filtered and the filtrate was concentrated and taken up in 5 mL of EtOH. This solution was diluted with 10 mL of H_2O and 1.1 g (0.011 mol, 10% excess) of KSCN and 2 mL of 12 N HCl was added. The imidazole-2-thione was formed by the same procedure used for **2a**. The crude reaction product was flash chromatographed on silica gel with EtOAc, and the chromatographed material was recrystallized from EtOAc–hexane to give 0.42 g (19%) of **5**: mp 143–145 °C; ^1H NMR [60 MHz, $CDCl_3$, $(CD_3)_2SO$] δ 3.9 (3 H, s, CH_3), 5.2 (2 H, s, CH_2), 6.45–6.8 (3 H, m, pyridyl, imidazole), 7.65 (1 H, dd, J = 12 Hz, 6 Hz, pyridyl), 8.2 (1 H, d, J = 3 Hz, pyridyl). Anal. ($C_{10}H_{11}N_3OS$) C, H, N.

Enzymology. In vitro IC_{50} determinations were made as previously reported.[1] The IC_{50} is defined as the concentration of compound that produces a 50% inhibition of product formation when compared to treated control.

Pharmacology. DA/NE Ratios. Mesenteric artery catecholamine levels were determined in SHR by a reported procedure.[9,10] Compounds were administered ip in two doses as suspensions in a 5% PEG 400, 1% methocel vehicle, 18 h apart.

Blood Pressure Measurements. These were determined as described previously.[1b] Compounds were administered ip as solutions in 20% DMF. Blood pressures and heart rates were monitored for 5–6 h and recorded at 0.5- intervals.

Registry No. 1, 95333-81-6; **2a**, 106984-85-4; **2b**, 106984-86-5; **2c**, 106984-87-6; **2c**·HCl, 108269-98-3; **3**, 106984-89-8; **3** (tosylate)·HCl, 108270-00-4; **4**, 106984-88-7; **5**, 108270-02-6; **6a**, 1121-60-4; **6b**, 500-22-1; **6c**, 872-85-5; **7a** (diethyl acetal), 6957-15-9; **7a** (imine, diethyl acetal), 6190-94-9; **7c**, 108269-96-1; **7c**·HCl, 108269-97-2; **8**, 1121-78-4; **8** (tosylate), 74838-56-5; **9**, 106984-92-3; **10**, 106984-95-6; **10** (2-alcohol, acetate), 106984-93-4; **10** (2-alcohol), 106984-94-5; **11**, 108269-99-4; **12**, 1628-89-3; **13**, 106984-91-2; **14**, 65873-72-5; DBH, 9013-38-1; $H_2NCH_2CH(OC_2H_5)_2$, 645-36-3; $H_2NCH_2CH(OCH_3)_2$, 22483-09-6; KSCN, 333-20-0; TosCl, 98-59-9; 5-[[(5,5-dimethoxyethyl)imino]methy]-2(1*H*)-pyridone, 108270-01-5; 1-benzyl-1,3-dihydro-2*H*-imidazole-2-thione, 23269-10-5; 1-(*p*-hydroxybenzyl)-1,3-dihydro-2*H*-imidazole-2-thione, 95333-64-5; fusaric acid, 536-69-6.

New Antitumor Agents Containing the Anthracene Nucleus

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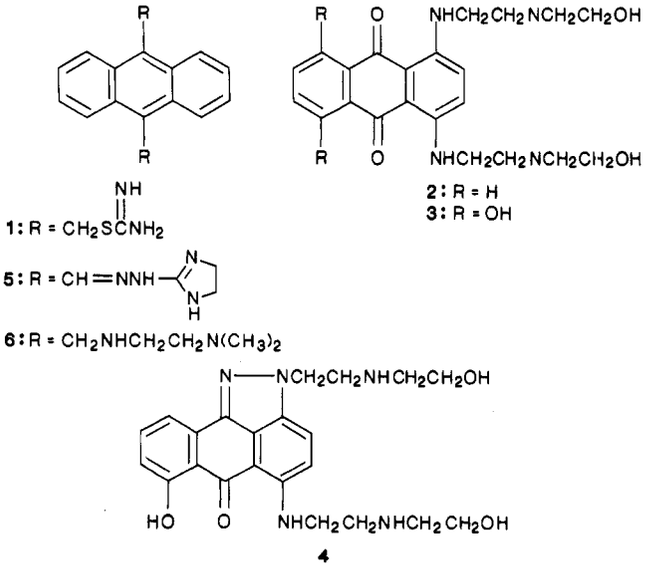
A series of 21 new compounds related to bisantrene was synthesized and tested in vitro by using clonogenic assays against a variety of human tumor cell lines, fresh human tumors, and P-388 leukemia. Those most closely related to bisantrene were less active than it was, but a subset of compounds with saturated side chains containing two basic nitrogens showed good activity. Two compounds of this subset, *N,N'*-bis[2-(dimethylamino)ethyl]-9,10-anthracenebis(methylamine) (**6**), and *N,N'*-bis(1-ethyl-3-piperidinyl)-9,10-anthracenebis(methylamine) (**19**), were very active in vitro against human tumor cell lines, but not active against fresh human tumors or P-388 leukemia cells. They had only marginal activity in mouse tumor models. Thus, the fresh human tumors and P-388 leukemia cells in vitro were better predictors than the established cell lines for the activity of these anthracene compounds in vivo against mouse tumors. These compounds appear to be distinct from bisantrene in aspects of mode of action. For example, **6** did not cause inhibition of macromolecular synthesis and promotion of DNA single strand breakage at cytotoxic drug concentrations. Toxicological studies showed that its rapid administration caused acute neurotoxicity resulting in apnea. It also produced skin ulcers on id administration, but they were less severe than those caused by bisantrene.

In recent years a variety of anthracene derivatives have received attention as potential antitumor drugs. The first such derivative was 2,2'-(9,10-anthrylenedimethylene)-bis[2-thiopseudourea] (**1**),[1,2] known as "pseudourea", which entered clinical trials, but was withdrawn because of phototoxicity.[3,4] Subsequently, a variety of substituted anthraquinones, including ametantrone (**2**) and mitoxantrone (Novantrone, **3**), have been tested in clinical trials.[5–8] The latter compound appears to be active principally in breast cancer, acute leukemias,[9] and non-Hodgkin's lymphomas.[10] It is less cardiotoxic than doxorubicin. A new system based on anthraquinone, the 5-substituted anthra[1,9-*cd*]pyrazol-6(2*H*)-one (e.g., **4**) also shows promise in cancer chemotherapy.[11] Diguanylhydrazones of anthracene-9,10-dicarboxaldehydes comprise a rather large group of compounds with antitumor activity in experimental systems.[12] The most active compound of this group, bisantrene (**5**), has proven active in patients with breast cancer and acute leukemia.[13–16]

Our initial interest in the anthracenes was to develop a more complete set of structure–activity relationships for compounds related to bisantrene and pseudourea. Although many guanylhydrazones had been prepared and tested, most of the structural variation was in the guanidinyl moiety or in substituents on the anthracene nucleus.[12]



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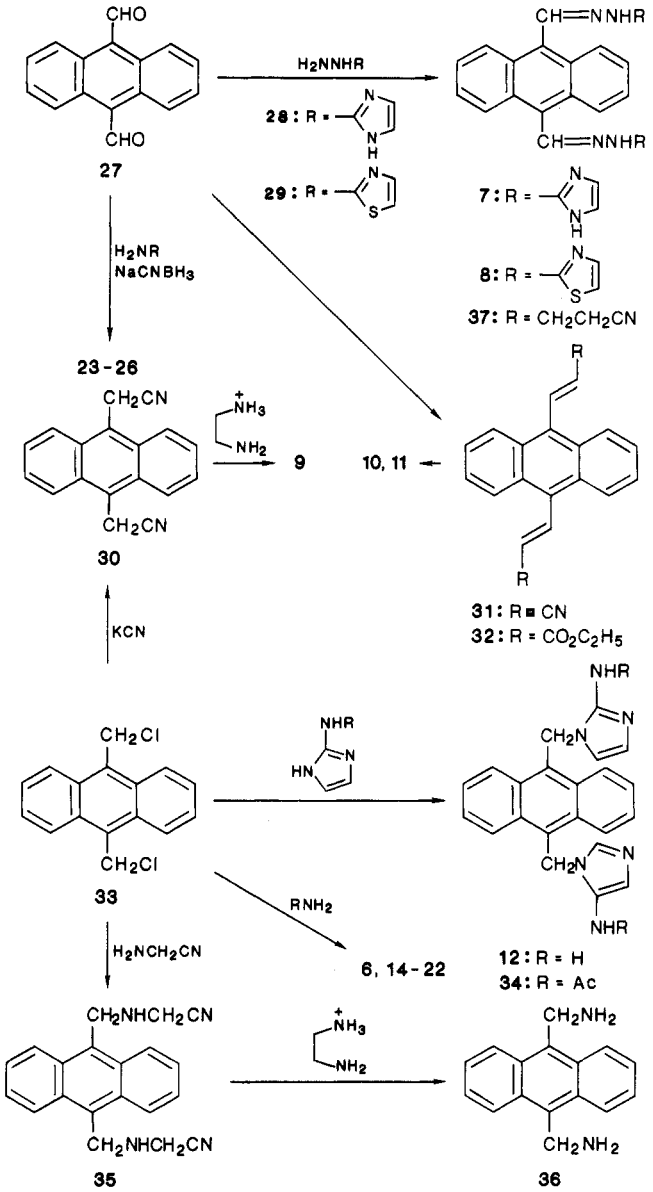
† Department of Pharmaceutical Sciences.
‡ Arizona Cancer Center.

