2-Fluoroethylamines. II.[1a] Biological Evaluation and Synthesis[1b]

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Nitrogen mustards containing fluorine were attached to cinnamic acid, hydrocinnamic acid, and phenylbutyric acid as carrier groups. These fluoro mustards and others, synthesized earlier, that were attached to benzaldehyde, phenylpyruvic acid, and phenylalanine, together with some intermediates were screened for antitumor activity. The majority were screened against Walker 256 in rat and KB cell culture. Selected mustards were screened against Sarcoma 180, lymphoid leukemia L1210, and Lewis lung carcinoma in mice. In general, replacement of chlorine by fluorine resulted in increased toxicity and the compounds did not meet the criteria for activity.

2-Fluoroethylamines have been studied the least of all 2-haloethylamines[2] as potential anticancer agents. However, the recent report[3] of the remarkable clinical application of "ftorpan" [5-(2-chloroethyl-2-fluoroethylamino)-6-methyluracil] has spurred interest in the synthesis of 2-fluoroethylamines in this laboratory[1a] and elsewhere.[4] We report here the synthesis and biological evaluation of the fluorine analogs of *p*-cinnamic acid mustards,[5] chlorambucil,[6] and related compounds.

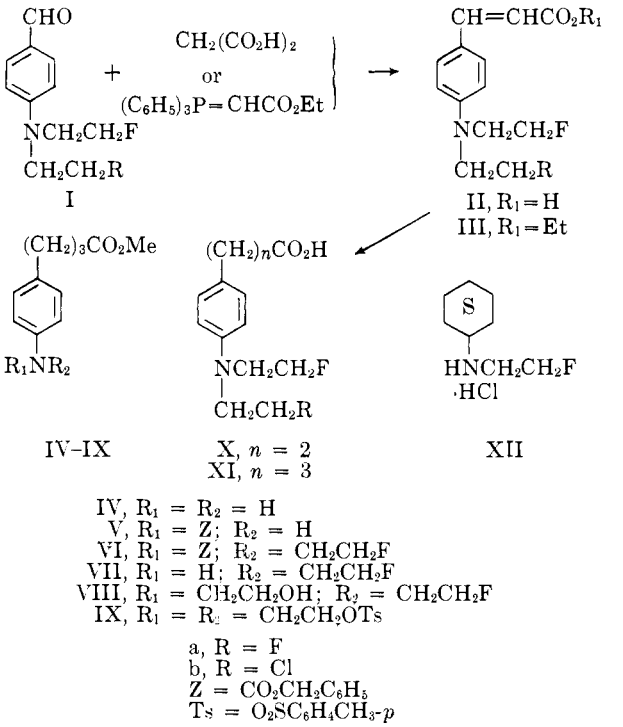
For the synthesis of the bisfluoro and the chlorofluoro mustards outlined in Chart I, we used either the intermediates or the general methods reported previously.[1a] *p*-[Bis(2-fluoroethyl)amino]benzaldehyde[1a] (Ia) reacted with malonic acid under either acid or base catalysis. In the presence of acetic acid, the reaction proceeded slowly to give a low yield of *p*-[bis(2-fluoroethyl)amino]cinnamic acid (IIa) of analytical purity. With the use of an equivalent amount of piperidine as catalyst, the condensation afforded much higher yields of IIa. No condensation of Ia with malonic acid took place under the milder conditions (room temperature, less piperidine) found suitable for *p*-[bis(2-chloroethyl)-amino]benzaldehyde.[7] The product, IIa, was apparently a mixture of *cis* and *trans* isomers that gave two spots on paper chromatograms. It was not converted to a single isomer by iodine in refluxing benzene and could not be separated by recrystallization. Hydrogenation of this mixture afforded the homogeneous hydrocinnamic acid Xa.

