Folate Antagonists. 7. Antimalarial, Antibacterial, and Antimetabolite Effects of 2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (1-3)

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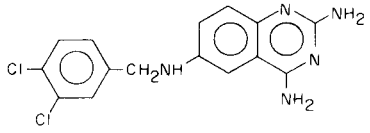
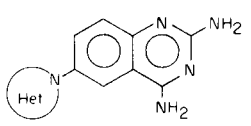
Various 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (IX) have been synthesized for antimalarial and antibacterial evaluation. Alkylation of 4-amino-3-cyano-1,2,5,6-tetrahydropyridine (VI) with the requisite α-chlorotoluene or picolyl chloride in 2-butanone afforded the corresponding 4-amino-3-cyano-1-(benzyl and pyridylmethyl)-1,2,5,6-tetrahydropyridines (VIII) (16-73%), which were cyclized to the pyrido[4,3-*d*]pyrimidines (IX) utilizing guanidine carbonate in dimethylformamide. Alternatively, VI was condensed with guanidine carbonate in ethyl cellosolve to give 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]-pyrimidine (VII) (52%), which upon treatment with the appropriate α-chlorotoluene in dimethylformamide gave other 2,4-diamino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (IX) (26-27%). Eight compounds were active orally against *Plasmodium berghei* in mice at doses ranging from 3.9 to 125 mg./kg./day for 6 days (0.6 to 19 times as potent as quinine hydrochloride), while three compounds displayed activity when administered in a single subcutaneous dose of 640 mg./kg. Four substances exhibited *in vitro* activity against *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, and *Streptococcus pyogenes* (C203), with MIC's ranging from < 0.25 to 10 μg./ml. Data on the inhibitory effects of various pyrido[4,3-*d*]pyrimidines against *Streptococcus faecalis* R (*S. faecium* var. *durans*, ATCC 8043), *S. faecalis* A (aminopterin, methotrexate-resistant), and *Lactobacillus plantarum* (ATCC 8014) is summarized.

2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (I)

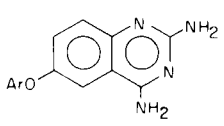


I

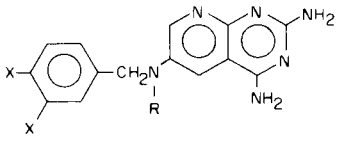
and related 2,4-diamino-6-[[aralkyl and (heterocyclic)-methyl]amino]quinazoline antifolates display strong antimalarial activity against sensitive and drug-resistant lines of *Plasmodium berghei* in mice, *P. gallinaceum* in chicks, and *P. cynomolgi* and *P. knowlesi* in rhesus monkeys (4,5). Nitrosation or alkylation at N^6 usually produces a marked enhancement of antimalarial potency (6-8), and good activity is retained among the 2,4-diamino-6-(heterocyclic)quinazolines (II) (9,10) and the 2,4-diamino-6-(aryloxy)quinazolines (III) (11,12). In contradistinction, antimalarial activity is abolished or drastically reduced among bioisosteres in the 2,4-diamino-6-[(benzyl)amino]-pyrido[2,3-*d*]pyrimidine (IV) (13) and fused 2,4-diaminothieno[2,3-*d*]pyrimidine (V) (1) series.



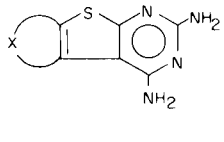
II



III



IV



V

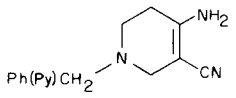
We now report the synthesis and biological properties of various 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (IX), a novel series of folate antagonists with interesting antimalarial, antibacterial, and antimetabolite effects.

Chemistry.

