for the preparation of X, gave 90 mg. (73%) of amorphous white solid, m.p. 102–108°. This material showed a single spot on TLC with 12 or 25% methanol in benzene. $\nu_{max.}^{KBr}$ 3500, 3400, 3350, 3150–3100 (NH, OH); 1670–1610, 1540 cm.$^{-1}$ (amide C=O, NH, pyrimidine); $\lambda_{max.}^{pH\ 1}$ 266 mμ (ε 7300); $\lambda_{max.}^{pH\ 13}$ 280 mμ (ε 6900); $\lambda_{max.}^{EtOH}$ 293 mμ (ε 7900).

Anal.—Calcd. for $C_{19}H_{26}N_4O_2$: C, 66.6; H, 7.65; N, 16.4. Found: C, 66.3; H, 7.70; N, 16.6.

To a solution of 225 mg. of a different preparation of amorphous XI in 2 ml. of 95% ethanol was added a solution of 229 mg. of picric acid in 4 ml. of ethanol. The crystalline picrate was collected and washed with alcohol; yield, 193 mg. (53% based on IX), m.p. 170–173°. Recrystallization from 50% ethanol gave yellow crystals, m.p. 171–172°.

Anal.—Calcd. for $C_{19}H_{26}O_2 \cdot C_6H_3N_3O_7$: C, 52.5; H, 5.11; N, 17.2. Found: C, 52.7; H, 5.40; N, 17.0.

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Synthesis and Pharmacological Properties of Some Fluorine-Containing Amide Derivatives

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Eight fluorine-containing compounds, seven of which are derivatives of bromal or dichloroacetaldehyde, were synthesized and their physical constants determined. These compounds have been screened for anticancer, antispasmodic, tranquilizing, and blood pressure effects. Acute toxicity studies have also been carried out.

VARIOUS amide derivatives of chloral, bromal, trichlorobutyraldehyde, and dichloroacetaldehyde (1–9) have been reported in the literature. Work with these compounds has shown their potentialities as fungicides (9) and sedative-hypnotics (10). Other than this, however, little has been reported in regard to the pharmacological activities exhibited by these compounds. In view of this fact, eight fluorinated amide derivatives have been synthesized and subjected to certain pharmacological screens.

Interest in these particular compounds stemmed from the fact that some of our anticancer drugs today are amide derivatives, an example is the phosphoramides. The fluorinated amide derivatives were chosen for study since certain fluorine-containing antimetabolites, such as 5-fluorouracil and 5-fluorodeoxyuridine, have found a place in cancer chemotherapy. Interest, too, was prompted by the fact that some amide derivatives, such as 3,4,5-trimethoxycinnamide and 2-ethyl-3-methylvaleramide, are being used as tranquilizers today.

Received January 8, 1965, from the School of Pharmacy, University of Arkansas, Little Rock.
Accepted for publication June 15, 1965.
This investigation was supported by grant GM-10371 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

EXPERIMENTAL

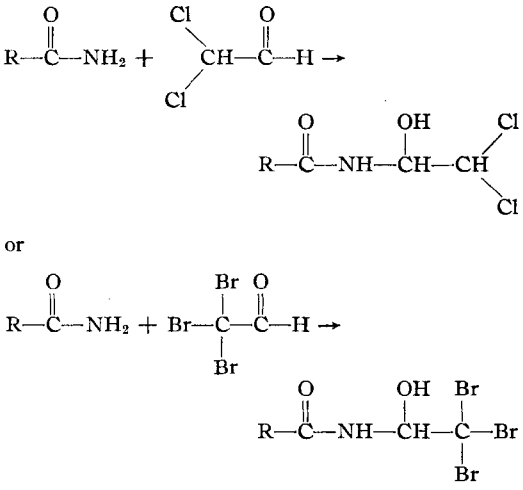
Materials

The intermediates used in this investigation were obtained through ordinary commercial sources. The fluoroacetamide, 4-fluorobenzoic acid, 3-aminobenzotrifluoride, and 4-fluorophenylacetic acid were purchased from Aldrich Chemical Co., Inc. The trifluoroacetamide and 2,4-dichlorobenzoyl chloride were obtained from the Matheson, Coleman, and Bell Division of the Matheson Co. The aldehydes used, bromal and dichloroacetaldehyde, were obtained from Eastman Kodak Co. and the Westvaco Chemical Co., respectively.

Synthesis

The amide derivatives of bromal and dichloroacetaldehyde were prepared by reacting the desired amide with the appropriate aldehyde in equimolar portions according to procedures previously reported (7, 8). The procedure (8) was modified for the preparation of the trifluoroacetamide derivative of bromal in that the condensation was carried out in a vacuum oven (Labline Duo-Vac, model 3620) at 20° and −20 lb. pressure rather than in a constant-temperature bath. The general reaction is shown in Scheme I, with R representing either a fluorinated alkyl or fluorinated aryl carbon chain.

