Potential Anticancer Agents. II. Schiff Bases from Benzaldehyde Nitrogen Mustards

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Schiff bases from benzaldehyde nitrogen mustards having methyl, methoxy, ethoxy, or chloro groups in 2, 3, or 3,5 positions, and amines such as 3-, 4-, and 3,5-halogenated anilines, 2-, 3-, and 4-aminobenzoic acids, 2-(*p*-aminophenyl)pyridine, and 2-(*p*-aminophenyl)-4-methylpyridine have been synthesized and screened for antitumor activity. A number of these compounds displayed significant activity against Dunning leukemia (solid), L1210 lymphoid leukemia, and Walker 256 (intramuscular). *m*-({4-[Bis(2-chloroethyl)amino]-3-methoxybenzylidene}amino)benzoic acid, N,N-bis(2-chloroethyl)-4-[N-(3,5-dichlorophenyl)formimidoyl]-*o*-anisidine, and N,N-bis(2-chloroethyl)-4-[N-(*m*-bromophenyl)formimidoyl]-*o*-anisidine were the most active compounds in the present series.

Many nitrogen mustards are cytotoxic toward rapidly proliferating tissues but the lack of specificity toward neoplastic tissues has limited their use as chemotherapeutic agents. Among several attempts to prepare compounds with greater specificity of action, the concept of "latent activity" has attracted a number of workers in this field.[2-7] Ross and Warwick[3,6] prepared a series of practically inert azo mustards (I) with the hope that these compounds might become activated by *in vivo* reduction of the azo linkage by enzymes. Indeed, several compounds in this series were found active against Walker rat carcinoma 256.

I

II

As an extension of this work Ross, *et al.*,[5] and, later, Popp[9,10] synthesized several Schiff bases (II) which are isosteres of the azo mustards (I). In an extensive research program, Popp observed that several of these compounds were active against Dunning leukemia in rats and a few compounds were considered for clinical trials.[11,12]

In view of the antitumor activity of these Schiff bases coupled with the suggested essential role of azomethine linkage in certain biological reactions,[13-15] it appeared worthwhile to explore this field further. We have previously reported the synthesis of Schiff bases from benzaldehyde nitrogen mustards and thiazole amines[16,17] as well as nitrogen mustard containing benzylidenehydrazides.[18] The present paper deals with the synthesis and antitumor evaluation of several Schiff bases from benzaldehyde nitrogen mustards and substituted anilines.

Chemistry.—The preparation of the Schiff bases reported in Table I involved the condensation of appropriate amines with benzaldehyde nitrogen mustards. In the majority of cases the Schiff bases were characterized as their monohydrochlorides which were formed by treating the hydrochloride salt of the requisite amine with aldehyde mustards in alcohol. However, several Schiff bases are recorded as free bases and were prepared by Popp's method.[9] The hydrochlorides of the Schiff bases prepared by the former method were extremely pure and the method was particularly useful when the amines were liquids, since it avoided the formation of gummy solids which are difficult to purify.

The required aldehyde mustards were prepared by the hydroxyethylation of appropriate anilines with ethylene oxide[19] and then treating the products with $POCl_3$ and DMF.[20] 2-(*p*-Aminophenyl)-4-methylpyridine was prepared by the reduction of the corresponding 2-(*p*-nitrophenyl)-4-methylpyridine which in turn was obtained from 4-methylpyridine following the method of Haworth, *et al.*,[21] used for the preparation of 2-(*p*-nitrophenyl)pyridine.

Biological Results

Representative Schiff bases were screened for their antitumor activity against Walker carcinosarcoma 256 (subcutaneous), Dunning leukemia (solid), L1210 lymphoid leukemia, and Walker 256 (intramuscular) under the auspices of the Cancer Chemotherapy National Service Center, Bethesda, Md., and the screening results are presented in Table II.

The data on toxicity indicate that Schiff bases derived from 4-[N,N-bis(2-chloroethyl)amino]-*m*-anisaldehyde (**4**, **8**, **20**, **35**, **44**, **47**) are relatively more toxic than the Schiff bases obtained from other aldehyde mustards.

