Method B. Inhibition of Chemically Induced Lysis of a Preformed Plasma Clot.—The clots were prepared from human plasma containing ^{125}I-labeled human fibrinogen by the addition of $CaCl_2$ and bovine thrombin. After thorough washing to remove loosely bound radioactivity, fibrinolysis was initiated by the addition of *o*-thymotic acid (6-methyl-3-isopropylsalicylic acid) to the suspending medium and was measured by the release of radioactivity into the medium. Lysis was prevented if an antifibrinolytic compound was present in the ambient solution. Inhibition of the release of radioactivity from the plasma clot into the ambient solution is directly proportional to inhibition of lysis. From these data, the relative potency of the antifibrinolytic compound was calculated.

Acknowledgments.—The authors acknowledge the valuable advice and encouragement of Dr. James M. Sprague during the course of this work, as well as the assistance of Mr. J. Schwering who prepared many chemical intermediates and Drs. G. S. Brenner and D. F. Hinkley who prepared the dimethyl cubane-1,4-dicarboxylate. Analytical data were obtained by Messrs. K. B. Streeter and Y. C. Lee and their staff, ir and nmr spectra by Mr. W. R. McGaughran, and glc analyses by Mr. A. Augenblick, to whom the authors are indebted for these services.

Potential Anticancer Agents. III. Schiff Bases from Benzaldehyde Nitrogen Mustards and Aminophenylthiazoles

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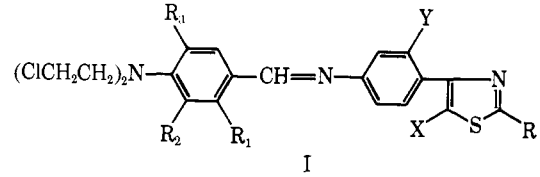
Received March 4, 1970

Several Schiff bases from different benzaldehyde nitrogen mustards and amines such as 4-(*p*-aminophenyl)-2,5-disubstituted-thiazoles and 4-[(4′-amino-2′-chloro)phenyl]-2-substituted-thiazoles have been synthesized and screened for antitumor activity. Many of the compounds displayed significant activity against L 1210 lymphoid leukemia, Walker 256 (intramuscular), and Dunning leukemia (solid).

The nonspecific cytotoxic effect of the N mustards has limited their use in the chemotherapy of cancer. The concept of "latent activity" whereby the drug is so designed as to be inactive *per se* but gets modified into an active form by processes taking place in the target cells has been very fruitful in the search for better antitumor agents. Ross and coworkers[2] synthesized azomustards while Popp[3] studied several Schiff bases of benzaldehyde N mustards and found them active enough in an experimental tumor system to merit clinical trials. Following this lead we have reported[4] in an earlier communication the synthesis and study of Schiff bases from substituted benzaldehyde N mustards and various arylamines. A number of compounds from this series displayed significant activity against Dunning leukemia (solid), lymphoid leukemia (L-1210), and Walker carcinosarcoma 256 (intramuscular). The substituent on the benzaldehyde N mustard greatly influenced the activity and specificity and the presence of a halogen in the *meta* position of the arylamine induced activity of a high order. Another significant observation in our earlier work was that among arylamines, the 4-(*p*-aminophenyl)thiazoles afforded more active Schiff bases. In view of these findings the work has now been extended and Schiff bases of structure I from substituted benzaldehyde N mustards and various 4-(*p*-aminophenyl)thiazoles have been prepared and screened to study the role of different substituents in the molecule.



I

X = Me and H
Y = Cl and H

R = M, $PhCH_2$, $PhOCH_2$ *o*- or *p*-toluoxymethyl, Ph, *p*-MePh, *p*-MeOPh, and *p*-ClPh
R_1 = H, OMe, Me, and F
R_2 = H, OMe and OEt
R_3 = H and OMe

Chemistry.—The general method adopted for the preparation of Schiff bases, *viz.*, heating a mixture of the amine and aldehyde in EtOH, though successful in certain cases was not particularly useful when the aldehyde was a liquid. In such cases the resulting compounds were invariably viscous oils which could not be induced to crystallize. In a few cases the modified method recommended by Tipson and Clapp[5] involving heating under reflux a mixture of amine and aldehyde in PhMe containing a few drops of piperidine was tried. The procedure, though successful when carried out with smaller amounts did not give pure products in larger quantities. The most suitable method found in the present work was the heating of pure amine hydrochloride with mustard aldehyde in EtOH.[6] In a short time the highly colored hydrochloride of the Schiff base separated out and the product was invariably found to be analytically pure with yields varying between 60 and 70% (Table I).

