with ether 3 times, and the ether extracts were combined, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by chromatography on a silica gel column and eluted with $CHCl_3$–EtOAc (9:1, v/v) mixed solvent. Fractions with R_f 0.65 on silica gel plate (same solvent system) were pooled and evaporated to dryness to yield 11 g (64%) of yellow oil. Samples for elemental analyses were purified by preparative TLC: NMR ($CDCl_3$) δ 1.22 (6 H, t, CH_3, J = 3.5 Hz), 1.75 (2 H, CH_2, m), 2.60 (2 H, CH_2, m), 3.08 (2 H, t, NH_2, J = 3.0 Hz), 4.24 (4 H, q, OCH_2, J = 3.5 Hz), 4.92 (2 H, s, OCH_2), 6.15 (3 H, m, aromatic), 6.98 (1 H, m, aromatic), 7.25 (5 H, s, aromatic), 7.65 (4 H, m, aromatic); mass spectrum, m/z (relative intensity) 544 (M^+·, 56), 212 (100), 122 (12), 104 (8.0), 91 (75). Anal. ($C_{31}H_{32}N_2O_7$) C, H, N.

***N*-(γ,γ-Dicarbethoxy-γ-aminobutyl)-*m*-(benzyloxy)aniline (19b).** This compound, a yellow oil, was prepared on a 5.5-g scale (95%) from **18b** by the same procedure used for **19a**: mp for the dihydrochloride 170 °C (decomposed); NMR ($CDCl_3$) δ 1.22 (6 H, t, CH_3, J = 3.5 Hz), 1.90 (4 H, m, CH_2CH_2), 2.65 (3 H, s, NH_2, NH), 3.06 (2 H, t, CH_2, J = 3.0 Hz), 4.15 (4 H, q, OCH_2, J = 3.5 Hz), 4.95 (2 H, s, OCH_2), 6.18 (3 H, m, aromatic), 6.95 (1 H, m, aromatic), 7.30 (5 H, s, aromatic). Anal. ($C_{23}H_{30}N_2O_5$·2HCl·0.5H_2O) C, H, N, Cl.

***N*$^{\delta}$-(*m*-Hydroxyphenyl)ornithine (1c).** Amine **19b** (9.5 g, 22.9 mmol) was refluxed in 100 mL of 0.5 N KOH for 4 h. After the solution cooled, the precipitates were collected and washed with a small amount of ethanol. The potassium salt was dissolved in 20 mL of water, acidified with concentrated HCl, and refluxed for 1.5 h. The solvent was evaporated to dryness, and the residue was suspended in 30 mL of MeOH and filtered to remove the potassium chloride. The filtrate was again evaporated to dryness, and the residue was dissolved in 30 mL of concentrated HCl and stirred at room temperature for 3 h. The reddish solution was extracted with $CHCl_3$ twice, and the aqueous layer was evaporated to dryness. The crude product was purified on a C_{18} reverse-phase column, using H_2O as eluent. The front band, R_f 0.55 (H_2O), fractions were pooled and lyophilized to give 0.7 g (21%) of the final product, **1c**. This compound is very hygroscopic and gives no specific melting point: mass spectrum (as $4Me_3Si$), m/z (relative intensity) 512 (M^+·, 22), 497 (M – CH_3, 1.7), 395 (M – CO_2Me_3Si, 3.1), 266 (33), 253 (17), 142 (66), 73 (100). Anal. ($C_{11}H_{18}N_2O_3Cl_2$·$^2/_3H_2O$) C, H, N, Cl.

In Vitro Growth Assays. A. Melanotic Melanoma (M_2R) and Amelanotic Melanoma (A_2). Toxicity was assayed by growth inhibition of two melanoma cell lines, M_2R and A_2. M_2R is a melanotic cell line developed from B16 murine melanoma and was obtained from J. P. Mather.[19] A_2 is an amelanotic cell line cloned from M_2R and has a 27-fold lower tyrosinase specific activity than M_2R[16] as assayed by the tyrosine hydroxylation method of Pomerantz.[20]

For growth studies, 1×10^4 cells suspended in growth medium (a 1:1 mixture of Dulbecco's modified Eagle's MEM and Ham's F-12 with 1.2 g/L of $NaHCO_3$, 15 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid, and 10% fetal bovine serum) were seeded into 60-mm tissue-culture dishes. Incubations were at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 24 h, the medium was aspirated off from above the cell monolayer and replaced with 4 mL of fresh medium containing various concentrations of the test substances. Growth was followed for at least 3 additional days by detaching the cells with trypsin and determining the cell number on a Coulter cell counter. Growth comparisons were made at a time when the control cultures had increased 8-fold, according to the following formula:

$$\text{growth (\% of control)} = \frac{\text{net increase in cell number of test culture}}{\text{net increase in cell number of control culture}} \times 100$$

B. Human Epidermoid Carcinoma of the Nasopharynx and P-388 Murine Leukemia. These assays were carried out by the screening program of the Developmental Therapeutic Program of NCI.

In Vivo Antitumor Testing. Antitumor activity was determined as percent T/C values, with T/C ≥ 125% defined as statistically significant. Dose–response studies were carried out for each compound according to published National Cancer Institute protocols.[18] Treatment begins 24 h after intraperitoneal (ip) tumor implant on days 1–9 with intraperitoneal doses of the compound under investigation. Normal saline (0.9% NaCl) was used as a vehicle.

(19) J. P. Mather and G. H. Sato, *Exp. Cell Res.*, **120**, 191 (1979).
(20) S. H. Pomerantz, *Science*, **164**, 838 (1969).

Antitumor Agents. 2.[1] Bisguanylhydrazones of Anthracene-9,10-dicarboxaldehydes

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9,10-Anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] (bisantrene, VI-1) showed anticancer activity in mice vs. both leukemias and solid tumors. Increases in life span vs. the following neoplasms were: P-388 leukemia, 137%; B-16 melanoma, 122%; Lieberman plasma cell tumor, >85%; colon tumor 26, 150%; Ridgway osteogenic sarcoma, 85%. There were significant numbers of long-term survivors. Both DNA and RNA synthesis were strongly inhibited. The drug was resistant to biodegradation and was bound strongly to tissues; in monkeys the half-life for disappearance from serum was 6 days. Related hydrazones were synthesized, and structure–activity relationships are discussed. Two routes to ring-substituted anthracene-9,10-dicarboxaldehyde intermediates were developed.

