solution and dried ($MgSO_4$), and the solvent was removed. The crystalline solid **4** which was obtained was washed with Et_2O and dried: 1.91 g (95%); mp 218 °C; $[\alpha]^{20}_D$ +166.9° (*c* 1.03, 10% HOAc) [authentic sample of enantiomer of **4**, mp 218 °C; $[\alpha]^{20}_D$ −160.9° (*c* 1.09, 10% HOAc)]. Anal. ($C_{18}H_{21}NO_4 \cdot 0.5H_2O$) C, H, N.

(+)-14-**Hydroxydihydromorphinone** (**5**). A solution of **4** (1.70 g, 5.4 mmol) in $CHCl_3$ (15 mL) was added, dropwise, to a stirred solution of BBr_3 (8.0 g, 32 mmol) in $CHCl_3$ (15 mL) at 10 °C over 10 min. The mixture was stirred for 50 min at 10–20 °C and poured into ice–H_2O (40 mL). The solution was made basic with NH_4OH, saturated with NaCl, extracted with $CHCl_3$ (8 × 50 mL), washed with saturated NaCl solution, and dried ($MgSO_4$). Removal of solvent gave solid **5**, which was recrystallized from EtOH: 1.23 g (76%); mp 250 °C dec; $[\alpha]^{20}_D$ +197.5° (*c* 0.87, EtOH) (lit.[12] for enantiomer of **5**, mp 244–246 °C). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

(+)-3,14-**Diacetoxydihydromorphinone** (**6**). A mixture of **5** (1.50 g, 4.98 mmol) in acetic anhydride (15 mL) was heated at 100 °C for 1 h and evaporated under reduced pressure. Water was added to the residue and it was basified (pH 9) with concentrated NH_4OH. The precipitate was filtered, washed with cold H_2O, and dried to give **6**: 1.74 g (90%); mp 218–219 °C; $[\alpha]^{20}_D$ +201.6° (*c* 1.31, $CHCl_3$) (lit.[12] for enantiomer of **6**, mp 214–215 °C). Anal. ($C_{21}H_{23}NO_6$) C, H, N.

(+)-*N*-**Cyano-3,14-diacetoxydihydronormorphinone** (**7**). A mixture of **6** (1.60 g, 4.15 mmol) and cyanogen bromide (1.00 g, 9.44 mmol) in $CHCl_3$ (100 mL) was refluxed for 8 h. Two additional amounts of cyanogen bromide were added; the first addition (1.50 g, 14.16 mmol) was followed by a 15-h refluxing period, and the second addition (0.5 g, 4.72 mmol) was followed by refluxing for another 9 h. The solution was washed with 10% HCl and H_2O and dried ($MgSO_4$). Removal of solvent gave **7**, which was recrystallized from EtOH–$CHCl_3$: 1.5 g (91%); mp 240–241 °C; $[\alpha]^{20}_D$ +220.5° (*c* 0.74, $CHCl_3$) (lit.[12] for enantiomer of **7**, mp 230–233 °C). Anal. ($C_{21}H_{20}N_2O_6$) C, H, N.

(+)-14-**Hydroxydihydronormorphinone** (**8**). A mixture of **7** (1.66 g, 4.18 mmol) in 25% H_2SO_4 (18 mL) was refluxed for 4.5 h under N_2. After cooling, the solution was made basic with NH_4OH, and the precipitated solid **8** was filtered, washed with H_2O, EtOH, and Et_2O, and dried: 1.15 g (95%); mp 290 °C; $[\alpha]^{20}_D$ +149.8° (*c* 1.02, 10% HOAc), (lit.[12] for enantiomer of **8**, mp 310–313 °C). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