The one compound reported in this paper that is neither a derivative of bromal or dichloroacetaldehyde, the *N*-(3-trifluoromethylphenyl)-2,4-dichlorobenzamide, was prepared by reacting 2,4-



Scheme I

dichlorobenzoyl chloride with 3-trifluoromethylaniline in chloroform according to the procedure of Kissman and Baker (11).

The fluorinated amide intermediates not available commercially, the 4-fluorobenzamide and the 4-fluorophenylacetamide, were prepared from the appropriate fluorinated acids using a modification of the procedure of Kissman and Baker (11) as follows. The fluorinated acid (0.1 mole) was dissolved in 150 ml. of absolute ether in a 500-ml. beaker, placed in an ice bath, and stirred constantly while phosphorus pentachloride (0.1 mole) was added slowly. The stirring was continued for approximately 0.5 hr., followed by evaporation of the ether with air. To insure removal of the ether, 50-ml. portions of chloroform were twice added to the residue and evaporated with air. An additional 200 ml. of chloroform was now added, the mixture placed in a salt ice bath, and 150 ml. of concentrated ammonium hydroxide added drop by drop, with constant stirring. The mixture was removed from the ice bath and allowed to react at room temperature for 0.5 hr., constant stirring being maintained. The amide, which precipitated from the mixture upon cooling, was filtered out and recrystallized from chloroform.

The analytical data for the eight compounds reported in this paper are given in Table I. Nitrogen assays were by the Kjeldahl method, and the halogen determinations were by Galbraith Laboratories, Inc., Knoxville, Tenn.

Anticancer Studies

The anticancer studies with Lymphoid Leukemia L-1210 and Sarcoma 180 were based on that set forth by the Cancer Chemotherapy National Service Center (12) and were performed as follows.

Propagation of Tumor Lines.—DBA/2 strain mice were used for propagation of the L-1210 tumor, and BDF/1 strain mice were used for propagation of

TABLE I.—BROMAL AND DICHLOROACETALDEHYDE AMIDES
RCONHCH(OH)CBr_3 OR RCONHCH(OH)$CHCl_2$

Compound[a]	Empirical Formula	M.p., °C.[b]	% Yield	Anal., % Calcd.	Found
Bromal fluoroacetamide	$C_4H_5Br_3FNO_2$	128–130	54	Br, 67.04	Br, 67.24
				Cl, ...	Cl, ...
				F, 5.31	F, 5.50
				N, 3.91	N, 4.14
Bromal trifluoroacetamide	$C_4H_3Br_3F_3NO_2$	130–132	29	Br, 60.91	Br, 60.93
				Cl, ...	Cl, ...
				F, 14.41	F, 14.78
				N, 3.55	N, 3.57
Bromal *p*-fluorobenzamide	$C_9H_7Br_3FNO_2$	138	59	Br, 57.14	Br, 57.16
				Cl, ...	Cl, 4.59
				F, 4.52	F, ...
				N, 3.33	N, 3.47
Bromal *p*-fluorophenylacetamide	$C_{10}H_9Br_3FNO_2$	150–155	52	Br, 55.30	Br, 55.47
				Cl, ...	Cl, ...
				F, 4.38	F, 4.48
				N, 3.23	N, 3.40
Dichloroacetaldehyde fluoroacetamide	$C_4H_6Cl_2FNO_2$	88–90	33	Br, ...	Br, ...
				Cl, 37.37	Cl, 37.46
				F, 10.00	F, 10.25
				N, 7.37	N, 7.17
Dichloroacetaldehyde *p*-fluorobenzamide	$C_9H_8Cl_2FNO_2$	98–100	48	Br, ...	Br, ...
				Cl, 28.17	Cl, 29.03
				F, 7.54	F, 8.16
				N, 5.56	N, 5.60
Dichloroacetaldehyde *p*-fluorophenylacetamide	$C_{10}H_{10}Cl_2FNO_2$	98–100	38	Br, ...	Br, ...
				Cl, 26.69	Cl, 28.65
				F, 7.14	F, 7.99
				N, 5.26	N, 5.54
Benzamide, *N*-(3-trifluoromethylphenyl)-2,4-dichloro-[c]	$C_{14}H_8Cl_2F_3NO$	125–126	95	Br, ...	Br, ...
				Cl, 21.26	Cl, 21.40
				F, 17.07	F, 17.21
				N, 4.19	N, 4.26

[a] The "Chemical Abstracts" names for these compounds, *N*-(2,2,2-tribromo-1-hydroxyethyl) amides and *N*-(2,2-dichloro-1-hydroxyethyl) amides, were not used in this table in order to conserve space. [b] Melting points were taken on a Thomas-Hoover capillary melting point apparatus. [c] Compound is not a derivative of bromal or dichloroacetaldehyde.

