# Folate Antagonists. 5. Antimalarial and Antibacterial Effects of 2,4-Diamino-6-(aryloxy and aralkoxy)quinazoline Antimetabolites (1-3)

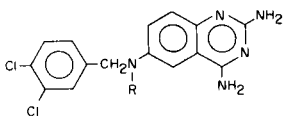
*Edward F. Elslager,* Jane Clarke, Judith Johnson, Leslie M. Werbel, and John Davoll*

Chemistry Department, Research and Development Division, Parke, Davis and Company, Ann Arbor, Michigan 48106 and Hounslow, Middlesex, England

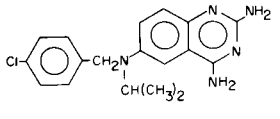
Received March 2, 1972

Various 2,4-diamino-6-(phenoxy, naphthyloxy, and phenalkoxy)quinazolines (VIII, XIV) were synthesized for antimalarial and antibacterial evaluation. Treatment of the anion of the requisite phenol or naphthol with 5-chloro-2-nitrobenzonitrile (V) gave the corresponding 2-nitro-5-(phenoxy and naphthyloxy)benzonitriles (VI) (33-78%). Alternatively, alkylation of 5-hydroxy-2-nitrobenzaldehyde (IX) with the appropriate phenalkyl halide afforded the 2-nitro-5-(phenalkoxy)benzaldehydes (X) (27-62%), which were converted to the 2-nitro-5-(phenalkoxy)benzonitriles (XII) *via* the intermediate oximes XI (62-87% overall). Reduction of the 2-nitrobenzonitriles (VI, XII) with stannous chloride-hydrochloric acid provided the corresponding 2-amino-5-(phenoxy, naphthyloxy, and phenalkoxy)benzonitriles (VII, XIII) (30-83%), which upon cyclization with chloroformamidine hydrochloride gave the 2,4-diaminoquinazolines VIII and XIV (12-85%). Against *Plasmodium berghei* in mice, eleven compounds were active orally at doses ranging from 6.3 to 174 mg./kg./day for 6 days, while seven substances displayed activity subcutaneously following single doses of 40-640 mg./kg. Fifteen compounds exhibited *in vitro* antibacterial activity against *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus, Escherichia coli* (Vogel), and *Shigella sonnei* (C-10) with MIC's ranging from $< 0.25$ to 20 μg./ml. (gradient plate). Data on the inhibitory effects of representative compounds against *Streptococcus faecalis* R *(S. faecium* var. *durans), S. faecalis* A (aminopterin, methotrexate-resistant), and *Lactobacillus plantarum* are presented, and overall structure-activity relationships are discussed.

An impressive array of 2,4-diaminoquinazoline antifolates, exemplified by 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia) (4,5), 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (Ib) (6-8), 2,4-diamino-6-[(*p*-chlorobenzyl)isopropylamino]quinazoline (II) (9), 2,4-

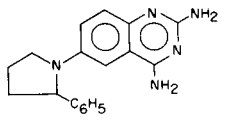


Ia, R = H
Ib, R = NO

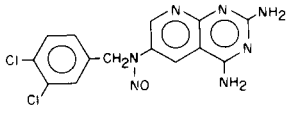


II

diamino-6-(2-phenyl-1-pyrrolidinyl)quinazoline (III) (10, 11), and 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]-pyrido[2,3-*d*]pyrimidine (IV) (1), exhibit potent antimalarial effects against sensitive and drug-resistant lines of



III



IV

*Plasmodium berghei* in mice, *P. gallinaceum* in chicks, and *P. cynomolgi* and *P. knowlesi* in rhesus monkeys (1,4-7, 9-11). Selected 2,4-diaminoquinazoline antimetabolites also exhibit significant antitrypanosomal, antifilarial, and antibacterial effects (1,4-6,8-11). It was, therefore, of interest to synthesize representative oxygen analogs and isosteres for antiparasitic and antibacterial evaluation. The present communication describes the preparation and biological properties of such 2,4-diamino-6-(aryloxy and aralkoxy)quinazolines.

## Chemistry.

The synthetic approach utilized for the preparation of the 2,4-diamino-6-(aryloxy)quinazolines (VIII) is outlined

in Scheme I. Treatment of the requisite phenol or naphthol with sodium hydride in dimethylformamide afforded a suspension of the sodium salt, which was allowed to react with 5-chloro-2-nitrobenzonitrile (V) (procedures I, II). The resulting 2-nitro-5-(phenoxy)benzonitriles (**1-6**,

SCHEME I

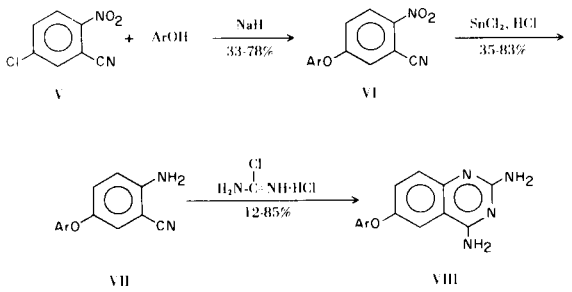
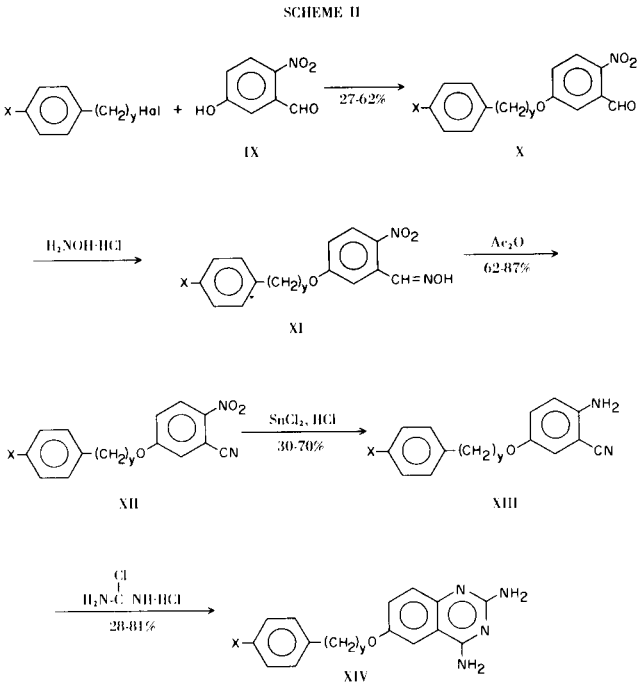


Table I) (50-78%) and 5-(naphthyloxy)-2-nitrobenzonitriles (**7-12**, Table II) (33-74%) were reduced with stannous chloride-hydrochloric acid in glacial acetic acid to the corresponding 2-amino-5-(phenoxy)benzonitriles (**13-18**, Table III) (50-83%) and 2-amino-5-(naphthyloxy)benzonitriles (**19-24**, Table IV) (35-76%) (procedure III), which were cyclized with chloroformamidine·HCl (12) to give the desired 2,4-diamino-6-(phenoxy)quinazolines (**25-30**, Table V) (32-85%) and 2,4-diamino-6-(naphthyloxy)-quinazolines (**31-36**, Table VI) (12-47%), respectively (procedures IV, V).