The compounds which have been shown to bind to DNA by intercalation[2] have generally been condensed tricyclic aromatics with at least one basic function. Since several of these compounds are active as antitumor agents,[2,3] we synthesized and tested widely differing polycyclic aromatics with various basic side chains. We recently reported the synthesis and antitumor properties of one of these compounds.[1,4] It has been named mitoxantrone (1) and is now undergoing clinical trials.[5]

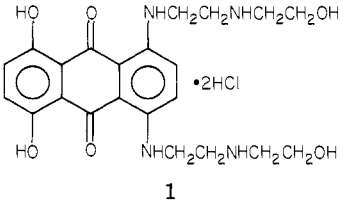

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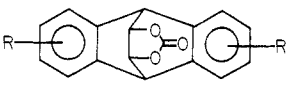
Table I. Cyclic Carbonates of *cis*-9,10-Dihydro-9,10-ethanoanthracene-11,12-diols



no.	R	method	% yield	mp, °C	formula	anal.[a] or lit. mp, °C
I-1	H	A	94	260-262	$C_{17}H_{12}O_3$	259-260[b]
I-2	1,2-benzo	A	80	220-224	$C_{21}H_{14}O_3$	219-224[c]
I-3	2-methyl[d]	A	24	225-227	$C_{18}H_{14}O_3$	C, H
I-4	2-methyl[d]	A	51	183-185	$C_{18}H_{14}O_3$	C, H
I-5	9-cyano	A	83	203-205	$C_{18}H_{11}NO_3$	C, H, N
I-6	9-methyl	A	75	253-254	$C_{18}H_{14}O_3$	C, H
I-7	1,4-dimethoxy[d]	A	50	283-285	$C_{19}H_{19}O_5 \cdot 0.25H_2O$	C, H
I-8	1,4-dimethoxy[d]	A	25	255-260	$C_{19}H_{16}O_5 \cdot 0.5H_2O$	C, H
I-9	2,3-benzo	A	11	250-251	$C_{21}H_{14}O_3$	C, H
I-10	1,4-diacetoxy	A	63	275-277	$C_{21}H_{16}O_7$	C, H
I-11	2,3-dimethyl	A	60	207-212	$C_{19}H_{16}O_3$	C, H
I-12	1,4-dimethyl	A	94	225-245	$C_{19}H_{16}O_3 \cdot 0.25H_2O$	C, H
I-13	2-*tert*-butyl	A	47	250-252	$C_{21}H_{20}O_3 \cdot 0.25H_2O$	C, H
I-14	2,6-difluoro	A	45	240-245	$C_{17}H_{10}F_2O_3$	C, H, F
I-15	1-chloro	A	90	242-250	$C_{19}H_{16}ClO_3$	H, Cl; C[e]
I-16	9,10-dimethyl	A	85	280-281	$C_{19}H_{16}O_3$	C, H
I-17	2-chloro	A	83	200-230	$C_{17}H_{11}ClO_3$	C, H, Cl
I-18	2-acetamido	A	66	268-271	$C_{19}H_{15}NO_4$	C, H, N

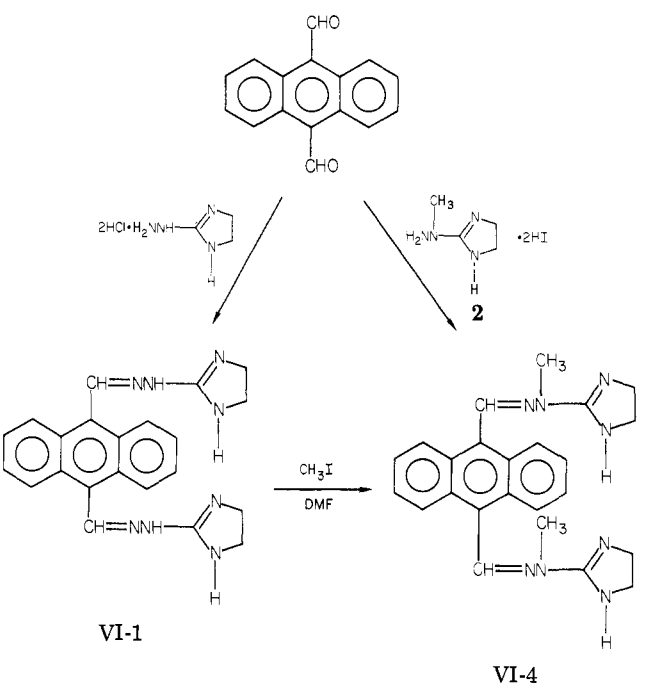
[a] Analytical results were within ±0.4% of the theoretical values for all elements listed, except as shown in subsequent footnotes. [b] Reference 7. [c] Reference 8. [d] Syn (or anti) racemic mixtures; the racemic mixtures I-3, I-4, I-7, and I-8 were obtained by fractional crystallization of the crude reaction mixtures from CH_2Cl_2–CH_3OH (1:3). [e] C: calcd, 68.3; found, 67.7.

Another compound synthesized as a potential DNA intercalator was 9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1H-imidazol-2-yl)hydrazone] dihydrochloride (VI-1, bisantrene hydrochloride; Scheme I). It inhibited splenomagaly in mice with Rauscher leukemia and showed a high degree of anticancer activity against both P-388 leukemia and B-16 melanoma in mice. The corresponding monosubstituted compound, 9-anthracenecarboxaldehyde (4,5-dihydro-1*H*-imidazol-2-yl)hydrazone hydrochloride, was inactive. Therefore, a broad synthetic program was initiated to prepare "two-armed" compounds analogous to VI-1.

This report describes the synthesis of a variety of -C=N- compounds derived from 9,10-anthracenedicarboxaldehyde and ring-substituted analogues and includes a discussion of the structure–activity relationships among these compounds and some older antitumor agents.

Chemistry. Anthracenedicarboxaldehydes formed bisguanylhydrazones (e.g., VI-1, Scheme I) readily and completely when the requisite aminoguanidines were used as their dihydrohalide salts. However, many aminoguanidines were available as monohydrohalides. With these salts, reaction was incomplete unless a 2nd equiv of acid was added. Methylation of VI-1 with excess methyl iodide in DMF gave a single product, VI-4. The indicated exocyclic N-methylation in VI-4 was established by an alternative synthesis from methylhydrazinoimidazoline, **2**.

Scheme I



The preparation of ring-substituted analogues of lead compound VI-1 required as intermediates the corresponding substituted 9,10-anthracenedicarboxaldehydes. Some previously described syntheses[6] of these dialdehydes were not satisfactory, so two other methods were developed as outlined in Scheme II.

Newman and co-workers[7,8] treated anthracene and sev-

(1) For part 1 of this series, see K. C. Murdock, R. G. Child, P. F. Fabio, R. B. Angier, R. E. Wallace, F. E. Durr, and R. V. Citarella, *J. Med. Chem.*, **22**, 1024 (1979); K. C. Murdock, and F. E. Durr, U.S. Patent 4 197 249 (1980).
(2) S. Neidle, *Progr. Med. Chem.* **16**, 151 (1979); H. S. Schwartz, *Adv. Cancer Chemother.*, **1**, 1 (1979).
(3) B. F. Cain and G. J. Atwell, *Eur. J. Cancer*, **10**, 539 (1974); M. Rozencweig, D. D. Von Hoff, R. L. Cysyk, and F. M. Muggia, *Cancer Chemother. Pharmacol.*, **3**, 135 (1979).
(4) R. E. Wallace, K. C. Murdock, R. B. Angier, and F. E. Durr, *Cancer Res.*, **39**, 1570 (1979).
(5) D. D. Von Hoff, E. Pollard, J. Kuhn, E. Murray, and C. A. Coltman, *Cancer Res.*, **40**, 1516 (1980); D. S. Alberts, K. S. Griffith, G. E. Goodman, T. S. Herman, and E. Murray, *Cancer Chemother. Pharmacol.*, **5**, 11 (1980).
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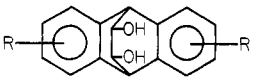
(7) M. S. Newman and R. W. Addor, *J. Am. Chem. Soc.*, **77**, 3789 (1955).

