Folate Antagonists. 8. Synthesis and Antimalarial Effects of 2,4-Diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines (1-3)

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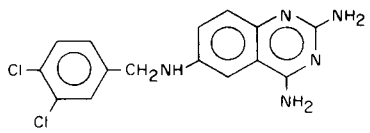
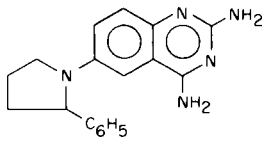
Received May 1, 1972

The antimalarial potency of 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (I) and related 2,4-diamino-6-[[aralkyl and (heterocyclic)methyl]amino]quinazolines (4,5) is usually markedly enhanced by N^6-nitrosation (6,7) or N^6-alkylation (8). Strong antimalarial



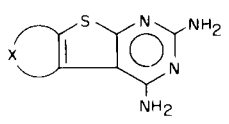
I

effects are also retained among certain cyclic relatives such as 2,4-diamino-6-(2-phenyl-1-pyrrolidinyl)quinazoline (II) (9,10), but are drastically reduced or abolished among

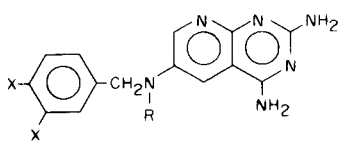


II

bioisosteres in the fused 2,4-diaminothieno[2,3-*d*]pyrimidine (III) (11) or the 2,4-diamino-6-[(benzyl)amino]pyrido[2,3-*d*]pyrimidine (IV) (12) series.

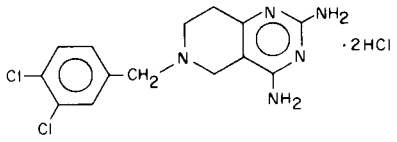


III



IV

In the previous communication on folate antagonists from these laboratories (1), it was reported that several 2,4-diamino-6-(benzyl and pyridylmethyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidines displayed good antimalarial activity orally against *Plasmodium berghei* in mice and seven compounds were more active than quinine hydrochloride. The most potent member of the series, namely 2,4-diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine dihydrochloride (V), was



V

approximately 19 times as active (SD_{90} = 3.9 mg./kg./day x 6 days) as quinine hydrochloride (SD_{90} = 74.5 mg./kg./day). Compound V 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, Q = 7.9), and was nearly as potent as cycloguanil hydrochloride (SD_{90} = 2.1 mg./kg./day, Q = 35).

A variety of substituted pyrrolo[3,4-*d*]pyrimidines (13-16), including several 2,4-diaminopyrrolo[3,4-*d*]pyrimidines (14-16), has recently been synthesized for biological evaluation. To date, none of the compounds reported has exhibited significant biological effects (14,16). However, unpublished antimalarial studies conducted in these laboratories showed that 2,4-diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride (VII), reported previously by our colleagues Cavalla and Willis (16), displayed significant antimalarial activity. When administered by drug-diet for 6 days to mice infected with *P. berghei*, VII produced a 90% suppression of the parasitemia in treated mice at a dose of 49 mg./kg./day and was thus approximately 1.5 times as potent as quinine hydrochloride. This observation provided the impetus for the synthesis of analogs of VII for antimalarial evaluation.

Chemistry.

The 2,4-diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines described in the present communication were prepared utilizing the route outlined in Scheme I. 4-Amino-1-benzyl-5-methyl-3-pyrroline-3-carbonitrile (VI) (17) was condensed with guanidine carbonate to give 2,4-diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine (VII) (16) (19%). Catalytic debenzylation of VII over Pd/C afforded 2,4-diamino-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride (VIII) (16) (73%), which was