(1) Miller, M. W.; Amidon, R. W.; Tawney, P. O. *J. Am. Chem. Soc.* **1955**, *77*, 2845.

0022-2623/87/1830-1313$01.50/0

Scheme I



We thought that it might be informative to change the hydrazone portion, in terms of type of atom, degree of unsaturation, and length of the chain separating the anthracene nucleus from the basic centers in the side chains. As discussed below, this investigation led us into the synthesis of bis[[2-(dialkylamino)ethyl]amino]methyl derivatives of anthracene, such as **6**. When in vitro antitumor activity was demonstrated for these compounds, a number of analogues of this type were synthesized.

Chemistry. The first bisantrene analogues prepared had the imidazoline ring replaced by the fully unsaturated imidazole and thiazole rings (compounds **7** and **8**, Table I). They were obtained by treating anthracene-9,10-dicarboxaldehyde (**27**, prepared readily from bis(chloromethyl) derivative **33**)[17] with the appropriate hydrazines (Scheme I). These hydrazines were not available, but they were prepared from the corresponding amines by diazotization followed by reduction.[18] 2-Hydrazinoimidazole (**28**) is a new compound, whereas 2-hydrazinothiazole (**29**) is known.[18]

Variations in the length and nature of the side chain, keeping the imidazoline ring constant, were obtained in the following ways. The dinitrile **30**[1] was converted into the bis(2-imidazolinyl)methyl analogue **9** upon heating with the monotoluenesulfonic acid salt of ethylenediamine. The analogue with a vinyl group between anthracene and imidazoline functionalities (**10**) was obtained by converting dialdehyde **27** into the bis(acrylonitrile) derivative **31** through Wittig–Horner reaction with diisopropoxyphosphonoacetonitrile, followed by a Pinner synthesis involving formation of the imidate with HCl in ethanol and treatment with ethylenediamine. An alternative method for the bis(acrylonitrile) derivative **31**, based on cyanoacetic acid in morpholine and DMF,[19] gave a lower yield (31% vs. 72%). The double bond in both the nitrile derivative **31** and the resulting imidazoline **10** is trans (*E*). The known bis(ethylacrylyl) derivative **32**[12] was obtained by treatment of the dialdehyde with ethyl bromoacetate and the zinc/copper couple followed by dehydration, but heating this derivative with ethylenediamine gave only amide **11**. We were unable to cyclize this amide to imidazoline **10**.

Another approach taken to the synthesis of bisantrene and pseudourea analogues was treating the 9,10-bis(chloromethyl) derivative **33** of anthracene with various amines. The first attempt was made with 2-aminoimidazole, in the expectation that the 2-amino group would be alkylated. However, the product **12** resulted from alkylation on one of the ring nitrogens. The structure of this product was verified by showing that its *N*-acetyl derivative **34**, prepared by treating **12** with acetic anhydride, was identical with the product obtained when **33** was treated with the known 2-(acetylamino)imidazole (Scheme I).[20] Treatment of **33** with 2-(dimethylamino)ethylamine was carried out in the expectation that the anticipated product **6** would resemble bisantrene in the distance separating the anthracene ring and the basic centers in the side chains. This product was obtained in good yield, and when it was shown

(2) Carter, S. K. *Cancer Chemother. Rep., Suppl.* **1968**, *1*, 153.
(3) Frei, E.; Luce, J. K.; Loo, T. L. *Cancer Chemother. Rep., Part 1* **1971**, *55*, 91.
(4) Wilson, W. L.; Weiss, A. J.; Andrews, N. C. *Cancer Chemother. Rep., Part 1* **1971**, *55*, 525.
(5) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1978**, *21*, 291.
(6) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, F. E.; Durr, F. E.; Citarella, R. V. *J. Med. Chem.* **1979**, *22*, 1024.
(7) Cheng, C. C.; Zee-Cheng, R. K. Y. *Prog. Med. Chem.* **1983**, *20*, 83.
(8) Wallace, R. E.; Murdock, K. C.; Angier, R. B.; Durr, F. E. *Cancer Res.* **1979**, *39*, 1570.
(9) Smith, I. E. *Cancer Treat. Rev.* **1983**, *10*, 103.
(10) Coltman, C. A., Jr.; McDaniel, T. M.; Balcerlzak, S. P.; Morrison, F. S.; Von Hoff, D. D. *Invest. New Drugs* **1983**, *1*, 65.
(11) Showalter, H. D. H.; Johnson, J. L.; Werbel, L. M.; Leopold, W. R.; Jackson, R. C.; Elslager, E. F. *J. Med. Chem.* **1984**, *27*, 255.
(12) Murdock, K. C.; Child, R. G.; Lin, Y.; Warren, J. D.; Fabio, P. F.; Lee, V. J.; Izzo, P. T.; Lang, S. A., Jr.; Angier, R. B.; Citarella, R. V.; Wallace, R. E.; Durr, F. E. *J. Med. Chem.* **1982**, *25*, 505.
(13) Alberts, D. S.; Mackel, C.; Pocelinko, R.; Salmon, S. E. *Cancer Res.* **1982**, *42*, 1170.
(14) Yap, H.; Yap, B.; Blumenschein, G. R.; Barnes, B. C.; Schell, F. C.; Bodey, G. P. *Cancer Res.* **1983**, *43*, 1402.
(15) Spiegel, R. J.; Blum, R. H.; Levin, M.; Pinto, C. A.; Wernz, J. C.; Speyer, J. L.; Hoffman, K. S.; Muggia, F. M. *Cancer Res.* **1982**, *42*, 354.
(16) Marty, M.; Guy, H.; Ferme, C.; Gisselbrecht, C.; Castaigne, S.; Boiron, M. *Cancer Chemother. Pharmacol.* **1982**, *9* (Suppl 15), Abstr 143.
(17) Klanderman, B. H. *J. Org. Chem.* **1966**, *31*, 2618.
(18) Lee, A. L.; Mackay, D.; Manery, E. L. *Can. J. Chem.* **1970**, *48*, 3554.
(19) Hann, R. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1379.
(20) Lawson, A. *J. Chem. Soc.* **1956**, 307.

to have interesting antitumor activity, a number of related compounds were prepared in the same way.. They are analogues **14–22** in Table I, and they were chosen to investigate the effect of alkyl substituent size and location on antitumor activity.

A final group of analogues was prepared from dialdehyde **27** by reductive amination, with sodium cyanoborohydride as the reducing agent. This method avoids a potential side reaction, since **33** is reactive enough to form ethers from alcohols in the presence of basic amines. Analogues **23–26** in Table I were prepared in this way. They have one basic nitrogen close in to the anthracene nucleus, but only a hydroxyl group (capable of hydrogen bonding with DNA) further out in the chain. The known Schiff's base **13** was prepared by condensing **27** and 2-(dimethylamino)ethylamine without reduction.[12]

A number of unsuccessful approaches to the preparation of bisantrene analogues were explored. Some of them seem worthy of brief mention because they illustrate the difficulties in working with the highly reactive benzylic-type side chains. For example, it was possible to prepare the bis[(cyanomethyl)aminomethyl] derivative **35** by treating **33** with aminoacetonitrile, but attempts to construct the imidazoline ring using the monotoluenesulfonic acid salt of ethylenediamine resulted in cleavage to the ditoluenesulfonic acid salt of the known 9,10-bis(aminomethyl)-anthracene (**36**, Scheme 1).[1] In another example, 3-hydrazinylpropionitrile condensed with 9,10-dialdehyde **27** to give the bishydrazone **37**, but treatment of this compound with the monotoluenesulfonic acid salt of ethylenediamine gave a product that was insoluble in all solvents tried. Attempts to solublize it by forming acid salts resulted in reversion to 9,10-dialdehyde **27**.