The cinnamic acid IIa was also prepared through the Wittig reaction by condensing Ia with triphenylcarbethoxymethylenephosphorane.[8] The cinnamate ester IIIa that resulted was hydrolyzed to afford a fair yield of IIa, also a mixture of isomers, but in somewhat different proportion than the previous one. The mixed chlorofluoro mustard, IIb, was readily obtained in high yields by condensation of *p*-[N-(2-chloroethyl)-N-(2-fluoroethyl)amino]benzaldehyde (Ib)[1a] with piperidine and malonic acid. The product IIb was homogeneous on paper chromatograms.

The *para*-substituted hydrocinnamic acid mustards Xa and Xb were readily prepared in excellent yields by hydrogenation of the bisfluoro- and chlorofluorocinnamic acids IIa and IIb with palladium-on-charcoal catalyst. The corresponding *meta*-substituted hydrocinnamic acids were prepared earlier[1a] in very low yields.

The bisfluoro XIa and chlorofluoro XIb analogs of chlorambucil were obtained by our general methods[1a] from methyl 4-(*p*-aminophenyl)butyrate (IV).[6] This was readily converted to the N-benzyloxycarbonyl derivative V, which was alkylated with 2-fluoroethyl

CHART I



IV, $R_1 = R_2 = H$
V, $R_1 = Z$; $R_2 = H$
VI, $R_1 = Z$; $R_2 = CH_2CH_2F$
VII, $R_1 = H$; $R_2 = CH_2CH_2F$
VIII, $R_1 = CH_2CH_2OH$; $R_2 = CH_2CH_2F$
IX, $R_1 = R_2 = CH_2CH_2OTs$

a, R = F
b, R = Cl
Z = $CO_2CH_2C_6H_5$
Ts = $O_2SC_6H_4CH_3$-*p*

(1)(a) Paper I: A. P. Martinez, W. W. Lee, and L. Goodman, *Tetrahedron*, **20**, 2763 (1964). (b) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH 43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

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TABLE I

PROPERTIES OF SOME 2-FLUOROETHYLAMINES AND INTERMEDIATES

Compd.	Yield,[a] %		M.p.,[a,b] °C.	R_f[c]	Formula	Calcd., %					Found, %				
						C	H	Cl	F	N	C	H	Cl	F	N
IIa	10[d]	(62)[d]	189–190 (185–186) (A)	0.22, 0.39 (A) 0.69, 0.83 (B) 0.52, 0.75 (C)	$C_{18}H_{18}F_2NO_2$	61.1	5.92		11.9	5.18	60.2	6.07		11.6	5.56
IIb		(91)	178.0–178.5 (173–174) (B)	0.20 (A) 0.78 (B)	$C_{18}H_{18}ClFNO_2$	57.4	5.56	13.1	6.99		57.2	5.62	12.9	7.12	
Xa	60	(90)	93.5–94.0 (91–92) (C)	0.27 (A) 0.81 (E)	$C_{18}H_{17}F_2NO_2$	60.7	6.67		14.8	5.44	60.5	6.43		14.5	5.43
Xb	60	(100)	83.5–84 (low)[e] (C)	0.30 (A) 0.84 (B)	$C_{18}H_{17}ClFNO_2$	57.0	6.26	13.0	6.24		56.8	6.07	13.3	7.07	
V		(75)	80.0–80.5 (77–78.5) (D)	0.22 (A) 0.91 (B)	$C_{19}H_{23}NO_4$	69.9	6.48				70.5	6.48			
VI		(88)	Oil	0.39 (A) 0.88 (B)											
VII·HCl	44	(71)	102.5–104 (low) (E)	0.29 (A)	$C_{13}H_{18}FNO_2 \cdot HCl$	56.7	6.95		6.90		56.5	6.84		7.04	
VIII		(92)	Oil	0.42 (A) 0.89 (B)											
XIb	32	(64)	49–50 (44–45) (F)	0.46 (A)	$C_{14}H_{19}ClFNO_2$	58.4	6.66	12.3	6.61		58.7	6.44	12.2	6.81	
IX		(87)	Oil	0.06 (A) 0.94 (B)											
XIa	16[f]		55–56 (F)	0.33 (A)	$C_{14}H_{19}F_2NO_2$	62.0	7.06		14.0	5.16	61.8	6.83		14.0	5.15
XII		(55)	189.5–190.0 (184–185) (G)		$C_8H_{16}FN \cdot HCl$	52.8	9.44		10.5		52.5	9.33		10.7	

