9-carbethoxy derivatives of 2-amino-6-chloropurine and of 2-amino-6-methylthiopurine lack significant activity toward L1210 lymphoid leukemia or Walker carcinosarcoma 256. The ethyl 6-chloropurine-9-carboxylate showed some inhibition of L1210 and of Walker carcinosarcoma 256; the substance was inactive toward KB cell culture. None of these compounds was as active an anticancer agent as 2-aminopurine-6-thione,[4] 2-amino-6-chloropurine,[5] or 6-chloropurine.[19]

Experimental Section[20]

Procedure A. Ethyl 2-Amino-6-chloropurine-9-carboxylate (5).—To a stirred solution of 2-amino-6-chloropurine (8.0 g, 48 mmoles) and NaOH (4.0 g, 100 mmoles) in 300 ml of H_2O was added ethyl chloroformate (10.8 g, 100 mmoles). The mixture was stirred for 1 hr, the pH was adjusted to 5 with glacial HOAc, and the precipitate was filtered and dried *in vacuo* to yield 10.3 g (93%) of product; nmr (DMSO-d_6), δ 1.41 (t, 3), 4.52 (q, 2 H), 8.49 (s, 1).

Procedure B. Ethyl 2-Amino-6-selenopurine-9-carboxylate (7).—To a refluxing solution of selenourea (0.102 g, 0.83 mmole) in 20 ml of anhydrous EtOH was added in one portion ethyl 2-amino-6-chloropurine-9-carboxylate (0.2 g, 0.83 mmole). The solution turned yellow and a precipitate appeared in 15–20 min. The solution was refluxed for 45 min more and cooled to room temperature, and the precipitate was filtered, washed with EtOH, and dried *in vacuo* to yield 0.11 g (47%) of analytically pure product.

Ethyl 2-Acetamido-6-benzylthiopurine-9-carboxylate (4) by Acetylation of 2.—A solution of 2 (0.14 g, 0.43 mmole) and Ac_2O (1 ml) in 4 ml of dry toluene was heated under reflux for 1.5 hr. Upon cooling and scratching, a precipitate of colorless crystals deposited which was washed with a small amount of cold Et_2O and dried *in vacuo* to yield 0.09 g (57%) of product: nmr (DMSO-d_6), δ 1.33 (t, 3), 2.4 (s, 3), 4.55 (q, 2), 4.75 (s, 2), 7.42 (m, 5), 8.65 (s, 1). A mixture melting point with **4** obtained by acylation of 2-acetamido-6-benzylthiopurine (**3**) with ethyl chloroformate by procedure A showed no depression and their ir spectra were superimposable.

Independent Synthesis of Ethyl 2-Amino-6-benzylthiopurine-9-carboxylate (2) by Alkylation of 2-(2-Amino-9-carbethoxypurin-6-yl)-2-thiopseudourea Hydrochloride (6).—Benzyl bromide (0.171 g, 1.00 mmole) was added to a stirred solution of **6** (0.317 g, 1.00 mmole) and Et_3N (0.202 g, 2.00 mmoles) in 10 ml of anhydrous DMF. The solution was stirred for 3.5 hr and poured into 50 ml of ice water and the pH was adjusted to 7 with glacial HOAc. The precipitate was filtered and dried *in vacuo* to yield 0.17 g (52%) of product. After recrystallization from EtOH, a mixture melting point with **2** prepared by procedure A was undepressed and their ir spectra were superimposable; nmr (DMSO-d_6), δ 1.42 (t, 3), 4.48 (q, 2), 4.60 (s, 2), 7.40 (m, 5), 8.35 (s, 1).

(19) F. M. Schabel, Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster, Jr., and J. R. Thomson, *Cancer Res.*, **21**, 690 (1961).

(20) Melting points, determined on a Fisher-Johns apparatus, were corrected. Uv spectra were obtained on a Perkin-Elmer 202 spectrophotometer and nmr spectra on a Varian A-60-A instrument.

