N-(*p*-Nitroanilino)-N′-phenylformamidine.—An excess (15 ml.) of aniline was added to 2 g. of Ia and the mixture was heated in a steam bath for 3 hr. After cooling, a dark red precipitate was obtained; it was removed by filtration and washed with ethyl ether. The product, m.p. 206–206.5°, was obtained by crystallization from ethanol, yield 47%.

Anal. Calcd. for $C_{13}H_{12}N_4O_2$: C, 60.96; H, 4.72; N, 21.87. Found: C, 61.04; H, 4.91; N, 21.37.

1-Aryl-4-arylamino-1H-1,2,4-triazolium (4) Chlorides (IV).—A suspension of 5 g. of the arylhydrazine hydrochloride (freshly recrystallized) in 50 ml. of EOF was heated, while being stirred, by distilling the ethanol and the low-boiling fractions as they were forming. In the case of IVa and b it was not convenient to distill the formed ethanol completely. The reaction mixture was filtered at once and the residue was washed on the filter with boiling EOF, in order to remove some by-products. In the case of phenylhydrazine hydrochloride the by-product (about 0.5 g.) was identified as III, m.p. 198–198.5°, already described.[12] In the case of IVc the reaction was interrupted when 2 ml. of ethanol had distilled and reaction mixture was filtered in order to obtain the crude material. For IVd, the substance went into solution at the beginning of the reaction; as soon as a precipitate formed, the reaction mixture was filtered immediately. Compounds IV consist of crystalline colorless powders, slightly soluble in water and ethanol, practically insoluble in ether and acetone. Solvents of recrystallization are listed in Table I.

Alkaline Degradation Products of IVa and b.—To a saturated aqueous solution of IVa and b, respectively, an equal volume of dilute aqueous ammonia was added in small portions with stirring. A yellow-orange precipitate was formed, which was filtered at once, washed thoroughly with water, and dried *in vacuo*. The crude material was crystallized repeatedly from ethyl acetate. Compound VI (R = H) was obtained as a white crystalline powder, m.p. 163–164° dec.

Anal. Calcd. for $C_{14}H_{10}N_6O$: C, 66.13; H, 5.55; N, 22.03. Found: C, 66.20; H, 5.63; N, 21.79.

Under the same conditions, VI (R = 4-CH_3) was obtained as a white crystalline powder, m.p. 160–161° dec.

Anal. Calcd. for $C_{16}H_{18}N_4O$: C, 68.06; H, 6.43; N, 19.84. Found: C, 67.99; H, 6.59; N, 19.99.

By oxidative hydrolysis of compounds VI (R = H and 4-CH_3) the corresponding formazans VII (R = H and 4-CH_3) were obtained as follows: 1 ml. of 33% hydrogen peroxide was added drop by drop with stirring to 300 mg. of VI suspended in 5 ml. of 2 *N* NaOH. The suspension was allowed to react while being stirred for 15–20 hr. A red mass was formed, which was filtered and crystallized.[5] The identity of these compounds was proved by comparison of their ultraviolet spectra with those of the authentic samples.

Hydrogenolysis of IVa and b.—A solution of 5 g. of IVa and b, respectively, in 500 ml. of ethanol was hydrogenated with 10% palladium on carbon (100 mg.) in a low-pressure apparatus. In 5–10 hr. the theoretical amount of hydrogen was absorbed. The completion of the reaction was indicated by the fact that a sample of the solution, treated with 2 *N* NaOH, no longer gave a red color. The reaction mixture was filtered to remove the catalyst, and the ethanol was evaporated to dryness. In the case of IVa the residual oil was taken up in a mixture of ethyl ether and water (1:1). From the aqueous layer, by evaporating to dryness, aniline hydrochloride was recovered. Removing the solvent from the ether layer, an oil was obtained. The picrate of this substance (lemon yellow crystals, m.p. 160–160.5°, from water) was compared with an authentic specimen obtained from 1-phenyl-1H-1,2,4-triazole (V, R = H), prepared according to the literature.[13] The two picrates were found to give identical infrared spectra.

Anal. Calcd. for $C_8H_7N_3 \cdot C_6H_3N_3O_7$: C, 44.94; H, 2.69; N, 22.46. Found: C, 44.83; H, 2.60; N, 22.62.

The hydrochloride of V (R = H), m.p. 179–179.5° (subl.), was also prepared.

Anal. Calcd. for $C_8H_5ClN_3$: C, 52.90; H, 4.44. Found: C, 53.03; H, 4.54.

In the case of IVb, the residue was solid and was taken up in 200 ml. of water; the suspension so obtained was stirred and filtered. The residue was crystallized from diluted methanol (1:3), giving white needles, m.p. 67–67.5°. Compared as above with an authentic sample,[6] the compound proved to be the 1-*p*-tolyl-1H-1,2,4-triazole (V, R = 4-CH_3).

