# 1,2-Bis(arylsulfonyl)hydrazines. 2. The Influence of Arylsulfonyl and Aralkylsulfonyl Substituents on Antitumor and Alkylating Activity

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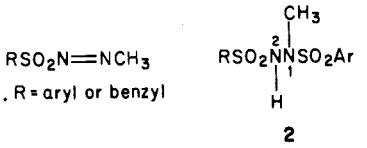
Several 1,2-bis(arylsulfonyl)-1-methylhydrazines were synthesized and evaluated for antineoplastic activity against the L1210 leukemia. The most active compound to emerge from this study, 2-[(4-chlorophenyl)sulfonyl]-1-methyl-1-(4-tolylsulfonyl)hydrazine, increased the survival time of tumor-bearing mice by 88%. The alkylating activity of the synthesized analogues and several compounds reported earlier was determined by measuring the absorbance at 540 nm of the alkylated product of 4-(4-nitrobenzyl)pyridine. The results obtained support the concept that the ability to alkylate is a necessary but not a sufficient condition for the expression of antitumor activity by agents of this class.

1-Methyl-1,2-bis(arylsulfonyl)hydrazines represent a new class of antineoplastic agents synthesized by our laboratory with demonstrable effectiveness against the L1210 leukemia in mice;[1] the formation of the putative alkylating species (1) was hypothesized to account for the observed




2

biological activity of compounds of this class. From purely chemical considerations, two factors that potentially influence the rate of breakdown of compounds of the general structure 2 to a reactive species are (a) the acidity of the hydrazine proton and (b) the leaving group ability of the arenesulfinate.

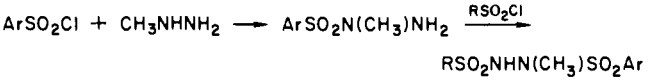
This paper describes the synthesis and evaluation against the L1210 leukemia of compounds in which (a) the arylsulfonyl or aralkylsulfonyl group attached to N-2 is varied while the leaving group is kept constant and (b) the leaving group is varied with a constant arylsulfonyl moiety attached to N-2. The investigations also provide evidence, using a modification of the method of Wheeler and Chumley,[2] that these compounds decompose in solution to generate species capable of alkylation.

**Chemistry.** Compounds **3–7** (Table I) and **8**[6] were prepared by the method shown in Scheme I. Compounds **3–5** and **8** were synthesized by reacting the appropriate arenesulfonyl chloride or aralkylsulfonyl chloride with 1-methyl-1-(4-tolylsulfonyl)hydrazine[3] in pyridine. Similarly, the reaction of the appropriate 1-methyl-1-(arylsulfonyl)hydrazine with *p*-toluenesulfonyl chloride in pyridine gave compounds **6** and **7**. 1-Methyl-1-(4-tolylsulfonyl)hydrazine, 1-methyl-1-[(4-chlorophenyl)sulfonyl]hydrazine, and 1-methyl-1-[(4-methoxyphenyl)sulfonyl]hydrazine were prepared by published methods.[3-5]

## Results and Discussion

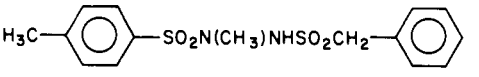
The tumor inhibitory properties of compounds **3–8** were determined by measuring their effects on the survival time of mice bearing the L1210 leukemia; the results are summarized in Table II. All of the compounds synthesized,

**Scheme I**




with the exception of **6**, displayed activity against the L1210 leukemia; the active agents increased the survival time of tumor-bearing mice between 41% and 88% at their optimal dosage levels. No clearcut correlation between the acidity of the hydrazine proton and antineoplastic activity could be discerned in this system. Thus, compound **8**, in which an aralkylsulfonyl group is attached to NH, was essentially equiactive with compounds **3–5** and **7**. In addition, no discernible correlation was observed between the leaving group ability of the arylsulfonyl substituent and activity against the L1210 leukemia. Thus, it is likely that the alkylation mechanism proposed in an earlier report[1] involves complex kinetics, an interpretation supported by the fact that the two arylsulfonyl moieties can function as leaving groups in two different reactions (i.e., an elimination reaction in which the putative alkylating species is generated, and a substitution reaction in which a nucleophile is alkylated). Furthermore, one cannot rule out the importance of pharmacodynamic mechanisms in the antitumor measurements.

