Antineoplastic and Biochemical Properties of Arylsulfonylhydrazones of 2-Formylpyridine N-Oxide[1]

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The structural parameters necessary for the antineoplastic potency of a new class of anticancer agents, arylsulfonylhydrazones of 2-formylpyridine N-oxide, were examined in mice bearing Sarcoma 180 ascites cells. The findings indicated that (a) replacement of the pyridine ring with benzene, quinoline, or isoquinoline resulted in loss of activity, (b) movement of the formylhydrazone side chain from the 2 to the 3 or 4 positions of the pyridine N-oxide produced inactive agents, (c) the pyridine N-oxide function was essential for anticancer activity, except for 4-substituted derivatives which were active without the N-oxide group, (d) replacement of the SO_2 group by CO resulted in complete loss of activity, and (e) a carbon atom could be inserted between the SO_2 and aryl ring with retention of anticancer potency. One of the most active members of this series, 1-oxidopyridine-2-carboxaldehyde p-toluenesulfonylhdrazone, exhibited antineoplastic activity against a broad spectrum of transplanted tumors including Sarcoma 180, Hepatoma 129, Ehrlich carcinoma, leukemia L1210, and a subline of Sarcoma 180 resistant to α-(N)-heterocyclic carboxaldehyde thiosemicarbazones. This agent caused inhibition of thymidine-^3H and uridine-^3H incorporation into DNA and RNA, respectively, of Sarcoma 180 ascites cells; protein biosynthesis was relatively insensitive to the action of this compound.

1-Oxidopyridine-2-carboxaldehyde p-toluenesulfonylhydrazone (1) was synthesized as an intermediate in the preparation of 1-oxido-2-pyridyldiazomethane,[2] which was used for the blocking of nucleoside hydroxyl groups,[2a] nucleotide phosphate groups,[2b] heterocyclic bases,[3] and thio functions.[4] Since it was conceivable that this agent might be similar in biochemical and biological action to the α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, which are known to be potent inhibitors of DNA biosynthesis, and thereby produce both antineoplastic and antiviral activity,[5] systematic modification of a series of arylsulfonylhydrazones of 2-formylpyridine N-oxide was carried out to define the importance of the pyridine ring, the pyridine N-oxide function, the sulfonyl group, and the aromatic moiety of the side chain for antineoplastic activity.

Chemistry. Arylsulfonylhydrazones of 2-formylpyridine N-oxide were synthesized by the two different routes shown in Scheme I; either pyridine-2-carboxaldehyde was first allowed to react with the appropriate sulfonyl hydrazide followed by N-oxidation with m-chloroperbenzoic acid or 2-formylpyridine N-oxide, obtained from 2-hydroxymethylpyridine N-oxide by MnO_2 oxidation, was allowed to react directly with various arylsulfonyl hydrazides. Both procedures produced approximately equivalent yields of final compounds. N-Oxidation of the various formylpyridine hydrazones occurred only at the pyridine ring nitrogen rather than at one of the hydrazone nitrogen atoms. Elemental analyses revealed that only one additional oxygen atom was incorporated into each molecule. The position of this oxidation was determined by proton NMR spectroscopy. It is well known[6] that N-oxidation of pyridine-type nitrogen atoms leads to marked upfield shifts of the resonances of protons adjacent to that nitrogen. For each of the pairs of pyridine–pyridine N-oxide studied, the α-proton resonance of the N-oxide appeared upfield approximately 0.3–0.5 ppm from that of the parent pyridine. For example, in the case of nicotinaldehyde derivatives, the two α-proton signals in **9** appeared at δ 8.93 and 8.78, whereas those of the analogous N-oxide **2** were found at δ 8.58 and 8.40.

