Antineoplastic Properties of Arylsulfonylhydrazones of 3-Formylpyridazine 2-Oxide and 4-Formylpyrimidine 3-Oxide[1]

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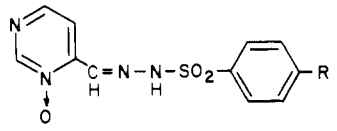
The antineoplastic activity of several arylsulfonylhydrazones of 4-formylpyrimidine 3-oxide and of 3-formylpyridazine 2-oxide has been investigated. Derivatives of the latter heteroaromatic *N*-oxide showed excellent antineoplastic potency against the murine neoplasm Sarcoma 180 but were inactive against leukemia L1210. In contrast, derivatives of 4-formylpyrimidine 3-oxide were inactive against both of these transplanted tumors.

Recent reports[3,4] from our laboratory have demonstrated the effectiveness of a variety of arylsulfonylhydrazones of 2-formylpyridine 1-oxide as antineoplastic agents in a number of experimental murine tumor systems. To determine whether the pyridine *N*-oxide portion of the molecule is essential for anticancer activity, arylsulfonylhydrazone derivatives of other heteroaromatic *N*-oxides have been synthesized and tested for their carcinostatic potency against Sarcoma 180 and leukemia L1210. This paper describes the antineoplastic activity and host toxicity of several arylsulfonylhydrazones of 4-formylpyrimidine 3-oxide, **1a–c**, and of 3-formylpyridazine 2-oxide, **2a–c**.



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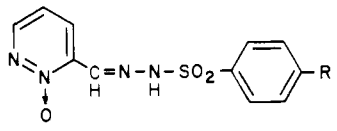

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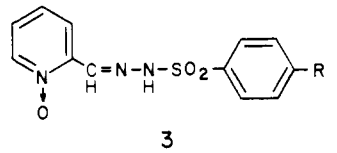

3

a, R = H
b, R = CH_3
c, R = OCH_3

Chemistry. Arylsulfonylhydrazones of 4-formylpyrimidine 3-oxide were obtained by oxidation of 4-methylpyrimidine 3-oxide[5] with freshly sublimed SeO_2 in *p*-dioxane at reflux temperature to provide 4-formylpyrimidine 3-oxide; this product was reacted without isolation with the appropriate arylsulfonylhydrazide to give **1a–c**. These compounds crystallized from the reaction mixture as analytically pure solids requiring no additional purification. Similar oxidation of 3-methylpyridazine 2-oxide,[6] using pyridine as solvent, provided the expected 3-formylpyridazine 2-oxide which was reacted directly with the appropriate arylsulfonylhydrazide to give the arylsulfonylhydrazones of 3-formylpyridazine 2-oxide, **2a–c**. The use of pyridine as solvent resulted in the presence of a significant quantity of selenium in the product which crystallized from the reaction mixture; removal of selenium required several recrystallizations to obtain analytical material, which greatly reduced the yields of these compounds. Physicochemical and ultraviolet spectral data for the synthesized compounds are listed in Table I.

Measurement of the UV spectra of these compounds as a function of time revealed that the pyridazine derivatives were readily hydrolyzed under neutral (pH 7.0) aqueous conditions, whereas the pyrimidines were quite stable in this environment. Both the pyrimidine and the pyridazine derivatives were, however, stable in dry nonhydroxylic solvents such as 1,2-dimethoxyethane (glyme).

Biological Results and Discussion. The tumor-inhibitory properties of the arylsulfonylhydrazones of 3-formylpyridazine 2-oxide and 4-formylpyrimidine 3-oxide were determined by measuring their effects on the survival time of mice bearing either Sarcoma 180 or leukemia L1210 ascites cells; the results of tests conducted with Sarcoma 180 are shown in Table II. The prolongation of life produced by the indicated daily dosage levels of each compound is listed and was employed as the measure of antineoplastic efficacy. The specific substitutions of the phenyl ring (R = H, CH_3, or OCH_3) were chosen because of the range of antitumor activity observed[3,4] for these derivatives of 2-formylpyridine 1-oxide, **3a–c**. Compounds **3a–c** showed excellent antitumor activity against Sarcoma 180 (T/C, >300); however, against leukemia L1210, **3a** showed only marginal activity (T/C, 126), **3b** displayed a moderate level of activity (T/C, 170), and **3c** demonstrated relatively good activity (T/C, 234).[3,4]

