A Systematic Investigation of Thioxanthen-9-ones and Analogs as Potential Antitumor Agents[1,2]

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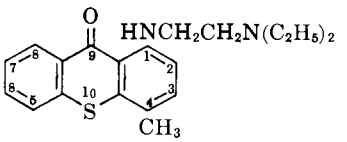
Received August 28, 1962

Thioxanthen-9-one drugs of the dialkylaminoalkylamino side chain type were prepared with various types of structural variation. These include: variation in the alkylenediamine side chain, ring atom replacements and ring substituents. These thioxanthen-9-ones and analogs were tested against transplanted mouse tumors Leukemia L-1210, Adenocarcinoma 755, and Sarcoma 180. Several compounds were found to have moderate but significant effects on Leukemia L-1210 and Adenocarcinoma 755. The only drug significantly better than the diethylaminoethylamino derivative was 1-(2-dimethylaminoethylamino)-4-methylthioxanthen-9-one. It increases the life span of leukemic mice up to 100% and, under optimal conditions, completely inhibits the growth of Adenocarcinoma 755 during the test period.

It has been known for a long time that xanthen-9-one and thioxanthen-9-one derivatives containing dialkylaminoalkylamino side chains show marked activity against schistosomiasis.[3,4] 1-(2-Diethylaminoethylamino)-4-methylthioxanthen-9-one[5] (I) has been found active on a number of mouse tumors[6] and has been studied clinically. Side effects, however, such as

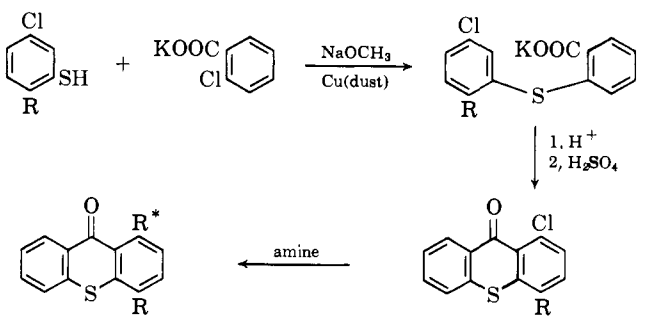


I

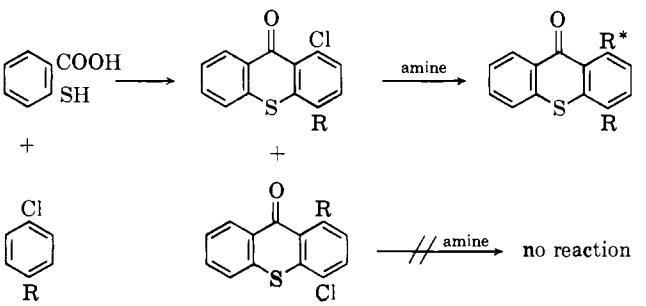
gastrointestinal irritation and CNS toxicity have severely limited clinical application. Design of drugs that absorb well while retaining activity has also been a problem. A program of synthesis and testing of thioxanthen-9-ones was initiated in this laboratory in an attempt to find structure–activity relationships. The thioxanthen-9-one drugs of the dialkylaminoalkylamino side chain type lend themselves to several types of structural variation, such as modification of the alkylenediamine side chain, ring atom replacements, and ring substitution changes.

Chemistry.—The aminothioxanthen-9-one derivatives were synthesized by two general methods.[7]

Method A.—R = CH_3 or Cl; R* = $HNCH_2CH_2R'$ (Table I) or R'' (Table II).



Method B.—R = CH_3, OCH_3 or OC_2H_5; R* = $HNCH_2CH_2R'$ (Table I) or R'' (Table II)



Tables I and II summarize the chemical structures of the thioxanthen-9-ones prepared.

Reduction of 1-(2-dimethylaminoethylamino)-4-methylthioxanthen-9-one with lithium aluminum hydride and sodium amalgam led to the corresponding thioxanthene and thioxanthen-9-ol, respectively (Table III). These reductions were based upon earlier experiments in which thioxanthen-9-one was reduced by these two reducing agents.[8,9] Alkylenediamine thioxanthen-9-one-10,10-dioxides were synthesized by the method of Mauss[7b] (Table III).

Xanthen-9-ones and 9-acridanones containing alkylenediamine side chains were prepared in a manner analogous to the thioxanthen-9-one preparations. Method A was used with 3-chloro-6-methylphenol or 3-chloro-6-methylaniline in place of 3-chloro-6-methylthiophenol[10] (Table IV). 5H-[1]-Benzothiopyrano[2,3-b]pyridin-5-ones and 5N-[1]benzothiopyrano[2,3-c]pyridin-5-ones were prepared by the methods of Mann and of Kruger[11,12] (Tables V and VI).

Experimental[13,14]

The preparation of the thioxanthen-9-ones and analogs reported in this paper (Tables I–VI) required several synthetic approaches, as will be exemplified.

(1) This investigation was supported by Grant CY-3287 from the National Cancer Institute.

(2) A summary of this work was presented at the 141st National Meeting of the American Chemical Society, Washington, D.C., 1962.

(3) W. R. Kikuth, R. Gönnert, and H. Mauss, *Naturwiss.*, **8**, 253 (1946).

(4) W. R. Kikuth and R. Gönnert, *Ann. Trop. Med. Parasitol.*, **42**, 256 (1948).