Alternatively, the 2,4-diamino-6-(aralkoxy)quinazolines (XIV) were synthesized according to Scheme II. Alkylation of 5-hydroxy-2-nitrobenzaldehyde (IX) with α-chlorotoluene, α,*p*-dichlorotoluene, or (2-bromoethyl)benzene gave the corresponding 2-nitro-5-(phenalkoxy)benzaldehydes (X) (27-62%). Treatment of X with hydroxylamine·HCl in pyridine afforded the 2-nitro-5-(phenalkoxy)benzaldehyde oximes (XI), which were converted to the corresponding 2-nitro-5-(phenalkoxy)benzonitriles (XII, **37-39**, Table VII) with acetic anhydride (62-87% overall, procedure VII). Reduction of XII with stannous chloride-hydrochloric acid in glacial acetic acid provided the 2-amino-5-(phenalkoxy)benzonitriles (XIII, **40-42**, Table VIII) (30-70%, procedure III), which upon ring-closure with chloroformamidine·HCl (12) gave the 2,4-diamino-6-(phenalkoxy)quinazolines (XIV, **43-45**, Table IX) (23-81%, procedures IV-VI). An abortive attempt to prepare 2-nitro-5-(phenethyloxy)benzonitrile (**39**) from the sodium salt of phenethyl alcohol and 5-chloro-2-nitrobenzonitrile resulted instead in the displacement of the nitro group to give 5-chloro-2-(2-phenethyloxy)benzonitrile (32%).

SCHEME II



## Biological Results.

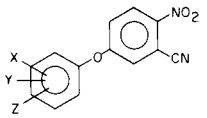
### Antimalarial Effects.

The 2,4-diamino-6-(aryloxy and aralkoxy)quinazolines described in the present communication were tested utilizing *P. berghei* in mice and *P. gallinaceum* in chicks. Compounds **27**, **30-36**, **43** and **45** were administered subcutaneously in a single dose to mice infected with a normal drug-sensitive strain of *P. berghei* (13,14) (Table X). Although four of the 2,4-diamino-6-(aryloxy)quinazolines (**27**, **31**, **32**, **36**) cured mice at one or more dose levels ranging from 80 to 640 mg./kg., surprisingly neither of the 2,4-diamino-6-(aralkoxy)quinazoline isosteres tested (**43**, **45**) was curative at doses of 160 or 640 mg./kg. 2,4-Diamino-6-[(1,6-dibromo-2-naphthyl)oxy]quinazoline (**31**), the most promising member of the series, cured mice at single subcutaneous doses ranging from 80 to 640 mg./kg., and displayed strong activity at 40 mg./kg. (ΔMST = 9.0, 9.3 days). No toxicity was encountered at 640 mg./kg., the highest level tested. Thus **31** proved to be more active than the reference drug 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia) (4,5), but was less potent than 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (Ib) (6-8). Compound **31** was also less toxic for mice than cycloguanil hydrochloride (Table X).

Eleven compounds (**25-31**, **33**, **34**, **43**, **44**) (Table X) were administered continuously for 6 days in the diet of mice infected with another normal drug-sensitive strain of *P. berghei* (15,16). Each compound displayed significant antimalarial activity at daily doses ranging from 6.3 to 174 mg./kg. Once again it was observed that the 2,4-diamino-6-(aryloxy)quinazolines exhibited more potent antimalarial effects than the 2,4-diamino-6-(benzyloxy)quinazoline iso-

TABLE I

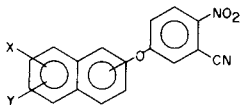
2-Nitro-5-(phenoxy)benzonitriles




| No. | X, Y, Z | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 2,4,5-$Cl_3$ | 143-145 | 58 | EtOH | I | $C_{13}H_5Cl_3N_2O_3$ | 45.4 | 45.7 | 1.5 | 1.6 | 8.2 | 8.4 |
| **2** | 3,4-$Cl_2$ | 110-111 | 78 | EtOH | I | $C_{13}H_6Cl_2N_2O_3$ | 50.5 | 50.6 | 2.0 | 2.0 | 9.1 | 9.2 |
| **3** | 3,5-$Cl_2$ | 123-125 | 62 | EtOAc-isooctane | II | $C_{13}H_6Cl_2N_2O_3$ | 50.5 | 50.7 | 2.0 | 2.1 | 9.1 | 9.0 |
| **4** | 4-Cl | 123-125 | 68 | EtOH | I | $C_{13}H_7ClN_2O_3$ | 56.8 | 56.8 | 2.6 | 2.6 | 10.2 | 10.2 |
| **5** | H | 91-94 | 50 | EtOH | I | $C_{13}H_8N_2O_3$ | 65.0 | 65.4 | 3.4 | 3.5 | 11.7 | 12.0 |
| **6** | 3,5-$(CF_3)_2$ | 113-115 | 54 | EtOH | II | $C_{15}H_6F_6N_2O_3$ | 47.9 | 48.0 | 1.6 | 1.7 | 7.4 | 7.5 |

TABLE II

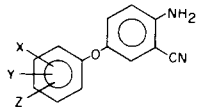
5-(Naphthyloxy)-2-nitrobenzonitriles




| No. | X, Y | Position of ring attachment | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **7** | 1,6-$Br_2$ | 2 | 210-213 | 74 | EtOH | II | $C_{17}H_8Br_2N_2O_3$ | 45.6 | 45.4 | 1.8 | 1.9 | 6.3 | 6.3 |
| **8** | 1-Br | 2 | 168-170 | 33 | EtOH | II | $C_{17}H_9BrN_2O_3$ | 55.3 | 55.6 | 2.5 | 2.5 | 7.6 | 7.5 |
| **9** | 6-Br | 2 | 164-166 | 51 | EtOH | II | $C_{17}H_9BrN_2O_3$ | 55.3 | 55.6 | 2.5 | 2.7 | 7.6 | 7.5 |
| **10** | 4-Cl | 1 | 125-126 | 63 | EtOH | II | $C_{17}H_9ClN_2O_3$ | 62.9 | 63.2 | 2.8 | 3.0 | 8.6 | 8.5 |
| **11** | H | 1 | 96-98 | 36 | EtOH | II | $C_{17}H_{10}N_2O_3$ | 70.3 | 70.5 | 3.5 | 3.4 | 9.7 | 9.7 |
| **12** | H | 2 | 93-97 | 36 | EtOH | II | $C_{17}H_{10}N_2O_3$ | 70.3 | 70.4 | 3.5 | 3.7 | 9.7 | 10.0 |