The first recorded pyrido[4,3-*d*]pyrimidine was synthesized in 1945 by Cook and Reed (14) by the condensation of ethyl 1-methyl-4-oxo-3-piperidinecarboxylate

TABLE I

4-Amino-3-cyano-1-(benzyl and pyridylmethyl)-1,2,5,6-tetrahydropyridines



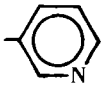
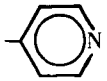
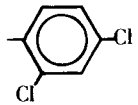
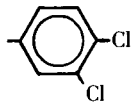
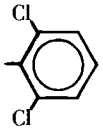
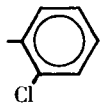
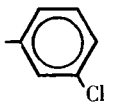
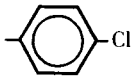
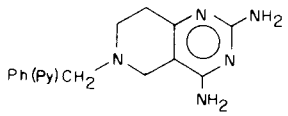
No.	Ph(Py)	M.p., °C	Yield purified, %	Purification solvent	Procedure	Formula	Analyses Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1		115-120	38	EtOH-Et_2O	I	$C_{12}H_{14}N_4$	67.27	67.10	6.59	6.70	26.15	26.42
2		153-156	16	EtOH	I	$C_{12}H_{14}N_4$	67.27	67.05	6.59	6.46	26.15	26.21
3		138-140	66	MeOH	II	$C_{13}H_{13}Cl_2N_3$	55.34	55.22	4.64	4.61	14.89	14.47
4		146.5-148	73	MeOH	II	$C_{13}H_{13}Cl_2N_3$	55.34	55.47	4.64	4.67	14.89	14.62
5		178.5-180.5	36	EtOAc-isooctane	II	$C_{13}H_{13}Cl_2N_3$	55.34	55.50	4.64	4.82	14.89	15.03
6		131.5-133.5	59	MeOH	II	$C_{13}H_{14}ClN_3$	63.03	63.11	5.70	5.73	16.96	16.95
7		143-145	60	EtOAc	II	$C_{13}H_{14}ClN_3$	63.03	63.02	5.70	5.71	16.96	17.01
8		150-152	57	MeOH	II	$C_{13}H_{14}ClN_3$	63.03	63.31	5.70	5.75	16.96	16.96

TABLE II

2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines



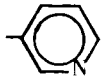
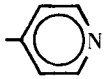
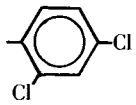
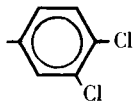
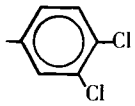
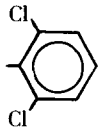
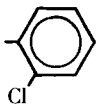
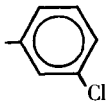
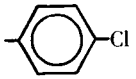
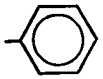
No.	Ph(Py)	M.p., °C	Yield purified, %	Purification solvent	Procedure	Formula	Analyses								
							Carbon, %		Hydrogen, %		Nitrogen, %		Other, %		
							Calcd.	Found	Calcd.	Found	Calcd.	Found		Calcd.	Found
9		288-290	23	MeOH-Et_2O	III	$C_{13}H_{16}N_6 \cdot 1.1\ H_2O$	56.55	56.49	6.64	6.42	30.44	30.92	H_2O	7.18	7.49
10		296-298	26	MeOH-H_2O	III	$C_{13}H_{16}N_6$	60.92	60.51	6.29	6.12	32.79	32.77			
11		241-245	25	DMF-H_2O	IV	$C_{14}H_{15}Cl_2N_5$	51.87	52.05	4.66	4.73	21.60	21.87			
12		206-210	15	EtOAc	IV	$C_{14}H_{15}Cl_2N_5 \cdot C_4H_8O_2$ (a)	52.44	52.38	5.62	5.50	16.98	17.07			
13		261-263	26	MeOH-Et_2O	V	$C_{14}H_{15}Cl_2N_5 \cdot 2HCl \cdot H_2O$	40.51	40.50	4.61	4.60	16.87	16.90	Cl^-	17.08	17.10
14		214-217	24	DMF-H_2O	III	$C_{14}H_{15}Cl_2N_5 \cdot 2H_2O$	46.68	46.66	5.32	5.02	19.44	19.73	H_2O	10.00	10.50
15		207-210	10	DMF-H_2O	IV	$C_{14}H_{16}ClN_5$	58.03	57.73	5.57	5.64	24.17	24.09			

