NMR (deuterochloroform): δ 2.00 (s, 3H, CH_3), 2.67 (t, 2H, CH_2CO_2R), 4.34 (t, 2H, CH_2OCOCH_3), 5.14 (s, 2H, $CH_2C_6H_5$), and 7.32 (s, 5H, ArH).

Anal.—Calc. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.34. Found: C, 65.01; H, 6.27.

***cis*-Benzyl 2-Acetoxycyclopropanecarboxylate (XII)**—A procedure similar to that described for XIII was utilized with 10.9 g (0.05 mole) of IX and 57.2 g (0.44 mole) of trifluoroperacetic acid. The 3.3 g of crude oily product was treated with 4.8 g of Girard's reagent P[2] and 3 ml of acetic acid in 40 ml of refluxing methanol for 4 hr. Ice water (50 ml) was added to the hot solution, and it was brought to neutrality with solid potassium carbonate. This solution was extracted with methylene chloride, and the extract was washed with water and dried over magnesium sulfate. Removal of the solvent and distillation of the residual liquid at 113° (0.035 mm) gave 2.5 g (25%) of product; IR (film): 1730 (ester C=O) cm^{-1}; NMR (deuterochloroform): δ 1.08–2.23 (m, 3H, ring H), 1.93 (s, 3H, CH_3), 4.00–4.44 (m, 1H, $CHOCOCH_3$), 5.16 (s, 2H, $CH_2C_6H_5$), and 7.38 (s, 5H, ArH).

Anal.—Calc. for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.49; H, 6.18.

[2] Eastman.

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Synthesis and Biological Evaluation of 2-(9-Acridinyl)ethyl-*N*-substituted Carbamates and Their Hydrochlorides and 10-*N*-Oxides

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Abstract □ The syntheses of 2-(9-acridinyl)ethyl-*N*-substituted carbamates and their hydrochlorides and 10-*N*-oxides are reported along with biological results in the areas of antineoplastic, antimalarial, and CNS activity screening. The compounds showed negative biological activity in the areas tested.

Keyphrases □ 2-(9-Acridinyl)ethyl-*N*-substituted carbamates, hydrochlorides, and 10-*N*-oxides—synthesis, pharmacological activity □ Antineoplastic activity—synthesis and screening of 2-(9-acridinyl)ethyl-*N*-substituted carbamates, hydrochlorides, and 10-*N*-oxides

Acridine derivatives have been tested extensively for potential medicinal activity. Antibacterial (1, 2), antimalarial (3), anthelmintic (4), analeptic (5), and antineoplastic (6–10) activities have been reported for many substituted acridines. The synthesis of new acridine derivatives containing urea, thiourea, thiocarbamate, and carbamate groupings has been reported from this laboratory (11, 12). While possessing little or no anticancer activity, a general pharmacological screen revealed activity in antibacterial, metabolic, parasitologic, and GI screening procedures for some derivatives.

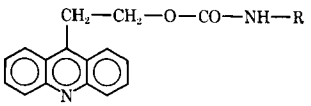
In a continuation of a study into the synthesis and potential biological activity of new acridine compounds, this paper reports the synthesis and antineoplastic screening data for 2-(9-acridinyl)ethyl-*N*-substituted carbamates and their hydrochlorides and 10-*N*-oxides. In addition, biological results for some compounds in antimalarial and central nervous system (CNS) activity screening procedures are reported.

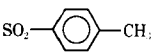
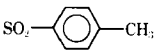
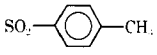
DISCUSSION

The incorporation of the carbamate and acridine moieties into one structure is of special interest, since each structure is singly present in compounds that have shown various degrees of antineoplastic activity (6–10, 13–15). The interaction of acridines with nucleic acids and inhibition of nucleic acid synthesis is well documented (16, 17). Acridines tend to accumulate selectively in tumor tissue (18, 19) showing inhibition of tumor growth, reduction of rate of growth, and, in some cases, regression of tumor size. Nevertheless, no clinically useful agent has been found among the acridines tested, most of which have been substituted 9-aminoacridines. Structures containing carbamate esters also have been reported to have potent antitumor activity (13, 14). Ethyl carbamate itself has been used both for the production of tumors in experimental animals and for the treatment of chronic leukemia and multiple myeloma (15). Some evidence also indicates that carbamates are involved in alkylation of DNA (20).

The mechanism by which these new acridine carbamates were expected to exert their antineoplastic effect was by selective absorption in cancerous tissue followed by a two-pronged attack upon the nucleic acids of the cancer cells. The acridine ring would be expected to interact with the nucleic acid bases, thus holding the carbamate portion in close proximity to the bases to facilitate alkylation by the carbamate.