The required aldehyde mustards were prepared by hydroxyethylation of various anilines with ethylene oxide[7] and then treating the products with $POCl_3$ in DMF.[8] The requisite 4-(*p*-aminophenyl)thiazoles

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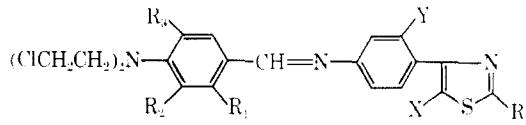
Table I



R = C_6H_5; X = CH; Y = H

No.	R_1	R_2	R_3	Mp, °C[a]	Formula[b,c]
1	H	H	H	248–249	$C_{27}H_{25}Cl_2N_3S \cdot HCl$
2	CH_3	H	H	240–242	$C_{28}H_{27}Cl_2N_3S \cdot HCl$[d]
3	F	H	H	219–221	$C_{27}H_{24}Cl_2FN_3S \cdot HCl$
4	OCH_3	H	H	221–223	$C_{28}H_{27}Cl_2N_3OS \cdot HCl$
5	H	OCH_3	H	197–198	$C_{28}H_{27}Cl_2N_3OS \cdot HCl$
6	OCH_3	H	OCH_3	213–215	$C_{29}H_{29}Cl_2N_3O_2S \cdot HCl$
7	H	OC_2H_5	H	170–171	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$
		R = *p*-$CH_3C_6H_4$; X = CH_3; Y = H			
8	H	H	H	205–207	$C_{28}H_{27}Cl_2N_3S \cdot HCl$
9	CH_3	H	H	265–267	$C_{29}H_{29}Cl_2N_3S \cdot HCl$[d]
10	F	H	H	229–230	$C_{28}H_{26}Cl_2FN_3S \cdot HCl$
11	OCH_3	H	H	205–207	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$
12	H	OCH_3	H	143–144	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$
13	OCH_3	H	OCH_3	213–215	$C_{30}H_{31}Cl_2N_3O_2S \cdot HCl$
14	H	OC_2H_5	H	185–187	$C_{30}H_{31}Cl_2N_3OS \cdot HCl$
		R = *p*-$CH_3OC_6H_4$; X = CH_3; Y = H			
15	H	H	H	220–222	$C_{28}H_{27}Cl_2N_3OS \cdot HCl$
16	CH_3	H	H	239–240	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$[d]
17	F	H	H	219–220	$C_{28}H_{26}Cl_2FN_3OS \cdot HCl$
18	OCH_3	H	H	212–214	$C_{29}H_{29}Cl_2N_3O_2S \cdot HCl$
19	H	OCH_3	H	140–142	$C_{29}H_{29}Cl_2N_3O_2S \cdot HCl$
20	OCH_3	H	OCH_3	198–200	$C_{30}H_{31}Cl_2N_3O_3S \cdot HCl$
21	H	OC_2H_5	H	155–157	$C_{30}H_{31}Cl_2N_3O_2S \cdot HCl$
		R = *p*-ClC_6H_4; X = CH_3; Y = H			
22	H	H	H	245–247	$C_{27}H_{24}Cl_3N_3S \cdot HCl$
23	CH_3	H	H	270–272	$C_{28}H_{26}Cl_3N_3S \cdot HCl$[d]
24	F	H	H	232–234	$C_{27}H_{23}Cl_3FN_3S \cdot HCl$
25	OCH_3	H	H	205–207	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$
26	H	OCH_3	H	220–222	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$
27	OCH_3	H	OCH_3	215–217	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
28	H	OC_2H_5	H	212–214	$C_{29}H_{28}Cl_3N_3OS \cdot HCl$
		R = CH_3; X = CH_3; Y = H			
29	H	H	H	210–212	$C_{22}H_{23}Cl_2N_3S \cdot HCl$
30	CH_3	H	H	221–222	$C_{23}H_{25}Cl_2N_3S \cdot HCl$[d]
31	F	H	H	214–215	$C_{22}H_{22}Cl_2FN_3S \cdot HCl$
32	H	OCH_3	H	165–167	$C_{23}H_{25}Cl_2N_3OS \cdot HCl$
33	OCH_3	H	OCH_3	210–212	$C_{24}H_{27}Cl_2N_3O_2S \cdot HCl$
34	H	OC_2H_5	H	156–157	$C_{24}H_{27}Cl_2N_3OS \cdot HCl$
		R = $C_6H_5CH_2$; X = CH_3; Y = H			
35	H	H	H	201–203	$C_{28}H_{27}Cl_2N_3S \cdot HCl$
36	CH_3	H	H	210–212	$C_{29}H_{29}Cl_2N_3S \cdot HCl$[d]
37	OCH_3	H	H	148–149	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$
38	H	OCH_3	H	207–209	$C_{29}H_{29}Cl_2N_3OS \cdot HCl$
39	OCH_3	H	OCH_3	198–199	$C_{30}H_{31}Cl_2N_3O_2S \cdot HCl$
40	H	OC_2H_5	H	140–142	$C_{30}H_{31}Cl_2N_3OS \cdot HCl$
		R = CH_3; X = H; Y = Cl			
41	H	H	H	208–210	$C_{21}H_{20}Cl_3N_3S \cdot HCl$
42	CH_3	H	H	215–217	$C_{22}H_{22}Cl_3N_3S \cdot HCl$
43	H	OCH_3	H	205–207	$C_{22}H_{22}Cl_3N_3OS \cdot HCl$
44	H	OC_2H_5	H	178–179	$C_{23}H_{24}Cl_3N_3OS \cdot HCl$
		R = $C_6H_5CH_2$; X = H; Y = Cl			
45	H	H	H	198–199	$C_{27}H_{24}Cl_3N_3S \cdot HCl$
46	CH_3	H	H	203–204	$C_{28}H_{26}Cl_3N_3S \cdot HCl$
47	F	H	H	172–174	$C_{27}H_{23}Cl_3FN_3S \cdot HCl$
48	H	OCH_3	H	198–200	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$[d]
49	OCH_3	H	OCH_3	180–181	$C_{29}H_{25}Cl_3N_3O_2S \cdot HCl$
50	H	OC_2H_5	H	140–142	$C_{29}H_{28}Cl_3N_3OS \cdot HCl$