[a] Melting points and yields are for the analytical samples. Corresponding values in parentheses are for less purified products of suitable purity for the next step. [b] Recrystallization solvents are indicated by letter after the melting point: A, methylene chloride–Skellysolve B; B, ethyl acetate–petroleum ether (b.p. 30–60°); C, ether–petroleum ether; D, methanol–water; E, ether with trace of methanol; F, Skellysolve B; G, ethanol. [c] The R_f values are given for the appropriate solvent systems listed in ref. 10. [d] Analytical sample obtained by method A (see Experimental Section); other sample by method B. [e] Low melting, but homogeneous by paper chromatography. [f] The yield is low because of the considerable amount of by-product which is completely removed only by several recrystallizations. When not completely free of by-product, IXa develops a reddish color rapidly.

p-toluenesulfonate to afford the oily fluoroethylamine VI. Removal of the benzyloxycarbonyl blocking group with HBr in glacial acetic acid gave oily methyl 4-[*p*-(2-fluoroethylamino)phenyl]butyrate (VII), characterized as the crystalline hydrochloride salt. Hydroxyethylation of VII to give VIII, followed by chlorination with phosphoryl chloride and then hydrolysis, afforded the chlorofluoro analog (XIb) of chlorambucil.

The bisfluoro analog (XIa) of chlorambucil was obtained in much poorer yield. Hydroxyethylation of methyl 4-(*p*-aminophenyl)butyrate, then tosylation afforded the bis(2-tosyloxyethyl)amine IX as a chromatographically homogeneous, but unanalyzed oil. Treatment with potassium fluoride[1a] afforded the ester of XIa and considerable amounts of a by-product, as indicated by paper chromatography. After hydrolysis, however, the desired XIa could be purified by crystallization. The experimental procedures used to prepare XIa and XIb have already been reported.[1a] The properties of XIa and XIb, together with those of other compounds prepared in this report are given in Table I.

In the course of preparing Ib, the hydrogenolysis of N-benzyloxycarbonyl-N-(2-fluoroethyl)aniline to N-(2-fluoroethyl)aniline has been a key step.[1a] In one experiment in which the hydrogenation pressure was inadvertently maintained at 4 atm. instead of 1 atm., the reaction product was N-(2-fluoroethyl)cyclohexylamine hydrochloride (XII).

Twenty-six of the 2-fluoroethylamines prepared here or previously[1a] were submitted to the Cancer Chemotherapy National Service Center (CCNSC) for antitumor screening. These compounds covered a wide range of carrier groups for the 2-fluoroethylamines. The screening results are given in Table II. For comparison, some data for several of the corresponding bischloro analogs are included at the bottom of Table II. The screening results are given in more detail for several compounds in Tables III and IV.

None of the fluoro mustards were active (by the criteria established by the CCNSC[9]) against any of the antitumor systems. This was quite different from the bischloro analogs, many of which were active against Walker 256 (see **27–30**, Table II), and some were also active against lymphoid leukemia L1210 and Sarcoma 180.

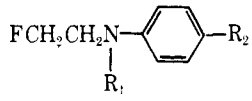
The data in Table II suggest that in most cases the toxicity of the mustards decreased in the order bisfluoro > chlorofluoro > bischloro, as in the case of the *m*-hydrocinnamic acid series (**15**, **14**, and **27**). However, this was not always the case; for the phenylalanine mustards it was reversed: bischloro > chlorofluoro ≅ bisfluoro (**29**, **20**, and **21**). The toxicity was not necessarily proportional to the amount, or percentage, of fluorine in the molecule. For example, the molecular weight of the azalactone (**7**) was about three times that of the cinnamic acid (**9**); however, the LD_{10} value of **7** was over 20 times higher.