The arylsulfonylhydrazone derivatives of 4-formylpyrimidine 3-oxide, **1a–c**, showed no antitumor activity against either Sarcoma 180 or leukemia L1210 at daily doses up to 80 or 100 mg/kg, respectively. In contrast, the arylsulfonylhydrazone derivatives of 3-formylpyridazine 2-oxide, **2a–c**, which also were not inhibitory to leukemia L1210, possessed marked activity against Sarcoma 180. Relative to **3a**, which was included at its optimum daily dosage[3] in these experiments as a positive control, **2a,b** appeared to be more potent as antitumor agents against this neoplasm, while compound **2c** appeared to be equiactive.

It was previously observed[3,4] that substitutions at the C-4 position of the pyridine ring of **3a** did not abolish antineoplastic activity against Sarcoma 180 but that replacement of the pyridine *N*-oxide moiety by quinoline *N*-oxide or isoquinoline *N*-oxide led to a complete loss of carcinostatic potency. The observed anticancer activity of compounds **2a–c** demonstrates that arylsulfonylhydrazone derivatives of heteroaromatic *N*-oxides other than pyridine *N*-oxide are capable of exerting significant biological activity. These findings, plus the observed inactivity of **1a–c**, suggest that the heteroaromatic *N*-oxide portion of the drug molecule must possess certain as yet undefined electronic and/or stereochemical properties in order to have tumor-inhibitory activity. The difference in stability between the pyridazine and pyrimidine derivatives in neutral aqueous solution and its relationship to the cancer inhibitory activity of the arylsulfonylhydrazones against Sarcoma 180 is currently under investigation.

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Table I. Physicochemical and Ultraviolet Spectral Data for Arylsulfonylhydrazones

compd	mp, °C[a]	yield, %	formula[b]	λ max, pH 7.0[c]	log ε	λ max, glyme	log ε
1a	110	69	$C_{11}H_{10}N_4O_3S$	375 sh	3.18	380	3.61
				326	4.23	303	4.15
				258	4.33	274	4.25
1b	113	62	$C_{12}H_{12}N_4O_3S$	377 sh	3.42	380	3.62
				325	4.24	305	4.15
				258	4.35	265	4.21
				223	4.32		
1c	107	67	$C_{12}H_{12}N_4O_4S$	380	3.35	380	3.55
				325	4.16	307	4.07
				257	4.33	271	4.20
				232	4.34	242	4.37
2a	150	40	$C_{11}H_{10}N_4O_3S$	345	4.29	362	3.63
				265	4.02	305	4.21
						274	4.14
						268	4.15
2b	168	25	$C_{12}H_{12}N_4O_3S$	349	4.29	364	3.64
				262	4.02	305	4.23
						274 sh	4.13
						270	4.14
2c	170	29	$C_{12}H_{12}N_4O_4S$	351	4.27	362	3.61
				233	4.22	307	4.20
						278 sh	4.08
						271	4.11
						238	4.34

[a] Rapid decomposition at indicated temperature. [b] All compounds were analyzed for C, H, and N. Analytical results were within ±0.4% of theoretical values. [c] Spectra were recorded beginning 2 min after dilution with 0.01 M phosphate buffer of a solution of sample in glyme; sh indicates shoulder.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by Baron Consulting Company, Orange, CT. Evaporations were conducted under reduced pressure at 40 °C with a rotary evaporator. TLC was performed on glass plates (0.25 mm) coated with aluminum oxide (Anasil AGF, Analabs) and compounds were visualized using a UV lamp. Proton magnetic resonance spectra were obtained with a Varian T-60 spectrometer (solutions in dimethyl-d_6 sulfoxide with tetramethylsilane as internal standard). The spectral data were as expected and are not included. Ultraviolet spectra were measured with a Beckman 25 spectrophotometer. Pertinent data for synthesized compounds are listed in Table I.

