Notes

Synthesis and Evaluation of 1-(Arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines as Antineoplastic Agents

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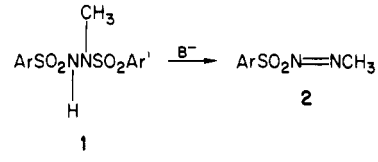
1-(Arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines, with the potential to function as biological methylating agents, were synthesized and evaluated as antineoplastic agents against the L1210 leukemia and the B16 melanoma in mice. All of the compounds of this class had significant activity against the B16 melanoma, with the most active compound, 2-[(methoxycarbonyl)sulfenyl]-1-methyl-1-[(4-methylphenyl)sulfonyl]hydrazine, producing percent T/C values for B16 melanoma tumor bearing mice of between 182 and 232 at dosage levels of from 12.5 to 50 mg/kg daily for 6 consecutive days. In contrast to the related class of agents, the *N,N'*-bis(sulfonyl)hydrazines reported earlier by this laboratory,[1] the 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines were found to be inactive against the L1210 leukemia in vivo.

This laboratory has recently described the synthesis of a variety of 1,2-bis(arylsulfonyl)-1-methylhydrazines (**1**) and have demonstrated their antineoplastic activity against the L1210 leukemia in mice.[1] The mechanism by which these compounds exerted their anticancer activity was postulated to be through base-catalyzed elimination of an arenesulfinate group under physiological conditions (Scheme I) to generate the putative alkylating species (**2**), which was hypothesized to be responsible for the observed biological activity. Replacement of the 2-arylsulfonyl group in compound **1** by a (methoxycarbonyl)sulfenyl moiety to form a 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazine (**3**) resulted in compounds that were capable of decomposing by the same mechanism to give the methylating species **4** (Scheme II). This intermediate could then function as a biological methylating agent in a manner analogous to species **2**.

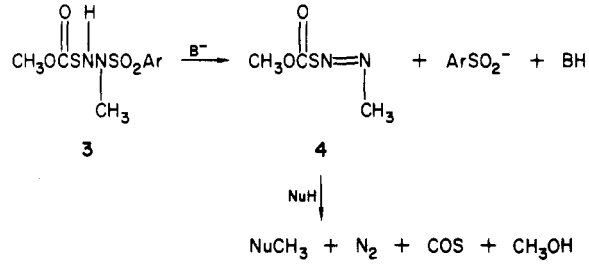
Compounds of the 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazine class may also possess the ability to react directly with the sulfur atom of biological molecules such as glutathione or various sulfur-containing proteins to give methyldiazene (**5**), the postulated alkylating species derived from procarbazine,[2] which can undergo further homolytic cleavage to generate the methyl radical, hydrogen radical, and nitrogen[3] as shown in Scheme III.

To examine the potential of this new class of methylating agents as antitumor drugs, we have synthesized a series of 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines and have evaluated their antineoplastic activity against the L1210 leukemia and the B16 melanoma. This paper also provides evidence, using a modification of the method of Wheeler and Chumley,[4] that compounds of this class decompose in solution to generate electrophiles capable of alkylating biological molecules.

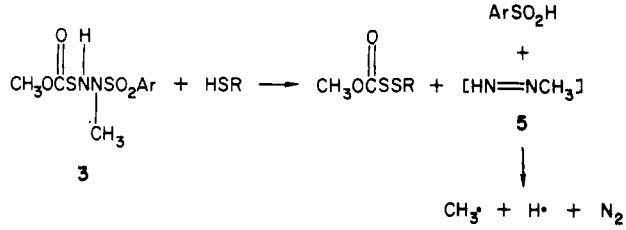
Scheme I



Scheme II



Scheme III



Chemistry

Compounds **6–11** were synthesized by the methodology shown in Scheme IV. The 1-(arylsulfonyl)-1-methylhydrazines **6a–11a** were prepared by using a modification of a literature procedure by reacting the appropriate arenesulfonyl chloride or aralkylsulfonyl chloride with methylhydrazine in a 1:2 molar ratio in tetrahydrofuran.[5,6] Reaction of these intermediates in ether with 1 equiv of pyridine and 1 equiv of (methoxycarbonyl)sulfenyl chloride at 5–10 °C afforded the appropriate products. 1-[(Meth-

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(2) Reed, D. J. In *Antineoplastic and Immunosuppressive Agents*; Sartorelli, A. C., Johns, D. G., Eds.; Springer-Verlag: Berlin, 1975; Part 2, pp 747–765.
(3) Tsuji, T.; Kosower, E. M. *J. Am. Chem. Soc.* **1971**, *93*, 1991.
(4) Wheeler, G. P.; Chumley, S. J. *J. Med. Chem.* **1967**, *10*, 259.
(5) Freidman, L.; Little, R. L.; Reichle, W. R. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 1055.
(6) Nurrenbach, A.; Pommer, H. *Justus Liebigs Ann. Chem.* **1969**, *721*, 34.