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Table I. Effects of Arylsulfonylhydrazones on the Survival Time of Mice Bearing Sarcoma 180 Ascites Cells

Compd	Max effective daily dose, mg/kg[a]	Av Δ wt, %[b]	Av survival time, days ± SE	50-Day survivors,[c] %	% T/C[d]
Control		+23.2	12.5 ± 0.3	0	100
1	60	-8.0	38.4 ± 1.6	0	307
2	60	0.0	13.6 ± 1.3	0	109
3	60	+17.7	15.4 ± 0.9	0	123
4	40	-5.2	42.4 ± 3.1	40	339
5	60	+20.0	15.4 ± 1.3	0	123
6	40	-0.3	35.2 ± 6.2	40	282
7	60	+10.2	11.2 ± 0.4	0	90
8	10	+20.1	15.4 ± 0.9	0	123
9	60	+12.6	13.2 ± 1.3	0	106
10	60	-0.9	28.2 ± 3.2	10	226
11	40	+19.5	12.4 ± 1.1	0	99
12	60	-3.3	21.2 ± 1.8	0	169
13	20	+18.6	13.4 ± 1.0	0	107
14	60	+17.4	11.8 ± 1.3	0	94
15	20	+15.1	14.4 ± 2.2	0	115
16	60	+2.1	13.2 ± 2.1	0	105
17	20	+2.6	13.6 ± 0.7	0	109
18	10	+1.6	13.4 ± 0.5	0	107

[a] Administered once daily for six consecutive days, beginning 24 h after tumor transplantation. The survival times for untreated tumor-bearing animals have been pooled from several experiments and represent 40 mice. Treated groups represent 5–10 animals. [b] Average change in body weight (%) from onset to termination of drug therapy. [c] Mice that survived more than 50 days were calculated as 50-day survivors in determination of the average survival time. [d] % T/C = treated/control × 100.

Biological Results and Discussion. The tumor inhibitory properties of various arylsulfonylhydrazones were determined by measuring their effects on the survival time of mice bearing Sarcoma 180 ascites cells; the results are shown in Table I. The prolongation of life produced by the maximum effective daily dose of each compound is listed; however, a range of daily dosage levels from 10 to 60 mg/kg was tested for each agent. The results indicated that 1-oxidopyridine-2-carboxaldehyde p-toluenesulfonylhydrazone (1) was a potent inhibitor of the growth of Sarcoma 180, increasing the survival time greater than threefold. Replacement of the pyridine ring of the parent

Table II. Effects of 1-Oxidopyridine-2-carboxaldehyde *p*-Toluenesulfonylhydrazone on the Survival Time of Mice Bearing Either Sarcoma 180, Sarcoma 180/IQ-1, Leukemia L1210, Ehrlich Carcinoma, or Hepatoma 129

Daily dosage, mg/kg[a]	Sarcoma 180		Sarcoma 180/IQ-1		Leukemia L1210		Ehrlich ascites carcinoma		Hepatoma 129	
	Av Δ wt, %[b]	Av survival time, days ± SE	Av Δ wt, %[b]	Av survival time, days ± SE	Av Δ wt, %[b]	Av survival time, days ± SE	Av Δ wt, %[b]	Av survival time, days ± SE	Av Δ wt, %[b]	Av survival time, days ± SE
Control	+23.2	12.9 ± 1.2	+29.6	14.4 ± 0.9	+13.6	8.2 ± 0.4	+26.8	14.4 ± 1.2	+13.5	18.0 ± 0.6
20	+21.6	24.9 ± 3.9 (10)[c]			+10.6	8.6 ± 0.4	+2.4	34.6 ± 6.5 (10)[c]	+11.0	22.2 ± 1.0
40	−3.1	36.3 ± 3.3 (20)[c]	+3.4	24.4 ± 1.2	+2.8	9.2 ± 0.2	−3.3	47.0 ± 3.0 (50)[c]	+2.3	24.8 ± 0.5
60	−8.0	38.4 ± 1.6	0.0	24.4 ± 0.5	−5.8	11.8 ± 1.7	−1.8	40.8 ± 4.8 (40)[c]	−1.6	29.4 ± 5.2 (20)[c]

[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 drug therapy. [c] Percent of mice that survived more than 50 days; these animals were calculated as 50-day survivors in determination of the average survival time.

