DL-3-(3-Carboxy-4-ethoxyphenyl)alanine Hydrochloride.—Diethyl acetamido(3-carboxy-4-ethoxybenzyl)malonate (4 g, 0.01 mole) was refluxed with 25 ml of 1.2 *N* HCl for 19 hr. The reaction mixture was concentrated *in vacuo* and the crystalline residue was recrystallized from isopropyl alcohol; yield 2.02 g (69.0%).

DL-3-(3-Carboxy-2-methoxyphenyl)alanine Hydrochloride.—Diethyl acetamido(3-carbomethoxy-2-methoxybenzyl)malonate (7 g, 0.0177 mole) was hydrolyzed with 100 ml of 1.2 *N* HCl for 20 hr. Concentration of the reaction mixture *in vacuo* gave a crystalline residue which was recrystallized from a mixture of isopropyl and ethyl alcohol; yield 2.7 g (55%).

DL-3-(3-Carboxy-4-methoxy-*m*-tolyl)alanine Hydrate.—A mixture of 4.5 g (0.0138 mole) of formamido-(3-carboxy-4-methoxy-5-methylbenzyl)malonic acid and 50 ml of 50% ethanol was refluxed for 24 hr. The white crystalline compound which precipitated was filtered off, washed with water, and dissolved in 0.1 *N* $NaHCO_3$ solution. The bicarbonate solution was charcoaled, filtered, and acidified. The precipitate was filtered off, washed with water, and dried; yield 1.8 g (41%).

DL-3-(3-Carboxamido-N-formyl-4-methoxyphenyl)alanine.—Formamido(3-carboxamido-4-methoxybenzyl)malonic acid (6 g, 0.0193 mole) was refluxed for 7 hr with 90 ml of 1:1 ethanol–water. The mixture was concentrated *in vacuo* and gave a syrupy residue which crystallized while standing under ether at ice-bath temperature. It melted at 118–119° after recrystallization from ethanol; yield 4 g (84%).

Anal. Calcd for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.25; N, 10.52. Found: C, 54.43; H, 5.59; N, 9.92.

DL-3-(3-Carboxamido-4-methoxyphenyl)alanine Hydrochloride.—3-(3-Carboxamido-N-formyl-4-methoxyphenyl)alanine (4 g, 0.015 mole) was refluxed for 7 hr with 200 ml of 0.0995 *N* HCl. The reaction mixture was concentrated to dryness *in vacuo*. The oily residue crystallized under a mixture of ether–ethanol and was recrystallized from alcohol; yield 1.5 g (36.5%), mp 243–244°.

DL-3-(3-Carboxamido 4-methoxyphenyl)alanine.—Formamido-(3-carboxamido-4-methoxybenzyl)malonate (7 g, 0.022 mole) was refluxed for 26 hr in a mixture of 300 ml of 1:1 ethanol–water. The solvents were evaporated *in vacuo*, and the oily residue was taken up in the minimum amount of hot ethanol and allowed to crystallize in the refrigerator. The crystalline product was removed by filtration and recrystallized from ethanol; yield 3.5 g (66.6%), mp 269–270°.

DL-4-Carboxy-*m*-tyrosine Hydrate.—DL-3-(Carboxy-3-methoxyphenyl)alanine (3.0 g, 0.0125 mole) was refluxed with 30 ml of 48% HBr for 5 hr and allowed to cool overnight. The crystalline precipitate was filtered off and dissolved in water. The pH of the resulting solution was adjusted to 3.2 with 10% NaOH. The precipitate was filtered off, washed with water, and then dissolved in 0.1 *N* $NaHCO_3$. The bicarbonate solution was clarified (charcoal), acidified to pH 3.2, and filtered. The filter cake was washed well with water and dried; yield 1.55 g (51%).