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Scheme IV

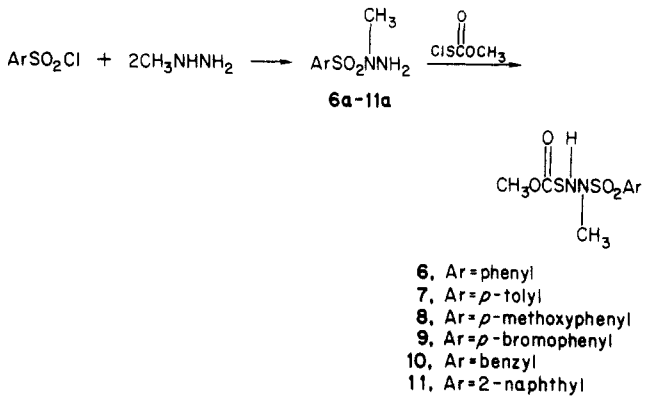


Table I. Effects of 1-(Arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines on the Survival Time of Mice Bearing the B16 Melanoma

compd	daily dose,[a] mg/kg	av Δ wt,[b] %	% T/C[c,d]
6	12.5	-5.2	169
	25	-13.0	183
	50	-33.4	49
7	12.5	+0.7	182
	25	-3.3	199
	50	-17.7	232
8	12.5	-0.2	148
	25	-6.4	160
	50	-18.7	177
9	12.5	-2.0	144
	25	-5.5	146
	50	-11.4	160
10	12.5	-2.5	152
	25	-14.8	157
	50	-27.4	59
11	12.5	-0.2	123
	25	+1.4	140
	50	-4.8	163
12[e]	12.5	+1.6	100
	25	+3.7	113
	50	-0.4	98
dacarbazine[e]	50	-2.3	135
	100	-4.4	161
	150	-5.7	177

[a] Administered once daily for six consecutive days, beginning 24 h after tumor transplantation. [b] Average change in body weight from onset to termination of therapy. [c] Percent T/C = average survival time of treated/control animals × 100. [d] Each value represents the average of two experiments of five mice/group. [e] Results from one experiment.

oxycarbonyl)sulfenyl]-2-[(4-methylphenyl)sulfonyl]-hydrazine (**12**), a desmethyl derivative, was similarly prepared from [(4-methylphenyl)sulfonyl]hydrazine[5] and (methoxycarbonyl)sulfenyl chloride.

Results and Discussion

The tumor-inhibitory properties of compounds **6–11** were determined by measuring their effects on the survival time of mice bearing the B16 melanoma, and the results are summarized in Table I. All of the compounds of this class displayed significant activity against the B16 melanoma, with the maximum percent T/C values of tumor-bearing animals produced by these agents being between 157 and 232. Although compound **7** was the most active member of the series, no clear-cut correlation was evident between the leaving group ability of the arylsulfonyl substitution and antineoplastic activity in this system. The clinically active methylating agent dacarbazine, tested for comparative purposes at daily dosage levels of from 50 to 100 mg/kg, gave percent T/C values of between 135 and 177.

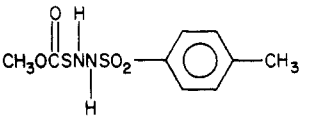
Table II. Effects of 1-(Arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines on the Survival Time of Mice Bearing the L1210 Leukemia

compd[a]	av Δ wt,[b] %	% T/C[c]
6	-10.9	113
7	-4.2	118
8	-6.7	149
9	-0.2	122
10	-8.0	100

[a] Administered once daily at dosage levels of from 12.5 to 50 mg/kg for six consecutive days, beginning 24 h after tumor transplantation. Only the findings with 50 mg/kg per day are presented; all 12.5 and 25 mg/kg per day were inactive against the L1210 leukemia. [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.

Compounds **6–10** were also evaluated for anticancer activity against mice bearing the L1210 leukemia, and the results are presented in Table II. All of the compounds tested were found to be inactive against this tumor, except for compound **8**, which displayed only limited activity. These findings contrast with our previous results[1] with the 1,2-bis(arylsulfonyl)hydrazine class of methylating agents, which showed significant activity against the L1210 leukemia. The difference between the 1,2-bis(arylsulfonyl)-hydrazines and the 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines as antineoplastic agents suggests that differences exist in the reactive species formed by decomposition in vivo; thus, the 1-(arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines would appear to represent a different class of compounds from the 1,2-bis(arylsulfonyl)hydrazines with potential clinical utility.

