Potential Anticancer Agents. I. Synthesis of Some Nitrogen Mustard Containing Benzylidenehydrazides

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In a previous paper from this laboratory we have described the synthesis of various Schiff bases from benzaldehyde nitrogen mustards and thiazole amines.[2] Earlier, Popp[3-5] had reported that several Schiff bases possess antitumor activity against a number of animal tumors. Since –CONHN=CH– is a structural modification of the azomethine linkage, it was thought worthwhile to study whether benzylidenehydrazides from benzaldehyde nitrogen mustards could also be evaluated as potential anticancer agents. Several investigators[6-10] have described the synthesis of such benzylidenehydrazides, and *p*-[bis(2-chloroethyl)aminobenzylidene]-*p*-aminobenzoic acid hydrazide prepared by Elderfield, *et al.*,[9,10] is reported to exhibit significant antitumor activity.

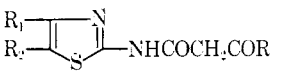
In the present paper we report the synthesis and evaluation of several nitrogen mustard containing benzylidenehydrazides (Table II and III) as possible anticancer agents. Most of the acid hydrazides employed in the synthesis of benzylidenehydrazides are known in literature, except N-(2-thiazolyl)malonamic acid hydrazides. These were prepared by the interaction of 2-aminothiazole with diethyl malonate to give ethyl N-(2-thiazolyl)malonamates,[11] which on subsequent treatment with hydrazine hydrate furnished the required hydrazides (Table I).

The condensation of the benzaldehyde nitrogen mustards[5,12] with the acid hydrazides was effected in alcohol in the presence of an acid catalyst.[8] Equimolar quantities of the aldehyde mustard and the acid hydrazide were treated in warm alcohol to give the benzylidenehydrazides recorded in the Tables II and III.

Biological Results.—Representative compounds were evaluated under the auspices of the Cancer Chemotherapy National Service Center, Bethesda, Md., against Dunning leukemia, Walker 256 (subcutaneous), L1210 lymphoid leukemia, and Walker 256 (intramuscular). The compounds are, in general, of low toxicity and some compounds are nontoxic even at high doses.

Benzylidenehydrazides obtained from N-(2-thiazolyl)malonamic acid hydrazides and substituted ben-

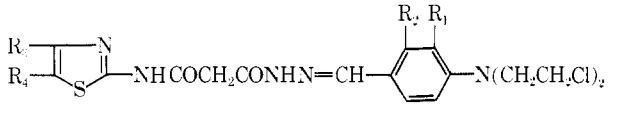
TABLE I
N-(2-THIAZOLYL)MALONAMIC ACID ESTERS AND HYDRAZIDES



No.	R	R_1	R_2	Yield, %	Mp °C	Formula	Analyses
1	OC_2H_5	H	C_2H_5	25	160–161[a]	$C_{10}H_{14}N_2O_3S$	C, H, N
2	OC_2H_5	*p*-$CH_3C_6H_4$	H	63	147–148[a]	$C_{15}H_{16}N_2O_3S$	N
3	OC_2H_5	*p*-ClC_6H_4	H	50	154–156[a]	$C_{14}H_{13}ClN_2O_3S$	N
4	$NHNH_2$	H	H	75	200[b]	$C_6H_8N_4O_2S$	C, H, N
5	$NHNH_2$	H	C_2H_5	70	245 dec[b]	$C_8H_{12}N_4O_2S$	C, H, N
6	$NHNH_2$	C_6H_5	H	72	257–258 dec[b]	$C_{12}H_{12}N_4O_2S$	N
7	$NHNH_2$	*p*-$CH_3C_6H_4$	H	76	270 dec[b]	$C_{13}H_{14}N_4O_2S$	C, H, N
8	$NHNH_2$	*p*-ClC_6H_4	H	68	266–268 dec[b]	$C_{12}H_{11}ClN_4O_2S$	N

[a] Recrystallized from EtOH. [b] Recrystallized from EtOH–H_2O (1:1).

