as previously described.[12] Binding of the 1,4-dihydropyridine (+)-[^{3}H]PN 200 110 (isopropyl 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-(methoxycarbonyl)-2,6-dimethyl-3-pyridinecarboxylate) and its competition by the isoxazolyl-1,4-dihydropyridines were carried out as previously described. Briefly, membrane protein (40–120 μg) was incubated in 5 mL of 50 mM tris[(hydroxymethyl)amino]methane (Tris) buffer at pH 7.2 for 90 min at 25 °C with 5×10^{-11} (+)-[^{3}H]PN 200 110 and varying concentrations of competing compounds. Duplicate tubes contained 10^{-7} M (+)-PN 200 110 to define nonspecific binding. Tubes were filtered and washed rapidly with two 5-mL portions of ice-cold Tris buffer in a Brandel cell harvester (Model M-24R, Biomedical Research Lab., Gaithesburg, MD). Trapped radioactivity was counted by liquid-scintillation spectrometry at an efficiency of 40–45%. Competing compounds were prepared in ethanol as 10^{-3} stock solutions. Concentrations of ethanol of 0.2% (v:v) did not affect specific binding. Binding data were analyzed by iterative curve-fitting programs (BDATA, CDATA, EMF Software, Knoxville, TN). (+)-[^{3}H]PN 200 110 with a specific activity of 70 Ci/mol [1 Ci = 3.7×10^{10} Bq) was purchased from Du Pont–New England Nuclear (Boston, MA).

Calculations. The Molecular Mechanics calculations were performed on a MacIntosh IIcx Computer, using Chem3D (Cambridge Scientific Computing, Inc). Chem3D Plus includes an implementation of the Allinger MM2 force field,[10a] by Ponder.[10b] All structures in Table I were minimized for total steric energy, until termination to root mean square gradients of <0.100. The Cartesian coordinates obtained for these calculations were used in subsequent quantum mechanical calculations. Molecular orbital calculations were of the intermediate neglect of differential overlap (INDO/I) type using the method of Ridley and Zerner.[15–17] Quantum mechanical calculations were run on a Hewlett-Packard 900/350 workstation, a component of the Computational Facility for Theoretical Chemistry in the University of Idaho. Calculations using this method were first applied to nifedipine, and the results obtained [3e] were essentially in agreement with the experimental observations of Rovnyak et al.[5a]

X-ray Data. Suitable crystals of **2** were obtained by slow evaporation from ethyl acetate–hexanes solution. Cell constants were determined by a least-squares fitting of setting angles of the diffractometer from 25 reflections between 55° and 85°. Data were collected by the ω scan technique[13] with graphite-monochromatized Cu Kα radiation (λ = 1.5418 Å). The measured intensities were corrected for Lorentz and polarization effects but not for absorption. The structure was solved with the SHELXTL program.[14] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were constrained to N–H and C–H distances of 0.96 Å; thermal parameters of all hydrogen atoms were set at 0.12 × the equivalent isotropic U of the atom to which it is bonded. Crystallographic data of **2** is listed in Table III. Largest peaks on the final Fourier difference map were 0.4 and −0.45 e/Å^3. The final R value for **2** was 6.91.

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Supplementary Material Available: ^{1}H NMR (300 MHz) of **2**, unit-cell diagram illustrating intermolecular hydrogen bonding in the X-ray of **2**, and X-ray data for **2**: atomic coordinates, bond lengths, bond angles, and torsion angles (7 pages). Ordering information is given on any current masthead page.

Synthesis and Evaluation of 1,2,2-Tris(sulfonyl)hydrazines as Antineoplastic and Trypanocidal Agents

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Several 1,2,2-tris(sulfonyl)hydrazines, conceived as prodrugs of 1,2-bis(sulfonyl)hydrazines, were synthesized and evaluated for antineoplastic and trypanocidal activities in mice. 1-Methyl-1,2,2-tris(methylsulfonyl)hydrazine emerged as an extremely efficacious antitrypanosomal agent, whereas 1-(2-chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine was inactive. In contrast, 1-(2-chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine displayed potent antineoplastic activity, producing several 60-day "cures" of mice bearing leukemia L1210, leukemia P388, or Sarcoma 180. Furthermore, the fact that the tris(sulfonyl) derivatives will not generate isocyanates, which contribute to the host toxicity of nitrosoureas like 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), makes them agents of significant promise in trypanosomal and cancer chemotherapy.

Recent studies in our laboratory have identified a number of 1,2-bis(sulfonyl)-1-methylhydrazines with antineoplastic activity.[1–3] The most active compound of this class to emerge from this study, 1,2-bis(methylsulfonyl)-1-methylhydrazine (1), displayed relatively high levels of activity against the L1210 leukemia and the B16 melanoma in mice.[3] More recently, we reported the antitrypanosomal activity of some of these agents against *T. brucei rhodesiense* in mice.[4] Compound 1 also emerged as the most

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effective agent of the series in this system.

$CH_3SO_2N(CH_3)NHSO_2CH_3$
1

1,2-Bis(sulfonyl)-1-methylhydrazines have been hypothesized to exert their antineoplastic and trypanocidal properties through the base-catalyzed generation of the putative methylating species $RSO_2N{=}NCH_3$. Thus, when compound 1 was aged in phosphate buffered saline (pH 7.6) at 37 °C, greater than 60% of its activity against *T. rhodesiense* was lost in only 5 min.[4] This phenomenon appeared to be due to the loss of alkylating ability, as evidenced by the observation that the abolition of antitrypanosomal activity was attended by an approximate 0.7–0.8 molar yield of methanol, the product of the reaction of the methylating species with water as shown in Scheme I.