None of the compounds displayed any appreciable activity against Walker 256 (subcutaneous). How-

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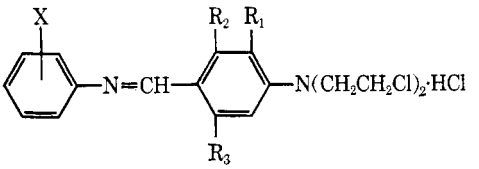
TABLE I

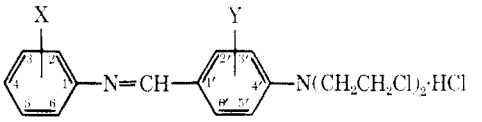


No.	X	R1	R2	R3	Mp, °C[a]	Formula	Analyses[b]
1	3-Cl	H	H	H	190–192	$C_{17}H_{17}Cl_3N_2 \cdot HCl$[c]	C, H, N
2	3-Cl	H	CH_3	H	229–230	$C_{18}H_{19}Cl_3N_2 \cdot HCl$[c]	C, H, N
3	3-Cl	H	OCH_3	H	156–157	$C_{18}H_{19}Cl_3N_2O \cdot HCl$[c]	C, H, N
4	3-Cl	OCH_3	H	H	182–184	$C_{18}H_{19}Cl_3N_2O \cdot HCl$[c]	C, H, N
5	3-Cl	OCH_3	H	OCH_3	201–202	$C_{19}H_{21}Cl_3N_2O_2 \cdot HCl$[c]	C, H, N
6	3-Cl	OC_2H_5	H	H	118–120	$C_{19}H_{21}Cl_3N_2O \cdot HCl$[c]	C, H, N
7	3-Cl	H	Cl	H	169–171	$C_{17}H_{16}Cl_4N_2 \cdot HCl$[c]	C, H, N
8	4-Cl	OCH_3	H	H	191–192	$C_{18}H_{19}Cl_3N_2O \cdot HCl$[c]	C, H, N
9	4-Cl	H	OCH_3	H	192–194	$C_{18}H_{19}Cl_3N_2O \cdot HCl$[c]	N
10	4-Cl	OCH_3	H	OCH_3	208–210	$C_{19}H_{21}Cl_3N_2O_2 \cdot HCl$[c]	N
11	4-Cl	OC_2H_5	H	H	168–170	$C_{19}H_{21}Cl_3N_2O \cdot HCl$[c]	N
12	3,5-Cl_2	H	H	H	220–221	$C_{17}H_{16}Cl_4N_2 \cdot HCl$[c]	C, H, N
13	3,5-Cl_2	H	CH_3	H	225–226	$C_{18}H_{18}Cl_4N_2 \cdot HCl$[c]	N
14	3,5-Cl_2	OCH_3	H	H	141–142	$C_{18}H_{18}Cl_4N_2O \cdot HCl$[c]	N
15	3,5-Cl_2	H	OCH_3	H	195–197	$C_{18}H_{18}Cl_4N_2O \cdot HCl$[c]	N
16	3,5-Cl_2	OC_2H_5	H	H	165–166	$C_{19}H_{20}Cl_4N_2O \cdot HCl$[c]	N
17	3,5-Cl_2	H	Cl	H	202–203	$C_{17}H_{15}Cl_5N_2 \cdot HCl$[c]	N
18	3-Br	H	H	H	210–211	$C_{17}H_{17}BrCl_2N_2 \cdot HCl$[c]	C, H N
19	3-Br	H	CH_3	H	202–203	$C_{18}H_{19}BrCl_2N_2 \cdot HCl$[c]	N
20	3-Br	OCH_3	H	H	185–186	$C_{18}H_{19}BrCl_2N_2O \cdot HCl$[c]	N
21	3-Br	H	OCH_3	H	195–196	$C_{18}H_{19}BrCl_2N_2O \cdot HCl$[c]	N
22	3-Br	OCH_3	H	OCH_3	205–206	$C_{19}H_{21}BrCl_2N_2O_2 \cdot HCl$[c]	N
23	3-Br	OC_2H_5	H	H	115–116	$C_{19}H_{21}BrCl_2N_2O \cdot HCl$[c]	N
24	3-Br	H	Cl	H	204–205	$C_{17}H_{16}BrCl_3N_2 \cdot HCl$[c]	N
25	3-F	H	H	H	128–130	$C_{17}H_{17}Cl_2FN_2 \cdot HCl$[c]	C, H, N
26	3-F	H	CH_3	H	220–221	$C_{18}H_{19}Cl_2FN_2 \cdot HCl$[c]	N
27	3-F	OCH_3	H	H	197–198	$C_{18}H_{19}Cl_2FN_2O \cdot HCl$[c]	N
28	3-F	H	OCH_3	H	172–173	$C_{18}H_{19}Cl_2FN_2O \cdot HCl$[c]	N