TABLE I (*Continued*)

No.	R_1	R_2	R_3	Mp, °C[a]	Formula[b,c]
		R = $C_6H_5OCH_2$; X = H; Y = Cl			
51	H	H	H	203–204	$C_{27}H_{24}Cl_3N_3OS \cdot HCl$
52	CH_3	H	H	218–220	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$[d]
53	F	H	H	194–196	$C_{27}H_{23}Cl_3FN_3OS \cdot HCl$
54	H	OCH_3	H	200–202	$C_{28}H_{26}Cl_3N_3O_2S \cdot HCl$
55	H	OC_2H_5	H	130–132	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
56	OCH_3	H	OCH_3	208–209	$C_{29}H_{28}Cl_3N_3O_3S \cdot HCl$
		R = *o*-$CH_3C_6H_4OCH_2$; X = H; Y = Cl			
57	H	H	H	210–212	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$[d]
58	CH_3	H	H	220–222	$C_{29}H_{28}Cl_3N_3OS \cdot HCl$
59	F	H	H	208–210	$C_{28}H_{25}Cl_3FN_3OS \cdot HCl$
60	H	OCH_3	H	198–199	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
61	H	OC_2H_5	H	165–167	$C_{30}H_{30}Cl_3N_3O_2S \cdot HCl$
62	OCH_3	H	OCH_3	185–187	$C_{30}H_{30}Cl_3N_3O_3S \cdot HCl$
		R = *p*-$CH_3C_6H_4OCH_2$; X = H; Y = Cl			
63	H	H	H	204–205	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$[d]
64	CH_3	H	H	210–212	$C_{29}H_{28}Cl_3N_3OS \cdot HCl$
65	F	H	H	220–222	$C_{28}H_{25}Cl_3FN_3OS \cdot HCl$
66	H	OCH_3	H	208–210	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
67	H	OC_2H_5	H	194–196	$C_{30}H_{30}Cl_3N_3O_2S \cdot HCl$
68	OCH_3	H	OCH_3	204–205	$C_{30}H_{30}Cl_3N_3O_3S \cdot HCl$
		R = C_6H_5; X = H; Y = Cl			
69	H	H	H	210–212	$C_{26}H_{22}Cl_3N_3S \cdot HCl$
70	CH_3	H	H	240–242	$C_{27}H_{24}Cl_3N_3S \cdot HCl$
71	F	H	H	206–208	$C_{26}H_{21}Cl_3FN_3S \cdot HCl$[d]
72	H	OCH_3	H	194–196	$C_{27}H_{24}Cl_3N_3OS \cdot HCl$
73	H	OC_2H_5	H	192–194	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$
74	OCH_3	H	OCH_3	202–204	$C_{28}H_{26}Cl_3N_3O_2 \cdot HCl$
		R = *p*-$CH_3C_6H_4$; X = H; Y = Cl			
75	H	H	H	203–205	$C_{27}H_{24}Cl_3N_3S \cdot HCl$
76	CH_3	H	H	249–250	$C_{28}H_{26}Cl_3N_3S \cdot HCl$[d]
77	F	H	H	235–237	$C_{27}H_{23}Cl_3FN_3S \cdot HCl$
78	H	OCH_3	H	210–212	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$
79	H	OC_2H_5	H	208–210	$C_{29}H_{28}Cl_3N_3OS \cdot HCl$
80	OCH_3	H	OCH_3	212–214	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