Antitumor Activity. The new anthracene derivatives described above were screened in vitro against a panel of four different human tumor cell lines according to established procedures for clonogenic assay in soft agar medium.[21] The results of these assays are given in Table II. As expected,[6,12,22] mitoxantrone, pseudourea, and bisantrene were highly cytotoxic, although **1** was tested only against two cell lines. Among the new analogues, those most closely related to bisantrene (**7–12**) showed relatively poor activity. Decreased cytotoxicity relative to that of the parent compound probably reflects two factors: inappropriately short chain length (**9** and **10**) and lower basicity of the side chain (**7**, **8**, and **11**). It is known that the imidazole ring (pK_a = 7.0) is much less basic than the imidazoline ring (pK_a = 12.1). The thiazole ring is even less basic, and this property was evident in the fact that **8** gave a dihydrochloride salt only in strongly acidic solution. These results suggest that the geometry and electronic character of bisantrene might be optimal, or nearly so, for anthracene derivatives of its type.

On the other hand, analogues with saturated side chains containing two basic nitrogens showed good activity in the clonogenic assays. As discussed below, they might constitute a new group of anthracene derivatives distinctly different from bisantrene. The best of these analogues were **19**, which had cytotoxicity comparable to that of mitoxantrone, and **6** and **14**, which had activities comparable to those of bisantrene. Analogues **15** and **22** also had significant activities, but other compounds of this type were active in only one assay, or none. Certain structural features of these analogues can be correlated qualitatively with antitumor potency. Thus, two methylene groups between the two nitrogens is optimal, but three are consistent with activity. Considerable bulk can be tolerated on the terminal nitrogen atom (e.g., **14**), but substitution on the nitrogen closer to the nucleus reduces potency: about 10-fold for a methyl group and drastically for a piperazine ring (compare **6** with **15** and **18**).

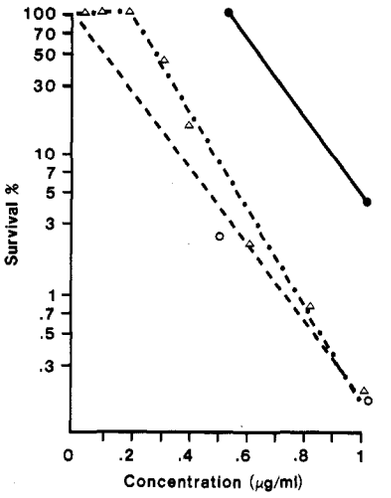


Figure 1. Survival of L-1210 cells 1 h after exposures to anthracene derivatives: (●) bisantrene, (○) mitoxantrone, (△) **6**.

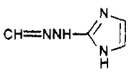
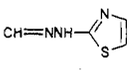
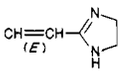
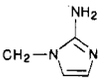
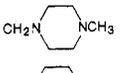
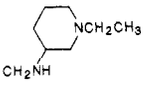
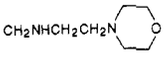
On the basis of their high degree of cytotoxicity in the clonogenic assays, **6** and **19** were chosen for further in vitro and in vivo studies. Compound **6** was highly active against L-1210 mouse leukemia cells in culture, as shown in Figure 1. Tumor colony formation was limited to 50% of control at a drug concentration of 0.3 $\mu g/mL \cdot h^{-1}$. Mitoxantrone (**3**) and bisantrene were evaluated in the same assay. The former was more active than **6** at lower doses, but not at higher doses. The latter was only about one-half as potent as **6** with respect to ID_{50} values. In contrast to its good potency against L-1210 leukemia cells, **6**, as well as **19**, were relatively inactive against P-388 leukemia cells. Compound **6** caused no tumor colony reduction, and **19** was associated with 53% reduction in tumor colonies only at 1 $\mu g/mL \cdot h^{-1}$. Both caused complete inhibition of colony growth at 10 $\mu g/mL \cdot h^{-1}$. In contrast, mitoxantrone and bisantrene were associated with 100% inhibition of colony formation even at the lowest doses (i.e., 0.1 $\mu g/mL \cdot h^{-1}$) tested.

Analogue **6** by the intraperitoneal (ip) route also showed marginal activity against ip L-1210 and P-388 leukemias in mice when given at doses of 60–100 mg/kg once, or for 5 consecutive days (Table III). The 5-day dosing schedule allowed greater total doses to be given without compounding the acute lethality. However, multiple daily dose schedules did not increase antileukemic activity. A single dose of 70 mg/kg was maximally effective, affording an increase of 40% in life span against L-1210 and 27% against P-388. There were no long-term survivors over a range of doses, and some early deaths occurred at doses above 70 mg/kg.

Analogue **19** was associated with slight, but inconsistent activity against P-388 leukemias in mice (Table III). In an initial trail a dose of 67 or 44 mg/kg given three times at 4-day intervals produced a 33% and 28% increase in life span, respectively. At higher doses early deaths due to toxicity were observed. However, in a second trial a dose of 100 mg/kg given on days 1, 5, and 9 gave no evidence of toxicity and **19** was found to be inactive at all doses. Finally, the compound demonstrated activity (40% increase in life span) against P-388 leukemia resistant to

(21) Salmon, S. E.; Hamburger, A. W.; Soehnlen, B. J. *N. Engl. J. Med.* **1978**, *298*, 1321.
(22) Von Hoff, D. D.; Coltman, C. A., Jr.; Forseth, B. *Cancer Chemother. Pharmacol.* **1981**, *6*, 141.

Table I. Preparation and Properties of 9,10-Disubstituted Anthracenes

compd	R	salt[a]	synth method[b]	% yield of salt	mp, °C (free base)	formula[c]	^{1}H NMR signals for R, and solvent impurity, δ[d]
7		2HCl	A	57	235–243	$C_{22}H_{20}N_8Cl_2 \cdot 0.75H_2O$	7.10 (s, 4), 9.40 (s, 2)
8			A[e]	97	dec 280	$C_{22}H_{16}N_6S_2 \cdot 0.1SnO_2$[f]	(Me_2SO-d_6) 6.82 (d, 2), 7.30 (d, 2), 9.19 (s, 2)
9		2HCl	B	42[g]	328–332 (dec 315)	$C_{22}H_{22}N_4 \cdot 0.25H_2O$[g]	(TFA-*d*) 3.40 (s, 8), 4.40 (s, 4)
10		2HCl	C	70	>360	$C_{24}H_{24}N_4Cl_2 \cdot 0.1EtOH$	4.05 (s, 8), 6.75 (d, J = 16.2 Hz, 2), 8.86 (d, J = 16.2 Hz, 2), 1.10, 3.55
11	CH=CHC(O)NHCH$_2$CH$_2$NH$_2$ (*E*)	2HCl	D	31	(dec 255)	$C_{24}H_{26}N_4O_2$[g,h]	(TFA-*d*) 3.67 (m, 4), 4.09 (m, 4), 6.66 (d, J = 16 Hz, 2), 8.70 (d, J = 16 Hz, 2)
12		2HCl	E	58	dec 280	$C_{22}H_{22}N_6Cl_2$	(TFA-*d*) 5.45 (m, 6), 6.10 (m, 2)
13	CH=NCH$_2$CH$_2$N(CH$_3$)$_2$		lit.[i]		(104–106)		
6	CH$_2$NHCH$_2$CH$_2$N(CH$_3$)$_2$	2F	F	79	174–176	$C_{32}H_{42}N_4O_8 \cdot 0.25H_2O$	2.97 (s, 12), 3.55 (t, 4), 3.92 (t, 4), 5.45 (s, 4)
14	CH$_2$NHCH$_2$CH$_2$N(*i*-Pr)$_2$	2F	F[j,k]	68	206–209	$C_{40}H_{58}N_4O_8$[l]	1.35 (s, 12), 1.45 (s, 12), 3.30–4.00 (m, 4), 5.65 (s, 4)
15	CH$_2$N(CH$_3$)CH$_2$CH$_2$N(CH$_3$)$_2$	2F	F	79	199–201	$C_{34}H_{46}N_4O_8$	2.75 (s, 6), 2.90 (s, 12), 3.70 (m, 4), 3.90 (m, 4), 5.60 (s, 4)
16	CH$_2$N(CH$_3$)CH$_2$CH$_2$NHCH$_3$	F	F[m]	34	(79–81) 221–222	$C_{28}H_{38}N_4O_4 \cdot 0.4EtOH$	2.70 (s, 6), 2.85 (s, 6), 3.50 (m, 4), 3.75 (m, 4), 5.65 (s, 4), 1.10, 3.55
17	CH$_2$NHCH$_2$CH$_2$NH$_2$	F	F[n]	51	185–187	$C_{24}H_{30}N_4O_4 \cdot 0.9H_2O$	3.25 (t, 4), 3.65 (t, 4), 5.45 (s, 4)
18		F	F[o]	74	(242–244) 243–245	$C_{30}H_{38}N_4O_4$	2.90 (s, 6), 3.55 (m, 16), 5.50 (s, 4)
19		F	F[p]	45	(101–103) 216–220	$C_{34}H_{46}N_4O_4 \cdot 0.75EtOH$	1.35 (t, 6), 2.00 (m), 3.30 (m), 4.10 (m), 5.40 (s, 4)
20		2F	F[q]	23	(123–126)	$C_{36}H_{46}N_4O_{10}$	3.50 (m, 12), 3.90 (m, 12), 5.45 (s, 4)
21	CH$_2$N(CH$_2$CH$_2$OH)CH$_2$CH$_2$NH$_2$	2C	F[r]	36	104–106	$C_{36}H_{50}N_4O_{10} \cdot 0.5MeOH$[s]	(Me_2SO-d_6) 2.80 (m, 4), 3.10 (m, 8), 3.60 (m, 4), 4.70 (s, 4), 3.20
22	CH$_2$NHCH$_2$CH$_2$CH$_2$N(CH$_3$)$_2$	F	F	56	190–191	$C_{30}H_{42}N_4O_4 \cdot 0.4EtOH$	2.15 (m, 4), 2.85 (s, 12), 3.22 (t, 4), 3.45 (t, 4), 5.40
23	CH$_2$NHCH$_2$CH$_2$OCH$_2$CH$_2$OH	F	G	78	(111–113) 187–189	$C_{28}H_{36}N_2O_8 \cdot 0.4EtOH$	3.50 (m, 4), 3.70 (s, 8), 3.92 (t, 4), 5.40 (s, 4), 1.10, 3.55
24	CH$_2$NHCH$_2$CH$_2$CH$_2$OH	F	G[t]	31	(115–117) 200–202	$C_{24}H_{32}N_2O_2$[g]	1.75 (m, 8), 3.50 (m, 8), 5.35 (s, 4)
25	CH$_2$NHCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OH	F	G[u]	56	(121–123) 210–212	$C_{26}H_{36}N_2O_2$[g]	1.56 (br s, 8), 1.80 (m, 4), 3.40 (m, 8), 5.37 (s, 4)
26	CH$_2$NHCH$_2$CH$_2$OH		G	87[g]	(177–179)	$C_{20}H_{24}N_2O_2 \cdot 0.1H_2O$	3.40 (t, 4), 3.90 (t, 4), 5.40 (s, 4)

