TABLE I
EFFECT OF METHOTREXATE DERIVATIVES ON ENZYMES AND BACTERIAL GROWTH

Compd	Dihydrofolate reductase	Thymidylate synthetase	*S. faecalis*	*P. cerevisiae*	*L. casei*
	Conen for 50% inhib, mμg/ml				
Methotrexate	9 (1)[a]	45,000 (1)	0.15 (1)	60 (1)	0.01 (1)
Dihydromethotrexate	16 (0.56)	1,125 (40)	0.011 (14)	24 (2.5)	0.008 (1.3)
Tetrahydromethotrexate	46 (0.20)	2,250 (20)	0.047 (3.4)	68 (1)	0.056 (0.18)

[a] Numbers in parentheses indicate potency relative to methotrexate.

Thymidylate synthetase from *Escherichia coli* B[12] was provided by Dr. M. Friedkin and Miss E. Donovan. Dihydrofolate reductase was obtained from a mouse tumor L1210-C95.[9] The enzymes were assayed as described.[9]

Fractions to be assayed microbiologically were diluted in potassium ascorbate (6 mg/ml, pH 6.0) and added aseptically to the assay medium.[13] The final concentration of ascorbate in the assay was 0.6 mg/ml. *Lactobacillus casei* (ATCC 7469), *Streptococcus faecalis* (ATCC 8043), and *Pediococcus cerevisiae* (ATCC 8081) were grown on the corresponding Difco assay media for 24 hr at 37°. The *L. casei* and *S. faecalis* media contained 1 mμg of folate/ml and the *P. cerevisiae* medium contained 1 mμg/ml of calcium *dl*-L-5-formyltetrahydrofolate. Growth was determined turbidimetrically.

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New Compounds

Synthesis of 6,8-Dibromo-3-substituted 2-(N,N-Dialkyl- (or N-Piperidino-) carboxamidomethylthio)-4(3H)-quinazolinones as Antimalarials

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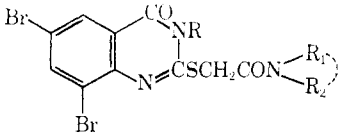
The antimalarial activity of febrifugine, an alkaloid of a 3-substituted 4(3H)-quinazolinone structure, has prompted the preparation and testing of a number of quinazolines,[1] and several patent claims have been made on quinazolines as intermediates for potential antimalarials.[2] Compounds having the side chain $-CH_2COCH_2R$ (where R = ω-N-morpholylpropyl or ω-N-piperidyl-*n*-butyl) at position 3 of the 4(3H)-quinazolinone nucleus were shown to have significant antimalarial activity.[3]

Since the activity of these compounds is influenced by various substituents and their positions, a number of derivatives have been synthesized in the course of our previous investigations[4] by introducing some new side chains into some 6,8-dibromo-substituted 2-mercapto-3-aryl- (or alkyl-) 4(3H)-quinazolones as antimalarials having the general structure **1**.

The standard tests for antimalarial activity in chicks infected with *Plasmodium gallinaceum* so far reported on these compounds indicate that they have no significant value pharmacologically.



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(2) B. R. Baker and M. V. Querry, U. S. Patent 2,796,417 (1957); *Chem. Abstr.*, **52**, 459 (1958); B. R. Baker, U. S. Patent 2,811,542 (1957); *Chem. Abstr.*, **52**, 5488 (1958).

(3) O. Y. Magidson and Y. K. Lu, *Zh. Obshch. Khim.*, **29**, 2843 (1959); *Chem. Abstr.*, **54**, 12144 (1960).

(4) P. N. Bhargava and M. R. Chaurasia, *J. Med. Chem.*, **11**, 404 (1968).

Experimental Section

6,8-Dibromo-3-phenyl-2-(N-piperidinocarboxamidomethylthio)-4(3H)-quinazolinone.—N-Chloroacetylpiperidine (2 ml) dissolved in EtOH, was added to a solution of 6,8-dibromo-2-thio-3-phenyl-2,4(1H,3H)-quinazolindione (4.5 g) in EtOH–NaOH. The resulting mixture was stirred thoroughly for 2 hr at 23–25°. On cooling the solution to 0° a crystalline product was formed. It was filtered and washed (H_2O, EtOH). Recrystallization of the product from EtOH–Me_2CO (1:2) gave a pure analytical sample.

Similarly various 6,8-dibromo-3-substituted 2-(N,N-dialkyl- (or N-piperidino-) carboxamidomethylthio)-4(3H)-quinazolinones have been prepared (see Tables I–V).