Since compounds **6–11** can be conceived as prodrugs of the putative methylating species **4**, a modification of the method of Wheeler and Chumley[4] was used to examine whether these compounds possessed alkylating ability. This method measured the absorbance at 540 nm of the alkylated product of 4-(4-nitrobenzyl)pyridine (data not shown). As expected, all of the compounds tested displayed significant alkylating ability under the conditions employed, except compound **12** (shown below), which would not be expected to be an effective generator of a reactive methylating species, and was inactive against the B16 melanoma. These results emphasize the importance of the methyl group attached to the hydrazide nitrogen, and are consistent with the hypothesis that these compounds act as methylating agents.



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It seems likely that the capacity of these compounds to act as biological alkylating agents involves complex kinetics, since two modes of activation are possible (see Schemes II and III) and may compete with each other. Investigations are currently ongoing in an effort to determine the mechanism(s) of action of these agents.

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 spectrometer with Me_4Si as an internal standard. (Methoxycarbonyl)sulfenyl chloride was purchased from the Fairfield Chemical Co. (Blythwood, SC). All other reagents were purchased from the Aldrich Chemical Co. (Mil-

waukee, WI). Elemental analyses were performed by the Baron Consulting Company (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]

Dosage levels of all title compounds were administered over a range of 12.5–50 mg/kg 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 intraperitoneally in a volume of 0.5 mL, and for experiments, 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.

General Procedure for the Preparation of 1-(Arylsulfonyl)-1-methylhydrazines (6a–11a). To an ice-cold stirred solution of the appropriate arenesulfonyl chloride or aralkylsulfonyl chloride (0.04 mol) in tetrahydrofuran (50 mL) was added methylhydrazine (4.4 mL, 0.08 mol) dropwise while the temperature was maintained between 0 and 5 °C. After the mixture was stirred an additional 4 h, the solvent was evaporated and 100 mL of cold water was added. The white crystalline solid was filtered, washed well with water, and air-dried. These intermediates were used without further purification for subsequent reactions.

General Procedure for the Preparation of 1-(Arylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazines (6–11). (Methoxycarbonyl)sulfenyl chloride (6.0 mmol) was slowly added dropwise to an ice-cold suspension of the appropriate 1-(arylsulfonyl)hydrazine or 1-(aralkylsulfonyl)hydrazine (6.0 mmol) in anhydrous ether (50 mL) and pyridine (6.0 mmol), while the temperature was maintained below 10 °C. After stirring at a temperature of between 0 and 5 °C for 15 min, the mixture was filtered and the etheral solution washed twice with ice-cold dilute hydrochloric acid (25-mL portions) followed by three washings with brine (25-mL portions). The organic phase was dried ($MgSO_4$) and the ether evaporated in vacuo to give an oil, which solidified upon trituration with light petroleum ether. Recrystallization from warm ethanol at 50 °C gave the pure compounds.

1-(Phenylsulfonyl)-1-methylhydrazine (6a): yield 62%; NMR ($CDCl_3$) δ 7.3–8.1 (m, 5 H, Ar), 3.7 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3).

1-Methyl-1-[(4-methylphenyl)sulfonyl]hydrazine[6] (7a): yield 83%; NMR ($CDCl_3$) δ 7.8 and 7.4 (2 d, 4 H, Ar), 3.7 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3).

1-[(4-Methoxyphenyl)sulfonyl]-1-methylhydrazine (8a): yield 83%; NMR ($CDCl_3$) δ 7.8 and 7.1 (2 d, 4 H, Ar), 3.9 (s, 3 H, OCH_3), 3.6 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3).

1-[(4-Bromophenyl)sulfonyl]-1-methylhydrazine (9a): yield 92%; NMR ($CDCl_3$) δ 7.6 (s, 4 H, Ar), 3.4 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3).

1-Methyl-1-(α-tolylsulfonyl)hydrazine (10a): yield 47%; NMR ($CDCl_3$) δ 7.4 (s, 5 H, Ar), 4.4 (s, 2 H, Ar CH_2), 3.2 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3).

1-Methyl-1-(2-naphthalenylsulfonyl)hydrazine (11a): yield 79%; NMR ($CDCl_3$) δ 8.4–7.3 (m, 7 H, Ar), 3.5 (br, 2 H, NH_2), 2.9 (s, 3 H, NCH_3).

