3100–3200 (NH), 1640 (CO) cm^{-1}. EI MS: m/e 290 (M^+). Anal. ($C_{17}H_{14}N_4O$) C, H, N.

Similarly, compounds **7**, **12**, and **13** were synthesized by reacting **4** and **9** with formic and glacial acetic acids, respectively (Table III).

Anthelmintic Test Methods. Antihookworm Screening. Hamsters of either sex (40–60 g) were infected orally with 60 (third stage) larvae of *A. ceylanicum*. After 17–20 days of inoculation, the animals were checked for infection by ovoscopic examination, and those found positive for infection were used for screening the test compounds with standard drugs as control. In each experiment, three to six animals were used per dose schedule and three were kept as controls in this screening and also in antioxyurid and antitapeworm screenings. The animals were starved overnight prior to administration of the compounds. The compounds, insoluble in water, were given orally as a suspension in Tween 80 at an initial dose of 250 mg/kg × 3 days. All the treated and control animals were starved overnight again and then sacrificed on day 3 posttreatment. The total number of worms present in the intestine of hamsters was counted on autopsy and the percent deparasitization was calculated by the formula $[(N - n)/N]$ 100, where N and n are the number of worms in control and treated animals, respectively.

Antioxyurid Screening. Swiss male albino mice (20–25 g) naturally infected with *Syphacia obvelata* were used for antioxyurid drug testing. Food was withheld for 5–6 h before the animals were fed with the test compounds. On the third day of drug treatment, animals were again starved for 5–6 h and then sacrificed to ascertain the number of worms in the caecum. Total clearance of the worms was the criterion of efficacy.

Antitapeworm Screening. Newly weaned University of Freiburg strain of male albino rats (25–30 g) or Swiss male mice (18–20 g) were infected by feeding orally 200 mature viable ova of *H. nana*. On the 17th day of infection, feces of all the animals were examined, and those showing *H. nana* eggs were used. Starved animals were treated with the compounds at an initial dose of 250 mg/kg. Treated animals were sacrificed in the same way as mentioned in the antioxyurid screening technique. The intestine of each animal was individually examined for the worms and scolices under a dissecting microscope. Because of the large variation in the number of adult worms recovered by feeding 200 viable eggs, total clearance of worms along with their scolices was taken as the criterion for denoting antitapeworm activity at a particular dose in each animal.

Antifilarial Screening. The micro- and macrofilaricidal activities of compounds were evaluated against *L. carinii* infection in cotton rats (*Sigmodon hispidus*) and *D. viteae* and *B. malayi* infections in *Mastomys natalensis*. *L. carinii* was transmitted to cotton rats through the vector *Liponyssus bacoti* by the method of Hawking and Sewell.[18] The *D. viteae* and *B. malayi* infections were transmitted to *Mastomys* through their respective vectors *Ornithodoros mobata* and *Aedes aegypti* by the methods of Worms et al.[19] and Murthy et al.,[20] respectively. At the end of prepatent period, animals showing 250 or more microfilariae per 5 μL of blood were chosen for screening. Five animals formed an experimental group. Blood samples of experimental and control animals were examined before starting the treatment. The compounds were suspended in water in the presence of Tween 80 and administered intraperitoneally, subcutaneously, or orally for 5 consecutive days. Blood smears of animals infected with *L. carinii* or *D. viteae* were examined for microfilariae at weekly intervals up to 6 weeks from the start of the treatment. On day 42, all the treated and control animals were sacrificed and the condition of adult male and female worms observed. The micro- and macrofilaricidal action of the compounds were assessed as described by Laemmler et al.[21]

For monitoring of the microfilaricidal activity, blood samples of the rodents infected with *L. carinii* or *D. viteae* were examined at weekly intervals up to day 91. In the case of *B. malayi* infection, blood was examined at fortnightly intervals up to day 91. To demonstrate the correct trend in the course of microfilariaemia in the treated animals, the microfilarial counts on days 8, 21, 42, 63, and 91 are incorporated in Table II.