		R = *p*-$CH_3OC_6H_4$; X = H; Y = Cl			
81	H	H	H	212–214	$C_{27}H_{24}Cl_3N_3OS \cdot HCl$
82	CH_3	H	H	242–244	$C_{28}H_{26}Cl_3N_3OS \cdot HCl$
83	F	H	H	218–220	$C_{27}H_{23}Cl_3FN_3OS \cdot HCl$[d]
84	H	OCH_3	H	225–227	$C_{28}H_{26}Cl_3N_3O_2S \cdot HCl$
85	H	OC_2H_5	H	180–182	$C_{29}H_{28}Cl_3N_3O_2S \cdot HCl$
86	OCH_3	H	OCH_3	212–214	$C_{29}H_{28}Cl_3N_3O_3S \cdot HCl$
		R = *p*-ClC_6H_4; X = H; Y = Cl			
87	H	H	H	208–210	$C_{26}H_{21}Cl_4N_3S \cdot HCl$
88	CH_3	H	H	260–262	$C_{27}H_{23}Cl_4N_3S \cdot HCl$
89	F	H	H	230–232	$C_{26}H_{20}Cl_4FN_3S \cdot HCl$
90	H	OCH_3	H	217–219	$C_{27}H_{23}Cl_4N_3OS \cdot HCl$[d]
91	H	OC_2H_5	H	148–150	$C_{28}H_{25}Cl_4N_3OS \cdot HCl$
92	OCH_3	H	OCH_3	210–212	$C_{28}H_{25}Cl_4N_3O_2S \cdot HCl$
	R	R_1	R_2		
		X = Y = R_3 = H			
93[e]	$C_6H_5CH_2$	CH_3	H	184	$C_{28}H_{27}Cl_2N_3S \cdot HCl$
94[e]	*p*-$CH_3OC_6H_4CH_2$	H	H	140	$C_{28}H_{27}Cl_2N_3OS \cdot HCl$
95[e]	$C_6H_5OCH_2$	H	OCH_3	102–104	$C_{28}H_{27}Cl_2N_3O_2S \cdot HCl$

[a] Melting points were taken in capillary tubes with a partial immersion thermometer and are corrected. [b] Pure compounds were obtained without recrystallization. [c] All compounds analyzed for N and S (see ref 10) except 71, 73, 86, 90, which were analyzed for N. [d] Also analyzed for C, H (see ref 10). [e] Reported earlier: S. S. Sabnis, *Indian J. Chem.*, 5, 619 (1967).

were prepared by the condensation of different halo ketones with necessary thioamides. Some of these thiazoles utilized have been reported from this laboratory[9] and those, not hitherto described, are given in Table III.

(9) B. S. Kulkarni, B. S. Fernandez, M. R. Patel, R. A. Beliare, and C. V. Deliwala, *J. Pharm. Sci.*, **58**, 852 (1969).

Biological Results.—Seventeen representative compounds were screened for their antitumor activity against Dunning leukemia (solid), L 1210 lymphoid leukemia, Walker carcinosarcoma 256 (intramuscular) and studied for toxicity under the auspices of Cancer Chemotherapy National Service Center, Bethesda, Md. The screening results of 13 active compounds are pre-

Table II

Screening Data[a,b]