Since compounds **3–8** were conceived as prodrugs of the putative alkylating species (1), a modification of the method of Wheeler and Chumley[2] was used to determine whether these compounds possessed alkylating ability. This methodology measured the absorbance at 540 nm of the alkylated product of 4-(4-nitrobenzyl)pyridine. The data for compounds **3–7**, as well as for **8–12** which were synthesized earlier,[1,6] are listed in Table III.

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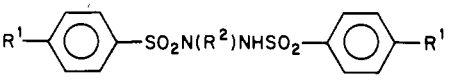
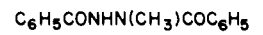



9, $R^1 = R^2 = CH_3$
10, $R^1 = Cl$, $R^2 = CH_3$
11, $R^1 = R^2 = H$




12

As expected, compounds **11** and **12** showed negligible alkylating ability under the conditions employed, and these agents were inactive against the L1210 leukemia. Of the 1-methyl-1,2-bis(arylsulfonyl)hydrazines tested, the com-

(1) Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1985**, *28*, 525.
(2) Wheeler, G. P.; Chumley, S. *J. Med. Chem.* **1967**, *10*, 259.
(3) Nürrenbach, A.; Pommer, H. *Justus Liebigs Ann. Chem.* **1969**, *721*, 34.
(4) Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.* **1985**, *28*, 149.
(5) Hrubiec, R. T.; Shyam, K.; Cosby, L. A.; Sartorelli, A. C. *J. Med. Chem.*, in press.
(6) Hrubiec, R. T.; Shyam, K.; Cosby, L. A. Furubayashi, R.; Sartorelli, A. C. *J. Med. Chem.*, in press.

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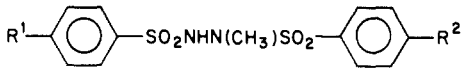
**Table I.** Physical Constants for 1,2-Bis(arylsulfonyl)-1-methylhydrazines



3-7

| compd | $R^1$ | $R^2$ | yield, % | mp, °C | formula | anal. |
|---|---|---|---|---|---|---|
| 3 | H | $CH_3$ | 41 | 171–173 | $C_{14}H_{16}N_2O_4S_2$ | C, H, N |
| 4 | $OCH_3$ | $CH_3$ | 35 | 159–160 | $C_{15}H_{18}N_2O_5S_2$ | C, H, N |
| 5 | Cl | $CH_3$ | 45 | 176–178 | $C_{14}H_{15}ClN_2O_4S_2$ | C, H, N |
| 6 | $CH_3$ | $OCH_3$ | 16 | 192–193 | $C_{15}H_{18}N_2O_5S_2$ | C, H, N |
| 7 | $CH_3$ | Cl | 70 | 174–176 | $C_{14}H_{15}ClN_2O_4S_2$ | C, H, N |

**Table II.** Effects of 1,2-Bis(arylsulfonyl)-1-methylhydrazines on the Survival Time of Mice Bearing the L1210 Leukemia

| compd | daily dose,[a] mg/kg | av Δ wt,[b] % | av survival time of treated animals, days ± SE | % T/C[c] |
|---|---|---|---|---|
| 3 | 50 | +2.0 | 15.0 ± 1.4 | 162 |
| | 100 | +5.4 | 10.6 ± 0.2 | 115 |
| | 150 | −1.7 | 10.8 ± 0.5 | 117 |
| 4 | 50 | +7.7 | 10.8 ± 0.6 | 116 |
| | 100 | +7.8 | 15.0 ± 2.3 | 162 |
| | 150 | +2.9 | 13.2 ± 2.2 | 143 |
| 5 | 50 | +3.7 | 17.4 ± 1.4 | 188 |
| | 100 | +10.0 | 10.8 ± 0.6 | 117 |
| | 150 | +6.0 | 11.2 ± 1.3 | 121 |
| 6 | 50 | +12.6 | 10.2 ± 0.5 | 111 |
| | 100 | +14.8 | 10.2 ± 0.2 | 111 |
| | 150 | +9.8 | 10.4 ± 0.2 | 113 |
| 7 | 50 | +5.1 | 10.4 ± 0.4 | 113 |
| | 100 | +5.5 | 13.0 ± 2.2 | 141 |
| | 150 | −4.6 | 10.0 ± 0.3 | 109 |

[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] % T/C = average survival time of treated/control animals × 100. Each value represents the average of five animals per group. The average survival time of untreated tumor-bearing animals was 9.2 ± 0.2 days.