Data on control animals, which were generally vehicle treated, are also given in Table II. The general dose schedule for both mebendazole and compound **5** were 200, 100, 50, 25, 12.5, 6.25, and 3.12 mg/kg of body weight. Only those doses of **5** and mebendazole showing maximum efficacy are included in Table II.

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Synthesis and Evaluation of 2-Substituted 1-Methyl-1-(4-tolylsulfonyl)hydrazines as Antineoplastic Agents

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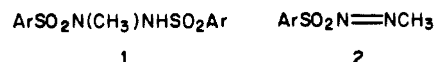
Several N-2 substituted 1-methyl-1-(4-tolylsulfonyl)hydrazines were synthesized and evaluated for antineoplastic activity against the L1210 leukemia and the B16 melanoma. The most active compound to emerge from this study, 2-(methylsulfonyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine, produced maximum percent T/C values with L1210 leukemia and B16 melanoma tumor bearing mice of 207 and 209, respectively. While the attachment of an aryl-, aralkyl-, or alkylsulfonyl moiety to N-2 resulted in retention of activity against both tumor systems, the corresponding benzoyl, 4-nitrobenzoyl, and (2-nitrophenyl)sulfenyl analogues only displayed activity against the L1210 leukemia.

A recent report from our laboratory has described the effectiveness of several 1,2-bis(arylsulfonyl)-1-methylhydrazines (**1**) against the L1210 leukemia in mice.[1] Base-catalyzed elimination in vivo to generate the putative alkylating species **2** was postulated to account for the observed biological activity of agents of this class. Since the acidity of the proton β to the leaving group would be expected to influence the rate of breakdown of these compounds to the active species **2**, we have synthesized a series of N-2 substituted 1-methyl-1-(4-tolylsulfonyl)hydrazines and have evaluated them for antitumor activity against both the L1210 leukemia and the B16 melanoma.



Chemistry. Compounds **4**–**12** (Table I) were prepared by reacting the appropriate acid chloride with 1-methyl-

(1) Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* 1985, *28*, 525.

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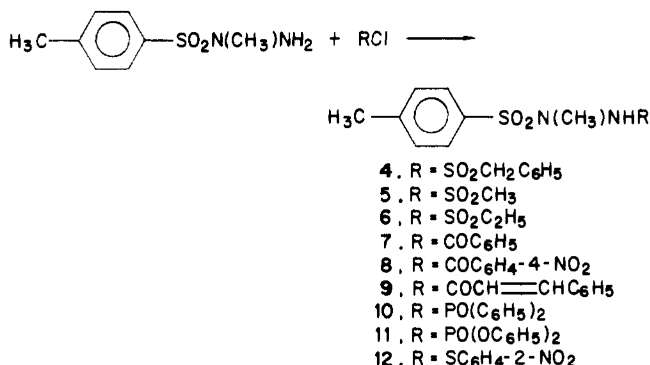
Table I. Effects of N-2 Substituted 1-Methyl-1-(4-tolylsulfonyl)hydrazines on the Survival Time of Mice Bearing the L1210 Leukemia

compd	R	optimum effective daily dose,[a] mg/kg	av Δ wt,[b] %	max % T/C[c]
3	$SO_2C_6H_4$-4-CH_3[d]	50	+3.7	147
4	$SO_2CH_2C_6H_5$	50	+10.8	174
5	SO_2CH_3[e]	30	−7.9	209
6	$SO_2C_2H_5$	NT[f]		
7	COC_6H_5	50	+7.5	167
8	COC_6H_4-4-NO_2	100	−6.3	133
9	$COCH{=}CHC_6H_5$	50	+14.8	94
10	$PO(C_6H_5)_2$	50	+5.8	100
11	$PO(OC_6H_5)_2$	150	+20.9	99
12	SC_6H_4-2-NO_2	50	+15.5	151

[a] Administered once daily for 6 consecutive days, beginning 24 h after tumor transplantation, with five animals being used per group. [b] Average change in body weight from onset to termination of therapy. [c] Percent T/C = average survival time of treated/control animals × 100. Each value represents the average of five animals per group. [d] Reported earlier.[1] [e] Percent T/C values at daily dosages of 10 and 20 mg/kg were 161 and 163, respectively. [f] Not tested.