TABLE II
N-(2-THIAZOLYL)MALONAMIC ACID {*p*-[N,N-BIS(2-CHLOROETHYL)AMINO]BENZYLIDENE}HYDRAZIDES



No.	R_3	R_2	R_1	R_4	Mp, °C[a]	Formula	Analyses
1	H	H	H	H	192–193	$C_{17}H_{19}Cl_2N_5O_2S$	C, H, N
2	H	H	H	C_2H_5	203–204	$C_{19}H_{23}Cl_2N_5O_2S$	C, H, N
3	H	H	C_6H_5	H	223–224	$C_{23}H_{23}Cl_2N_5O_2S$	C, H, N
4	H	H	*p*-ClC_6H_4	H	210–212	$C_{23}H_{22}Cl_3N_5O_2S$	N
5	H	H	*p*-$CH_3C_6H_4$	H	217–219	$C_{24}H_{25}Cl_2N_5O_2S$	N
6	H	CH_3	C_6H_5	H	200–201	$C_{24}H_{25}Cl_2N_5O_2S$	Cl, N
7	H	CH_3	*p*-$CH_3C_6H_4$	H	193–195	$C_{25}H_{27}Cl_2N_5O_2S$	N
8	OCH_3	H	*p*-$CH_3C_6H_4$	H	126–127	$C_{25}H_{27}Cl_2N_5O_3S$	N

[a] Pure compounds were obtained without recrystallization.

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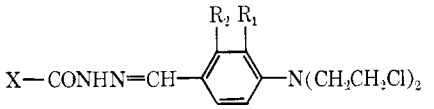
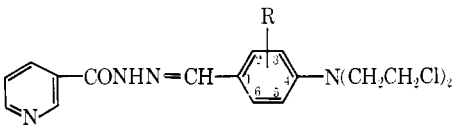
TABLE III
p-[N,N-BIS(2-CHLOROETHYL)AMINO]BENZYLIDENEHYDRAZIDES



No.	X	R_1	R_2	Mp, °C[a]	Formula	Analyses
1	4-Nitrophenyl[b]	H	H	228–229	$C_{18}H_{18}Cl_2N_4O_3$	C, H, N
2	4-Nitrophenyl	H	CH_3	203–204	$C_{19}H_{20}Cl_2N_4O_3$	N
3	4-Nitrophenyl	H	OCH_3	213–214	$C_{19}H_{20}Cl_2N_4O_4$	N
4	4-Nitrophenyl	H	Cl	204–206	$C_{18}H_{17}Cl_3N_4O_3$	N
5	4-Nitrophenyl	OC_2H_5	H	132–133	$C_{20}H_{22}Cl_2N_4O_4$	N
6	3-Chlorophenyl	H	H	164–165	$C_{18}H_{18}Cl_3N_3O$	C, H, N
7	3-Chlorophenyl	H	CH_3	192–193	$C_{19}H_{20}Cl_3N_3O$	N
8	3-Chlorophenyl	H	OCH_3	198–200	$C_{19}H_{20}Cl_3N_3O_2$	N
9	3-Chlorophenyl[b]	OCH_3	H	159–160	$C_{19}H_{20}Cl_3N_3O_2$	N
10	3-Chlorophenyl	H	Cl	218–219	$C_{18}H_{17}Cl_4N_3O$	N
11	3-Chlorophenyl	OC_2H_5	H	145–146	$C_{20}H_{22}Cl_3N_3O_2$	N
12	3,4,5-Trimethoxyphenyl[b]	H	H	192–193	$C_{21}H_{25}Cl_2N_3O_4$	C, H, N
13	3,4,5-Trimethoxyphenyl	H	CH_3	205–206	$C_{22}H_{27}Cl_2N_3O_4$	N
14	3,4,5-Trimethoxyphenyl	H	OCH_3	197–198	$C_{22}H_{27}Cl_2N_3O_5$	N
15	3,4,5-Trimethoxyphenyl	OC_2H_5	H	175–176	$C_{23}H_{29}Cl_2N_3O_5$	N
16	3,4,5-Trimethoxyphenyl	H	Cl	208–209	$C_{21}H_{24}Cl_3N_3O_4$	N
17	3-Pyridyl[b]	H	H	152–153	$C_{17}H_{18}Cl_2N_4O$	C, H, N
18	3-Pyridyl[b]	H	CH_3	164–165	$C_{18}H_{20}Cl_2N_4O$	N
19	3-Pyridyl[b]	H	OCH_3	179–180	$C_{18}H_{20}Cl_2N_4O_2$	N
20	3-Pyridyl	OC_2H_5	H	122–124	$C_{19}H_{22}Cl_2N_4O_2$	N
21	3-Pyridyl[b]	H	Cl	175–176	$C_{17}H_{17}Cl_3N_4O$	N
22	3-Pyridyl[b]	H	NO_2	215–217	$C_{17}H_{17}Cl_2N_5O_3$	N
23	4-Pyridyl[b]	H	CH_3	207–208	$C_{18}H_{20}Cl_2N_4O$	C, H, N
24	4-Pyridyl	H	OCH_3	218–220	$C_{18}H_{20}Cl_2N_4O_2$	N
25	4-Pyridyl	H	Cl	221–222	$C_{17}H_{17}Cl_3N_4O$	N
26	4-Pyridyl	H	NO_2	265–266	$C_{17}H_{17}Cl_2N_5O_3$	N
27	2-Pyridyl[b]	H	H	147–148	$C_{17}H_{18}Cl_2N_4O$	C, H, N
28	2-Pyridyl	H	CH_3	140–141	$C_{18}H_{20}Cl_2N_4O$	N
29	2-Pyridyl	H	OCH_3	154–155	$C_{18}H_{20}Cl_2N_4O_2$	N
30	2-Pyridyl	OCH_3	H	125–128	$C_{18}H_{20}Cl_2N_4O_2$	N
31	2-Pyridyl	H	Cl	129–130	$C_{17}H_{17}Cl_3N_4O$	N
32	2-Pyridyl	OC_2H_5	H	124–125	$C_{19}H_{22}Cl_2N_4O_2$	N
33	2-Pyridyl	H	NO_2	191–192	$C_{17}H_{17}Cl_2N_5O_3$	N
34	3-(2-Hydroxy-4,6-dimethyl)-pyridyl[b]	H	H	218–219	$C_{19}H_{22}Cl_2N_4O_2$	C, H, N
35	3-(2-Hydroxy-4,6-dimethyl)-pyridyl	H	CH_3	210–212	$C_{20}H_{24}Cl_2N_4O_2$	C, H, N