(+)-**Naloxone** (**9**). A mixture of **8** (1.13 g, 3.9 mmol), allyl bromide (520 mg, 4.3 mmol), and K_2CO_3 (594 mg, 4.3 mmol) in DMF (15 mL) was heated at 90–95 °C (bath temperature) for 3 h with stirring under N_2. The cooled mixture was diluted with H_2O, saturated with NaCl, extracted with $CHCl_3$, washed with saturated NaCl solution, and dried ($MgSO_4$). After solvent removal, the residue was purified by silica gel TLC using Et_2O–hexane (85:15) as a solvent to give **9** (970 mg, 76%), which was recrystallized from ethyl acetate as colorless prisms: mp 178–179 °C; $[\alpha]^{20}_D$ +197.4° (*c* 1.05, $CHCl_3$) [lit.[13] (for enantiomer of **9**) mp 184 and 177–178 °C; $[\alpha]^{20}_D$ −194.5° (*c* 0.93, $CHCl_3$)[7]. (+)-Naloxone (**9**) was chromatographically and spectroscopically indistinguishable from an authentic sample of the (−) enantiomer except for its opposite optical rotation. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

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Spiro[isobenzofuran-1(3*H*),4′-piperidines]. 3. Diuretic and Antihypertensive Properties of Compounds Containing a Sulfur Attached to Nitrogen

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The synthesis and antihypertensive and diuretic activity of several *N*-sulfur derivatives of 3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidine] are reported. Benzenesulfenamide **3** possessed marked, species-specific diuretic and antihypertensive activity in rats.

We recently reported the synthesis and antitetrabenazine activity of a series of 3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidines] **1**[1] and their corresponding derivatives **2** with an additional heteroatom attached directly to the piperidine nitrogen.[2] In both series potent antitetrabenazine activity was shown to be associated with

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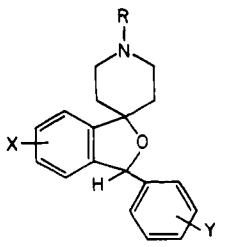
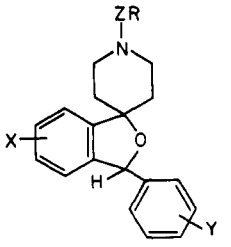


1

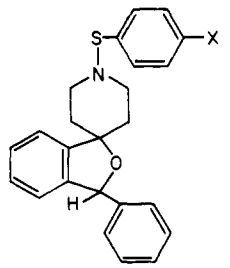
1a, R = X = Y = H



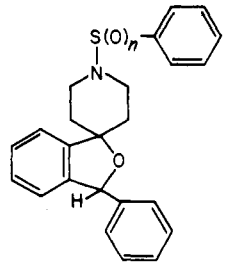
2, Z = O, NH

those compounds possessing a sterically unhindered, basic nitrogen.

During the course of these investigations, it was found, however, that when the size of the piperidine substituent was increased beyond lower alkyl [e.g., 1, R = CH_2CH_2-C_6H_5, $(CH_2)_3C(=O)$-*p*-FC_6H_4], the resultant compounds possessed noteworthy CNS depressant activity.[3] We, therefore, decided to explore whether the introduction of a larger heteroatom such as sulfur interposed between the nitrogen and a phenyl ring would furnish compounds retaining CNS depressant properties. Consequently, sulfenamides **3**–**5**, sulfinamide **6**, and sulfonamide **7** were



3, X = H
4, X = NO_2
5, X = OCH_3



6, *n* = 1
7, *n* = 2

prepared. In this paper the synthesis and biological activity of these *N*-sulfur derivatives are described.

Chemistry. The *N*-sulfur derivatives of 3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidine] were prepared by reacting secondary amine **1a** with the appropriate arylsulfenyl, -sulfinyl, or -sulfonyl chloride in dichloromethane in the presence of triethylamine. The arylsulfenyl chlorides were in turn synthesized by treating the corresponding thiophenol with chlorine gas in carbon tetrachloride.[4] Benzenesulfinyl chloride was prepared by the action of thionyl chloride on benzenesulfinic acid sodium salt.[5]