Scheme I



1-(4-tolylsulfonyl)hydrazine[2] in a 1:1 molar ratio in pyridine as shown in Scheme I. Compound **3** was synthesized as previously described by this laboratory.[1]

Biological Results and Discussion

The antineoplastic properties of compounds **3–5** and **7–12** were determined by measuring their effects on the survival time of mice bearing the L1210 leukemia and the B16 melanoma. Compound **6** was tested against the B16 melanoma only. The results of these tests are summarized in Tables I and II.

All of the 1,2-bis(sulfonyl)-1-methylhydrazines examined displayed appreciable levels of activity against both the L1210 leukemia and the B16 melanoma. Replacement of the 4-tolylsulfonyl moiety attached to N-2 in compound **3** by benzylsulfonyl to give compound **4** produced a slight enhancement of activity against the L1210 leukemia and a comparable level of activity against the B16 melanoma. Replacement of the 4-tolylsulfonyl portion of the molecule by methylsulfonyl, however, not only increased the survival time of tumor bearing mice significantly but also resulted in a significant increase in potency. Thus, while compound **3** was found to be active in the 50–150 mg/kg dosage range,

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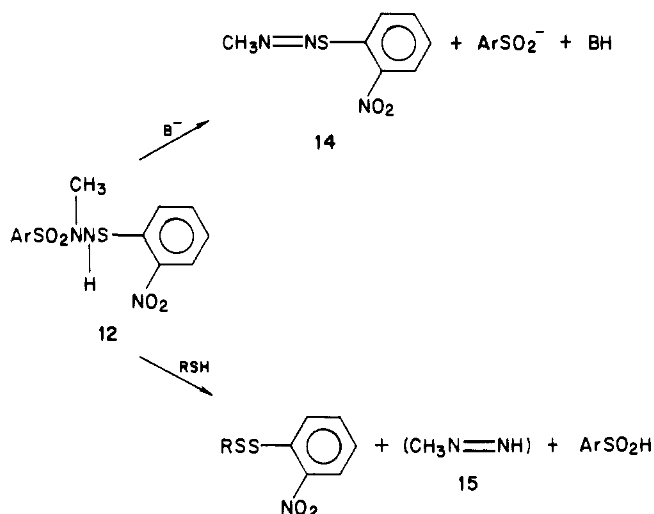
Table II. Effects of N-2 Substituted 1-Methyl-1-(4-tolylsulfonyl)hydrazines on the Survival Time of Mice Bearing the B16 Melanoma

compd	optimum effective daily dose,[a] mg/kg	av Δ wt,[b] %	max % T/C[c]
3	150	−2.7	165
4	50	−2.4	172
5[d]	10	−1.9	207
6	50	−1.1	174
7	150	+6.8	96
8	50	+3.9	97
9	150	+1.8	94
10	150	+0.7	112
11	100	+7.4	91
12	100	−2.0	99

[a] Administered once daily for 6 consecutive days, beginning 24 h after tumor transplantation, with five animals being used per group. [b] Average change in body weight from onset to termination of therapy. [c] Percent T/C = average survival time of treated/control animals × 100. Each value represents the average of five animals per group. [d] Percent T/C values at daily dosages of 20 and 30 mg/kg were both 206.