TABLE I
6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-METHYLPHENYL-CARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	58	87	$C_{23}H_{17}Br_2N_3O_2S$
o-$CH_3C_6H_4$	40	246	$C_{24}H_{19}Br_2N_3O_2S$
m-$CH_3C_6H_4$	50	83	$C_{24}H_{19}Br_2N_3O_2S$
p-$CH_3C_6H_4$	55	98	$C_{24}H_{19}Br_2N_3O_2S$
p-ClC_6H_4	50	95	$C_{23}H_{16}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	55	104	$C_{24}H_{19}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	60	218	$C_{25}H_{21}Br_2N_3O_3S$
n-C_4H_9	35	200	$C_{21}H_{21}Br_2N_3O_2S$
$C_6H_5CH_2$	53	221	$C_{24}H_{19}Br_2N_3O_2S$

[a] Crystallization solvent: EtOH. [b] All compounds were analyzed for N, S. The analytical results were within ±0.3% of the calculated values.

TABLE II
6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-ETHYLPHENYL-CARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	65	106	$C_{24}H_{19}Br_2N_3O_2S$
o-$CH_3C_6H_4$	50	105	$C_{25}H_{21}Br_2N_3O_2S$
m-$CH_3C_6H_4$	40	295 dec	$C_{25}H_{21}Br_2N_3O_2S$
p-$CH_3C_6H_4$	75	121	$C_{25}H_{21}Br_2N_3O_2S$
m-ClC_6H_4	45	248 dec	$C_{24}H_{18}Br_2ClN_3O_2S$
p-ClC_6H_4	65	110	$C_{24}H_{18}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	55	114	$C_{25}H_{21}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	70	104	$C_{26}H_{23}Br_2N_3O_3S$
$C_6H_5CH_2$	35	258 dec	$C_{25}H_{21}Br_2N_3O_2S$

[a] Crystallization solvent: EtOH. [b] All compounds were analyzed for Br, N. The analytical results were within ±0.3% of the calculated values.

TABLE III

6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-BENZYLPHENYLCARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	70	113	$C_{29}H_{21}Br_2N_3O_2S$
o-$CH_3C_6H_4$	45	245	$C_{30}H_{23}Br_2N_3O_2S$
m-$CH_3C_6H_4$	50	84	$C_{30}H_{23}Br_2N_3O_2S$
p-$CH_3C_6H_4$	60	88	$C_{30}H_{23}Br_2N_3O_2S$
m-ClC_6H_4	65	103	$C_{29}H_{20}Br_2ClN_3O_2S$
p-ClC_6H_4	55	96	$C_{29}H_{20}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	65	93	$C_{30}H_{23}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	75	111	$C_{31}H_{25}Br_2N_3O_3S$
n-C_4H_9	35	219	$C_{27}H_{25}Br_2N_3O_2S$
$C_6H_5CH_2$	40	238 dec	$C_{30}H_{23}Br_2N_3O_2S$

[a] Crystallization solvent: EtOH. [b] All compounds were analyzed for Br, N. The analytical results were within ±0.3% of the calculated values.

TABLE IV

6,8-DIBROMO-3-SUBSTITUTED 2-(N,N-DIETHYLCARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	60	187	$C_{20}H_{19}Br_2N_3O_2S$
o-$CH_3C_6H_4$	50	162	$C_{21}H_{21}Br_2N_3O_2S$
m-$CH_3C_6H_4$	30	275 dec	$C_{21}H_{21}Br_2N_3O_2S$
p-$CH_3C_6H_4$	55	188	$C_{21}H_{21}Br_2N_3O_2S$
m-ClC_6H_4	40	270 dec	$C_{20}H_{18}Br_2ClN_3O_2S$
p-ClC_6H_4	35	295 dec	$C_{20}H_{18}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	45	>320	$C_{21}H_{21}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	35	235 dec	$C_{22}H_{23}Br_2N_3O_3S$
CH_3	25	305 dec	$C_{15}H_{17}Br_2N_3O_2S$
C_2H_5	30	>320	$C_{16}H_{19}Br_2N_3O_2S$
n-C_4H_9	45	285 dec	$C_{18}H_{23}Br_2N_3O_2S$
$C_6H_5CH_2$	25	248 dec	$C_{21}H_{21}Br_2N_3O_2S$

[a] Crystallization solvent: Me_2CO–EtOH–EtOAc (3:1:1). [b] All compounds were analyzed for N, S. The analytical results were within ±0.3% of the calculated values.

TABLE V

6,8-DIBROMO-3-SUBSTITUTED 2-(N-PIPERIDINOCARBOXAMIDOMETHYLTHIO)-4(3H)-QUINAZOLINONES[a]

R	% yield	Mp, °C	Formula[b]
C_6H_5	60	240	$C_{21}H_{19}Br_2N_3O_2S$
o-$CH_3C_6H_4$	35	238 dec	$C_{22}H_{21}Br_2N_3O_2S$
m-$CH_3C_6H_4$	40	270 dec	$C_{22}H_{21}Br_2N_3O_2S$
p-$CH_3C_6H_4$	45	250 dec	$C_{22}H_{21}Br_2N_3O_2S$
m-ClC_6H_4	50	268 dec	$C_{21}H_{18}Br_2ClN_3O_2S$
p-ClC_6H_4	55	260 dec	$C_{21}H_{18}Br_2ClN_3O_2S$
p-$OCH_3C_6H_4$	65	116	$C_{22}H_{21}Br_2N_3O_3S$
p-$OC_2H_5C_6H_4$	50	290 dec	$C_{23}H_{23}Br_2N_3O_3S$
CH_3	30	280 dec	$C_{16}H_{17}Br_2N_3O_2S$
n-C_4H_9	25	305 dec	$C_{19}H_{23}Br_2N_3O_2S$
$C_6H_5CH_2$	35	275 dec	$C_{22}H_{21}Br_2N_3O_2S$

[a] Crystallization solvent: Me_2CO–EtOH (2:1). [b] All compounds were analyzed for N, S. The analytical results were within ±0.3% of the calculated values.

