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Antitubercular 2,8-Bis(alkylaminomethyl)phenazines

K. C. Murdock, Yang-i Lin, J. P. Thomas, and S. A. Lang, Jr.*

Infectious Disease Therapy Research Section, Lederle Laboratories, a Division of American Cyanamid Company, Pearl River, New York 10965. *Received December 5, 1977*

The preparation and antitubercular properties of a series of 2,8-bis(alkylaminomethyl)phenazines are described. These compounds all inhibited the growth of *Mycobacterium smegmatis* ATCC 607 in vitro. 2,8-Bis(dibutylaminomethyl)phenazine (**5c**) was also active against a lethal *Mycobacterium tuberculosis* H37Rv infection in mice.

Certain acridines and phenazines bearing a pair of basic side chains were earlier found to have antiviral activity associated with an ability to induce interferon formation.[1] Though later syntheses of several 2,8-bis(dialkylaminomethyl)phenazines revealed no additional antiviral activities, 2,8-bis(dibutylaminomethyl)phenazine (**5c**) was found to have antitubercular activity in mice. We now report these syntheses and efforts to extend this antitubercular lead.

Chemistry. A key intermediate, 2,8-bis(bromomethyl)phenazine (**4**), was prepared from di-*p*-tolylamine as shown in Scheme I. 2,2′-Dinitro-*p*-tolylamine[2] (**1**) was separated from an *N*-nitroso coproduct **2**, which sometimes cocrystallized with **1**, by selective flotation of **1** in a CCl_4–hexane mixture. NMR data from the *N*-nitroso compound **2** indicated a steric inhibition of free rotation about the bonds between the amino N atom and the aryl groups.

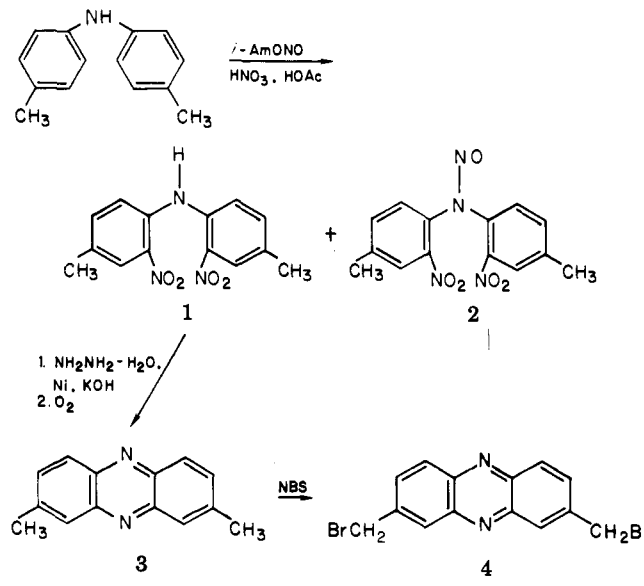
A reductive cyclization method[3] using hydrazine was applied to the dinitro compound **1** to give a synthesis of 2,8-dimethylphenazine (**3**) that is more direct than earlier routes.[2,4] Potassium nitrite and 9,10-dihydro-2,8-dimethylphenazine were coproducts formed in the cyclization. The dihydro derivative was conveniently oxidized to **3** in situ with molecular oxygen.

2,8-Bis(dialkylaminomethyl)phenazines **5** were prepared by the reaction of 2,8-bis(bromomethyl)phenazine (**4**) with the appropriate amine at room temperature, as shown in Scheme II.

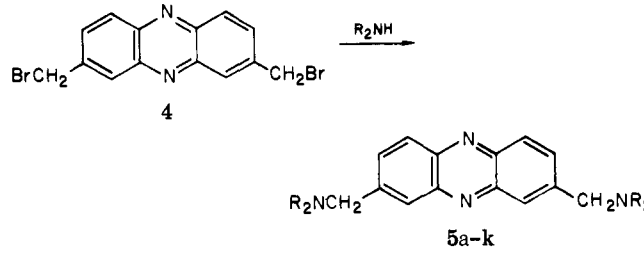
Biology. The compounds were evaluated for activity in vitro against *Mycobacterium smegmatis* ATCC 607. Minimum inhibitory concentrations (MIC) were determined by the agar dilution method[5] in trypticase soy agar (BBL) medium and are shown in Table I.