SCHEME I

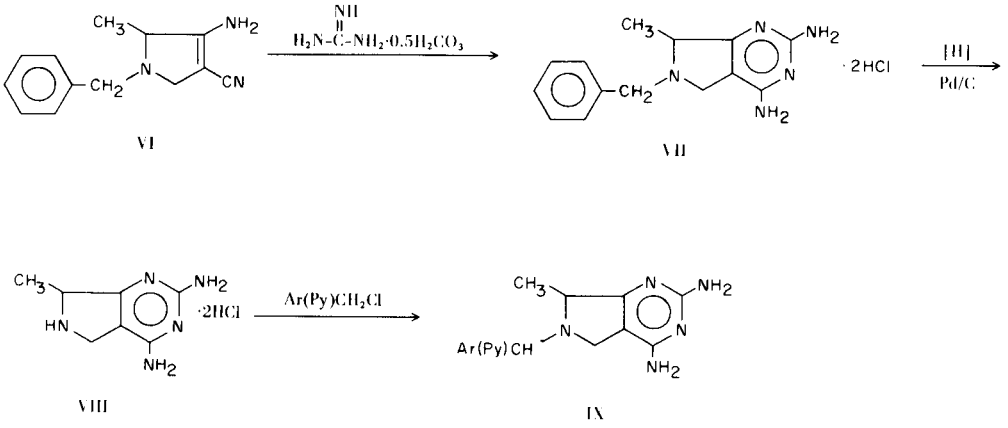
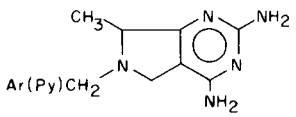
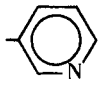
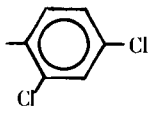
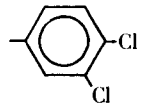
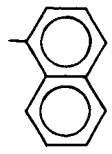


TABLE I

2,4-Diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines



No.	Ar(Py)	M.p., °C	Yield purified %	Purifcn solvent	Formula	Analyses Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1		260-262 dec.	15	EtOH	$C_{13}H_{16}N_6$	60.92	60.90	6.29	6.43	32.79	33.07
2		193-195	21	MeCN	$C_{14}H_{15}Cl_2N_5$	51.87	51.70	4.67	4.73	21.61	21.97
3		234-235	21	MeCN	$C_{14}H_{15}Cl_2N_5$	51.87	51.91	4.67	4.70	21.61	21.53
4		171-175	13	MeCN	$C_{18}H_{19}N_5$	70.79	70.70	6.27	6.35	22.93	22.71

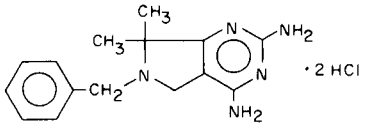
converted to the base and treated with the requisite α-chlorotoluene derivative or 3-picolyl chloride utilizing sodium hydride in dimethylformamide to give the desired 2,4-diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines (IX) (**1-4**, Table I) (13-21% yield). Since experimental details for the preparation of VI-VIII were omitted previously (16), they are included here.

Biological Results.

Antimalarial Effects.

The 2,4-diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines (IX) (**1-4**, Table I) described in the present communication, together with 2,4-diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride (VII) and 2,4-di-

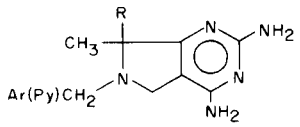
amino-6-benzyl-6,7-dihydro-7,7-dimethyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride (X) described previously (16), were tested for antimalarial activity utilizing *P. berghei* in mice and *Plasmodium gallinaceum* in chicks.

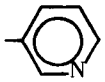
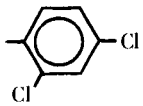
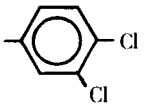
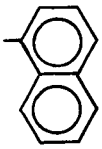
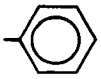
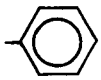


X

TABLE II

Oral and Parenteral Antimalarial Effects of 2,4-Diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines Against *Plasmodium berghei* in Mice



			Drug-diet, 6 days			Single s.c. dose					
			No. of	SD_{90}, (a)		ΔMST; T or C (c) after mg./kg. dose:					
No.	Ar(Py)	R	mice	mg./kg./day	Q (b)	640	320	160	80	40	20
1		H					4.9	1.3	1.1	0.5	0.3
2		H				12.2; C2	11.9 12.3	1.9 1.9	0.5 0.5	0.3 0.5	0.3 0.3
3		H	21	58	1.3	0.5	0.3	0.3	0.3	0.1	0.1
4		H						1.1	0.7	0.7	0.3
VII		H	21	49	1.5	T5	T5	0.9; T2	0.3	0.3	0.1
X		CH_3	14	>289	<0.3						
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 was 6.1 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.