Table III. Effects of Various Dosage Levels of 1-Oxidopyridine-2-carboxaldehyde *p*-Toluenesulfonylhydrazone on the Incorporation of Thymidine-^3H, Uridine-^3H, and DL-Leucine-^{14}C into DNA, RNA, and Protein, Respectively, of Sarcoma 180 Ascites Cells

Dose, mg/kg	% control incorporation[a]		
	Thymidine-^3H into DNA	Uridine-^3H into RNA	Leucine-^{14}C into protein
10	71	74	
20	71	71	95
40	67	65	93
80	59		73

[a] Mice bearing 6-day implants of Sarcoma 180 ascites cells received a single intraperitoneal injection of an indicated dose of 1-oxidopyridine-2-carboxaldehyde *p*-toluenesulfonylhydrazone. One hour later, each animal received the appropriate radioactive tracer by intraperitoneal injection, which was allowed 1 h to be incorporated. The specific activities for control samples were 20.3 cpm/nmol, 11.7 cpm/nmol, and 6520 cpm/mg for thymidine-^3H, uridine-^3H, and leucine-^{14}C incorporation, respectively. The values indicated represent the mean of results from the tumor cells of 4–16 mice analyzed separately.

compound with benzene (**15**), nitrobenzene (**16**), quinoline (**17**), or isoquinoline (**18**) resulted in complete loss of anticancer activity. Movement of the formylhydrazone side chain from the 2 (**1**) to the 3 (**2**) or 4 (**3**) positions of the pyridine *N*-oxide produced essentially inactive agents. The pyridine *N*-oxide function appeared to be essential for anticancer activity in compounds containing the formylhydrazone side chain in position 2, since compound **8** was also essentially inactive. However, the 4-substituted derivative **10**, which did not contain the *N*-oxide function, showed good antineoplastic potency in this tumor system, increasing the average survival time of tumor-bearing mice from 12.5 days for untreated control animals to 28.2 days. The findings suggest that compounds **1** and **10** may represent different classes of antitumor agents. Replacement of the SO_2 group by CO (compounds **7**, **11**, and **14**) resulted in complete loss of activity, indicating the essentiality of the SO_2 function.

Changes in the terminal portion of the side chain were possible with retention of tumor-inhibitory activity. Thus, compound **4**, a derivative in which the tolyl group of **1** was replaced by a phenyl ring, possessed antineoplastic potency essentially equivalent to that of the parent molecule **1**. Introduction of a $-CH_2-$ group in between the phenyl and SO_2 functions also resulted in an active compound (**6**); however, replacement of the relatively bulky phenyl moiety by a methyl group on the terminal position of the side chain (**5**) resulted in a material with no anticancer activity.

Since 1-oxidopyridine-2-carboxaldehyde *p*-toluenesulfonylhydrazone (**1**) was one of the most active agents of this series in the Sarcoma 180 test system, it was also examined in a variety of other transplanted murine neoplastic tumor systems, i.e., leukemia L1210, Ehrlich carcinoma, Hepatoma 129, and a subline of Sarcoma 180 resistant to α-(N)-heterocyclic carboxaldehyde thiosemicarbazones (Sarcoma 180/IQ-1) (Table II). Compound **1** showed activity in each of these test systems indicating that this class of agents possessed a wide spectrum of antineoplastic potency.