Synthesis of Potential Antineoplastic Agents. XVII. N,N-Bis(2-fluoroethyl)anilines[1]

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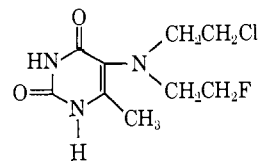
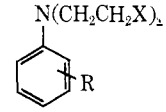
Prior to the report[2] of the clinical application of the drug ftorpan (I), essentially no work had been reported on the synthesis of 2-fluoroethylamines as potential antineoplastic agents. Since that time, however, a number of reports[3–5] of 2-fluoroethylamines have appeared.[6] In addition to the activity of I additional



I

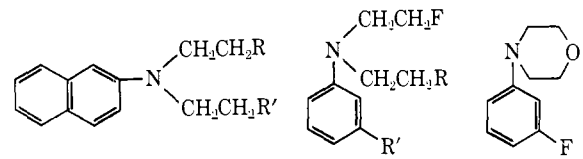


II, X = F
III, X = OH
IV, X = Cl
V, X = OTs

rationale for the synthesis of 2-fluoroethylamines has been presented.[5] We report here the synthesis and some screening results on a number of substituted N,N-bis(2-fluoroethyl)anilines (II).

In an initial approach to the synthesis of II it was found that N,N-bis(2-chloroethyl)aniline (IV, R = H) and N,N-bis(2-chloroethyl)-*m*-toluidine (IV, R = 3-CH_3) could be converted to the corresponding fluoroethylanilines (II) by refluxing with anhydrous potassium fluoride in methanol. However, attempts to extend the generality of this reaction to other chloroethylanilines (IV) failed.

As an alternative approach it was decided to attempt to replace the *p*-tolylsulfonyloxy grouping because of the ease with which they could be prepared and because several of the desired compounds had already been reported.[7] These tosylates (V) were prepared by reaction of III with *p*-toluenesulfonyl chloride in the presence of pyridine; the new compounds so prepared are included in Table I.



VI, R = R′ = OTs
VIII, R = F; R′ = OCH_3
IX, R = F; R′ = OC_2H_5

VII, R = OCH_3
XI, R = OCH_2-$CH_2OCH_2CH_2OH$

X

In initial examples it was found that the tosylate could be readily replaced by fluorine by refluxing V with excess anhydrous KF in methanol. This displacement was accomplished without difficulty to give VI (R = H, R = 3-CH_3, and R = 4-CH_3). These and other N,N-bis(2-fluoroethyl)anilines are included in Table II. However, when applied to V (R = 3-F and R = 3-NO_2) and to VI this method led to the isolation of a N-(2-fluoroethyl)-N-(2-methoxyethyl)aniline (VII and VIII, respectively). These and other related compounds are included in Table III. Use of absolute ethanol in place of methanol with VI led to IX. Several other attempted displacements in methanol gave oils from which pure products could not be separated. Treatment of N,N-bis(2 fluoroethyl)aniline (IX) or compounds of the type VII with refluxing methanol or

(1) (a) Part XVI: P. Schuyler, F. D. Popp, and A. C. Noble, *J. Med. Chem.*, **9**, 774 (1966). (b) Supported in part by research grants from the American Cancer Society and from the National Cancer Institute, U. S. Public Health Service.

(2) (a) L. P. Larinov, M. D. Chadakova, and E. I. Arkhangel'skaia, *Vopr. Onkol.*, **7**, 112 (1961); *Cancer Chemotherapy Abstr.*, **2**, 872 (1961); (b) see also H. Dubicki, F. Zielinski, and F. W. Starks, *J. Pharm. Sci.*, **53**, 1422 (1964).

(3) (a) A. P. Martinez, W. W. Lee, and L. Goodman, *Tetrahedron*, **20**, 2763 (1964); (b) A. P. Martinez, W. W. Lee, and L. Goodman, *J. Med. Chem.*, **8**, 741 (1965).

(4) (a) Z. B. Papanastassiou and R. J. Bruni, *J. Org. Chem.*, **29**, 2870 (1964); (b) Z. B. Papanastassiou, R. J. Bruni, F. P. Fernandes, and P. L. Levins, *J. Med. Chem.*, **9**, 357 (1966).

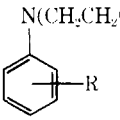
(5) G. R. Pettit and R. L. Smith, *Can. J. Chem.*, **42**, 572 (1964).

(6) Our work in this area began before these reports[3–5] appeared.

(7) G. M. Timmis, British Patent 662,645 (1951).