Scheme II



compound **5** displayed its effectiveness in the 10–30 mg/kg range and was toxic at higher dosage levels. Furthermore, the methylsulfonyl analogue was ca. 4 times more toxic to B16 melanoma cells in culture than compound **3**, with the LD_{50} for compound **3** being 6.2×10^{-4} M (average of two determinations), and the LD_{50} for compound **5** being 1.6×10^{-4} M (average of two determinations). Since methanesulfinate would be expected to be a poorer leaving group than 4-toluenesulfinate, the lower reactivity and presumably greater selectivity of the alkylating species **13** generated from compound **5** compared to that generated from compound **3** could be a possible reason for the enhanced potency of compound **5**. Replacement of the aryl-

or alkylsulfonyl moiety by a benzoyl or 4-nitrobenzoyl group led to a retention of activity against the L1210 leukemia but loss of the activity against the B16 melanoma. The cinnamoyl analogue **9** and the two phosphorus compounds **10** and **11** were inactive in both tumor systems. The reasons for these results are not obvious and will be explored in the future.

The (2-nitrophenyl)sulfenyl analogue **12** could conceivably be activated by two mechanisms, as shown in Scheme II. The first involves base-catalyzed elimination of an arenesulfinate group to generate species **14**, which may

function as an alkylating agent in a manner analogous to alkanediazohydroxides, the alkylating species generated by *N*-alkyl-*N*-nitrosoureas.[3-5] In the second mechanism, nucleophilic attack by an endogenous thiol such as glutathione can be visualized to generate methyldiazene (**15**), the reactive species hypothesized to account for the alkylating ability of procarbazine.[6] Compound 12, like the aroyl analogues 7 and 8, was active against the L1210 leukemia but inactive against the B16 melanoma.

Experimental Section

Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian T-60A or EM-390 spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by the Baron Consulting Co. (Orange, CT). Where analyses are indicated by the symbols of elements, the analytical results for those elements were within ±0.4% of theoretical values.

Antitumor Activity. The B16 melanoma was propagated as a solid tumor in C57Bl mice. Transplantation was carried out by removing tumors from donor mice bearing 14-day subcutaneous tumor growths. The tissue was fragmented to make a well-dispersed cellular suspension and diluted with Fischer's medium without serum so that 1 g of tissue was suspended in 5 mL of solution. A portion (0.2 mL) of the resulting cell suspension was injected intraperitoneally into each recipient animal. The L1210 leukemia was maintained and transplanted as reported earlier.[1]

Compounds were administered over a wide range of dosage levels by intraperitoneal injection, beginning 24 h after tumor implantation, once daily for 6 consecutive days. The test compounds were injected as fine suspensions following homogenization in 2–3 drops of 20% aqueous Tween 80 and then made up to volume with isotonic saline. All drugs were administered in a volume of 0.5 mL and for any one experiment animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Laboratory Chow pellets and water ad libitum. Control tumor-bearing animals given injections of comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percent change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatments.

Determination of Cytotoxicity. The cytotoxicity of compounds 3 and 5 was determined by measuring their effects on the colony-forming ability of B16 melanoma cells. The cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum. Exponentially growing B16 melanoma cells (10^5 cells) were inoculated into 25-cm^2 flasks 2 days before addition of drug. The cell monolayer was exposed to various concentrations of compound for 1 h. After exposure, the layer was washed twice with phosphate-buffered saline to remove the compound. The cells were harvested with 2 mM EDTA in phosphate-buffered saline, and 200 cells were plated in a 60-mm dish with 5 mL of medium. After incubation for 12 days, the colonies were stained with crystal violet and counted. The surviving fraction of drug-treated cells was calculated by normalizing the results to those of vehicle treated cells that had a cloning efficiency of 48–76%. The LD_{50} was determined from the individual dose–response curves.

General Procedure for the Preparation of N-2 Substituted 1-Methyl-1-(4-tolylsulfonyl)hydrazines (5–12). The appropriate acid chloride (0.01 mol) was added in portions to a cold stirred suspension of 1-methyl-1-(4-tolylsulfonyl)hydrazine (0.01 mol) in pyridine (2 mL) while the temperature was maintained between 5 and 15 °C. The reaction mixture was allowed to stand in the refrigerator for an additional 24 h and then poured into a mixture of ice and concentrated hydrochloric acid (25 mL, 1:1, v/v). The solid that separated was filtered immediately, washed with H_2O, pressed dry, and recrystallized from an appropriate solvent.