Table I—2-(9-Acridinyl)ethyl-*N*-substituted Carbamates, Hydrochlorides, and 10-*N*-Oxides



Compound	R	Melting Point	Method of Synthesis[a]	Yield, %	λ_{max} (ethanol), nm	Analysis, %	Calc.	Found
1	H	213–215°	A[b]	50	252 (2.0×10^5)	C	72.16	71.92
						H	5.30	5.51
						N	10.52	10.40
2	C_2H_5	125–126°	B	74	252 (1.2×10^5)	C	69.64	69.72
						H	5.84	5.88
						N	9.06	8.97
3	C_6H_5	183–184°	B	94	252 (8.1×10^4)	C	77.17	77.30
						H	5.30	5.39
						N	8.18	8.17
4		133–135°	B	92	250 (1.3×10^5)	C	63.29	63.07
						H	4.62	4.71
						N	6.42	6.28
	10-Hydrochlorides							
5	H	200° dec.	C	95	252 (1.7×10^5)	C	63.47	63.53
						H	4.99	5.04
						N	9.25	9.23
6	C_2H_5	126–130°	C	97	252 (8.2×10^4)	C	65.35	65.52
						H	5.79	5.86
						N	8.47	8.44
7	C_6H_5	159–160°	C	97	252 (1.4×10^5)	C	69.71	69.92
						H	5.05	5.15
						N	7.40	7.33
8		135° dec.	C	98	252 (1.4×10^5)	C	60.46	60.58
						H	4.63	4.77
						N	6.13	6.06
	10-*N*-Oxides							
9	H	169–171°	D	30	266 (8.0×10^4)	C	68.07	67.70
						H	4.99	4.94
						N	9.92	9.55
10	C_2H_5	142–145°	D	40	266 (4.9×10^4)	C	69.64	69.72
						H	5.84	5.88
						N	9.06	8.97
11	C_6H_5	218–220°	D	72	265 (9.0×10^4)	C	73.73	73.84
						H	5.06	5.19
						N	7.82	7.71
12		180–181°	D	10	266 (1.7×10^5)	C	63.29	63.07
						H	4.62	4.71
						N	6.42	6.28

[a] Letters refer to the experimental procedures. [b] Procedure modified from general method described in Ref. 17.

The hydrochloride salts of the various acridine carbamates were prepared to increase polarity of the molecules and, therefore, their water solubility. The 10-*N*-oxide derivatives were also synthesized, since it has been reported that oxidation of the acridine ring nitrogen reduces the toxicity of the agent by increasing renal clearance (21).

EXPERIMENTAL[1]

Chemistry—The carbamates were synthesized by reaction of 2-(9-acridinyl)ethyl alcohol with sodium cyanate, trifluoroacetic acid (22), and/or the respective isocyanate (23). The starting alcohol was obtained in a few synthetic steps from commercial materials (24, 25). The hydrochlorides were prepared from the respective carbamates with ethereal hydrogen chloride solution, and the 10-*N*-oxides were prepared using *m*-chloroperbenzoic acid. The synthesized compounds are shown in Table I, together with their melting points, UV maxima, and elemental analysis data.

2-(9-Acridinyl)ethyl Carbamate (Procedure A)—A solution of 4.5 g (0.02 mole) of 2-(9-acridinyl)ethyl alcohol in 25 ml of methylene chloride was treated with 2.6 g (0.04 mole) of sodium cyanate. The resulting suspension was slowly stirred while 3.2 ml (0.04 mole) of trifluoroacetic acid was added dropwise over 5 min. The reaction mixture was then stirred at room temperature for 2 hr. After washing with one 5-ml portion of water, the methylene chloride layer was separated and dried over anhydrous sodium sulfate. The filtered solution was then evaporated to dryness on a steam bath, followed by neutralization of the residue with a saturated solution of sodium bicarbonate. The resulting precipitate was recrystallized from aqueous ethanol to give the 2-(9-acridinyl)ethyl carbamate.

*2-(9-Acridinyl)ethyl-*N*-substituted Carbamates (Procedure B)*—The respective isocyanate (0.03 mole) was added to a solution of 4.5 g (0.02 mole) of 2-(9-acridinyl)ethyl alcohol in 100 ml of dry benzene. The solution was heated at reflux for 4 hr. The reaction mixture was evaporated to dryness, and the residue was recrystallized from aqueous ethanol (Compound 2), benzene–cyclohexane (Compound 3), or acetone–petroleum ether (Compound 4).

