Folate Antagonists. 19. Synthesis and Antimalarial Effects of 6-(Arylthio)-2,4-pteridinediamines[1-3]

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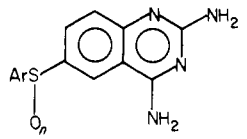
A series of 6-(arylthio)-2,4-pteridinediamines (IIIa) was prepared by allowing 6-chloro-2,4-pteridinediamine to react with the requisite benzenethiols in dimethyl sulfone at 190–200 °C. Attempts at oxidation to the corresponding sulfoxide (IIIb) or sulfone (IIIc) were unsuccessful. The compounds exhibited a spectrum of antibacterial activity similar to, but below the potency of, the related quinazolinediamines and pteridinediamines. Unlike these related types, however, they were devoid of antimalarial activity when tested against a normal drug-sensitive strain of *Plasmodium berghei* in mice by the parenteral route.

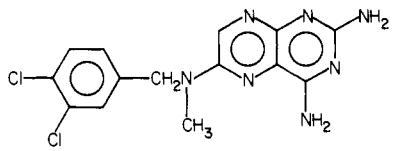
The nonclassical thioquinazoline analogues of methotrexate represented by the 2,4-diamino-6-[(phenyl and naphthyl)thio]quinazolines (Ia) and their sulfinyl (Ib) and



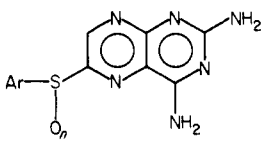
Ia, $n = 0$
b, $n = 1$
c, $n = 2$

sulfonyl (Ic) analogues are among the most potent antimalarial drugs ever reported in experimental animal models.[4,5]

We demonstrated recently that certain nonclassical pteridine analogues of methotrexate represented by N^6-(arylmethyl)-N^6-methyl-2,4,6-pteridinetriamines such as II exhibited potent suppressive antimalarial effects against



II



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

lines of *Plasmodium berghei* in mice.[6] It was thus of interest to examine the antimalarial properties of the 6-(arylthio)-2,4-pteridinediamines (IIIa), as well as their sulfinyl (IIIb) and sulfonyl (IIIc) derivatives, related to I. The preparation of the 6-(arylthio) analogues and efforts to obtain the oxidized forms are described in this report.

Chemistry. The 6-(arylthio)-2,4-pteridinediamines (IIIa, 1–7) were prepared by allowing 6-chloro-2,4-pteridinediamine[7,8] to react with the requisite benzenethiols in dimethyl sulfone at 190–200 °C (Scheme I). The analogous 6-[[(4-chlorophenyl)methyl]thio]-2,4-pteridinediamine (8) was prepared similarly from 4-chlorobenzenemethanethiol, for comparison with the corresponding 6-[(phenylmethyl)thio]-2,4-quinazolinediamines which exhibited antimalarial activity but were much less effective than I.[9] After this work had been completed, the synthesis of 3 and some related analogues was reported,[10] without biological activity, using slightly different conditions.

The derivative, N',N''-[6-[(3,4-dichlorophenyl)thio]-2,4-pteridinediyl]bis[N,N-dimethylmethanimidamide] (9) was obtained by treating pteridinediamine 2 with dimethylformamide dimethyl acetal in DMF. Efforts to oxidize 2 to the corresponding sulfoxide (IIIb) with either H_2O_2 in HOAc or the bromine complex of 1,4-diazabicyclo[2.2.2]octane[11] in 70% HOAc and to the sulfone (IIIc) with excess H_2O_2 in HOAc apparently resulted only in the rupture of the pteridine ring.

Suppressive Antimalarial Screening in Mice. The 6-(arylthio)-2,4-pteridinediamines 1–9 were tested against a normal drug-sensitive strain of *P. berghei* in mice by the parenteral route.[12,13] The compounds were dissolved or suspended in sesame or peanut oil and were administered to mice in a single subcutaneous dose 72 h postinfection. Extension of the mean survival time of the treated mice is interpreted as evidence of antimalarial activity.[14]

Compounds are arbitrarily considered to be "active" when they produce at least a 100% increase in the mean survival time of treated mice. Animals that survive to 60 days are considered "cured". The mean survival time of infected control mice in the present study ranged from 6.1

(1) This is paper 50 of a series on antimalarial drugs. For paper 49, see ref 6.
(2) This investigation was supported by the U.S. Army Medical Research and Development Command (Contract DA 17-72-C-2077). This is contribution no. 1618 to the Army Research Program on Malaria.
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(12) The parenteral antimalarial screening in mice was carried out in the laboratory of Dr. Leo Rane of the University of Miami. Test results were provided through the courtesy of Drs. T. R. Sweeney and E. A. Steck of the Walter Reed Army Institute of Research.
(13) For a description of the test method, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, 10, 431 (1967).
(14) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, 10, 431 (1967).