29	3-F	OCH_3	H	OCH_3	198–199	$C_{19}H_{21}Cl_2FN_2O_2 \cdot HCl$[c]	N
30	3-F	OC_2H_5	H	H	200–202	$C_{19}H_{21}Cl_2FN_2O \cdot HCl$[c]	N
31	3-F	H	Cl	H	198–200	$C_{17}H_{16}Cl_3FN_2 \cdot HCl$[c]	N
32	3-CF_3	OCH_3	H	OCH_3	198–200	$C_{20}H_{21}Cl_2F_3N_2O_2 \cdot HCl$[c]	N
33	2-COOH	H	H	H	166–167	$C_{18}H_{18}Cl_2N_2O_2$[d]	C, H, N
34	2-COOH	H	CH_3	H	194–195	$C_{19}H_{20}Cl_2N_2O_2$[d]	N
35	2-COOH	OCH_3	H	H	147–148	$C_{19}H_{20}Cl_2N_2O_3$[d]	N
36	2-COOH	H	OCH_3	H	113–115	$C_{19}H_{20}Cl_2N_2O_3$[d]	N
37	2-COOH	OCH_3	H	OCH_3	182–183	$C_{20}H_{22}Cl_2N_2O_4$[d]	N
38	2-COOH	OC_2H_5	H	H	159–160	$C_{20}H_{22}Cl_2N_2O_3$[d]	N
39	2-COOH	H	Cl	H	182–184	$C_{18}H_{17}Cl_3N_2O_2$[d]	N
40	3-COOH	H	H	H	245–246	$C_{18}H_{18}Cl_2N_2O_2 \cdot HCl$[c]	C, H, N
41	3-COOH	H	CH_3	H	254–255	$C_{19}H_{20}Cl_2N_2O_2 \cdot HCl$[c]	N
42	3-COOH	OCH_3	H	H	217–218	$C_{19}H_{20}Cl_2N_2O_3 \cdot HCl$[c]	N
43	3-COOH	H	OCH_3	H	215–216	$C_{19}H_{20}Cl_2N_2O_3 \cdot HCl$[c]	N
44	3-COOH	OCH_3	H	OCH_3	219–220	$C_{20}H_{22}Cl_2N_2O_4 \cdot HCl$[c]	N
45	3-COOH	OC_2H_5	H	H	222–224	$C_{20}H_{22}Cl_2N_2O_3 \cdot HCl$[c]	N
46	3-COOH	H	Cl	H	243–244	$C_{18}H_{17}Cl_3N_2O_2 \cdot HCl$[c]	N
47	4-COOH	OCH_3	H	H	222–223	$C_{19}H_{20}Cl_2N_2O_3 \cdot HCl$[c]	C, H, N
48	4-COOH	H	OCH_3	H	198–199	$C_{19}H_{20}Cl_2N_2O_3 \cdot HCl$[c]	N
49	4-COOH	OCH_3	H	OCH_3	228–229	$C_{20}H_{22}Cl_2N_2O_4 \cdot HCl$[c]	N
50	4-COOH	OC_2H_5	H	H	220–223	$C_{20}H_{22}Cl_2N_2O_3 \cdot HCl$[c]	N
51	4-COOH	H	Cl	H	248–250	$C_{18}H_{17}Cl_3N_2O_2 \cdot HCl$[c]	N
52	4-(2-Pyridyl)	H	H	H	130–131	$C_{22}H_{21}Cl_2N_3$[d]	C, H, N
53	4-(2-Pyridyl)	H	CH_3	H	Oil	$C_{23}H_{25}Cl_2N_3$[d]	N
54	4-(2-Pyridyl)	H	OCH_3	H	Oil	$C_{23}H_{23}Cl_2N_3O$[d]	N
55	4-(2-Pyridyl)	H	Cl	H	Oil	$C_{22}H_{20}Cl_3N_3$[d]	N
56	4-[2-(4-Methylpyridyl)]	H	H	H	Oil	$C_{23}H_{23}Cl_2N_3$[d]	N
57	4-[2-(4-Methylpyridyl)]	H	OCH_3	H	150–152	$C_{24}H_{25}Cl_2N_3O$[d]	C, H, N

[a] Melting points were taken in open capillary tubes in a sulfuric acid bath and are uncorrected. [b] Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [c] Prepared by method A; pure compounds were obtained without recrystallization. [d] Prepared by method B and characterized as free Schiff bases; recrystallized from EtOH.

