

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 Section²⁰

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 H₂O 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 (90%) 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 Ac₂O (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 Et₂O 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 Et₃N (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

Received July 1, 1968

Many of the disubstituted dimethylaminoazobenzenes (DAB) (Table I) have been tested for rat hepatocarcinogenic activity.^{1,2} With the exception of the

TABLE I. SUBSTITUTED 4-DIMETHYLAMINOAZOBENZENES

Compd	Mp, °C	Yield, crude (%)	Formula	Analyses
2',3'-Me ₂ DAB	120–121	70	C ₁₆ H ₁₅ N ₃	C, H, N
3',4'-Me ₂ DAB	Ref 2			
3',4'-Et ₂ DAB ^a	82–83	25	C ₁₈ H ₂₅ N ₃	C, H, N
2',3'-Cl ₂ DAB	218–220	35	C ₁₄ H ₁₃ Cl ₂ N ₃	C, H, N
3',4'-Cl ₂ DAB	159–160	55	C ₁₄ H ₁₃ Cl ₂ N ₃	C, H, N

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

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 F₂DAB have zero activity on the Miller scale.¹ Later work² showed mild carcinogenic activity for 3',4'-Me₂DAB and we have since verified this activity. We have now shown that 2',3'-Me₂DAB is extremely active. Since 4'-Et-DAB shows greater activity than DAB itself,^{2–4} we have extended our work to a related disubstituted compound, 3',4'-Et₂DAB, and it has been found to be fairly active. Neither 2',3'-Cl₂ nor 3',4'-Cl₂DAB was found to have any activity under our testing conditions.

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 $\pm 0.4\%$ of the theoretical values.

N,N-Dimethyl-*p*-(3-*o*-xylylazo)aniline.—2,3-Dimethylamine hydrochloride (Eastman Kodak) (31 g) was dissolved in a mixture of 80 ml of concentrated HCl and 200 ml of H₂O and diazotized at 0° using 14 g of NaNO₂. One-half hour after the final addition a solution of 24 g of C₆H₅NMe₂, 200 ml of EtOH, 120 ml of H₂O, and 72 g of NaOAc was added, and the solution was stirred for another 30 min and made basic with NH₄OH. 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 C₆H₆ 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 Miller¹ 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; cerelese, 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 9/10 at 6 months. 3',4'-Me₂DAB 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'-Me₂DAB gave 10/10 in 1 month with gross tumors in rats surviving to 2 months, 3',4'-Et₂DAB 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. Sugimura, M. L. Crossley, and C. J. Kensler, *J. Natl. Cancer Inst.*, **15**, 67 (1954).

(4) E. V. Brown and A. A. Hamdan, *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'-Cl₂- and 3',4'-Cl₂DAB groups showed 0/10 tumors in 12 months.

2',3 -Me₂DAB 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% H₂O₂ 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 SnCl₂ 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.

Experimental Section³

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

(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 $\pm 0.4\%$ 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.

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
	400	6/6	8.8/9.2	95
III	400	6/6	8.7/8.8	98
	200	6/6	9.2/8.8	104
	100	6/6	8.5/8.8	96
	400	4/4	8.3/9.4	88
IV	200	4/4	9.0/9.4	95
	100	4/4	9.3/9.4	98
	400	6/6	9.7/9.3	104
	400	4/4	9.0/8.8	102
V	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
	400	6/6	8.8/8.6	102
VII	400	4/4	8.8/9.4	93
	200	4/4	9.8/9.4	104
	100	4/4	9.8/9.4	104
	400	1/4	9.0/9.4	104
VIII	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 BDF₁ mice.

ml of H₂O 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 (C₆H₆) gave an analytical sample, mp 133–134°. *Anal.* (C₁₃H₉OS) 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 C₆H₆; mp 148–148.5°. *Anal.* (C₁₅H₁₀O₂S) 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 H₂O₂ (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.* (C₁₄H₁₀O₂S) C, H, S.

Methyl 2-Nitro-9-oxofluoren-6-yl Sulfone (IV). **A.**—Oxidation of methyl 2-nitro-9-oxofluoren-6-yl sulfoxide with H₂O₂ (30%), as above, gave a product with mp 258–259° (100%). Recrystallization (C₆H₅CH₃) gave an analytical sample with the same melting point. *Anal.* (C₁₄H₉NO₂S) N.

B.—To 25 ml of yellow fuming HNO₃ (*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 (C₆H₅CH₃) 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 SnCl₂·2H₂O, 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 (C₆H₅CH₃ and EtOH) gave mp 241–242°. *Anal.* (C₁₄H₁₁NO₂S) C, H, N.

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