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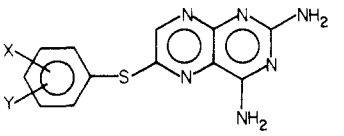
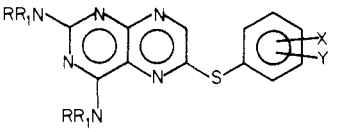
Table I. 6-[(Phenyl and naphthyl)thio]-2,4-pteridinediamines



no.	X, Y	mp, °C	yield purified, %	purifn solvent	formula	anal.
1	2,4,5-Cl_3	>300	51	DMF	$C_{12}H_7Cl_3N_6S$	C, H, N
2	3,4-Cl_2	>300	30	DMF	$C_{12}H_8Cl_2N_6S$	C, H, N
3	2-Cl	>300	17	EtOH	$C_{12}H_9ClN_6S$	C, H, N, Cl
4	3-CF_3	277-279 dec	41	EtOH	$C_{13}H_9F_3N_6S$	C, H, N
5	4-OCH_3	306-309 dec	47	DMF	$C_{13}H_{12}N_6OS$	C, H, N
6	2,3-(-CH=CHCH=CH-)	>300	88	DMF	$C_{16}H_{12}N_6S$	C, H, N
7	3,4-(-CH=CHCH=CH-)	302-305 dec	54	DMF	$C_{16}H_{12}N_6S$	C, H, N

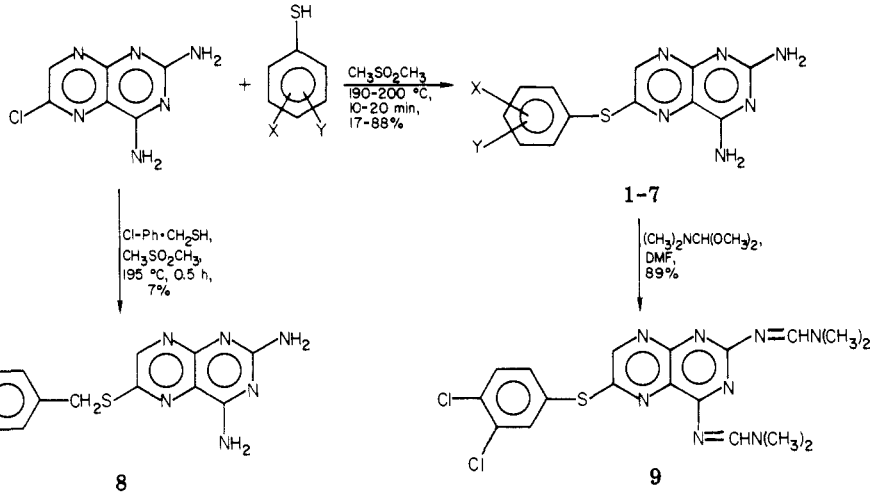
Table II. In Vitro Antibacterial Effects of 6-[(Phenyl and naphthyl)thio]-2,4-pteridinediamines



			min inhibitory concn,[a] μg/mL		
no.	RR_1	X, Y	*S.f.* MGH-2[b]	*S.a.* UC-76[c]	*S.a.* S18713
1	H_2	2,4,5-Cl_3	5.0	>25	>25
2	H_2	3,4-Cl_2	2.0	>25	>25
4	H_2	3-CF_3	<0.25	<0.25	<0.25
5	H_2	4-OCH_3	<0.25	2.0	2.0
6	H_2	2,3-(-CH=CHCH=CH-)	<0.25	<0.25	1.5
7	H_2	3,4-(-CH=CHCH=CH-)	1.5	>25	>25
9	$(CH_3)_2NCH=$	3,4-Cl_2	<0.25	>25	>25

[a] Gradient plate test. [b] *S.f.* = *Streptococcus faecalis.* [c] *S.a.* = *Staphylococcus aureus.*

Scheme I



to 6.3 days. Using these criteria, the present compounds were devoid of antimalarial activity at doses up to 640 mg/kg.

Antibacterial Activity. Most of the pteridines (**1**, **2**, **4–7**, and **9**) were also tested in vitro against a spectrum of pathogenic bacteria, including *Streptococcus faecalis* (MGH-2), normal (UC-76) and drug-resistant (S18713) *Staphylococcus aureus*, *Escherichia coli* (Vogel), *Pseudomonas aeruginosa* (28), and *Shigella sonnei* (C-10). A modification of the gradient plate procedure of Szybalski[15] and Webb and Washington[16] was employed. Although none was particularly effective against *P. aeruginosa* (28), *E. coli* (Vogel), or *S. sonnei* (C-10), all the compounds tested inhibited the growth of *S. faecalis* (MGH-2), and three compounds (**4–6**) exhibited activity against *S. aureus* (UC-76) and *S. aureus* (S18713). These results are presented in Table II.