[a] F = fumarate; C = citrate. [b] See Experimental Section. [c] All compounds were analyzed for C, H, N, and the results were within ±0.4% of theoretical values, except where listed in subsequent footnotes. [d] NMR samples are dissolved in Me_2SO-d_6–TFA-*d* unless stated otherwise. [e] The portionwise procedure described for **7** was not needed. The bis(2-hydrazinothiazolyl)chlorostannate (ref 18) and **27** were refluxed in EtOH, and the product was collected by filtration and washed with 5% NaOH and H_2O. [f] Also found ash. [g] Free base. [h] N: calcd, 13.92; found, 13.10. [i] Reference 12, lit. mp 108–109 °C. [j] Starting diamine, *N*,*N*′-diisopropylethylenediamine, was prepared according to McKay and Gilpin.[34] [k] Crude product is treated with excess (>4 equiv) fumaric acid, which precipitates the difumarate salt. The salt is neutralized, and the free base is extracted into ether, dried, and evaporated. Treatment with 2 equiv of fumaric acid gives the product. Quantities <250

Table I (Continued)

mg produce the tetrafumarate salt. [l] Analysis for tetrafumarate salt. [m] Free base recrystallized from EtOH–hexanes. [n] The cooled reaction mixture was treated with 5% NaOH and filtered. The solid was dissolved in $CHCl_3$, washed with H_2O, dried, and evaporated to yield the solid free base. [o] The product precipitated from the reaction mixture and was collected, washed with 5% NaOH and H_2O, and dried. The free base was dissolved in $CHCl_3$ to prepare the fumarate salt. [p] Recrystallized the free base from acetonitrile. [q] Free base chromatographed on C-18, acetonitrile–H_2O (6:4). [r] MeOH was used to prepare the salt. [s] N: calcd, 6.91; found, 6.03. [t] Free base chromatographed on silica gel, $CHCl_3$–EtOH (8:2). [u] Free base recrystallized from 95% EtOH.

Table II. Activity of Anthracene Derivatives against Cloned Human Tumor Cells[a]

	concentration (μM) for 50% inhibition of tumor cell growth (ID_{50}) against the following types[b]			
compd	myeloma	ovarian	endometrial	colon
mitoxantrone (3)	0.02	0.09		0.04
bisantrene (5)	0.23	2.40	0.38	0.44
pseudourea (1)		0.05	0.16	
6	0.16	1.60	0.36	0.18
	0.007	0.19		0.22
7	6.26	8.80	200	5.90
8	un[c]	4.36	5.60	un
9	un	un	un	un
10	3.16	un	un	un
11	165	un	un	un
12	un	111	un	un
13	7.63	un		15.5
14	0.29	0.70		0.14
15	3.51	3.42		1.93
16	692	4.14		0.42
17	0.10	un		10.4
18	0.04	un		5.63
19	0.02	0.03		0.02
20		5.76		5.04
21	11	un	56.8	un
22	0.23	6.57		2.91
23	un	18.2		un
24	4.67	24.7		13.4
25	27.8	74.9		un

[a] Clonogenic assays were conducted according to the procedure of Salmon et al.[21] [b] Complete names for the tumor types are 8226 myeloma, UACC-66 ovarian, HEC 1A endometrial, and WIDR colon. [c] Experiments in which 50% inhibition was unachievable are denoted by un.

Table III. Antileukemic Activity of Compound 6 against L-1210 and P-388 Leukemias in Mice

	daily ip dose, mg/kg	days of treatment	% ILS of leukemic mice		
drug			P-388	P-388/ ADR[a]	L-1210
6[b]	60	1	11	--	17
	70	1	27	--	40
	80	1	36	--	14
	90	1	-10	--	3
	100	1	-33	--	nd
	30	5	0	--	5
	37.5	5	0	--	11
	60	5	25	--	27
	70	5	10	--	30
	80	5	0	--	15
19[c]	125	3	-81	--	
	100	3	-34	--	
	67	3	33	--	
	44	3	28	--	
19 (trial 2)	100	3	9	40	
	67	3	9	15	
	44	3	-5	15	
	30	3	4	5	

[a] P-388 cells that are resistant to adriamycin. [b] Adult male DBA/2J mice (Jackson Laboratories) were given 10^5 L-1210 cells ip on day 0.[35] Twenty-four hours after tumor implantation, a solution of 6 in PBS was given ip at doses of 30–100 mg/kg once or daily for 5 days. A control group of 10 mice received saline. There were no early deaths in this group and the median survival time was 18.5 days. These results represent the mean of three experiments (n = 10 mice/group per experiment). [c] Groups of six adult female CD2F1 mice (Charles River) were given 4.4×10^6 cells ip on day 0. A solution of 19 in water was given ip at doses of 30–125 mg/kg on days 1, 5, and 9. The median survival time of the control group was 10.5 days.

adriamycin at a dose of 100 mg/kg on days 1, 5, and 9.

Toxicology. The lethal dose posology for 6 is summarized in Table IV. The dose of 6 causing death in 60% of the mice (LD_{60}) was 100 mg/kg. In this study, it was given ip as a rapid injection. Deaths occurred within 20–30 min after 100 mg/kg doses and within 10–12 days after acute doses of 80–90 mg/kg. With five daily doses (each above 80 mg/kg), death occurred immediately after the second, third, or fourth injections. A 100 mg/kg dose given as a rapid iv push by the tail vein was lethal immediately to 80% of the mice. When daily doses were fractionated into four injections, significantly more 6 could be delivered without causing lethality. Thus an LD_{30} dose of 6 was 45 mg/kg, given four times daily for 5 days (total dose 900 mg/kg). All injections of 6 greater than 70 mg/kg were also associated with significant weight loss of approximately 10% per week following dosing (Table IV). A typical prodromal syndrome after injection of large doses included lethargy, labored breathing, convulsions, and apnea, followed by death. The heart was palpable throughout the apneic period. Postmortem examination of mice dying from the 120 mg/kg dose revealed dark red and irregular pulmonary surfaces, livers were pale, but other organs were not grossly affected.