Scheme I

$$CH_3N{=}NSO_2CH_3 + H_2O \rightarrow CH_3SO_2H + N_2 + CH_3OH$$

The 1,2-bis(arylsulfonyl)-1-methylhydrazines examined (e.g., 1,2-bis(phenylsulfonyl)-1-methylhydrazine), which were less efficacious as antitrypanosomal agents, decayed considerably faster than compound 1 (data not shown). These observations suggested that the synthesis of more stable prodrugs of 1,2-bis(sulfonyl)-1-methylhydrazines would lead to an increase in the $t_{1/2}$ of these agents in the blood stream, with a resulting increase in antineoplastic and trypanocidal activities.

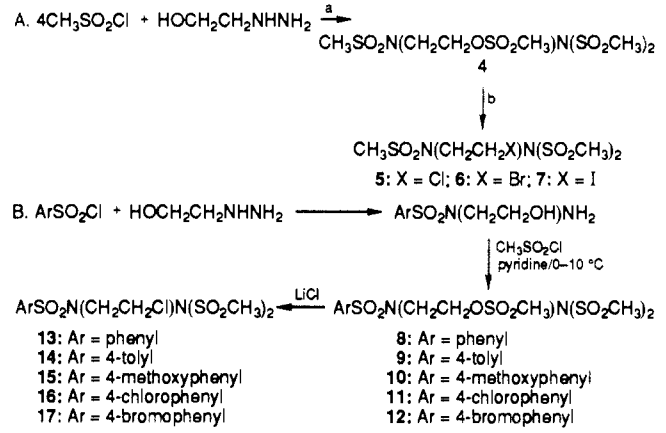
Since several *N*-(2-chloroethyl)-*N*-nitrosoureas[5,6] and (2-chloroethyl)triazenes[7,8] exhibited great antineoplastic activity, a few 1,2-bis(sulfonyl)-1-(2-chloroethyl)hydrazines, conceived as chloroethylating agents, were synthesized and evaluated for antineoplastic activity against leukemia L1210 and the B16 melanoma.[3] Although the synthesized compounds showed positive activity against the L1210 leukemia in mice, they displayed considerable host toxicity and were active only over a narrow dosage range. From our studies on 1,2-bis(sulfonyl)-1-methylhydrazines it was reasonable to assume that the rate of decomposition of 1,2-bis(sulfonyl)-1-(2-chloroethyl)hydrazines in aqueous media would also be quite rapid. Prodrugs of 1,2-bis(sulfonyl)-1-(2-chloroethyl)hydrazines with a decreased capacity to undergo decomposition were postulated to have the potential advantage of not only improved distribution characteristics but also superior therapeutic properties, since such agents would be expected to deliver the alkylation stress at more sustained rates than the parent compound.

This paper reports the synthesis of 1,2,2-tris(sulfonyl)-hydrazines and their evaluation as antineoplastic and antitrypanosomal agents. These compounds are believed to undergo spontaneous hydrolysis in aqueous solution at physiological pH and temperature to generate 1,2-bis(sulfonyl)hydrazines as shown in Scheme II.

Scheme II

$R'SO_2N(R)N(SO_2CH_3)_2 + H_2O \rightarrow$
R′ = methyl, aryl
R = methyl, 2-chloroethyl
$R'SO_2N(R)NHSO_2CH_3 + CH_3SO_2OH$

Scheme III[a]



[a] a = pyridine/0–5 °C; b = LiCl, LiBr, or KI.

The 1,2-bis(sulfonyl)-1-methylhydrazines and 1,2-bis(sulfonyl)-1-(2-chloroethyl)hydrazines that have been generated are hypothesized to act as monofunctional and bifunctional alkylating agents, respectively, in a manner previously described.[1,3]

Chemistry

The 1,2,2-tris(sulfonyl)hydrazines examined are listed in Table I. 1-Methyl-1,2,2-tris(methylsulfonyl)hydrazine (2) was prepared by reacting methylhydrazine with an excess of methanesulfonyl chloride in pyridine. 1-(2-Chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine (5) was synthesized as shown in Scheme IIIA. The reaction of (2-hydroxyethyl)hydrazine with an excess of methanesulfonyl chloride in pyridine gave 1-[2-[(methylsulfonyl)-oxy]ethyl]-1,2,2-tris(methylsulfonyl)hydrazine (4). The reaction of this compound with lithium chloride in acetone gave compound 5. The use of lithium bromide or potassium iodide in lieu of lithium chloride in the second step in Scheme IIIA gave the 2-bromoethyl (6) or the 2-iodoethyl (7) analogues, respectively. The 1-arylsulfonyl-1-(2-chloroethyl)-2,2-bis(methylsulfonyl)hydrazines (13–17) were synthesized by reacting the corresponding 1-(arylsulfonyl)-1-[2-[(methylsulfonyl)oxy]ethyl]-2,2-bis(methylsulfonyl)hydrazines (8–12) with lithium chloride in acetone (Scheme IIIB). The 1-[2-(methylsulfonyl)oxy]-ethyl compounds, in turn, were prepared by reacting the appropriate 1-(arylsulfonyl)-1-(2-hydroxyethyl)hydrazides with an excess of methanesulfonyl chloride in pyridine. The 1-(arylsulfonyl)-1-(2-hydroxyethyl)hydrazides and 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (3) were synthesized by using or adapting published procedures.[3]