The Hepatocarcinogenicity of Some Disubstituted 4-Dimethylaminoazobenzenes

ELLIS V. BROWN

Chemistry Department, University of Kentucky, Lexington, Kentucky 40506

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Many of the disubstituted dimethylaminoazobenzenes (DAB) (Table I) have been tested for rat hepatocarcinogenic activity.[1,2] With the exception of the fluoro derivatives none of the methyl- or halogen-substituted compounds has been more active than DAB itself. In fact preliminary work indicated that disubstituted compounds with the exception of F_2DAB have zero activity on the Miller scale.[1] Later work[2] showed mild carcinogenic activity for 3′,4′-Me_2DAB and we have since verified this activity. We have now shown that 2′,3′-Me_2DAB is extremely active. Since 4′-EtDAB shows greater activity than DAB itself,[2-4] we have extended our work to a related disubstituted compound, 3′,4′-Et_2DAB, and it has been found to be fairly active. Neither 2′,3′-Cl_2 nor 3′,4′-Cl_2DAB was found to have any activity under our testing conditions.

TABLE I: SUBSTITUTED 4-DIMETHYLAMINOAZOBENZENES

Compd	Mp, °C	Yield, crude %	Formula	Analyses
2′,3′-Me_2DAB	120–121	70	$C_{16}H_{19}N_3$	C, H, N
3′,4′-Me_2DAB	Ref 2			
3′,4-Et_2DAB[a]	82–83	25	$C_{18}H_{23}N_3$	C, H, N
2′,3′-Cl_2DAB	218–220	35	$C_{14}H_{13}Cl_2N_3$	C, H, N
3′,4-Cl_2DAB	159–160	55	$C_{14}H_{13}Cl_2N_3$	C, H, N

[a] J. P. Lambooy, *J. Am. Chem. Soc.*, **71**, 3756 (1949).

Experimental Section

All melting points were determined on a Fisher-Johns apparatus and are corrected. The C, H, N analyses were performed in this department on an F and M Model 185 analyzer by Mr. Daryl Sharp. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

N,N-Dimethyl-*p*-(3-*o*-xylylazo)aniline.—2,3-Dimethylaniline hydrochloride (Eastman Kodak) (31 g) was dissolved in a mixture of 80 ml of concentrated HCl and 200 ml of H_2O and diazotized at 0° using 14 g of $NaNO_2$. One-half hour after the final addition a solution of 24 g of $C_6H_5NMe_2$, 200 ml of EtOH, 120 ml of H_2O, and 72 g of NaOAc was added, and the solution was stirred for another 30 min and made basic with NH_4OH. Filtration, washing, and drying afforded the crude azo compound. The others were made in the same way. Crystallization from EtOH and in some cases chromatography on alumina from C_6H_6 gave the pure materials.

Biological Properties.—Young male rats of the Sprague–Dawley strain, approximately 8 weeks old and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of ten animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller[1] to which had been added one of the azo compounds at a level of 0.06%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; cerelose, 770 g; Osborne and Mendel salt mixture, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, obtained from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate, 67,500 IU.

A group received DAB at the 0.06% while the control group received only the basal diet. All the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Results and Discussion

DAB gave tumor incidences of 6/10 at 4 months and and 9/10 at 6 months. 3′,4′-Me_2DAB gave 0/10 at 2 months, 8/10 at 6 months, and 10/10 gross tumors at 8 months. On the other hand, 2′,3′-Me_2DAB gave 10/10 in 1 month with gross tumors in rats surviving to 2 months, 3′,4′-Et_2DAB gave 0/9 tumors in 4 months,

(1) J. A. Miller and E. C. Miller, *Advan. Cancer Res.*, **1**, 339 (1953).

(2) J. A. Miller, E. C. Miller, and G. C. Finger, *Cancer Res.*, **17**, 387 (1957).

(3) K. Sugiura, M. L. Crossley, and C. J. Kensler, *J. Natl. Cancer Inst.*, **15**, 67 (1954).