Conclusions

In general the 6-(arylthio)-2,4-pteridinediamines exhibit a spectrum of antibacterial activity similar to, but below the potency of, the quinazolinediamines[4,5] and pteridine-

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triamines[6] reported previously. The lack of antimalarial activity is difficult to reconcile. Possibly the presence of nitrogen in position 5 still allows the formation and interconversion of one-carbon units in reduced forms of these compounds analogous to various 5,6,7,8-tetrahydrofolate coenzymes, as opposed to the 2,4-diaminoquinazoline antimetabolites in which the absence of N-5 could preclude such changes, thus inhibiting the critical biochemical pathway.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are corrected. The progress of the reactions was followed by TLC using silica gel plates (Eastman) and a solvent mixture of MeOH–EtOAc–Et_3N, 25:75:1. Satisfactory infrared spectra were obtained for all compounds.

Preparation of 6-[(Phenyl and naphthyl)thio]-2,4-pteridinediamines (Table I; 1–7). The preparation of 6-[(2,4,5-trichlorophenyl)thio]-2,4-pteridinediamine (Table I; 1) is described as an example. A mixture of 1.0 g (0.005 mol) of 6-chloro-2,4-pteridinediamine, 1.24 g (0.006 mol) of 2,4,5-trichlorobenzenethiol, and 10 g of dimethyl sulfone was stirred at 192–200 °C for 20 min, cooled slightly, and poured into H_2O. The warm mixture was made basic with 50% aqueous NaOH and filtered to collect the precipitate that had formed. The filter cake was washed with H_2O and then with 20 mL of acetone and was then recrystallized from DMF to give 0.97 g (51%) of the product, mp >300 °C.

6-[[(4-Chlorophenyl)methyl]thio]-2,4-pteridinediamine (Scheme I; 8). A mixture of 3 g (0.0153 mol) of 6-chloro-2,4-pteridinediamine, 2.45 g (0.0155 mol) of 4-chlorobenzenemethanethiol, and 22 g of dimethyl sulfone was heated at 195 °C (external temperature) for 0.5 h. The temperature of the reaction mixture rose to 203 °C. The mixture was allowed to cool to 130 °C, poured into H_2O, and filtered. The filter cake was washed successively with acetone, 1 N NaOH, H_2O, and acetone again. The dark material was then extracted with EtOH continuously in a Soxhlet extractor for 25 h. The extract was evaporated in vacuo to give 0.5 g of crude product. Recrystallization from DMF gave 0.32 g (6.6%) of the title compound, mp 275–277 °C dec. Anal. ($C_{13}H_{11}ClN_6S$) C, H, N.

***N',N''*-[6-[(3,4-Dichlorophenyl)thio]-2,4-pteridinediyl]-bis[*N,N*-dimethylformamidine] (Scheme I; 9).** A mixture of 0.73 g (0.002 mol) of 6-[(3,4-dichlorophenyl)thio]-2,4-pteridinediamine, 5.14 g (0.04 mol) of *N,N*-dimethylformamide dimethyl acetal, and 15 mL of DMF was stirred at room temperature for 20 h, chilled, and filtered to give 0.80 g (89%) of the product, mp 231–234 °C. Anal. ($C_{18}H_{18}Cl_2N_8S$) C, H, N.

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Diuretic Agents Related to Indapamide. 1. Synthesis and Activity of 1-Substituted 2-(4-Chloro-3-sulfamoylbenzamido)isoindolines

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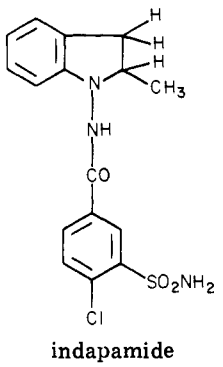
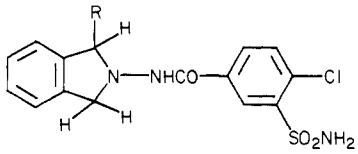
The synthesis of isoindoline analogues of indapamide is described. These substances (**4a,c,d**) were tested for diuretic activity, and **4a** was found to be comparable in potency to indapamide.

Indapamide is a diuretic agent widely used in the therapy of hypertension.[1] Comparing its structure to that of clopamide and other 4-chloro-3-sulfamoylbenzhydrazides having diuretic properties,[2] we considered the possibility that the indoline moiety of indapamide might not be essential for drug–receptor interaction. We therefore synthesized **4a** to evaluate its diuretic activity.



indapamide



4a, R = CH_3
c, R = CH_2OH
d, R = COOH

The two potential metabolites of **4a**, namely **4c** (R = CH_2OH) and **4d** (R = COOH), were also synthesized and tested.

Chemistry. The starting material for the synthesis of **4a** was the unknown 1-methyl-2-aminoisoindoline (**3a**), which was prepared by condensing the dibromide **1a**[3] with *tert*-butyl carbazate in DMF to give the carbamate **2a**; **3a**·HCl was recovered when **2a** was treated with dilute HCl. The overall yield was 86%.

Final condensation of **3a** with 4-chloro-3-sulfamoylbenzoyl chloride[4] yielded 67% of **4a**. This was accom-

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