$CH_3SO_2N(CH_3)N(SO_2CH_3)_2$
2

$CH_3SO_2N(CH_2CH_2Cl)NHSO_2CH_3$
3

Results and Discussion

The trypanocidal properties of compounds 1–3 and 5 were determined by measuring their effects on the survival time of CD-1 mice infected with *T. rhodesiense* (Y Tat 1.1), a pleiomorphic strain that produces a nonrelapsing disease in mice. Since compounds 3 and 5 were inactive, further testing of the chloroethyl analogues for antitrypanosomal activity was abandoned. Both compounds 1 and 2 were found to be highly active as trypanocides. Although compound 2 (mean extension of life = 7.7 days) was less potent than compound 1 (mean extension of life = 12 days)[4] at 0.2 mmol/kg, the former was considerably less toxic to the host. Thus, compound 2 not only produced

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Table I. Effects of Sulfonylhydrazine Derivatives on the Survival Time of Mice Bearing the L1210 Leukemia

compd	optimum effective daily dose, mg/kg[a,b]	av Δ wt, %[c]	max % T/C[d]	60-day survivors, %
1	40	−5.8	180	0
2	150	−7.7	186	0
3	15[e]	−3.3	130	40
4	100	−4.2	198	0
5	60[f]	−7.2	–	100
6	150	−2.0	213	0
7	150	+8.0	110	0
13	150	+0.5	187	40
14	150	−12.0	210	60
15	200	+5.9	–	100
16	200	−3.4	203	60
17	150	+0.9	241	60

[a] Administered once daily for 6 consecutive days, beginning 24 h after tumor implantation, with 5–10 mice being used per group. [b] Average day of death for tumored control mice was 8.8. [c] Average percent change in body weight from onset to termination of therapy. Average percent change in body weight for vehicle-treated controls was +7.7. [d] %T/C = average survival time of treated/control animals × 100; 60-day survivors are listed separately and are not included in this calculation. [e] Administered as a single intraperitoneal dose. %T/C against L1210 leukemia at 5 mg/kg × 6 = 145. [f] %T/C against P388 leukemia = 218 (80% 60-day survivors) at 60 mg/kg × 6; against Sarcoma 180 (100% 60-day survivors at 60 mg/kg × 6). Average days of death of tumored control mice were as follows: 12.4, P388 leukemia; 15.8, Sarcoma 180. Average percent changes in body weight for vehicle-treated controls were +9.3 for P388 leukemia and +20.9 for Sarcoma 180.

100% 30-day "cures" in mice bearing *T. rhodesiense* but did so at a dosage level (1 mmol/kg) that was considerably lower than its LD_{50} (approximately 10 mmol/kg). In contrast, compound 1, which produced 50% "cures" at 0.4 mmol/kg had an LD_{50} of approximately 0.6 mmol/kg. Thus, a dramatic decrease in host toxicity was achieved by introducing an additional methanesulfonyl group at N-2.

The antineoplastic activity of compounds 1–7 and 13–17 was assessed primarily by measuring their effects on the survival time of mice bearing the L1210 leukemia; the results of these tests are summarized in Table I. Whereas 1-methyl-1,2,2-tris(methylsulfonyl)hydrazine (2) displayed the same level of activity as the corresponding bis(methylsulfonyl) analogue 1, the same modification in the 1-(2-chloroethyl) series yielded a marked increase in therapeutic activity. For example, 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (3) produced %T/C values in mice bearing the L1210 leukemia of 131, 146, and 130 (two 60-day survivors) at single intraperitoneal doses of 5, 10, and 15 mg/kg, respectively. At the maximum daily dose (5 mg/kg × 6), which resulted in no toxic deaths, this compound produced a %T/C of 145 with no long term survivors, whereas the corresponding tris(sulfonyl) analogue, compound 5, produced 100% 60-day "cures" at 60 mg/kg × 6. Replacement of the chloroethyl group in compound 5 by 2-bromoethyl (6) or 1-[2-[(methylsulfonyl)oxy]ethyl] (4) resulted in active but less efficacious compounds against the L1210 leukemia, with maximum %T/C values being 213 and 198, respectively. Activity was completely abolished, however, when the chloroethyl group was replaced by 2-iodoethyl (7). The effect of the halogen leaving group on activity, i.e., Cl > Br > I, follows the same trend as that observed by Johnston et al.[5] in their study on a series of *N*-(2-haloethyl)-*N*-nitrosoureas. The few aromatic analogues examined (13–17) were also capable of producing "cures" in mice bearing the L1210 leukemia but were considerably less potent than compound 5.

Compound 5 also produced 80% 60-day "cures" of mice bearing the P388 leukemia and 100% 60-day "cures" of Sarcoma 180 ascites tumor-bearing animals at a daily dose of 60 mg/kg for 6 consecutive days. Thus, the relatively great efficacy of this compound against three transplanted tumor systems makes compound 5 an agent of some promise in cancer chemotherapy.