TABLE III

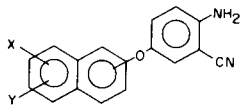
2-Amino-5-(phenoxy)benzonitriles




| No. | X, Y, Z | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **13** | 2,4,5-$Cl_3$ | 125-127 | 58,81 | EtOH | III | $C_{13}H_7Cl_3N_2O$ | 49.8 | 49.4 | 2.3 | 2.2 | 8.9 | 9.0 |
| **14** | 3,4-$Cl_2$ | 121-123 | 76 | EtOH | III | $C_{13}H_8Cl_2N_2O$ | 55.9 | 56.2 | 2.9 | 2.9 | 10.0 | 10.2 |
| **15** | 3,5-$Cl_2$ | 119-122 | 74 | 2-PrOH | III | $C_{13}H_8Cl_2N_2O$ | 55.9 | 56.1 | 2.9 | 3.1 | 10.0 | 10.3 |
| **16** | 4-Cl | 124-127 | 73 | EtOH | III | $C_{13}H_9ClN_2O$ | 63.8 | 63.4 | 3.7 | 4.0 | 11.4 | 11.5 |
| **17** | H | 104-107 | 83 | EtOH | III | $C_{13}H_{10}N_2O$ | 74.3 | 74.0 | 4.8 | 4.8 | 13.3 | 13.6 |
| **18** | 3,5-$(CF_3)_2$ | 109-111 | 50 | EtOH-$H_2O$ | III | $C_{15}H_8F_6N_2O$ | 52.0 | 51.9 | 2.3 | 2.5 | 8.1 | 8.1 |

TABLE IV

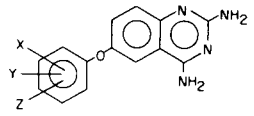
2-Amino-5-(naphthyloxy)benzonitriles




| No. | X, Y | Position of ring attachment | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **19** | 1,6-$Br_2$ | 2 | 179-181 | 62 | EtOH-$H_2O$ | III | $C_{17}H_{10}Br_2N_2O$ | 48.8 | 49.1 | 2.4 | 2.5 | 6.7 | 6.6 |
| **20** | 1-Br | 2 | 148-152 | 40 | EtOH-$NH_4OH$ | III | $C_{17}H_{11}BrN_2O$ | 60.2 | 60.5 | 3.3 | 3.5 | 8.3 | 8.2 |
| **21** | 6-Br | 2 | 157-160 | 76 | EtOH-$NH_4OH$-$H_2O$ | III | $C_{17}H_{11}BrN_2O$ | 60.2 | 60.0 | 3.3 | 3.5 | 8.3 | 7.9 |
| **22** | 4-Cl | 1 | 94-96 | 51 | 2-PrOH | III | $C_{17}H_{11}ClN_2O$ | 69.3 | 69.4 | 3.8 | 4.0 | 9.5 | 9.1 |
| **23** | H | 1 | 114-116 | 35 | EtOH-$H_2O$ | III | $C_{17}H_{12}N_2O$ | 78.4 | 78.7 | 4.6 | 4.8 | 10.8 | 10.9 |
| **24** | H | 2 | 108-110 | 40 | $C_6H_6$-petr ether | III | $C_{17}H_{12}N_2O$ | 78.4 | 78.4 | 4.6 | 4.7 | 10.8 | 10.7 |

TABLE V

2,4-Diamino-6-(phenoxy)quinazolines

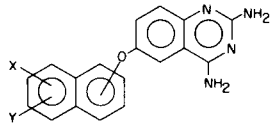


| No. | X, Y, Z | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **25** | 2,4,5-$Cl_3$ | 299-301 | 76 | EtOH-$H_2O$ | IV | $C_{14}H_9Cl_3N_4O$ | 47.3 | 47.5 | 2.6 | 2.5 | 15.8 | 15.6 |
| **26** | 3,4-$Cl_2$ | 237-238 | 63 | HOAc-EtOH-$H_2O$ | IV | $C_{14}H_{10}Cl_2N_4O$ | 52.4 | 52.6 | 3.1 | 3.1 | 17.4 | 17.2 |
| **27** | 3,5-$Cl_2$ | 235-236 | 59 | DMF-$H_2O$ | V | $C_{14}H_{10}Cl_2N_4O\cdot H_2O$ (a) | 49.6 | 49.3 | 3.6 | 3.6 | 16.5 | 16.3 |
| **28** | 4-Cl | 224-226 | 77 | EtOH-$H_2O$ | IV | $C_{14}H_{11}ClN_4O$ | 58.6 | 58.9 | 3.9 | 4.1 | 19.5 | 20.0 |
| **29** | H | 205-207 | 85 | EtOH-$H_2O$ | IV | $C_{14}H_{12}N_4O$ | 66.7 | 66.7 | 4.8 | 4.8 | 22.2 | 22.3 |
| **30** | 3,5-$(CF_3)_2$ | 243-245 | 32 | MeCN-EtOH | IV | $C_{16}H_{10}F_6N_4O$ | 49.5 | 49.3 | 2.6 | 2.7 | 14.4 | 14.5 |

(a) $H_2O$: Calcd., 5.3; Found, 5.4.

TABLE VI

2,4-Diamino-6-(naphthyloxy)quinazolines

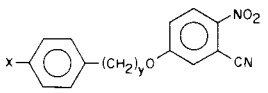


| No. | Position of ring attachment | X, Y | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **31** | 2 | 1,6-$Br_2$ | 309-312 dec. | 12 | HOAc | V | $C_{18}H_{12}Br_2N_4O\cdot 1.7C_2H_4O_2\cdot 0.9H_2O$ (a,b) | 44.4 | 44.6 | 3.6 | 3.6 | 9.7 | 9.6 |
| **32** | 2 | 1-Br | 309-312 dec. | 30 | EtOH-$H_2O$ | V | $C_{18}H_{13}BrN_4O$ | 56.7 | 56.6 | 3.4 | 3.6 | 14.7 | 14.6 |
| **33** | 2 | 6-Br | 250-252 dec. | 46 | EtOH | IV | $C_{18}H_{13}BrN_4O\cdot 1.3C_2H_6O$ | 56.1 | 56.3 | 4.8 | 4.6 | 12.7 | 12.7 |
| **34** | 1 | 4-Cl | 273-278 dec. | 47 | DMF-$H_2O$ | V | $C_{18}H_{13}ClN_4O\cdot H_2O$ (c) | 60.9 | 61.0 | 4.3 | 4.3 | 15.8 | 15.7 |
| **35** | 1 | H | 236-238 dec. | 44 | EtOH-$H_2O$ | IV | $C_{18}H_{14}N_4O$ | 71.5 | 71.8 | 4.7 | 4.7 | 18.5 | 18.4 |
| **36** | 2 | H | 232-235 | 33 | EtOH | V | $C_{18}H_{14}N_4O\cdot C_2H_6O$ | 68.9 | 68.9 | 5.8 | 5.7 | 16.1 | 16.0 |

(a) Br: Calcd., 27.6; Found, 27.7. (b) $H_2O$: Calcd., 2.8; Found, 2.8. (c) $H_2O$: Calcd., 5.1; Found, 5.0.

TABLE VII

2-Nitro-5-(phenalkoxy)benzonitriles

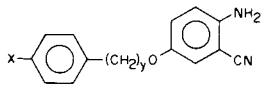


| No. | X | y | M.p., °C | Yield (a) purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **37** | Cl | 1 | 111-112 | 62 | EtOH | VII | $C_{14}H_9ClN_2O_3$ | 58.2 | 58.5 | 3.1 | 3.2 | 9.7 | 9.5 |
| **38** | H | 1 | 114-116 | 87 | EtOH | VII | $C_{14}H_{10}N_2O_3$ | 66.1 | 66.2 | 4.0 | 4.3 | 11.0 | 10.9 |
| **39** | H | 2 | 80-82 | 64 | 2-PrOH | VII | $C_{15}H_{12}N_2O_3$ | 67.2 | 67.3 | 4.5 | 4.7 | 10.4 | 10.6 |

(a) Overall yield based on the 2-nitro-5-(phenalkoxy)benzaldehyde.