(5) Miracil D®.

(6) E. Hirschberg, A. Gellhorn, M. R. Murray and E. F. Elslager, *J. Natl. Cancer Inst.*, **22**, 567 (1959).

(7) (a) F. Ullman and O. v. Glenck, *Ber.*, **49**, 2487 (1916); (b) H. Mauss, *ibid.*, **81**, 19 (1948); (c) S. Archer and C. M. Suter, *J. Am. Chem. Soc.*, **74**, 4296 (1952); (d) T. M. Sharp, *J. Chem. Soc.*, 2961 (1951).

(8) A. Mustafa and M. K. Hilmy, *J. Chem. Soc.*, 1343 (1952).

(9) H. F. Oehlschlaeger and I. R. MacGregor, *J. Am. Chem. Soc.*, **72**, 5332 (1950).

(10) S. Archer, L. B. Rochester and M. Jackson, *ibid.*, **76**, 588 (1954).

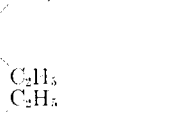
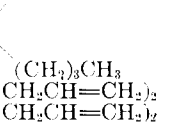
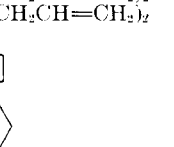
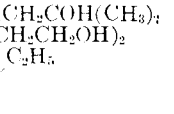
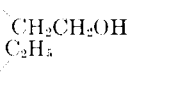
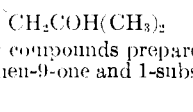
(11) F. G. Mann and J. A. Reid, *J. Chem. Soc.*, 2057 (1952).

(12) S. Kruger and F. G. Mann, *ibid.*, 3905 (1954).

(13) Melting points are corrected and were measured on a Thomas-Hoover capillary melting point apparatus.

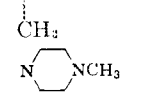
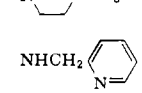
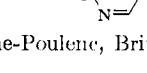
(14) Microanalyses were performed by the Berkeley Analytical Laboratory, Berkeley, Calif. and by Micro-Analysis, Inc., Wilmington, Delaware.

TABLE I
1,1-SUBSTITUTED

No.	R	R′	M.p., °C.	Yield,[a] %	Method of prepn.	Empir. formula	C Calcd.	C Found
1	CH_3	NH_2	250–251	64	A	$C_{16}H_{16}N_2OS\cdot HCl$	59.89	59.82
							Monoalkylaminoethyl	
2	H	NHC_2H_5	252–253	..	B	$C_{17}H_{18}N_2OS\cdot HCl$	60.97	60.82
3	CH_3	NHC_2H_5	97–97.5	52	A	$C_{18}H_{20}N_2OS$	69.19	69.12
4	CH_3	$NH(CH_2)_2CH_3$	244.5–246	76	A	$C_{19}H_{22}N_2OS\cdot HCl$	62.88	62.76
5	CH_3	$NH(CH_2)_3CH_3$	239.5–241	69	A	$C_{20}H_{24}N_2OS\cdot HCl$	63.72	63.59
6	CH_3	$NHCH(CH_3)_2$	250–251.5	72	A	$C_{19}H_{22}N_2OS\cdot HCl$	62.88	62.94
7	CH_3	$NHCH_2CH(CH_3)_2$	252.5–254	64	A	$C_{20}H_{24}N_2OS\cdot HCl$	63.72	63.79
							Dialkylaminoethyl	
8	H	$N(CH_3)_2$	112–113	..	B	$C_{17}H_{18}N_2OS$	68.42	68.19
9[b,c]	CH_3	$N(CH_3)_2$	115–117	..	B	$C_{18}H_{20}N_2OS$	69.19	69.17
10	OCH_3	$N(CH_3)_2$	249–250	..	B	$C_{18}H_{20}N_2O_2S\cdot HCl$	58.90	59.02
11	OC_2H_5	$N(CH_3)_2$	112.5–113.5	..	B	$C_{19}H_{22}N_2O_2S$	66.64	66.43
12	Cl	$N(CH_3)_2$	155–156	81	A	$C_{17}H_{17}ClN_2OS$	61.34	61.26
13[d]	CH_3	$N(C_2H_5)_2$	196–197	..	..	$C_{20}H_{24}N_2OS\cdot HCl$	...	...
14	OCH_3	$N(C_2H_5)_2$	89.5–90.5	..	B	$C_{20}H_{24}N_2O_2S$	67.38	67.26
15	OC_2H_5	$N(C_2H_5)_2$	195.5–197	..	B	$C_{21}H_{26}N_2O_2S\cdot HCl$	61.98	61.71
16	CH_3		92–93.5	65	A	$C_{18}H_{22}N_2OS$	69.90	69.97
17	CH_3		68–69	61	A	$C_{22}H_{28}N_2OS$	71.15	71.17
18	CH_3	$N(CH_2CH{=}CH_2)_2$	81.5–82.5	48	A	$C_{22}H_{24}N_2OS$	72.49	72.21
19	Cl	$N(CH_2CH{=}CH_2)_2$	119.5–120.5	65	A	$C_{21}H_{21}ClN_2OS$	65.52	65.41
							Cycloalkyleneiminoethyl	
20[e]	CH_3		242–243	70	A	$C_{20}H_{22}N_2OS\cdot HCl$	64.07	64.08
21[b]	CH_3		260.5–262.5	73	A	$C_{21}H_{24}N_2OS\cdot HCl$	...	...
22	CH_3		241.5–242	76	A	$C_{22}H_{26}N_2OS\cdot HCl$	65.50	65.55
							Hydroxylated Alkylamino	
23[e]	CH_2	$NHCH_2COH(CH_3)_2$	228.5–229.5	..	..	$C_{20}H_{24}N_2O_2S\cdot HCl$	...	...
24	CH_3	$N(CH_2CH_2OH)_2$	250–252	41	A	$C_{20}H_{24}N_2O_3S\cdot HCl$	59.40	59.29
25[b]	CH_3		185–186.5	55	A	$C_{20}H_{24}N_2O_2S\cdot HCl$	...	...
26[e]	CH_3		157.6–160.4	..	..	$C_{22}H_{28}N_2O_2S\cdot HCl$	...	...