Table II. Effect of Aging on the Antitrypanosomal Activity of 1-Methyl-1,2,2-tris(methylsulfonyl)hydrazine[a]

aging time, min	mean increase in survival,[b] days ± SE
0	6.3 ± 1.2
100	3.7 ± 0.6
200	1.2 ± 0.5

[a] Compound 2 was aged by adding 100 μL of a 1 M solution in dry dimethyl sulfoxide to 9.9 mL of 200 mM potassium phosphate buffer (pH 7.6, 37 °C). [b] CD-1 mice were treated 3 days after i.p. injection with 10^6 *T. rhodesiense* (Y Tat 1.1) by dosing (i.p.) with 20 μL of this solution per g body weight to give an effective initial dose of 0.2 mmol/kg at various times after mixing. The number of days the mice survived beyond equivalently infected mice receiving the vehicle (phosphate buffer and dimethyl sulfoxide) only was used as a measure of biological activity. Control animals died 1 day after receiving the vehicle. Three to four mice were used per group.

The most striking difference observed between the 1,2-bis(sulfonyl)- and the 1,2,2-tris(sulfonyl)hydrazines was the increase in antineoplastic activity exhibited by the latter compounds. Although the presence of three methylsulfonyl groups resulted in a considerable drop in potency, the overall effect was a marked increase in therapeutic efficacy. Decomposition studies were carried out on representative agents of each class in an effort to determine whether a correlation existed between the activity of the 1,2-bis(sulfonyl)hydrazines and the 1,2,2-tris(sulfonyl)hydrazines and their stability in aqueous media. The generation of methanol in aqueous solutions free from strong competing nucleophiles (200 mM potassium phosphate buffer, pH 7.6, at 37 °C) was used as a measure of the spontaneous decomposition of 1,2-bis(methylsulfonyl)-1-methylhydrazine (1) to a reactive methylating species. In the case of compound 1, maximum generation of methanol occurred in 15 min, which was also the time required for the complete loss of antitrypanosomal activity.[4] For compound 2, half-maximum generation of methanol occurred in ca. 100–110 min at 37 °C, pH 7.6. This was also the time required for compound 2 to lose one-half of its biological activity upon aging (Table II). Measurement of the yields of methanol from compounds 1 and 2 in 200 mM potassium phosphate buffer (pH 7.6, 37 °C) gave mean values of 73% (SE = ±9.3) and 57% (SE = ±5.3), respectively, implying a 78% yield of compound 1 from compound 2 upon hydrolysis. The alternative hydrolysis product, 2,2-bis(methylsulfonyl)-1-methylhydrazine, would not be expected to generate a methylating species and may account for the lower yield of methanol obtained with compound 2. This decrease in the yield of the alkylating species may be responsible for the difference in potency observed between the bis(sulfonyl)- and the tris(sulfonyl)hydrazines, not only in the *N*-methyl series but also in the *N*-(2-chloroethyl) series. However, since the increase in the stability of the tris compounds in aqueous media might well permit a better distribution of the drug(s), this effect would be expected to counter, at least in part, the decrease in potency resulting from the lower yield of the alkylating species. Improved drug distribution may also contribute to the observed decreased toxicity by decreasing areas of local damage.

N-(2-Chloroethyl)-*N*-nitrosoureas are bifunctional alkylating agents with a broad spectrum of antitumor activity. Decomposition studies have established that ni-

trosoureas such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) generate a large number of products, including chloroethylating,[9,10] hydroxyethylating,[11] and carbamoylating[9,10,12] species. The antitumor activity of nitrosoureas has been suggested to result from chloroethylation and not hydroxyethylation. Hydroxyethylation of DNA, which has been postulated to occur via 4,5-dihydro-1,2,3-oxadiazole intermediates,[11,13-15] is believed to be a mutagenic and/or carcinogenic event.[16,17] Carbamoylation reactions, which do not appear to contribute to antitumor activity, are thought to interfere with DNA repair.[18] Therefore, agents which retain chloroethylating activity but are devoid of hydroxyethylating and carbamoylating activities could be potentially superior to the nitrosoureas as therapeutic agents.[19] Compound 5 may be such an agent since isocyanates and 4,5-dihydro-1,2,3-oxadiazoles that are formed from nitrosoureas are unlikely to be products of the decomposition of this compound in vivo. Furthermore, the therapeutic synergism exhibited by combinations of alkylating agents and the differences in susceptibility of human cancers to different alkylating agents make it reasonable to assume that a structurally distinct effective agent of this type would be a useful addition to our therapeutic arsenal against cancer.[20,21]

Experimental Section

Melting points were recorded with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer with Me_4Si as an internal standard. 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.

Synthesis. 1-Methyl-1,2,2-tris(methylsulfonyl)hydrazine (2). To an ice-cold stirred solution of methylhydrazine (4.6 g, 0.10 mol) in dry pyridine (30 mL) was added methanesulfonyl chloride (44.6 g, 0.39 mol) dropwise, while maintaining the temperature between 0 and 10 °C. The reaction mixture was left in a freezer (−10 °C) for 2 days. It was then stirred with a mixture of ice and concentrated hydrochloric acid (1:1, v/v, 100 mL). The precipitate that formed was collected, washed with cold water, and dried. This product was stirred with chloroform (200 mL) and filtered. The undissolved material, consisting mainly of 1,2-bis(methylsulfonyl)-1-methylhydrazine, was discarded and the filtrate was treated with decolorizing carbon, filtered and evaporated to dryness in vacuo to give a yellow solid, which was crystallized twice from ethanol (Norit A) to give 5.1 g (18%) of the title compound: mp 123–124 °C; ^{1}H NMR (acetone-d_6) δ 3.6 [s, 6 H, $N^2(SO_2CH_3)_2$], 3.5 (s, 3 H, NCH_3), and 3.2 (s, 3 H, $N^1SO_2CH_3$). Anal. ($C_4H_{12}N_2O_6S_3$) C, H, N.