TABLE VIII

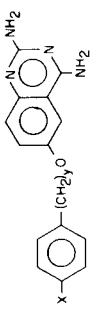
2-Amino-5-(phenalkoxy)benzonitriles



| No. | X | y | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found | Nitrogen, % Calcd. | Nitrogen, % Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **40** | Cl | 1 | 93-96 | 30 | EtOH-$H_2O$ | III | $C_{14}H_{11}ClN_2O$ | 65.0 | 65.0 | 4.3 | 4.2 | 10.8 | 10.8 |
| **41** | H | 1 | 109-110 | 70 | EtOH | III | $C_{14}H_{12}N_2O$ | 75.0 | 75.4 | 5.4 | 5.7 | 12.5 | 12.9 |
| **42** | H | 2 | 168-170 | 48 | 2-PrOH | III | $C_{15}H_{14}N_2O \cdot HCl$ | 65.6 | 65.9 | 5.5 | 5.3 | 10.2 | 9.9 |

TABLE IX

2,4-Diamino-6-(phenalkoxy)quinazolines



| No. | X | y | M.p., °C | Yield purified, % | Purification solvent | Procedure | Formula | Analyses Carbon, % Calcd. | Found | Hydrogen, % Calcd. | Found | Nitrogen, % Calcd. | Found |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **43** | Cl | 1 | 258-260 dec. | 23 | DMF-$H_2O$ | V | $C_{15}H_{13}ClN_4O \cdot 0.25H_2O$ (a) | 59.0 | 59.3 | 4.5 | 4.4 | 18.4 | 18.1 |
| **44** | H | 1 | 194-198 | 81 | EtOH-$H_2O$ | IV | $C_{15}H_{14}N_4O$ | 67.7 | 67.9 | 5.3 | 5.7 | 21.0 | 21.1 |
| **45** | H | 2 | 295-297 dec. | 28 | DMF-$H_2O$ | VI | $C_{16}H_{16}N_4O \cdot HCl$ (b) | 60.7 | 60.6 | 5.4 | 5.3 | 17.7 | 17.6 |

(a) $H_2O$: Calcd., 1.5; Found, 1.3. (b) $Cl^-$: Calcd., 11.2; Found, 10.9.

steres of Ia (Table X). The aryloxyquinazolines **25**, **26**, **28**, and **29** proved to be the most potent members of the series, and caused a 90% suppression of the parasitemia at daily oral doses of 6.3 to 9.8 mg./kg. These four substances ranged from 7.6 to > 11.8 times as potent as quinine hydrochloride ($SD_{90}$ = 74.5 mg./kg.), and each compound compared favorably with 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (Ia) (4,5).

2,4-Diamino-6-(3,5-dichlorophenoxy)quinazoline (**27**) and 2,4-diamino-6-[(α,α,α,α′,α′,α′-hexafluoro-3,5-xylyl)-oxy]quinazoline (**30**) were also evaluated against *P. gallinaceum* infections in white Leghorn cockerels (17). Chicks were given an intravenous injection of 0.2 ml. of heparinized blood infected with *P. gallinaceum* and having a minimum of 80-90% parasitized red blood cells. The parasitized blood was drawn by cardiac puncture from donor birds infected 72 hours earlier with *P. gallinaecum*. Donor strains were maintained in separate groups of chicks, 14-16 days old, that also received inoculations of heparinized infected blood. In every experiment 100% of the untreated control birds died within 72-96 hours post-infection. Candidate substances were administered to chicks in a single subcutaneous dose in peanut oil immediately after infection. In this test, as in the parenteral mouse test, 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 controls. A compound was arbitrarily considered to be active against malaria if it produced increases in the survival times of treated chicks that were at least 100% over the survival times of untreated controls. Chicks surviving to 30 days post infection are termed "cured", although data to establish parasitological cure based on sub-inoculation is unavailable (17).

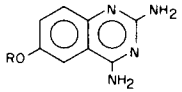
Compound **27** cured 2 of 5 birds following a single subcutaneous dose of 320 mg./kg., and prolonged the mean survival time of the other three chicks 17.1 days. At doses of 160 and 80 mg./kg., the survival time was increased 15.4 and 11.6 days, respectively. A single 120 mg./kg. subcutaneous dose of compound **30** failed to cure any of the five birds, but afforded a 6.8 day increase in the mean survival time of the chicks. The mean survival time of control chicks was 3.6 days in both experiments.

Antibacterial Activity.

The 2,4-diamino-6-(aryloxy and aralkoxy)quinazolines (**25-36**, **43-45**) were tested *in vitro* against a spectrum of pathogenic bacteria including *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Pseudomonas aeruginosa* (28), *Escherichia coli* (Vogel), and *Shigella sonnei* (C-10) (Table XI). A modification of the gradient plate procedure of Szybalski

TABLE X

Oral and Parenteral Effects of 2,4-Diamino-6-(aryloxy and aralkoxy)quinazolines Against *Plasmodium berghei* in Mice



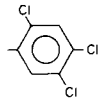
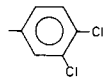
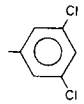
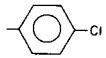
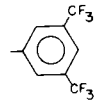
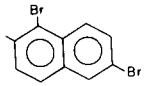
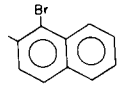
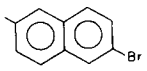
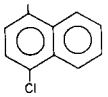
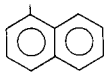
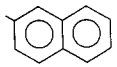
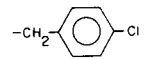
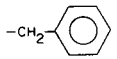
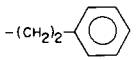
| No. | R | No. of mice | Drug Diet, 6 days $SD_{90}$, (a) mg./kg./day | Q (b) | *P. berghei* Single s.c. dose ΔMST; T or C (c) after mg./kg. dose 640 | 320 | 160 | 80 | 40 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| **25** |  | 21 | 9.5 | 7.8 | | | | | | |
| **26** |  | 21 | 9.8 | 7.6 | | | | | | |
| **27** |  | 14 | 25 | 3.0 | C5<br>C5 | 12.1 | 5.1<br>4.9 | 0.5 | 0.3<br>0.3 | 0.3 |
| **28** |  | 28 | <6.3 | >12 | | | | | | |
| **29** |  | 28 | 9.4 | 7.9 | | | | | | |
| **30** |  | 14 | 14 | 5.1 | T5 | | 1.3;T3 | | 0.8;Tl | |
| **31** |  | 14 | 41 | 1.8 | C5 | C5<br>C5 | C5<br>C5 | 14.8;C2<br>15.9;C2 | 9.0<br>9.3 | 2.8<br>3.1 |
| **32** |  | | | | C5 | 13.3 | 7.9<br>8.1 | 4.5<br>4.9 | 1.1<br>1.1 | 0.5<br>0.3 |