The effects of compound **1** on the biosynthesis of DNA, RNA, and protein of Sarcoma 180 ascites cells was ascertained by measuring the effects of the administration of a single intraperitoneal dose of this agent to tumor-bearing animals (Table III). The incorporation of

Table IV. Time Course of the Effects of 1-Oxidopyridine-2-carboxaldehyde *p*-Toluenesulfonylhydrazone on the Incorporation of Thymidine-^3H, Uridine-^3H, and DL-Leucine-^{14}C into Macromolecules of Sarcoma 180 Ascites Cells

Pretreatment (h) before radioactive precursor	% control incorporation[a]		
	Thymidine-^3H into DNA	Uridine-^3H into RNA	Leucine-^{14}C into protein
1	59		73
3	53	62	70
6	63	50	89
12	57	102	

[a] Mice bearing 6-day implants of Sarcoma 180 ascites cells received a single intraperitoneal injection of 80 mg of 1-oxidopyridine-2-carboxaldehyde *p*-toluenesulfonylhydrazone per kilogram of body weight. At the indicated times, thereafter, animals received the appropriate radioactive tracer by intraperitoneal injection, which was allowed 1 h to be incorporated. Control specific activities were as indicated in Table III.

thymidine-^3H into DNA and of uridine-^3H into RNA was essentially equally susceptible to the action of this agent, whereas protein biosynthesis was relatively resistant. The duration of inhibition of these metabolic processes by 1-oxidopyridine-2-carboxaldehyde *p*-toluenesulfonylhydrazone (1) was determined and the results are shown in Table IV. The inhibition of DNA biosynthesis by compound 1 persisted for up to 12 h, while the rate of incorporation of uridine-^3H into RNA recovered to that of untreated control cells by 12 h after drug.

The activity of 1 against Sarcoma 180/IQ-1, coupled with (a) differences in structure–activity requirements and (b) lack of potent inhibition of DNA synthesis in intact Sarcoma 180 cells by 1, contrasts with the α-(N)-heterocyclic carboxaldehyde thiosemicarbazones and indicates differences in the biochemical mechanism of the

Scheme I

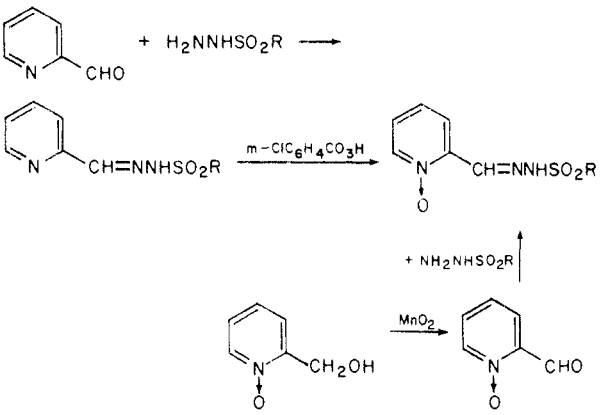
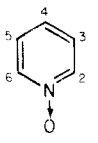
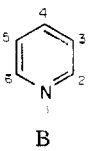
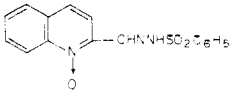
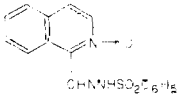


