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-styrylquinoline	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./ml.
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	29	$C_{18}H_{16}N_2$	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 C. A. R. Kon, *J. Chem. Soc.*, 2147 (1948).

Aminostyrylquinolines[1]

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Received July 17, 1964

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

(1) This work was supported in part by grants from the American Cancer Society and Public Health Service Research Grants CA 03717-01-7 from the National Cancer Institute.

styryl ring, and the styryl group has been attached at the 4- or sometimes the 2-position on the quinoline ring.[2]

We have prepared isomers of the active 4-(4-aminostyryl)quinoline by changing the position of the attachment of the aminostyryl group on the quinoline or changing the position of the amino group on the styryl ring. All of the compounds listed in Table I were prepared by reduction of the corresponding nitro compounds with stannous chloride in concentrated hydrochloric acid at 80–110°, and were recrystallized repeatedly before analysis. The three compounds having a 4-aminostyryl group on the benzene ring of the quinoline appear to be more toxic than the others in rats, but not in KB cell cultures, and did not show superior antitumor activity against the Walker 256 tumor. The two compounds containing a 4-amino group on a styryl group attached at the 4-position on the pyridine ring were most effective in cell culture inhibition and strongly inhibited growth of the Walker tumor.

(2) C. T. Bahner, C. Cook, J. Dale, J. Fain, F. Hannan, P. Smith, and J. Wilson, *J. Org. Chem.*, **23**, 1060 (1958).

6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione. A Potential Urinary Tract Antibacterial

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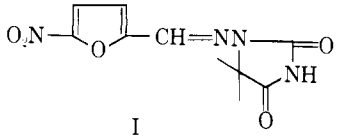
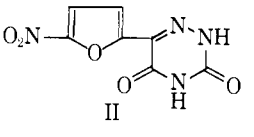
Received July 2, 1964

The significant utility of nitrofurantoin[1] for the control of bacterial infections of the urinary tract[2] has inspired the search for other nitrofuran structures for this application. The criteria considered significant include: resistance to metabolic degradation as evidenced by a high level of renal excretion, an adequate antibacterial spectrum, an adequate therapeutic index, and low incidence of emesis.

Incorporation of the hydrolytically vulnerable azomethine linkage of nitrofurantoin (I) in an imidic 1,2,4-triazine seemed likely to aid resistance to metabolic degradation and yet permit high kidney clearance. 6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione (II), fitting these tentative requirements, was prepared and evaluated.



I



II

2-Furoylformic acid semicarbazone was treated with sodium alkoxide in propylene glycol to yield 6-(2-furyl)-*as*-triazine-3,5(2H,4H)-dione. This furan derivative was nitrated in acetic anhydride to give II.

Some biological observations made on II are presented in Tables I–III in comparison with nitrofurantoin. These data indicate II to be a significant candidate for further investigation as a urinary tract antibacterial agent.

TABLE I

SENSITIVITY OF BACTERIA ISOLATED FROM URINARY TRACT INFECTIONS TO NITROFURANTOIN SODIUM (I) AND II[a]

Bacterial species	I: No. sensitive / No. tested	I: Limits of zones[b]	II: No. sensitive / No. tested	II: Limits of zones[b]
Escherichia coli	26/26	9–24	25/26	0–22
Proteus sp.	1/14	0–10	0/14	0
Aerobacter	12/15	0–15	11/15	0–14
Pseudomonas sp.	0/16	0	1/10	0–9
Alcaligenes faecalis	1/2	0–11	1/2	0–12
Staphylococcus aureus	6/6	17–22	6/6	13–23
Streptococcus (group D)	7/8	0–21	8/8	14–24

[a] Bacteriological data supplied by Dr. J. O'Connor. [b] Impregnated paper disks (30 γ). Zone diameters in mm. include the 6-mm. disk, except negative reactions are recorded as 0. Averages of 6 determinations.

TABLE II

URINARY EXCRETION OF I AND II[a]

Animal	Dose, mg./kg.	% excreted in urine: I		II	
Mouse	10	22.3[b]	(25.3)[c]	21.3[b]	(23.0)[c]
Rat	10	44.7	(42.3)	50.2	(42.8)
Monkey	10	16.0	(16.3)	18.3	(21.7)

[a] These data supplied by Dr. R. Bender. [b] Per cent of oral dose as determined by antibacterial assay; 24-hr. urine collection. [c] Per cent of oral dose as determined by ultraviolet spectroscopy; 24-hr. urine collection. Ultraviolet curves of excreted urine resemble that for drugs administered.

TABLE III

ACUTE TOXICITY[a]

Animal	I	II
	Oral median lethal dose, mg./kg.	
Mouse	605	940
Rat	981	950
	Median emetic dose, mg./kg.	
Dog	25	100

[a] Toxicological data supplied by Dr. A. R. Borgmann.

Experimental

2-Furoylformic Acid Semicarbazone.—2-Furoyl cyanide[3] (44 g.) was hydrolyzed with concentrated hydrochloric acid to 2-furoylformic acid by the method of Fischer.[3] The crude acid was dissolved in 50 ml. of ethanol and added to a solution of 44 g. of semicarbazide hydrochloride in 450 ml. of water. The solid semicarbazone was filtered, washed with water, alcohol, and ether. The crude product (40.5 g.) was purified by solution in a mixture of 1500 ml. of water and 100 ml. of concentrated ammonium hydroxide and treating with charcoal. Acidification of the filtered solution gave white, felted needles which were filtered and dried at 110°; yield 33 g. (46%), m.p. 174° dec. (Fisher–Johns, corrected). An analytical sample was recrystallized from 50% aqueous 2-propanol.

Anal. Calcd. for $C_7H_7N_3O_4$: C, 42.64; H, 3.58; N, 21.32. Found: C, 42.79, 42.68; H, 3.69, 3.67; N, 21.12, 21.25.

6-(2-Furyl)-*as*-triazine-3,5(2H,4H)-dione.—2-Furoylformic acid semicarbazone (20 g.) in 450 ml. of propylene glycol was mixed with a sodium ethoxide solution prepared from 7.5 g. of sodium in 150 ml. of absolute ethanol and refluxed for 24 hr.

(1) K. Hayes, U. S. Patent 2,610,181 (1952).

(2) (a) C. Norfleet, Jr., P. Beamer, and H. Carpenter, Transactions, Southeast Section of the American Urological Association, Boca Raton, Fla., 1952, p. 26; (b) S. Mintzer, E. Kadison, W. Shlaes, and O. Felsenfeld, *Antibiot. Chemotherapy*, **3**, 151 (1953); (c) B. Waisbren, *A.M.A. Arch. Internal Med.*, **101**, 397 (1958).

(3) (a) E. Fischer and F. Brauns, *Ber.*, **46**, 892 (1913). (b) To avoid the use of HCN, 2-furoyl cyanide can be conveniently prepared in 48% yield from the acid chloride and cuprous cyanide by the procedure of T. Oakwood and C. Weisgerber for benzoyl cyanide ("Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 112).