Table II—Physical and Analytical Data for Probe Molecules and New Compounds [a]

Compound	Melting Point	Optical Rotation [b], $[\alpha]_D^{25}$	Formula		Analysis, % Calc.	Found
I	112–114° [c]	+29°	$C_{28}H_{34}INO_4$	C	58.4	58.16
				H	5.9	6.12
				I	22.1	21.90
				N	2.4	2.11
II	116–118° [c]	−30°	$C_{28}H_{34}INO_4$	C	58.4	58.20
				H	5.9	5.77
				I	22.1	22.46
				N	2.4	2.34
III	166–168°	+74°	$C_{23}H_{32}INO_4 \cdot H_2O$	C	52.0	51.78
				H	6.4	6.24
				I	23.9	24.19
				N	2.6	2.47
IV	164–168° [c]	−72°	$C_{23}H_{32}INO_4 \cdot H_2O$	C	52.0	51.83
				H	6.4	6.57
				I	23.9	23.73
				N	2.6	2.49
V	202–204°	0°	$C_{27}H_{28}INO_4$	C	58.2	58.21
				H	5.0	4.99
				I	22.8	22.66
				N	2.5	2.25
VI	84–86°	0°	$C_{27}H_{31}NO_4$	C	74.8	74.57
				H	7.2	7.33
				N	3.2	3.03

[a] Reported only for new compounds and for VI because of a discrepancy in melting-point data for this compound. [b] All rotations determined as (c 0.5, ethanol). [c] Obtained as amorphous solids.

from light. The solvent was then removed under reduced pressure, and the racemic derivative was crystallized from either ethanol or water while enantiomeric derivatives yielded soft, tar-like materials. The enantiomeric tars were taken up in acetone, which was then evaporated to produce fine amorphous powders. Final yields were: racemic material, 75%; I, 67%; and II, 17%. Physical data for probe compounds are recorded in Table II.

Quaternization with Ethyl Iodide—Either (*S*)- or (*R*)-laudanosine (3.57 g, 0.01 mole) was dissolved in ethanol (100 ml), ethyl iodide (1.6 ml, 0.02 mole) was added, and the mixture was refluxed gently for 12 hr. The solution was evaporated, and the residue was recrystallized (six times) from ethanol until its NMR spectrum and TLC showed only the *trans*-product. Final yields were: III, 14%; and IV, 10%.

Quaternization of VI—To a solution of VI (2.17 g, 0.005 mole) in methanol (50 ml) was added methyl iodide or methyl iodide-d_3 (0.01 mole). The mixtures were refluxed gently for 6 hr under nitrogen while protected from light. The solvent was removed by evaporation, and the initial reaction product mixture residues were used for NMR studies.

***cis–trans*-Equilibration Reactions**—A chloroformic solution of *trans*-(±)-*N*-benzyllaudanosinium iodide or of the residue obtained from quaternization of VI was subjected to the conditions specified previously (12, 16), except that reactions were carried out in a stainless steel bomb apparatus. Residues obtained after solvent evaporation were utilized for NMR studies.

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Phenazines with Two Cationic Side Chains as Potential Antimalarials

SHANTI N. SAWHNEY and DAVID W. BOYKIN ˣ

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Abstract □ 1,9-Phenazine-bis(dialkylaminocarboxamides) were prepared for screening as potential antimalarials. No significant activity against *Plasmodium berghei* was observed. The phenazine targets were prepared from 1,9-phenazinedicarboxylic acid by standard methods. The reaction between 1,9-phenazinedicarboxylic acid and thionyl chloride in the presence of dimethylformamide unexpectedly gave 4-chloro-1,9-phenazinedicarbonyl chloride.

Keyphrases □ Phenazines—with two cationic side chains, synthesized and screened as potential antimalarials □ Antimalarials, potential—synthesis and screening of phenazines with two cationic side chains

As part of a research program to develop new antimalarials (1), some selected phenazine derivatives were synthesized because of indications of antimalarial activity for several phenazine types. Clofazimine, which has proven safe for human use against leprosy, has been reported to suppress parasitemia completely in mice infected with *Plasmodium berghei* (2). Modest activity against *P. berghei* in mice has been observed for several simple phenazines, including 7-chloro-2-phenazinenitrile, methylene violet/Bernthsen, and diazine black[1].

While the mode of antimalarial action of these compounds is unknown, the fact that phenazine antibiotics such as myxin and iodimin (3), as well as other phenazines (4, 5), related phenoxazines (6) (actinomycins), and related

[1] Dr. E. A. Steck, Walter Reed Army Institute of Research, Washington, D.C., personal communication.