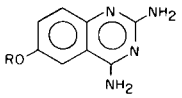
TABLE X (continued)

| No. | R | No. of mice | Drug Diet, 6 days $SD_{90}$, (a) mg./kg./day | Q (b) | *P. berghei* Single s.c. dose ΔMST; T or C (c) after mg./kg. dose: 640 | 320 | 160 | 80 | 40 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| **33** |  | 14 | 31 | 2.4 | 6.5 | 4.9<br>5.1 | 0.5<br>0.9 | 0.3<br>0.3 | 0.3<br>0.3 | 0.1<br>0.3 |
| **34** |  | 14 | 90 | 0.8 | | 5.5 | 3.9<br>3.9 | 1.7<br>1.9 | 0.9<br>1.1 | 0.3<br>0.5 |
| **35** |  | | | | | | 7.3 | 2.3<br>2.5 | 0.5<br>0.7 | 0.5<br>0.5 |
| **36** |  | | | | | 13.9;C4 | 8.9;C2<br>9.6;C2 | 8.1<br>8.3 | 1.1<br>0.9 | 0.5<br>0.5 |
| **43** |  | 14 | 26 | 2.9 | 13.3;T3 | 11.2<br>11.5 | 7.7<br>7.2 | 3.7<br>3.6 | 1.7<br>1.8 | 1.3<br>1.2 |
| **44** |  | 14 | 174 | 0.4 | | | | | | |
| **45** |  | | | | | | 2.7 | 0.9 | 0.3 | 0.3 |
| Ia·HOAc | | 14 | 9.5 | 7.9 | C5 | C5<br>C5 | 9.9;C3<br>9.9;C3 | 12.9<br>13.1 | 7.1<br>7.3 | 2.5<br>2.7 |
| Ib·HOAc | | 40 | 0.27 | 270 | | C5 | | C5 | | 22.4;Cl |
| Cycloguanil·HCl | | 40 | 2.1 | 35 | T5 | C3,T2<br>C2,T3 | C5<br>C5 | 21.6;C2<br>21.6;C2 | 13.4;Cl<br>13.4;Cl | 7.9<br>8.1 |

(a) $SD_{90}$ represents the daily dose (mg./kg.) required for 90% suppression of the parasitemia in treated mice relative to control mice. The $SD_{90}$ was estimated graphically using semi-log paper. (b) The quinine equiv Q is the ratio of the $SD_{90}$ of quinine·HCl (74.5 mg. base/kg. per day) to the $SD_{90}$ of the test substance under comparable experimental conditions. (c) 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 post infection and termed "cured"; data to establish parasitological cure based on subinoculation is unavailable. Each entry at each dose level represents results with a 5-animal group.

TABLE XI

*In vitro* Antibacterial Effects of 2,4-Diamino-6-(aryloxy and aralkoxy)quinazolines



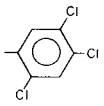
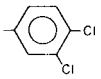
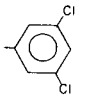
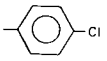
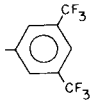
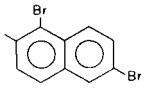
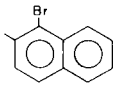
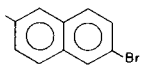
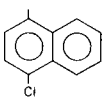
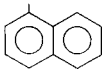
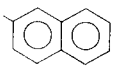
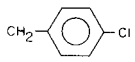
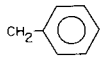
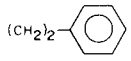
| No. | R | Minimum inhibitory concentration, μg./ml. *Streptococcus faecalis* MGH-2 | *Staphylococcus aureus* UC-76 | *Staphylococcus aureus* S18713 | *Pseudomonas aeruginosa* 28 | *Escherichia coli* Vogel | *Shigella sonnei* C-10 |
|---|---|---|---|---|---|---|---|
| 25 |  | <0.25 | <0.25 | <0.25 | >25 | 15 | 15 |
| 26 |  | <0.25 | <0.25 | <0.25 | >25 | 15 | 15 |
| 27 |  | <0.25 | <0.25 | <0.25 | >25 | 5 | 20 |
| 28 |  | <0.25 | <0.25 | <0.25 | >25 | 5 | 10 |
| 29 |  | <0.25 | <0.25 | <0.25 | >25 | 10 | 15 |
| 30 |  | <0.25 | <0.25 | <0.25 | >25 | >25 | >25 |
| 31 |  | <0.25 | <0.25 | <0.25 | >25 | >25 | >25 |
| 32 |  | <0.25 | <0.25 | <0.25 | >25 | 10 | 15 |
| 33 |  | 1.0 | 2.0 | 2.0 | >25 | >25 | >25 |
| 34 |  | <0.25 | <0.25 | <0.25 | >25 | 1.0 | 15 |
| 35 |  | <0.25 | <0.25 | <0.25 | >25 | 1.5 | 2.0 |

TABLE XI (continued)

| No. | R | Minimum inhibitory concentration, μg./ml. *Streptococcus faecalis* MGH-2 | *Staphylococcus aureus* UC-76 | *Staphylococcus aureus* S18713 | *Pseudomonas aeruginosa* 28 | *Escherichia coli* Vogel | *Shigella sonnei* C-10 |
|---|---|---|---|---|---|---|---|
| **36** |  | <0.25 | <0.25 | <0.25 | >25 | 15 | 20 |
| **43** |  | <0.25 | 1.0 | 2.5 | >25 | 10 | 10 |
| **44** |  | <0.25 | 1.5 | 2.5 | >25 | >25 | >25 |
| **45** |  | <0.25 | 1.5 | 2.0 | >25 | >25 | >25 |

(18) and Webb and Washington (19) was employed throughout (11). Each of the 2,4-diamino-6-(aryloxy)quinazolines (**25-36**), with the exception of 2,4-diamino-6-[(6-bromo-2-naphthyl)oxy]quinazoline (**33**), inhibited *S. faecalis* MGH-2, *S. aureus* UC-76, and *S. aureus* S18713 at concentrations of <0.25 μg./ml., and nine compounds (**25-29**, **32**, **34-36**) inhibited the growth of *E. coli* (Vogel) and *S. sonnei* (C-10) at drug concentrations ranging from 1-20 μg./ml. (Table XI). None was active against *P. aeruginosa* (28) at 25 μg./ml. The three 2,4-diamino-6-(aralkoxy)-quinazoline isosteres **43-45** also exhibited strong antibacterial effects against *S. faecalis* MGH-2 at 0.25 μg./ml., but were in general less active than the 2,4-diamino-6-(aryloxy)quinazolines against *S. aureus* (UC-76), *S. aureus* (S18713), *E. coli* (Vogel), and *S. sonnei* (C-10). 2,4-Diamino-6-(3,4-dichlorophenoxy)quinazoline (**26**) was tested against *S. aureus* infections in mice utilizing published procedures (20). When administered in single oral doses of 125 or 250 mg./kg., this substance protected 50% and 90% of the mice from death, respectively.

Antimetabolite Studies.

