aminetetraacetic acid. Cells were washed in PBS and resuspended, and 50-μL volumes containing 1.5×10^6 cells were added to each tube. They were incubated for 30 min at ambient temperature, washed two times, and then resuspended in 100-μL volumes of PBS. An equal volume of fluorescein isothiocyanate (FITC) conjugated rabbit anti-horse IgG (Miles-Yeda, Ltd, Israel) at a dilution of 1:30 was added to the lymphocytes or FITC rabbit anti-rat IgG (Cappel Laboratories, Cochranville, PA) at a dilution of 1:25 to the SW1116 cells. They were allowed to incubate for an additional 30 min at ambient temperature, washed two times with PBS, and resuspended in a 1:1 mixture of 50% glycerol and PBS. Fluorescence was scored 0–4+ with a Zeiss fluorescence microscope with epiillumination and a halogen light source. End point titers were recorded at the dilution of antibody that gave 0 (i.e., background) fluorescence.

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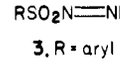
Synthesis and Evaluation of *N,N'*-Bis(arylsulfonyl)hydrazines as Antineoplastic Agents

Krishnamurthy Shyam, Lucille A. Cosby, and Alan C. Sartorelli*

Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06510. *Received July 23, 1984*

Several *N,N'*-bis(arylsulfonyl)hydrazines, with the potential to function as biological methylating agents, were synthesized and evaluated for antineoplastic activity against the L1210 leukemia and other transplanted rodent tumors. In general, the *N*-methyl-*N,N'*-bis(arylsulfonyl)hydrazines that possess the capacity to generate an alkylating species under physiological conditions showed significant antineoplastic activity, while *N,N'*-bis(phenylsulfonyl)-hydrazine and *N*-methyl-*N,N'*-dibenzoylhydrazine were inactive.

N-Acyl-*N'*-(arylsulfonyl)hydrazines (**1**) are decomposed by bases to form aldehydes in moderate yields (Scheme I);[1] this reaction often proceeds at high temperatures.[2] Replacement of the acyl group in compound **1** by an arylsulfonyl moiety, to form an *N,N'*-bis(arylsulfonyl)-hydrazine, enhances the acidity of the proton β to the leaving group and a reaction analogous to the one depicted in Scheme I can occur with greater facility. The reaction intermediate in this case would be **3** and the product, an arenesulfinic acid. While species **3** would be expected to

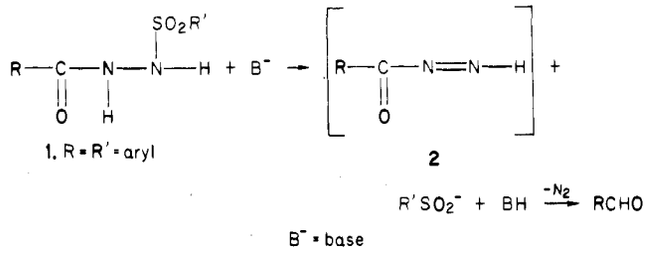


3, R = aryl

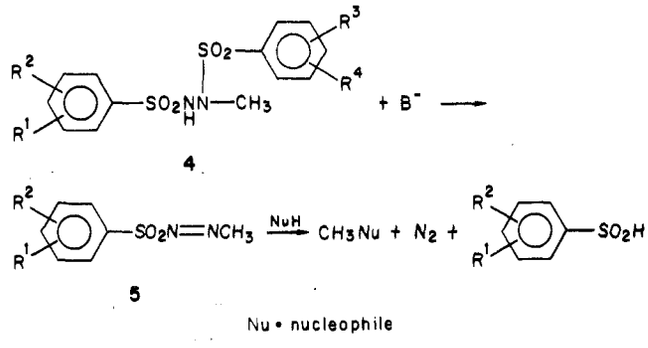
lose a molecule of nitrogen readily to give the corresponding arenesulfinic acid, a compound or intermediate such as **5**, generated from the precursor molecule **4**, would be less prone to such decomposition. Furthermore, since arenesulfinate is a good leaving group, compound **4** may function as an alkylating agent in a manner analogous to the *N*-alkyl-*N*-nitrosoureas, which generate as biological alkylating agents alkanediazohydroxides[3] (Scheme II).