Anal. Calcd. for $C_9H_9N_3$: C, 67.94; H, 5.70; N, 26.41. Found: C, 67.81; H, 5.60; N, 26.51.

By evaporating the filtrate to dryness, *p*-toluidine hydrochloride was obtained.

Acknowledgment.—The authors are indebted to Professor V. Palma (The "Mario Negri" Institute of Pharmacological Research, Milan) for his cooperation and for making the screening data available.

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(12) W. Baker, W. D. Ollis, and V. D. Poole, *J. Chem. Soc.*, 3389 (1950).

(13) G. Pellizzari, *Gazz. chim. ital.*, **24**, 222 (1894).

Fluorine-Containing Potential Anticancer Agents. III.[1a] Syntheses of Some Trifluoromethylpyrazolo[3,4-*d*]pyrimidines[1b]

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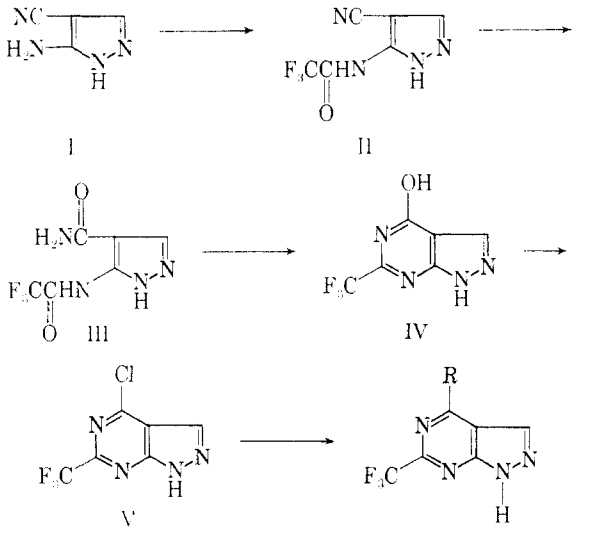
The purpose of this work was to prepare derivatives of 6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine as part of a general program in these laboratories to synthesize potential anticancer agents containing the fluoro and trifluoromethyl groups. Prior to this work fluorine-containing derivatives of the pyrazolo[3,4-*d*]-pyrimidine ring system had not been reported.

The method of synthesis outlined in Scheme I is analogous to the route used by Cheng and Robins[2]

SCHEME I

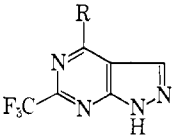


VI, R = NH_2
VII, R = $NHCH_3$
VIII, R = NHC_2H_5
IX, R = NHC_4H_9
X, R = $NHCH_2C_6H_5$
XI, R = SH

(1) (a) Paper II of this series: H. Nagano, S. Inoue, A. J. Saggiomo, and E. A. Nodiff, *J. Med. Chem.*, **7**, 215 (1964). (b) This investigation was supported by Research Grant CY-4270, from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. (c) Reprint requests should be addressed to this author.

(2) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 191 (1958).

TABLE I
4-SUBSTITUTED DERIVATIVES OF 6-TRIFLUOROMETHYLPYRAZOLO[3,4-*d*]PYRIMIDINE



Compd. no.	R	M.p., °C.	Recrystn. solvent	Yield, %	Formula	% calcd. C	% calcd. H	% calcd. N	% found C	% found H	% found N
IV	OH	306–308	Water	80	$C_6H_3F_3N_4O$	35.30	1.48	27.45	35.75	1.41	26.76
V[a]	Cl	133–135	Benzene–ligroin	80	$C_6H_2ClF_3N_4$	32.38	0.90		32.50	1.18	
VI	NH_2	320	Ethanol	61	$C_6H_4F_3N_5$	35.47	1.98		35.81	2.16	
VII[b]	$NHCH_3$	316–317	Ethanol	94	$C_7H_6F_3N_5$	38.71	2.78	32.25	38.86	2.94	32.10
VIII	NHC_2H_5	295–296	Ethanol	91	$C_8H_8F_3N_5$	41.55	3.48	30.29	41.61	3.31	30.10
IX	NHC_4H_9	230–231	Methanol–water	97	$C_{10}H_{12}F_3N_5$	46.32	4.66		46.66	4.75	
X[c]	$NHCH_2C_6H_5$	215–216	Methanol–water	90	$C_{13}H_{10}F_3N_5$	53.24	3.43	23.88	53.36	3.46	24.04
XI[d]	SH	258–262	Methanol–water	73	$C_6H_3F_3N_4S$	32.73	1.37		33.19	1.69	

[a] Calcd.: Cl, 15.93. Found: Cl, 16.27. [b] Calcd.: F, 26.29. Found: F, 26.55. [c] Calcd.: F, 19.42. Found: F, 19.30. [d] Calcd.: S, 14.55. Found: S, 14.33.