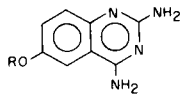
Several of the 2,4-diamino-6-(aryloxy and aralkoxy)-quinazolines (**25**, **26**, **28**, **29**, **33**, **34**, **44**) were evaluated as inhibitors of *Streptococcus faecalis* R (*S. faecium* var. *durans*, ATCC 8043), *S. faecalis* A (methotrexate, aminopterin-resistant mutant), and *Lactobacillus plantarum* (ATCC 8014) (Table XII) in anticipation that antimetabolite studies utilizing bacterial systems might aid in elucidating relationships between structure and the antimalarial and antibacterial effects summarized above. Details of the experimental procedures employed have been described previously (4).

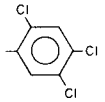
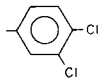
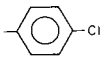
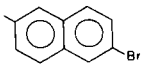
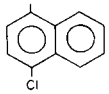
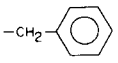
Each of the 2,4-diamino-6-(aryloxy and aralkoxy)-quinazolines tested showed strong inhibitory effects against *S. faecalis* R utilizing FA as the substrate (Table XII). These substances inhibit one or both reduction stages and are competitive with FA. Compounds **25**, **26**, **28**, **29**, **33**, **34**, and **44** produced 50% inhibition at concentrations of 0.1 to 15 ng./ml., and thus were equipotent with or more potent than the reference drugs pyrimethamine, trimethoprim, cycloguanil hydrochloride, aminopterin, methotrexate, and the benzylaminoquinazolines Ia and b. In general, these inhibitory effects are fairly well reversed by 5-CHO-$FAH_4$. This suggests that these substances, like trimethoprim and the benzylaminoquinazolines (Ia and b), function not only as reductase inhibitors, but also have significant effects either on the folate transport mechanism or elsewhere in the folate cycle. Compound **28** retains some inhibitory effects against *S. faecalis* R even in the presence of 5-CHO-$FAH_4$, adenosine, and thymidine (Table XII), which indicates that it may also exert some activity outside the folate cycle. By contrast, the inhibitory effects of **29** were completely reversed utilizing this substrate.

Six quinazolines (**25** **26**, **28**, **29**, **34**, **44**) produced 50% inhibition of *L. plantarum* at concentrations ranging from 134-630 ng./ml., and thus showed activity comparable with pyrimethamine, trimethoprim, cycloguanil hydrochloride, and the quinazolines Ia and b. However, none was as potent as methotrexate (50% inhibition at 3 ng./ml.).

TABLE XII

Inhibitory Effects of 2,4-Diamino-6-(aryloxy and aralkoxy)quinazolines Against *Streptococcus faecalis* R, *Lactobacillus plantarum*, and *Streptococcus faecalis* A



| | | Concentration (ng./ml.) causing 50% inhibition | | | |
|---|---|---|---|---|---|
| | | *S. Faecalis* R | | *L. plantarum* | *S. faecalis* A |
| No. | R | FA (a) | 5-CHO-$FAH_4$ (b) | 5-CHO-$FAH_4$ + adenosine + thymidine (c) | None | FA (d) |
| **25** |  | 0.4 | 40 | | 510 | 28 |
| **26** |  | 1 | >40 | | 304 | 60 |
| **28** |  | 2 | 33 | 13,000 | 260 | 56 |
| **29** |  | 3 | 90 | >40,000 | 286 | 148 |
| **33** |  | 4 | >40 | | 2,860 | 1,300 |
| **34** |  | 0.1 | >4 | | 134 | 11 |
| **44** |  | 15 | 117 | | 630 | 500 |
| | Pyrimethamine | 4 | 3,100 | | 590 | 680 |
| | Trimethoprim | 12 | 70 | >40,000 | 74 | 284 |
| | Cycloguanil hydrochloride | 8 | 11,400 | >400,000 | 480 | 560 |
| | Aminopterin | 2 | 4 | >40,000 | | >40,000 |
| | Methotrexate | 0.2 | 0.6 | >40,000 | 3 | 3,800 |
| | Ia base | 6 | 112 | 2,400 | 550 | 294 |
| | Ib base | 4 | 88 | 28,600 | 720 | 150 |

(a) 0.4 ng./ml. FA; (b) 0.4 ng./ml. 5-CHO-$FAH_4$; (c) 0.4 ng./ml. 5-CHO-$FAH_4$ + 10 μg./ml. adenosine + 10 μg./ml. thymidine; (d) 500 ng./ml. FA.

Against the methotrexate, aminopterin-resistant *S. faecalis* A, five compounds (**25**, **26**, **28**, **29**, **34**) caused 50% inhibition at concentrations of 11-148 ng./ml. utilizing 500 ng./ml. FA as the substrate (Table XII). These inhibitory concentrations are equal to or less than those required for pyrimethamine, trimethoprim, cycloguanil hydrochloride, and the quinazolines Ia and b. The *S. faecalis* A to *S. faecalis* R inhibition ratios (28 to 110) for these compounds are relatively low compared with those observed for aminopterin (> 20,000) or methotrexate (19,000). This indicates that there is relatively little cross resistance between these substances and aminopterin or methotrexate utilizing *S. faecalis*.

*Pediococcus cerevisiae* (ATCC 8081) requires 0.4 ng./ml. final strength medium of the *L*-form of 5-CHO-$FAH_4$ (4). This organism does not do well using an oxidized form of folate. Therefore, it is likely that inhibition of this organism does not involve folate reductase inhibition. It may represent the blockade of a transport mechanism or inhibition at subsequent stages of the folate cycle. 2,4-Diamino-6-(*p*-chlorophenoxy)quinazoline (**28**) was tested against *P. cerevisiae* utilizing 5-CHO-$FAH_4$ as the substrate and produced 50% inhibition at 53 ng./ml. Half inhibition in the presence of 1 μg./ml. of thymidine occurred at 800 ng./ml., while in the presence of 1 μg./ml. each of adenosine and thymidine the inhibitory concentration was 1400 ng./ml. These results suggest that **28** may be a thymidylate synthetase inhibitor.

Although the 2,4-diamino-6-(aryloxy and aralkoxy)-quinazolines that show potent inhibitory effects against *S. faecalis* R often display good antimalarial effects (**25**, **26**, **28**, **29**), there are notable exceptions (**34**, **44**) (Tables X *vs.* XII). Compounds that exhibited the most potent inhibitory effects against *S. faecalis* R (**25**, **26**, **28**, **29**, **33**, **34**, Table XII) were likewise potent inhibitors of *S. faecalis* MGH-2, *S. aureus* UC-76 and S18713, *E. coli* (Vogel), and *S. sonnei* C-10 (Table XI).

## EXPERIMENTAL (21)

2-Nitro-5-(phenoxy)benzonitriles (**1-6**, Table I) and 5-(Naphthyloxy)-2-nitrobenzonitriles (**7-12**, Table II).

Procedure I.

Sodium hydride (50% in oil; 1.9 g., 0.04 mole) was washed twice by decantation with petroleum ether (b.p. 40-60°) and 20 ml. of dimethylformamide was added. The suspension was stirred magnetically and was treated with a solution of 7.4 g. (0.0375 mole) of 2,4,5-trichlorophenol in 20 ml. of cold dimethylformamide while maintaining the temperature below 35° by external cooling. To this suspension was then added in one portion a solution of 5.5 g. (0.03 mole) of 5-chloro-2-nitrobenzonitrile in 20 ml. of dimethylformamide. No apparent evolution of heat occurred, and the mixture was heated at 55-60° for 2 hours. The cloudy light red solution was cooled and poured into a mixture of 500 ml. of water and 50 ml. of 2 *N* sodium hydroxide. A yellow oil separated which soon crystallized. The mixture was allowed to stand overnight and the crude yellow product was collected, triturated with water, and dried. The yield was 8.0 g. (78%), m.p. 122-136°. Recrystallization from 300 ml. of ethanol gave 6.0 g. (58%) of 2-nitro-5-(2,4,5-trichlorophenoxy)benzonitrile (**1**) as colorless needles, m.p. 143-145°.