TABLE II (continued)

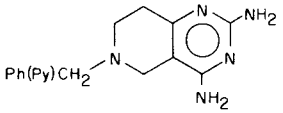
2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines

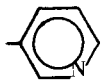
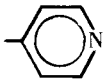
No.	Ph(Py)	M.p., °C	Yield purified, %	Purification solvent	Procedure	Formula	Analyses Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found		Other, % Calcd.	Other, % Found
16		217-220	19	MeOH	III	$C_{14}H_{16}ClN_5$	58.03	57.82	5.57	5.62	24.17	24.45			
17		212-215	8	EtOH	IV	$C_{14}H_{16}ClN_5 \cdot C_2H_6O$ (b)	57.23	57.35	6.60	6.85	20.85	20.89			
18		255-258	27	MeOH-Et_2O	V	$C_{14}H_{17}N_5 \cdot 2HCl \cdot 3H_2O$	43.99	44.10	6.59	6.80	18.32	18.69	Cl^-	18.55	18.60

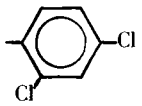
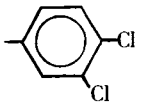
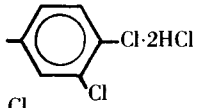
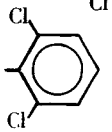
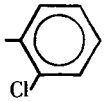
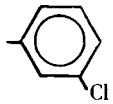
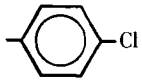
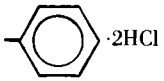
(a) The nmr spectrum confirmed the presence of ethyl acetate. (b) The nmr spectrum confirmed the presence of ethanol.

TABLE III

Oral and Parenteral Effects of 2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines Against *Plasmodium berghei* in Mice



No.	Ph(Py)	No. of mice	Drug diet, 6 days SD_{90} (a) mg./kg./day	Q (b)	Single s.c. dose ΔMST; C or T (c) after mg./kg. dose: 640	320	160	80	40	20
9		14	34	2.1	6.1	1.9 2.1	0.7 0.5	0.7 0.5	0.3 0.5	0.3 0.3
10		14	125	0.6		1.3	0.7	0.5	0.3	0.1

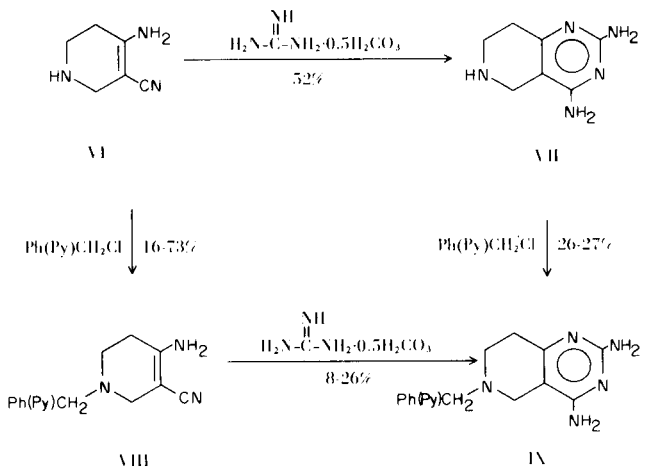
11		14	13	5.7	12.6	5.6 5.8	2.2 2.6	1.0 1.4	0.6 0.8	0.2 0.4
12					3.3; Tl	1.2	0.4	0.4	0.2	0.2
13		35	3.9	19						
14		14	14	5.3			5.0	4.2 4.2	2.8 2.8	0.4 0.6
15		14	54	1.4	6.8	2.6 2.8	1.4 1.2	0.2 0.4	0.2 0.4	0.2 0.2
16		14	>34	<2.2		0.7	0.7	0.7	0.5	0.5
17		14	21	3.5			2.6	2.2	0.6	0.2
18		21	57	1.3						
Quinine·HCl		224	74.5	1.0						
Cycloguanil·HCl		40	2.1	35	T5	C3, T2 C2, T3	C5 C5	21.6; C2 21.6; C2	13.4; Cl 13.4; Cl	7.9 8.1
I·HOAc		14	9.5	7.9	C5	C5 C5	9.9; C3 9.9; C3	12.9 13.1	7.1 7.3	2.5 2.7