Pharmacology. As very little was known concerning the pharmacophoric properties of the arylsulfenamide and arylsulfinamide linkages, compounds **3**–**7** were submitted to a wide variety of pharmacological tests to evaluate their biological activity. CNS depressant activity was evaluated in the Irwin profile.[6] All of the *N*-sulfur derivatives **3**–**7** were only marginally active in this assay at the screening dose of 30 mg/kg (ip). It seems that although these compounds probably possess the steric requirements for CNS depressant activity, the absence of a strongly basic nitrogen makes it difficult for them to bind properly into the appropriate receptor sites. Antidepressant activity was evaluated in the tetrabenazine assay.[7] None of the compounds in this series exhibited any appreciable antitetrabenazine activity at the normal screening dose of 25 mg/kg (ip). This result is, of course, not surprising in view of the previously mentioned criteria for optimum antitetrabenazine activity. However, when **3**–**7** were examined for antihypertensive and diuretic properties, some very interesting activity was uncovered.

Antihypertensive activity was evaluated using spontaneous hypertensive rats (SHR). The data summarized in Table I indicate that the unsubstituted benzenesulfenamide **3** exhibited a marked hypotensive response. In contrast, both the *p*-nitrobenzenesulfenamide **4** and the *p*-methoxybenzenesulfenamide **5** possessed diminished antihypertensive activity. Likewise, sulfinamide **6** and sulfonamide **7**, while retaining some hypotensive activity, were not nearly as potent as **3**.

Table I. Indirect Hypotensive Screen (Spontaneous Hypertensive Rat)

Compd	Dose, mg/kg po	Drop of systolic pressure, mmHg on day 3, 2 h postdrug
Guanethidine	10	34
3	50	66
3	25	44
4	25	9[a]
5	50	14[a]
6	100	22
7	50	13[a]

[a] Generally a drop in systolic pressure of greater than 15 mmHg is considered significant. These values are, therefore, probably not statistically significant.

Table II. Diuretic Activity: Acute Sodium Loaded Rat

Compd	Dose, mg/kg po	Vol	Ratio of drug/urea, 0–5 h[b] Na^+	K^+	Cl^- [a]
Chlorothiazide	50	1.6	2.2	1.1	ND
3	50	1.71	1.83	0.97	1.85
	25	2.2	2.5	1.2	ND
	10	1.49	1.69	0.94	1.62
	5	1.20	1.31	0.86	1.43
	2	1.00	1.20	0.74	1.19
4	50	1.73	2.41	1.2	2.30
	25	0.59	0.76	0.58	0.75
5	50	1.47	1.78	0.80	1.47
	25	1.4	0.97	0.84	ND
6	50	1.74	2.20	1.00	ND
	25	0.81	1.22	0.68	1.18
7	50	0.63	0.70	0.57	0.79

[a] ND = not determined. [b] A drug to urea ratio greater than or equal to one for diuresis and/or sodium is indicative of significant diuretic activity.

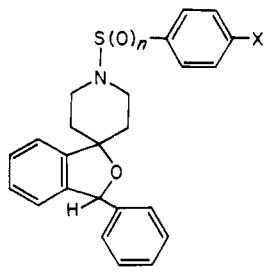
Diuretic activity was evaluated in acute sodium loaded rats (Table II). It is interesting that diuresis diminished in going from sulfenamide **3** to sulfinamide **6** and then virtually disappeared in proceeding to sulfonamide **7**. The degree of oxidation of the sulfur attached to the piperidine ring thus seems to have a profound effect on diuretic activity. Within the group of sulfenamides **3**–**5**, it is apparent that aryl substitution with both a powerful elecron-withdrawing substituent (**4**) and a powerful electron-donating substituent (**5**) served to decrease diuretic potency.

As benzenesulfenamide **3** showed both potent hypotensive and diuretic properties in rats, follow-up investigations in other models and species were performed. Compound **3** produced a marked hypotensive response at both 50 and 25 mg/kg (po) in the DOCA rat.[8] However, when it was examined in the acute anesthetized dog (10 mg/kg iv), no antihypertensive activity was observed. Likewise, the potent diuretic activity of **3** in rats could not be corroborated by studies in either dogs (1, 2, 10, 20, and 50 mg/kg po and 20 mg/kg iv) or rhesus monkeys (10 and 20 mg/kg po).