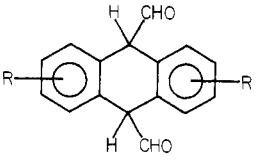
Table II. *cis*-9,10-Dihydro-9,10-ethanoanthracene-11,12-diols



no.	R	method	% yield	mp, °C	formula	anal.[a] or lit. mp, °C
II-1	H	B	89	202-204	$C_{16}H_{14}O_2$	202-203[b]
II-2	1,2-benzo	B	90	196-198	$C_{20}H_{16}O_2$	196-198[c]
II-3	2-methyl[d]	B	95	227-228	$C_{17}H_{16}O_2$	C, H
II-4	2-methyl[d]	B	76	153-156	$C_{17}H_{16}O_2$	C, H
II-5	9-methyl	B	92	138-140	$C_{17}H_{16}O_2$	C, H
II-6	1,4-dimethoxy[d]	B	81	187-188	$C_{18}H_{18}O_4$	C, H
II-7	1,4-dimethoxy[d]	B	84	189-192	$C_{18}H_{18}O_4 \cdot 0.25H_2O$	C, H
II-8	1,4-dihydroxy	B	80	280-285	$C_{16}H_{14}O_4 \cdot H_2O$	C, H
II-9	2,3-benzo	B	58	238-241	$C_{20}H_{16}O_2$	C, H
II-10	2,3-dimethyl	B	69	225-230	$C_{18}H_{18}O_2$	H; C[e]
II-11	1,4-dimethyl	B	96	158-160	$C_{18}H_{18}O_2$	C, H
II-12	2-*tert*-butyl	B	97	198-200	$C_{20}H_{22}O_2$	C, H
II-13	2,6-difluoro	B	81	139-141	$C_{16}H_{12}F_2O_2$	C, H; F[f]
II-14	1-chloro	B	85	180-182	$C_{16}H_{13}ClO_2$	C, H, Cl
II-15	9,10-dimethyl	B	96	201-203	$C_{18}H_{18}O_3$	C, H
II-16	2-chloro	B	75	195-210	$C_{16}H_{13}ClO_2$	C, H, Cl

[a-d] See footnotes *a-d* in Table I. [e] C: calcd, 81.2; found, 80.5. [f] F: calcd, 13.9; found, 13.2

Table III. 9,10-Dihydro-9,10-anthracenecarboxaldehydes



no.	R	method	% yield	mp, °C	formula	anal.[a]
III-1	H (cis)	C & D	94	144-146	$C_{16}H_{12}O_2$	C, H
III-2	H (trans)	E[b]	77	132-135	$C_{16}H_{12}O_2$	C, H
III-3	1,2-benzo (cis)	C	50	170-172	$C_{20}H_{14}O_2$	C, H
III-4	2-methyl (cis)	D	96	125-126	$C_{17}H_{14}O_2$	C, H
III-5	1,4-dimethoxy (cis)	C	28	130-140	$C_{18}H_{16}O_4$	C, H
III-6	2,3-benzo (cis)	C	39	170-172	$C_{20}H_{14}O_2$	C, H
III-7	2,3-dimethyl (cis)	D	66	113-117	$C_{18}H_{16}O_2$	H; C[c]
III-8	1,4-dimethyl (cis)	D	95	159-160	$C_{18}H_{16}O_2$	C, H
III-9	1-chloro (cis)	C	34	144-146	$C_{16}H_{11}ClO_2 \cdot 0.25H_2O$	C, H, Cl
III-10	9,10-dimethyl (cis)	C	96	193-195	$C_{18}H_{16}O_2$	C, H
III-11	2-chloro (cis)	C	33	113-115	$C_{16}H_{11}ClO_2 \cdot 0.25H_2O$	C, H, Cl
III-12	1,5-dichloro[d]	E	55	179-188	$C_{16}H_{10}Cl_2O_2$	C, H, Cl

[a] Analytical results were within ±0.4% of the theoretical values for all elements listed, except as shown in subsequent footnotes. [b] See ref 9. [c] C: calcd, 81.8; found, 81.0. [d] Reference 40.

eral derivatives, **3**, with vinylene carbonate. Subsequent hydrolysis gave cyclic diols **5**, which were then oxidized to dicarboxylic acids[7] or dihydrodialdehydes **6**.[8] We found that a slight modification of their lead tetraacetate oxidation procedure smoothly converted cyclic diols **5** to aromatic dialdehydes **7** in generally satisfactory yields. In addition, it was found that aqueous sodium periodate under controlled conditions oxidized cyclic diols **5** exclusively to the dihydro dialdehydes **6**, which could then be oxidized to the aromatic dialdehydes **7** by mild oxidizing agents. In two cases, as shown in Tables I and II, the cyclic carbonates **4** and cyclic diols **5** were separated into syn and anti isomers. However, such separations were not required, since mixtures of isomers were also converted to the symmetrical aromatic dialdehydes **7**.

A second dialdehyde synthesis utilized the more readily available anthraquinones rather than anthracenes and has been described in detail by Lin and co-workers.[9a] Reaction of anthraquinones **8** with dimethylsulfonium methylide gave dioxiranes **9**, which were then rearranged to 10-(hydroxymethyl)anthracene-9-carboxaldehydes, **10**, with LiBr in acetonitrile. Oxidation of **10** with Me_2SO then gave the desired aromatic dialdehyde **7**. Rearrangement of oxiranes **9** with BF_3 gave dihydrodialdehydes **6**.

Structure-Activity Relationships. Thirty different aminoguanidine derivatives of 9,10-anthracenedicarboxaldehyde are listed in Table VI. All but four (VI-

(8) M. S. Newman and Z. U. Din, *J. Org. Chem.*, **36**, 966 (1971).

(9) (a) Y.-i. Lin, S. A. Lang, Jr., C. M. Seifert, R. G. Child, G. O. Morton, and P. F. Fabio, *J. Org. Chem.*, **44**, 4701 (1979). (b) Dimethyl derivative i was prepared from VI-4 by method X (93% yield), mp 240-245 °C dec. Anal. ($C_{26}H_{30}N_8 \cdot 2HI \cdot 0.5H_2O$) C, H, N.