Pharmacologic antagonism studies were conducted in normal DBA male mice to determine if antiinflammatory, antihistamine, sympathomimetic, cholinergic, or anticonvulsant drugs could reduce the lethality of 6 given by rapid iv injection. There was no effect of antihistamines, antiinflammatory corticosteroids, or epinephrine on the lethality produced by 100 mg/kg doses. Physostigmine salicylate significantly enhanced the acute lethality, causing 5/5 deaths immediately. However, intravenous pretreatment with pentobarbital sodium and diazepam appeared to significantly reduce lethality. Only one of five mice died and this death occurred 5 h after the injection of 6. These preliminary experiments suggest that 6 causes acute neurotoxicity at high ip doses. The observations of immediate

Table IV. High-Dose Toxicity of Compound 6 Ip in Mice[a]

dose/ injection, mg/kg	no. daily doses	days of treatment	lethality, % (median day of death)	% av weekly wt change
80	1	1	10 (12)	-3
90	1	1	20 (10)	-5
100	1	1	60 (0)	
60	1	5	0	0
70	1	5	10 (0)	-7
80	1	5	20 (4)	-10
90	1	5	20 (3)	-11
100	1	5	80 (8)	-15
30	4	5	0	-3
37.5	4	5	20 (2)	-7
45	4	5	30 (2)	-12
52.5	4	5	100 (4)	

[a] Adult male DBA/2J mice, 30–35-g weight at time of dosing. All drug doses given ip in 0.89% sodium chloride as a rapid injection at 0.1 mL/10 g body weight. At least 10 mice were treated in each group on day 0 and were observed for survival twice daily and weighed twice weekly.

Table V. Skin Toxicity of Intradermal 6 in Balb/c Mice[a]

dose, mg id	extent of skin lesions, cm²		
	induration	erythema	ulceration
0.25	0.7	0.3	0.2
0.75	1.7	1.3	1.0
1.25	4.3	2.2	1.9
2.00	5.3	3.1	2.6
2.5	6.1	5.0	3.8

[a] The compound was given 10–100 mg/kg as an id injection in 0.05-mL volume. For a detailed description of the assay, see ref 36.

death with rapid iv administration and the increased total dose tolerance with 5-day dosing are consistent with concentration-dependent neurotoxicity. Such behavior has been seen before when concentrated solutions of anthracyclines are given ip or iv.[23] A slower administration rate or more dilute solutions of **6** might reduce lethality.

Because bisantrene (but not mitoxantrone) is a potent vesicant,[13] analogue **6** was tested in an intradermal skin ulceration assay. As shown in Table V, it caused significant, dose-dependent skin ulcers when given as an id injection at 10–100 mg/kg. Visible skin ulcers formed in 1 day and involved concentric circular areas of induration, erythema, and ulceration. At the highest dose, maximal lesions developed by day 5 and healed by day 25. Lesions produced by lower doses healed more rapidly. This level of toxicity is similar to that produced by doxorubicin, but much less than that produced by bisantrene.

Macromolecular Synthesis. The effects of mitoxantrone, bisantrene, and **6** on DNA, RNA, and protein synthesis were determined by measuring the incorporation of radiolabeled precursors into L-1210 murine leukemia cells and 8226 human multiple myeloma cells, following the method described by Bhuyan and co-workers.[24] Among the radiolabeled compounds, [^{14}C]thymidine was used to measure DNA synthesis, [^{3}H]uridine was used for RNA synthesis, and [^{14}C]valine was used for protein synthesis. The results of these assays are given in Table VI, which shows significant inhibition by **6** of both DNA and RNA only at a 1-h concentration of 10 μg/mL. This result is surprising in view of the fact that **6** is cytotoxic at much lower doses. It produces a 50% cell kill at 0.3 μg/mL and a 1-log-reduction cell kill at 0.5 μg/mL in L-1210 leukemia, as indicated by single and double asterisks in Table VI. These cell kill data are taken from Figure 1. In contrast to **6**, both bisantrene and mitoxantrone inhibit DNA and RNA synthesis at concentrations corresponding to their cytotoxic ones. As expected for this class of agents, protein synthesis was not strongly inhibited at the lower doses.

Similar results were obtained in the 8226 myeloma cell assay, except that low doses of **6** appeared to stimulate the incorporation of [^{14}C]valine. This occurred consistently with a 1-h exposure to concentrations of **6** ≤ 1.0 μg/mL and may represent enhanced protein synthesis for attempted repair following exposure to cytotoxic concentrations of **6**. This increase was maximal (>200% of control) with a 1-h exposure to a 0.3 μg/mL concentration of **6**, the approximate IC_{50} in this human cell line. It should also be recalled that the macromolecular synthesis endpoints are measured immediately following drug exposure, whereas the cytotoxicity endpoints are measured after 12–14 days of incubation following the 1-h exposure to the drug. Thus, some divergence may be anticipated if different cells require substantial time for maximal damage expression.

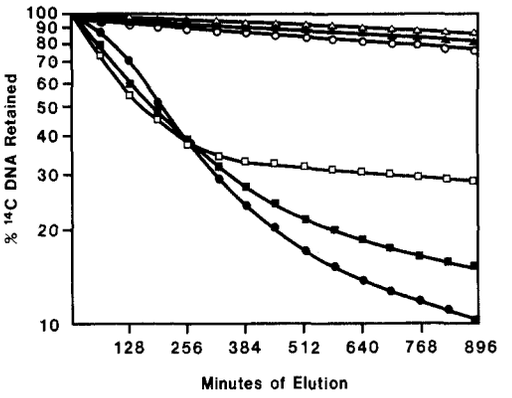


Figure 2. Alkaline elution assay for DNA single strand breaks in labeled L-1210 cells exposed 1 h to anthracene derivatives or X-rays: (○) control, (●) X-rays (6.5 cGy), (□) bisantrene, 1 μg/mL, (■) bisantrene, 10 μg/mL, (△) **6**, 1 μg/mL, (▲) **6**, 10 μg/mL.

Alkaline DNA Elution Studies. Both bisantrene and mitoxantrone cause single strand breaks in DNA, as determined by rapid elution of [^{14}C]thymidine-labeled DNA through 2.0-μm polyvinyl chloride filters.[25] This is typically compared to untreated controls, following alkaline treatment (pH 12.1) of labeled DNA isolated from L-1210 cells grown in the presence and absence of the drug.[26] Both mitoxantrone and bisantrene are known to cause DNA single strand breaks (SSB), which are either partially associated with proteins (**3**) or totally associated with proteins (**5**).[27] To see the SSB's, the elutions are typically preceded by a 1-h treatment with proteinase K (0.5 mg/mL; (Type XI fungal, E. Merck Co., Darmstadt, Germany) to remove the protein from such protein-associated SSB's. Analogue **6** was evaluated in this SSB assay with bisantrene and X-rays (6.5 cGy) as positive controls for drug-induced and radiation-induced DNA strand breaks, respectively. The results are shown in Figure 2. There was no evidence of DNA damage from **6** in any of triplicate experiments when it was given at doses that caused pronounced cytotoxicity. In contrast, bisantrene produced significant DNA scission at cytotoxic drug concentrations, comparable to that of X-rays. Additional alkaline DNA elution studies using L-1210 cells postirradiated with 30 cGy and no proteinase K after drug treatment gave no evidence for DNA–protein cross-links induced by **6**. (Data are not shown.) In this regard, it is important to recall that bisantrene-induced DNA single strand breaks are completely associated with such DNA–protein cross-links.[27] These intercalator-induced, protein-associated DNA breaks may represent aberrant DNA–topoisomerase II complexes.[28] Because **6** did not induce these lesions at cytotoxic concentrations, a different mechanism of action may be operating for these substituted anthracene derivatives.

Conclusions. The potent antitumor activity of compounds **6**, **19**, and certain analogues in human tumor colony-forming assays in vitro but marginal activity against L-1210 and P-388 leukemias in mice suggest that this type of anthracene derivative merits further study. However, it will be necessary to determine the factors responsible for differences in their in vitro activity against the estab-

(23) Venditti, J. M. *Cancer Chemother. Rep., Part 3* **1971**, *2*, 35.
(24) Bhuyan, B. K.; Fraser, T. J. *Cancer Res.* **1974**, *34*, 778.
(25) Kohn, K. W.; Ewig, R. A. G.; Erickson, L. C.; Ewelling, L. A. *DNA Repair*; Frieberg, E. C., Hanawalt, P. C., Eds.; Marcel Dekker: New York, 1981; Vol. 1, Part B, p 379.
(26) Ross, W. E.; Glaubiger, B. L.; Kohn, K. W. *Biochem. Biophys. Acta* **1978**, *519*, 23.
(27) Bowden, G. T.; Garcia, D.; Peng, Y. M.; Alberts, D. S. *Cancer Res.* **1982**, *42*, 2660.
(28) Yang, L.; Rowe, T. C.; Nelson, E. M.; Liv, L. F. *Cell* **1985**, *41*, 127.