TABLE II.—ANTICANCER STUDIES

Compd.	Leukemia-1210 Mean Survival Time, Days (Control Group)	Leukemia-1210 Mean Survival Time, Days (Test Group)	Sarcoma-180 Wt. of Tumor, Gm. (Control Group)	Sarcoma-180 Wt. of Tumor, Gm. (Test Group)	% Control Growth
Bromal fluoroacetamide	6.50	6.50	0.995	[a]	[a]
Bromal trifluoroacetamide	6.50	[a]	0.995	1.163	121.7
Bromal *p*-fluorobenzamide	6.50	6.40	0.995	0.476	49.8
Bromal *p*-fluorophenylacetamide	6.67	6.75	0.995	1.071	91.5
Dichloroacetaldehyde fluoroacetamide	6.67	6.50	0.511	0.395	77.3
Dichloroacetaldehyde *p*-fluorobenzamide	6.50	6.50	0.316	0.387	122.0
Dichloroacetaldehyde *p*-fluorophenylacetamide	6.20	6.20	0.316	0.426	134.0
Benzamide, *N*-(3-trifluoromethylphenyl)-2,4-dichloro-	6.50	7.25	0.995	0.668	69.95

[a] No results obtained as death of animals occurred due to toxicity of drug.

the S-180 tumor. The tests were carried out using BDF/1 mice. The tumor lines were obtained from Microbiological Associates, Inc., Washington, D. C.

Lymphoid Leukemia (L-1210) Test.—The animal with the donor tumor was sacrificed in a humane way on the day of growth designated in the individual protocol. The ascitic fluid was withdrawn aseptically by inserting the needle through the abdominal muscle from which the skin had been removed. The fluid was held in a sterile glass container in an ice bath, with samples being pooled if more than one donor was needed.

A cell morphology study was made by placing 1 drop of the ascitic fluid on a glass slide, making a smear, allowing to dry, and staining with Wright's stain. If the leukocyte count indicated that the fluid contained more than 95% lymphoblasts, the fluid was considered suitable for use in the tests. A cell count was then made by placing ascitic fluid in a small crucible or test tube containing dry heparin, diluting with 10 to 100 vol. of saline, and making a cell count as for white blood cells.

Implantations of the ascitic fluid into test animals were made by injection intraperitoneally using a 23-gauge needle. A sufficient number of sterile syringes and needles were used to avoid refilling from the pool of the donor fluid. No more than 60 min. was allowed to elapse from the time the fluid was taken from the donor until it was implanted in the test animals.

Treatment of the test animals with the experimental compounds was begun 24 hr. after transplant. Drug concentration was 500 mg./Kg. body weight, dosage being administered intraperitoneally each day for 7 days. The results were evaluated according to the mean survival time as follows and recorded in Table II:

$$\text{mean survival time (days)} = \frac{{}^*S + ASa - (B + 1)\,NT}{Sa - NT}$$

where

A = day prior to which deaths are considered due to drug toxicity (for L-1210, A = day 6)
B = day beyond which control group survivors are considered "no take" (for L-1210, B = day 18)
$^*S$ = the sum from day A through day B, if there are "no takes" in the treated group. If there are no "no takes," $^*S$ is the sum of daily survivors from day A onward
Sa = number of survivors prior to day A (number surviving at end of day $A - 1$)
NT = number of "no takes"

Solid Tumor (S-180) Tests.—The animal with the donor tumor was sacrificed on the day of growth designated in the individual protocol. The tumor was aseptically excised and debrided of necrotic tissue, then placed in a sterile Petri dish with a small amount of buffered physiological saline, or equivalent, on ice. For transplantation, a 13-gauge trocar was used after loading with a single fragment by aspiration or handling with sterile forceps. A fresh sterile trocar was used for every ten tumor fragments. No more than 30 min. was allowed to elapse from the time the tumor was removed from the donor animal until it was transplanted into the axillary region of the test animals.

Treatment of the test animals was begun 24 hr. after transplant. The drug being tested was administered intraperitoneally each day for 7 days (500 mg./Kg.). The animals were sacrificed on the eighth day after implant and the weights of the tumors compared with that of the controls. Results were reported as per cent of control growth. (See Table II.)