[a] Pure compound was obtained without recrystallization. [b] Screened for antitumor activity.

TABLE IV
SUMMARY OF THE SCREENING RESULTS AGAINST DUNNING LEUKEMIA AND WALKER 256 (SUBCUTANEOUS)[a]



	Dunning leukemia (solid)				Walker 256 (subcutaneous)			
R	Dose, mg/kg/day	Survivors	Cures[b]	T/C,[c] %	Dose, mg/kg/day	Survivors	Tumor wt[d] T/C, g	T/C, %
H	400.0	6/6	2	150[e]	50.0	6/6	0.2/7.3	2
2-CH_3	400.0	4/6	2	187[e]	50.0	6/6	1.1/7.3	15
2-OCH_3	200.0	6/6	4	187[e]	50.0	6/6	6.8/10.7	63
2-Cl	200.0	7/7	0	106[e]	50.0	6/6	9.2/10.7	85
2-NO_2					50.0	6/6	5.3/5.5	96

[a] For testing procedures see *Cancer Chemotherapy Rept.*, **25**, 1 (1962). [b] Survivors at 30 days without measurable tumors. [c] Ratio of mean survival time of test animals (T) to control animals (C). [d] T stands for test animals, C for controls. [e] Mean survival time of control is 16 days.

zoic acid hydrazides were inactive against all the tumor systems studied. Similarly, compounds from picolinic acid hydrazide and isonicotinic acid hydrazide did not show any appreciable activity. However, derivatives of nicotinic acid hydrazide demonstrated significant antitumor activity against Dunning leukemia and Walker 256 (subcutaneous) and their screening results are included in Table IV.

{*p*-[Bis(2-chloroethyl)amino]benzylidene}nicotinic acid hydrazide (**17**, Table III) gave two cures against Dunning leukemia at 400.0 mg/kg/day and also produced 98% inhibition against Walker 256 (subcu-

taneous) tumor at 50.0 mg/kg/day. However, introduction of substituents in the phenyl ring of this compound either retains or increases its activity against Dunning leukemia but lowers the activity against Walker 256 tumor.

Experimental Section

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. Melting points were taken in an open capillary tube in sulfuric acid bath and are uncorrected.

Ethyl N-(4-*p*-Tolyl-2-thiazolyl)malonamate.—A mixture of 1.9 g (0.01 mole) of 2-amino-4-*p*-tolylthiazole[13] and 9.6 g (0.06 mole) of diethyl malonate was refluxed for 2.5 hr in an oil bath at 160°. The reaction mixture was cooled, diluted with hexane, and kept in an ice box for 1 hr. The compound which separated on cooling was collected and recrystallized from EtOH to give 1.9 g (63%) of the ester, mp 147–148°. *Anal.* ($C_{15}H_{16}N_2O_3S$) C, H, N.