TABLE I

PREPARATION OF TOSYLATES FROM ALCOHOLS

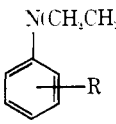


Substituent	Mp, °C[a]	Yield, %	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
3-Cl	86–87	71	55.00	5.00	2.67	55.07	5.03	2.74
3-NO_2	110–111[b]	86	53.91	4.90	5.24	53.46	4.57	5.58
2-OCH_3	93–96	10	57.78	5.63	2.70	58.00	5.50	2.80
4-$CO_2C_2H_5$	105–107	90	57.73	5.56	2.49	57.79	5.63	2.44
3-F	93–95	83	56.78	5.16	2.76	56.94	5.27	2.64
4-F	84–87	62	56.79	5.16	2.76	56.67	5.33	2.67
3-CH_3	80–83	95	59.62	5.81	2.78	59.90	5.65	3.19
4-CH_3	132–134	86	59.62	5.81	2.78	59.29	5.69	2.76
3-CH_3-4-CHO	117–119[c]	72[d]	58.74	5.50	2.64	58.64	5.36	2.52

[a] Recrystallized from methanol unless otherwise noted. [b] Recrystallized from benzene–hexane. [c] Recrystallized from ethanol. [d] Prepared by formylation of the 3-CH_3 compound as described by A. Cohen and R. S. Tipson, *J. Med. Chem.*, **6**, 822 (1963), for the preparation of the 4-CHO compound.

TABLE II

PREPARATION OF N,N-BIS(2-FLUOROETHYL)ANILINES FROM TOSYLATES

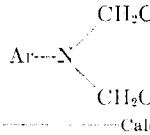


Substituents	Bp (mm) or mp, °C	Yield, %	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % F	Found, % C	Found, % H	Found, % N	Found, % F
H	131–133 (5.5)	81[a]	64.86	7.03	7.57	20.54	65.10	7.32	7.49	20.20
3-CH_3	105–110 (0.3)	70[a]	66.30	7.59	7.03	19.07	66.35	7.80	7.18	19.25
4-CH_3	86–89 (0.1–0.2)	82[a]	66.30	7.59	7.03	19.07	66.59	7.70	7.04	19.00
3-NO_2	50–54[b]	77[c]	52.17	5.25	12.17	16.51	52.16	5.34	11.87	15.96
4-F	88–92 (0.5)	33[c]	59.10	5.95	6.89	28.05	58.79	6.21	6.91	27.77
4-F·HCl salt	142–144[d]	e	50.11	5.46	5.84	23.78[f]	50.26	5.68	5.64	23.69
4-OCH_3	102–110 (0.2–0.5)	89[c]	61.38	7.02	6.51	17.65	61.27	7.03	6.48	17.74
4-OCH_3·HCl salt	148–150[d]	e	52.48	6.41	5.56	15.09[g]	52.41	6.51	5.41	15.24
3-F	92–95 (0.3)	30[c]	59.10	5.95	6.89	28.05	58.91	6.09	6.96	28.11
3-Cl	91–95 (0.2)	27[c]	54.66	5.50	6.38	17.30[h]	54.72	5.65	6.14	17.09
4-$CO_2C_2H_5$	54–55[i]	60[c]	60.68	6.66	5.45	14.77	60.54	6.71	5.38	14.55
4-Cl	52–54[i]	30[c]	54.66	5.50	6.38	17.30	54.56	5.41	6.27	17.12
4-CH=2,4-DNPH[k]	193–196[l]	e, m	51.90	4.36	17.80	9.66	51.94	4.49	17.75	9.84
3-CH_3-4-CH=2,4-DNPH[n]	148–150[o]	e	53.07	4.70	17.19	9.33	53.34	4.88	17.15	9.24

[a] Reaction in methanol. [b] Recrystallized from water. [c] Reaction in 2,2′-oxydiethanol. [d] Recrystallized from acetone. [e] Prepared from base. [f] *Anal.* Calcd: Cl, 14.79. Found: Cl, 14.86. [g] *Anal.* Calcd: Cl, 14.09. Found: Cl, 14.19. [h] *Anal.* Calcd: Cl, 16.14. Found: Cl, 16.21. [i] Recrystallized from ether. [j] Purified by chromatography. [k] 2,4-Dinitrophenylhydrazone of 4-CHO compound; 4-CHO reported by ref 3a. [l] Recrystallized from ethanol–DMF. [m] Base obtained[c] in 50% yield. [n] 2,4-Dinitrophenylhydrazone of 3-CH_3-4-CHO compound; obtained[c] as an oil in 50% yield. [o] Recrystallized from ethanol.