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine (3). To an ice-cold stirred solution of (2-hydroxyethyl)hydrazine (7.6 g, 0.1 mol) in dry pyridine (32 mL) was added methanesulfonyl chloride (35.5 g, 0.31 mol) in portions while maintaining the temperature between 0 and 10 °C. After an additional 60 min the reaction mixture was left in a freezer (−10 °C) overnight. It was then stirred with a mixture of ice and concentrated hydrochloric acid (2:1, v/v, 200 mL) until a clear solution was obtained. This solution was saturated with sodium chloride and extracted with ethyl acetate (3 × 200 mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness to give a thick semisolid which upon trituration with anhydrous ether gave a yellow solid. The ether layer was decanted, and the solid was shaken with chloroform (25 mL) and filtered. The filtrate was discarded, and the residue, which was predominantly 1,2-bis(methylsulfonyl)-1-[2-[(methylsulfonyl)oxy]-ethyl]hydrazine, was dissolved in acetone (150 mL) containing dry lithium chloride (10 g), and the mixture was heated under reflux for 3 days. The reaction mixture was filtered and the filtrate evaporated to dryness in vacuo. To the residue was added ethyl acetate (200 mL), and the mixture was warmed to 50 °C and filtered. The filtrate was concentrated to one-fifth its volume and passed through a column of silica gel (70–270 mesh, 60 Å) with ethyl acetate as eluant. On evaporation of the solvent, a light yellow solid was obtained which was recrystallized from ethanol (Norit A) to give 5.1 g (20%) of the title compound: mp 138–139 °C; ^{1}H NMR (acetone-d_6) δ 8.6 (broad s, 1 H, NH), 3.9 (m, 4 H, CH_2CH_2), 3.2 and 3.1 (2s, 6 H, $2CH_3$). Anal. ($C_4H_{11}ClN_2O_4S_2$) C, H, N.

1-[2-[(Methylsulfonyl)oxy]ethyl]-1,2,2-tris(methylsulfonyl)hydrazine (4). To an ice-cold stirred solution of (2-hydroxyethyl)hydrazine (6.08 g, 0.08 mol) in dry pyridine (40 mL) was added methanesulfonyl chloride (41.2 g, 0.36 mol) dropwise while maintaining the temperature between 0 and 5 °C. After the reaction mixture was kept stirring at this temperature range for an additional 3 h, it was left in a freezer (−10 °C) for 48 h. It was then triturated with a mixture of ice and concentrated hydrochloric acid (1:1, v/v, 100 mL). A thick semisolid separated and settled to the bottom of the flask. The clear supernatant was carefully decanted, and the semi-solid was warmed to 60 °C in glacial acetic acid (150 mL) and cooled to 5 °C. The solid that separated was filtered, washed with cold glacial acetic acid (20 mL), dried, and recrystallized from ethanol–acetone (1:3, v/v) using Norit A as a decolorizing agent to give 9.6 g (31%) of the title compound: mp 160–162 °C; ^{1}H NMR (acetone-d_6) δ 4.5 and 4.1 (2t, 4 H, CH_2CH_2), 3.6 [s, 6 H, $N^2(SO_2CH_3)_2$], 3.3 (s, 3 H, $N^1SO_2CH_3$), and 3.2 (s, 3 H, OSO_2CH_3). Anal. ($C_6H_{16}N_2O_9S_4$) C, H, N.

1-(2-Chloroethyl)-1,2,2-tris(methylsulfonyl)hydrazine (5). A mixture of compound 4 (2.0 g, 0.005 mol), lithium chloride (2.0 g, 0.047 mol), and dry acetone (50 mL) was heated under reflux for 4 days. The reaction mixture was cooled to room temperature and filtered, and the filtrate was evaporated to dryness in vacuo. The residue was warmed with chloroform (100 mL) to 50 °C and filtered, and the filtrate was evaporated to dryness in vacuo. Recrystallization of the residue from ethanol gave 1.1 g (65%) of the title compound: mp 154–155 °C; ^{1}H NMR ($CDCl_3$) δ 3.6–4.0 (m, 4 H, CH_2CH_2), 3.5 [s, 6 H, $N^2(SO_2CH_3)_2$], and 3.2 (s, 3 H, $N^1SO_2CH_3$). Anal. ($C_5H_{13}ClN_2O_6S_3$) C, H, N.

1-(2-Bromoethyl)-1,2,2-tris(methylsulfonyl)hydrazine (6). 1-(2-Bromoethyl)-1,2,2-tris(methylsulfonyl)hydrazine was prepared in a manner analogous to that of the corresponding 2-chloroethyl analogue 5 by reacting compound 4 with lithium bromide in acetone for 48 h: yield, 35%; mp 147–148 °C; ^{1}H NMR ($CDCl_3$) δ 4.0 and 3.6 (2t, 4 H, CH_2CH_2), 3.5 [s, 6 H, $N^2(SO_2CH_3)_2$], and

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3.2 (s, 3 H, $N^1SO_2CH_3$). Anal. ($C_5H_{13}BrN_2O_6S_3$) C, H, N.

