TABLE I: ANALYTICAL DATA AND PHYSICAL PROPERTIES OF THIOSEMICARBAZONES SYNTHESIZED

Compd[a]	Mp, °C	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % S	Found, % C	Found, % H	Found, % N	Found, % S	$\lambda_{max}^{95\%EtOH}$, mμ	ε
2-Keto-3-methoxybutyraldehyde bis(thiosemicarbazone) (III)	208–212	$C_7H_{14}N_6OS_2$	32.1	5.4	32.1	24.4	32.8	5.5	31.4	24.1	233	9,100
											271	7,350
											348	47,450
2-Keto-3-methoxyethoxybutyraldehyde bis(thiosemicarbazone) (IV)	213–216	$C_9H_{18}N_6O_2S_2$	35.3	5.9	27.4	20.9	35.4	6.1	26.9	20.9	237	9,250
											267	7,500
											347	48,200
2-Keto-3-acetoxybutyraldehyde bis(thiosemicarbazone)[b] (V)	210–212	$C_8H_{14}N_6O_2S_2$	33.1	4.9	29.0	22.1	33.3	4.9	28.2	22.2	247	14,200
											346	28,500
Pyruvaldehyde bis(N^4-dimethylthiosemicarbazone) (VI)	115 dec	$C_9H_{18}N_6S_2$	39.4	6.6	30.6	23.4	39.1	6.7	30.3	22.7	232	15,400
											328	24,300
											410	3,320

[a] Abbreviations in common use for these compounds are: III, KMTS; IV, MKTS; V, KATS; and VI, $PTSM_2$. [b] Original sample prepared by B. D. Aspergren.

TABLE II: THE ANTITUMOR ACTIVITY[a] OF SEVERAL THIOSEMICARBAZONES COMPARED TO KETHOXAL BIS(THIOSEMICARBAZONE)

Expt	Drug	Dose, mg/kg	Dosing period, days[b]	Survival	Δ body wt, g	Δ tumor size, mm	% inhib[c]
A	...	Control	.... (17)	9/10	70.0	33.9	...
	I	25 oral	4–17	5/5	61.4	−1.8	>100
	V	25 oral	4–17	4/5	51.5	33.3	2
B	...	Control	.... (17)	9/10	45.2	32.5	...
	I	25 ip	5–15	5/5	18.0	0.7	98
	V	25 ip	5–15	5/5	8.2	7.0	79
C	...	Control	.... (18)	9/9	56.2	29.8	...
	I	25 oral	6–17	10/10	47.2	5.3	82
	III	25 oral	6–17	10/10	41.5	0.6	98
D	...	Control	.... (20)	9/9	66.8	38.0	...
	I	25 ip	6–19	5/5	29.2	2.2	94
	III	25 ip	6–19	4/4	22.8	−1.9	105
E	...	Control	.... (17)	4/4	88.8	42.3	...
	I	25 oral	4–15	5/5	45.8	3.0	93
	IV	25 oral	4–15	5/5	42.0	14.6	65

[a] Against a nitrogen mustard resistant variant of Walker 256 carcinosarcoma in Sprague–Dawley rats. [b] Days after tumor implantation. Dosed daily except Sundays. Parentheses indicate the day on which final measurements were made. [c] % inhibition = [change in average diameter (control tumors − treated tumors) × 100]/[change in average diameter (control tumors)].

It was found that this agent was equally active by either route of administration and that it was equal to or slightly more active than I, the parent compound, while the toxicities of the two drugs were similar.

In expt E we found that 2-keto-3-methoxyethoxybutyraldehyde bis(thiosemicarbazone) (IV) had less antitumor activity but that its toxicity to rats was essentially the same as that of I.

These data and those previously reported[2] show that certain modifications of the ketoaldehyde portion of I can be made without great loss of activity but that such changes are limited. Substitution of a methoxy group for the ethoxy group of I seemed to cause an increase in antitumor activity (*cf.* III) but the substitution of an acetoxy or methoxyethoxy group for the ethoxy radical caused a loss of biological activity (*cf.* IV and V). Previous work had indicated that pyruvaldehyde bis(thiosemicarbazone) and ethoxypyruvaldehyde bis(thiosemicarbazone) were considerably less active than I, which of course suggests that the nature of the ketoaldehyde is important in determining the antitumor activity of bis(thiosemicarbazones).