Although the results in Table II show that the 2-fluoroethylamines are not active against any of the test systems investigated, this does not mean that the fluoro mustards are devoid of activity. This is illustrated in Tables III and IV. In Table III, the chlorofluoro mustard of phenylalanine caused disappearance of the tumors at doses of 12.5 and 6.25 mg./kg. in the Walker 256 tests. However, these dosages are close to the LD_{10} dosages so that the therapeutic index ($T.I.$) = $LD_{10}/ED_{90} \simeq 12.5/5 \simeq 2.5$. This is less than

(9) *Cancer Chemotherapy Rept.*, **25**, 1 (1962). A compound is active against Walker 256 if it has a therapeutic index $T.I. \geq 4$, where $T.I. = LD_{10}/ED_{90}$. A compound is confirmed active in (a) KB cell culture if the average $ED_{50} \leq 4$ γ/ml. for results from two laboratories, (b) Sarcoma 180 and Lewis lung carcinoma if the average $T/C \leq 42\%$ in three confirmation tests, and (c) lymphoid leukemia L1210 if $T/C \geq 125\%$ in a confirmation test.

TABLE II

ANTITUMOR SCREENING[a] RESULTS FOR SOME 2-FLUOROETHYLAMINES



No.	R_1[b]	R_2	Toxicity[c] LD_{10}, mg./kg.	Activity[d] in test systems[a] WA	KB	SA	LE	LL
1	H·HCl	H	7.9	−	−		−	−
2	Q	CHO	7.9	−	−	−	−	
3	P	CH=NC H_4Br-*p*	10	−	−	−		
4	Q	CH=N$C_6H_4NO_2$-*p*	3.8	−	−			
5	Q	CH=NC H_4OMe-*p*	3.8	−	−			
6	P	CH=CC(O)OC(C_6H_5)=N	42	−	−	−	−	
7	Q	CH=CC(O)OC(C_6H_5)=N	>100	−	−			
8	P	$CH=CHCO_2H$	14	−	−			
9	Q	$CH=CHCO_2H$	3.8	−				
10	P	$CH_2CH_2CO_2H$	14	−	−	−		−
11	Q	$CH_2CH_2CO_2H$	3.8	−				
12	H·HCl	*m*-$CH_2CH_2CO_2H$			−			
13	H·HCl	*m*-$CH_2CH_2CO_2Me$	3.8	−	−	−	−	−
14	P	*m*-$CH_2CH_2CO_2H$	7.9	−				
15	Q	*m*-$CH_2CH_2CO_2H$	3.8	−	−			
16	P	$(CH_2)_3CO_2H$	14	−	−	−	−	
17	Q	$(CH_2)_3CO_2H$	3.8	−	−			
18	H·HCl	$(CH_2)_3CO_2Me$	14	−	−	−	−	
19	$HOCH_2CH_2$	$(CH_2)_3CO_2Me$	>50	−	−			
20	P	$CH_2CH(NH_2)CO_2H$	12.5	−	−			
21	Q	$CH_2CH(NH_2)CO_2H$	14	−	−	−		
22	P	CH_2COCO_2H	14	−				
23	Q	CH_2COCO_2H	3.8	−	−			
24	P	$CH_2CH(NHCOC_6H_5)CO_2H$	14	−				
25	Q	$CH_2CH(NHCOC_6H_5)CO_2H$	17	−				
26	Compd. XII		22	−	−	−		
		Bischloro Analogs of Some of the Fluoro Mustards						
27	15		41, 100[e]	+			+	
28	17 (chlorambucil)		15.5[f]	+[f]				
29	21 (sarcolysin)		6.5[f]	+[f]			+[g]	
30	23		13	+	−	+	+	