TABLE III

PRODUCTS OF SOLVENT INTERACTION IN THE PREPARATION OF N,N-BIS(2-FLUOROETHYL)ANILINES

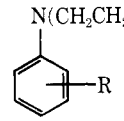


Compd[a]	Bp (mm) or mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Calcd, % F	Found, % C	Found, % H	Found, % N	Found, % F
XI, R′ = 3-CH_3	b	63.13	8.48	4.91	6.66	63.12	8.43	5.22	6.92
VIII	173–175 (2)	72.85	7.34	5.67	7.68	72.58	7.31	6.05	7.15
VII, R′ = 3-F	116–120 (4.5)	61.38	7.02	6.51	17.65	61.44	6.73	6.74	18.06
VII, R′ = 3-F·HCl salt	130–132[c]	52.50	6.32	5.58	15.12	52.43	6.34	5.46	15.23
VII, R′ = 3-NO_2	b	54.53	6.24	11.57	7.84	54.37	5.99	11.51	8.10
IX	143–144 (0.2)	73.53	7.72	5.36		73.65	7.59	5.39	
XI, R′ = 4-F	165 (0.18)	58.12	7.32	4.84	13.13	57.75	7.65	5.02	12.92

[a] For the R and Ar components, see the structural formulas in text. [b] Purified by chromatography; decomposed on attempted distillation. [c] Recrystallized from acetone.

TABLE IV

ANTINEOPLASTIC ACTION[a] OF



X	R	KB cell culture[b] ED50, μg/ml	KB cell culture[b] Slope	T/C (%)/dose (mg/kg) WA[c]	T/C (%)/dose (mg/kg) Other systems[d]
OTs	H	7.1×10^{-1}	−0.3	0/50[e]	SA 33/500, LE 122/50
				0/12.5[e]	FV 89/25, WC 66/16
				2/6.25[f]	
F	H	2.8×10^{1}	−0.6	97/20	DL 96/7.5
F	3-Cl	4.7×10^{1}	−0.6	118/1.5	
F	3-F	M1.0 × 10^{2}		117/2	
VII[h]	R′ = F	5.1×10^{1}	−0.4		
F	3-CH_3	5.5×10^{1}	−1.0	94/41	
F	3-NO_2			109/0.3	LE 112/20
OTs	4-$CO_2C_2H_5$	1.8×10^{1}	−0.5	88/100	
F	4-$CO_2C_2H_5$	M1.0 × 10^{2}		100/1.25	
F	4-Cl	7.2×10^{1}	−0.7	100/21	
OTs	4-F				LE 127/400
F	4-OCH_3	4.5×10^{1}	−0.7	59/1.2	
OTs	4-CH_3	L1.0 × 10^{4}		0/200[g]	
				8/100[g]	
				65/50	
F	4-CH_3	7.1×10^{1}	−0.7	86/8.5	
VIII[h]		3.1×10^{1}	−1.1		

[a] Data from CCNSC. [b] ED_{50} = dose that inhibits growth to 50% of control growth; slope = difference in result for a tenfold difference in dose. [c] Walker carcinosarcoma 256 (sc). [d] SA = Sarcoma 180, LE = L1210 lymphoid leukemia, FV = Friend virus leukemia, WC = Walker carcinosarcoma 256/cytoxan (sc), DL = Dunning leukemia (solid). [e] Six cures. [f] Four cures. [g] Three cures. [h] Refers to formulas in text.

with refluxing methanol containing trace amounts of sodium methoxide for extended periods did not lead to any displacement of fluorine.