Experimental Section

Source of α-Ketoaldehydes.—Pyruvaldehyde was obtained as a 30% solution from Aldrich Chemical Co. 2-Keto-3-methoxybutyraldehyde and 2-keto-3-methoxyethoxybutyraldehyde were obtained from B. D. Tiffany, and 2-keto-3-acetoxybutyraldehyde from B. D. Aspergren, both of The Upjohn Co. laboratories.

Preparation of Thiosemicarbazones.—All bis(thiosemicarbazones) were prepared by small variations of the general method described earlier.[2] Recrystallization was accomplished from hot ethanol (III–V) by adding an equal volume of water or directly from absolute methanol (VI).

Testing of Drugs.—Drugs were prepared for testing and tested in the manner described earlier.[2] All other experimental conditions were also the same as previously reported.

Acknowledgment.—We wish to express our appreciation to E. E. Smith for his assistance with the animal studies.

Synthesis of Potential Antineoplastic Agents. XIII. 1-{*p*-[Bis(2-chloroethyl)amino]benzyl}-pyridinium *p*-Toluenesulfonate and Related Compounds[1,2]

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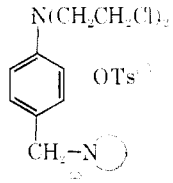
As part of our program directed toward the synthesis of various nitrogen mustard derivatives as potential antineoplastic agents it was desirable to have the

(1) Part XII: F. D. Popp and D. W. Alwani, *Can. J. Chem.*, **42**, 1506 (1964).

(2) This work was supported in part by research grants from the American Cancer Society (T-177D) and from the National Cancer Institute, U. S. Public Health Service (CA 06606-03).

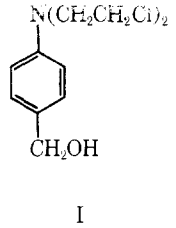
TABLE I



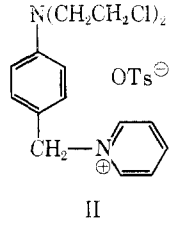
–N⊕	Yield, %	Mp, °C	Calcd, % C	H	N	Cl	S	Found, % C	H	N	Cl	S
Pyridine	89	122–124[a]	57.37	5.44	5.82	14.72	6.66	57.31	5.34	5.69	14.38	6.42
4-Acetylpyridine	76	126–127[b]	57.36	5.39	5.35			57.46	5.43	5.32		
3-Acetylpyridine	75	138–140[c]	57.36	5.39	5.35	13.54		57.48	5.15	5.64	13.59	
Ethyl isonicotinate	40	77–79[d]	56.14	5.46	5.06	12.81		55.68	5.75	4.91	12.69	
3-Picoline	80	136–137[b]	58.65	4.92	5.70	14.43	6.52	58.55	5.03	5.54	14.34	6.50
Quinoline	60	137–138[b]	61.01	5.31	5.27	13.34		61.20	5.37	5.35	13.49	
Lepidine	45	148–149[d]	61.64	5.54	5.14	13.00		61.81	5.30	5.30	12.98	
Isoquinoline	66	188–190[e]	61.01	5.31	5.27	13.34		60.84	5.24	5.40	13.26	

[a] Recrystallized from dioxane. [b] Recrystallized from acetone. [c] Recrystallized from ethanol–ether. [d] Recryscallized from ethyl acetate. [e] Recrystallized from benzene–dimethylformamide.

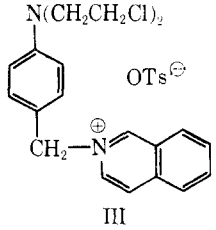
p-toluenesulfonate of *p*-[bis(2-chloroethyl)amino]benzyl alcohol (benzyl alcohol mustard) (I).[3] In an attempt to synthesize this compound by the reaction of I with *p*-toluenesulfonyl chloride in the presence of pyridine, it was noted that instead 1-{*p*-[bis(2-chloroethyl)-amino]benzyl}pyridinium *p*-toluenesulfonate (II) was obtained. Reaction of II with isoquinoline gave the corresponding isoquinolinium salt III which was also obtained by reaction of I with *p*-toluenesulfonyl chloride in the presence of isoquinoline. In a similar manner the reaction of I with *p*-toluenesulfonyl chloride in the presence of other bases gave the related com-



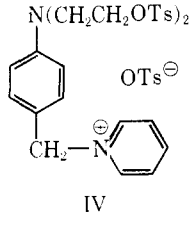
I



II



III



IV

pounds shown in Table I. Use of triethylamine, benzyldimethylamine, dimethylaniline, ethyl picolinate, ethyl quinaldate, and 6-methylquinoline gave oils which could not be induced to crystallize and which were not investigated further.