The 2,8-bis(alkylaminomethyl)phenazines **5** were evaluated for oral activity against lethal *Mycobacterium tuberculosis* H37Rv infections in mice by procedures previously described.[6,7] In brief, each test compound was mixed on a weight basis with powdered mouse food. Drug–diet treatment began with the day of infection and ended 14 days later. A compound which promoted survival in at least two of the five mice in a test group for 30 days in two consecutive tests was accepted as active. In this series of compounds, none was active except **5c**. As judged by survival ratios in Table II, the activity of **5c** was marginal.

Scheme I



Scheme II



Results and Discussion

As shown in Table I, all of the 2,8-bis(disubstituted amino)phenazines inhibited the growth of *M. smegmatis* ATCC 607 in vitro. In mice, however, the only significant protection against a lethal infection with *M. tuberculosis* H37Rv bacilli was shown when the substituent was *n*-butyl (**5c** in Table II). The marginal potencies in this series and the apparently narrow structural range compatible with activity precluded further synthetic interest.

Experimental Section

Melting points were observed on a Mel-Temp apparatus. Solvents were dried over molecular sieves before use. Evaporations

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Table I. In Vitro Antitubercular Activity of 2,8-Bis(alkylaminomethyl)phenazines

Compd	R	MIC,[a] μg/mL
5a	*N*-Piperidinyl	62
5b	$N(C_2H_5)_2 \cdot 2HCl$	5
5c	$N(n\text{-}Bu)_2 \cdot 2HCl$	2.5
5d	3,5-Dimethyl-1-piperazinyl·4HCl	62
5e	4-Benzyl-1-piperazinyl·4HCl	10
5f	4-(2-Pyridyl)-1-piperazinyl·4HCl	125
5g	$N(n\text{-}C_6H_{13})_2$	62
5h	$N(n\text{-}C_7H_{15})_2$	62
5i	$N(n\text{-}C_8H_{17})_2$	62
5j	$N(n\text{-}C_5H_{11})_2$	62
5k	4-Methyl-1-piperazinyl	62
INH[b]		0.05
Ethambutol		0.5

[a] Minimum inhibitory concentration. [b] Isonicotinoyl hydrazide.

Table II. Efficacy of Compound 5c and Isonicotinoyl Hydrazide (INH) against Lethal *M. tuberculosis* H37Rv Infections in Mice[a]

Compd	Drug-diet, mg/kg/day	Alive/total mice at 30-days postinfection		
		Test 1	Test 2	Pool
5c	75	2/5[b]	2/5[b]	4/10
	19	1/5	0/5	1/10
	5	0/5	0/5	0/10
INH[c]	9	5/5	5/5	10/10
	2.5	3/5	0/5	3/10

[a] Infected untreated controls: 39/40 mice died with an average survival time of 16 (9–24) days. [b] Probability = 0.01. [c] Isonicotinoyl hydrazide.

were performed under reduced pressure. Solids were pressed with KBr for IR spectral determinations on a Perkin-Elmer Model 21 spectrophotometer. NMR data were obtained with a Varian Model HA-100 spectrophotometer using Me_4Si as an internal standard. An AEI MS-9 instrument was used to obtain mass spectral data. Confirmatory spectral data were obtained for all new compounds. Purifications were followed by TLC on phosphordoped silica gel or Al_2O_3, observing with a UV lamp at 254 nm.

2,2′-Dinitro-di-*p*-tolylamine[2] **(1) and 2,2′-Dinitro-*N*-nitroso-*p*-tolylamine (2).** A solution of di-*p*-tolylamine (20 g, 0.105 mol) in 40 mL of concentrated nitric acid and 200 mL of acetic acid was maintained at 5–7 °C and stirred during the gradual (5 min) addition of 10 mL of isoamyl nitrite, whereupon a sudden exothermic crystallization occurred. The mixture was rechilled and another 10 mL of isoamyl nitrite added. After another 10 min at 5–10 °C, the orange precipitate was collected and washed with ether to give 25.2 g (87%) of **1**, mp 194–196 °C. Recrystallization (HOAc) returned 21.6 g of orange rods, mp 198–200 °C (lit.[2] mp 197–198 °C).