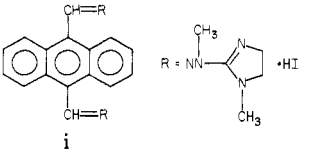


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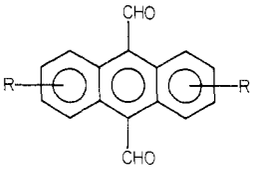
Table IV. 9,10-Anthracenedicarboxaldehydes



no.	R	method	% yield	mp, °C	formula	anal.[a] or lit. mp, °C
IV-1	H	F[b]	85	245-247	$C_{16}H_{16}O_2$	244-245[c]
IV-2	1,2-benzo	F	75	193-198	$C_{20}H_{12}O_2$	C, H
IV-3	2-methyl	F	76	162-164	$C_{17}H_{12}O_2$	C, H
IV-4	1,4-dimethoxy	F	5	208-212	$C_{18}H_{14}O_4 \cdot 0.25H_2O$	C, H
IV-5	2,3-benzo	F	20	215-217	$C_{20}H_{12}O_2$	C, H
IV-6	2,3-dimethyl	F	47	203-204	$C_{18}H_{14}O_2$	C, H
IV-7	1,4-dimethyl	F	10	158-162	$C_{18}H_{14}O_2 \cdot 0.75H_2O$	C, H
IV-8	2-*tert*-butyl	F	31	125-126	$C_{20}H_{18}O_2$	C, H
IV-9	2,6-difluoro	F	32	240-242	$C_{16}H_8F_2O_2 \cdot 1.5H_2O$	C, H; F[d]
IV-10	2-ethyl	G	25	99-100	$C_{18}H_{14}O_2$	H; C[e]
IV-11	1-chloro-2-methyl	G	90	175-177	$C_{17}H_{11}ClO_2$	*f*
IV-12	1-chloro	F	96	186-189	$C_{16}H_9ClO_2$	C, H
IV-13	2-chloro	F	94	193-196	$C_{16}H_9ClO_2 \cdot 0.25H_2O$	C, H
IV-14	1-fluoro	G	21	226-238	$C_{16}H_9FO_2 \cdot 0.75H_2O$	C, H, F
IV-15	2-fluoro	G	82	214-222	$C_{16}H_9FO_2$	*f*
IV-16	1,5-difluoro	G	61	248-250	$C_{16}H_8F_2O_2$	C, H, N, F

[a] Analytical results were within ±0.4% of the theoretical values for all elements listed, except as noted in subsequent footnotes. [b] Also prepared as in ref 40. [c] Reference 6. [d] F: calcd, 12.8; found, 11.7. [e] C: calcd, 82.4; found, 81.9. [f] The crude product was used in the next step.

10,11,13,22) were accepted as active. None was clearly superior to the lead compound, VI-1; however, based on "percent increase in life span" (ILS) and "cures" in the P-388 leukemia and B-16 melanoma tests, the following compounds show activities approximately equivalent to VI-1: hydrazinoimidazoline derivatives VI-4 and -7, hydrazinotetrahydropyrimidine derivatives VI-2,5,8,9, hydrazinoazepine derivative VI-3, and substituted aminoguanidine derivatives VI-15 (2-methyl), VI-17 (2,2-dimethyl), VI-18 (2,3-dimethyl), and VI-25 (2-furfuryl). These results do not suggest a clear structure–activity relationship. However, a steric factor is suggested, since the most active compounds are those in which the aminoguanidine moiety is substituted with small groups or is involved in a cyclic structure. Conversely, larger substituents cause a decrease in activity that is moderate for most monosubstituted aminoguanidine moieties but very large for the marginally active 2,3-diisopropylaminoguanidine derivative VI-20 and the bulky cyclic compounds VI-10 and -11. Complete methylation of the imidazoline nitrogen atoms of VI-1, as in VI-13 or its isomer i,[9b] eliminates all activity.

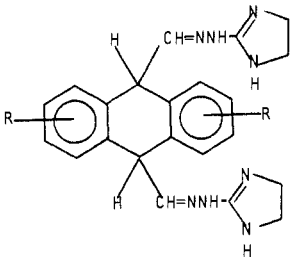
Further substitution of the anthracene nucleus (Tables VII and IX) also has not produced any compound clearly superior to lead compound VI-1. Mono- and dihalogen substitution in various positions caused no significant change in activity (VII-1 to -6 and IX-1). This was also true for substitution with a 2-methyl group (VII-7). However, dimethyl derivatives VII-8 and -9, dimethoxy derivative VII-13, and 2-ethyl derivative VII-10 showed decreased activities, while 2-*tert*-butyl derivative VII-11 was completely inactive, indicating again a steric effect. If intercalation is indeed involved in the mechanism of action of these compounds, the inactivity of compounds such as VII-11 would be understandable, since the requisite sandwiching of the flat anthracene nucleus between adjacent base pairs of a DNA chain would be expected to be much diminished by a side chain bulky in three dimensions. The addition of another coplanar aromatic ring to give tetracyclic analogues VII-14 and -15 had only a slight effect on activity. Equilibrium dialysis studies[10] showed binding of VI-1 to DNA, which is probably intercalative. Further studies are in progress.

An examination of the structures of other derivatives of 9,10-anthracenedicarboxaldehyde, listed in Table VI, shows that most of the derivatives with significant antitumor activity were hydrazones derived from aminoguanidines. Inactive compounds were derived from substituted hydrazines (VI-33–35,39,44), acylhydrazines (VI-37,38), semicarbazides and thiosemicarbazides (VI-41–43), tosylhydrazide (VI-32), and dimethylethylenediamine (VI-36). The exceptions were aminoamidine derivative VI-31, which was moderately active against P-388 leukemia and marginally active against B-16 melanoma, and bisthiosemicarbazone VI-40, which has modest activity only against P-388 leukemia. The finding of activity in the nonbasic bisthiosemicarbazone VI-40 led us to synthesize the bis(dimethylaminopropyl) derivative VIII-1 with the hope of improving the activity of VI-40. This derivative (VIII-1) did indeed show considerably greater activity and potency than VI-40, indicating again the need for basic side chains to produce highly active compounds. Additional analogues of VIII-1 were prepared (Table VIII), but none was as active as the lead compound VI-1.

A number of 9,10-dihydro derivatives of lead compound VI-1 and its analogues were prepared and tested (Table V). None was superior to VI-1, and most of them were either less active or less potent. Activity may depend on aromatization by dehydrogenation in vivo since the 9,10-dimethyl derivative V-6 was inactive.

(10) G. Nicolau, W. H. Wu, S. Gordon, D. Cosulich, and W. McWilliams, *Antimicrob. Agents Chemother.*, abstr 26 (1980). Dialysis of ^{14}C-labeled VI-1 at 2×10^{-5} M against calf thymus DNA at 10^{-4} M (based on nucleotide content) required 72 h to reach equilibrium, when 66% of the radioactivity in the DNA compartment was bound to the DNA. The membrane used was Spectra/por #2 natural cellulose in 0.1 M NaCl with 0.01 M sodium cacodylate buffer, pH 6.00. We thank Dr. Gabriela Nicolau for these data. Further DNA binding studies are in progress.