(4) E. V. Brown and A. A. Hapeman, *ibid.*, **27**, 663 (1961).

9/9 in 10 months, and gross tumors in all rats surviving to 12 months. The control group, the 2′,3′-Cl_2- and 3′,4-Cl_2DAB groups showed 0/10 tumors in 12 months. 2′,3-Me_2DAB is by far the most active of the DAB derivatives so far tested and compares in activity with some of the heterocyclic analogs.[5]

Acknowledgment.—The authors are indebted to Dr. Daniel L. Weiss, Department of Pathology, University of Kentucky College of Medicine, for the microscopic evaluation of the tumors.

(5) E. V. Brown and J. J. Duffy, *J. Natl. Cancer Inst.*, **40**, 891 (1968).

Derivatives of Fluorene. XXVII. New Thiofluorenes Related to Metabolism of the Carcinogen N-2-Fluorenylacetamide. II[1a]

MOSES J. NAMKUNG AND T. LLOYD FLETCHER

Chemistry Research Laboratory of the Department of Surgery, University of Washington School of Medicine, Seattle, Washington 98105

Received June 21, 1968

An earlier publication[1b] described the synthesis of N-2-(3-methylthiofluorenyl)acetamide and related compounds for structure confirmation of a substance which was isolated by degradation of the reaction product of the carcinogen N-acetoxy-N-2-fluorenylacetamide with methionine, methionylglycine, or proteins prepared under physiological conditions.[2] In the course of this work, some new fluorenyl sulfones and thiofluorenes were prepared for the antitumor testing program of the CCNSC.

3-Mercapto-9-oxofluorene (I) was prepared by alkaline hydrolysis of the corresponding ethyl xanthate.[1b] The acetylmercapto derivative (II) was then prepared. Oxidation of 3-methylthio-9-oxofluorene[1b] with 30% H_2O_2 in AcOH gave the corresponding sulfone (III). Methyl 2-nitro-9-oxofluoren-6-yl sulfone (VI) was obtained by peroxide oxidation of methyl 2-nitro-9-oxofluoren-6-yl sulfoxide[1b] and also by vigorous nitration of 3-methylthio-9-oxofluorene. Increasingly vigorous nitrating (and oxidizing) conditions altered 3-methylthio-9-oxofluorene stepwise to the sulfoxide,[1b] nitro sulfoxide,[1b] and nitro sulfone. Reduction of the 2-nitro-9-oxofluoren-6-yl sulfone with $SnCl_2$ gave the corresponding amine (V); reduction with hydrazine hydrate in diethylene glycol gave 6-mesyl-2-aminofluorene (VI). Each of these amines was acetylated.

Antitumor activities of these compounds are shown in Table I. Compound V exhibited slightly activity; the other compounds were inactive.

TABLE I

ANTITUMOR ACTIVITY[a]

Compd	Daily dose, mg/kg	Survivors	Survival, days T/C	T/C, %
I	400	6/6	8.3/8.6	96
	400	4/4	9.5/9.4	101
	200	4/4	9.8/9.4	104
	100	4/4	10.3/9.4	109
II	400	4/4	9.5/9.4	101
	200	4/4	9.0/9.4	95
	100	4/4	10.3/9.4	109
III	400	6/6	8.8/9.2	95
	400	6/6	8.7/8.8	98
	200	6/6	9.2/8.8	104
	100	6/6	8.5/8.8	96
IV	400	4/4	8.3/9.4	88
	200	4/4	9.0/9.4	95
	100	4/4	9.3/9.4	98
V	400	6/6	9.7/9.3	104
	400	4/4	9.0/8.8	102
	200	4/4	9.0/8.8	102
	100	4/4	10.3/8.8	117
VI	400	0/4		
	200	4/4	9.8/9.4	104
	100	4/4	8.8/9.4	93
VII	400	6/6	8.8/8.6	102
	400	4/4	8.8/9.4	93
	200	4/4	9.8/9.4	104
	100	4/4	9.8/9.4	104
VIII	400	1/4	9.0/9.4	
	200	4/4	9.8/9.4	104
	100	4/4	8.8/9.4	93

[a] The screening data in this table were kindly supplied by Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md. Assays were performed as reported in *Cancer Chemotherapy Rept.*, **25**, 1 (1962). The tumor system used was L1210 lymphoid leukemia tested in BDF_1 mice.