Compound IV and the corresponding quinolinium compound were prepared in a similar manner.

Although screening data are not available on all of the compounds included in Table I the available figures from the CCNSC are included in Table II. In addition to these results, 1-{*p*-[bis(2-chloroethyl)-amino]benzyl}quinolinium *p*-toluenesulfonate was tested for intravenous toxicity to mice. The compound had LD_{50} 56 mg/kg and at 32 mg/kg caused decreased locomotor activity, nonparalytic eyelid ptosis, dyspnea, and vasodilation. The first two effects were still noted at 10 mg/kg while no effects were noted at 3.2 mg/kg. At an estimated exposure concentration of 0.53 mg/l. this compound caused decreased locomotor activity and increased respiratory depth in mice after 10-min inhalation time with no effects noted 5 min following the end of inhalation and normal lung appearance on autopsy.

(3) R. H. Iwamoto, E. M. Acton, L. O. Ross, W. A. Skinner, B. R. Baker, and L. Goodman, *J. Med. Chem.*, **6**, 43 (1963).

Experimental Section[4]

***p*-[Bis(2-chloroethyl)amino]benzyl Alcohol (I).**—The following procedure was more successful in our hands than that reported by Iwamoto, *et al.*[3] To a suspension of 12.3 g (0.05 mole) of *p*-[bis(2-chloroethyl)amino]benzaldehyde[5] in 200 ml of methanol, cooled in an ice bath, was added with stirring and in small portions (over 25–30 min) 3.8 g (0.1 mole) of $NaBH_4$. The mixture was stirred for 2 hr in the ice bath and then for 2 hr at room temperature. It was then poured into ice with vigorous stirring and the white solid that separated in 85–99% yield was filtered, dried, and used without further purification; mp 51–52°, lit.[3] mp 52–53°.

1-{*p*-[Bis(2-chloroethyl)amino]benzyl}pyridinium *p*-Toluenesulfonate (II).—To a mixture of 4.92 g (0.02 mole) of I and 4.2 g (0.022 mole) of *p*-toluenesulfonyl chloride, 5–8 ml of pyridine was added with stirring. The mixture was stirred overnight at room temperature, poured into ice, and extracted ($CHCl_3$). Concentration of the dried chloroform extract gave a solid which was recrystallized from dioxane to give 8.6 g (89%) of solid, mp 122–124°.

Anal. Calcd for $C_{23}H_{26}Cl_2N_2O_3S$: C, 57.38; H, 5.44; Cl, 14.73; N, 5.82; S, 6.66. Found: C, 57.31; H, 5.34; Cl, 14.38; N, 5.69; S, 6.42.

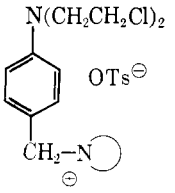
All of the compounds included in Table I were prepared by a similar method. In the cases of solid amines chloroform was used as a solvent. In a number of cases concentration of the $CHCl_3$ gave a gum which could be induced to crystallize by trituration with an appropriate solvent. Carrying out the reaction in an ice bath rather than at room temperature did not have any appreciable effect.

1-{*p*-[Bis(2-chloroethyl)amino]benzyl}isoquinolinium *p*-Toluenesulfonate (III).—In addition to being prepared as described for II and included in Table I this compound was also prepared as follows. A mixture of 4.81 g (0.01 mole) of II and 1.43 g (0.011 mole) of isoquinoline in 50 ml of benzene–dimethylformamide was refluxed for 1 hr. After cooling 3.62 g (68%) of solid was obtained, mp 188–190°. This material was identical with that described in Table I.

(4) All melting points were determined in capillaries and are corrected. Analyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(5) R. H. Wiley and G. Irick, *J. Org. Chem.*, **26**, 593 (1961).