1-(2-Iodoethyl)-1,2,2-tris(methylsulfonyl)hydrazine (7). 1-(2-Iodoethyl)-1,2,2-tris(methylsulfonyl)hydrazine was prepared in a manner analogous to that of the corresponding 2-chloroethyl compound **5** by reacting compound **4** with potassium iodide in acetone for 48 h: yield, 66%; mp 136–138 °C; ^1H NMR ($CDCl_3$) δ 4.0 and 3.4 (2t, 4 H, CH_2CH_2), 3.5 [s, 6 H, $N^2(SO_2CH_3)_2$], and 3.2 (s, 3 H, $N^1SO_2CH_3$). Anal. ($C_5H_{13}IN_2O_6S_3$) C, H, N.

2,2-Bis(methylsulfonyl)-1-[2-[(methylsulfonyl)oxy]ethyl]-1-(phenylsulfonyl)hydrazine (8). To an ice-cold stirred mixture of 1-(2-hydroxyethyl)-1-(phenylsulfonyl)hydrazine (10.8 g, 0.05 mol) and dry pyridine (25 mL) was added methanesulfonyl chloride (29.6 g, 0.26 mol) dropwise, while maintaining the temperature between 0 and 10 °C. After an additional 3 h of stirring at this temperature range, the reaction mixture was left in a freezer (−10 °C) for 48 h. It was then triturated with a mixture of ice and concentrated hydrochloric acid (1:1, v/v, 100 mL). A thick semisolid separated and settled to the bottom of the flask. The clear supernatant was decanted, and the residue was boiled with ethanol (100 mL). The solid that separated was filtered, while the ethanol mixture was still hot, washed with ethanol, and dried. It was recrystallized from a mixture of ethanol and acetone (Norit A) to give 3.1 g (14%) of the title compound: mp 107–108 °C; ^1H NMR (acetone-d_6) δ 8.0 and 7.7 (d and m, 5 H, aromatic H), 4.3 and 4.0 (2t, 4 H, CH_2CH_2), 3.6 [s, 6 H, $N(SO_2CH_3)_2$], and 3.0 (s, 3 H, OSO_2CH_3). Anal. ($C_{11}H_{18}N_2O_9S_4$) C, H, N.

2,2-Bis(methylsulfonyl)-1-[2-[(methylsulfonyl)oxy]ethyl]-1-(4-tolylsulfonyl)hydrazine (9). 1-(2-Hydroxyethyl)-1-(4-toluenesulfonyl)hydrazine (6.9 g, 0.03 mol) was reacted with methanesulfonyl chloride (14.1 g, 0.12 mol) in dry pyridine (12 mL), and the product was isolated in a manner identical to that described for compound **8**: yield, 4.7 g (34%); mp 153–155 °C; ^1H NMR (acetone-d_6) δ 7.9 and 7.4 (2d, 4 H, aromatic H), 4.4 and 4.0 (2t, 4 H, CH_2CH_2), 3.6 [s, 6 H, $N(SO_2CH_3)_2$], 3.0 (s, 3 H, OSO_2CH_3), and 2.4 (s, 3 H, $ArCH_3$). Anal. ($C_{12}H_{20}N_2O_9S_4$) C, H, N.

2,2-Bis(methylsulfonyl)-1-[2-[(methylsulfonyl)oxy]ethyl]-1-[(4-methoxyphenyl)sulfonyl]hydrazine (10). To an ice-cold stirred mixture of 1-(2-hydroxyethyl)-1-[(4-methoxyphenyl)sulfonyl]hydrazine (10.0 g, 0.04 mol) and dry pyridine (25 mL) was added methanesulfonyl chloride (29.6 g, 0.26 mol) in portions, while maintaining the temperature between 0 and 5 °C. After an additional 2 h of stirring at this temperature range, the reaction mixture was left in the freezer (−10 °C) for 48 h. It was then triturated with a mixture of ice and concentrated hydrochloric acid (1:1, v/v, 100 mL), the clear supernatant was decanted, and the thick semisolid that separated was boiled with ethanol (100 mL) and cooled to 5 °C. A yellow solid separated that was stirred with methylene chloride (200 mL) and filtered. The filtrate was evaporated to dryness in vacuo to give the crude title compound, which was recrystallized from a mixture of ethanol and acetone (Norit A): yield, 6.7 g (34%); mp 144–145 °C; ^1H NMR (acetone-d_6) δ 7.9 and 7.1 (2d, 4 H, aromatic H), 4.3 and 4.0 (2t, 4 H, CH_2CH_2), 3.9 (s, 3 H, OCH_3), 3.6 [s, 6 H, $N(SO_2CH_3)_2$], and 3.0 (s, 3 H, OSO_2CH_3). Anal. ($C_{12}H_{20}N_2O_{10}S_4$) C, H, N.

2,2-Bis(methylsulfonyl)-1-[(4-chlorophenyl)sulfonyl]-1-[2-[(methylsulfonyl)oxy]ethyl]hydrazine (11). To an ice-cold stirred mixture of 1-[(4-chlorophenyl)sulfonyl]-1-(2-hydroxyethyl)hydrazine (12.5 g, 0.05 mol) and dry pyridine (20 mL) was added methanesulfonyl chloride (23.8 g, 0.21 mol) dropwise, while maintaining the temperature between 0 and 10 °C. After an additional 2 h of stirring at this temperature range, the reaction mixture was left in a freezer (−10 °C) for 48 h. It was then triturated with a mixture of ice and concentrated hydrochloric acid (1:1, v/v, 100 mL). The solid that separated was filtered, stirred with chloroform (300 mL) for 10 min, treated with Norit A, and filtered. On evaporation of the filtrate to dryness in vacuo, a solid was obtained that was recrystallized from ethyl acetate–petroleum ether (Norit A) to give 6.3 g (26%) of the title compound: mp 152–153 °C; ^1H NMR (acetone-d_6) δ 8.1 and 7.7 (2d, 4 H, aromatic H), 4.5 and 4.1 (2t, 4 H, CH_2CH_2), 3.6 [s, 6 H, $N(SO_2CH_3)_2$], and 3.1 (s, 3 H, OSO_2CH_3). Anal. ($C_{11}H_{17}ClN_2O_9S_4$) C, H, N.