Table V. 9,10-Dihydro-9,10-anthracenedicarboxaldehyde Bis(imidazolin-2-ylhydrazones)



No.	R	Method	% Yield	Mp, °C	Formula	Analyses[a]	% Increase in Median Life Span (Optimum Dose, mg/kg) P-388 Leukemia[b]	B-16 Melanoma[c]	"Cures" B-16
V-1	H	H	13	258-262	$C_{22}H_{24}N_8 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl	72 (12.5)	100 (12.5)	0
V-2	1,4-dimethoxy	H	10	250-255	$C_{24}H_{28}N_8O_2 \cdot 2HCl \cdot 1.0H_2O$	C,H,N,Cl	0 (200)	NT (not tested)	
V-3	2,3-benzo	H	8	290-295	$C_{26}H_{26}N_8 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl	105 (225)	NT	
V-4	2,3-dimethyl	H	35	285-290	$C_{24}H_{28}N_8 \cdot 2HCl \cdot 0.5H_2O$	C,H,Cl;N[d]	73 (50)	NT	
V-5	1-chloro	H	51	200 (dec)	$C_{22}H_{23}N_8Cl \cdot 2HCl \cdot 0.5H_2O$	C;H,N,Cl[e]	100 (50)	153 (50)	3/10
V-6	9,10-dimethyl	H	44	275-277	$C_{24}H_{28}N_8 \cdot 2HCl \cdot 1.25H_2O$	C,H,N;Cl[f]	0 (200)	0 (50)	
V-7	2-chloro	H	77	190 (dec)	$C_{22}H_{25}N_8Cl \cdot 2HCl$	C,H,Cl;N[g]	100 (50)	230 (12.5)	5/10
V-8	1,5-dichloro[i]	H	15	205-210	$C_{22}H_{22}N_8Cl_2 \cdot 2HCl \cdot C_3H_7OH$	C,H,Cl;N[h]	0 (200)	0 (50)	

[a] Same as Table IV. [b] BDF_1 or CDF_1 mice were injected ip with 10^6 ascitic leukemia cells and dosed ip on days 1, 5, and 9. There were no 30-day survivors. [c] BDF_1 mice were implanted ip with a homogenate from 0.05 g of tumor and dosed ip on days 1-9. "Cures" = number of survivors/total at 60 days. When the "cures" were 50% or greater, the experiment was continued until a specific ILS could be calculated. [d] N: calcd, 22.0; found, 21.2. [e] H: calcd, 5.0; found, 5.7. N: calcd, 21.6; found, 20.0. Cl: calcd, 20.6; found, 19.0. [f] Cl: calcd, 13.5; found, 14.2. [g] N: calcd, 20.6; found, 19.8. [h] N: calcd, 18.6; found, 17.5. ^1H NMR showed the presence of the C_3H_7OH. [i] Reference 40.

Although none of the analogues in Tables V–IX was considered to be clearly superior to VI-1, there was the possibility that some of the compounds might prove to be superior if tested further. Therefore, eight of the most interesting compounds were compared against L-1210 leukemia, colon tumor 26, Madison lung carcinoma 109, and the advanced disease forms of P-388 leukemia, B-16 melanoma, and L-1210 leukemia. The results of these tests (Table X) also indicate that none of these compounds is broadly superior to the lead compound VI-1.

Details of a more extensive pharmacological testing of VI-1 will be reported separately.[11] It was found that it gave an ILS of >85% in mice with the Lieberman plasma cell tumor, and 150% and 85% in mice with colon tumor 26 and Ridgway osteogenic sarcoma, respectively. It was active by intraperitoneal, intravenous, or subcutaneous injection, but was inactive orally. An adriamycin-resistant subline of P-388 leukemia was completely cross-resistant to VI-1. DNA and RNA syntheses were strongly inhibited in L-5178Y lymphoma cells in vitro.[11] In cultures of human diploid fibroblasts (WI-38) and colon carcinoma cells (WiDr), it was lethal in both rapidly dividing and nondividing phases of the cell cycle, suggesting that it might be effective against slow-growing tumors.[12] It was mutagenic in the Ames test with *Salmonella typhimurium* strain TA 98, but the mutagenic potency was much less than that of daunorubicin. Thus, there were 0.3 to 0.4 reverse mutations per nanomole for VI-1 as compared with 0.2 to 0.3 for mitoxantrone (1)[13] and 100 ± 30 for daunorubicin.[14]

The drug is surprisingly stable and persistent in vivo. In the rat, dog, and monkey, the half-lives for disappearance from serum of a single intravenous injection of ^{14}C-labeled VI-1 were 1.5, 3, and 6 days, respectively. It was well distributed throughout the organs and glands studied.

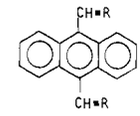
(11) R. V. Citarella, R. E. Wallace, K. C. Murdock, R. B. Angier, and F. E. Durr, *Antimicrob. Agents Chemother.*, abstr 23 (1980); *Cancer Res.*, **42**, 440 (1982).

(12) R. E. Wallace, R. V. Citarella, and F. E. Durr. *Antimicrob. Agents Chemother.*, abstr 28 (1980).

(13) For these data we thank Dr. Jane S. Allen, American Cyanamid Co. Agricultural Research Center, Princeton, N.J.

(14) G. L. Tong, H. Y. Wu, T. H. Smith, and D. W. Henry, *J. Med. Chem.*, **22**, 912 (1979).