**Table III.** Comparison of the Degree of Alkylation of 4-(4-Nitrobenzyl)pyridine and Antineoplastic Activity of 1,2-Bis(sulfonyl)hydrazines and 1,2-Dibenzoyl-1-methylhydrazine

| compd | rel alkylating act.[a] | antineoplastic act.[b] (max % T/C at dosage levels examined) |
|---|---|---|
| 11 | 0 | 101 |
| 12 | 10 | 97 |
| 6 | 85 | 113 |
| 4 | 91 | 162 |
| 9 | 100 | 147 |
| 3 | 112 | 162 |
| 5 | 126 | 188 |
| 8 | 136 | 174 |
| 7 | 163 | 141 |
| 10 | 190 | 124 |

[a] Relative alkylating activity = (absorbance at 540 nm of test sample/absorbance at 540 nm of compound 9) × 100. The absorbance value used in each case was the average of two determinations, with the values being within ±5% of each other. [b] Activity against the L1210 leukemia (Table II and ref 1 and 6).

pounds that gave the highest and lowest absorbance values (i.e., alkylating activity), compounds **10** and **6**, respectively, were also inactive against this tumor. It is conceivable that compound **10** is inactivated prior to reaching an intracellular site of action due to its rapid decomposition to a reactive electrophile. Compounds **5** and **7**, two other chloro-containing compounds that decomposed less rapidly than compound **10**, exhibited antineoplastic activity against the L1210 leukemia. The reason for the inactivity of compound **6** is not evident, especially since compound **4**, which does not give an appreciably different degree of alkylation with the trapping agent 4-(4-nitrobenzyl)-pyridine, is an active anticancer agent (% T/C = 147 at 50 mg/kg).[1] In general, however, there appeared to be a correspondence between the capacity to generate a reactive species and inhibitory activity against the L1210 leukemia. The lack of an absolute correlation, however, implies that other factors are operative, including the necessity for an optimal rate of breakdown to a reactive species to achieve antineoplastic activity.

## Experimental Section

Melting points were recorded on a Thomas-Hoover 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 I.

**General Procedure for the Preparation of 1-Methyl-1,2-bis(arylsulfonyl)hydrazines.** The appropriate arenesulfonyl chloride (0.01 mol) was added in portions to an ice-cold stirred solution or suspension of the appropriate 1-methyl-1-(arylsulfonyl)hydrazine (0.01 mol) in pyridine (2 mL) while the temperature was maintained between 0 and 5 °C. After an additional 3 h, the reaction mixture was poured into a mixture of ice and concentrated hydrochloric acid (25 mL, 1:1, v/v). The solid that separated was filtered immediately, heated with glacial acetic acid (3 mL), and cooled. The precipitate obtained was filtered, washed with cold water, and dried. Recrystallization from chloroform–carbon tetrachloride (6:1, v/v) gave the pure compound.

**Antitumor Activity.** The ascites cell form of the L1210 leukemia was 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 $10^6$ cells/mL. A portion (0.1 mL) of the resulting cell suspension (containing $10^5$ cells) was injected intraperitoneally into each recipient animal. Dosage levels of all compounds 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 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 through 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 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.

**Determination of Alkylating Activity of Compounds 3–12.** A solution of the test sample (6 μmol) in acetone (1 mL), distilled water (2 mL), and Tris-HCl buffer (pH 7.4; 1 mL) was incubated with 4-(4-nitrobenzyl)pyridine (148 μmol in 0.4 mL of acetone) at 37 °C for 5 min. Following addition of acetone (2 mL) and 0.25 M sodium hydroxide solution (1.5 mL), the material was extracted with ethyl acetate (5 mL). The absorbance was determined at 540 nm 0.5 min after the addition of the sodium hydroxide solution.

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# *Book Reviews*

**Modern Practice of Gas Chromatography.** Edited by Robert L. Grob. Wiley-Interscience, New York. 1985. 895 pp. 16.5 × 24 cm.

This book provides a good introduction and overview to the theory and practice of gas chromatography. Its primary strengths are its good coverage of the basic theory and instrumentation and some application chapters covering environment, food, petroleum, polymer, drug, and clinical analyses. Little attention is given to capillary GC, including some of the current topics like injection techniques, wide-bore columns, bonded phases, and column switching.