2,2-Bis(methylsulfonyl)-1-[(4-bromophenyl)sulfonyl]-1-[2-[(methylsulfonyl)oxy]ethyl]hydrazine (12). This compound was prepared by reacting 1-[(4-bromophenyl)sulfonyl]-1-(2-hydroxyethyl)hydrazine (5.2 g, 0.018 mol) and methanesulfonyl chloride (9.0 g, 0.079 mol) in dry pyridine (15 mL) in a manner analogous to that described for compound **11**; yield, 2.5 g (27%); mp 154–155 °C; ^1H NMR (acetone-d_6) δ 7.9–8.0 (2d, 4 H, aromatic H), 4.4 and 4.1 (2t, 4 H, CH_2CH_2), 3.6 [s, 6 H, $N(SO_2CH_3)_2$], and 3.0 (s, 3 H, OSO_2CH_3). Anal. ($C_{11}H_{17}BrN_2O_9S_4$) C, H, N.

2,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-1-(phenylsulfonyl)hydrazine (13). A mixture of compound **8** (2.0 g, 0.0044 mol), dry lithium chloride (2.0 g, 0.047 mol), and dry acetone (50 mL) was heated under reflux for 5 days. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. To the residue was added chloroform (100 mL), and the mixture was stirred for 10 min and filtered. The filtrate was evaporated to dryness, and the semisolid residue obtained was dissolved by boiling in a minimum quantity of ethanol and filtered. On cooling, the title compound was obtained as white crystals: yield, 0.68 g (39%); mp 114–115 °C; ^1H NMR (acetone-d_6) δ 8.0 and 7.7 (d and m, 5 H, aromatic H), 3.6–4.0 (m, 4 H, CH_2CH_2), and 3.6 (s, 6 H, $2CH_3$). Anal. ($C_{10}H_{15}ClN_2O_6S_3$) C, H, N.

2,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-1-(4-tolylsulfonyl)hydrazine (14). A mixture of compound **9** (2.0 g, 0.0043 mol), dry lithium chloride (2.0 g, 0.047 mol), and dry acetone (50 mL) was heated under reflux for 4 days. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue was warmed with chloroform (100 mL) to 40 °C and filtered, and the filtrate was evaporated to dryness. The residue was boiled with ethanol (150 mL) and cooled to 10 °C. The unreacted sulfonate which crystallized was removed by filtration, and the filtrate was evaporated to dryness in vacuo. The residue thus obtained was recrystallized from chloroform–petroleum ether (Norit A) to give 1.2 g (69%) of the title compound: mp 99–101 °C; ^1H NMR ($CDCl_3$) δ 7.9 and 7.4 (2d, 4 H, aromatic H), 3.6–3.9 (m, 4 H, CH_2CH_2), 3.5 [(s, 6 H, $(SO_2CH_3)_2$], and 2.4 (s, 3 H, $ArCH_3$). Anal. ($C_{11}H_{17}ClN_2O_6S_3$) C, H, N.

The following 1-(2-chloroethyl)-1,2,2-tris(sulfonyl)hydrazines were synthesized by using procedures similar to those described above.

2,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-1-[(4-methoxyphenyl)sulfonyl]hydrazine (15): yield, 68%; mp 109–110 °C; ^1H NMR ($CDCl_3$) δ 7.9 and 7.0 (2d, 4 H, aromatic H), 3.9 (s, 3 H, OCH_3), 3.5–3.8 (m, 4 H, CH_2CH_2), and 3.5 (s, 6 H, $2CH_3$). Anal. ($C_{11}H_{17}ClN_2O_7S_3$) C, H, N.

2,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-1-[(4-chlorophenyl)sulfonyl]hydrazine (16): yield, 69%; mp 122–123 °C; ^1H NMR ($CDCl_3$) δ 7.9 and 7.5 (2d, 4 H, aromatic H), 3.6–4.0 (m, 4 H, CH_2CH_2), and 3.5 (s, 6 H, $2CH_3$). Anal. ($C_{10}H_{14}Cl_2N_2O_6S_3$) C, H, N.

2,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-1-[(4-bromophenyl)sulfonyl]hydrazine (17): yield, 45%; mp 117–118 °C; ^1H NMR (acetone-d_6) δ 7.9–8.0 (2d, 4 H, aromatic H), 3.7–4.1 (m, 4 H, CH_2CH_2), and 3.6 (s, 6 H, $2CH_3$). Anal. ($C_{10}H_{14}BrClN_2O_6S_3$) C, H, N.