Table VI. Inhibition of Macromolecular Synthesis by Anthracene Derivatives[a]

compd	concn, μg/mL	% inhibition of radiolabeled precursor uptake L-1210 mouse leukemia 8226 human myeloma cells					
		DNA	RNA	protein	DNA	RNA	protein
bisantrene	0.01	1.8	0	0	0	0	0
	0.1	9.3	24.1	5.6	33.3	21.0	20.2
	1*	43.6	21.7	48.5	79.9	91.6	41.9
	10**	66.8	96.1	89.0	97.7	92.9	63.5
mitoxantrone	0.01	0	0	0	1.8	0	0
	0.1*	55.0	30.8	11.9	1.4	0	13.5
	1**	71.7	67.5	40.4	49.3	86.9	36.5
	10**	93.2	97.8	94.1	67.6	92.8	59.5
6	0.01	0	0	0	0	0	(67.5)[b]
	0.3*	0	0	9.8	8.4	0	(218.5)
	0.5**	5.8	7.2	21.8	24.3	7.3	(63.5)
	1**	48.7	6.9	31.2	21.1	70.4	(45.9)
	10**	75.7	96.3	87.3	82.2	91.4	17.6

[a] Inhibition assays are based on a 1-h exposure time. Details of the assay are given in ref 20. (*) Exposures producing >50% cell kill. (**) Exposures producing >1 log cell kill. [b] Numbers in parentheses indicate an increase over control levels by the stated percentage.

lished tumor cell lines, fresh human tumors, and P-388 leukemia as evaluated in clonogenic assays. The fresh human tumors are more heterogeneous and therefore may contain both sensitive and resistant populations; however, this mechanism of drug resistance cannot explain the resistance of the P-388 leukemia cells in vitro to both compounds **6** and **19**. Other factors such as intracellular drug uptake or metabolism might determine the extent of resistance. On the other hand, in contrast to bisantrene and mitoxantrone, cytotoxic amounts of **6** do not inhibit DNA or RNA synthesis, or cause single strand breaks in DNA. Thus **6** and its congeners may have a unique mechanism of action, or a more prolonged time (>1 h) is required for expression of cytotoxic damage. This difference in mechanism might also determine their more limited spectrum of activity. Further studies on the DNA-binding properties of **6** and **19**, including spectrophotometric and Tm measurments and computer modeling, are in progress. The results of these studies and the data from the fresh human tumor assays will be published elsewhere.

The data from this study do provide new leads for improved design of cytotoxic drug screening. Human tumor cell lines can be used as a rapid and relatively inexpensive initial drug screening model. Those drugs found to be especially potent against human tumor cells can then be tested against P-388 leukemia and fresh human tumors by using clonogenic assays. Drugs that continue to express a high degree of potency (i.e., >50% inhibition of tumor colony growth) in these in vitro models could then be tested in vivo against P-388 leukemia and other mouse tumor models. On the basis of results of in vitro testing of our antracene compounds, it is unlikely that human tumor cell lines can accurately predict the in vivo cytotoxicity of myelosuppressive type drugs.

Experimental Section

Melting points were taken on a Mel-temp apparatus and are uncorrected. IR spectral determinations were obtained on a Beckman FT-1100 spectrophotometer. ^{1}H NMR spectra (90 MHz) and ^{13}C NMR spectra were obtained on a JEOL FX-90Q spectrometer. Elemental analyses were performed by the University of Arizona Microanalytical Center or by MicAnal Inc., Tucson, AZ, and are within ±0.4% of the calculated values, except where noted otherwise.

Method A. 9,10-Anthracenedicarboxaldehyde Di-1*H*-imidazol-2-ylhydrazone Dihydrochloride (7). Except for 150 mg, all of the gummy product from the preparation of **28** was dissolved in EtOH–H_2O (8:2, 10 mL) and heated to reflux. A solution of 9,10-anthracenedicarboxaldehyde (**27**, 115 mg, 0.49 mmol)[17] in $CHCl_3$ (16 mL) was added in 2-mL portions every 0.5 h, after it was confirmed (TLC) that there was no **27** remaining from the previous addition. Unreacted **27** was detected 0.5 h after the final 2-mL portion was added. At this time, the remaining mixture of **28** was added and refluxing was continued for 0.5 h. The $CHCl_3$ and EtOH were evaporated off, and the precipitate in the remaining H_2O was collected by filtration to yield 132 mg (57%) of an orange-brown solid.

Method B. 2,2′-(9,10-Anthrylenedimethylene)bis[2-imidazoline] Dihydrochloride (9). 9,10-Anthracenediacetonitrile (**30**, 400 mg, 1.56 mmol) (prepared by the procedure of Miller et al.[1] except that the composition of the reaction mixture solvent was modified to acetone–H_2O (5:1)) and ethylenediamine monotoluenesulfonic acid salt[29] (1.45 g, 6.24 mmol) was heated at 200 °C for 1 h. H_2O (4 mL) was added to the partly cooled reaction mixture, and after further cooling in an ice bath, the solid was collected and washed with cold H_2O. The solid was recrystallized from MeOH to give 715 mg (67%) of the product as the ditoluenesulfonic acid salt, an off-white solid: mp 280–282 °C. A suspension of the solid in H_2O (10 mL) and 10% NaOH (4 mL) was stirred for several minutes and filtered, and the solid was dried in vacuo to yield 332 mg (62%) of the free base. The dihydrochloride salt of the compound was prepared by dissolving the free base in MeOH that had been saturated previously with HCl gas and then precipitating the salt with Et_2O.

Method C. (*E*,*E*)-2,2′-(9,10-Anthrylenedivinylene)bis[2-imidazoline] Dihydrochloride (10). (a) (*E*,*E*)-9,10-Anthracenediacrylonitrile (31). NaH (225 mg, 4.75 mmol, 50% suspension in oil) was washed with petroleum ether and then suspended in THF (25 mL) at 0 °C. A solution of diisopropyl cyanomethylphosphonate (1.10 g, 5.35 mmol) in THF (25 mL) was added and the mixture was stirred at room temperature for 45 min. The clear solution was cooled to 0 °C and a solution of **27** (500 mg, 215 mmol) in THF (75 mL) was added over 15 min. The resulting green solution was stirred at room temperature for 3 h, and during that time a yellow precipitate formed. The mixture was poured into H_2O (300 mL) and extracted with $CHCl_3$ (4 × 100 mL). The combined $CHCl_3$ extract was washed with 10% NaOH (50 mL) and H_2O (50 mL), dried (Na_2SO_4), and concentrated in vacuo to 75 mL. The mixture was concentrated further to 25 mL on a hot plate and then cooled. The yellow solid which contained a small amount of cis product (^{1}H NMR) was recrystallized from $CHCl_3$ to give 436 mg (72%) of pure trans product: mp 337–339 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.32 (d, J = 16.7 Hz, 2), 7.65 (m, 4), 8.27 (m, 4), 8.68 (d, J = 16.7 Hz, 2). Anal. ($C_{20}H_{12}N_2$·0.075$CHCl_3$) C, H, N.

(b) (*E*,*E*)-2,2′-(9,10-Anthrylenedivinylene)bis[2-imidazoline] Dihydrochloride (10). A solution at 0 °C of **31** (100 mg, 0.36 mmol) in dry $CHCl_3$ (60 mL), containing 2 mL of EtOH, was saturated with HCl gas. The red solution was stirred at 0 °C for 4 h and then at room temperature overnight. Some imidate had precipitated and was collected by filtration. The remaining imidate was obtained by adding Et_2O (100 mL) to the filtrate. The solids were combined to give 145 mg (88%) of a yellow solid: mp 338–340 °C dec; ^{1}H NMR ($CDCl_3$–Me_2SO-d_6) δ 1.65 (t, 6), 4.71 (q, 4), 6.87 (d, J = 16.2 Hz, 2), 7.67 (m, 4), 8.26

(29) Oxley, P.; Short, W. F. *J. Chem. Soc.* **1947**, 497.

(m, 4), 8.86 (d, J = 16.2 Hz, 2).

A mixture of ethylenediamine (70 mg, 1.16 mmol) and the imidate (100 mg, 0.22 mmol) in EtOH (5 mL) was refluxed for 12 h under N_2. The mixture was cooled and the yellow solid was collected and dried in vacuo 2 h at 110 °C to give 69 mg (70%) of product.

Method D. (*E*,*E*)-*N*,*N*′-Bis(2-aminoethyl)-9,10-anthracenediacrylamide (11). (a1) Diethyl (*E*,*E*)-9,10-Anthracenediacrylate (32). The procedure of Murdock and co-workers[12] was used with THF as the solvent. ^{1}H NMR (Me_2CO-d_6) δ 6.36 (d, J = 16 Hz, 2), 8.55 (d, J = 16 Hz, 2).