Procedure II.

To a stirred solution of 11.3 g. (0.063 mole) of 4-chloro-1-naphthol in 130 ml. of cold dimethylformamide at 5-10°, was added 2.7 g. (0.063 mole) of a 57% dispersion of sodium hydride in mineral oil. The solution was allowed to warm to room temperature and a solution of 10.0 g. (0.055 mole) of 5-chloro-2-nitrobenzonitrile in 15 ml. of dimethylformamide was added. The mixture was stirred overnight and extracted once with isooctane. The dimethylformamide layer was separated and concentrated to 25 ml. *in vacuo*. The residue was pipetted, with stirring, into an ice-water mixture. The dark gold precipitate that formed was collected, washed with water, and recrystallized from ethanol (charcoal) to give 11.2 g. (63%) of 5-[(4-chloro-1-naphthyl)-oxy]-2-nitrobenzonitrile (**10**), m.p. 125-126°.

2-Amino-5-(phenoxy)benzonitriles (**13-18**, Table III), 2-Amino-5-(naphthyloxy)benzonitriles (**19-24**, Table IV), and 2-Amino-5-(phenalkoxy)benzonitriles (**40-42**, Table VIII).

Procedure III.

A solution of 3.4 g. (0.01 mole) of 2-nitro-5-(2,4,5-trichlorophenoxy)benzonitrile (**1**) in 15 ml. of boiling glacial acetic acid was cooled to 90° and added in one portion, with stirring, at 30° to a solution of stannous chloride dihydrate (7.5 g., 0.033 mole) in 23 ml. of concentrated hydrochloric acid and 8 ml. of glacial acetic acid. A white solid separated. The mixture was stirred and heated at 50° for 3 hours and cooled. Water (35 ml.) was added, and the mixture was slurried with a solution of 35 g. of sodium hydroxide in water (60 ml.) and sufficient crushed ice to maintain the temperature below 5°. The white precipitate was collected, washed with water, and dried to give 2.3 g. (75%) of crude product, m.p. 121-123°. Crystallization from ethanol (decolorizing charcoal) gave 1.8 g. (58%) of 2-amino-5-(2,4,5-trichlorophenoxy)-benzonitrile (**13**) as colorless needles, m.p. 125-127°. Repeat on a 0.04 mole scale afforded 10.2 g. (81%), m.p. 122-126°.

2,4-Diamino-6-(phenoxy)quinazolines (**25-30**, Table V), 2,4-Diamino-6-(naphthyloxy)quinazolines (**31-36**, Table VI), and 2,4-Diamino-6-(phenalkoxy)quinazolines (**43-45**, Table IX).

Procedure IV.

2-Amino-5-(2,4,5-trichlorophenoxy)benzonitrile (**13**) (10.7 g., 0.034 mole) and chloroformamidine hydrochloride (12) were stirred and heated in 34 ml. of redistilled diglyme at 150° (bath temperature) for 1 hour. When the temperature reached 145°, hydrogen chloride was evolved and a yellow solution formed. Soon thereafter, the mixture set to a white crystalline mass. The mixture was cooled and diluted with 100 ml. of anhydrous ether, and the product was collected, washed with anhydrous ether, and dried. The hydrochloride salt thus obtained weighed 12.0 g. The hydrochloride salt was dissolved in 1.1 l. of boiling 50% ethanol (decolorizing charcoal), the charcoal was removed by filtration, and the filtrate was basified with 20 ml. of concentrated ammonium hydroxide. The mixture was refrigerated at 0° for 18 hours, and the product was collected, washed with ethanol, and dried *in vacuo* at 100° for 4 hours. The colorless prisms of 2,4-diamino-6-(2,4,5-trichlorophenoxy)quinazoline (**25**) weighed 9.2 g. (76%), m.p. 299-301°.

Procedure V.

A mixture of 2.4 g. (0.0071 mole) of 2-amino-5-[(1-bromo-2-naphthyl)oxy]benzonitrile (**20**) and 0.9 g. (0.0078 mole) of chloroformamidine hydrochloride (12) in 4.4 ml. of dry diglyme was heated to 145° and kept at that temperature for 45 minutes. A dark solution resulted and then a dark gold solid precipitated. The mixture was cooled and diluted with ether. The solid was collected and dissolved in dimethylformamide containing concentrated ammonium hydroxide. The dark solution was treated with decolorizing charcoal, filtered through Supercel, and poured into cold dilute sodium hydroxide. The precipitate was collected, washed with water, dried, and recrystallized from glacial acetic acid. The acetate salt thus obtained was dissolved in hot 95% aqueous ethanol, and the solution was made basic with 3 ml. of concentrated ammonium hydroxide to afford 0.8 g. (30%) of 2,4-diamino-6-[(1-bromo-2-naphthyl)oxy]quinazoline (**32**) as pale yellow crystals, m.p. 309-312° dec.

Procedure VI.

A mixture of 2.2 g. (0.008 mole) of 2-amino-5-(phenethyloxy)-benzonitrile (**42**) and 1.0 g. (0.0088 mole) of chloroformamidine hydrochloride (12) in 5 ml. of diglyme was heated to 150° in an oil bath. A brisk evolution of hydrogen chloride produced a homogeneous solution from which a new solid was deposited. After 0.5 hour the mixture was allowed to cool to room temperature, 30 ml. of ether was added, and the insoluble material was collected and dried. The solid was recrystallized (charcoal) from ethanol containing an excess of triethylamine. The crude product was recrystallized twice from a mixture of dimethylformamide-water. Following the second recrystallization the pale yellow crystals were washed with anhydrous ethanol and ether. After drying 0.7 g. (28%) of 2,4-diamino-6-(phenethyloxy)quinazoline monohydrochloride (**45**) was obtained as off-white crystals, m.p. 295-297° dec.

2-Nitro-5-(phenalkoxy)benzonitriles (**37-39**, Table VII).

Procedure VII.

5-(Benzyloxy)-2-nitrobenzaldehyde (22) (11.9 g., 0.046 mole), hydroxylamine hydrochloride (3.5 g., 0.05 mole), and pyridine (23 ml.) were heated on a steam bath for 2 hours. The mixture was cooled and poured into water. The water was decanted and the residual oil was extracted with 50 ml. of ether. The ether solution was washed successively with 0.5 *N* hydrochloric acid and aqueous sodium bicarbonate and dried over magnesium sulfate. The ether was evaporated and the residue solidified to give 12.2 g. (96%) of the crude oxime as off-white crystals.