Antitrypanosomal Activity. The antitrypanosomal activity of compounds **1–3** and **5** was determined in female CD-1 mice of 6–8 weeks of age and 25–30 g body weight. Mice were infected intraperitoneally with 10^6 *T. rhodesiense* (Y Tat 1.1). If untreated, mice die in 4 days after infection, when the parasitemia reaches (1–2) × 10^9 cells/mL. Infected mice were treated with a single dose of the test agent when the parasitemia reached approximately 10^6 cells/mL. The number of days the mice survived beyond that of the untreated controls receiving vehicle only was used as a measure of trypanocidal activity. The level of parasitemia in treated mice was measured at regular intervals as described previously[4] to distinguish between parasite-related and test agent toxicity-related deaths; no toxic deaths were observed in these studies. Mice surviving 30 days or more were invariably cured and did not subsequently relapse. Compounds were administered by intraperitoneal injection dissolved in 50 μL of dimethyl sulfoxide. The loss of biological activity of compounds **1** and **2** upon aging of aqueous solutions was measured by adding 100 μL of 1 M solution in dimethyl sulfoxide to 9.9 mL of 200 mM potassium phosphate buffer (pH 7.6) at 37 °C. Aliquots were removed at various time points and 20 μL of this solution per g of body weight was injected intraperitoneally into mice infected with *T. rhodesiense* (10^8 cells/mL of blood) to give an effective initial dose of

0.2 mmol/kg of the test agent. The number of days the mice survived beyond that of mice treated with phosphate buffer containing 1% dimethyl sulfoxide was used as a measure of biological activity.

Measurement of Methanol Generation. The generation of methanol was assayed by placing 2.2 mL aliquots of a 1:200 dilution of aged solutions of the composition described above into a Gilson Oxygraph. Twenty microliters of *Pichia pastoris* alcohol oxidase (666 units/mL) was then added and the resultant O_2 consumption was used as a measure of methanol content.

Antineoplastic Activity. The ascites cell forms of leukemias L1210 and P388 were obtained from the Frederick Cancer Research Facility DCT Tumor Repository of the National Cancer Institute and Sarcoma 180 ascites cells were obtained from stocks available at the Yale Comprehensive Cancer Center; these lines were maintained by serial passage in tissue culture. Every 8 weeks, the tumor cells were injected intraperitoneally into five donor mice (CD_2F_1) 8-10 weeks of age and were allowed to grow for 7 days. The peritoneal fluid was withdrawn and the suspension centrifuged for 5 min (1600g). The supernatant was decanted and 1 $\times 10^5$ cells/mL were seeded in 10 mL of RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% glutamine, and once again maintained in culture. For the assay, 0.1 mL of the cell suspension containing 10^5 cells (10^6 cells in the case of the P388 leukemia and Sarcoma 180) was injected into each recipient mouse. The test compounds were administered over a wide range of dosage levels in the case of the L1210 leukemia and for compound **5** at 60 mg/kg for Sarcoma 180 and the P388 leukemia, beginning 24 h after tumor implantation, once daily for 6 consecutive days. Compound **3** was also administered as single doses as noted in Table I. All drugs were administered intraperitoneally as solutions in dimethyl sulfoxide in a volume not exceeding 0.025 mL. For any one experiment, animals were distributed into groups of five mice of comparable weight and maintained throughout the course of the experiment on Purina Laboratory Chow pellets and water ad libitum. Control tumor-bearing mice given comparable volumes of vehicle were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of these neoplasms to these agents was based upon the prolongation of survival time afforded by the drug treatments.

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Monophosphoric Acid Diesters of 7β-Hydroxycholesterol and of Pyrimidine Nucleosides as Potential Antitumor Agents: Synthesis and Preliminary Evaluation of Antitumor Activity

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7β-Hydroxycholesterol, which has been shown to be selectively cytotoxic toward tumor cells cultered in vitro, was converted into the corresponding water-soluble phosphoric acid ester and linked to a pyrimidine nucleoside such as 5-fluoro-2′-deoxyuridine or 2′-deoxyuridine. 2-Chlorophenyl phosphorodichloridate (**3**), without activation, was used directly to phosphorylate the protected oxygenated sterol. The intermediate phosphorylated the 5′-OH group of nucleoside selectively, leading to compounds **1a** and **1b** after deprotection. These compounds were screened for their antiproliferative activity toward EL-4 murine leukemia cells in vitro and for their antitumor activity against the mice bearing Krebs II ascitic carcinoma in vivo.

Introduction

Sterols and triterpenes bearing several oxygen functions have demonstrated a wide variety of biological activities[1,2] expressed, in particular, by the inhibition of several steps in the biosynthesis of cholesterol.[3,4] Our laboratory has long been interested in the selective cytotoxicity of these compounds. A series of polyoxygenated sterols and triterpenes either isolated from traditional antitumor remedies or synthesized have been shown to be much more toxic toward tumor cells than toward normal ones.[5,6] Another important effect of this class of compounds is their action on the cell membrane.[7-9] This effect is different from that of some classical antitumor drugs such as nucleoside analogues, whose action focuses mainly on the cell cycle. 7β-Hydroxycholesterol (7β-OHC), one of these oxysterols, has been intensively studied because of its high activity and its relatively easy synthesis.[10] Unfortunately, the low water solubility of 7β-OHC makes its use difficult for in vivo studies. For this reason, we have used more water-soluble derivatives. The sodium bis(hemisuccinate) of 7β-hydroxycholesterol (BHS-7β-OHC) has been synthesized, and its antitumor activity on mice bearing Krebs II ascitic carcinoma gave very encouraging results.[11] However, its moderate water-solubility (<2%) did not satisfy our requirements in further biological assays and this led us to undertake the synthesis of more elaborate

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