Table VI. 9,10-Anthracenedicarboxaldehyde Bishydrazones



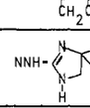
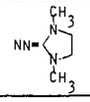
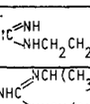
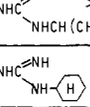
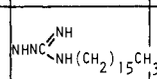
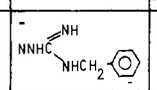
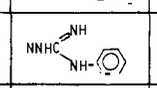
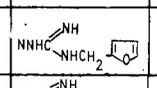
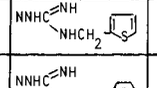
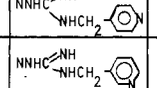
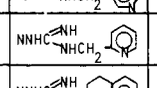
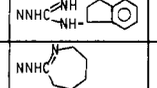
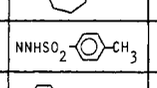
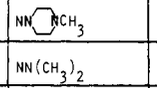
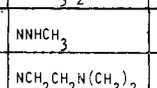
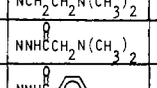
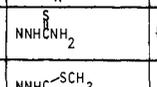
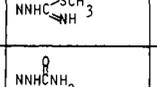
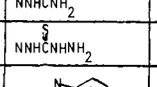
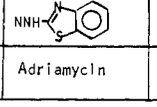
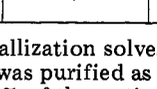
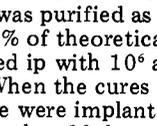
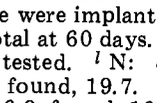
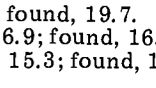
	R	Method[a]	% Yield[b]	Mp, °C	Formula	Analyses[c]	Median % Increase in Life Span (Optimum Doses mg/kg)			
							P-388 Leukemia[d]	"Cures"[e]	B-16 melanoma[f]	"Cures"[g]
VI-1		J	97	288-289	$C_{22}H_{22}N_8 \cdot 2HCl \cdot 0.5H_2O$	CHNCl	137 (12.5)	17/42	122 (6)	2/10
VI-2		J	41	215-230	$C_{24}H_{26}N_8 \cdot 2HCl \cdot 1.5H_2O$	CHNCl	82 (12.5)	1/12	106 (12)	0
VI-3		N	87	301-302	$C_{26}H_{30}N_8 \cdot 2HI$	CHNI	90 (12.5)	0	173 (12)	1/10
VI-4		N, X	77	298-300	$C_{24}H_{26}N_8 \cdot 2HI \cdot H_2O$	CHNI	124 (6.25)	1/6	95 (3)	0
VI-5		H	75	293-295	$C_{26}H_{30}N_8 \cdot 2HCl \cdot 1.5H_2O$	CHNCl	148 (6.25)	1/6	80 (1.5)	0
VI-6		N	47	246-251	$C_{28}H_{34}N_8 \cdot 2HI \cdot 1.75H_2O$	CHNI	35 (25)	1/12	97 (25)	0
VI-7		H	95	310-315	$C_{26}H_{30}N_8 \cdot 2HCl \cdot H_2O$	CHCl;N[h]	120 (12.5)	0	242 (6)	5/10
VI-8		H	75	340·	$C_{28}H_{34}N_8 \cdot 2HCl \cdot 0.5H_2O$	CHCl;N[i]	205 (100)	3/6	143 (25)	4/10
VI-9		H	68	350° dec.	$C_{28}H_{30}N_8 \cdot 2HCl$	CHCl;N[j]	100 (12.5)	1/6	127 (1.5)	4/10
VI-10		H	23	285-290	$C_{40}H_{58}N_8 \cdot 2HCl \cdot 0.5H_2O$	CHNCl	NT		0 (50)	0
VI-11		H	23	275-280	$C_{34}H_{46}N_8 \cdot 2HCl \cdot 1.25H_2O$	CHNCl	0 (50)	0	NT	
VI-12		N	98	286-288	$C_{34}H_{30}N_8 \cdot 2HI \cdot 0.5H_2O$	CHNI	30 (200)	0	NT	
VI-13		N	89	286-288	$C_{26}H_{30}N_8 \cdot 2HI$	CHNI	0 (200)	0	NT[k]	
VI-14		M	80	298-300	$C_{18}H_{18}N_8 \cdot 2HCl \cdot 0.25H_2O$	CHNCl	132 (25)	3/6	67 (3)	0
VI-15		N	44	252-254	$C_{20}H_{22}N_8 \cdot 2HI \cdot H_2O$	CHNI	157 (25)	6/24	145 (12)	8/20
VI-16		U	84	324-325	$C_{20}H_{22}N_8 \cdot 2HBr$	CHNBr	91 (12.5)	0	50 (6)	0
VI-17		N	87	320-322	$C_{22}H_{26}N_8 \cdot 2HI$	CHNI	103 (6.25)	3/18	125 (3)	2/10
VI-18		N(H_2O)	61	281-283	$C_{22}H_{26}N_8 \cdot 2HI$	CHNI	118 (6.25)	1/6	111 (3)	0
VI-19		K		234-235	$C_{22}H_{26}N_8O_2 \cdot 2HCl$	CHNCl	90 (25)	0	80 (12)	1/10
VI-20		N	81	278-280	$C_{30}H_{42}N_8 \cdot 2HI$	CHNI	50 (50)	0	0	0
VI-21		P EtOH Ether	53	>300°	$C_{30}H_{38}N_8 \cdot 2HI$	CHN	73 (50)	0	80 (12)	0

TABLE VI (cont'd)

No.	R	Method[a]	% Yield[b]	Mp, °C	Formula	Analyses[c]	Median % Increase in Life Span (Optimal Doses mg/kg)			
							P-388 Leukemia[d]	"Cures"[e]	B-16 melanoma[f]	"Cures"[g]
VI-22		H	12	235-240	$C_{50}H_{82}N_8 \cdot 2HCl$	CHCl;N[l]	0 (200)	0	0 (50)	0
VI-23		N	70	279-282	$C_{32}H_{30}N_8 \cdot 2HI$	CHNI	68 (25)	0	47 (12)	0
VI-24		J	69	239-240	$C_{30}H_{26}N_8 \cdot 2HCl \cdot 2H_2O$	CHNCl	67 (50)	0	50 (6)	0
VI-25		J	90	130-140	$C_{28}H_{26}N_8O_2 \cdot 2HCl \cdot 0.5H_2O$	CHNCl	91 (50)	0	167 (12.5)	6/10
VI-26		J	90	160-200	$C_{28}H_{26}N_8S_2 \cdot 2HCl \cdot 0.5H_2O$	CHN; Cl[m]	90 (50)	0	75 (12)	0
VI-27		V	93	299-302	$C_{30}H_{28}N_{10} \cdot 4HCl \cdot 0.5H_2O$	CHNCl	90 (12.5)	0	78 (25)	0
VI-28		V	92	298-302	$C_{30}H_{28}N_{10} \cdot 4HCl$	CHN; Cl[n]	114 (25)	1/6	77 (25)	0
VI-29		V	97	285-290	$C_{30}H_{28}N_{10} \cdot 4HCl$	CHNCl	95 (50)	0	74 (25)	0
VI-30		N	57	262-263	$C_{36}H_{34}N_8 \cdot 2HI$	CHNI	80 (200)	0	62 (25)	0
VI-31		J	47	220-225	$C_{28}H_{32}N_6 \cdot 2HCl \cdot 0.5H_2O$	CHNCl	95 (25)	0	35 (12)	0
VI-32		P (DMF)	67	220-221	$C_{30}H_{26}N_4S_2O_4$	CHNS	0 (200)	0	NT	
VI-33		J	97	268-270	$C_{26}H_{32}N_6 \cdot 2HCl \cdot 0.75H_2O$	CHNCl	0 (200)	0	NT	
VI-34	$NN(CH_3)_2$	P	70	177-179	$C_{20}H_{22}N_4$	CHN	0 (200)	0	NT	
VI-35	$NNHCH_3$	P	69	172-174	$C_{18}H_{18}N_4$	CHN	0 (200)	0	NT	
VI-36	$NCH_2CH_2N(CH_3)_2$	Q	70	108-109	$C_{24}H_{30}N_4$	CHN	0 (200)	0	NT	
VI-37		R	56	277-279	$C_{24}H_{28}N_6O_2 \cdot 2HCl \cdot 0.5H_2O$	CHN;Cl[o]	0 (200)	0	NT	
VI-38		P (DMF)	100	327-330	$C_{28}H_{20}N_6O_2$	CHN	0 (200)	0	NT	
VI-39		P	94	267-272	$C_{26}H_{20}N_6$	CH;N[p]	0 (200)	0	NT	
VI-40		P (DMF-H_2O)	16	275-277	$C_{18}H_{16}N_6S_2$	CH;NS[q]	71 (300)	0	0 (300)	
VI-41		P (DMF EtOH Ether)	50	225-227	$C_{20}H_{20}N_6S_2 \cdot 2HI$	HNS;C[r]	0 (200)	0	NT	
VI-42		P	69	>300	$C_{18}H_{16}N_6O_2$	C;HN[s]	0 (200)	0	NT	
VI-43		P (DMF)	66	252-253	$C_{18}H_{18}N_8S_2 \cdot H_2O$	CHNS	0 (200)	0	NT	
VI-44		P	62	299-300	$C_{30}H_{20}N_6S_2$	CH;NS[t]	0 (200)	0	NT	
	Adriamycin						159 (5)	1/6	275 (0.8)	18/20