Experimental Section[3]

3-Mercapto-9-oxofluorene (I).—To 3 g of ethyl 9-oxofluoren-3-yl xanthate in 30 ml of EtOH, a solution of 3 g of NaOH in 15 ml of H_2O was added and the mixture was boiled for 2 min and filtered hot. The precipitate in the acidified filtrate was filtered off and dried, giving 2 g, mp 125–130°. Two recrystallizations (C_6H_6) gave an analytical sample, mp 133–134°. *Anal.* ($C_{13}H_8OS$) C, H, S.

3-Acetylthio-9-oxofluorene (II).—Acetylation of 1 g of the foregoing compound gave 1 g, mp 147–148°. An analytical sample was prepared by recrystallizations from ligroin (*d* 0.69–0.71) and then from C_6H_6; mp 148–148.5°. *Anal.* ($C_{15}H_{10}O_2S$) C, H, S.

Methyl 9-Oxofluoren-3-yl Sulfone (III).—To a solution of 2 g of 3-methylthio-9-oxofluorene in 20 ml of glacial AcOH, 20 ml of H_2O_2 (30%) was added. The mixture was boiled for 2 min and cooled giving a yellow precipitate, mp 194–195°. An analytical sample, with unchanged melting point, was obtained by recrystallization (AcOH). *Anal.* ($C_{14}H_{10}O_3S$) C, H, S.

Methyl 2-Nitro-9-oxofluoren-6-yl Sulfone (IV). **A.**—Oxidation of methyl 2-nitro-9-oxofluoren-6-yl sulfoxide with H_2O_2 (30%), as above, gave a product with mp 258–259° (100%). Recrystallization ($C_6H_5CH_3$) gave an analytical sample with the same melting point. *Anal.* ($C_{14}H_9NO_5S$) N.

B.—To 25 ml of yellow fuming HNO_3 (*d* 1.49–1.50), 4 g of 3-methylthio-9-oxofluorene was added with stirring. The temperature rose to 60° and brown fumes were given off. The mixture was then heated to 75° and allowed to cool. The precipitate was filtered off, washed, and dried, giving 4.3 g, mp 225–248°. Two recrystallizations ($C_6H_5CH_3$) and one from AcOH raised the melting point to 258–259°. A mixture of this with the product in A had the same melting point.

Methyl 2-Amino-9-oxofluoren-6-yl Sulfone (V).—A mixture of 3 g of methyl 2-nitro-9-oxofluoren-6-yl sulfone, 8 g of $SnCl_2 \cdot 2H_2O$, 10 ml concentrated HCl, and 5 ml of EtOH was boiled for 10 min and worked up as usual to obtain 2.2 g of product, mp 237–241°. Recrystallizations ($C_6H_5CH_3$ and EtOH) gave mp 241–242°. *Anal.* ($C_{14}H_{11}NO_3S$) C, H, N.

N-2-(6-Mesyl-9-oxofluorenyl)acetamide (VII).—Acetylation

(1) (a) Supported in part by a grant (CA-01744) from the National Cancer Institute, National Institutes of Health, and in part by Research Career Development Award 5-K3-CA-14,991 (T. L. F.). (b) T. L. Fletcher, M. J. Namkung, and H.-L. Pan, *J. Med. Chem.*, **10**, 936 (1967).

(2) P. D. Lotlikar, J. D. Scribner, J. A. Miller, and E. C. Miller, *Life Sci.*, **5**, 1263 (1966).

(3) Melting points were taken on a Fisher-Johns block and are corrected to standards. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Absorption bands of ir spectra were as expected; bands near 1300 and 1145 cm^{-1} were assigned to the sulfone group.