No.[c]	Test system[d]	Dose (mg/kg)	Survivors	Cures	Animal[e] wt diff (g) ($T - C$)	Tumor wt[f] or survival days[g] T/C	T/C %
32	3LE	100.0	2/4		−3.2	7.0/8.7	
		50.0	4/4		−7.2	8.5/8.7	97
		25.0	4/4		−6.4	13.5/8.7	155
		37.5	6/6		−6.4	14.2/10.0	142
		25.0	6/6		−3.8	13.8/10.0	138
		16.6	6/6		−4.2	12.3/10.0	123
		11.1	6/6		−3.2	11.8/10.0	118
		11.0	6/6		−4.0	17.8/8.9	200
		7.0	5/6		−3.2	12.0/8.9	134
		4.6	6/6		−2.5	11.3/8.9	126
		3.0	6/6		−2.4	11.5/8.9	129
35	AA	330.0	3/3		4		
		100.0	3/3		8		
		70.0	3/3		22		
		33.0	3/3		18		
	5WM	330.0	6/6		−1	2.9/8.9	36[h]
36	AA	330.0	3/3		8		
		330.0	3/3		8		
		30.0	3/3		15		
		10.0	3/3		13		
	5WM	330.0	6/6		−6.0	1.2/8.0	15[i]
38	AA	100.0	0/3				
		33.0	3/3		−14		
		10.0	3/3		0		
		3.0	3/3		8		
	3LE	400.0	0/6				
		200.0	6/6		−6.0	8.7/8.9	97
		100.0	6/6		−6.4	12.5/8.9	140
		150.0	6/6		−6.9	9.8/9.0	108
		110.0	6/6		−7.4	12.5/9.0	138
		66.0	6/6		−5.9	12.2/9.0	135
		44.0	6/6		−4.9	11.0/9.0	122[j]
	5WM	40.0	6/6		−27	0.6/5.3	11[k]
39	3LE	150.0	6/6		−2.3	14.3/9.3	153
		75.0	6/6		−2.8	13.2/9.3	141
		150.0	3/6		−5.8	7.7/10.0	
		100.0	6/6		−5.2	8.8/10.0	88
		66.0	6/6		−4.8	10.2/10.0	102
		44.0	6/6		−4.3	12.7/10.0	127
		50.0	6/6		−1.9	13.5/10.4	129[l]
46	5WM	400.0	6/6		−7.0	0.4/5.2	7
		400.0	6/6		−9.0	0.7/6.0	11
		400.0	6/6		−5.0	0.8/6.8	11
		400.0	6/6		−8.0	1.2/7.3	16
		400.0	6/6		−11.0	0.3/6.3	4[k]
49	5LE	300.0	6/6		−3.1	17.0/9.3	182
		150.0	6/6		−2.4	14.3/9.3	153
		75.0	6/6		−2.9	13.8/9.3	148[l]
52	AA	330.0	3/3				
		100.0	3/3				
		33.0	3/3				
		10.0	3/3				
	5WM	330.0	6/6		−7.0	1.9/6.3	30
54	AA	330.0	0/3		0		
		110.0	1/3		−34.0		
		36.0	3/3		−8.0		
		12.0	3/3		10.0		

Table II (*Continued*)