*2-(9-Acridinyl)ethyl-*N*-substituted Carbamate Hydrochlorides (Procedure C)*—A solution of 0.02 mole of the respective carbamate in 30 ml of ether was saturated with hydrogen chloride gas. A precipitate, which formed immediately, was collected by filtration and purified by trituration in acetone (Compounds 5–7) or ethanol (Compound 8) to yield the desired hydrochloride salt.

*2-(10-Oxido-9-acridinyl)ethyl-*N*-substituted Carbamates (Procedure D)*—A solution of 0.025 mole of *m*-chloroperbenzoic acid in 5–10 ml of chloroform was added to a solution of 0.02 mole of the respective carbamate dissolved in 25 ml of chloroform. The mixture was stirred for 3 hr at room temperature followed by evaporation to dryness. The resulting residue was washed with four 10-ml portions of ether followed by recrystallization of the residue with cyclohexane–chloroform.

[1] Melting points were taken in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were obtained from Atlantic Microlabs, Atlanta, Ga. IR spectra were recorded on a Perkin-Elmer model 237 B spectrophotometer and were as expected. UV spectra were recorded on a Perkin-Elmer model 202 spectrophotometer.

Table II—Antineoplastic Screening Results

Compound	L-1210 Dose, mg/kg[a]	Survivors	Weight Difference	T/C, %
1[b]	400	3/3	−0.2	105
	200	3/3	+0.5	
	100	3/3	−0.5	105
2[c]	400	3/3	+0.4	94
	200	3/3	−0.3	97
	100	3/3	−0.6	105
3[c]	400	3/3	−1.0	90
	200	3/3	+0.6	94
	100	3/3	+0.5	94
4[d]	400	0/3	−1.2	
	200	3/3	−0.4	109
	100	3/3	−0.0	97
5[e]	400	3/3	−0.1	
	200	3/3	−1.2	103
	100	3/3	−0.1	93
	50	3/3	−0.3	93
	25	3/3	−0.5	96
6[f]	400	3/3	−1.1	97
	200	3/3	−0.1	90
	100	3/3	−0.3	102
7[g]	400	3/3	−1.0	97
	200	3/3	+0.3	94
	100	3/3	−0.4	
8[h]	400	0/3	−1.2	
	200	3/3	−2.0	97
	100	3/3	−0.2	97
9[c]	400	2/3	−0.7	95
	200	3/3	−0.8	98
	100	3/3	+0.2	95
	50	3/3	+0.5	98
10[c]	400[i]	5/6	+0.3	90
	200	6/6	−0.0	102
	100	6/6	+0.5	96
11[c]	400[i]	6/6	−1.0	102
	200	6/6	+1.4	99
	100	6/6	+0.2	91

[a] Doses were administered every 4th day for two doses unless otherwise noted. [b] Cell culture (KB): ED_{50} at 44 μg/ml. [c] Cell culture (KB): ED_{50} at 1.0×10^2 μg/ml. [d] Cell culture (KB): ED_{50} at 73 μg/ml. [e] Cell culture (KB): ED_{50} at 14 μg/ml. [f] Cell culture (KB): ED_{50} at 94 μg/ml. [g] Cell culture (KB): ED_{50} at 90 μg/ml. [h] Cell culture (KB): ED_{50} at 23 μg/ml. [i] Doses were administered every 4th day for three doses.

Biological[2]—Compounds 1–11 were tested against L-1210 leukemia according to the standard protocol[3] of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health. None of the compounds screened exhibited anticancer activity patterns considered adequate to justify expanded testing or further extension of the present group (Table II). Activity is defined as percent T/C (test/control) of 125 or greater. The term "survivors" in Table II is a measure of toxicity and was determined at Day 5 after the first injection.

Compounds 3 and 6 were tested for antimalarial activity. The activity was assessed against *Plasmodium berghei* in mice by the method of Osdene *et al.* (28). Both compounds were found to be inactive.

Compound 4 was tested for potential CNS activity by the method of Iturrian and Johnson (29). In the procedure, the carbamate was administered to audioconditioned mice at a level of 150 mg/kg. Data showed that the compound failed to block the running or tonic components of the audiogenic seizure.

Compound 12 was not submitted for biological testing due to insufficient quantities available from the synthetic program.

[2] The antineoplastic screening data were made available through courtesy of Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. The antimalarial test results were provided by the Walter Reed Army Institute of Research. The CNS activity data were provided by the Department of Pharmacology, School of Pharmacy, University of Georgia.

[3] For general screening procedure and data interpretation, see Refs. 26 and 27.

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