(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 equivalent 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 6.3 days. T signifies the number of toxic deaths occurring on days 2-5 after infection which are attributed to drug action. C indicates the number of mice surviving at 60 days 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.

with benzamidine. Subsequently, several hundred other 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines have been synthesized by ring closure of various 4-oxo-3-piperidinecarboxylates, 3-cyano-4-piperidones, and 3-cyano-4-iminopiperidines utilizing amidines, ureas, thioureas, and guanidines (15-17). However, only one 2,4-diaminoderivative has been reported, namely 2,4-diamino-6-phenethyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (17). This substance was obtained by fusing 4-amino-3-cyano-1-phenethyl-1,2,5,6-tetrahydropyridine with guanidine base at 140° (17). The biological properties of the compound were not disclosed.

The 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines described in the present communication were synthesized utilizing the two routes displayed in Scheme I. The key intermediate, 4-amino-3-cyano-1,2,5,6-tetrahydropyridine (VI) (18,19), was prepared by the cyclization of 3,3'-iminodipropionitrile employing potassium *t*-butoxide as described previously (18,19). Alkylation of VI with the requisite α-chlorotoluene or picolyl chloride in 2-butanone containing anhydrous potassium carbonate afforded the corresponding 4-amino-3-cyano-1-(benzyl and pyridylmethyl)-1,2,5,6-tetrahydropyridines (VIII) (**1-8**, Table I) (16-73% yield, procedures I and II). Ring-closure with

SCHEME I



guanidine carbonate in dimethylformamide gave the 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines **9-12** and **14-17** (Table II) in 8-26% yield (procedures III and IV).

Alternatively, 4-amino-3-cyano-5,6,7,8-tetrahydropyridine (VI) (18,19) was condensed with guanidine carbonate in 1,2-bisethoxyethane to give 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (VII) (52%). Alkylation of VII with α-chlorotoluene or α,3,4-trichlorotoluene in dimethylformamide containing potassium carbonate afforded 2,4-diamino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (**18**) (27%) and 2,4-diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (**13**) (26%), respectively (procedure V, Table II). Spectral data (ir, uv) were in agreement with the structures assigned.

Biological Results.

Antimalarial Effects.

Antimalarial studies with the 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines described in the present communication were conducted utilizing *P. berghei* in mice and *P. gallinaceum* in chicks. Compounds **9-12** and **14-17** were administered in single subcutaneous doses ranging from 20 to 640 mg./kg. to mice infected with a normal drug-sensitive strain of *P. berghei* (20,21) (Table III). Three of the pyrido[4,3-*d*]pyrimidines (**9,11,15**) increased the mean survival time of mice by 100% or more at a dose of 640 mg./kg. and are thus considered active. However, none was curative at any dose level, and these substances were significantly less active than the reference drugs cycloguanil hydrochloride and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline acetate (I) (4,5).

Nine compounds (**9-11, 13-18**) (Table III) were administered continuously for 6 days in the diet of mice infected with another normal drug-sensitive strain of *P. berghei* (22,23). Eight substances (**9-11,13-15.17,18**) caused a 90% suppression of the parasitemia relative to control animals at daily doses ranging from 3.9 to 125 mg./kg., and seven of them (**9,11,13-15,17,18**) were more potent than quinine hydrochloride. 2,4-Diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (**13**), the most promising member of the series, was approximately 19 times as active (SD_{90} = 3.9 mg./kg./day) as quinine hydrochloride (SD_{90} = 74.5 mg./kg./day). It was also more potent than the reference drug 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline acetate (I) (SD_{90} = 9.5 mg./kg./day), and nearly as potent as cycloguanil hydrochloride (SD_{90} = 2.1 mg./kg./day) (Table III).