In a repetition of this experiment a yellow coproduct **2** contaminated the recrystallized product (20.9 g, mp 188–196 °C). These yellow crystals were separated by utilizing their great density in a "sink-or-float" procedure in 400 mL of CCl_4 and 90.4 mL of hexane in a separatory funnel. Recrystallization (CH_3NO_2) afforded 1.27 g of yellow prisms of **2**, mp 157–167 °C dec. In TLC on silica gel with C_6H_6–CCl_4 (1:1), the R_f of **2** was lower than that of **1**. The NMR of **2** showed a nonequivalence of the 6 and 6′ H atoms (δ 7.58 and 7.35 in DMF-d_7, both as doublets with J = 0.13 Hz) inferring restricted rotation about the *N*-aryl bonds. Anal. ($C_{14}H_{12}N_4O_5$) C, H, N.

2,8-Dimethylphenazine (3). To a solution of KOH (7.5 g, 0.13 mol) in 375 mL of absolute ethanol in a 3-L flask was added 2,2′-dinitro-di-*p*-tolylamine (25.0 g, 0.087 mol), 99–100% hydrazine hydrate (12.5 mL, 0.25 mol), and Raney nickel (0.625 g of an aqueous sludge, W. R. Grace and Co., No. 28). The mixture was heated on a steam bath until the orange dinitro compound had dissolved (5 min) and the vigorous effervesence had subsided. An insoluble dihydrophenazine coproduct (**3a**) was aromatized by bubbling in a vigorous stream of oxygen for 30 min while refluxing was continued. (Oxidation using air gave unsatisfactory results.) The hot mixture was filtered and the black nickel (0.44 g) washed once with hot ethanol. There was a greater weight of insoluble material at this point when the oxidation period was either too short (5 min) or too long (90 min). The filtrate was concentrated to 75 mL and the product allowed to crystallize, finally at 0 °C. The solids were collected and washed with ice-cold ethanol and then water (to remove potassium nitrite), leaving 8.4 g of a dark brown solid, mp 154–157 °C.

Sublimation [170–175 °C (0.05 mm)] yielded 7.8 g (43%) of orange crystals, mp 156–158 °C (lit.[2,4] mp 155 and 156 °C). Anal. ($C_{14}H_{12}N_2$) C, H, N.

In one run the hot, concentrated ethanol filtrate deposited an 8% yield of potassium nitrite (IR ≡ an authentic sample) before **3** began to crystallize.

9,10-Dihydro-2,8-dimethylphenazine (3a). A prior synthesis of 2,8-dimethylphenazine (**3**) without the oxygen treatment gave **3** in only 26% yield, along with a 25% yield of a dihydro derivative **3a** which remained after **3** was removed by washing with CH_2Cl_2. Recrystallization from CH_3CN gave leaflets which melted at 343–345 °C in a sealed tube evacuated to 0.1 mm but otherwise melted at ca. 170–195 °C: IR 2.98 μm (s, –NH); MS *m/e* 210 (M^+). Anal. ($C_{14}H_{14}N_2$) C, H, N.

When the dihydro compound was crystallized from DMF, the resulting crystals were initially black, suggesting a quinhydrone-type intermediate, but after standing 3 days with the mother liquor they became yellow. The mixture was chilled at 0 °C before the crystals were collected: mp 158–159 °C; the IR of these crystals was identical with that of **3**.

2,8-Bis(bromomethyl)phenazine (4). To a 3-L three-necked flask fitted with a mechanical stirrer and containing a solution of dimethylphenazine (**3**, 8.3 g, 0.04 mol) in carbon tetrachloride (600 mL) was added NBS (14.2 g, 0.08 mol). The suspension was irradiated from below with a 500-W Photoflood lamp, causing the solution to reflux vigorously. (An aluminum foil skirt was hung down from the flask to conserve heat and light.) The stirrer was turned slowly enough not to create a deep vortex, i.e., so that the length of the light path through the solution was nearly the same at the edges and the center. At 1.5 h a KI–starch test on an aliquot was negative. Heating was discontinued and the mixture allowed to stand at room temperature overnight. The solids were collected and washed with CCl_4. Succinimide was removed by slurrying with three portions of ice-cold 1 N NaOH solution. The product was then washed with ice-cold water to give 8.76 g (60%) of crystals which sintered and decomposed above 155 °C. The absence of an IR peak at 5.89 μm was the criterion for complete removal of succinimide. Anal. ($C_{14}H_{10}N_2Br_2$) C, H, N, Br.