General Procedure for Preparation of Arylsulfonylhydrazones of 4-Formylpyrimidine 3-Oxide, 1a–c. Selenium dioxide (490 mg, 4.5 mmol) was added to a solution of 4-methylpyrimidine 3-oxide[5] (500 mg, 4.5 mmol) in *p*-dioxane (37 mL); this mixture was heated at reflux temperature for 5 h. The reaction mixture was treated with charcoal, filtered, and immediately evaporated to a syrupy residue which was dissolved in chloroform (50 mL). The chloroform solution was filtered, the filter cake washed with chloroform (15 mL), and the filtrate evaporated to a syrup. This syrup was dissolved in methanol (4 mL) and filtered, and the filtrate was cooled to 3 °C. A solution of the appropriate arylsulfonylhydrazide (4 mmol) in methanol (ca. 5 mL) was then added, and the resulting solution was stirred at ambient temperature for 30 min, followed by refrigeration (4 °C) for 15 h. The resulting crystalline solid was collected by filtration, washed with cold methanol (4 mL), and air-dried to provide analytical material.

General Procedure for the Preparation of Arylsulfonylhydrazones of 3-Formylpyridazine 2-Oxide, 2a–c. To a solution of 3-methylpyridazine 2-oxide[6] (550 mg, 5 mmol) in pyridine (8 mL) was added selenium dioxide (500 mg, 4.5 mmol); the resulting mixture was heated at reflux temperature for 6 h. The reaction mixture was treated with charcoal and filtered, and the filtrate was evaporated to a residue which was coevaporated with toluene (3 × 15 mL). The resulting residue was extracted with chloroform (4 × 10 mL), and the combined chloroform extracts were evaporated to a syrup which was dissolved in methanol (4 mL) and cooled (3 °C). Addition of a solution of the appropriate arylsulfonylhydrazide as described for 1 gave a crude product. Recrystallization two or three times from dimethylformamide–water provided analytical samples.

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

compd	daily dose, mg/kg[a]	av Δ wt, %[b]	av survival time, days ± SE	50-day survivors, %[c]	% T/C[d]
control		+13.5	14.4 ± 1.0	0	100
1a	40	+16.3	12.8 ± 0.8	0	89
	60	+11.2	13.9 ± 0.5	0	97
	80	+9.6	16.1 ± 1.5	0	112
1b	40	+19.6	16.5 ± 1.0	0	115
	60	+16.5	14.4 ± 0.5	0	100
	80	+15.2	14.8 ± 0.7	0	103
1c	40	+20.3	13.8 ± 1.6	0	96
	60	+10.8	14.4 ± 1.3	0	100
	80	+13.9	13.6 ± 0.2	0	94
2a	40	−2.9	27.9 ± 2.8	10	194
	60	−3.8	33.1 ± 3.0	10	230
	80	−3.5	40.6 ± 3.3	40	274
2b	40	−0.2	23.7 ± 3.1	10	164
	60	−3.2	36.6 ± 3.8	20	254
	80	−4.9	45.3 ± 2.3	60	315
2c	40	−1.6	33.2 ± 3.2	10	231
	60	−3.2	31.1 ± 3.2	20	218
	80	−4.3	27.7 ± 3.2	0	193
3a	40	−2.6	33.8 ± 3.4	20	235

[a] Administered once daily for six consecutive days, beginning 24 h after tumor implantation. Treated groups represent 10 animals each except for 1b and 1c where treated groups represent five animals each. [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 = average survival time (treated/control) × 100.

Antitumor Activity. The ascites-cell forms of Sarcoma 180, propagated in female CD-1 mice, and leukemia L1210, grown in male CDF-1 mice, were employed. Transplantation was carried out using donor mice bearing 7-day tumor growths; experimental details have been described earlier.[7] 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. Dosage levels of each compound were administered in the range of 40–80 mg/kg per day for experiments

with Sarcoma 180 and 60–100 mg/kg per day for those with leukemia L1210; each agent was injected intraperitoneally for six consecutive days beginning 24 h after tumor implantation. Determination of the sensitivity of ascitic neoplasms to these agents was based upon the prolongation of survival time afforded by drug treatments.

References and Notes

(1) This work was supported by U.S. Public Health Service Grants CA-02817 and CA-16359 from the National Cancer Institute and a grant from the Bristol-Myers Co.
(2) Norwich-Eaton Pharmaceuticals, Norwich, N.Y. 13815.
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Antimycotic Imidazoles. 2. Synthesis and Antifungal Properties of Esters of 1-[2-Hydroxy(mercapto)-2-phenylethyl]-1*H*-imidazoles[1]