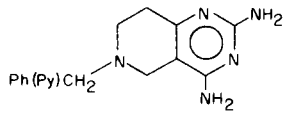
2,4-Diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine base (**12**) was also evaluated against *P. gallinaceum* infections in white Leghorn cockerels (21,24). When administered subcutaneously in a single dose of 100 mg./kg., **12** was active and prolonged the mean survival time of the chicks 5.2 days beyond the survival time of control animals. Control chicks survived 3.4 days.

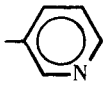
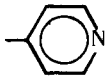
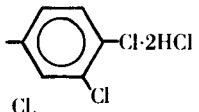
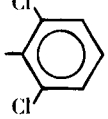
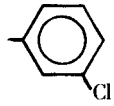
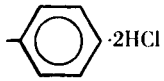
Antibacterial Studies.

Two of the pyrido[4,3-*d*]pyrimidines (**11,17**) were tested *in vitro* against a spectrum of pathogenic bacteria

TABLE IV

Inhibitory Effects of 2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines Against *Streptococcus faecalis* R, *Lactobacillus plantarum*, and *Streptococcus faecalis* A



No.	Ph(Py)	Concentration (ng./ml.) causing 50% inhibition *S. faecalis* R FA (a)	5-CHO-FAH_4 (b)	*L. plantarum* None	*S. faecalis* A FA (c)
9		30	3760	>40,000	2500
10		52	>4000	>40,000	6000
13		16	>400	7200	700
14		1	>40	266	56
16		7	>40	8800	560
18		150	3560	>40,000	3400
Pyrimethamine		4	3100	590	680
Trimethoprim		12	70	74	284
Cycloguanil hydrochloride		8	11,400	480	560
Aminopterin		2	4		>40,000
Methotrexate		0.2	0.6	3	3800
I base		6	112	550	294

(a) 0.4 ng./ml. FA; (b) 0.4 ng./ml. 5-CHO-FAH_4; (c) 500 ng./ml. FA.

including *Streptococcus faecalis* (MGH-2), normal (Uv-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Pseudomonas aeruginosa* (28), *Escherichia coli* (Vogel), and *Shigella sonnei* (C-10). A modification of the gradient plate procedure of Szybalski (25) and Webb and Washington (26) was employed throughout (10). 2,4-Diamino-6-(2,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**11**) caused 50% inhibition of *S. faecalis* MGH-2, *S. aureus* UC-76, and *S. aureus* S18713 at concentrations of <0.25 μg./ml., 5.0 μg. ml., and 5.0 μg./ml., respectively. The *p*-chlorobenzyl analog **17** produced 50% inhibition of *S. faecalis* MGH-2 at 1.0 μg./ml. and of *S. aureus* UC-76 at 10 μg./ml. Neither compound was active against the other test organisms at 25 μg./ml.

In an allied study, 2,4-diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (**13**) and 2,4-diamino-6-benzyl-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (**18**) were tested against *Streptococcus pyogenes* (C203), *S. aureus* (UC-

76), *Proteus mirabilis* (MGH-1), *P. aeruginosa* (28), *Salmonella typhimurium* (V-31), and *Mycobacterium tuberculosis* ($H_{37}Rv$) utilizing serum broth dilution techniques (27). Compounds **13** and **18** produced complete inhibition of *S. pyogenes* (C203) at a concentration of 0.63 μg./ml. and 10 μg./ml., respectively. Neither substance was active against the other bacteria at a concentration of 25 μg./ml.

Antimetabolite Studies.

In anticipation that antimetabolite studies utilizing bacterial systems might assist in clarifying relationships between structure, antimalarial activity, and antibacterial effects within this series, six of the 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (**9**,**10**,**13**,**14**,**16**,**18**) 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 IV). Details of the experimental procedures employed have been described previously (4). Data on the reference drugs aminopterin, methotrexate, pyrimethamine, trimethoprim, cycloguanil hydrochloride, and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (I) are included for comparative purposes.