The book is valuable for one beginning in GC, but those practicing in this field will need to use monographs for in-depth discussion of special GC topics.

*Clinical Chemistry* **R. Giese**
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**Annual Reports in Organic Synthesis.** Edited by Martin J. O'Donnell and Louis Weiss. Academic Press, New York. 1985. xiii + 465 pp. 15 × 23 cm. ISBN-0-12-040815-5. $29.00

This excellent and relatively inexpensive series of summaries of synthetic methods continues in a 1984 edition. The organization is highly logical and simple and encourages the practicing organic chemist to keep the book on the bench shelf and consult it very frequently. Not the least of its attractions is the section on protecting groups, to which one might also add the section on useful synthetic preparations and a list of reviews that the editors have found particularly attractive.

In view of the burgeoning synthetic literature, which as the compilers correctly point out, is becoming more and more difficult to access completely, this compilation is of immediate practical value to all synthetic organic chemists. It deserves wide circulation.

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**Advances in Drug Research. Volume 14.** Edited by Bernard Testa. Academic Press, London. 1985. ix + 339 pp. 15.5 × 23.5 cm. ISBN 0-12-013314-8. $69.50

The four chapters in this volume deal with widely varied but generally interesting topics. This has characterized the series over the years. The first chapter covers the topic of deuterium effects in the metabolism of drugs. It is well written and documented. The organization is based on chemical class with generous use of structures.

The second chapter is a definitive treatment of drug design based upon the realization of the three dimensions occupied by drugs and receptors. The chapter begins with a background of classical considerations and then swiftly moves into computer-based displays of molecules in space. Stereochemical considerations of several drug classes are described followed by applications to drug design in five categories. The author makes generous use of colored plates reproduced from computer-modeling exercises. The bibliography is extensive. This is an excellent treatise for anyone working in this area.

The third chapter is a short but useful review of the mechanisms of action of antiinflammatory drugs. The final chapter is an extensive review of benzodiazepine receptors and structure–activity relationships. This is a very thorough analysis of the literature that is cited on over 22 pages. Numerous tables document the search for structure–activity relationships and will prove useful to investigators in this area.

Overall, the volume continues the fine tradition of this series, and it belongs in libraries serving medicinal chemists, pharmacologists, and specialists in these areas.

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**The Alkaloids, Chemistry and Pharmacology. Volume 26.** Edited by A. Brossi. Academic Press, New York. 1985. xi + 401 pp. 16 × 23 cm. ISBN 0-12-469526-4. $95.00

Any new volume of *The Alkaloids* is always welcome, and this latest addition to the series is no exception. It covers the following topics: the simple indole alkaloids (H.-P. Husson); sulfur-containing alkaloids (J.T. Wróbel); pyridine and piperidine alkaloids (G. M. Strunz and J. A. Findlay); benzophenanthridine alkaloids (V. Šimánek); Lycopodium alkaloids (D. B. MacLean); peptide alkaloids (U. Schmidt, A. Lieberknecht, and E. Haslinger); and pyrrolizidine alkaloids (J. T. Wróbel).

The editor has succeeded in assembling a distinguished constellation of international authors. The discussions and drawings are uniformly of high quality. *The Alkaloids* series continues to be the most useful compendium on alkaloid chemistry and pharmacology available.

*Department of Chemistry* **Maurice Shamma**
*The Pennsylvania State University*
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**Polycyclic Hydrocarbons and Carcinogenesis.** Edited by Ronald G. Harvey. American Chemical Society, Washington, DC. 1985. vii + 406 pp. 15.5 × 23 cm. ISBN 0-8412-0924-3. $74.95

This book was developed from a symposium on the subject sponsored by the Division of Organic Chemistry of the ACS in August 1984. Sometimes publications of symposia are outdated, contain only abstracts or short summaries of some of the papers, and are not well integrated. However, this publication stands as a remarkable exception to this occasional problem. All of the chapters provide thorough, well-referenced coverage of their topic. Even additional chapters on relevant topics are included that were not part of the original symposium. The integration of the chapters is thorough with appropriate cross references in the discussions. This timely and authoritative book therefore belongs on the shelves of anyone concerned with this subject.