fluxed 2 hr and allowed to cool. The crystals which formed were dissolved in MeOH, neutralized with NaOH, and purified by fractional recrystallization (C_6H_6–Me_2CO) yielding two isomers.

The substituted 2-benzylidene-1,3-indanedione was prepared by dissolving equal molar quantities of the aldehyde and indanedione in absolute EtOH, heating until crystal formation occurred, cooling, filtering, and recrystallizing from 95% EtOH and Me_2CO.

6-Dimethylaminochrysene and Other Analogs of 4-(4-Dimethylamino)stilbene[1]

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6-Aminochrysene (I) has been reported to be active against mammary carcinoma.[2] Noting that a *cis*-4-aminostilbene structure can be seen in one of the Kekule formulas for this compound, we prepared 6-dimethylaminochrysene (Table I) by methylating I with MeI for

TABLE I

ANALOGS OF 4-(4-DIMETHYLAMINO)STILBENE

No.	Compd	Mp, °C[a]	Formula[b]
1	6-Dimethylaminochrysene	101–102[c]	$C_{20}H_{17}N$
2	2-(4-Aminophenyl)indole	215[d]	$C_{14}H_{12}N_2$
3	3-(4-Dimethylaminobenzylidene)oxindole	241–242[e]	$C_{17}H_{16}N_2O$
4	1-(4-Dimethylaminocinnamylidene)indene	205–208[f]	$C_{20}H_{19}N$
5	5-(4-Dimethylaminobenzylidene)hydantoin	286–288[g]	$C_{12}H_{13}N_3O_2$
6	2′-Chloro-4-methylaminostilbene[h]	40–41[i]	$C_{15}H_{14}NCl$[j]
7	2,5-Dimethoxystilbene[h]	51[i]	$C_{16}H_{16}O_2$[j]

[a] Determined with Mel-Temp apparatus. Recrystallization solvents are given as footnotes for each compound. [b] All compounds were analyzed for C and H by Galbraith Laboratories except where indicated otherwise. Analytical results obtained were within ±0.3% of the theoretical values. [c] Pentane and absolute EtOH. [d] *i*-PrOH. [e] 95% EtOH and C_6H_6. [f] Absolute ethanol. Chromatographed on Florisil with C_6H_6, then recrystallized. [g] 95% EtOH. [h] Test results not available for these compounds in the Walker system. [i] Purified by chromatographing on Florisil with C_6H_6. [j] Analysis by Weiler and Strauss.

testing against the Walker 256 tumor. It was effective at dose levels of 240–1500 mg/kg without killing any of the animals, whereas the NH_2 compound was more toxic, killing two of the test animals at 625 mg/kg, but was more effective than the $N(CH_3)_2$ compounds at lower dose levels.

2-(4-Aminophenyl)indole, prepared by catalytic reduction of the 4-nitro compound[3] with Pd catalyst in EtOAc, can be considered as a *trans*-4-aminostilbene, but was inactive against the Walker tumor (Table II).

3-(4-Dimethylaminobenzylidene)oxindole, an analog of 1-(4-dimethylaminobenzylidene)-2-indanone, was prepared by the usual KOH-catalyzed condensation method. It was inactive against the Walker tumor.

(1) This investigation was supported by Public Health Service Research Grants CA-03717-05–11 from the National Cancer Institute.

(2) J. Gelzer and P. Loustalot, *European J. Cancer*, **3**, 79 (1967).

(3) C. E. Blades and A. L. Wilds, *J. Org. Chem.*, **21**, 1013 (1956).

TABLE II

No.[a]	KB cell test,[b] ED_{50}, μg/ml	Effect[c] Tumor wt T/C	Effect[c] Tumor wt mg/kg	Lethality No. killed	Lethality mg/kg
1		0.5	240	0/3	1500
		0.2	600		
2		1.0	400[d]	0/6	400[d]
3	100	0.8	1280	0/3	1280
4	100	1.0	1500	0/3	1500
5		0.9	1600	0/3	1600
6	6				

[a] See Table I for names of compounds. [b] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at Southern Research Institute and University of Miami Cell Culture Laboratory. [c] We are grateful to Professor Sir Alexander Haddow, Mr. J. E. Everett, and Mr. 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 admintered as a single intraperitoneal 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. [d] We are grateful to CCNSC for screening tests against Walker 256 in random-bred albino rats, using four daily intraperitoneal injections in CMC or peanut oil administered 3 days after implantation and sacrificed 7 days after implantation.

The importance of space relations in determining the activity of 1-(4-dimethylaminobenzylidene)indene is demonstrated by the inactivity of 1-(4-dimethylaminocinnamylidene)indene in which the conjugated series of double bonds has been lengthened to the extent of one more ethylene group. 5-(4-Dimethylaminobenzylidene)hydantoin was inactive and nontoxic.

2′-Chloro-4-methylaminostilbene and 2,5-dimethoxystilbene were prepared by treating the appropriate aldehyde with Grignard reagent prepared from benzyl chloride or 2-chlorobenzyl chloride. The 2′-chloro compound was more toxic in KB cell culture than the methoxy compound.