Each of the pyridopyrimidines tested displayed moderate to strong inhibitory effects against *S. faecalis* R utilizing folic acid (FA) as the substrate (Table IV). These substances inhibit one or both reduction stages and are competitive with FA. Three compounds (**13**,**14**,**16**) produced 50% inhibition at concentrations of 1-16 ng./ml., and thus were equipotent with or more potent than pyrimethamine, trimethorpim, cycloguanil hydrochloride, aminopterin, and the quinazoline I. In general activity was fairly well reversed by 5-CHO-FAH_4 (Table IV), suggesting that these pyridopyrimidines function primarily as reductase inhibitors.

2,4-Diamino-6-(2,6-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**14**) caused 50% inhibition of *L. Plantarum* at a concentration of 266 ng./ml., a level of activity comparable with that shown by pyrimethamine, cycloguanil hydrochloride, and the 2,4-diaminoquinazoline I (Table IV). Compounds **13** and **16** showed modest activity, while **9**, **10**, and **18** were inactive at 40,000 ng./ml.

Against the methotrexate, aminopterin-resistant *S. faecalis* A, three compounds (**13**, **14**, **16**) produced 50% inhibition at concentrations of 56-700 ng./ml. utilizing FA as the substrate (Table IV). These inhibitory concentrations are comparable with or less than those required for pyrimethamine, trimethoprim, cycloguanil hydrochloride, or compound I (284-680 ng./ml.). The *S. faecalis* A to *S. faecalis* R inhibition ratios (23-115) for all six pyridopyrimidines are relatively low compared with those observed for aminopterin (> 20,000) and methotrexate (19,000). This indicates that there is relatively little cross-resistance between these pyridopyrimidines and aminopterin or methotrexate utilizing *S. faecalis*.

Based on available data, it must be concluded that the relative inhibitory potency of the 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines against *S. faecalis* R, *S. faecalis* A, and *L. plantarum* does not provide a reliable basis for predicting the relative magnitude of antimalarial effects (4).

EXPERIMENTAL (28)

4-Amino-3-cyano-1-(benzyl and pyridylmethyl)-1,2,5,6-tetrahydropyridines (VIII) (**1-8**, Table I). Procedure I.

To a cold solution of 25.0 g. of 3-picolyl chloride hydrochloride in 50 ml. of water was added 7.6 ml. of 50% aqueous sodium hydroxide. The free base was extracted with ether and the combined ether extracts were washed with saturated sodium chloride solution and dried over magnesium sulfate. The ether was removed *in vacuo* to give 18.7 g. (0.146 mole) of 3-picolyl chloride. This was combined with 15.0 g. (0.122 mole) of 4-amino-3-cyano-1,2,5,6-tetrahydropyridine (VI) (19), 20.0 g. (0.145 mole) of anhydrous potassium carbonate, and 600 ml. of 2-butanone. The mixture was stirred under reflux for 18 hours, cooled, and filtered. The filtrate was concentrated to dryness *in vacuo* leaving an oily residue which was triturated with ether and extracted with 400 ml. of chloroform. The chloroform solution was decanted from the viscous insoluble gum, washed twice with water, once with a saturated sodium chloride solution, and dried over magnesium sulfate. Removal of the chloroform *in vacuo* gave an oily residue which crystallized. The solid was triturated with an ethanol-ether mixture and dried to give 10.0 g. (38%) of 4-amino-3-cyano-1-(3-pyridylmethyl)-1,2,5,6-tetrahydropyridine (**1**) as yellow crystals, m.p. 115-120°.

Procedure II.