TABLE II
ANTITUMOR SCREENING DATA

Compd. no.	Test system	Daily dose, mg./kg.[b]	Survivors	Test/control	Test/control, %	ED_{50},[a] γ/ml.
IV	SA-180	100	6/6	663/916	72	
	CA-755	100	10/10	1118/1525	77	
	LE-1210	29	6/6	8.8/8.6	102	
		45	6/6	9.2/8.6	106	
		67	6/6	9.3/8.6	108	
		100	6/6	8.0/8.6	93	
V	SA-180	55	5/6	1148/1288	89	
		55	5/6	304/739	41	
		110	3/6	975/1642		
	LE-1210	44	6/6	8.6/8.2	104	
		66	6/6	8.5/8.2	103	
		100	4/6	8.2/8.2	100	
VI	LE-1210	44	6/6	8.0/9.0		
		44	6/6	9.0/7.6		
		66	6/6	8.1/7.6		
		66	5/6	8.3/9.0		
		100	6/6	7.8/9.0		
		100	6/6	7.8/7.6		
VII	SA-180	125	6/6	1147/886	129	
	CA-755	100	9/10	1361/1486	91	
	LE-1210	100	6/6	7.8/8.1	96	
	KB (cell culture)	w				1.0×10^2
VIII[c]	KB (cell culture)	w				3.9×10^1
X[d]	SA-180	125	6/6	840/886	94	
	CA-755	100	6/10	2134/1486		
		50	10/10	1706/1502	113	
	LE-1210	100	6/6	8.0/8.1	98	
	KB (cell culture)	w				5.1×10^0
XI	SA-180	110	6/6	1677/1642	102	
	LE-1210	44	4/6	8.2/8.2	100	
		66	6/6	7.5/8.2	91	
		100	5/6	7.8/8.2	95	

[a] ED_{50}: the dose that inhibits growth to 50% of control growth. [b] w = materials tested by weight. [c] This compound has a slope (change of response for each 1-log change of dose) of −0.75. [d] This compound has a slope of −0.52.

for the preparation of 6-alkyl-4-substituted pyrazolo[3,4-*d*]pyrimidines. 5-Amino-4-cyanopyrazole[3] (I) was acylated by trifluoroacetic anhydride to give the corresponding 4-cyano-5-trifluoroacetamidopyrazole (II). Cheng and Robins found that the 5-acylamino-4-cyanopyrazoles gave the desired 6-alkyl-4-hydroxypyrazolo[3,4-*d*]pyrimidines directly when treated with hydrogen peroxide in alkaline solution at 70–80°. They were unable, however, to isolate the probable intermediate, 5-acylaminopyrazole-4-carboxamide, during the cyclization process. In contrast, when alkaline peroxide was employed at 10–15° with the trifluoroacetamido derivative (II), only the cyclization intermediate, 5-trifluoroacetamido-4-pyrazolecarboxamide (III), was obtained. Subsequently, thermal ring closure of III provided 4-hydroxy-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (IV).

Chlorination of IV with phosphorus oxychloride in dimethylaniline afforded 4-chloro-6-trifluoromethylpy-

(3) R. K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).

razolo[3,4-*d*]pyrimidine (V). The reaction of V with ammonia and various primary amines provided the corresponding 4-amino (VI) and 4-alkylamino (VII–X) derivatives, respectively. The 4-mercapto derivative (XI) was synthesized from V and thiourea. The physical properties and chemical analyses of these compounds appear in Table I.

Screening by the Cancer Chemotherapy National Service Center has revealed no significant antineoplastic activity in this group thus far. A summary of this test data is presented in Table II.

Experimental[4]

4-Cyano-5-trifluoroacetamidopyrazole (II).—To 300 ml. of cooled trifluoroacetic anhydride was added in portions with stirring 5-amino-4-cyanopyrazole[3] (42 g., 0.39 mole). The mixture was heated for a short time at 40° and was then poured onto flaked ice. Crystallization of the resulting solid from water provided II (73 g., 91%) as colorless needles, m.p. 204–205°.

Anal. Calcd. for $C_6H_3F_3N_4O$: C, 35.30; H, 1.48. Found: C, 36.09; H, 1.68.

5-Trifluoroacetamido-4-pyrazolecarboxamide (III).—To 255 ml. of 10% potassium hydroxide solution and 550 ml. of 3% hydrogen peroxide at 10–15° was added with stirring 73 g. (0.36 mole) of II. The yellow solution was kept at 10–15° for 2 hr. and was then acidified with glacial acetic acid. A recrystallization of the precipitate from water afforded III (67 g., 84%) as colorless prisms, m.p. 221°.