Table II

Screening Data[a,b]



No.[c]	X	Y	Test system[d]	Dose, mg/kg	Survivors	Cures	Animal wt dif, g (T − C)[e]	Tumor wt (g)[f] or survival days[g] T/C	T/C, %	Specificity test	Confidence, %
1	3-Cl	H	AA	100.0	3/3		22				
				33.0	3/3		24				
				10.0	3/3		20				
				3.0	3/3		22				
			WM	400.0	4/6		−18	0.5/4.4	11		
				400.0	5/6		−16	0.8/6.4	12		
				200.0	6/6		−3	4.2/8.7	48		
				200.0	6/6		−4	4.6/6.2	74		
				200.0	6/6		0	2.1/5.2	40		
				200.0	6/6		−2	2.6/8.2	31[h]		
2	3-Cl	2′-CH_3	AA	100.0	3/3		9				
				33.0	3/3		15				
				10.0	3/3		19				
				3.0	3/3		31				
			WA	50.0	6/6		0	1.8/5.5	32		
			WM	200.0	6/6		−9	0.4/4.4	9		
				200.0	6/6		−26	0.5/5.4	9		
				200.0	6/6		−17	0.3/6.1	4		
				200.0	6/6		−19	0.5/7.3	6		
				200.0	6/6		−16	0.7/6.8	10		
				200.0	6/6		−16	0.2/8.0	2[i]		
										<12, >0	99.7
3	3-Cl	2′-OCH_3	AA	100.0	3/3		3				
				33.0	3/3		18				
				10.0	3/3		26				
				3.0	3/3		28				
			WA	50.0	6/6		−22	9.9/14.9	66		
			WM	400.0	6/6		−15	0.1/4.4	2		
				400.0	6/6		−17	0.8/6.4	12		
				400.0	6/6		−17	1.0/6.8	14		
				400.0	6/6		−17	1.0/8.7	11		
				400.0	6/6		−13	0.4/7.1	5		
4	3-Cl	3′-OCH_3	AA	100.0	0/3						
				33.0	0/3		15				
				10.0	2/3		6				
				3.0	3/3		12				
			WA	1.25	6/6		−14	5.7/6.8	83		
			DL	5.0	5/6	4	−20	30.0/16.0	187[j]		
			WM	50.0	4/6		−23	0.2/7.0	2		
				75.0	4/6		−18	0.6/7.0	8		
				50.0	4/6		−16	0.5/7.0	7		
				33.0	4/6		−14	0.5/7.0	7		
				22.0	6/6		−8	1.1/7.0	15		
				12.0	6/6		−7	0.5/5.9	8		
										<11, >0	99.7
8	4-Cl	3′-OCH_3	AA	100.0	0/3						
				33.0	3/3		8				
				10.0	3/3		23				
				3.0	3/3		22				
			WA	20.0	6/6		−26	11.2/14.9	75		
			LE	400.0	1/4		−7.5	7.0/9.0			
				200.0	4/4		−4.0	13.3/9.0	147		
				100.0	4/4		−0.1	9.8/9.0	120		
				300.0	0/4						
				200.0	4/4		−5.9	11.5/9.8	117		
				133.0	4/4		−2.6	10.5/9.8	107		
				89.0	3/4		−5.1	12.3/9.8	125		
			WM	15.0	6/6		−10	0.3/5.4	5		
				15.0	6/6		−7	0.7/6.8	10		
				15.0	6/6		−7	1.1/8.7	12		

TABLE II (*Continued*)