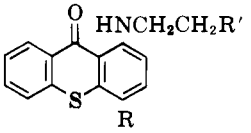
[a] No yields were recorded for compounds prepared by Method B as the starting material is a mixture of indeterminate amounts of 1-chloro-4-substituted thioxanthen-9-one and 1-substituted-4-chloro thioxanthen-9-one. [b] See footnote 7c. [c] See footnote 7d. [d] Ob-

TABLE II
1-SUBSTITUTED

No.	R″	M.p., °C.	Yield, %	Method of prepn.	Empir. formula	C Calcd.	C Found
27	$NHCHCH_3CH_2N(CH_3)_2$	104–105	80	A	$C_{19}H_{22}N_2OS$	69.90	69.81
28	$NHCH_2CHCH_3N(CH_3)_2$	104–105	66	A	$C_{19}H_{22}N_2OS$	69.90	69.78
29[a]	$NHCH_2CHCH_3N(C_2H_5)_2$	209–210	74	A	$C_{21}H_{26}N_2OS\cdot HCl$	64.51	64.38
30[b]	$NHCH_2CHOHCH_2N(C_2H_5)_2$	174–175	66	A	$C_{21}H_{26}N_2O_2S\cdot HCl$	61.98	61.66
31[c]	$NH(CH_2)_3N(CH_3)_2$	70.5–71.5	73	A	$C_{19}H_{22}N_2OS$	69.90	69.82
32		175–176	[d]	B	$C_{19}H_{22}N_2OS\cdot 2HCl\cdot {}^3/_2CH_3OH$	55.03	55.07
33[e]		139–140	65	A	$C_{19}H_{20}N_2OS$	70.33	70.39
34		174–174.5	59	A	$C_{20}H_{16}N_2OS$	72.27	72.31

[a] Rhône-Poulenc, British Patent 698,003 (1953). [b] See footnote 7c. [c] See footnote 6. [d] See footnote a, Table I. [e] S. Kushner,

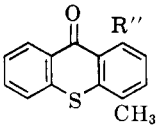
THIOXANTHEN-9-ONES



H		Cl		N		S		Antitumor Activity (T/C)		
Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	L-1210	S-180	AC-755
5.34	5.49	11.05	11.13	8.73	8.56	9.99	10.01	1.04	0.73	0.38
amino chain										
5.72	5.74	10.59	10.48	8.37	8.28	9.58	9.47	1.24	0.55	0.67
6.45	6.70	...	...	8.97	9.13	10.26	10.34	1.49	0.63	0.15
6.39	6.48	9.77	9.92	7.72	7.78	8.83	8.91	1.37	0.76	0.56
6.69	6.77	9.41	9.58	7.44	7.62	8.51	8.58	1.11	0.95	0.89
6.39	6.36	9.77	9.70	7.72	7.57	8.83	8.77	1.05	0.77	0.66
6.69	6.57	9.41	9.24	7.44	7.34	8.51	8.31	1.15	0.80	0.72
amino chain										
6.08	6.12	...	...	9.39	9.32	10.75	10.82	1.14	0.89	0.91
6.45	6.52	...	...	8.97	8.97	10.26	10.24	1.95	0.53	0.13
5.80	6.02	9.72	9.68	..	..	8.78	8.68	1.13	0.66	0.90
6.48	6.49	...	...	8.18	8.18	9.36	9.09	1.07	0.89	0.70
5.15	5.15	10.66	10.81	8.42	8.31	9.64	9.48	1.52	0.43	0.57
..	..	...	...	..	..	...	...	1.79	0.64	0.21
6.79	6.57	...	...	7.86	7.83	8.99	9.00	1.01	0.92	0.96
6.69	6.70	8.71	8.92	6.88	6.85	7.88	7.82	1.17	1.05	0.93
6.79	6.71	...	...	8.58	8.55	9.82	9.85	1.21	0.74	0.86
7.39	7.29	...	...	7.90	8.09	9.05	8.88	1.21	0.78	0.65
6.64	6.56	...	...	..	..	8.80	8.98	1.09	0.88	0.77
5.50	5.46	...	...	7.28	7.57	8.33	8.53	0.95	1.13	0.87
amino chain										
6.19	6.13	...	...	..	..	8.55	8.33	1.22	0.68	0.53
..	..	...	...	..	..	...	...	1.20	0.73	0.54
6.75	6.80	8.80	8.97	7.21	6.95	7.94	8.10	0.99	0.77	0.69
ethylamino chain										
..	..	...	...	..	..	...	...	1.18	0.83	1.11
5.11	5.20	8.77	8.99	6.92	7.06	7.93	7.98	1.20	0.72	0.85
..	..	...	...	..	..	...	...	1.37	0.65	0.77
..	..	...	...	..	..	...	...	1.15	0.94	0.60

tained from the Cancer Chemotherapy National Service Center, NSC 14574. [e] Obtained from Winthrop Laboratories.