2-(α-Tolylsulfonyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine (4). In the general procedure described above, the solid that separated after pouring the reaction mixture into a mixture of ice and concentrated hydrochloric acid was filtered immediately, heated with glacial acetic acid (3 mL) to 60 °C, and cooled. The precipitate that was obtained was filtered, washed with cold water, and dried. The compound was recrystallized from $CHCl_3$–CCl_4: yield 62%; mp 185–187 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.2–7.8 (m, 9 H, Ar), 5.4 (br s, 1 H, NH), 4.5 (s, 2 H, CH_2), 3.1 (s, 3 H, NCH_3), 2.4 (s, 3 H, Ar CH_3). Anal. ($C_{15}H_{18}N_2O_4S_2$) C, H, N.

2-(Methylsulfonyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine (5): yield 50%; recrystallization solvent $CHCl_3$–CCl_4; mp 144–145 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ 7.7 and 7.3 (2 d, 4 H, Ar), 5.2 (br s, 1 H, NH), 3.1–3.2 (2 s, together 6 H, NCH_3 and SO_2CH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_9H_{14}N_2O_4S_2$) C, H, N.

2-(Ethylsulfonyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine (6): yield 21%; recrystallization solvent $CHCl_3$–CCl_4; mp 135–136 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ 7.7 and 7.3 (2 d, 4 H, Ar), 5.2 (br s, 1 H, NH), 3.3 (q, 2 H, SCH_2), 3.1 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3), 1.4 (t, 3 H, $SCCH_3$). Anal. ($C_{10}H_{16}N_2O_4S_2$) C, H, N.

2-Benzoyl-1-methyl-1-(4-tolylsulfonyl)hydrazine (7): yield 78%; recrystallization solvent EtOH; mp 188–190 °C; ^1H NMR (acetone-d_6, 60 MHz) δ 9.4 (br s, 1 H, NH), 7.2–7.8 (m, 9 H, Ar), 3.2 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{15}H_{16}N_2O_3S$) C, H, N.

1-Methyl-2-(4-nitrobenzoyl)-1-(4-tolylsulfonyl)hydrazine (8): yield 83%; recrystallization solvent acetone; mp 242–243 °C; ^1H NMR (acetone-d_6, 60 MHz) δ 11.0 (br s, 1 H, NH), 7.4–8.4 (m, 8 H, Ar), 3.2 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{14}H_{15}N_3O_5S$) C, H, N.

2-Cinnamoyl-1-methyl-1-(4-tolylsulfonyl)hydrazine (9): yield 86%; recrystallization solvent $CHCl_3$–CCl_4; mp 161–163 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 6.2–8.2 (m, together 12 H, Ar, NH and vinyl), 3.2 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{17}H_{18}N_2O_3S$) C, H, N.

2-(Diphenylphosphinyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine (10): yield 52%; recrystallization solvent CCl_4; mp 58–61 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.0–8.2 (m, 14 H, Ar), 4.5 (d, 1 H, NH), 3.2 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{20}H_{21}N_2O_3PS$) C, H, N.

2-(Diphenoxyphosphinyl)-1-methyl-1-(4-tolylsulfonyl)hydrazine (11): yield 54%; recrystallization solvent CCl_4; mp 130–132 °C; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.0–7.9 (m, 14 H, Ar), 5.4 (d, 1 H, NH), 3.0 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{20}H_{21}N_2O_5PS$) C, H, N.

1-Methyl-2-[(2-nitrophenyl)sulfenyl]-1-(4-tolylsulfonyl)hydrazine (12): yield 34%; recrystallization solvent: EtOH; mp 110–111 °C; ^1H NMR ($CDCl_3$) δ 7.2–8.4 (m, 8 H, Ar), 5.6 (br s, 1 H, NH), 3.0 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{14}H_{15}N_3O_4S_2$) C, H, N.

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