The above crude oxime (12.2 g.) was refluxed with 29 ml. of acetic anhydride and 0.2 g. of anhydrous sodium acetate for 3 hours. The solution was cooled and poured into 40 ml. of an ice-water mixture. The solid that separated was collected, washed with water, and dried. Crystallization from 250 ml. of ethanol gave 10.2 g. (90% from oxime, 87% overall) of 5-(benzyloxy)-2-nitrobenzonitrile (**38**) as colorless laths, m.p. 114-116°.

5-[(*p*-Chlorobenzyl)oxy]-2-nitrobenzaldehyde.

5-Hydroxy-2-nitrobenzaldehyde (3.5 g., 0.021 mole) was added to a solution of 25 ml. of ethanol, 1.2 g. of potassium hydroxide, and 2 ml. of water. A thick suspension of the potassium salt formed. α,*p*-Dichlorotoluene (3.7 g., 0.023 mole) was added, and the mixture was heated under reflux for 5.5 hours, cooled slightly, and filtered. The filtrate was chilled, and the solid that separated was collected, washed with ethanol, and dried to give 2.8 g. (62%) of the desired product, m.p. 103-105° with preliminary softening.

*Anal.* Calcd. for $C_{14}H_{10}ClNO_4$: C, 57.6; H, 3.5; N, 4.8. Found: C, 57.8; H, 3.7; N, 5.0.

5-(Phenethyloxy)-2-nitrobenzaldehyde.

To a stirred solution of 6.8 g. (0.122 mole) of potassium hydroxide in 7.5 ml. of water and 80 ml. of 95% ethanol was added 19.5 g. (0.116 mole) of 5-hydroxy-2-nitrobenzaldehyde. To the thick yellow suspension that formed was added 23.7 g. (0.128 mole) of (2-bromoethyl)benzene and the mixture was stirred under reflux for 5.5 hours. An additional 3.2 g. (0.017 mole) of (2-bromoethyl)benzene was added, and the mixture was refluxed for 2 hours and filtered hot. The dark filtrate was concentrated to dryness and the residue was taken up in toluene. The toluene solution was extracted with small portions of water (until the aqueous layer was colorless), washed once with saturated sodium chloride solution, and dried over magnesium sulfate. The toluene was removed under reduced pressure to give an oily residue which solidified. Recrystallization from 95% ethanol followed by a recrystallization from 2-propanol yielded 8.3 g. (27%) of the desired product, m.p. 89-91°.

*Anal.* Calcd. for $C_{15}H_{13}NO_4$: C, 66.4; H, 4.8; N, 5.2. Found: C, 66.5; H, 5.0; N, 5.2.

5-Chloro-2-(phenethyloxy)benzonitrile.

To a stirred solution of 3.7 g. (0.03 mole) of phenethyl alcohol in 7 ml. of dimethylformamide was added in small portions 1.3 g. (0.03 mole) of a 57% suspension of sodium hydride in mineral oil. A thick suspension of the sodium salt resulted. To this stirred mixture, a solution of 5.0 g. (0.0274 mole) of 5-chloro-2-nitrobenzonitrile in 5 ml. of dimethylformamide was added dropwise (exothermic) and the brown solution was allowed to stir overnight at room temperature. The mixture was diluted with toluene and washed several times with water and once with a saturated sodium chloride solution. The toluene layer was separated, dried over magnesium sulfate, and concentrated to dryness. The oily residue was triturated with isooctane and taken up in toluene. The soluble portion was purified by passage over a chromatographic column of silica (*p*H 7) (Silicar CC-7) and elution with toluene. Concentration of the fractions gave a yellow oil which crystallized on standing. Trituration with 2-propanol followed by recrystallization from ethanol yielded 2.5 g. (32%) of product, m.p. 69-71°.

*Anal.* Calcd. for $C_{15}H_{12}ClNO$: C, 69.9; H, 4.7; N, 5.4. Found: C, 69.9; H, 4.9; N, 5.5.

Acknowledgments.

The authors are indebted to Dr. Leo Rane of the University of Miami and Miss Bronislawa Olszewski and Dr. Paul E. Thompson of Parke, Davis and Company for the antimalarial testing. We also thank Dr. M. W. Fisher, Dr. C. L. Heifetz, and Dr. O. D. Bird for the antibacterial and antimetabolite studies, Mr. C. E. Childs and associates for the microanalyses, and Dr. J. M. Vandenbelt and co-workers for determination of the spectral data.

## REFERENCES

(1) For previous paper on folate antagonists, see J. Davoll, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, 15, 000 (1972).

(2) This is communication 27 of a series on antimalarial drugs. For paper 26, see (1).

(3) This investigation was supported in part by U. S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is contribution No. 1049 to the Army Research

Program on Malaria.

(4) J. Davoll, A. M. Johnson, H. J. Davies, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 000 (1972).

(5) P. E. Thompson, A. Bayles, and B. Olszewski, *Exp. Parasitol.*, **25**, 32 (1969).

(6) J. Davoll, A. M. Johnson, J. Dickinson, O. D. Bird, J. Clarke, and E. F. Elslager, *J. Med. Chem.*, **15**, 000 (1972).

(7) P. E. Thompson, A. Bayles, and B. Olszewski, *Am. J. Trop. Med. Hyg.*, **19**, 12 (1970).

(8) P. E. Thompson and A. Bayles, *J. Parasitol.*, **56**, 616 (1970).

(9) E. F. Elslager, O. D. Bird, J. Clarke, S. C. Perricone, D. F. Worth, and J. Davoll, *J. Med. Chem.*, **15**, 000 (1972).

(10) E. F. Elslager, J. Davoll, L. M. Werbel, and D. F. Worth, Abstracts of Papers, Third International Congress of Heterocyclic Chemistry, Sendai, Japan, August 23-27, 1971, pp. 366-369.

(11) E. F. Elslager, J. Clarke, L. M. Werbel, D. F. Worth, and J. Davoll, *J. Med. Chem.*, **15**, 000 (1972).

(12) A. Hantzsch and A. Vagt, *Ann. Chem.*, **314**, 366 (1900).

(13) The parenteral antimalarial screening was carried out by Dr. Leo Rane of the University of Miami, and test results were supplied through the courtesy of Dr. David P. Jacobus, Dr. T. R. Sweeney, and Dr. E. A. Steck of the Walter Reed Army Institute of Research.

(14) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(15) For a description of test methods, see references 5 and 7.

(16) Selected compounds were studied orally against *P. berghei* in mice by Miss Bronislawa Olszewski and Dr. P. E. Thompson, Department of Pharmacology, Parke, Davis and Co., Ann Arbor, Michigan.

(17) Antimalarial screening against *Plasmodium 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. David P. Jacobus, Dr. T. R. Sweeney, and Dr. E. A. Steck of the Walter Reed Army Institute of Research.

(18) W. Szybalski, *Microbial Genetics Bull.*, **5**, 16 (1951).

(19) A. H. Webb and L. Washington, *Bacteriol. Proc.*, 52 (1966).

(20) A. L. Erlandson, M. W. Fisher, L. A. Gagliardi, and M. R. Gaetz, *Antibiot. Chemother.*, **10**, 84 (1960).

(21) Melting points (corrected) were taken on a Thomas-Hoover capillary melting point apparatus.

(22) E. Giovannini and P. Portmann, *Helv. Chimica Acta*, **31**, 1381 (1948).