No.[c]	X	Y	Test system[d]	Dose, mg/kg	Survivors	Cures	Animal wt dif, g (T − C)[e]	Tumor wt (g)[f] or survival days[g] T/C	T/C, %	Specificity test	Confidence, %
8	4-Cl	3′-OCH	WM	15.0	6/6		−7	0.8/6.8	11		
				15.0	6/6		5	1.1/8.0	13		
				15.0	6/6		−7	0.9/7.1	12[i]		
										<21, >0	99.7
14	3,5-Cl_2	3′-OCH_3	AA	100.0	3/3		11				
				33.0	3/3		0				
				10.0	3/3		14				
				3.0	3/3		16				
			WA	50.0	6/7		−48	2.2/13.8	15		
			LE	400.0	4/4		−3.3	14.3/10.0	143		
				200.0	4/4		−4.8	12.3/10.0	123		
				100.0	3/4		−4.7	15.7/10.0	157		
				50.0	4/4		−3.1	12.3/10.0	123		
				150.0	4/4		−6.2	10.8/9.3	116		
				100.0	4/4		−5.9	12.5/9.3	134		
				66.0	4/4		−5.5	13.0/9.3	139		
				42.0	4/4		−6.3	12.8/9.3	137		
				50.0	6/6		−5.9	11.8/9.6	112		
				30.0	6/6		−3.7	12.5/9.6	130		
				18.0	6/6		−3.4	11.3/9.6	117		
				10.0	6/6		−2.7	11.2/9.6	116		
			WM	40.0	6/6		−20	0.9/6.9	15		
				40.0	6/6		−15	1.0/7.5	13		
				40.0	6/6		−18	0.5/7.4	6		
				40.0	6/6		−24	0.2/5.9	3		
				40.0	5/6		−20	0.2/5.2	3		
				40.0	6/6		−20	0.1/6.0	1[i]		
										<27, >0	99.7
20	3-Br	3′-OCH_3	AA	100.0	0/3		24				
				33.0	0/3						
				10.0	3/3		−8				
				3.0	3/3		11				
			WA	4.0	6/6		−2	0.9/5.5	16		
			LE	400.0	4/4		−1.6	10.5/8.5	123		
				200.0	4/4		−0.8	10.0/8.5	117		
				100.0	4/4		−1.9	10.0/8.5	117		
				50.0	4/4		−4.4	10.8/8.5	127		
				75.0	3/4		−3.6	12.7/7.7	130		
				50.0	4/4		−3.5	11.0/9.7	113		
				33.0	4/4		−4.3	10.5/9.7	108		
				22.0	4/4		−0.8	10.8/9.7	111		
				25.0	6/6		−4.7	12.0/8.8	136		
				15.0	6/6		−3.9	13.0/8.8	147		
				9.0	6/6		−3.4	14.0/8.8	159		
				5.0	6/6		−2.6	12.8/8.8	145		
			WM	25.0	6/6		−19	0.5/6.1	8		
				25.0	6/6		−10	0.3/8.7	3		
				25.0	6/6		−21	0.2/6.8	2		
				25.0	6/6		−11	0.7/8.7	8		
				25.0	6/6		−15	0.4/7.1	5		
				25.0	6/6		−16	1.0/6.2	16[i]		
										<20, >0	99.7
33	2-COOH	H	AA	100.0	3/3		8				
				33.0	3/3		24				
				10.0	3/3		29				
				3.0	3/3		27				
			WA	50.0	6/6		1	9.6/10.7	89		
			DL	200.0	7/7	7	−20	30.0/16.0	187		
				100.0	7/7	3	−7	29.0/16.0	181[j]		
			WM	100.0	6/6		−4	1/2/6.7	17		
				100.0	6/6		0	2.2/5.7	38		
				100.0	6/6		3	2.0/4.4	45		
				100.0	6/6		0	1.8/5.9	30		
				100.0	6/6		−3	1.1/5.2	21		
				100.0	6/6		−1	0.5/5.4	9[i]		
										<19, >0	99.7

Table II (*Continued*)