Methylating agents form a useful group of antineoplastic agents, with procarbazine, streptozotocin, and dacarbazine being clinically active methylating agents. To exploit further the impact of methylation in cancer chemotherapy, we have synthesized a new class of potential methylating agents and have tested them for antineoplastic activity against the L1210 leukemia and other transplanted rodent tumors.

Scheme I



Scheme II



Chemistry. Bis(arylsulfonyl)hydrazines (**6–11**) were prepared by reacting the appropriate arenesulfonyl chloride with hydrazine or methylhydrazine in a 2:1 molar ratio

(1) McFadyen, J. S.; Stevens, T. S. *J. Chem. Soc.* **1936**, 584.
(2) Mosettig, E. *Org. React.* **1954**, *8*, 234.
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(4) Dzhidzhelava, A. B.; Konovalova, M. Y.; Kostenko, V. I.; Dykhanov, N. N. *Zh. Obshch. Khim.* **1966**, *36*, 1368; *Chem. Abstr.* **1967**, *66*, 10458t.
(5) Mukherjee, R. *J. Chem. Soc. D* **1971**, 1113.

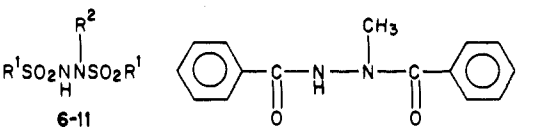
0022-2623/85/1828-0525$01.50/0

Table I. Effects of *N,N'*-Bis(arylsulfonyl)hydrazines and *N*-Methyl-*N,N'*-dibenzoylhydrazine on the Survival Time of Mice Bearing the L1210 Leukemia

compd	daily dose,[a,b] mg/kg	av Δ wt,[c] %	av survival time of control animals, days ± SE	av survival time of treated animals, days ± SE	% T/C[d]
6	50	−0.3	9.5 ± 0.3	9.2 ± 0.2	97
	100	+5.8		9.2 ± 0.2	97
	150	+0.8		9.6 ± 0.6	101
7[e]	50	−4.8	9.0 ± 0.0	13.2 ± 0.7	147
	100	−5.2		11.6 ± 0.2	129
	150	−13.5		9.4 ± 0.4	104
8[f]	50	+3.7	9.5 ± 0.3	14.0 ± 1.3	147
	100	+0.8		13.6 ± 2.4	143
9[g]	50	+10.9	9.0 ± 0.0	13.4 ± 1.0	149
	100	+1.9		13.2 ± 1.1	147
	150	−1.6		12.2 ± 1.2	136
10	50	+4.1	9.5 ± 0.3	9.2 ± 0.2	97
	100	+4.0		11.4 ± 0.8	120
	150	+1.3		11.8 ± 1.1	124
11	50	+6.9	9.0 ± 0.0	11.4 ± 1.1	127
	100	+3.0		10.2 ± 0.4	113
	150	−3.3		14.0 ± 2.3	156
12	50	+4.1	9.5 ± 0.3	9.0 ± 0.0	95
	100	+2.5		9.2 ± 0.2	97
	150	−2.0		9.2 ± 0.2	97

[a] Administered once daily for six consecutive days, beginning 24 h after tumor transplantation. [b] Number of mice per group equals five. [c] Average change in body weight from onset to termination of therapy. [d] % T/C = average survival time of treated/control animals × 100. [e] Maximum % T/C vs. Sarcoma 180 = 203 at 100 mg/kg. [f] Maximum % T/C vs. Sarcoma 180 = 197 at 50 mg/kg. [g] Maximum % T/C vs. P388 leukemia = 136 at 150 mg/kg.

Table II. Physical Constants for *N,N'*-Bis(arylsulfonyl)hydrazines and *N*-Methyl-*N,N'*-dibenzoylhydrazine



6-11

12

compd	R^1	R^2	yield, %	mp, °C	formula	anal.
6	phenyl	H	72	228–229[a]	$C_{12}H_{12}N_2O_4S_2$	C, H, N, S
7	phenyl	CH_3	36	172–173	$C_{13}H_{14}N_2O_4S_2$	C, H, N, S
8	*p*-tolyl	CH_3	28	152–154	$C_{15}H_{18}N_2O_4S_2$	C, H, N
9	*p*-methoxyphenyl	CH_3	37	195–197	$C_{15}H_{18}N_2O_6S_2$	C, H, N
10	*p*-chlorophenyl	CH_3	13	202–204	$C_{13}H_{12}Cl_2N_2O_4S_2$	C, H, N
11	2-naphthyl	CH_3	37	194–195	$C_{21}H_{18}N_2O_4S_2$	C, H, N
12			76	142–144[b]	$C_{15}H_{14}N_2O_2$	C, H, N

[a] Lit.[4] 231 °C. [b] Lit.[5] 145 °C.

in pyridine. A similar procedure was employed for the preparation of *N*-methyl-*N,N'*-dibenzoylhydrazine (**12**) in which benzoyl chloride was used in place of arenesulfonyl chloride.