9-(4-Aminobenzylidene)fluorenes[1]

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Haddow, *et al.*,[2] found that 9-(4-dimethylaminobenzylidene)fluorene (I) had some antitumor effect. We have synthesized I and three of its analogs shown in Table I. The Walker 256 tumor inhibition test as now carried out is apparently less sensitive than the test originally used, since the only carcinostatic result obtained was a 38% reduction in the size of tumors treated with 9-(4-methylaminobenzylidene)fluorene. The ED_{50} values in the standard KB tissue culture tests were of the same order of magnitude as for the 1-(4-methylaminobenzylidene)indene.[3]

(1) This investigation was supported by Public Health Service Research Grants CA-03717-07 and -08 from the National Cancer Institute.

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TABLE I

Deriv of fluorene[a]	Mp, °C[b]	KB cell test, ED_{50},[d] μg/ml	Effect[c] T/C	Effect[c] mg/kg	Lethality[d] No. killed	Lethality[d] mg/kg
9-(4-Methylaminobenzylidene)-	135–136[e]	30	0.6	1600		
9-(4-Dimethylaminobenzylidene)-	140–142[e,f]	35	1.4	1600	0/3	1600
2-Dimethylamino-9-(4-dimethylaminobenzylidene)-	188–191[g]	38	1.1	1600		
2-Dimethylamino-9-(4-dimethylaminobenzylidene)-	148–150[h]	32				

[a] All new compounds were analyzed for C and H; analytical results were within ±0.3% of the theoretical values. [b] Determined with Mel-Temp melting point apparatus. [c] We are grateful to Professor Sir Alexander Haddow, Mr. J. E. Everett, and Mr. 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 interperitoneal 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 untreated hosts are reported as the ratio T/C. [d] Results of the standard *in vitro* KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center at the University of Miami Cell Culture Laboratory. [e] Orange. [f] Reference 2. [g] Red. [h] Yellow.

Experimental Section

Our efforts to prepare these compounds by KOH-catalyzed condensation in EtOH were unsuccessful, but good results were obtained in DMSO. In a typical experiment a solution of 0.025 mole of 4-dimethylaminobenzaldehyde and 0.025 mole of 2-dimethylaminofluorene in 125 ml of DMSO at 90° was poured into a solution of 0.025 mole of KOH in 50 ml of DMSO at 163° and heated 30 min in a bath at 110°. The solvent and unreacted aldehyde were distilled off under vacuum and the product was extracted from the residue by isohexane,[4] recrystallized (EtOH), and purified further by use of a Florisil column. Two types of crystals were obtained, both of which had the correct analysis for the expected product and the same uv absorption spectrum in EtOH or AcOH. Ir spectra in Nujol were similar. The two solids may be *cis* and *trans* isomers.

(4) A mixture of isomeric branched chain hexanes.

Potential Antiradiation Agents. II.[1] Guanidinoalkanethiosulfuric Acids

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The mechanism by which 2-(2-aminoethyl)-2-thiopseudourea dihydrobromide (I) confers antiradiation properties is believed to involve an intratransguanylation to form an intermediate *gem*-diaminothiazolidine (II) which subsequently ring opens to give 2-guanidinoethanethiol (III).[2] Compound III and its corresponding disulfide, bis(2-guanidinoethyl) disulfide (IV), have been studied extensively and have been shown to offer excellent protection against the effects of ionizing radiation.[3] A logical variation of III possessing the $H_2NC(=NH)NHCCS$– moiety is the thiosulfate inner salt, 2-guanidinoethanethiosulfuric acid (**1**), first synthesized by Kaluszyner.[4] While he indicated that **1**

$H_2NC(=NH)NHCH_2CH_2SSO_3H$

1

was a good antiradiation agent, no further details of its actual effectiveness were provided. Compound **1** was reported by Mangina[5] to have radioprotective effect on the yeast, *Saccharomyces vini*. Westland, *et al.*,[6] found that several 2-(1-alkylguanidino)ethylthiosulfuric acids, prepared by the sulfitolysis of bis(2-guanidinoethyl) disulfides, had *in vitro* antibacterial action against *Streptococcus pyogenes* and *Staphylococcus aureus*. The phosphate analog of **1**, 2-guanidinoethanethiol S-phosphate (V), prepared by Åkerfeldt[7]

$H_2NC(=NH)NH—CH_2CH_2SPO_3H_2$

V

as the ammonium lithium salt, was reported by him to be a good radioprotector at doses lower than are required for 2-mercaptoethylamine (MEA).[8] Compound V, however, was found to be more toxic than MEA.

We report here the synthesis and the antiradiation activity of **1** and six variants of its structure in which the carbon skeleton consists of 2, 3, or 4 CH_2 groups and in which some of the guanidino groups are alkyl substituted.

Chemistry.—Kaluszyner's method[4] for the preparation of **1** from 2-aminoethanethiosulfuric acid was slightly modified and adapted for the synthesis of the members of this series. It consists of heating a solution of an S-methyl derivative of a thiopseudourea with the Na salt of an aminoalkanethiosulfuric acid. When

$$RNHC(SCH_3)=NH + R'NHCH_2CH_2SSO_3^- \longrightarrow RNHC(=NH)NCH_2CH_2SSO_3^- + CH_3SH$$

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