No.[c]	X	Y	Test system[d]	Dose, mg/kg	Survivors	Cures	Animal wt dif, g (T − C)[e]	Tumor wt (g)[f] or survival days[g] T/C	T/C, %	Specificity test	Confidence, %
34	2-COOH	2′-CH_3	AA	100.0	3/3		11				
				33.0	3/3		22				
				10.0	3/3		27				
				3.0	3/3		31				
			WA	50.0	6/6		−12	7.8/10.7	72		
			DL	100.0	6/6	6	0	30.0/16.0	187		
				50.0	6/6	5	0	30.0/16.0	187		
			WM	100.0	6/6		−13	0.8/8.7	9		
				100.0	6/6		−17	1.2/7.8	15		
				100.0	6/6		−12	1.3/4.0	32[h]		
35	2-COOH	3′-OCH_3	AA	100.0	0/3		0				
				33.0	1/3		4				
				10.0	3/3		2				
				3.0	3/3		16				
			WA	3.0	7/7		−11	11.6/13.8	84		
			LE	25.0	6/6		−6.0	10.2/8.1	125		
				16.0	6/6		−5.8	10.0/8.1	123		
				10.0	6/6		−5.5	12.5/8.1	154		
				7.1	6/6		−5.5	10.3/8.1	127		
				40.0	4/4		−8.0	11.5/8.2	140		
				20.0	4/4		−4.1	13.3/8.2	162		
				10.0	4/4		−2.1	10.8/8.2	131		
				5.0	4/4		−2.3	10.0/8.2	121		
				25.0	6/6		−5.3	8.8/9.0	97		
				15.0	6/6		−4.3	13.2/9.0	146		
				9.0	6/6		−2.4	12.8/9.0	142		
				5.3	6/6		−2.2	11.0/9.0	122		
				50.0	4/4		−7.3	9.3/9.3	100		
				33.0	4/4		−5.5	15.3/9.3	164		
				22.0	4/4		−4.8	14.0/9.3	150		
			WM	15.0	6/6	4	−19	0.0/3.2	0		
				15.0	6/6		−12	0.6/6.0	10		
				15.0	6/6		−15	1.0/7.5	13		
				15.0	6/6		−13	0.9/7.4	12		
				15.0	6/6		−9	0.6/4.4	13		
				15.0	6/6		−13	0.2/5.9	3[i]		
										<20, >0	99.7
44	3-COOH	3′-OCH_3	AA	100.0	0/3		0				
				33.0	1/3		−20				
				10.0	3/3		6				
				3.0	3/3		14				
			WA	4.0	7/7		−13	8.5/13.8	61		
			WM	12.0	6/6	6	−15	0.0/3.2	0		
				12.0	6/6		−14	1.0/6.0	16		
				12.0	6/6		−5	0.1/7.5	1		
				12.0	6/6		−15	0.5/7.4	6		
				12.0	6/6		−7	0.4/4.4	9		
				12.0	6/6		−13	0.5/5.9	8[i]		
										<19, >0	99.7
47	4-COOH	3′-OCH_3	AA	100.0	0/3						
				33.0	2/3		−5				
				10.0	2/3		−11				
				3.0	3/3		9				
			DL	6.0	6/6	3	−11	30.0/16.0	187		
			LE	50.0	5/6		−4.5	13.2/9.6	137		
				25.0	6/6		−4.0	12.3/9.6	128		
				12.5	6/6		−0.4	10.8/9.6	112		
				6.0	6/6		−0.5	10.3/9.6	107[h]		
			WM	50.0	4/6		−23	0.6/8.8	6		
				25.0	6/6		−11	0.9/8.8	10		
				12.5	6/6		−6	1.0/8.8	11		
				6.2	6/6		0	1.1/8.8	12		
				3.1	6/6		3	1.8/8.8	20		
										<12, >0	99.7
52	4-(2-Pyridyl)	H	AA	100.0	3/3		20				
				33.0	3/3		15				
				10.0	3/3		19				
				3.0	3/3		16				

TABLE II (*Continued*)

No.[c]	X	Y	Test system[d]	Dose, mg/kg	Survivors	Cures	Animal wt dif, g (T − C)[e]	Tumor wt (g)[f] or survival days[g] T/C	T/C, %	Specificity test	Confidence, %
52	4-(2-Pyridyl)	H	WM	400.0	6/6		−2	1.0/4.0	22		
				400.0	6/6		−10	1.0/5.4	18		
				400.0	6/6		−12	2.5/6.1	40		
				400.0	6/6		−7	2.4/7.3	32[k]		

[a] Only a part of the data is presented. [b] Assays were performed according to specifications established by CCNSC as reported in *Cancer Chemotherapy Rept.*, **25**, 1 (1962). [c] Numbers refer to those from Table I. [d] AA = toxicity; WA = Walker carcinosarcoma 256 (subcutaneous); LE = L1210 lymphoid leukemia; WM = Walker 256 (intramuscular). [e] Average weight change of test group (T) minus average weight change of control (C) animals. [f] Tumor weight for WA and WM test systems. [g] Survival days for DL and LE test systems. [h] Activity not confirmed. [i] Activity confirmed. [j] At lower doses the compound is inactive. [k] Further testing is in progress.

ever, against Dunning leukemia all four Schiff bases (**4**, **33**, **34**, **47**) tested exhibited significant activity; **33** and **34** cured all the rats at dose levels of 200 and 100 mg/kg/day, respectively.