TABLE II: SCREENING DATA[a]



−N◯	Test system[b]	Dose, mg/kg	Survivors	Cures	Animal wt dif (T − C)[c]	Tumor wt — Test	Tumor wt — Control	T/C, %	Cell culture — ED_{50}[d]	Cell culture — Slope[e]
Pyridine	AA	100	0/3							
	AA	33	3/3							
	AA	10	3/3							
	WA	41	6/6	6	−56	0.0	5.0	0		
	WA	20.5	6/6	6	−29	0.0	5.0	0		
	WA	10.2	6/6	0	−25	1.1	5.0	22		
	WA	8.0	6/6	0	−17	3.0	8.0	37		
	WA	6.0	6/6	0	−5	4.5	8.0	56		
	WA	5.1	6/6	0	−17	2.5	5.0	50		
	WA	4.0	6/6	0	−7	5.9	8.0	73		
	WA	2.0	6/6	0	−2	8.5	8.0	106		
	KB								1.6	−0.56
	KB								4.3	−0.49
3-Acetylpyridine	AA	33	0/3		−13					
	AA	10	3/3		2					
	AA	3.0	3/3		6					
Ethyl isonicotinate	AA	50	3/3		3					
	AA	10	3/3		8					
	AA	3.0	3/3		20					
Quinoline	AA	33	0/3							
	AA	10	3/3							
	AA	3.0	3/3							
	WA	13	6/6	6	−59	0.0	8.9	0		
	WA	6.5	6/6	4	−40	0.9	8.9	10		
	WA	3.25	6/6	0	−19	3.0	8.9	33		
	WA	1.6	6/6	0	−15	6.9	8.9	77		
	KB								4.3	−0.33
Lepidine	AA	33	0/3							
	AA	10	3/3		−9					
	AA	3.0	3/3		1					
Isoquinoline	AA	100	0/3							
	AA	33	3/3							
	AA	10	3/3							
	AA	3.0	3/3							
	WA	41	6/6	2	−29	0.6	5.0	12		
	WA	20.5	5/6	0	−10	4.7	5.0	94		
	WA	10.2	6/6	0	−13	4.3	5.0	86		
	WA	5.1	6/6	0	−6	5.2	5.0	104		
	KB								L1.0	
	KB								1.6	−0.46

[a] Tests carried out by the CCNSC according to their normal screening protocol. [b] AA = toxicity test, WA = Walker carcinosarcoma 256, KB = cell culture. [c] T = test animal, C = control animal. [d] ED_{50} = dose (μg/ml) that inhibits growth to 50% of control growth. [e] Slope = difference in response for a tenfold difference in dose.

1-{*p*-[Bis(2-*p*-tolylsulfonyloxyethyl)amino]benzyl}pyridinium *p*-Toluenesulfonate (IV).—*p*-[Bis(2-*p*-tolylsulfonyloxyethyl)amino]benzaldehyde[6] was reduced with $NaBH_4$ as described for the preparation of I. The crude alcohol, mp 78–80°, was treated with *p*-toluenesulfonyl chloride and pyridine as described for the preparation of II to give a 66% yield of the hemihydrate of IV, mp 79–81° (from acetone).

Anal. Calcd for $C_{37}H_{40}N_2O_9S_3 \cdot 0.5H_2O$: C, 58.32; H, 5.42; N, 3.68; S, 12.63. Found: C, 58.20; H, 5.31; N, 3.65; S, 12.64.

1-{*p*-[Bis(2-*p*-tolylsulfonyloxyethyl)amino]benzyl}quinolinium *p*-toluenesulfonate was obtained in 56% yield as its hemihydrate, mp 97–99° (from acetone–dimethylformamide), in a procedure similar to the preparation of IV.

Anal. Calcd for $C_{41}H_{42}N_2O_9S_3 \cdot 0.5H_2O$: C, 60.52; H, 5.34; N, 3.45; S, 11.85. Found: C, 60.38; 60.24; H, 5.50, 5.57; N, 3.46; S, 11.55.

(6) A. Cohen and R. S. Tipson, *J. Med. Chem.*, **6**, 822 (1963).

2,4-Bis(arylamino)pyrimidines as Antimicrobial Agents

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Although a large number of 2,4-diaminopyrimidines with hydrogen, alkyl, or aryl substitution in the 5 and 6 positions (type A) have been synthesized and screened

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