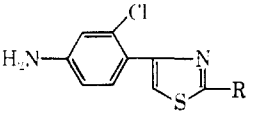
No.[c]	Test system[d]	Dose (mg/kg)	Survivors	Cures	Animal[e] wt diff (g) ($T - C$)	Tumor wt[f] or survival days[g] T/C	T/C %
	3LE	400.0	0/4				
		200.0	4/4		−8.1	11.3/9.5	118
		100.0	4/4		−2.8	12.5/9.5	131
		150.0	4/4		−7.0	11.5/8.6	133
		100.0	4/4		−1.7	11.0/8.6	127
		66.6	4/4		−5.8	12.0/8.6	139
		44.4	4/4		−4.8	11.0/8.6	127
	5WM	22.5	6/6		−1.0	0.6/6.0	10
		11.2	6/6		6.0	0.9/6.0	15
		5.6	6/6		2.0	3.5/6.0	58
		2.8	5/6		8.9	6.0/6.0	100
		45.0	6/6		−12.0	0.7/8.7	8
		22.5	6/6		−3.0	1.1/5.3	20
		11.2	6/6		−1.0	0.6/5.3	11
		5.6	6/6		−1.0	2.0/5.3	37
		2.8	6/6		−2.0	3.8/5.3	71
72	3LE	200.0	3/4		−9.4	8.0/8.4	95
		100.0	4/4		−6.1	14.0/8.4	166
		150.0	6/6		−7.7	13.3/9.3	138
		100.0	6/6		−7.3	13.3/9.6	138
		66.0	6/6		−6.6	13.3/9.6	138
		44.0	6/6		−5.8	13.3/9.6	138
		44.0	6/6		−3.2	13.0/8.9	146
		29.0	6/6		−3.1	12.0/8.9	134
		19.0	6/6		−1.7	11.5/8.9	129
		12.0	6/6		−0.8	11.3/8.9	126
		30.0	6/6		−6.7	14.2/9.1	156
		20.0	6/6		−4.3	12.8/9.1	140
		13.0	6/6		−4.5	12.5/9.1	137
		8.0	6/6		−3.7	11.7/9.1	128
93	AA	100.0	3/3		12.0		
		33.0	3/3		15.0		
		10.0	3/3		20.0		
		3.0	3/3		23.0		
	DL	200.0	7/7	4	−15.0	30/15	200
		100.0	7/7		−5.0	20/15	133
		50.0	7/7		−6.0	19/15	126
		25.0	7/7		3.0	15/15	100
	5WM	400.0	6/6		−4	1.4/6.7	20
		400.0	6/6		−10	0.4/7.0	5
		400.0	6/6		−9	0.9/5.7	15
		400.0	6/6		−17	0.6/4.8	12
		400.0	6/6		−13	0.5/4.9	10
		400.0	6/6		−17	1.4/8.8	15[h]
94	AA	100.0	3/3		11.0		
		33.0	3/3		11.0		
		10.0	3/3		16.0		
		3.0	3/3		13.0		
	5WM	400.0	6/6		−1	2.4/6.7	35
		400.0	6/6		−1	1.6/7.0	22
		400.0	6/6		−7	1.6/5.7	28
		400.0	6/6		−12	0.8/4.8	16
		400.0	6/6		−8	0.7/4.9	14
		400.0	6/6		−8	2.3/8.8	26[h]
95	AA	100.0	0/3		−18		
		33.0	3/3		−18		
		10.0	3/3		4		
		3.0	3/3		24		
	DL	44.0	2/6		0	0/16	0
		22.0	6/6	2	0	23/16	143
		11.0	6/6	6	0	30/16	187
		5.5	6/6	5	0	30/16	187
		5.5	7/7		−3	21/16	131
		2.75	7/7		−3	18/16	112
		1.37	7/7		−4	17/16	106
		0.68	7/7		−5	16/16	100

Table II (Continued)

No.[a]	Test system[d]	Dose (mg/kg)	Survivors	Cures	Animal[e] wt diff (g) (T − C)	Tumor wt[f] or survival days[g] T/C	T/C %
	5WM	75.0	5/6		−19	0.5/6.7	7
		50.0	5/6		−22	0.4/6.7	5
		33.0	6/6		−13	0.3/6.7	4
		22.0	6/6		19	0.8/6.7	11
		100.0	2/6		16	0.6/7.0	
		12.0	5/6		−17	0.9/7.0	12
		6.0	6/6		−3	1.9/7.0	27
		3.0	6/6		−3	3.4/7.0	48
	3LE	400.0	0/4				
		200.0	3/4		−6.2	10.0/9.6	104
		100.0	4/4		−5.5	14.0/9.6	145
		50.0	4/4		−6.2	10.3/9.6	107
		150.0	4/4		−5.6	10.8/8.5	127
		100.0	4/4		−5.5	13.3/8.5	156
		66.0	4/4		−4.6	14.8/8.5	164
		44.4	4/4		−5.5	11.8/8.5	138
		50.0	6/6		−4.5	8.7/9.4	92
		33.0	6/6		−3.3	10.5/9.4	111
		22.0	5/6		−3.6	10.8/9.4	114
		15.0	6/6		−2.6	13.8/9.4	146
		40.0	4/4		−5.2	13.3/8.5	156
		20.0	4/4		−3.6	10.5/8.5	123
		10.0	4/4		−1.8	10.3/8.5	121

[a] Only a part of the data is presented. [b] Assays were performed according to specifications established by CCNSC as reported in *Cancer Chem. Rep.*, **25**, 1 (1962). [c] Numbers refer to those from Table I. [d] AA, toxicity; LE, L1210 lymphoid leukemia; WM, Walker 256 (intramuscular). [e] Average wt change of test group minus average wt change of control animals in grams; T, test; C, control. [f] Tumor wt for WM test system. [g] Survival days for DL and LE test systems. [h] Single treatment. [i] At lower doses the compound is inactive. [j] Further testing in progress. [k] Activity confirmed.