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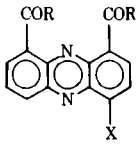


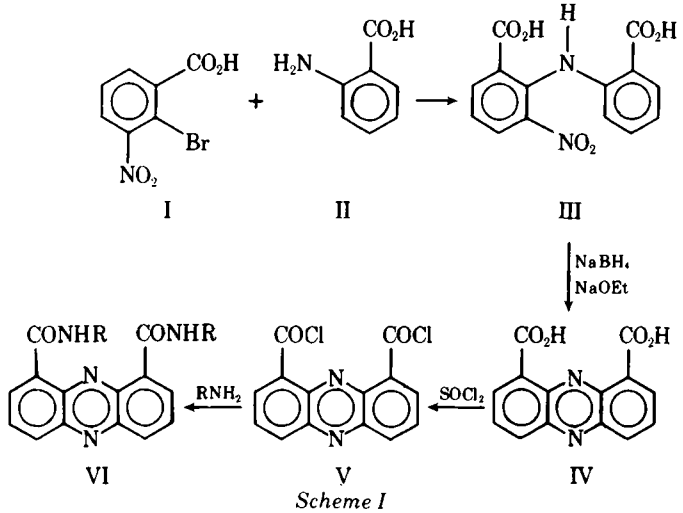
Table I—1,9-Substituted Phenazines

Number	R	X	Melting Point	Yield, %	Recrystallization Solvent	Formula	Analysis, %	Calc.	Found
IX	$CONH(CH_2)_3N(CH_3)_2$	H	179–180°	60	Benzene	$C_{24}H_{32}N_6O_2$	C	66.03	65.88
							H	7.29	7.42
							N	19.25	19.17
X	$CONH(CH_2)_3N(C_2H_5)_2$	H	149–150°	84	Benzene–*n*-hexane	$C_{28}H_{40}N_6O_2$	C	68.26	68.43
							H	8.18	8.25
							N	17.06	17.08
XI	$CONH(CH_2)_3\underset{\displaystyle CH_3}{\underset{\vert}{N}}(CH_2)_2N(CH_3)_2$	H	105–107°	66	Cyclohexane	$C_{30}H_{46}N_8O_2$	C	65.42	65.19
							H	8.42	8.49
							N	20.35	20.29
XII	$CONH(CH_2)_3\underset{\displaystyle C_2H_5}{\underset{\vert}{N}}(CH_2)_2N(C_2H_5)_2$	H	88–90°	87	Cyclohexane	$C_{36}H_{58}N_8O_2$	C	68.10	68.00
							H	9.21	9.22
							N	17.65	17.64
XIII	$CONH(CH_2)_3N(CH_3)_2$	Cl	210–211°	65	Benzene	$C_{24}H_{31}ClN_6O_2$	C	61.20	60.88
							H	6.65	7.05
							Cl	7.53	7.52
							N	17.84	17.69
XIV	$CONH(CH_2)_3\underset{\displaystyle C_2H_5}{\underset{\vert}{N}}(CH_2)_2N(C_2H_5)_2$	Cl	88–90°	65	*n*-Hexane	$C_{36}H_{57}ClN_8O_2$	C	64.60	64.57
							H	8.58	8.61
							N	16.74	16.81

thiaxantenones (6), intercalate with DNA permits the working hypothesis that DNA binding might be involved in their antimalarial action. Recent work (7) on other antimalarials, however, emphasized the need for caution in such speculations. Nevertheless, in the absence of necessary biochemical information, it was decided to make phenazines with cationic side chains at the 1- and 9-positions for evaluation as possible antimalarials. This approach appeared promising in view of the reports (8–10) of the enhancement of DNA binding brought about by attachment of cationic side chains to planar aromatic rings that intercalate with DNA.

RESULTS AND DISCUSSION

The synthesis of the phenazines (VI) was achieved by employing known approaches to the phenazine ring system, followed by side-chain attachment in a conventional manner (Scheme I). The first step was an Ullman-type condensation between 2-bromo-3-nitrobenzoic acid and anthranilic acid to produce 2-nitrodiphenylamine-6,2′-dicarboxylic acid. The reductive cyclization of 2-nitrodiphenylamines by sodium borohydride is a well-established phenazine synthesis (11, 12). Decarboxylation occurred during this reaction since, in addition to IV, 1-phenazinecarboxylic acid was isolated.



Scheme I

The subsequent steps in the synthesis of VI involved classical amide preparation procedures. One aliphatic diamine employed to make the side chains was a new compound, but it was prepared by standard methods and the details are given under *Experimental*.

In the conversion of 1,9-phenazinedicarboxylic acid into its diacid chloride, when dimethylformamide was added as a catalyst, aromatic ring chlorination unexpectedly occurred in addition to conversion into the dicarbonyl chloride. The product of the dimethylformamide–thionyl chloride reaction was 4-chloro-1,9-phenazinedicarbonyl chloride. This structural assignment was based on hydrolysis of the dicarbonyl chloride to 4-chloro-1,9-phenazinedicarboxylic acid. The latter structure was demonstrated by decarboxylation of the dicarboxylic acid to the known 1-chlorophenazine (13). This heteroaromatic ring chlorination reaction by dimethylformamide–thionyl chloride appears to be unreported and is being investigated. Because of the availability of 4-chloro-1,9-phenazinedicarboxylic acid, it was decided to make target compounds from it for evaluation as antimalarials as well.