[a] Recrystallization solvents are shown in parentheses. In other cases the compound was analyzed without recrystallization or was purified as detailed under Experimental Section. [b] Yield of analytical material. [c] Analytical results were within ±0.4% of theoretical value for all elements listed, except as shown in subsequent footnotes. [d] BDF_1 or CDF_1 mice were injected ip with 10^6 ascitic leukemia cells and dosed ip on days 1, 5, and 9. [e] "Cures" = number of survivors/total at 30 days. When the cures were 50% or greater, the experiment was continued until a specific ILS could be calculated. [f] BDF_1 mice were implanted ip with a homogenate from 0.05 g of tumor and dosed ip on days 1-9. [g] Cures = number of survivors/total at 60 days. [h] N: calcd, 20.6; found, 20.0. [i] N: calcd, 19.9; found, 19.4. [j] N: calcd, 20.3; found, 19.5. [k] NT = not tested. [l] N: calcd, 12.9; found, 12.3. [m] S: calcd, 10.4; found, 10.9; Cl: calcd, 11.5; found, 11.0. [n] Cl: calcd, 21.1; found, 19.7. [o] Cl: calcd, 13.8; found, 14.3. [p] N: calcd, 20.2; found, 18.7. [q] N: calcd, 22.1; found, 21.3; S: calcd, 16.9; found, 16.0. [r] C: calcd, 36.2; found, 36.7. [s] H: calcd, 4.6; found, 5.1. N: calcd, 24.1; found, 22.9. [t] N: calcd, 15.3; found, 14.7. S: calcd, 12.1; found, 10.8.

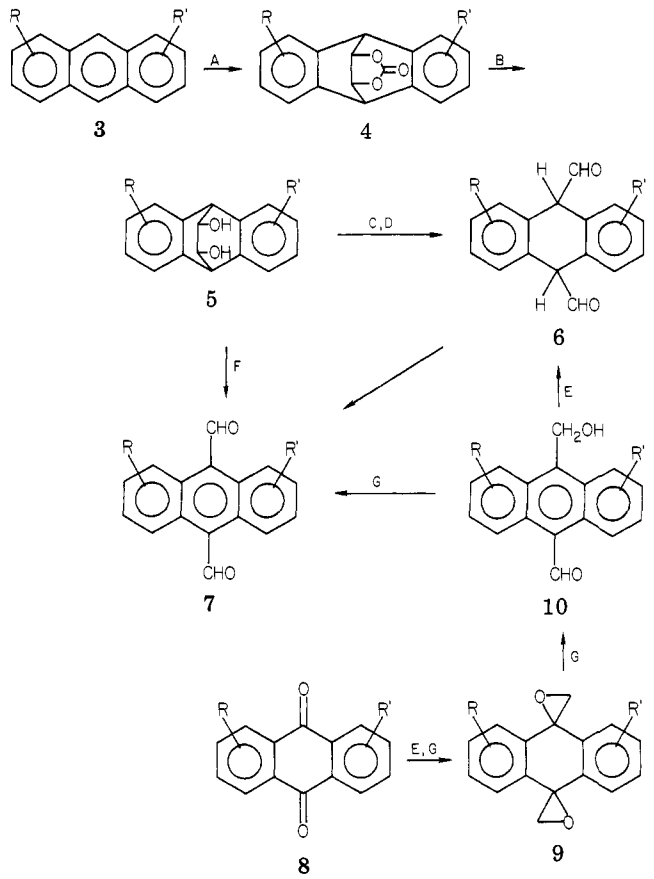
However, as might be expected for a highly polar bisguanidine salt, only very low levels were found in the brain or in fat deposits. Excretion of unchanged VI-1 was mostly in feces. No evidence was found for any significant metabolic transformation of the drug.[10]

Both bisantrene (VI-1) and mitoxantrone (1) were found to be noncardiotoxic in beagle dogs, in marked contrast to adriamycin.[15] Clinical trials of bisantrene hydrochloride (CL 216,942; NSC 337766) are in progress.

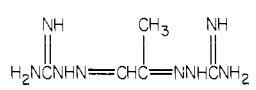
Related Compounds Not Derived from Anthracene-9,10-dicarboxaldehyde. Although they were con-

(15) B. M. Sparano, G. Gordon, C. Hall, M. J. Iatropoulos, and J. F. Noble, *Antimicrob. Agents Chemother.*, abstr 31 (1980).

Scheme II[a]



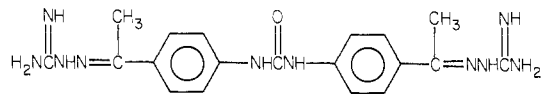
ceptually unconnected, it is interesting to compare the present series of polycyclic bisguanylhydrazones with previously reported anticancer compounds having some structural similarities. The compound with greatest structural simplicity and the only one with confirmed clinical effectiveness is methylglyoxal bis(guanylhydrazone) (11).[16,17] It was active against several leukemias and



11

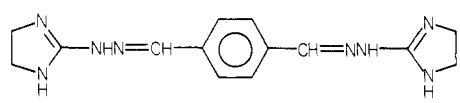
lymphomas in mice, though even close analogues were inactive. It inhibited the biosynthesis of spermidine,[18] and spermidine blocked the antiproliferative and immunosuppressive effects of 11.[19,17] In our hands it was inactive vs. P-388 leukemia in mice. It was active in patients with acute myelocytic leukemia, lymphoma,[20] carcinoma of the esophagus,[21] and some solid tumors.[22]

The bis(guanylhydrazone) of 4,4'-diacetyldiphenylurea, 12, was very active against several leukemias and other

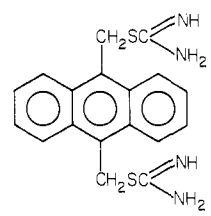


12

tumors in mice and had a high therapeutic index, but it was inactive in man.[17,23] It differed from 11 in many pharmacological respects. The antileukemic action was associated with an immunostimulatory effect.[24] It also bound to DNA (apparently not by intercalation) and inhibited DNA polymerase.[25] Analogues[26] of 12 and other bisguanidines and bisamidines[17] showed some activity but were less active than 12. The bishydrazone 13[27] from 2-hydrazinoimidazoline and terephthalaldehyde was inactive in our P-388 leukemia test but was marginally active vs. B-16 melanoma (ILS 35% at 25 mg/kg).[27,28]



13

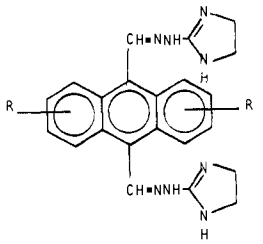


14

Although all of these compounds had two strongly basic termini, none has a system of three or more fused aromatic rings, in contrast to VI-1 and to various DNA intercalating agents,[2] such as adriamycin, *m*-AMSA,[29] and mitoxantrone.[30] However, we recently ran across some older literature on a strongly basic anthracenylbis(isothiourea), 14, prepared a sample,[31] and in our test systems found it to be substantially active against P-388 leukemia and moderately active vs. B-16 melanoma (ILS 145% and 63% at