[a] Screening was performed under the auspices of the Cancer Chemotherapy National Service Center according to its protocol.[9] The test systems included: WA, Walker 256 (subcutaneous) in rats, p. 11 of the protocol; KB, KB cell culture, p. 22; SA, Sarcoma 180 in mice, p. 1; LE, lymphoid leukemia L1210 in mice, p. 3; LL, Lewis lung carcinoma in mice, p. 5. [b] P = CH_2CH_2Cl, Q = CH_2CH_2F. [c] These LD_{10} values were determined in rats with no tumors as the prescribed preliminary step before the Walker 256 (WA) tests; see ref. 9. [d] The criteria for activity are given in ref. 9. A minus (−) indicates that the compound was inactive; a blank, that the compound was not tested. [e] Values from different laboratories. [f] From Table 22 (p. 63) of H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Rept.*, **17**, 1 (1962). [g] Table 14 (p. 51) of reference in footnote *f*.

4, the cut-off value for active compounds. In Table IV, *p*-[bis(2-fluoroethyl)amino]benzaldehyde at a dosage of 31 mg./kg. caused decrease in the tumor size in the Sarcoma 180 tests. However, this decrease was not sufficient for the compound to be considered active.

These screening results indicate that there is no advantage in substituting fluorine atoms for chlorine atoms in nitrogen mustards. This substitution generally results in increased toxicity and decreased activity.

Experimental Section[10]

***p*-[Bis(2-fluoroethyl)amino]cinnamic Acid (IIa). A.**—A mixture of 1.72 (8.07 mmoles) of *p*-[bis(2-fluoroethyl)amino]benzaldehyde[11a] (Ia), 0.84 g. (8.07 mmoles) of powdered malonic acid, and 1.7 g. of glacial acetic acid was stirred in a stoppered flask for 7 days at room temperature. The mixture was diluted with 25 ml. of toluene and evaporated to dryness at 50° (1 mm.), and the residue was partitioned between 50 ml. each of methylene chloride and water. The water was back-extracted with 50 ml. of methylene chloride. The combined organic extracts were dried, decolorized with charcoal, and concentrated to 25 ml. This was diluted with 10 ml. of Skellysolve B and cooled. The crystals were collected, washed with cold (−15°) methylene chloride, and dried to afford 0.35 g. (17%) of IIa, m.p. 185.5–186.5°. Recrystallization from ethanol afforded 0.20 g. (10%) of analytically pure (see Table II) IIa: m.p. 189.0–190.0°; λ_{max}^{Nujol} 3.6–4.3, 5.92 (CO_2H), 6.25, 6.55 μ (aryl and C=C).

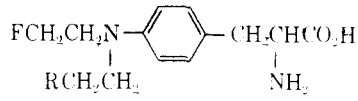
B.—A solution of 2.00 g. (9.4 mmoles) of the benzaldehyde Ia, 2.50 g. (24 mmoles) of malonic acid, and 1.0 ml. (10 mmoles) of piperidine in 25 ml. of dioxane[5] was heated at reflux for 3 hr. with protection from moisture. Evaporation *in vacuo* left a residue that was worked up as in A to give, from 25 ml. of methylene chloride, a yield of 1.50 g. (62%) of IIa, m.p. 185–186°, which moved as two spots in solvent B. Two recrystallizations from ethanol raised the melting point to 191.5–192°, but its infrared spectrum was unchanged and it still gave two spots in solvent B. Boiling this material in benzene containing a trace of iodine did not change the melting point, infrared spectrum, nor behavior in solvent B.

C.—A solution of 3.48 g. (10 mmoles) of triphenylcarbethoxymethylenephosphorane[8a] and 2.13 g. (10 mmoles) of the benz-

(10) Melting points were determined with the Fisher-Johns apparatus and were not corrected. Paper chromatography was done by the descending technique on Whatman No. 1 paper, except for solvent A which was run on Schleicher and Schuell No. 2496 acetylated paper. The solvent systems are: A, benzene–methanol–water (2:6:1); B, 1-butanol water (saturated); C, 5% aqueous Na_2HPO_4, pH 8.9.