The targets (IX–XIV) shown in Table I were screened against *P. berghei* by the method of Osdene *et al.* (14). These compounds exhibited only very limited activity. The maximum observed increase in mean survival time of test animals over controls was less than 1 day. Preliminary viscosity studies with IX and XIII indicate that these compounds bind very strongly to DNA, and these results will be reported elsewhere.

EXPERIMENTAL[2]

Melting points under 300° were taken using an oil bath melting-point apparatus; melting points above 300° were obtained using a solid block apparatus. All melting points are uncorrected. Satisfactory IR spectra were recorded on all new compounds. The expected carbon and PMR spectra were recorded on selected compounds in ^{2}H-chloroform and ^{2}H-dimethyl sulfoxide using tetramethylsilane as the standard.

The amines used in side-chain preparation were commercially available (*N,N*-dimethylpropanediamine and *N,N*-diethylpropanediamine), previously reported [*N*-(2-dimethylaminoethyl)-*N*-methyl-1,3-propanediamine] (15), or synthesized as described here.

[2] Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga.

N - (2 - Diethylaminoethyl) - N - ethyl - 1,3 - propanediamine—*N,N,N'*-Triethylethylenediamine (36 g, 0.25 mole) was added gradually to acrylonitrile (26.5 g, 0.4 mole) with stirring at room temperature. The mixture was left at room temperature for 1 hr and then heated under reflux on a steam bath for 22 hr. Acrylonitrile was distilled off at reduced pressure, and the residual liquid was fractionally distilled under vacuum. The fraction of bp 81–83°/0.2 mm was collected in a 45-g yield (90%). The picrate of *N,N,N'*-triethyl-*N'*-(2-cyanoethyl)-ethylenediamine was prepared in alcohol and crystallized from acetone, mp 188–189°.

Anal.—Calc. for dipicrate $C_{23}H_{29}N_9O_{14}$: C, 42.14; H, 4.46; N, 19.23. Found: C, 42.05; H, 4.44; N, 19.16.

A solution of this cyanoamine (19.7 g, 0.1 mole) in dry ether (50 ml) was added slowly, with vigorous stirring, to a suspension of lithium aluminum hydride (4 g) in dry ether (200 ml) and was then cooled in an ice bath. The addition took about 30 min. Stirring was continued for 2 hr with cooling in ice. Water (5 ml), 20% NaOH (5 ml), and water (15 ml) were added successively. The ether layer was decanted, and the residual white solid was washed twice with ether by decantation. The combined ether extracts were dried over potassium hydroxide, and the ether was removed under reduced pressure. The residual liquid was distilled under vacuum, and the fraction of bp 76–77°/0.2 mm was collected in a 14.5-g yield (72%). The picrate was prepared in alcohol and crystallized from methyl ethyl ketone, mp 211–212°.

Anal.—Calc. for tripicrate $C_{29}H_{36}N_{12}O_{21}$: C, 39.19; H, 4.08; N, 18.91. Found: C, 39.14; H, 4.12; N, 18.96.

2-Nitrodiphenylamine-6,2'-dicarboxylic Acid (III)—2-Bromo-3-nitrobenzoic acid (I) (24.6 g, 0.1 mole), anthranilic acid (II) (15.1 g, 0.11 mole), potassium carbonate (50 g), and copper powder (0.5 g) were mixed throughly and added to isoamyl alcohol (27.5 ml). The mixture was heated gradually with stirring until the bath temperature reached 150°, when it melted and the alcohol began to reflux. Heating was continued for 1 hr at 160–170°, and the alcohol was removed by steam distillation. The residue was dissolved in water and filtered. The filtrate was shaken with chloroform, and the aqueous layer was added gradually to stirred 1 *N* HCl (1 liter); stirring was continued for 0.5 hr. The yellow solid was filtered, washed with water, dried, and crystallized from acetic acid in a 16-g yield (53%), mp 283–285° [lit. (12) mp 285–290°].

1,9-Phenazinedicarboxylic Acid (IV)—The diphenylamine (III) (9.1 g, 0.03 mole) was added with stirring to absolute ethanol (250 ml) containing dissolved sodium (8.9 g) and sodium borohydride (6.3 g). The mixture was refluxed, with vigorous stirring, for 16 hr when a greenish-brown suspension resulted. Water (50 ml) was added, and the solid was filtered and washed with a small volume of water. The residue on the filter was dissolved in water (800 ml) by heating, cooled in ice, and acidified with concentrated hydrochloric acid. The precipitated acid was filtered, washed with water, dried, boiled with ethylene glycol monomethyl ether (50 ml), and filtered hot. The residual product was crystallized from dimethylformamide in a 3-g yield (38%), mp 362°.