Table V. Physical Constants for Aldehyde Acylhydrazones


A


B


C

Compd		Method of prepn	Yield, %	Mp, °C dec	Formula	Analyses
	Group A (Pyridine *N*-Oxides)					
1	2-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	B	76	138–139	*a*	C, H, N
2	3-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	B	79	165–166	C$_{13}$H$_{13}$N$_3$O$_3$S·H$_2$O	C, H, N
3	4-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	B	42	143–144	C$_{13}$H$_{13}$N$_3$O$_3$S	C, H, N
4	2-CHNNHSO$_2$-C$_6$H$_5$	B	85	112–114	C$_{12}$H$_{11}$N$_3$O$_3$S	C, H, N
5	2-CHNNHSO$_2$CH$_3$	B	75	136–137	C$_7$H$_9$N$_3$O$_3$S·0.5H$_2$O	C, H, N
6	2-CHNNHSO$_2$CH$_2$-C$_6$H$_5$	A	66	132	C$_{13}$H$_{13}$N$_3$O$_3$S	C, H, N
7	2-CHNNHC(=O)-C$_6$H$_4$-*p*-CH$_3$	B	54	255–256	C$_{14}$H$_{13}$N$_3$O$_2$	C, H, N
	Group B (Pyridines)					
8	2-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	A	62	112–113	C$_{13}$H$_{13}$N$_3$O$_2$S	C, H, N
9	3-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	A	94	155–156	C$_{13}$H$_{13}$N$_3$O$_2$S·0.5H$_2$O	C, H, N
10	4-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	A	93	115–116	C$_{13}$H$_{13}$N$_3$O$_2$S	C, H, N
11	4-CHNNHC(=O)-C$_6$H$_4$-*p*-CH$_3$	A	94	188–189	C$_{14}$H$_{13}$N$_3$O·0.5H$_2$O	C, H, N
12	4-CHNNHSO$_2$CH$_2$-C$_6$H$_5$	A	93	120–121	C$_{13}$H$_{13}$N$_3$O$_2$S	C, H, N
13	4-CHNNHSO$_2$CH$_3$	A	97	125	C$_7$H$_9$N$_3$O$_2$S·0.5H$_2$O	C, H, N
14	2-CHNNHC(=O)-C$_6$H$_4$-*p*-CH$_3$	A	97	161–163	C$_{14}$H$_{13}$N$_3$O	C, H, N
	Group C (Benzenes)					
15	1-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$	A	93	127–128	C$_{14}$H$_{14}$N$_2$O$_2$S·0.5H$_2$O	C, H, N
16	1-CHNNHSO$_2$-C$_6$H$_4$-*p*-CH$_3$, 2-NO$_2$	A	88	154–155	C$_{14}$H$_{13}$N$_3$O$_4$S	C, H, N
17		C	90	119	C$_{16}$H$_{13}$N$_3$O$_3$S	C, H, N
18		C	94	114	C$_{16}$H$_{13}$N$_3$O$_3$S	C, H, N

[a] The properties were the same as previously reported.[2b]

cytotoxic action of these two classes of agents.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The ir absorption spectra were obtained with a Perkin-Elmer Model 257 spectrophotometer with thin films of liquids and KBr pellets of solids. NMR spectra were determined with a Varian T-60A or a Jeol C60H spectrometer with Me_4Si as an internal standard. The spectral data were as expected; therefore, routine data are not included. Elemental analyses were performed by the Baron Consulting Co., Orange, Conn., or by Het-Chem-Co., Harrisonville, Mo. Where analyses are indicated only by symbols of the element, the analytical results for those elements were within ±0.4% of the theoretical values. Pertinent data for the compounds synthesized are listed in Table V.

Antitumor Activity. The ascites cell forms Sarcoma 180, Sarcoma 180/IQ-1 (a subline of Sarcoma 180 selected for resistance to 1-formylisoquinoline thiosemicarbazone), and Ehrlich carcinoma were propagated in female CD-1 mice, while leukemia L1210 and Hepatoma 129 were grown in male BDF1 and C3H mice, respectively. Transplantation was carried out using an appropriate donor mouse bearing a 7-day tumor growth. The experimental details have been described earlier.[7] Mice were weighed throughout 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. Dose levels were administered in the range of 10–60 mg/kg/day for six consecutive days for each compound. Determination of the sensitivity of ascitic neoplasms to these agents was based upon the prolongation of survival time afforded by the drug treatments.

Incorporation of Radioactive Precursors into DNA, RNA, and Protein. The effect of 1-oxidopyridine-2-carboxaldehyde *p*-toluenesulfonylhydrazone on macromolecular synthesis was determined by injecting either 200 μg of thymidine-^3H (2.2×10^4 cpm/μg), 200 μg of uridine-^3H (1.3×10^4 cpm/μg), or 125 μg of DL-leucine-^{14}C (1.7×10^4 cpm/μg) intraperitoneally into mice bearing 6-day accumulations of Sarcoma 180 ascites cells that were either untreated or treated for various periods of time with a single intraperitoneal injection of 10–80 mg/kg of drug. The radioactive tracers were allowed 1 h to be incorporated and the specific radioactivity was then measured by previously described methodology.[8,9]

General Procedure for Preparation of Tosylhydrazones (Method A). The appropriate aldehyde (0.05 mol) was dissolved in methanol (15 ml) and cooled in an ice bath. To the stirred, cold solution was added the hydrazide (0.06 mol) in methanol (50 ml). The solution was removed from the ice bath and allowed to warm to room temperature with stirring. After 15–60 min, the reaction mixture was placed in a refrigerator overnight. The product was filtered, washed with cold methanol, and dried in vacuo.