N-(4-*p*-Tolyl-2-thiazolyl)malonamic Acid Hydrazide.—A solution of 1.5 g (0.005 mole) of ethyl N-(4-*p*-tolyl-2-thiazolyl)-malonamate in a small quantity of EtOH was treated with 0.6 ml of 58% hydrazine hydrate and the solution was heated under reflux for 10 min. The reaction mixture gradually deposited a crystalline solid which was filtered off and washed with a little EtOH to give 1.1 g (76%) of the crude hydrazide. It was crystallized from EtOH–H_2O (1:1), mp 270° dec. *Anal.* ($C_{13}H_{14}N_4O_2S$) C, H, N.

Other malonamic acid esters and hydrazides were similarly prepared and are listed in Table I.

The following hydrazides were prepared as described in the literature: 4-nitrobenzoic acid hydrazide,[14] 3-chlorobenzoic acid hydrazide,[15] 3,4,5-trimethoxybenzoic acid hydrazide,[16] nicotinic acid hydrazide,[17] isonicotinic acid hydrazide,[18] picolinic acid hydrazide,[19] and 2-hydroxy-4,6-dimethylnicotinic acid hydrazide.[20,21]

4-[N,N-Bis(2-chloroethyl)amino]-*o*-anisaldehyde.—To 22 ml of DMF cooled in an ice bath was added 14 ml of $POCl_3$ with stirring at 7–10°. Then a mixture of 10.5 g (0.05 mole) of N,N-bis(2-hydroxyethyl)-*m*-anisidine[22] dissolved in 30 ml of DMF was added slowly at 5–10°. The mixture was then heated for 1 hr on a water bath and poured onto ice and kept overnight at 4°. The solid was filtered off, washed thoroughly with ice water, and dried. Crystallization from hexane yielded 11.0 g (80%) of the aldehyde mustard, mp 96–97°. *Anal.* ($C_{12}H_{15}Cl_2NO_2$) C, H, N.

The 2,4-dinitrophenylhydrazone, prepared in EtOH, was recrystallized from Me_2CO, mp 215–216°. *Anal.* ($C_{18}H_{19}Cl_2N_5O_5$) C, H, N.

Other aldehyde mustards employed in the present work are reported in the literature and were prepared according to the known methods.[5,12]

N-(4-Phenyl-2-thiazolyl)malonamic Acid {*p*-[N,N-Bis(2-chloroethyl)amino]benzylidene}hydrazide.—To a solution of 0.20 g (0.001 mole) of N-(4-phenyl-2-thiazolyl)malonamic acid hydrazide in a minimum of EtOH at 70° was added a solution of 0.25 g (0.001 mole) of the 4-[N,N-bis(2-chloroethyl)amino]-benzaldehyde[12] in EtOH. Two drops of concentrated HCl were then added to this solution and the mixture was allowed to stand. Within a short time, a crystalline solid separated out. This was filtered off and washed with a little EtOH to give 0.30 g (60% yield) of product, mp 223–224°. *Anal.* ($C_{23}H_{23}Cl_2N_5O_2S$) C, H, N.

All other benzylidenehydrazides were similarly prepared and are recorded in Tables II and III.

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Some New Salts of Lucanthone as Potential Anticancer Agents

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Since the report by Mauss, *et al.*,[1a] that lucanthone, 1-(2-diethylaminoethylamino)-4-methylthioxanthen-9-one,[1b] possessed schistosomicidal activity, numerous analogs have been synthesized. Many of the derivatives have also been tested against a variety of experimental tumors *in vitro* and *in vivo*. Hirschberg and coworkers[2] have reported that lucanthone exhibits antitumor activity against a variety of transplantable mouse tumors such as Sarcoma 180, lymphoid leukemia L1210 ascites, and Adenocarcinomas 755 and E0771. More recently, Blanz and French[3] also showed that lucanthone hydrochloride possessed antitumor activity when tested with a number of structural analogs in three tumor (Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210) mouse screening experiments. However, the hydrochloride of the chemotherapeutic agent is somewhat limited in usefulness by its high toxicity. For a number of years, we have studied the effects of numerous chemicals as potential detoxifying adjuvants for toxic chemotherapeutic agents. The results have indicated that certain sulfonic acids[4] possessed significant detoxifying action when administered concomitantly with the toxic chemotherapeutic agent (streptomycin), so that mice tolerated twice the lethal dose. This study stimulated our interest in the possibility of sulfonic acid salts of lucanthone as potential anticancer agents with maximum therapeutic effectiveness and with little or no toxicity. This report includes the preparation of five sulfonic acid salts of lucanthone with analyses and tests for acute toxicity in mice and *in vivo* antitumor activity of certain derivatives against Sarcoma 180, Adenocarcinoma 755, and Leukemia 1210.

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