Anal.—Calc. for $C_{14}H_8N_2O_4$: C, 62.68; H, 3.00; N, 10.44. Found: C, 62.52; H, 3.01; N, 10.42.

After removal of the greenish-brown suspension, the alkaline filtrate was diluted with water. Ethanol was removed by distillation under reduced pressure. On acidification, the aqueous solution gave an acid which, after crystallization from ethylene glycol monomethyl ether, melted at 238–239°. Some of this material was also obtained from the ethylene glycol monomethyl ether filtrate during purification of IV. The total yield was 1.6 g. This compound was found to be 1-carboxyphenazine [lit. (16) mp 239°]. Its mixed melting point with an authentic sample remained undepressed, and the IR spectra of two samples were superimposable.

Phenazine-1,9-dicarboxylic Acid Chloride (V)—Compound IV (3 g) was suspended in dry benzene (60 ml), and thionyl chloride (5 ml) was added. The mixture was refluxed for 1 hr. Thionyl chloride (1 ml) was added, and refluxing was continued for 1 hr more when a clear solution resulted. On cooling, the acid chloride crystallized. It was filtered, washed with a small amount of dry ether, and crystallized from benzene in a 3-g yield (89%), mp 193–194°.

Anal.—Calc. for $C_{14}H_6Cl_2N_2O_2$: C, 55.10; H, 1.98; N, 9.18. Found: C, 55.33; H, 2.02; N, 9.08.

4-Chlorophenazine-1,9-dicarboxylic Acid Chloride (VIII)—To IV (2 g) suspended in dry benzene (10 ml) were added thionyl chloride (2 ml) and dimethylformamide (0.4 g), and the mixture was refluxed gently for 3 hr. The solution was allowed to stand at room temperature, and the acid chloride crystallized. It was filtered, washed with cold benzene, and crystallized from benzene in a 1.3-g yield (50%), mp 188–190°.

Anal.—Calc. for $C_{14}H_5Cl_3N_2O_2$: C, 49.51; H, 1.48; Cl, 31.32; N, 8.25. Found: C, 49.65; H, 1.49; Cl, 31.24; N, 8.23.

4-Chlorophenazine-1,9-dicarboxylic Acid (VII)—The acid chloride (VIII) (1 g) was boiled with water (20 ml) for 3 hr and then allowed to stand overnight. The solid was separated, dissolved in a 5% $NaHCO_3$ solution by heating, and precipitated by acidification with hydrochloric acid. Then it was filtered, washed with water, and crystallized twice from acetic acid in a 0.5-g yield (57%), mp 298°.

Anal.—Calc. for $C_{14}H_7ClN_2O_4$: C, 55.55; H, 2.32; N, 9.25. Found: C, 55.70; H, 2.34; N, 9.22.

Decarboxylation of VII—The acid (VII) (1.5 g), copper powder (0.4 g), and dry redistilled quinoline (10 ml) were heated at 175–185° under nitrogen for 2 hr. After cooling, the mixture was added to an excess of 1 *N* HCl, and a dark-brown solid separated. It was filtered, washed with 1 *N* HCl and water, and dried. This material was vacuum sublimed at 125–130°/3 mm. The sublimate was dissolved in chloroform and chromatographed over alumina. Then it was eluted with benzene, and the yellow band that eluted first was collected. The solvent was removed, and the light-yellow residue was crystallized from methanol in a 0.3-g yield (30%), mp 119–120° [lit. (13) mp 122–123°].

Anal.—Calc. for $C_{14}H_7ClN_2$: C, 67.14; H, 3.29; N, 13.05. Found: C, 67.09; H, 3.30; N, 13.03.

Target Compounds (IX–XIV)—The synthesis of XI is given as a typical example of the synthesis of all target amides. A solution of V (1.3 g, 0.004 mole) in hot dry benzene (100 ml) was added gradually to a stirred solution of *N*-methyl-*N*-(2-dimethylaminoethyl)-1,3-propanediamine (3 g, 0.019 mole) in benzene (30 ml), and the mixture was stirred at room temperature for 1 hr. The mixture was then refluxed on a steam bath for 2 hr.

After cooling, the benzene solution was washed with 10% Na_2CO_3 solution (4 × 25 ml). The aqueous layer was then extracted with hot benzene. The combined benzene extracts were dried (calcium sulfate), the benzene was distilled off, the residual semisolid mass was triturated with hexane, and a greenish-yellow solid was obtained. It was filtered, washed with a small amount of hexane, dried, and crystallized from cyclohexane in a 1.6-g yield (66%), mp 105–107°.

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