Anal. Calcd. for $C_6H_5F_3N_4O_2$: C, 32.44; H, 2.27; N, 25.22. Found: C, 32.70; H, 2.13; N, 24.49.

4-Hydroxy-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (IV).—The carboxamide III (32 g., 0.14 mole) was heated at 210–260° for 0.5 hr. The product was extracted with hot methanol and the extract was decolorized with carbon. The concentrated filtrate slowly deposited IV as pale green prisms.

4-Chloro-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (V).—A mixture of IV (5.6 g., 0.027 mole) and phosphorus oxychloride (25 ml.) in N,N-dimethylaniline (5.6 ml.) was heated at reflux for 2 hr. The excess phosphorus oxychloride was removed by distillation under reduced pressure and the residue was poured onto crushed ice. The mixture was extracted with ether which was then removed by distillation. Recrystallization of the ether residue afforded V as colorless needles.

4-Amino-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (VI).—A solution of V (1 g., 0.0045 mole) and ammonia (3 g.) in 25 ml. of ethanol was heated in a stainless steel reactor at 100° for 3 hr. The solvent was removed under reduced pressure and the residue was washed with water. Crystallization gave VI as colorless needles.

4-Alkylamino-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidines (VII–X).—To a solution of 4-chloro-6-trifluoromethylpyrazolo-[3,4-*d*]pyrimidine (V) (2.5 g., 0.011 mole) in methanol (20 ml.) was added a 30% solution of methylamine (2.5 g., 0.024 mole) in methanol (20 ml.). The mixture was heated at reflux for 3 hr. The crystals that separated were collected and washed with water. Recrystallization from ethanol gave 4-methylamino-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (VI) as white crystals.

The other 4-alkylamino derivatives listed in Table I were prepared from the appropriate amines by essentially the same method.

4-Mercapto-6-trifluoromethylpyrazolo[3,4-*d*]pyrimidine (XI).—A mixture of the 4-chloro derivative V (3 g., 0.013 mole) and thiourea (1.2 g., 0.016 mole) in methanol (100 ml.) was heated at reflux for 3 hr. The solvent was removed under reduced pressure. The residue was then triturated with a small amount of water. The product was precipitated from a sodium hydroxide solution with acetic acid. The mixture was extracted with ether and the ethereal extracts were dried over anhydrous sodium sulfate. After the removal of the ether under reduced pressure, a recrystallization of the residue provided XI as yellow needles.

(4) All melting points were determined in a Thiele-Dennis apparatus. Much of this work was completed in 1961. The samples and melting point apparatus used at that time were not available for melting point correction at the submission date of this manuscript. Elemental analyses were conducted by Schwarzkopf Microanalytical Laboratory.

Acknowledgment.—The authors gratefully acknowledge the technical assistance of Mrs. Joanne Rosenbloom.

TABLE I: AMINOSTYRYLQUINOLINES

Position of Amino groups on styryl ring in 4-styryl-quinoline	M.p., °C.[a]	Yield, %	Formula	—Calcd., %— C	H	—Found,[b] %— C	H	Tumor wt., T/C[c]	Dose, mg./kg.	Killed	Dose, mg./kg.	ED_{90},[d] g./pt.
4-Aminostyryl group												
2-	181–182.5	53	$C_{17}H_{14}N_2$	82.90	5.73	82.79	5.64	1.2	250	0/3	250	20
3-	*e*							1	50	3/3	500	22
2- and 4-	196.5–197.0	20	$C_{17}H_{14}N_2$	78.14	5.78	78.02	5.65	0.07	250	2/6	250	11
4-	*f*							0	100	2/3	200	4
								0.12	75	1/3	150	
4-Aminostyryl group												
6-	*g*							1	15	2/3	50	24
7-	208–209.5	51	$C_{17}H_{14}N_2$	82.90	5.73	82.80	5.73	1	20	1/3	50	25
8-								1	15	1/3	50	120

[a] Determined by use of Thiele tube. [b] Analyses by Weiler and Strauss, Oxford, England. [c] We are grateful to Professor A. Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor-bearing animals were sacrificed approximately 8 days later, and the average weights of tumors in treated and control hosts are reported as the ratio T/C. [d] Results of the standard KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at University of Miami Cell Culture Laboratory and Southern Research Institute. [e] H. Koenigs, *Ber.*, **21**, 2189 (1889). [f] See ref. 2. [g] D. M. Brown and G. A. R. Kon, *J. Chem. Soc.*, 2147 (1948).

Aminostyrylquinolines[1]

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Styrylquinolines effective against Walker 256 tumor have had an amino group on the 4-position of the

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