Experimental Section

The structures of all compounds are supported by their IR

Table III[a]



Compd	X	n	Mp,[b] °C	Yield, %[c]	Formula	Analyses[d]
3	H	0	130-133	80.8	$C_{24}H_{23}NOS$	C, H, N, S
4	NO_2	0	168-170	19.4[e]	$C_{24}H_{22}N_2O_3S$	C, H, N, S
5	OCH_3	0	140-143	26.1	$C_{25}H_{25}NO_2S$	C, H, N, S
6	H	1	167-171	78.3	$C_{24}H_{23}NO_2S$	C, H, N, S
7	H	2	204-206	64.9[f]	$C_{24}H_{23}NO_3S$	C, H, N, S

[a] All compounds exhibited IR and ^1H NMR spectra consistent with the assigned structures. [b] Melting points are uncorrected. [c] Yield of analytically pure material recrystallized from ethanol; yields were not optimized. [d] Analytical results within ±0.4% of theoretical values. [e] Yield of material chromatographed on silica gel using $CHCl_3$ as eluent and fractionally crystallized from ethanol. [f] Yield of material boiled with ethanol, cooled, and filtered.

(Perkin-Elmer 457) and ^1H NMR (JEOL C6OHL) (in $CDCl_3$ relative to an internal Me_4Si standard). Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Labs, Skokie, Ill. Results are within ±0.4% of theoretical values.

The following reagents were prepared according to the cited literature references: benzenesulfenyl chlorides[4] and benzenesulfinyl chloride.[5] All of these materials were utilized in crude form.

1′-Benzenesulfenyl-3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidine] (3). To a solution of 31.85 g (0.12 mol) of **1a** in 600 mL of dichloromethane containing 14.57 g (0.144 mol) of triethylamine was added dropwise with stirring under nitrogen a solution of 19.09 g (0.132 mol) of benzenesulfenyl chloride in 240 mL of dichloromethane. The reaction mixture was stirred for 3 h at room temperature, diluted with 1 L of dichloromethane, and washed with water and 1 N NaOH. The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. Trituration of the residual solid with ether–petroleum ether gave 41.37 g (92.3%) of nearly colorless crystals. Recrystallization from ethanol afforded 36.21 g (80.8%) of **3** as fine colorless plates, mp 130–133 °C. The properties of compounds **4–7**, prepared in an analogous manner, are included in Table III.

SHR Test for Antihypertensive Activity. Compounds were screened for antihypertensive activity using genetically spontaneous hypertensive rats (SHR) by a standard indirect tail-cuff method.[9] Two rats were placed into a wire basket in an incubator set to 40 °C. Each cage of rats was left to condition in the incubator for 20 min; then the rats were removed and placed in individual trigonal or trapezoidal cages made of Lucite. A tubular inflatable cuff was placed around the base of the tail. A microphone (Narco Bio-Systems or Biodynamics) was placed under the ventral surface of the tail and the tail was strapped down. When the microphone was properly located, the pulse could be detected and was amplified by a universal type amplifier. Each microphone was connected to an individual channel on a recorder, and the pulse was recorded. The cuff was inflated to approximately 300 mmHg. The pulse was thereby obliterated. The pressure in the cuff was slowly released and, as the pressure fell below the systolic pressure, the pulse could again be detected by the microphone. The blood pressure of each rat was determined three to five times from which a mean value was derived. Before any animal was included in the screening program, a 2-week training period was employed to acclimate the animal to the test environment. Also, this procedure permitted the establishment of baseline blood pressures for each animal. Any animal whose blood pressure was less than 175 mmHg was withdrawn from the test group.

