glass funnel and 53.5 ml. of 1.0 *N* hydrochloric acid was added to the filtrate. A cream colored solid precipitated and was collected by filtration, washed with ether, and dried *in vacuo*. The dried solid was washed again with ether and then three times with a solution of chloroform:methanol:water (60:45:10). The solid was dried again *in vacuo* and then stirred for 3 hours in the chloroform:methanol:water mixture. The solid was collected by filtration, dried *in vacuo*, and allowed to equilibrate in the air to give 0.5 g. (43%) of product, m.p. 237-240° dec.

Anal. Calcd. for $C_{20}H_{19}N_5O_5S \cdot 1.3\ H_2O \cdot 0.25$ HCl: C, 50.68; H, 4.64; N, 14.78; Cl, 1.86; H_2O, 4.93. Found: C, 51.02; H, 4.31; N, 14.86; Cl, 1.79; H_2O, 5.20.

Acknowledgements.

The authors are indebted to Dr. Leo Rane of the University of Miami for the antimalarial testing, and to Mr. C. E. Childs and associates for the microanalyses.

REFERENCES AND NOTES

(1) This is paper **47** of a series on antimalarial drugs. For paper **46** see J. L. Johnson, B. Whitney and L. M. Werbel, *J. Heterocyclic Chem.*, **17**, 501 (1980).

(2) This investigation was supported by U.S. Army Medical Research and Development Command Contract DA-17-72-MD-2077. This is contribution No. 1562 to the Army Research Program in malaria.

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(8) The parenteral antimalarial screening in mice was carried out by Dr. Leo Rane and Mrs. D. S. Rane at the University of Miami 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.

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