Table III



No.	R	Yield,[a] %	Mp, °C[b]	Formula[c]
1	Me[d]	40	265–267	$C_{10}H_9ClN_2S \cdot HCl$
2	$PhCH_2$[d]	48	193–195	$C_{16}H_{13}ClN_2S \cdot HCl$
3	$PhOCH_2$[d]	55	196–198	$C_{16}H_{13}ClN_2OS \cdot HCl$
4	o-$MeC_6H_4OCH_2$[d]	53	190–192	$C_{17}H_{15}ClN_2OS \cdot HCl$
5	p-$MeC_6H_4OCH_2$[d]	50	178–179	$C_{17}H_{15}ClN_2OS \cdot HCl$
6	Ph[e]	65	242–243	$C_{15}H_{11}ClN_2S \cdot HCl$
7	p-MePh[e]	60	233–235	$C_{16}H_{13}ClN_2S \cdot HCl$
8	p-MeOPh[e]	58	218–220	$C_{16}H_{13}ClN_2OS \cdot HCl$
9	p-ClPh[e]	65	260–262	$C_{15}H_{10}Cl_2N_2S \cdot HCl$

[a] Yields are the results of single experiments and are based on ω-chloro-4-amino-2-chloroacetophenone. [b] See footnote *a*, Table I. [c] All compounds analyzed for N and S (ref 10). [d] From EtOH and Et_2O. [e] From EtOH.

sented in Table II. Compounds **1**, **45**, **69**, and **70** were inactive.

Schiff bases derived from 4-[*N*,*N*-bis(2-chloroethyl)amino]-*m*-anisaldehyde (**32**, **38**, **54**, **72**) are in general significantly active against L 1210 lymphoid leukemia. Compounds **38** and **54** have also shown good activity against Walker carcinosarcoma 256 (intramuscular) at low doses. However, the related Schiff bases from other aldehyde mustards (**35**, **36**, **46**, **49**, **52**) are either inactive or poorly active against L 1210. The MeO group present in 4-[*N*,*N*-bis(2-chloroethyl)amino]-*m*-anisaldehyde appears to play a key role in deciding the antitumor activity against L 1210 lymphoid leukemia. The Schiff bases **35**, **36**, **46**, **52**, **93**, and **94** which are devoid of MeO did not exhibit much activity against L 1210 lymphoid leukemia in spite of high tumor inhibition in Walker carcinosarcoma 256 (intramuscular), where three compounds (**46**, **93**, and **94**) have shown confirmed activity.

Compounds **93** and **95** showed significant activity against Dunning leukemia (solid). The most active Schiff base **95** gave 6/6 cures at 11.0 mg/kg per day.

The presence of Cl in **54** did not increase the activity against 3 LE in comparison with **95** but it did reduce the toxicity. The addition of Me at the 5 position in the thiazole ring did not alter the activity against 3 LE (*cf.* **36** and **93**).

Experimental Section[10]

2-(*p*-Chlorophenyl)-4-[(4-amino-2-chloro)phenyl]thiazole.—A mixture of ω-chloro-4-amino-2-chloroacetophenone (0.204 g, 0.001 mole), *p*-chlorothiobenzamide (0.17 g, 0.001 mole), and abs EtOH (10 ml) was heated gently at reflux temp for 30 min. A crystalline solid, obtained from this soln upon cooling in ice bath, was filtered off and washed (EtOH, Et_2O) to give the required monohydrochloride which was recrystd (EtOH).

All the other 4-[(4-amino-2-chloro)phenyl]-2-substituted-thiazoles were prepared similarly and are listed in Table III.

2-(*p*-Chlorophenyl)-5-methyl-4-(*p*-aminophenyl)thiazole was prepared by the condensation of ω-chloro-*p*-aminopropiophenone with *p*-chlorothiobenzamide in refluxing EtOH. It was recrystd (EtOH), yield 56%, mp 268–269°. *Anal.* ($C_{16}H_{13}ClN_2S \cdot HCl$) N, S.

2-(Phenoxymethyl)-4-{*p*-({4-[*N*,*N*-bis(2-chloroethyl)amino]-3-methoxybenzylidene}amino)-2-chlorophenyl]thiazole Monohydrochloride.—To a soln of 4-[(4-amino-2-chloro)phenyl]-2-(phenoxymethyl)thiazole·HCl (0.353 g, 0.001 mole) in dry warm EtOH was added a concd EtOH soln of 4-[*N*,*N*-bis(2-chloroethyl)amino]-*m*-anisaldehyde (0.276 g, 0.001 mole). The resulting dark red solution on standing overnight in an ice bath deposited a crystalline solid which was collected by filtration and

(10) Analyses of the elements were within ±0.4% of the theoretical values.

washed with dry EtOH and Et_2O to give the pure hydrochloride (0.358 g, 70%) of the desired Schiff base.

All the other Schiff bases were similarly prepared and are recorded in Table I.