In a standard 3-day test, systolic blood pressure readings were made at 0 time (control) on days 1 and 3 and at 2 h after administration of the compound on day 3. Dosing was orally at the dose listed in Table I at 0 h on days 1, 2, and 3 on groups of six animals per test. Activity was determined by comparison of the treatment blood pressure values with the 0 time (control) blood pressure readings. Comparisons were made using the paired *t* test method for evaluation of statistical significance.[10]

Acute Sodium Loaded Rat. A Diuretic Screen.[11] Groups of female Wistar rats (150–200 g) were used, and they were food deprived 16 h prior to testing. Drugs were prepared in 1% saline and administered in a dosage volume of 15 mL/kg orally. After dosing each animal was placed in an individual metabolic cage. Water was permitted ad libitum. Urine was collected from 0 to 5 h after dosing.

Each test consisted of a vehicle control, a positive control group of urea treated (1000 mg/kg), and the potential diuretic agent (50 mg/kg).

The individual urine samples were analyzed for sodium, potassium, and chloride. Sodium and potassium values were determined using a flame photometer (IL Model 343). Chloride determinations were made by a chloride analyzer (IL Model 279).

The mean values obtained for sodium, potassium, chloride, and diuresis are expressed in a ratio to the sodium, potassium, chloride, and diuresis values obtained for the urea treated group. This ratio is called the "drug to urea ratio". A drug to urea ratio greater than or equal to 1 for diuresis and/or sodium is indicative of diuretic activity. If a compound was active at 50 mg/kg orally (0–5 h), it was tested at a lower dose to confirm activity and to indicate relative potency.

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Antitubercular 2,8-Bis(alkylaminomethyl)phenazines

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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.

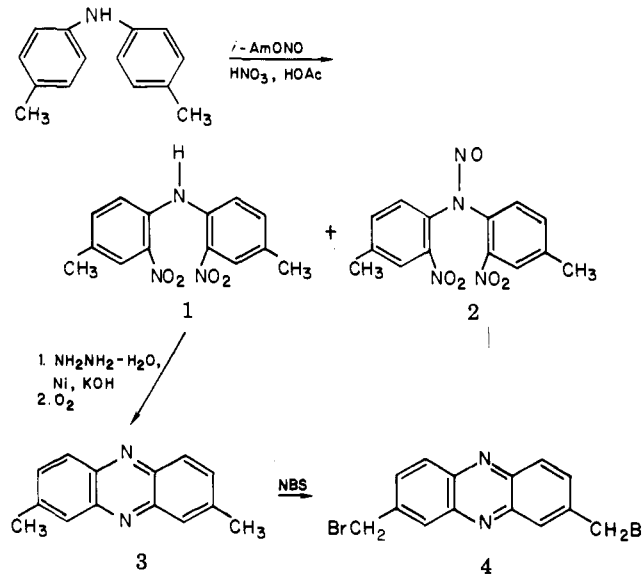
A reductive cyclization method[3] using hydrazine was applied to the dinitro compound **1** to give a synthesis of 2,8-dimethylphenazine (**3**) that is more direct than earlier routes.[2,4] Potassium nitrite and 9,10-dihydro-2,8-dimethylphenazine were coproducts formed in the cyclization. The dihydro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

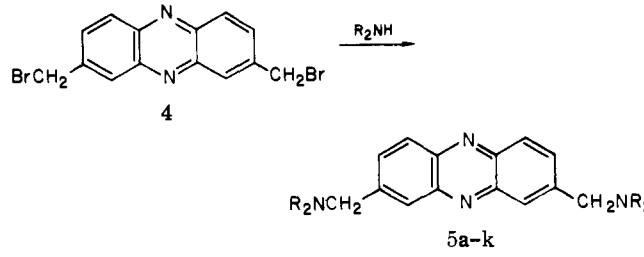
Biology. The compounds were evaluated for activity in vitro against *Mycobacterium smegmatis* ATCC 607. Minimum inhibitory concentrations (MIC) were determined by the agar dilution method[5] in trypticase soy agar (BBL) medium and are shown in Table I.

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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