METHYLTHIOXANTHEN-9-ONES

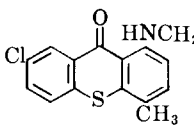
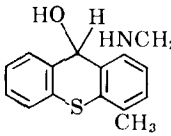
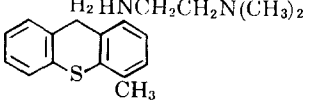
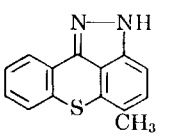


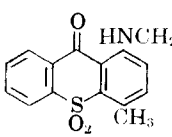
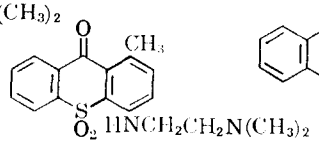
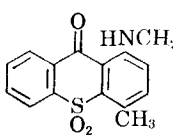
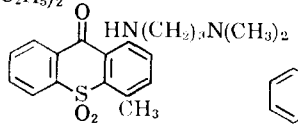
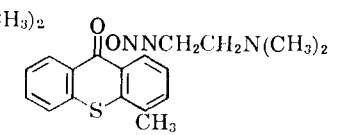
H		Cl		N		S		Antitumor Activity (T/C)		
Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	L-1210	S-180	AC-755
6.79	6.83	...	...	8.58	8.58	9.82	9.78	1.10	0.81	0.42
6.79	6.77	...	...	..	..	9.82	9.69	1.20	0.73	0.65
6.96	7.11	9.06	8.91	7.16	6.95	8.20	8.01	1.26	0.84	0.88
6.69	6.84	8.71	8.51	6.88	6.64	7.88	7.77	1.12	0.81	0.59
6.79	6.79	...	...	8.58	8.49	9.82	9.82	1.01	0.85	0.84
6.75	6.66	15.84	16.15	..	..	7.16	7.06	1.04	0.80	0.79
6.21	6.27	...	...	8.64	8.84	9.88	9.65	0.99	0.91	1.04
4.85	4.84	...	...	8.43	8.54	9.65	9.54	1.11	0.90	0.62

U. S. Patent 2,656,357 (1953).

TABLE III

MISCELLANEOUS THIOXANTHEN-9-ONE ANALOGS

No.	35	36	37	38	39
Compound					
M.p., °C.	128–129	166.5–168.5	161.5–162.5	84.5–85.5	245–247
Yield, %	73	83	90	60	79
Method of prepn.	A	A	[a]	[a]	[b]
Emp. formula	$C_{18}H_{19}ClN_2OS$	$C_{17}H_{16}Cl_2N_2OS$	$C_{18}H_{22}N_2OS$	$C_{18}H_{22}N_2S$	$C_9H_{10}N_2S$
Carbon, % Calcd.	62.32	55.86	68.75	72.44	
Carbon, % Found	62.22	55.75	68.53	72.28	
Hydrogen, % Calcd.	5.52	4.41	7.05	7.43	
Hydrogen, % Found	5.52	4.52	7.09	7.35	
Nitrogen, % Calcd.	8.08		8.91	9.39	
Nitrogen, % Found	8.16		9.06	9.39	
Sulfur, % Calcd.	9.24	8.77	10.20		
Sulfur, % Found	9.21	8.74	10.00		
Antitumor Activity (T/C) L-1210	1.12	1.15	1.13	1.07	1.06
Antitumor Activity (T/C) S-180	0.99	0.65	0.83	0.80	0.88
Antitumor Activity (T/C) AC-755	0.66	1.28	0.85	1.19	0.56

No.	40	41	42	43	44
Compound					
M.p., °C.	155.5–157	127.5–129	118–119	251–253	103–104
Yield, %	83	80	78	69	53
Method of prepn.	[c]	[c]	[c]	[c]	[a]
Emp. formula	$C_{18}H_{20}N_2O_3S$	$C_{18}H_{20}N_2O_3S$	$C_{20}H_{24}N_2O_3S$	$C_{19}H_{22}N_2O_3S\cdot HCl$	$C_8H_9N_3O_2S$
Carbon, % Calcd.	62.77	62.77		57.78	63.31
Carbon, % Found	62.52	62.92		57.28	63.22
Hydrogen, % Calcd.	5.85	5.85		5.87	5.58
Hydrogen, % Found	5.92	6.10		5.81	5.55
Chloride, % Calcd.				8.98	
Chloride, % Found				8.82	
Nitrogen, % Calcd.	8.13	8.13		7.09	12.30
Nitrogen, % Found	8.26	8.08		6.99	12.04
Sulfur, % Calcd.	9.31	9.31		8.12	
Sulfur, % Found	9.39	9.28		7.98	
Antitumor Activity (T/C) L-1210	1.01	1.06	0.99	1.00	1.11
Antitumor Activity (T/C) S-180	0.70	0.88		0.88	0.87
Antitumor Activity (T/C) AC-755	1.12	1.10	1.06	0.67	0.73

[a] The preparation of these compounds is described in the Experimental section. [b] See footnote 7a. [c] Prepared by the method of Mauss (see footnote 7b).