6,8-Dibromo-3-benzyl-2-carboxymethylthio-4(3H)-quinazolinone.—An equimolar quantity of sodium monochloroacetate was added to a 6,8-dibromo-2-thio-3-benzyl-2,4(1H,3H)-quinazolindione in EtOH–NaOH, and the mixture was shaken for 6 hr. It was acidified with HCl, the precipitate obtained was dissolved in $NaHCO_3$, filtered, and reprecipitated with HCl. The product was crystallized (EtOH); yield 60%, mp 237. *Anal.* ($C_{17}H_{12}Br_2N_2O_3S$) C, H, N, S.

6,8-Dibromo-3-phenyl-1-ethyl-2-thio-2,4(1H,3H)-quinazolinedione.—A mixture of 3,5-dibromo-N-ethylanthranilic acid (1.6 g), pyridine (0.4 g), EtOH (5 ml), and phenyl isothiocyanate (0.68 g) was refluxed at 90° for 6 hr. The crystalline product was filtered and recrystallized from C_6H_6 and EtOH mixture (3:1) to give 60% yield of the required product, white needles, mp 242°. *Anal.* ($C_{16}H_{12}Br_2N_2OS$) C, H, N.

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4-Dialkylaminoalkylamino-3-phenylpyridines[1]

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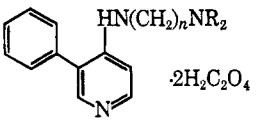
As part of a program devoted to the synthesis of novel antimalarial agents we have prepared the series of N-substituted 3-phenyl-4-aminopyridines listed in Table I. Treatment of 3-phenylpyridines bearing appropriate substituents in the 4 position with the diamines corresponding to the side chains was the general synthetic approach. The oily free bases were characterized by their nmr spectra and as oxalic acid salts. None of the tabulated compounds were active when screened against *Plasmodium berghei* in mice.[2]

TABLE I

4-DIALKYLAMINOALKYLAMINO-3-PHENYLPYRIDINE DIOXALATES



n	R	Mp, °C	Formula	Analyses[3]
2	Et	177–178	$C_{17}H_{23}N_3 \cdot 2H_2C_2O_4$	C, H, N
3	Et	155–157	$C_{18}H_{25}N_3 \cdot 2H_2C_2O_4$	C, H, N
4	Et	140–141	$C_{19}H_{27}N_3 \cdot 2H_2C_2O_4$	C, H, N
5	Et	162–164	$C_{20}H_{29}N_3 \cdot 2H_2C_2O_4$	C, H, N
6	Me	145–148	$C_{19}H_{27}N_3 \cdot 2H_2C_2O_4$	C, H, N

Experimental Section[3]

4-Methoxy-3-phenylpyridine was prepared by the procedure of Ahmad and Hey.[4] The improved Gomberg reaction procedure of Cadogan[5] was used in the final step of the sequence.

4-Hydroxy-3-phenylpyridine.—4-Methoxy-3-phenylpyridine (15 g, 81 mmoles) was refluxed for 3 hr with 100 ml of 58% HI. The solution was cooled, diluted with 80 ml of H_2O, and treated with Na_2SO_3 until the dark color had faded to orange-yellow. The solution was made slightly alkaline, and the oily solid that came out of solution was collected and washed thoroughly with Et_2O. The filtrate was extracted with Et_2O to remove unhydrolyzed starting material. From the ethereal washings and extracts was recovered 4.86 g (32%) of starting material. The remaining crystalline solid (5.3 g, 56% yield based on recovered starting material) had mp 210–225°. Recrystallization from hot H_2O gave pure product, mp 228–230°. *Anal.* ($C_{11}H_9NO$) C, H.

4-Chloro-3-phenylpyridine.—4-Hydroxy-3-phenylpyridine (0.20 g, 1.17 mmoles) was refluxed for 1.5 hr with *ca.* 2 ml of $POCl_3$; the mixture was cooled and poured into ice water.

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(2) The antimalarial tests were performed by Dr. Leo Rane of the University of Miami [T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967)]. Testing results were supplied through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.

(3) Melting points were taken in a Mel-Temp apparatus and are corrected. Microanalyses were performed by the Stanford Research Institute Analytical Laboratories. Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

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