1-(Phenylsulfonyl)-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazine (6): yield 83%; mp 62–63 °C dec; NMR ($CDCl_3$) δ 7.6 (br m, 5 H, Ar), 5.5 (br, 1 H, NH), 3.9 (s, 3 H, OCH_3), 3.1 (s, 3 H, NCH_3). Anal. ($C_9H_{12}N_2O_4S_2$) C, H, N.

2-[(Methoxycarbonyl)sulfenyl]-1-methyl-1-[(4-methylphenyl)sulfonyl]hydrazine (7): yield 74%; mp 92–93 °C dec; NMR ($CDCl_3$) δ 7.7 and 7.3 (2 d, 4 H, Ar), 5.5 (br, 1 H, NH), 3.9 (s, 3 H, OCH_3), 3.1 (s, 3 H, NCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_{10}H_{14}N_2O_4S_2$) C, H, N.

1-[(4-Methoxyphenyl)sulfonyl]-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazine (8): yield 76%; mp 74–75.5 °C dec; NMR ($CDCl_3$) δ 7.8 and 7.0 (2 d, 4 H, Ar), 5.5 (br, 1 H, NH), 3.9 (s, 3 H, OCH_3), 3.8 (s, 3 H, Ar OCH_3), 3.1 (s, 3 H, NCH_3). Anal. ($C_{10}H_{14}N_2O_5S_2$) C, H, N.

1-[(4-Bromophenyl)sulfonyl]-2-[(methoxycarbonyl)sulfenyl]-1-methylhydrazine (9): yield 79%; mp 98.5–100 °C dec; NMR ($CDCl_3$) δ 7.7 (s, 4 H, Ar), 5.5 (br, 1 H, NH), 3.9 (s, 3 H, OCH_3), 3.1 (s, 3 H, NCH_3). Anal. ($C_9H_{11}BrN_2O_4S_2$) C, H, N.

2-[(Methoxycarbonyl)sulfenyl]-1-methyl-1-(α-tolylsulfonyl)hydrazine (10): yield 41%; mp 82–84 °C dec; NMR ($CDCl_3$) δ 7.4 (s, 5 H, Ar), 5.6 (br, 1 H, NH), 4.5 (s, 2 H, Ar CH_2), 3.9 (s, 3 H, OCH_3), 3.0 (s, 3 H, NCH_3). Anal. ($C_{10}H_{14}N_2O_4S_2$) C, H, N.

2-[(Methoxycarbonyl)sulfenyl]-1-methyl-1-(2-naphthalenylsulfonyl)hydrazine (11): yield 85%; mp 91–92 °C dec; NMR ($CDCl_3$) δ 8.5–7.6 (m, 7 H, Ar), 5.5 (br, 1 H, NH), 3.9 (s, 3 H, OCH_3), 3.1 (s, 3 H, NCH_3). Anal. ($C_{13}H_{14}N_2O_4S_2$) C, H, N.

Preparation of 1-[(Methoxycarbonyl)sulfenyl]-2-[(4-methylphenyl)sulfonyl]hydrazine (12). This compound was synthesized following the general procedure employed for the preparation of the methylhydrazines **6–11**, using [(4-methylphenyl)sulfonyl]hydrazine: yield 63%; mp 98–100 °C dec; NMR ($CDCl_3$) δ 7.8 and 7.4 (2 d, 4 H, Ar), 7.6 (br, 1 H, Ar SO_2NH), 6.6 (br, 1 H, $HNSCO_2$), 3.9 (s, 3 H, OCH_3), 2.5 (s, 3 H, Ar CH_3). Anal. ($C_9H_{12}N_2O_4S_2$) C, H, N.

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Registry No. **6**, 102235-36-9; **6a**, 102235-37-0; **7**, 102235-38-1; **7a**, 22547-51-9; **8**, 102235-39-2; **8a**, 98394-35-5; **9**, 102235-40-5; **9a**, 102235-41-6; **10**, 102235-42-7; **10a**, 102235-43-8; **11**, 102235-44-9; **11a**, 102235-45-0; **12**, 102235-46-1; $ClSC(O)OCH_3$, 26555-40-8; CH_3NHNH_2, 60-34-4; phenylsulfonyl chloride, 98-09-9; *p*-tolylsulfonyl chloride, 98-59-9; *p*-methoxyphenylsulfonyl chloride, 98-68-0; *p*-bromophenylsulfonyl chloride, 98-58-8; benzylsulfonyl chloride, 1939-99-7; 2-naphthylsulfonyl chloride, 93-11-8; [(4-methylphenyl)sulfonyl]hydrazide, 1576-35-8.