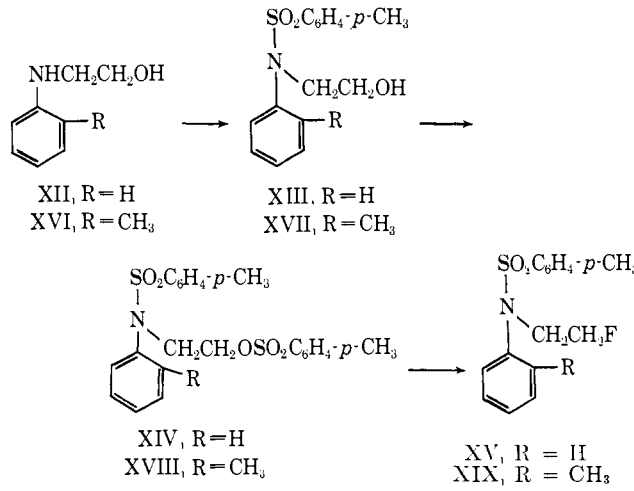
An attempt to use dimethylformamide (DMF) as a solvent for the reaction of V (R = 3-F) with KF gave rise to the morpholine X. Use of DMF as a solvent with V (R = 2-Cl) also gave a morpholine.

Use of 2,2′-oxydiethanol as the solvent for reaction of V with KF gave the desired product (II) when R was 3-F, 3-NO_2, 4-F, 4-OCH_3, 3-Cl, 4-$CO_2C_2H_5$, 4-Cl, 4-CHO, 3-CH_3-4-CHO, and 3-CH_3. In the case of V (R = 3-CH_3, 4-F, and 3-NO_2) some compound of the type XI was isolated in addition to II.

In an attempt to avoid the interaction of a hydroxylic solvent to lead to materials of the type VII and XI, the use of N-methylpyrrolidone as a solvent for the conversion of V to II was tried in several cases. In each case the yields of II were very much inferior to those obtained with the hydroxylic solvents.

Available screening results[8] are included in Table IV. None of the N,N-bis(2-fluoroethyl)anilines tested exhibited any appreciable antineoplastic activity although many were quite toxic. The few N,N-bis(2-tosylethyl)anilines screened exhibited activity as might be predicted.[7]

2-Anilinoethanol (XII) and the *o*-methyl compound (XVI) were subjected to a similar sequence of reactions. The reaction of XII and XVI with equimolar quantities of *p*-toluenesulfonyl chloride took place at the nitrogen to give XIII and XVII, respectively. Use of a 2:1 molar ratio of *p*-toluenesulfonyl chloride, however, led to the compounds XIV and XVIII. Treatment of XIV and XVIII with anhydrous KF in DMF caused



displacement of the *p*-tolylsulfonyloxy group to yield the 2-fluoroethyl compounds XV and XIX. Neither XV nor XIX exhibited any appreciable activity against KB cell culture[8] and XIX was completely inactive against Walker 256 carcinosarcoma while XV was only very slightly active (T/C = 77%) at 25 mg/kg against Walker 256 carcinosarcoma.[8]

Experimental Section[9]

N,N-bis(2-*p*-toluenesulfonoxyethyl)anilines (V).—To a chilled mixture of 0.025 mole of III[10] in 0.25 mole of pyridine was added, in small portions, 0.05 mole of *p*-toluenesulfonyl chloride. Con-

(8) We would like to thank the Cancer Chemotherapy National Service Center (CCNSC) for screening results.

(9) Analyses by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points were taken in capillaries and are corrected.

(10) These compounds were prepared by standard procedures from the anilines and ethylene oxide or were obtained commercially.

tinuous stirring was employed and the reaction was carried out in an ice–salt bath. The mixture was poured into 100 ml of ice and water with stirring. After standing overnight, the products were isolated as solids or as oils which crystallized on standing. Recrystallization gave the compounds listed in Table I. In addition to these compounds several known compounds[2] of this type were prepared by the same procedure. Alternately the pyridine was added slowly to a cooled mixture of the aniline and *p*-toluenesulfonyl chloride.