Compounds **1-4** and VII 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* (18,19) (Table II). The most promising compound, 2,4-diamino-6-(2,4-dichlorobenzyl)-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine (**2**), cured two of five mice at a dose of 640 mg./kg. and increased the mean survival time of the mice an average of 12.1 days at 320 mg./kg. Compounds **1**, **3**, **4**, and VII lacked appreciable parenteral activity, and VII surprisingly was toxic for mice through 160 mg./kg. None of the 2,4-diaminopyrrolo[3,4-*d*]-pyrimidines was as active as the reference drugs cycloguanil hydrochloride and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline acetate (I) (4,5) (Table II).

Three pyrrolopyrimidines (**3**, VII, and X) (Table II) were administered continuously for 6 days in the diet of mice infected with another normal drug-sensitive strain of *P. berghei* (20,21). The two 7-methyl derivatives (**3**, VII) produced a 90% suppression of the parasitemia relative to control animals at daily doses of 58 and 49 mg./kg., respectively, and were 1.3 and 1.5 times as potent as quinine hydrochloride. By contrast, the 7,7-dimethyl analog X lacked appreciable antimalarial effects even at high dose levels (289 mg./kg./day) (Table II).

2,4-Diamino-6-(3,4-dichlorobenzyl)-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine (3) was also evaluated against *P. gallinaceum* infections in white Leghorn cockerels (19,22). However, the drug lacked significant antimalarial activity in this test model when administered in a single subcutaneous dose of 120 mg./kg.

Antibacterial and Antimetabolite Studies.

Three of the 2,4-diaminopyrrolo[3,4-*d*]pyrimidines (**3**,VII,X) were tested *in vitro* against a spectrum of pathogenic bacteria comprising *Streptococcus pyogenes* (C203), *Staphylococcus aureus* (UC-76), *Proteus mirabilis* (MGH-1), *Psuedomonas aeruginosa* (28), *Salmonella typhimurium* (V-31), and *Mycobacterium tuberculosis* (H_{37}-Rv) utilizing broth dilution techniques (23). Compound **3** produced complete inhibition of *S. pyogenes* (C203) at a concentration of 10 μg./ml., but was inactive against the other bacteria at concentrations of 25 μg./ml. The other substances (VII,X) were inactive against all of the organisms at a concentration of 25 μg./ml.

2,4-Diamino-6-(3,4-dichlorobenzyl)-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine (**3**) was evaluated for inhibitory effects against *Streptococcus faecalis* R (*S. faecium* var. *durans*, ATCC 8043), *S. faecalis* A (methotrexate, aminopterin-resistant mutant), and *Lactobacillus plantarum* (ATCC 8014) (4). The compound produced 50% inhibition of *S. faecalis* R at 600 ng./ml., *L. plantarum* at 32 μg./ml., and *S. faecalis* A at 13 μg./ml. However, **3** was much less potent as a folate antagonist than the isosteric 2,4-diamino-6-(3,4-dichlorobenzyl)-5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine (V) (1) and 2,4-diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (I) (4).

The results of the present investigation and earlier studies with various 2,4-diaminothieno[2,3-*d*]pyrimidine (III) systems (11) suggest that a six-membered ring fused with the 2,4-diaminopyrimidine moiety plays an important role in conferring optimal antimalarial properties among the 2,4-diaminoquinazoline antifolates and their analogs.

EXPERIMENTAL (24)

2,4-Diamino-6-[(aryl and pyridyl)methyl]-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidines (IX) (**1-4**, Table I).