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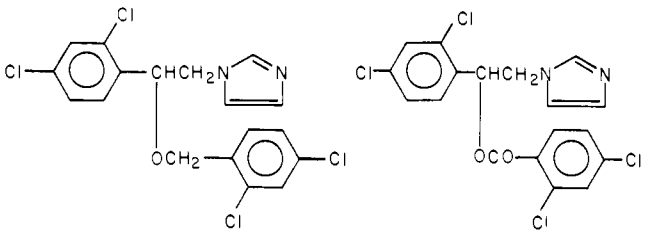
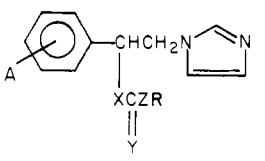
The synthesis of carboxylic and (thio)carbonate esters of 1-[2-hydroxy(mercapto)-2-phenylethyl]-1*H*-imidazoles, some of which are formally related to miconazole and its analogues by replacement of an ether with an ester linkage, is described. In antifungal bioassays a number of compounds display in vitro and, in a few cases, in vivo activities comparable to that of miconazole. In this series lipophilicity within a relatively narrow range is shown to be a necessary, although not sufficient, criterion for in vitro and, in particular, in vivo antifungal activity.

In connection with a program directed toward the development of new broad-spectrum antifungal agents, we became interested in compounds obtained by the formal replacement of the ether linkage in the potent drug, miconazole[2] (I), by an ester function, II (e.g., **6**). Since it



I

6



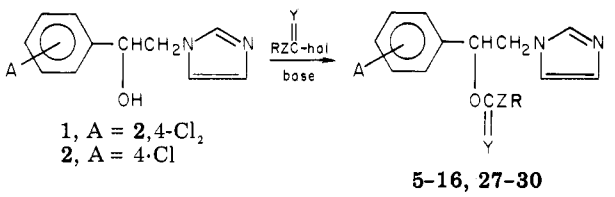
II

5–16, X = Y = O; Z = bond
17–26, X = S; Y = O; Z = bond
27–29, X = Y = Z = O
30, X = Y = O; Z = S
31, 32, X = S; Y = Z = O
33, 34, X = Z = S; Y = O
35–44, X = Y = S; Z = O

has been found by others[2,3] and by ourselves[4] that maximum antifungal activity in 1-phenylethylimidazoles is associated with 2,4-dichloro substitution in the benzene ring, we retained this subunit in the majority of compounds prepared. The nature of the ester linkage was extended beyond simple esters to include carbonates and various thio derivatives (see II).

Chemistry. Phenylethyloxy esters were prepared by standard esterification procedures from the known[2] alcohols **1** and **2** and the corresponding acyl halide (Scheme I). Phenylethylthio esters were obtained by a different route (Scheme II); the xanthates **35–44** were obtained via

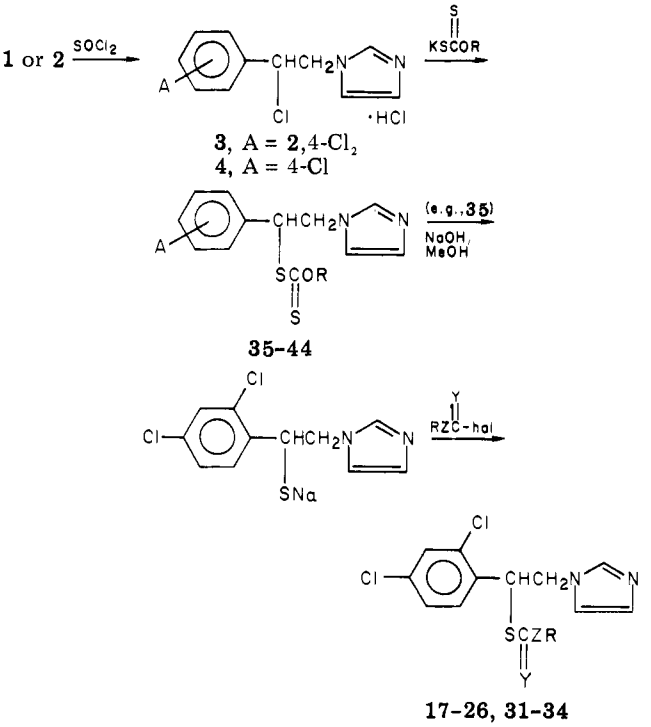
Scheme I



1, A = **2**,4-Cl_2
2, A = 4-Cl

5–16, 27–30

Scheme II



3, A = **2**,4-Cl_2
4, A = 4-Cl

35–44

17–26, 31–34

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