(a2). To a refluxing mixture of Zn/Cu couple[30] (1.70 g) and **27** (500 mg, 2.1 mmol) in benzene (30 mL) was added dropwise ethyl bromoacetate (1.70 g, 10.1 mmol) in benzene (15 mL) over a 40-min period. Refluxing was continued an additional 40 min until the mixture became colorless. The mixture was cooled to room temperature and 10% H_2SO_4 (40 mL) was added slowly to decompose the couple. The mixture was filtered and the filter was washed with ether (20 mL). The phases of the combined filtrate and washing were separated, and the organic layer was washed with 5% H_2SO_4 (3 × 10 mL), H_2O (10 mL), saturated $NaHCO_3$ (10 mL), and H_2O (10 mL). The organic layer was dried (Na_2SO_4) and evaporated to a yellow oil. To the yellow oil in CH_2Cl_2 (20 mL) at 0 °C was added dropwise a solution of methanesulfonyl chloride (3.2 mL, 42 mmol) in CH_2Cl_2 (5 mL) over a 5-min period. A solution of triethylamine (8.8 mL, 63 mmol) in CH_2Cl_2 (10 mL) then was added dropwise over a 15-min period. The mixture was stirred an additional 15 min at 0 °C and then poured into ice H_2O (20 mL), and the layers were separated. The aqueous layer was acidified with 10% HCl (10 mL) and extracted with CH_2Cl_2 (5 mL). The combined CH_2Cl_2 layer was washed with 10% HCl (10 mL), saturated $NaHCO_3$ (10 mL), and H_2O 910 mL) and dried over Na_2SO_4. The material was chromatographed on silica gel and petroleum ether–ether (9:1) to yield 299 mg (38%) of a yellow solid identical with that obtained above.

(b) (*E*,*E*)-*N*,*N*′-Bis(2-aminoethyl)-9,10-anthracenediacrylamide (11). Diester **32** (100 mg, 0.27 mmol) and ethylenediamine (1 mL) were heated together at 110 °C. A precipitate began to form after 1 h. The mixture was evaporated to dryness, dissolved in hot MeOH (30 mL), filtered hot, and then concentrated to 10 mL. The cooled mixture was filtered and the yellow solid, 34 mg (31%), was dried 5 h at 110 °C in vacuo.

Method E. 1,1′-(9,10-Anthrylenedimethylene)bis[2-aminoimidazole] Dihydrochloride (12). To dry (CaH_2) Me_2SO (2 mL) under N_2 was added NaH (64 mg, 1.33 mmol, 50% oil dispersion), followed by a solution of 2-aminoimidazole (96 mg, 1.16 mmol) in dry Me_2SO (3 mL). After 10 min **33** (144 mg, 0.53 mmol) was added and the mixture was stirred overnight. The black mixture was poured into ice water (50 mL) and the precipitate was collected and washed with H_2O and then hexanes. The solid was dissolved in MeOH that had been saturated previously with HCl gas. The solution was treated with charcoal and filtered through Celite, and the product was precipitated from the filtrate by the addition of Et_2O. The solid was collected by filtration and dried in vacuo to yield 135 mg (58%) of a tan solid.

Method F. *N*,*N*′-Bis[2-(dimethylamino)ethyl]-9,10-anthracenebis(methylamine) Fumarate (1:2) (6). A mixture of **33** (50 g, 0.18 mol) and *N*,*N*-dimethylethylenediamine (144 g, 1.64 mol) in toluene (500 mL) was refluxed under N_2 for 6 h. The resulting solution was cooled in an ice bath, and the crystals that formed were removed by filtration. The filtrate was extracted with 5% NaOH (2 × 400 mL) and H_2O (2 × 250 mL), dried over Na_2SO_4, and evaporated to an oil, which was kept under high vacuum at 40 °C for 1 h to yield 67.9 g of a yellow-orange oil.

To a stirred solution of the oil in EtOH (1 L) at 40–50 °C was added dropwise a solution of fumaric acid (41.8 g, 0.36 mol) in EtOH (1.5 L). The mixture was stirred at 40–50 °C for 15 min following the completion of the addition and then cooled finally to 0 °C. The solid was collected by filtration, washed with 100 mL of cold EtOH, and dried for 5 h at 110 °C in vacuo to yield 87.8 g (79%) of a white solid.

Method G. *N*,*N*′-Bis[2-(2-hydroxyethoxy)ethyl]-9,10-anthracenebis(methylamine) Fumarate (1:1) (23). 2-(2-Aminoethoxy)ethanol (900 mg, 8.56 mmol) was converted to the hydrochloride salt by dissolving it in EtOH saturated with HCl gas and evaporating the solution to dryness. To the residue were added 2-(2-aminoethoxy)ethanol (1.35 g, 12.84 mmol) and **27** (500 mg, 2.14 mmol) in 60 mL of EtOH. After the mixture was stirred at room temperature for 3 h, $NaBH_3CN$ (300 mg, 4.88 mmol) was added and the stirring was continued for an additional 24 h. The flask was cooled to 0 °C and 15 mL of 1 N HCl was slowly added. The EtOH was evaporated off and the aqueous solution was basified with 5% NaOH and extracted with $CHCl_3$ (3 × 25 mL). The combined $CHCl_3$ layer was washed with H_2O (10 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from $CHCl_3$ to give 833 mg (95%) of a light yellow solid. The fumarate salt was prepared as described for **6** by adding a solution of fumaric acid (116 mg, 1.0 mmol) in 5 mL of EtOH to a warm solution of the free base (408 mg, 1.0 mmol) in 10 mL of EtOH. The resulting precipitate was collected and dried 1 h at 110 °C in vacuo to yield 450 mg (82%) of the fumarate salt.

2-Hydrazinoimidazole Dihydrochloride (28). To concentrated HCl (8 mL) at 0 °C was added 2-aminoimidazole sulfate (1.0 g, 3.78 mmol), and a solution of $NaNO_2$ (600 mg, 8.68 mmol) in H_2O (2 mL) was added under the surface, while the temperature was kept between −10 and −5 °C. The mixture was stirred 20 min at 0 °C while a solution of sulfurous acid was prepared by saturating H_2O (20 mL) with SO_2 gas at 0 °C. A brisk stream of gas was maintained while the cold diazonium salt solution was added over a 15-min period to the sulfurous acid. The ice bath was removed and the SO_2 was bubbled into the solution for 0.5 h. After being allowed to stand overnight at room temperature, the solution was concentrated until a precipitate formed. The mixture was filtered, and the filtrate was evaporated to a sticky gum. The gum was dissolved in H_2O–EtOH (8:2, 40 mL) and treated with Bio-Rad AG1-X8 200–400-mesh (OH) resin until the pH was between 8 and 9. The resin was removed by filtration and washed with H_2O–EtOH (8:2, 50 mL), and the combined filtrate and washing was acidified with excess concentrated HCl and evaporated to a sticky gum. Since 2-aminoimidazole does not completely diazotize,[31,32] this material is a mixture of 2-hydrazinoimidazole and 2-aminoimidazole.

***N*,*N*′-[9,10-Anthrylenebis(methyleneimidazole-1,2-diyl)]-bis[acetamide] Dihydrochloride (34).** An aqueous solution of **12** (50 mg, 0.11 mmol) was basified with dilute NaOH, and the precipitate was collected and dried in vacuo overnight. The solid in Ac_2O (1 mL) was heated at 50–60 °C for 45 min. The mixture was treated with dilute NaOH and the gummy solid was collected, washed with H_2O, and dried. The solid was treated as described in method E with MeOH that had been saturated previously with HCl gas, to yield 15 mg of a hygroscopic solid after drying at 110 °C in vacuo for 5 h: mp 223–225 °C dec, mixture melting point with material prepared by method E with 2-(acetylamino)-imidazole hydrochloride[20] 223–225 °C dec.

***N*,*N*′-Bis(cyanomethyl)-9,10-anthracenebis(methylamine) (35).** NaH (349 mg, 7.28 mmol, 50% suspension in oil) was washed with petroleum ether and then stirred for 10 min in Me_2SO (10 mL) under N_2. Aminoacetonitrile hydrochloride (670 mg, 7.28 mmol) was added slowly, and the mixture was stirred for 5 min. Compound **33** (500 mg, 1.82 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was filtered into 60 mL of ice water. The solid that formed in the filtrate was collected by filtration and recrystallized twice from acetone. The solid was dried 5 h at 110 °C in vacuo to give 184 mg (32%): mp 186–188 °C; ^{1}H NMR (Me_2SO-d_6) δ 3.80 (d, 4), 4.70 (d, 4), 7.60 (m, 4), 8.45 (m, 4), 2.04 (s, acetone), due to H_2O in the NMR solvent the 2 NH protons were hidden by the H_2O peak. Anal. ($C_{20}H_{18}N_4$·0.4$(CH_3)_2CO$) C, H, N.

9,10-Anthracenebis(methylamine) Toluenesulfonate (1:2) (36). A mixture of **35** (100 mg, 0.32 mmol) and ethylenediamine monotoluenesulfonic acid salt[29] (600 mg, 2.56 mmol) was heated at 190–195 °C for 45 min. The resulting solid was treated with 5 mL of *i*-PrOH and cooled to room temperature. The solid was collected and then recrystallized from *i*-PrOH to give 172 mg

(30) LeGoff, E. *J. Org. Chem.* **1964**, *29*, 2048.