Effects on Dog Blood Pressure

Mongrel dogs of either sex weighing between 7.3 to 17.3 Kg. were anesthetized with pentobarbital (35 mg./Kg.) intravenously and through carotid cannulation were attached to blood pressure recording device. Injections of the compounds were made *via* the femoral vein in doses ranging from 12.5 to 50 mg./Kg. All of the compounds tested had a slight hypotensive effect on blood pressure. The hypotensive effects were not decreased by atropinization of the dog subsequently followed by the compounds.

Antispasmodic Effects on Rat and Rabbit Intestine

The Magnus (13) method was employed for testing antispasmodic action. The animals were fasted for 24 hr. and then killed by a sharp blow on the back of the head. The ileum was removed and its lumen washed out by forcing Locke-Ringer's solution (14) from the oral to the aboral end. The ileum was stored in Locke-Ringer's solution in a refrigerator and used within 12 hr. The bathing

solution for all isolated rat strips was Tyrode's solution (15) and for rabbit intestine Locke-Ringer's was used. A temperature of 38° was maintained. Approximately 30 min. was allowed for acclimatization and for spontaneous movements to develop. The compounds that were water insoluble were suspended in a methylcellulose solution (1:20 concentration) and the suspension added to the tissue bath to give a uniform concentration of the compound throughout the vessel. The one water-soluble compound, *N*-(2,2-dichloro-1-hydroxyethyl)-4-fluorobenzamide, was dissolved in distilled water (1:20 concentration) and then used. The contractions were recorded on a smoked kymograph. All of the eight compounds tested in concentrations of 0.16 to 0.48 mg./ml. decreased the height of contractions, but none were of sufficient activity to warrant further investigation.

Acute Toxicity Determinations

The LD_{50} of the eight compounds was determined using the method of Deichman and LeBlanc (16). Swiss mice weighing between 25 and 35 Gm. were injected intraperitoneally and survival time after 24 hr. was determined. Four concentrations were injected into different mice at intervals of two dosage levels. Once the dose that kills was found, the dosage was dropped two levels, and if the animal survived, the LD_{50} was taken as the dose in between as established by Deichman and LeBlanc. The sixth animal was then given this dosage, allowing a determination of the approximate LD_{50} of the test compound. The results are given in Table III.

Tranquilizing Effects

Preliminary screening of these compounds for tranquilization effects was carried out using the method of Dunham and Miya (17). Preliminary screening shows that these compounds induce ataxia and decreased motor activity. The activity of these compounds was compared to chlorpromazine. Of these compounds examined, *N*-(2,2,2-tribromo-1-hydroxyethyl)-2,2,2-trifluoroacetamide and *N*-(2,2-dichloro-1-hydroxyethyl)-2-fluoroacetamide warrant further investigation. The remaining compounds are not comparable to chlorpromazine in potency of action.

RESULTS

The eight compounds screened in this report showed the following results.

Anticancer tests.—One of the compounds, the bromal *p*-fluorobenzamide derivative, gave a control growth of 49.8% and will be investigated further. The other compounds did not seem to warrant further study in this area.

Antispasmodic Tests.—The eight compounds showed varying effects on smooth muscle ranging from little to no effect. At the present time there seems to be very little use for these agents as antispasmodics.

TABLE III.—ACUTE TOXICITY

Compd.	Acute Toxic Dose, Gm./Kg.
Bromal fluoroacetamide	0.52
Bromal trifluoroacetamide	0.10
Bromal *p*-fluorobenzamide	Greater than 7.1
Bromal *p*-fluorophenylacetamide	Greater than 4.7
Dichloroacetaldehyde fluoroacetamide	0.35
Dichloroacetaldehyde *p*-fluorobenzamide	0.78
Dichloroacetaldehyde *p*-fluorophenylacetamide	0.999
Benzamide, *N*-(3-trifluoromethylphenyl)-2,4-dichloro-	Greater than 7.1

Blood Pressure Studies.—The compounds caused a decrease in blood pressure which was not blocked by atropine. These results lead us to believe that the action of these agents is directly on the smooth muscle.

Acute Toxicity.—Some of the compounds tested in this screening procedure were shown to be relatively nontoxic compared to the effective doses found for some of the compounds. This would make it possible for the agent to be used quite safely with this range between the effective dose and the lethal dose if a use for the compounds should be found. The exceptions are the bromal trifluoroacetamide, bromal fluoroacetamide, and dichloroacetaldehyde fluoroacetamide derivatives.

Tranquilizer Tests.—The screening data from this area of testing showed these compounds to cause decreased motor activity. With the exception of the bromal trifluoroacetamide derivative and the dichloroacetaldehyde fluoroacetamide derivative, these compounds when compared to chlorpromazine do not warrant further investigation.

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