Method A.—This is a general procedure used by Archer and Suter for preparing dialkylaminoalkylamino thioxanthen-9-ones.[7c] The only change of procedure was that, wherever possible, free bases were isolated instead of salts.

1-(2-Diallylaminoethylamino)-4-methylthioxanthen-9-one.—A mixture of 7.8 g. (0.03 mole) of 1-chloro-4-methylthioxanthen-9-one,[7d] 8.5 g. (0.06 mole) of diallylaminoethylamine, and 20 ml. of dry pyridine was heated under reflux for 20 hr. The dark red solution was allowed to cool and was treated with 5 ml. of 50% potassium hydroxide. The residue was steam distilled to remove the volatile bases. After cooling the residue, the supernatant liquid was carefully decanted. The residual oil was dissolved in 200 ml. of 10% acetic acid and filtered. The filtrate was made alkaline with potassium hydroxide solution. The thioxanthen-9-one was extracted in 100 ml. of chloroform, dried with anhydrous magnesium sulfate and evaporated *in vacuo* to a red-orange oil. The residue was crystallized from methanol and yielded 5.2 g. (48%) of product, m.p. 81.5–82.5°.

Method B. 1-(2-Dimethylaminoethylamino)-4-methylthioxanthen-9-one.—Fifty grams (0.57 mole) of dimethylaminoethylamine and 50 ml. of pyridine, and a mixture of 30 g. of 1-chloro-4-methylthioxanthen-9-one and its isomer, 4-chloro-1-methylthioxanthen-9-one, were heated for 24 hr. The mixture was cooled to room temperature, treated with 5 ml. of potassium hydroxide, and then steam distilled to remove the volatile bases. After the residue was cooled and the supernatant liquid was carefully decanted, the remaining oil was extracted with two 100-ml. portions of 10% acetic acid. The extracts were filtered from the unreacted 1-methyl-4-chlorothioxanthen-9-one and made alkaline with 50% potassium hydroxide solution. The oily amine quickly solidified. It was washed with two 100-ml. portions of water and dried. After crystallizing from ethanol, 14.3 g. of the amine was obtained, m.p. 115–117°.

1-(2-Dimethylaminoethylamino)-4-methylthioxanthen-9-ol.—Sodium (1.7 g., 0.074 g. atom) was melted under 20 ml. of dry xylene. To this was added 60 g. of mercury, slowly at first, with stirring. After the amalgam solidified, the xylene was decanted. A suspension of 3.1 g. (0.01 mole) of 1-(2-dimethylaminoethylamino)-4-methylthioxanthen-9-one in 25 ml. of ethanol was added to the amalgam and vigorously shaken for 30 min. During the exothermic reaction the yellow thioxanthen-9-one dissolved and a white suspension settled on cooling. The whole reaction product was poured into 500 ml. of water. The mercury was separared from the organic suspension by decantation. The white product was filtered and washed well with water. After crystallization from methanol, 2.8 g. (90%) of product was obtained, m.p. 161.5–162.5°.

1-(2-Dimethylaminoethylamino)-4-methylthioxanthene.—To 5.5 g. (0.017 mole) of 1-(2-dimethylaminoethylamino)-4-methylthioxanthen-9-one dissolved in 50 ml. benzene was added 100 ml. of ether containing 6 g. (0.016 mole) of lithium aluminum hydride. The color of the reaction mixture changed to a dark claret and then rapidly to a colorless slurry. The reaction mixture was refluxed for 5 hr., treated very carefully dropwise with 10 ml. of water, filtered through "Celite," dried over anhydrous magnesium sulfate, and evaporated to dryness. After crystallizing from ethanol, 3.3 g. (60%) of product was obtained, m.p. 84.5–85.5°.

1-(2-Dimethylaminoethylnitrosoamino)-4-methylthioxanthen-9-one.—1-(2-Dimethylaminoethylamino)-4-methylthioxanthen-9-one (3.1 g., 0.01 mole) was added to 25 ml. of water containing 2 ml. of concd. hydrochloric acid. The suspension was cooled to −5° and 0.69 g. (0.01 mole) sodium nitrite dissolved in 10 ml. of water was added dropwise over a period of 30 min. The suspension slowly dissolved on stirring; a pale yellow solution remained which was stirred for 1 hr. at room temperature. The acid solution was treated with sodium bicarbonate until the reaction mixture was nearly neutral. The compound was extracted with two 25-ml. portions of chloroform and the combined extracts dried with anhydrous magnesium sulfate. The extract was evaporated to a syrup and then triturated with absolute ethanol until the compound crystallized. After crystallization from 20 ml. of boiling ethanol the yield of the nitroso derivative was 2.0 g. (53%), m.p. 103–104°.

1-(2-Dimethylaminoethylamino)-4-methylxanthen-9-one.—In a Carius tube were placed 13 g. (0.055 mole) of 1-chloro-4-methylxanthen-9-one[10] and 20 g. (0.23 mole) of dimethylaminoethylamine. The tube was sealed and heated to a temperature of 150–160° for 6 hr. The cooled product then was treated with 10 ml. of 50% potassium hydroxide and steam distilled to remove the volatile amine. The yellow oil, which soon solidified, was filtered, washed well with water and dried. After crystallization from ethanol, the yield of the amine was 12.5 g. (77%), m.p. 114–114.5°.