2,8-Bis(piperidinomethyl)phenazine (5a). A suspension of 1.9 g (0.005 mol) of **4** in 15 mL of dry ether and 8 mL of piperidine was stirred at room temperature overnight. The solid was collected and washed with ether and then with concentrated NH_4OH, leaving 0.89 g of product, mp 148–149 °C. Solvent removal from the ethereal filtrate (finally at 0.1 mm) left a thick residue which crystallized from petroleum ether as pale yellow needles, mp 146–147 °C (0.87 g). The total yield was 91%. Anal. ($C_{24}H_{30}N_4$) C, H, N.

2,8-Bis(diethylaminomethyl)phenazine Dihydrochloride (5b). A suspension of **4** (1.3 g, 3.55 mmol) in Et_2NH (20 mL) was stirred for 2 h. The Et_2NH·HBr was removed, the filtrate evaporated, and the residual syrup dissolved in petroleum ether, filtered, and evaporated (finally at 0.05 mm). To a solution of the residue in 2 mL of ethanol was added 1 mL of 7.5 N ethanolic HCl. The resulting crystals were collected and washed with acetone. Recrystallization from EtOH–acetone returned 1.06 g of product, mp 233–234 °C dec. Anal. ($C_{22}H_{30}N_4 \cdot 2HCl \cdot 0.25H_2O$) C, H, N, Cl, H_2O.

2,8-Bis(dibutylaminomethyl)phenazine Dihydrochloride (5c). A mixture of 3.66 g of **4** and 10.3 g of Bu_2NH in 50 mL of dry CH_3CN was stirred at 24 °C for 5 days and then evaporated. A solution of the residue in 100 mL of $CHCl_3$ was washed with 50 mL of 1 N NaOH and twice with H_2O, then dried ($MgSO_4$), and evaporated. To a solution of the residual gum in 750 mL of

dry ether was added 6 mL of 3.75 N HCl in 2-propanol. The resulting yellow crystals (4.1 g, mp 170 °C) were dissolved in CH_2Cl_2 (25 mL) and filtered through a column containing 10 g of silica gel, eluting with more CH_2Cl_2. Evaporation of the eluates and extraction of the residue with acetone gave 1.60 g of yellow crystals, mp 190–195 °C. Anal. ($C_{30}H_{46}N_4$·HCl) C, H, N, Cl.

2,8-Bis[(3,5-dimethyl-4-piperazinyl)methyl]phenazine Tetrahydrochloride (5d). A suspension of 4 (7.32 g, 0.020 mol) in a mixture of 2,6-dimethylpiperazine (4.56 g, 0.40 mol), triethylamine (25 mL), and dichloromethane (50 mL) was stirred at room temperature for 5 days. The solution was diluted with 500 mL of hexane. The precipitate was removed by filtration, and the brown oil obtained by evaporation was chromatographed on silica gel. Elution with chloroform–hexane (6:1) gave a homogeneous brown oil which was dissolved in acetone (50 mL) and treated with 15 mL of a saturated solution of HCl in 2-propanol. The light yellow precipitate was collected: yield 7.2 g (50%); mp 300 °C dec. Anal. ($C_{26}H_{36}N_6$·4HCl) C, H, N, Cl.

2,8-Bis(4-benzyl-1-piperazinylmethyl)phenazine Tetrahydrochloride (5e). A suspension of 4 (7.32 g, 0.020 mol) in a mixture of *N*-benzylpiperazine (7.04 g, 0.040 mol), triethylamine (25 mL), and dichloromethane (50 mL) was treated as outlined for **5d** to afford 3.9 g (26%) of **5e** as a white solid, mp >300 °C dec. Anal. ($C_{36}H_{40}N_6$·HCl) C, H, N, Cl.

2,8-Bis[[4-(2-pyridyl)-1-piperazinyl]methyl]phenazine Tetrahydrochloride (5f). A suspension of 4 (7.32 g, 0.020 mol) in a mixture of *N*-(2-pyridyl)piperazine (6.64 g, 0.04 mol), triethylamine (25 mL), and dichloromethane (50 mL) was treated as described for **5d** to afford 2.5 g (17%) of **5f** as a light tan solid, mp >250 °C dec. Anal. ($C_{32}H_{34}N_8$·4HCl) C, H, N, Cl.