Acknowledgments.—The authors are greatly indebted to Dr. H. B. Wood, Chief, Drug Development Branch, Cancer Chemotherapy National Service Center for his cooperation and for making the screening data available and to Mr. M. T. Jaokar for microanalyses. They are grateful to Dr. N. K. Dutta, Director, Haffkine Institute, Bombay, for providing facilities to carry out the present work.

Synthesis of Dideoxyzearalanone and Hydroxyl Derivatives

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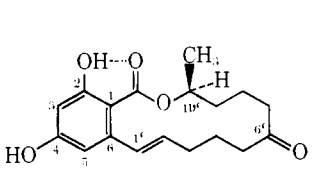
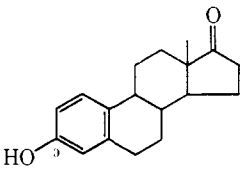
Received May 4, 1970

Syntheses are reported of 2,4-dideoxyzearalanone and each of the possible monophenolic derivatives of this compound. The preparation of 5-hydroxyzearalanone is also described. None of the new compounds exceeded the parent in respect to estrogenic activity.

The isolation of zearalenone (**1**) and a preliminary account of its marked uterotrophic activity and anabolic properties were reported by Stob and coworkers.[1] The structure of this fungal metabolite was deduced by chemical and spectroscopic means,[2] total syntheses of zearalenone have been announced[3] and its absolute configuration has also been determined.[4]



1



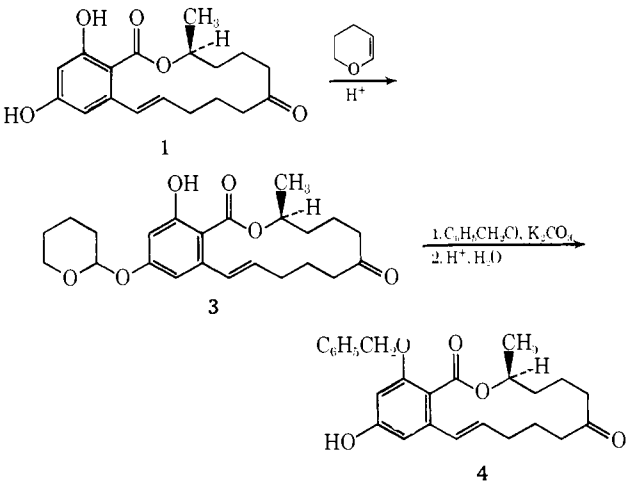
2

We have synthesized a number of structural variants of zearalenone in a joint project conducted several years ago with a group similarly engaged in the laboratories of the Commercial Solvents Corporation. The purpose of the present report is to summarize our findings in respect to the effect of phenolic OH addition or removal on the biological activity of the parent compound. This study was undertaken in a systematic way since the structural relationships of zearalenone to a typical estrogen such as estrone (**2**) are not obvious by inspection of models of their formulae. We selected as our synthetic goals the parent 2,4-dideoxyzearalanone (**6**) and each of the possible monosubstituted phenolic analogs, as well as 5-hydroxyzearalanone.

Chemistry.—Hydrogenolysis of the phenolic hydroxyls in zearalenone was possible using the technique of Musliner and Gates.[5] This involved preparation of the 1-phenyl-5-tetrazolyl ethers and their hydrogenolysis with 5% Pd–C which concurrently caused reduction of the olefin functionality. Our preferred conditions were 95% EtOH as solvent, and 3.5 kg/cm² at about 70° for 48 hr. These conditions are more drastic than those which were of general utility for Musliner and Gates.

In order to attain selective removal of the 2- and 4-hydroxyls of zearalenone it was necessary to prepare the required monophenyltetrazolyl derivatives. This was accomplished from the 2-benzyl and 4-tetrahydropyranyl ethers whose preparation is shown in Scheme I.

SCHEME I



The key reaction in this sequence was the selective monotetrahydropyranyl ether formation at C-4 in good yield using excess dihydropyran. This selectivity is attributed to the equilibrium nature of the reaction. Steric hindrance at C-2 and a loss of H bonding to the lactone CO are considered to disfavor derivatization of the 2-OH. It is also worth noting that benzyl ether formation is possible using K_2CO_3 in MeOH without any appreciable opening of the lactone ring. Formation of the phenyltetrazolyl ethers **5**, **7**, and **9** went well from the corresponding phenols using anhyd K_2CO_3 and 1-phenyl-5-chlorotetrazole in refluxing dry Me_2CO for 16 hr. The properties of these derivatives and of their

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