N,N-Bis(2-fluoroethyl)anilines (II).—A mixture of 0.01 mole of N,N-bis(2-*p*-toluenesulfonyloxyethyl)aniline (V) and 0.1 mole of anhydrous KF in approximately 50 ml of anhydrous solvent was heated on a steam bath with stirring for 4–24 hr. The reaction mixture was cooled and, after removal of any precipitated potassium tosylate, poured into 200 ml of ice water. After standing the product was obtained by filtration or extraction. The compounds reported in Table II were then obtained by recrystallization, distillation, or alumina chromatography. When the solvent used was methanol, ethanol, or 2,2′-oxydiethanol products of the type included in Table III were sometimes isolated. With DMF as solvent, V (R = 3-F) gave morpholine X, bp 100–105° (0.2 mm), in 66% yield. The hydrochloride of X had mp 166–167° from acetone–ether.

Anal. Calcd for $C_9H_{11}ClFNO$: C, 55.30; H, 5.98; N, 6.44; Cl, 16.32. Found: C, 55.25; H, 5.87; N, 6.55; Cl, 16.51.

Also with DMF as solvent and V (R = 2-Cl), a morpholine, bp 102–105° (0.3 mm), was obtained in 50% yield. This material solidified, mp 62–64°.

Anal. Calcd for $C_9H_{11}ClNO$: C, 60.70; H, 6.09; N, 7.11; Cl, 18.00. Found: C, 60.88; H, 5.94; N, 7.06; Cl, 17.75.

The hydrochloride had mp 134–136° (from acetone–ether).

Anal. Calcd for $C_9H_{13}Cl_2NO$: C, 51.30; H, 5.59; N, 5.98; Cl, 30.28. Found: C, 51.28; H, 5.62; N, 5.98; Cl, 30.19.

In addition to the procedure noted above II (R = H and R = 3-Cl) was also prepared as follows. A mixture of 21.8 g (0.1 mole) of N,N-bis(2-chloroethyl)aniline (IV) in 250 ml of absolute methanol and 58 g (1.0 mole) of anhydrous KF was refluxed with stirring for 8 hr. The mixture was cooled to room temperature and poured with stirring into cold water. The mixture was extracted with $CHCl_3$ and the dried extract was distilled to give a 77% yield of a product, identical with that prepared by the above procedure and included in Table II.

N-(*p*-Toluenesulfonyl)-N-(2-hydroxyethyl)aniline (XIII).—To 13.7 g (0.1 mole) of 2-anilinoethanol (XII) was added, in one batch with cooling, 20.95 g (0.11 mole) of *p*-toluenesulfonyl chloride. The mixture was stirred for 10–15 min. To the cold mixture was then added dropwise with stirring 53 ml of pyridine. The mixture was allowed to stir for 50–60 min in an ice bath and then poured with vigorous stirring into crushed ice. The thick paste obtained was dissolved in acetone and precipitated with anhydrous ether to give a quantitative yield, mp 71–73°.

Anal. Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81. Found: C, 62.01; H, 5.80; N, 4.95.

N-(*p*-Toluenesulfonyl)-N-(2-*p*-toluenesulfonyloxyethyl)aniline (XIV).—Use of 41.9 g (0.22 mole) of *p*-toluenesulfonyl chloride in the above sequence gave a solid which was recrystallized from methanol to give the product, mp 120–122°, in 90% yield. Recrystallization from methanol gave material, mp 125–126°.

Anal. Calcd for $C_{22}H_{23}NO_5S_2$: C, 59.30; H, 5.20; N, 3.14; S, 14.39. Found: C, 59.15; H, 5.00; N, 3.12; S, 14.55.

N-(2-Fluoroethyl)-N-(*p*-toluenesulfonyl)aniline (XV).—A mixture of 9.0 g (0.02 mole) of XIV and 5.8 g (0.1 mole) of anhydrous KF in 150 ml of DMF was refluxed for 48 hr. The mixture was filtered, cooled, and poured into cold water to give the product as a solid. The solid was dissolved in cold methanol and dilution with water gave the product, mp 73–74°, in 51% yield.

Anal. Calcd for $C_{15}H_{16}FNO_2S$: C, 61.41; H, 5.49; N, 4.77. Found: C, 61.42; H, 5.68; N, 4.94.

N-(*p*-Toluenesulfonyl)-N-(2-hydroxyethyl)-*o*-toluidine (XVII). Using XVI and an equimolar quantity of *p*-toluenesulfonyl chloride as described in the preparation of XIII, this compound was obtained in quantitative yield. Recrystallization from cyclohexane gave a solid, mp 77–79°.