To a suspension of 3.2 g. (0.0135 mole) of 2,4-diamino-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride (VIII) in 100 ml. of methanol was added 1.5 g. (0.027 mole) of sodium methoxide. The flask was swirled briefly and the resulting solution was evaporated to dryness at room temperature. The beige solid residue was taken up in 50 ml. of *N,N*-dimethylformamide, warmed on the steam bath, and filtered to remove the insoluble inorganic material. The filtrate was transferred to a reaction flask and stirred for 1 hour in a 50-60° oil bath with 0.57 g. (0.0135 mole) of 57% sodium hydride, then cooled to room temperature. To this was added 2.6 g. (0.0135 mole) of α,2,4-trichlorotoluene. The reaction mixture was stirred at room temperature overnight, then warmed in a 50-60° oil bath for 1 hour. The reaction mixture was cooled to room temperature and filtered. The filtrate was diluted with 400 ml. of water causing the precipitation of a beige crystalline solid which was collected and air dried. A second crop was obtained by adding a small amount of 50% aqueous sodium hydroxide to the filtrate. This was collected, combined with the first crop and recrystallized from acetonitrile. The material was collected, triturated with ether and dried *in vacuo* at 100° to give 0.9 g. (21%) of 2,4-diamino-6-(2,4-dichlorobenzyl)-6,7-dihydro-7-methyl-5*H*-pyrrolo-[3,4-*d*]pyrimidine (**2**) as off-white crystals, m.p. 193-195°.

2,4-Diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine Dihydrochloride (VII).

A solution of 42.6 g. (0.2 mole) of 4-amino-1-benzyl-5-methyl-3-pyrroline-3-carbonitrile (VI) (17) and 27.0 g. (0.15 mole) of guanidine carbonate in 300 ml. of β-ethoxyethanol was stirred under reflux for 6 hours and then allowed to stir at room temperature overnight. The reaction mixture was diluted with 500 ml. of 2-propanol and 2 l. of ether and filtered. The filtrate was acidified with 100 ml. of 2-propanol saturated with hydrogen chloride, and the hygroscopic beige solid that formed was collected and placed in a vacuum desiccator. After additional drying in a vacuum oven the material was equilibrated with the atmosphere, whereupon it became a moist semi-solid. This was triturated first with warm 2-propanol and then with warm ethanol. The off-white solid which resulted was suspended in boiling 2-propanol. Methanol was added gradually to effect a complete solution. The solution was cooled and the product was obtained by addition of ether to give 13.3 g. (19%) of 2,4-diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine dihydrochloride hydrate, m.p. 253-256° dec. Lit. (16) reports m.p. 251-254° dec.

Anal. Calcd. for $C_{14}H_{17}N_5 \cdot 2HCl \cdot H_2O$: C, 48.56; H, 6.11; N, 20.22; Cl^-, 20.48; H_2O, 5.19. Found: C, 48.53; H, 5.69; N, 19.89; Cl^-, 20.43; H_2O, 5.11.

2,4-Diamino-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]pyrimidine Dihydrochloride (VIII).

2,4-Diamino-6-benzyl-6,7-dihydro-7-methyl-5*H*-pyrrolo[3,4-*d*]-pyrimidine dihydrochloride monohydrate (VII) (14.4 g., 0.046 mole) was catalytically debenzylated at room temperature in 200 ml. of water and 80 ml. of 2-propanol over 0.5 g. of 20% palladium on carbon. The initial pressure was 51 psig. Hydrogen take up was complete after 80 minutes. The catalyst was removed by filtration and the solvent was removed *in vacuo*. The residue was reprecipitated from methanol with ether to give 8.0 g. (73%) of product, m.p. 299-301° dec. Lit. (16) reports m.p. 296-299° dec.

Anal. Calcd. for $C_7H_{10}N_5 \cdot 2HCl$: C, 35.32; H, 5.51; N, 29.42; Cl^-, 29.79. Found: C, 35.53; H, 5.47; N, 29.55; Cl^-, 29.88.

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 Mrs. Jane Clarke 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.

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(1) For the previous paper on folate antagonists, see E. F. Elslager, J. Clarke, P. Jacob, L. M. Werbel and J. D. Willis, *J. Heterocyclic Chem.*, 9, 1113 (1972).

(2) This is communication 30 of a series of antimalarial drugs. For paper 29, see (1).

(3) This investigation was supported by U.S. Army Medical Research and Development Command Contract DA-49-193-MD-2754. This is contribution No. 1071 to the Army Research Program on Malaria.

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

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

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(22) 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.

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