1-(2-Dimethylaminoethylamino)-4-methyl-9-acridanone.—Dimethylaminoethylamine (20 g., 0.23 mole), 0.1 g. of copper dust, and 5.7 g. (0.023 mole) of 1-chloro-4-methyl-9-acridanone, prepared by the method of Archer, *et al.*,[10] were heated in a Carius tube at 220–230° for 4 hr. The content of the tube was treated with 5 ml. of 50% potassium hydroxide and steam distilled remove the volatile amines. The residue was extracted with two 100-ml. portions of boiling 10% acetic acid. The extracts were filtered and made basic with 50% potassium hydroxide solution. The orange solid that was isolated was filtered and washed well with water. The amine, 4.5 g. (66%), was isolated after being crystallized from ethanol, m.p. 243–245°.

3-Carboxy-2-(5-chloro-2-methylphenylthio)-pyridine.—A mixture of 2-chloronicotinic acid, 5 g. (0.032 mole)[11] and 10 g. (0.63 mole) of 2-methyl-5-chlorothiophenol was heated at 140° until it effervesced and a clear solution was obtained. Heating was then continued at 185–190° for 1 hr. The mixture, which solidified on cooling, was dissolved in sodium bicarbonate solution and then extracted with ether. The aqueous layer, when acidified with glacial acetic acid, deposited the sulfide, 8.0 g. (89%), m.p. 200–202°.

Anal. Calcd. for $C_{13}H_{10}ClNO_2S$: C, 55.81; H, 3.60; Cl, 12.67; N, 5.01; S, 11.46. Found: C, 55.76; H, 3.56; Cl, 12.87; N, 5.09; S, 11.52.

6-Chloro-9-methyl-5H-[1]benzothiopyrano[2,3-b]pyridin-5-one.—Thionyl chloride (30 ml.) and 7.1 g. (0.025 mole) of 3-carboxy-2-(5-chloro-2-methylphenylthio)-pyridine were refluxed for 1.5 hr. and the excess thionyl chloride was removed. The crude acid chloride solidified on standing. It then was dissolved in 60 ml. of dry nitrobenzene, and 16 g. (0.12 mole) of anhydrous aluminum chloride was added. The reaction mixture was heated on a steam bath for 2.5 hr., poured on ice, and steam distilled to remove the nitrobenzene. The residue was filtered, dried, and crystallized from ethanol, m.p. 177–178.5°. The material was sublimed at 165–175° (1 mm.) furnishing 5.0 g. (76%) of product, m.p. 177–178°.

Anal. Calcd. for $C_{13}H_8ClNOS$: C, 59.67; H, 3.08; Cl, 13.55; N, 5.35; S, 12.55. Found: C, 59.74; H, 3.14; Cl, 13.83; N, 5.47; S, 12.35.

6-(2-Dimethylaminoethylamino)-9-methyl-5H-[1]benzothiopyrano[2,3-b]pyridin-5-one.—Dimethylaminoethylamine (25 g., 0.28 mole) and 5.0 g. (0.019 mole) of 6-chloro-9-methyl-5H-[1]-benzothiopyrano[2,3-b]pyridin-5-one were heated at 165–170° in a Carius tube for 4 hr. The material was added to 500 ml. of water containing 5 ml. of 50% potassium hydroxide. The dark orange precipitate was filtered and washed well with water. The crude amine then was extracted with two 100-ml. portions of boiling 10% acetic acid. The extracts were filtered and made basic with 50% potassium hydroxide solution. The precipitate which resulted was filtered, washed well with water and dried. After crystallizing from methanol the amine (3.3 g., 55%) was obtained, m.p. 153–154.5°.

4-Carboxy-3-(5-chloro-2-methylphenylthio)-pyridine.—A solution of 3-aminoisonicotinic acid (27.7 g., 0.2 mole)[15] in concd. hydrochloric acid (40 ml.) was stirred at 5° while a solution of sodium nitrite (15.9 g., 0.23 mole) in water (110 ml.) was added dropwise. Ten minutes after complete addition, urea was added to destroy the excess nitrous acid and the solution was added slowly, with stirring, to one of sodium hydroxide (44 g., 1.10 moles) and 2-methyl-5-chlorothiophenol (28 g., 0.19 mole) in water (220 ml.) at 95°, heating being maintained until evolution of nitrogen ceased. The filtered, chilled solution was brought to pH 5 by addition of acetic acid, whereupon the sulfide was precipitated, m.p. 252–254° dec. After crystallization from acetic acid, 18 g. (34%) of product was obtained, m.p. 253–255° dec.

Anal. Calcd. for $C_{13}H_{10}ClNO_2S$: C, 55.81; H, 3.60; Cl, 12.67; N, 5.01; S, 11.46. Found: C, 55.80; H, 3.55; Cl, 12.79; N, 5.12; S, 11.60.