Biological Results and Discussion

The tumor-inhibitory properties of compounds **6–12** were determined by measuring their effects on the survival time of mice bearing the L1210 leukemia. Compounds **7** and **8** were also evaluated for anticancer activity against Sarcoma 180 tumor-bearing mice and compound **9** against the P388 leukemia. The results of these tests are summarized in Table I.

In general, the *N*-methyl-*N,N'*-bis(arylsulfonyl)-hydrazines showed significant activity against the L1210 leukemia, with the most active compounds increasing the survival time of the treated mice by approximately 40–50%. The inactivity of compound **6** emphasized the importance of the methyl group on the hydrazide nitrogen and is consistent with the mechanism of activation proposed in Scheme II. Compound **12**, which also was inactive against the L1210 leukemia, should not be an effective generator of an alkylating species, since the hydrazide proton is not sufficiently acidic to be abstracted at physiological temperature and pH and, in addition, the benzoyl anion is a relatively poor leaving group. The inactivity of compound **10** is not explained by these considerations and may be due to pharmacodynamic mechanisms. The compounds of this class active against the L1210 leukemia also showed significant anticancer activity against Sarcoma 180 and the P388 leukemia.

Experimental Section

Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were determined with a Varian T-60A spectrometer with Me_4Si as an internal standard. The spectral measurements were as expected; therefore, routine data are not included. 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 the theoretical values. Pertinent physical data for the compounds synthesized are listed in Table II.

Antitumor Activity. The ascites cell forms of the L1210 leukemia, Sarcoma 180, and P388 leukemia were propagated in CDF_1 mice. Transplantation was carried out by withdrawing peritoneal fluid from donor mice bearing 7-day tumor growths. The suspension was centrifuged for 2 min (1600*g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to a level of 10^7 cells/mL for Sarcoma 180 and P388 leukemia and 10^6

cells/mL for the L1210 leukemia. A portion (0.1 mL) of the resulting cell suspension was injected intraperitoneally into each recipient animal. Dosage levels of all compounds except 8 were administered over a range of 50–150 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. 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 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 ascitic neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatments.

General Procedure for the Preparation of *N,N'*-Bis(arylsulfonyl)hydrazines. The appropriate arenesulfonyl chloride (0.02 mol) was added in portions to an ice-cold, stirred solution of hydrazine or methylhydrazine (0.01 mol) in pyridine (4 mL) over a period of 20 min. After an additional 30 min, the reaction mixture was poured into a mixture of 25 mL of ice and concentrated hydrochloric acid (1:1, v/v). The solid that separated was filtered immediately, washed with cold water, and dried. Recrystallization from glacial acetic acid afforded the analytically pure product.

N-Methyl-*N,N'*-dibenzoylhydrazine (12) was prepared with use of benzoyl chloride in a procedure analogous to the one described for the *N,N'*-bis(arylsulfonyl)hydrazines.

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Registry No. 6, 6272-36-2; 7, 94905-07-4; 8, 94905-08-5; 9, 94905-09-6; 10, 94905-10-9; 11, 94905-11-0; 12, 21150-15-2; hydrazine, 302-01-2; methylhydrazine, 60-34-4; benzenesulfonyl chloride, 98-09-9; 4-methylbenzenesulfonyl chloride, 98-59-9; 4-methoxybenzenesulfonyl chloride, 98-68-0; 4-chlorobenzenesulfonyl chloride, 98-60-2; 2-naphthalenesulfonyl chloride, 93-11-8; benzyl chloride, 98-88-4.

Book Reviews

Development of Target-Oriented Anticancer Drugs (Progress in Cancer Research and Therapy. Volume 28). Edited by Yung-Chi Cheng, Barry Goz, and Mimi Minkoff. Raven Press, New York. 1983. xv + 246 pp. 18 × 26 cm. ISBN 0-89004-161-X. $39.00.