A mixture of 9.0 g. (0.073 mole) of 4-amino-3-cyano-1,2,5,6-tetrahydropyridine (VI) (19), 11.0 g. of anhydrous potassium carbonate, and 14.2 g. (0.073 mole) of α-2,4-trichlorotoluene in 500 ml. of 2-butanone was stirred under reflux for 19 hours, cooled, and filtered. The filtrate was concentrated to dryness and the residue was recrystallized twice from methanol to give 12.5 g. (66%) of 4-amino-3-cyano-1-(2,4-dichlorobenzyl)-1,2,5,6-tetrahydropyridine (**3**) as colorless needles, m.p. 138-140°.

2,4-Diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines (IX) (**9-18**, Table II). Procedure III.

A mixture of 9.7 g. (0.045 mole) of 4-amino-3-cyano-1-(3-pyridylmethyl)-1,2,5,6-tetrahydropyridine (**1**) and 10.0 g.(0.056 mole) of guanidine carbonate in 15 ml. of dimethylformamide was stirred under reflux for 3 hours and cooled. A brown solid formed. The mixture was diluted with 150 ml. of cold water and the solid was collected, triturated with methanol, and dried. The crude product was dissolved in a mixture of 10 ml. of methanol and 7 ml. of 2*N* hydrochloric acid and reprecipitated by addition to a cold dilute sodium hydroxide solution. Another reprecipitation from glacial acetic acid-10% ammonium hydroxide afforded a beige crystalline solid which was collected and washed successively with water, methanol, and ether. After drying *in vacuo* at 50° for 18 hours, 2,4-diamino-5,6,7,8-tetrahydro-6-(3-pyridyl-

methyl)pyrido[4,3-*d*]pyrimidine (**9**) was obtained as the hydrate (2.9 g., 23%), off-white crystals, m.p. 288-290°.

Procedure IV.

4-Amino-3-cyano-1-(2,4-dichlorobenzyl)-1,2,5,6-tetrahydropyridine (**3**) (12.2 g., 0.043 mole) and guanidine carbonate (9.7 g., 0.054 mole) in 40 ml. of dimethylformamide were stirred and heated under reflux for 3.5 hours. The mixture was cooled and 75 ml. of 95% ethanol was added. The mixture was chilled in ice and the yellow solid was collected, washed with water, and dried. Recrystallization from dimethylformamide-water gave 3.5 g. (25%) of 2,4-diamino-6-(2,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (**11**) as pale yellow crystals, m.p. 241-245° with preliminary softening.

Procedure V.

A mixture of 20.0 g. (0.078 mole) of 2,4-diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride hydrate (VII), 17.2 g. (0.088 mole) of α,3,4-trichlorotoluene, and 48.0 g. (0.35 mole) of potassium carbonate in 500 ml. of dimethylformamide was stirred at room temperature for 18 hours. The mixture was filtered and the residue was triturated with water. The solid was extracted with hot chloroform and the solution was treated with an excess of hydrogen chloride in ether. The resulting pale yellow gelatinous precipitate was collected and crystallized from methanol-ether to give 8.4 g. (26%) of 2,4-diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride monohydrate (**13**) as an off-white solid, m.p. 261-263°.

2,4-Diamino-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine Dihydrochloride (VII).

A mixture of 100 g. (0.81 mole) of 4-amino-3-cyano-1,2,5,6-tetrahydropyridine (VI) (19) and 150 g. (0.83 mole) of guanidine carbonate in 2 l. of 1,2-bisethoxyethane was stirred under reflux for 18 hours. The red solution was treated with an excess of hydrogen chloride in ether, and the orange-yellow precipitate was collected and triturated successively with 1 l. of 2-propanol and 1 l. of ether. The product was collected and dried to give 108 g. (52%) of product, m.p. 269-271°. An analytical sample was recrystallized from aqueous 2-propanol yielding yellow crystals, m.p. 272-275°.

Anal. Calcd. for $C_7H_{11}N_5 \cdot 2HCl \cdot H_2O$: C, 32.83; H, 5.90; N, 27.34. Found: C, 32.80; H, 6.30; N, 27.70.

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