(31) Beaman, A. G.; Tautz, W.; Gabriel, T.; Duschinsky, R. *J. Am. Chem. Soc.* **1965**, *87*, 389.

(32) Agrawal, K. C.; Bears, K. B.; Sehgal, R. K.; Brown, J. N.; Rist, P. E.; Rupp, W. D. *J. Med. Chem.* **1979**, *22*, 583.

(72%) of a tan solid: mp 320–322 °C dec; IR (KBr) no CN; ^{1}H NMR (Me_2SO-d_6–TFA-d) δ 2.30 (s, 6), 5.20 (s, 4), 7.20 (d, 4), 7.65 (d, 4), 7.80 (m, 4), 8.60 (m, 4).

3,3′-[9,10-Anthrylenebis(methylidenehydrazino)]dipropionitrile (37). Acrylonitrile (0.95 mL, 14.43 mmol) was added to hydrazine hydrate (755 mg, 12.98 mmol) while the temperature was kept below 35 °C with the aid of occasional cooling with an ice bath.[33] The mixture was stirred at room temperature for 2 h, and then any unreacted starting materials were removed by evaporating the solution at 40 °C (35 mmHg). A solution of the residue and **27** (975 mg, 4.17 mmol) in EtOH (25 mL) was stirred at room temperature for 3 h and then refluxed for 1 h. The mixture was cooled and filtered, and the solid was dried 2 h at 110 °C in vacuo to yield 1.35 g (88%) of yellow solid: mp 156–158 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.85 (t, 4), 3.60 (q, 4), 7.55 (m, 4), 7.85 (t, 2), 8.55 (m, 4), 8.80 (s, 2). Anal. ($C_{22}H_{20}N_6$) C, H, N.

Alkaline Elution Assays.[25] One million L-1210 cells in logarithmic growth, prelabeled with [^{14}C]thymidine (0.1 μCi/mL of 55 mCi/mmol activity), were exposed to drug for 1 h, then washed in fresh medium (RPMI 1640, Gibco N.Y.), and irradiated on ice with 6.5 or 30 cGy (Varian Linac 4 mEv) for SSB or DNA–protein cross-link assays, respectively. The cells were then loaded on 2.0 μm, 25 mm PVC filters (Millipore Corp, San Francisco, CA) and lysed with pH 10.0 solution of 0.2% *N*-laurolysarcosine. Proteinase K (0.5 mg/mL) (E. Merck Co, Darmstadt, Germany) was then pumped through the filters for 1 h (2.5 mL/h in SSB assays. DNA from the cells was then eluted over 16 h at 2.5 mL/h with a pH 12.1 solution of tetrapropylammonium hydroxide (Eastman Organic Chemicals, Rochester, NY) and 1% sodium dodecyl sulfate. Hourly fractions were collected and ^{14}C radioactivity was counted by scintillation in three volumes of scintillation fluid. The rapidly eluting fractions (DNA SSB's) were compared to the percent of radioactivity ([^{14}C]DNA) retained on the filter (nonsheared DNA).

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Registry No. **6**, 108365-87-3; **6**·2fumarate, 108366-14-9; **7**, 108365-88-4; **7**·2HCl, 108394-56-5; **8**, 108365-89-5; **9**, 108365-90-8; **9**·2HCl, 108366-10-5; **9**·2H_3C-*p*-$C_6H_4SO_3H$, 108366-26-3; **10**, 108365-91-9; **10**·2HCl, 108366-11-6; **11**, 108365-92-0; **11**·2HCl, 108366-12-7; **12**, 108365-93-1; **12**·2HCl, 108366-13-8; **13**, 78363-52-7; **14**, 108365-94-2; **14**·2fumarate, 108366-15-0; **15**, 106712-13-4; **15**·2fumarate, 108366-16-1; **16**, 108365-95-3; **16**·fumarate, 108394-57-6; **17**, 108365-96-4; **17**·fumarate, 108366-17-2; **18**, 108365-97-5; **18**·fumarate, 108366-18-3; **19**, 108365-98-6; **19**·fumarate, 108366-19-4; **20**, 108365-99-7; **20**·2fumarate, 108366-20-7; **21**, 108366-00-3; **21**·2citrate, 108366-21-8; **22**, 108366-01-4; **22**·fumarate, 108366-22-9; **23**, 108366-02-5; **23**·fumarate, 108366-23-0; **24**, 108366-03-6; **24**·fumarate, 108366-24-1; **25**, 108366-04-7; **25**·fumarate, 108366-25-2; **26**, 19926-09-1; **27**, 7044-91-9; **27** (ethyl acetate deriv.), 108366-28-5; **28**, 59214-44-7; **29**, 30216-51-4; **30**, 62806-30-8; **31**, 108366-05-8; **32**, 108366-06-9; **32** (imidate, 108366-27-4; **33**, 10387-13-0; **34**, 108365-86-2; **34**·2HCl, 108366-07-0; **35**, 108366-08-1; **36**, 6705-67-5; **37**, 108366-09-2; H_2NCH_2CN·HCl, 6011-14-9; H_2C=CHCN, 107-13-1; 4-$H_2N(CH_2)_2NHO_3SC_6H_4Me$, 14034-59-4; $NCCH_2P(O)(OPr\text{-}i)_2$, 58264-04-3; $H_2N(CH_2)_2NH_2$, 107-15-3; $BrCH_2CO_2Et$, 105-36-2; $H_2N(CH_2)_2NMe_2$, 108-00-9; $H_2N(CH_2)_2N(Pr\text{-}i)_2$, 121-05-1; $MeNH(CH_2)_2NMe_2$, 142-25-6; $MeNH(CH_2)_2NHMe$, 110-70-3; $HO(CH_2)_2NH(CH_2)_2NH_2$, 111-41-1; $H_2N(CH_2)_3NMe_2$, 30734-81-7; $H_2N(CH_2)_2O(CH_2)_2OH$, 929-06-6; $H_2N(CH_2)_4OH$, 13325-10-5; $H_2N(CH_2)_5OH$, 2508-29-4; 2-amino-imidazole, 7720-39-0; *N*-methyl-piperazine, 109-01-3; *N*-ethyl-3-aminopiperidine, 6789-94-2; *N*-(2-aminoethyl)morpholine, 2038-03-1; 2-aminoimidazole sulfate, 42383-61-9.

(33) Dorn, H.; Zubek, A. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 39.
(34) McKay, A. F.; Gilpin, J. R. *J. Am. Chem. Soc.* **1956**, *78*, 486.
(35) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1.
(36) Dorr, R. T.; Alberts, D. S.; Chen, H-S. G. *J. Pharmacol. Methods* **1980**, *4*, 237.

Antianaphylactic Benzophenones and Related Compounds

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The synthesis and biological properties of 85 benzophenones and related compounds are described. The majority of the compounds inhibit the release of leukotrienes (LT) C_4 and D_4 in vitro from sensitized guinea pig chopped lung. In addition, some of the compounds inhibited the release of LTs from passively sensitized human chopped lung and protected guinea pigs from the effects of anaphylaxis in a modified Herxheimer test.

To our knowledge, until the work described herein was undertaken, the possibility that 2-hydroxybenzophenones could be antiasthmatic agents had not been considered, despite the fact that compounds of this type contain structural features, namely, an oxygen atom ortho to an aromatic carbonyl group, that are found in the antiasthmatic agent disodium cromoglycate. Work in another field[1] made available a number of 2-hydroxybenzophenones and their oximes, which were submitted for screening in tests designed to reveal antianaphylactic activity.[2] This revealed that oxime **6a** and ketone **20** (Table I) possessed interesting antiallergic activity, and these two "lead" compounds formed the basis of the study reported here. Subsequent to the completion of this work, a paper[3] has appeared claiming *antiinflammatory* activity in a limited number of benzophenones, including 2-hydroxy derivatives.

Chemistry. The compounds were prepared by standard methods of organic chemistry. Further details are to be found in Tables I–III and the Experimental Section.

Antianaphylactic Activity. The following tests were used for the detection of antiallergic activity: (1) the guinea pig chopped lung test (in vitro) (GPCL),[4,5] (2) the

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(1) Saunders, J. C.; Williamson, W. R. N, *J. Med. Chem.* **1979**, *22*, 1554.
(2) Ross, W. J.; Harrison, R. G.; Jolly, M. R. J.; Neville, M. C.; Todd, A.; Verge, J. P.; Dawson, W.; Sweatman, W. J. F. *J. Med. Chem.* **1979**, *22*, 412.
(3) Barton, H. *Pol. J. Pharmacol. Pharm.* **1979**, *31*, 169.
(4) Mongar, J. L.; Schild, H. O. *J. Physiol. (London)* **1956**, *131*, 207.

0022-2623/87/1830-1321$01.50/0