TABLE III

WALKER 256 SCREENING DATA FOR PHENYLALANINE FLUORO MUSTARDS



R	Test systems[a]	Dose, mg./kg.	Survivors	Wt. change (T − C),[b] g.	Tumor wt. (T/C),[b] g.	% T/C[b]
Cl	AA	100	0/3			
		33.0	0/3	−19		
		10.0	3/3	−3		
		3.00	3/3	+1		
Cl	WA	12.5	6/6	−25	0.0/8.1	0
		6.25	6/6	−17	0.0/8.1	0
		3.12	6/6	−13	3.4/8.1	41
		1.56	6/6	−5	9.6/8.1	118
F	AA	100	1/3			
		33.0	0/3			
		10.0	3/3	0		
		3.00	3/3	6		
F	WA	14.0	6/6	−4	8.3/8.1	107
		7.00	6/6	−2	6.4/8.1	79
		3.50	6/6	−2	9.2/8.1	113
		1.70	6/6	−5	6.4/8.1	79

[a] AA, animals without tumors; WA, Walker 256. [b] T stands for test animals; C, for controls.

TABLE IV

SCREENING DATA FOR *p*-[BIS(2-FLUOROETHYL)AMINO]BENZALDEHYDE

Test system[a]	Dose, mg./kg.	Survivors	Wt. change (T − C),[b] g.	Tumor wt.[c] or survival (days) (T/C)[b]	% T/C[b]
SA	500	0/6			
	125	0/6			
	31.0	6/6	−14	720/1263	57
LE	25.0	0/6			
	6.00	6/6	−0.6	7.6/8.4	90
AA	50.0	0/3			
	10.0	2/3			
	3.00	3/3			
WA	7.90	0/6			
	3.95	6/6	6	7.3/6.6	110
	1.97	6/6	−5	5.4/6.6	81
	0.98	6/6	6	5.9/6.6	89
KB	slope = −0.20 and ED_{50} = 93 γ/ml.				

[a] SA, Sarcoma 180; LE, lymphoid leukemia L1210; AA, animals without tumors; WA, Walker 256; KB, KB cell culture. [b] T stands for test animals; C, for controls. [c] Tumor weight is in milligrams for SA and grams for WA.

aldehyde Ia in 50 ml. of benzene was heated at reflux for 6 hr. and then evaporated *in vacuo* to give a yellow oil (IIIa). This was heated with 50 ml. of concentrated HCl for 3 hr. on a steam bath and again evaporated. The residue was dissolved in 125 ml. of methylene chloride, washed with water, dried, and again evaporated. The residue was crystallized from 50 ml. of ethanol to afford 0.59 g. (23%) of IIa, m.p. 180–183°. It moved as two spots in solvent B with R_f 0.71 (+++) and 0.85 (++). Here the slower moving component was the major product in methods A and B, the faster one predominated.

***p*-[Bis(2-fluoroethyl)amino]hydrocinnamic Acid (Xa).**—A mixture of 0.10 g. of 5% palladium on charcoal, 25 ml. of ethanol, and 0.30 g. (1.2 mmoles) of the cinnamic acid IIa (prepared in B: 2 spots in solvent B) was hydrogenated at room temperature and 1 atm. The reaction was complete in 3 hr. The catalyst was removed and the solvent was evaporated to leave a residue. This was taken up in Skellysolve B and re-evaporated to leave 0.27 g. (90%) of Xa, homogeneous on paper chromatograms. Recrystallization of 0.15 g. from ether–petroleum ether (b.p. 30–60°) afforded 0.10 g. of Xa as shiny white plates: m.p. 93.5–94.0°; λ_{max}^{Nujol} 5.80 (CO_2H), 6.15, 6.52 μ (aryl).

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