2,8-Bis(dihexylaminomethyl)phenazine (5g). A suspension of 4 (7.32 g, 0.020 mol) in a mixture of di-*n*-hexylamine (7.4 g, 0.040 mol), triethylamine (25 mL), and dichloromethane (25 mL) was stirred at room temperature for 7 days and then poured into 600 mL of hexane. The precipitate was removed by filtration. After removal of the volatile materials under reduced pressure, the brown oil was chromatographed on a column of silica gel (100 g). Elution with chloroform afforded 4.7 g (41%) of **5g** as an oil. Anal. ($C_{38}H_{62}N_4$) C, H, N.

2,8-Bis(diheptylaminomethyl)phenazine (5h). This was prepared from 4 using the procedure described for the preparation of **5g**: yield, 6.6 g (52%) of **5h** as an oil. Anal. ($C_{42}H_{70}N_4$) C, H, N.

2,8-Bis(dioctylaminomethyl)phenazine (5i). This was prepared from 4 using the procedure described for the preparation of **5g**: yield, 7.3 g (53%) of **5i** as an oil. Anal. ($C_{46}H_{78}N_4$) H, N; C: calcd, 80.4; found, 79.4.

2,8-Bis(dipentylaminomethyl)phenazine (5j). A suspension of 4 (7.32 g, 0.020 mol) in a mixture of di-*n*-pentylamine (20 g, 0.127 mol) and dichloromethane (50 mL) was stirred at room temperature for 6 days and then poured into 600 mL of hexane. The precipitate was removed by filtration. After removal of the volatile materials under reduced pressure, the brown oil was chromatographed on a column of Woelm silica gel (100 g). The procedure described for **5g** afforded 9.8 g (94%) of **5j** as an oil. Anal. ($C_{34}H_{54}N_4$) C, H, N.

2,8-Bis(4-methyl-1-piperazinylmethyl)phenazine (5k). A solution of 10.02 g (0.1 mol) of 1-methylpiperazine in 60 mL of CH_2Cl_2 was stirred and chilled with an ice bath during the addition of 5.49 g (0.015 mol) of 4 and for another 10 min, when all of the solid had dissolved. After 2 h at 22 °C the solution was stirred vigorously with 7 mL of 10 N NaOH. The CH_2Cl_2–H_2O mixture was dried ($MgSO_4$), filtered, and then evaporated, finally at 60 °C (0.02 mm). Recrystallization of the residual solid from *n*-heptane gave 3.66 g of golden crystals, mp 140–142 °C, which were very soluble in H_2O. Anal. ($C_{24}H_{32}N_6$) C, H, N.

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2-(Alkoxyaryl)-2-imidazoline Monoamine Oxidase Inhibitors with Antidepressant Activity

Morton Harfenist,* Francis E. Soroko, and Gerald M. McKenzie[1]

The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709. *Received August 24, 1976*

Unlike the related noncyclic amidines which are broad-spectrum cestocides, a number of 2-imidazolines substituted in the 2 position by alkoxyaryl groups were not highly active in screening tests against the mouse tapeworms *Hymenolepis nana* and *Oochoristica symmetrica*. Certain of the 2-(4-alkoxynaphthyl)-2-imidazolines and 2-(6-alkoxy-2-naphthyl)-2-imidzolines, however, had activity interpreted as antidepressant in the mouse. This activity paralleled in vitro irreversible inhibitory activity against mouse brain MAO for those where no substitution is present on the imidazoline ring. This irreversibility probably has a different origin from that postulated to explain the irreversible MAO inhibition of properglic, cyclopropyl, and other "chemically reactive" MAO inhibitors.

Some time ago, one of us reported[2] that certain 4-alkoxy-*N*,*N*-dialkyl-1-naphthamidines had activity against the mouse pinworms *Syphacia obvelata* and *Aspiculuris tetraptera*. More recently our laboratories have reported the activity of one of these, *N*,*N*-dibutyl-4-hexyloxy-1-naphthamidine (generic name bunamidine), which is widely used as a broad-spectrum cestocidal compound especially useful because it is effective against *Echinococcus granulosus*.[3] Since the standard synthetic methods[4,5] generally used in amidine synthesis gave vanishingly small yields of such amidines, these had to be made either by heating an amine metal salt with the nitrile[6a] or by reaction of the amine and nitrile in the presence of aluminum chloride.[6b] Both of these methods

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