This book is a collection of papers presented in a symposium sponsored by the Cancer Research Center and other departments of North Carolina School of Medicine at Chapel Hill in 1983. It is divided into three parts: membrane transport, enzyme activity, and gene function.

An introductory chapter entitled "Cancer Chemotherapy and the Medicinal Chemist" was provided by J. A. Montgomery, who emphasized that drug development is a closely integrated team effort which should consist of medicinal chemists, chemotherapists, and biochemical pharmacologists. Montgomery believed, with examples of work done in his laboratory, that the most approachable area of research is the design of enzyme inhibitors. This is certainly true, provided we recognize the fact that interference with enzyme functions does not necessarily produce anticancer or even cytotoxic agents and sometimes may even produce toxicity.

Four papers were presented under the membrane transport approach. I. D. Goldman et al. used computer simulations and computer analyses to study the cellular pharmacology of 4-aminoantifolates and found that many previously reported concepts with regard to the antifolate pharmacology were incorrect. They concluded that, among factors which contribute to the antifolate action, a study of the structural sites of antifolate molecules for binding to DHFR may not be as important as to exploit the sites that govern the transport and polyglutamylation. Of course, as the authors pointed out, that structural modifications that favorably alter one parameter could deleteriously alter another parameter, and therefore structural modifications that enhance the transport or polyglutamylation in tumors may not necessarily provide similar advantages in host tissues. A. R. P. Paterson et al. found that nitrobenzylthioinosine (NBMPR) is a potent inhibitor of nucleoside transport, and since neoplastic cells differ from host tissues in the NBMPR sensitivity, the difference may be used to preferentially destroy the neoplastic cells with combinations of NBMPR-P and high doses of cytotoxic adenosine analogues, such as tubercidin or nebularine. W. H. Prusoff reported the historical development of nitrosourea nucleosides as anticancer agents and offered a number of approaches to the development and delivery of nitrosourea drugs. Concepts in new folate analogues design were presented by F. M. Sirotnak, who suggested that positions 5, 10, and the γ-carboxyl group (the site for polyglutamylation) of antifolates could be the logical points for structural modification. The author also pointed out that much information gathered are still controversial and further study is needed.

Under the area of enzyme activity approach, seven groups of investigators presented their views. J. Jolivet and B. A. Chabner discussed their studies on methotrexate polyglutamates (MTXPG) in cultured human breast cancer cells and confirmed that the MTXPGs, which retained much longer intracellularly than MTX, are stronger inhibitors of human thymidylate synthetase in vitro than the parent antifolate. The prolonged tissue retention of the MTXPGs may also be responsible for some chronic drug toxicities. Since leucovorin (5-CHO-FH_4) prevents the formation of higher MTXPG derivatives from MTX by interfering with the action of folylpolyglutamate synthetase, the enzyme responsible for polyglutamation, this preventive action may be related to the mechanism of leucovorin rescue. J. J. McGuire et al. gave a detailed report on their study of folylpolyglutamate synthetase (FPGS) and various agents tested for substrate and/or inhibitory activity of rat liver FPGS. They believed that searching for certain prodrugs, which are substrates for FPGS and are converted to potent enzyme inhibitors in vivo, could be an approach for new drug development. An example of such a prodrug was illustrated by the authors as 5,8-dideazaisopteroylglutamate (IAHQ). Y.-C. Cheng and R. W. Brockman suggested the use of the principle of collateral sensitivity—the phenomenon of increased sensitivity of tumor cells resistant to one drug to a second drug with a different mode of action—could be a fruitful approach for new drug development, provided that the biochemical mechanisms of resistance (such as transport defects for a drug, increased enzymic catebolism of a drug, deficiency of drug-activating enzyme, increase in the level of target enzyme, etc.) be understood and more information on mechanisms of clinical resistance be gathered. This valuable and practical approach, therefore, is useful only in designing compounds with known mechanism of action. Y. M. Rustum, using the example of a FU-prodrug 5'-deoxy-5-fluorouridine (dFUR, not to be confused with the well-known 5-FUDR, which is 2'-deoxy-5-fluorouridine), emphasized the development of prodrugs that should be activated in target tissues rather than in biological systemic compartments for selective anticancer activity. T. M. Saverese et al. discussed various bio-