Whereas Schiff bases derived from 4-[N,N-bis(2-chloroethyl)amino]-*m*-anisaldehyde (Y = 3′-OCH_3, Table II) are in general significantly active against L1210 lymphoid leukemia, related Schiff bases from other aldehyde mustards are either inactive or poorly active. The methoxy group present in 4-[N,N-bis(2-chloroethyl)amino]-*m*-anisaldehyde appears to play a key role in deciding the antitumor activity of the resulting Schiff base against L1210 lymphoid leukemia; the Schiff bases **33** and **34** which are devoid of such a group failed to demonstrate any appreciable activity against L1210 lymphoid leukemia despite their high antitumor activity against Dunning leukemia. Compounds **1**, **2**, **3**, **4**, **44**, and **52** were inactive against L1210 lymphoid leukemia even at the maximal dose of 400 mg/kg/day.

Walker 256 (intramuscular), in general, appears to be a tumor very sensitive to the Schiff bases; a large number of compounds demonstrate significant activity in this test system. The most active Schiff base is **44** which gave 6/6 cures at 12.0 mg/kg/day. In this compound the position of a COOH group *meta* to the azomethine linkage appears to be critical for maximum biological activity; the *ortho* and *para* analogs of this compound (**35** and **47**) are less active.

Experimental Section

The various benzaldehyde nitrogen mustards employed in the present work were prepared according to literature methods.[12,18,20,22]

2-(*p*-Nitrophenyl)-4-methylpyridine.—A solution of *p*-nitrobenzenediazonium chloride[23] prepared from 35 g (0.25 mole) of *p*-nitroaniline and 25 g (0.33 mole) of $NaNO_2$ was slowly added to 250 ml of γ-picoline with stirring at 25–30°. The reaction mixture was heated on a water bath for 1 hr, poured onto ice water, and left overnight. The resulting tarry solid was separated by decantation, extracted (C_6H_6), dried (Na_2SO_4), and treated with charcoal. The residue left over after evaporation of the solvent was crystallized from hexane to give 10.0 g of a solid, mp 115–120°.

The above solid (2 g) was dissolved in $CHCl_3$ and added to 30 g of activated alumina in a column and the chromatogram was eluted with C_6H_6. The first six 25-ml portions of benzene were collected and evaporated to give 0.72 g of product, mp 149–150°. *Anal.* ($C_{12}H_{10}N_2O_2$) C, H, N.

2-(*p*-Aminophenyl)-4-methylpyridine.—To a suspension of 1 g of 2-(*p*-nitrophenyl)-4-methylpyridine in EtOH (*ca.* 10 ml) was added 2 g of Raney Ni and the mixture was shaken under H_2 at 2.1 kg/cm² for 4 hr. The alcoholic solution was separated and the solvent was evaporated. The residue was crystallized from hexane to give 0.4 g (40%) of the amine, mp 92–94°. *Anal.* ($C_{12}H_{12}N_2$) C, H, N.

2-(*p*-Aminophenyl)pyridine[24] and 3,5-dichloroaniline[25–27] were prepared according to literature methods and all other amines were obtained from commercial sources.

N,N-Bis(2-chloroethyl)-4-[N-(*m*-chlorophenyl)formimidoyl]-*o*-anisidine Monohydrochloride (Method A).—To a solution of 0.164 g (0.001 mole) of *m*-chloroaniline hydrochloride in minimum warm absolute EtOH was added a warm alcoholic solution of 0.276 g (0.001 mole) of 4-[N,N-bis(2-chloroethyl)amino]-*m*-anisaldehyde.[12] The resulting dark red solution on standing and cooling in ice deposited a crystalline solid which was filtered and washed with EtOH to give 0.280 g (70%) of the hydrochloride salt of the desired Schiff base (**4**, Table I).

2-[*p*-({*p*-[Bis(2-chloroethyl)amino]benzylidene}amino)phenyl]-pyridine (Method B).—A mixture of 0.170 g (0.001 mole) of 2-(*p*-aminophenyl)pyridine, 0.246 g (0.001 mole) of 4-[N,N-bis(2-chloroethyl)amino]benzaldehyde,[20] and 6 ml of alcohol was refluxed for 30 min and allowed to stand. The yellow crystals that separated were filtered, washed, and recrystallized from EtOH to give 0.26 g (65%) of the desired Schiff base (**52**, Table I).

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