6-Chloro-9-methyl-5H-[1]benzothiopyrano[2,3-c]pyridin-5-one.—A mixture of 4-carboxy-3-(5-chloro-2-methylphenylthio)-pyridine (17 g., 0.06 mole) and 75 ml. of thionyl chloride was heated for 1 hr. on a steam bath and the excess of thionyl chloride removed *in vacuo*. Nitrobenzene (150 ml.) and aluminum chloride (45 g., 0.34 mole) were added to the residue and the mixture

(15) S. Gabriel and J. Colman, *Ber.*, **35**, 2832 (1902).

TABLE IV
9-ACRIDANONES AND

No.	R″	X	M.p., °C.	Yield, %	Emp. Formula
45	$NH(CH_2)_2N(CH_3)_2$	O	114–114.5	77	$C_{18}H_{20}N_2O_2$
46[b]	$NH(CH_2)_2N(C_2H_5)_2$	O	76.5–77.5	70	$C_{20}H_{24}N_2O_2$
47	$NH(CH_2)_2N(CH_2CH{=}CH_2)_2$	O	58.5–59.5	53	$C_{24}H_{24}N_2O_2$
48	$NH(CH_2)_2NHC_2H_5$	O	261–263	77	$C_{18}H_{20}N_2O_2 \cdot HCl$
49	$NHCHCH_3CH_2N(CH_3)_2$	O	91.5–92.5	72	$C_{19}H_2N_2O_2$
50	$NH(CH_2)_2NHC_2H_5$	NH	281–283	31	$C_{18}H_{21}N_3O \cdot 2HCl \cdot {}^1/_3H_2O$
51	$NH(CH_2)_2N(CH_3)_2$	NH	243–245	66	$C_{18}H_{21}N_3O$

[a] The preparation of these compounds or close analogs is described in the Experimental section. [b] See footnote 7b.

TABLE V
5H-[1]BENZOTHIOPYRANO

No.	R″	R	M.p., °C.	Yield, %	Emp. formula	C Calcd.	C Found
52	$NH(CH_2)_2NHC_2H_5$	CH_3	292–293.5	46	$C_{17}H_{19}N_3OS \cdot HCl$	58.36	58.20
53	$NH(CH_2)_2N(CH_3)_2$	CH_3	153–154.5	55	$C_{17}H_{19}N_3OS$	65.15	64.97
54[b]	$NH(CH_2)_2N(C_2H_5)_2$	CH_3	101.5–103	51	$C_{19}H_{23}N_3OS$	66.83	66.80
55	$NH(CH_2)_2N(CH_3)_2$	Cl	173.5–174.5	60	$C_{16}H_{16}ClN_3OS$	57.56	57.72

[a] The preparation of these compounds or close analogs is described in the Experimental section. [b] M. M. Coombs and W. H. Gray.

TABLE VI
5H-[1]-BENZOTHIOPYRANO

No.	R″	M.p., °C.	Yield, %	Emp. formula	C Calcd.	C Found
56	$NH(CH_2)_2N(CH_3)_2$	171.5–173.5	38	$C_{17}H_{19}N_3OS$	65.15	65.21
57	$NH(CH_2)_2N(C_2H_5)_2$	138–138.5	42	$C_{19}H_{23}N_3OS$	66.83	66.80

[a] The preparation of these compounds or close analogs is described in the Experimental section.

was then heated at 100° for 3 hr., poured on ice and steam distilled to remove the nitrobenzene. The hot solution was filtered, cooled and strongly basified with sodium hydroxide. The precipitated solid was collected, washed, dried and sublimed at 160–170° (1 mm.), furnishing a yellow product, 11.0 g. (70%), m.p. 186–188°.

Anal. Calcd. for $C_{13}H_8ClNOS$: C, 59.67; H, 3.08; Cl, 13.55; N, 5.35; S, 12.25. Found: C, 59.63; H, 2.90; Cl, 13.76; N, 5.34; S, 12.19.

6-(2-Dimethylaminoethylamino)-9-methyl-5H-[1]benzothiopyrano[2,3-c]pyridin-5-one.—6-Chloro-9-methyl-5H-[1]-benzothiopyrano[2,3-c]pyridin-5-one (4.5 g., 0.017 mole) and 20 g. (0.23 mole) of dimethylaminoethylamine were heated to 160–165° in a Carius tube for 4 hr. The reaction mixture was poured into water containing 5 ml. of 50% potassium hydroxide and the amine solidified. The solid material was washed well and extracted with two 50-ml. portions of 10% acetic acid. The extracts were filtered and made basic with 50% potassium hydroxide. After the mixture stood for 2 hr. in the cold, it was filtered, the solid washed well with water and dried. The amine was crystallized from methanol (2.0 g., 38%), m.p. 171.5–173.5°.

Antitumor Activity: Methods.—The mice were maintained on a standard laboratory diet (Diablo Labration). The drug doses were determined by preliminary toxicity tests and in the experiments were given at maximum tolerated doses starting 24 hr. after tumor inoculation and continued throughout the experiment. Six to ten mice were used in each experiment; the tumors used are listed.

Leukemia L-1210.—BDF_1 mice were given inoculations intraperitoneally of approximately 10^6 leukemia cells. Mean survival time of the treated animals relative to the controls was used to evaluate the results of treatment.

Sarcoma 180.—Swiss mice were given inoculations in the groin of 0.2 ml. of a suspension, in normal saline, containing approximately 150 mg. of crushed tumor tissue per ml. At the termination of the experiment (7 days) the tumors were excised and weighed individually to a precision of ±1 mg.