General Procedure for the Preparation of *N*-Oxides (Method B). The appropriate hydrazone (0.03 mol) was added slowly to a cold (ice bath) stirred solution of *m*-chloroperoxybenzoic acid (0.05 mol) in ether–methanol (6:1 v/v, 60 ml). The stirred reaction mixture was allowed to warm to room temperature over about 2 h; stirring was continued at room temperature for a total of 40 h. The solid was filtered, washed with ether, suspended in a mixture of methanol–1,2-dimethoxyethane (1:5, 35 ml), and refluxed with stirring for 20 min. The solid was filtered, washed with ether, and air-dried.

General Procedure for Preparation of Arylsulfonylhydrazones (Method C). To an appropriate heterocyclic carboxaldehyde *N*-oxide (0.005 mol) solution in 10 ml of ethanol was added a solution of an arylsulfonyl hydrazide (0.05 mol) dissolved in 10 ml of ethanol. The mixture was warmed slowly for a few minutes with stirring and then cooled. The hydrazone which crystallized was filtered, washed with ethanol, and dried. In most cases it was not necessary to recrystallize the compound.

Nicotinaldehyde *p*-Toluenesulfonylhydrazone 1-*N*-Oxide (2). Acetic anhydride (8.6 g) was added to 90% H_2O_2 (2.5 ml) with cooling (water bath). (*Caution*: mixture is potentially explosive.) The peracetic acid solution was added slowly to a stirred suspension of nicotinaldehyde *p*-toluenesulfonylhydrazone (4.0 g) in ether (25 ml) and 1,2-dimethoxyethane (6 ml) in an ice bath. After stirring for 28 h, the mixture was filtered. The solid was washed with ether and then extracted several times with boiling 1,2-dimethoxyethane to remove starting material. The chromatographically pure product [TLC, SilicAR 7 GF, EtOAc–*n*-PrOH–H_2O, (4:2:1)] weighed 3.4 g (79%), mp 165–166°.

References and Notes

(1) Presented in part before the Division of Medicinal Chemistry at the 170th National Meeting of the American Chemical Society, Chicago, Ill., August 1975; this work was supported by U.S. Public Health Service Grants CA-02817 and CA-16359 (A.C.S.) and CA-11935 (A.D.B.) from the National Cancer Institute.

(2) (a) D. G. Bartholomew and A. D. Broom, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., March 1974, Abstracts, MEDI 66. (b) T. Endo, K. Ikeda, Y. Kamura, and Y. Mizuno, *J. Chem. Soc., Chem. Commun.*, 676 (1973).

(3) Y. Mizuno, T. Endo, T. Miyaska, and K. Ikeda, *J. Org. Chem.*, **39**, 1250 (1974).

(4) Y. Mizuno and K. Ikeda, *Chem. Pharm. Bull.*, **22**, 2889 (1974).

(5) K. C. Agrawal and A. C. Sartorelli in "Handbook of Experimental Pharmacology", Vol. 38, Part II, A. C. Sartorelli and D. G. Johns, Ed., Springer-Verlag, Berlin, 1975, pp 793–807.

(6) L. M. Jackman and S. Sternhall, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, New York, N.Y., 1969, and references cited therein.

(7) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, 11, 700 (1968).

(8) B. A. Booth, K. C. Agrawal, E. C. Moore, and A. C. Sartorelli, *Cancer Res.*, **34**, 1308 (1974).

(9) B. A. Booth and A. C. Sartorelli, *J. Biol. Chem.*, **236**, 203 (1961).