Anal. Calcd for $C_{16}H_{19}NO_3S$: C, 62.92; H, 6.27; N, 4.59; S, 10.50. Found: C, 62.90; H, 6.32; N, 4.76; S, 10.60.

N-(2-Fluoroethyl)-N-(*p*-toluenesulfonyl)-*o*-toluidine (XIX). Use of XVI and *p*-toluenesulfonyl chloride in a 2:1 molar ratio as described for the preparation of XIV gave a quantitative yield of crude product which was then refluxed in methanol with anhydrous KF to give the product, mp 131–133° (from acetone–ether).

Anal. Calcd for $C_{16}H_{18}FNO_2S$: C, 62.54; H, 5.90; N, 4.56; F, 6.18. Found: C, 62.28; H, 5.65; N, 4.34; F, 5.94.

4-Hydroxy-2-butanone Thiosemicarbazone, a Potential Anticancer Agent

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The high antimycobacterial activity of *p*-acetaminobenzaldehyde thiosemicarbazone reported by Domagk[1] stimulated various workers to prepare numerous thiosemicarbazones as potential antimicrobial,[2,3] antiviral,[4] antifungal,[5] and anticancer agents.[6] For several years we have synthesized and studied the effects of a number of thiosemicarbazone derivatives as potential chemotherapeutic agents. The results have indicated that certain aliphatic thiosemicarbazones may possess anticancer activity *in vivo*.[6] This report describes the synthesis, purification, chemical and physical properties, and tests for acute toxicity of 4-hydroxy-2-butanone thiosemicarbazone as a potential anticancer agent against Lewis lung carcinoma in BDF1 mice. Studies with the compound reported herein have shown that it has an effect against this tumor.

Experimental Section

4-Hydroxy-2-butanone Thiosemicarbazone.—A hot solution of thiosemicarbazide (9.1 g, 0.1 mole) in distilled water (150 ml) was added to a mixture of 8.8 g (0.1 mole) of 4-hydroxy-2-butanone and 5 ml of glacial acetic acid in ethanol (100 ml) and the resulting mixture refluxed for 3 hr. After cooling, the insoluble condensation product was filtered, washed with water and petroleum ether (bp 30–60°), and dried. The product was purified by recrystallizing twice from 70% ethanol to give a 90% yield of shiny white crystals, mp 142–145°.

Anal. Calcd for $C_5H_{11}N_3OS$: C, 37.24; H, 6.88; N, 26.07. Found: C, 36.99; H, 6.80; N, 26.01.

Toxicity and Antitumor Studies. Acute toxicity studies were performed in the BDF1 strain of mice as maintained at the National Institutes of Health, Bethesda, Md., according to a procedure described previously.[6] This strain of mice was also used in the antitumor studies. All of the animals tolerated 500 mg/kg. The compound was tested for antitumor activity against four tumor systems, Sarcoma 180, Dunning ascites leukemia, Leukemia 1210, and Lewis lung carcinoma by screeners under contract to the Cancer Chemotherapy National Service Center. The testing procedures employed have been described previously.[7]

(1) G. Domagk, R. Behnisch, F. Mietzsch, and H. Schmidt, *Naturwissenschaften*, **33**, 315 (1946).

(2) J. Bernstein, H. L. Yale, K. Losee, M. Holsing, J. Martins, and W. A. Lott, *J. Am. Chem. Soc.*, **73**, 906 (1951).

(3) L. E. Weller, H. M. Sell, and R. Y. Horsfall, *ibid.*, **76**, 1950 (1954).

(4) D. Hamre, J. Bernstein, and R. Donovick, *Proc. Soc. Exptl. Biol. Med.*, **78**, 275 (1950).

(5) B. Prescott, C. P. Li, W. R. Piggott, W. B. Hill, and E. C. Martino, *Proc. 3rd Interna. Congr. Chemotherapy*, **2**, 1358 (1964).

(6) B. Prescott and C. P. Li, *J. Med. Chem.*, **7**, 383 (1964).

(7) J. Leiter, A. R. Rourke, S. A. Schepartz, and I. Wodinsky, *Cancer Res.*, **20**, 731 (1960).