Adenocarcinoma 755.—BDF_1 mice were given inoculations in the groin of 0.2 ml. of a suspension, in normal saline, containing approximately 200 mg./ml. of crushed tumor tissue. At the termination of the experiment (11 days) the tumors were excised and weighed individually to a precision of ±1 mg.

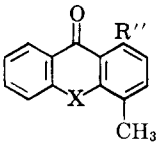
Results.—The antitumor activity of the thioxanthen-9-ones is given in the last three columns of the Tables.[16] The numbers are T/C (treated/control) values. In Adenocarcinoma 755 and Sarcoma 180 this is the ratio of the mean tumor weight of the treated group to the mean tumor weight of the control group; a T/C value of $\leqq 0.5$ is considered positive. In Leukemia L-1210 the T/C value is the ratio of the mean survival time of the treated animals to the mean survival time of the control animals; a T/C value of $\geqq 1.25$ is considered positive.

Discussion

As a result of this investigation of the antitumor activity of the thioxanthen-9-ones, some generalities may be formulated: (a) The carbon chain between the nitrogen atoms of the side chain on the thioxanthen-9-one molecule must be two carbon atoms in length. (b) The terminal alkyl group (s) must be small. Dimethyl is the best one found so far. (c) The ring attached nitrogen atom must bear one free hydrogen atom. (d) There must be an unsubstituted sulfur

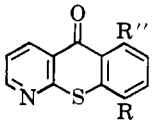
(16) Detailed presentation of the antitumor activity of these compounds is in press in the *Cancer Chemotherapy Screening Data* supplement to *Cancer Research*.

XANTHEN-9-ONES[a]



C		H		Cl		N		Antitumor Activity (T/C)		
Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	L-1210	S-180	AC-755
72.94	72.92	6.80	6.68	...	...	9.45	9.42	1.09	0.77	0.88
...	...	..	..	...	...	...	...	1.00	0.77	0.99
75.80	75.83	6.94	6.94	...	...	8.04	8.01	0.90	0.94	0.94
65.15	65.13	6.38	6.47	8.44	8.64	10.38	10.66	0.99	0.70	0.82
73.52	73.39	7.14	7.12	...	...	9.03	9.05	1.13	0.81	0.80
57.29	57.30	6.41	6.50	18.79	18.62	11.14	11.14	1.09	0.96	0.94
73.18	73.02	7.17	7.17	...	...	14.23	13.94	1.09	1.00	0.79

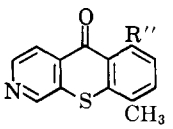
[2,3-b]PYRIDIN-5-ONES[a]



H		Cl		N		S		Antitumor Activity (T/C)		
Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	L-1210	S-180	AC-755
5.76	5.94	10.13	10.16	12.01	12.12	9.16	8.97	1.07	0.82	0.58
6.11	6.21	...	...	13.41	14.04	10.28	10.24	1.12	1.02	0.69
6.79	6.64	...	...	12.31	12.26	9.39	9.24	1.03	0.95	1.16
4.83	4.77	10.62	10.92	12.59	12.66	9.61	9.42	1.27	0.80	1.06

U. S. Patent 2,656,357 (1953).

[2,3-c]PYRIDIN-5-ONES[a]



H		N		S		Antitumor Activity (T/C)		
Calcd.	Found	Calcd.	Found	Calcd.	Found	L-1210	S-180	AC-755
6.11	6.12	13.41	13.63	10.28	10.09	1.32	1.20	1.42
6.79	6.61	12.31	12.33	9.39	9.26	1.30	0.95	0.97

atom in position 10. 9-Acridanone and xanthen-9-one analogs, as well as thioxanthen-9-one-10,10-dioxides, are inactive. (e) There must be a carbonyl group in position 9 of the thioxanthen-9-one molecule. Reduction of the carbonyl group to the thioxanthen-9-ol or thioxanthene eliminated the antitumor activity. (f) There must be a compact, fairly durable substituent in ring position 4. (g) A few polysubstitution patterns were studied. Additional chloro substitution in position 7 of the thioxanthen-9-one molecule yielded compounds poorly absorbed by mice; hence it was generally impossible to discern intrinsic activity. Hirschberg, *et al.*,[6] have shown that chloro substitution at position 6 as well as methyl in position 4 inhibit activity of the thioxanthen-9-ones. The preparation of more complexly substituted compounds with good absorption characteristics is desirable; however, the synthetic problems become increasingly difficult. (h) 5H-[1]-Benzothiopyrano[2,3-b]pyridin-5-ones and 5H-[1]benzothiopyrano[2,3-c]pyridin-5-ones show little, if any, activity on the tumor systems. Thus it appears that ring atom replacement of the original thioxanthen-9-one molecule generally eliminates antitumor activity.

One may speculate on the possibility of the potential hydrogen bonding or the metal chelating ability *via* the carbonyl group and the dialkylaminoethylamino chain being essential to carcinostatic activity. A sharply demarcated range of "fit" is indicated and π-bonding (electron exchange) components may be present in the biological interaction.

Acknowledgment.—We wish to express our appreciation to Maris V. Nora and Douglas French for their assistance in the preparation of many of the thioxanthen-9-ones and to Mrs. Arvia Hosking and Mrs. June French for the antitumor evaluations.