(16) B. L. Freedlander and F. A. French, *Cancer Res.*, 18, 360 (1958).
(17) E. Mihich, in "Antineoplastic and Immunosuppressive Agents", Part II, Springer Verlag, New York, 1975, p 766; *Handb. Exp. Pharmacol.*, **38**, 766 (1975).
(18) H. G. Williams-Ashman and A. Schenone, *Biochem. Biophys. Res. Commun.*, **46**, 288 (1972).
(19) E. Mihich, *Cancer Res.*, **23**, 1375 (1963); *Pharmacologist*, **5**, 270 (1963).
(20) W. Regelson and J. F. Holland, *Cancer Chemother. Rep.*, 11, 81 (1961) and **27**, 15 (1963); E. J. Freirich, E. Frei III, and M. Karon, *Cancer Chemother. Rep.*, **16** 183 (1962); R. H. Levin, E. Henderson, M. Karon, and E. J. Freireich, *Clin. Pharmacol. Ther.*, **6**, 31 (1965); M. Boiron, C. Jacquillat, M. Weil, and B. J. Bernard, *Cancer Chemother. Rep.*, **45**, 69 (1965); M. Weil, C. Jacquillat, M. Boiron, and J. Bernard, *Eur. J. Cancer*, **5**, 271 (1969).
(21) G. Falkson, *Cancer Chemother. Rep.*, **55**, 209 (1971).
(22) W. A. Knight III, R. B. Livingston, C. Fabian, and J. Costanzi, *Proc. Am. Assoc. Cancer Res.*, **20**, 319 (1979); D. D. Von Hoff, G. J. Harris, and J. Kuhn, *Cancer Chemother.*, **2**, 138 (1980).
(23) E. Mihich et al., *Cancer Res.*, **28**, 354, 553 (1968).
(24) E. Mihich, *Cancer Res.*, **29**, 848 (1969).
(25) C. Dave, J. Ehrke, and E. Mihich, *Cancer Res.*, **33**, 2129 (1973).
(26) W. Korytnyk, N. Angelino, C. Dave, and L. Caballes, *J. Med. Chem.*, **21**, 507 (1978).
(27) This compound (13) was synthesized by Dr. A. S. Tomcufcik in 85% yield using method V: mp 350–353 °C. Anal. ($C_{14}H_{18}N_8 \cdot 2HBr \cdot H_2O$) C, H, N.
(28) We found the analogous bishydrazone from aminoguanidine and terephthalaldehyde to be inactive vs. P-388 leukemia and B-16 melanoma. It had been prepared earlier by F. Baiocchi et al. [*J. Med. Chem.*, **6**, 431 (1963)] who found it to be inactive vs. L-1210 leukemia in mice and KB cells in tissue culture.
(29) M. J. Waring, *Eur. J. Cancer*, **12**, 995 (1976); V. S. Sethi, *Ann. N.Y. Acad. Sci.*, **284**, 508 (1977); P. E. Gormley, V. S. Sethi, and R. L. Cysyk, *Cancer Res.*, **38**, 1300 (1978).
(30) R. K. Johnson, R. K.-Y. Zee-Cheng, W. W. Lee, E. M. Acton, D. W. Henry, and C. C. Cheng, *Cancer Treat. Rep.*, **63**, 425 (1979); F. Dall'Acqua, Institute of Pharmaceutical Chemistry, University of Padua, Italy, private communication; W. O. Foye, O. Vajragupta, and S. K. Sengupta, *J. Pharm. Sci.*, **71**, 253 (1982).
(31) M. W. Miller, R. W. Amidon, and P. O. Tawny, *J. Am. Chem. Soc.*, **77**, 2845 (1955).

Table VII. 9,10-Anthracenedicarboxaldehyde Bis(imidazolin-2-ylhydrazones)



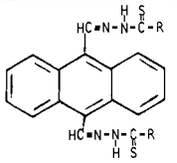
No.	R	Method	% Yield	Mp, °C	Formula	Analysis	Median % Increase in Life Span (Optimum Dose, mg/kg) P-388 Leukemia[b]	"Cures"[c]	B-16 Melanoma[d]	"Cures"[e]
VI-1 (Table VI)	H	J	97	288-289	$C_{22}H_{22}N_8 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl	137 (12.5)	17/42	122 (6)	2/10
VII-1	1-chloro	H	50	230 (dec)	$C_{22}H_{21}N_8Cl \cdot 2HCl \cdot 0.5H_2O$	C,H;N,Cl[f]	186 (6.25)	3/6	153 (1.5)	3/10
VII-2	2-chloro	H	22	280 (dec)	$C_{22}H_{21}N_8Cl \cdot 2HCl$	C,H,Cl;N[g]	95 (12.5)	1/6	206 (6.25)	4/10
VII-3	1-fluoro	H	79	215-226	$C_{22}H_{21}N_8F \cdot 2HCl$	C,H,N,F,Cl	115 (3.1)	0	100 (3.1)	1/10
VII-4	2-fluoro	H	75	300 (dec)	$C_{22}H_{21}N_8F \cdot 2HCl \cdot 0.5H_2O \cdot 0.5C_3H_7OH$[h]	C,H,N	123 (6.25)	3/12	183 (3.1)	4/10
VII-5	2,6-difluoro	H	56	>320	$C_{22}H_{20}N_8F_2 \cdot 2HCl$	C,H;N,Cl[j]	135 (3.1)	1/6	111 (3.1)	1/10
VII-6	1,5-difluoro	H	93	309 (dec)	$C_{22}H_{20}N_8F_2 \cdot 2HCl$	C,H,F,Cl;N[k]	140 (100)	1/6	194 (50)	5/10
VII-7	2-methyl	H	50	300-302	$C_{23}H_{22}N_8 \cdot 2HCl \cdot H_2O$	C,H,N;Cl[l]	145 (6.25)	1/12	172 (6.25)	4/10
VII-8	2,3-dimethyl	H	90	300-305	$C_{24}H_{26}N_8 \cdot 2HCl$	C,H,N,Cl	77(160)	0	76 (3.1)	0
VII-9	1,4-dimethyl	H	82	185-190	$C_{24}H_{26}N_8 \cdot 2HCl \cdot H_2O$	C,H;N,Cl[m]	57 (18.8)	0	NT	0
VII-10	2-ethyl	H	55	287-290	$C_{24}H_{26}N_8 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl	64 (3.1)	0	97 (6.25)	1/10
VII-11	2-t-butyl	H	95	240-245	$C_{26}H_{32}N_8 \cdot 2HCl \cdot 1.5H_2O$	C,H;N,Cl[n]	0	0	0	0
VII-12	1-chloro-2-methyl	H	95	215-220	$C_{23}H_{23}N_8Cl_2 \cdot 2HCl \cdot 2.5H_2O$	C,H;N,Cl[o]	130 (12.5)	0	220 (12.5)	4/10
VII-13	1,4-dimethoxy	H	75	300-305	$C_{24}H_{26}N_8O_2 \cdot 2HCl \cdot 0.5H_2O$	C,H,N,Cl	50 (50)	0	64 (12.5)	0
VII-14	1,2-benzo	H	53	240-245	$C_{26}H_{24}N_8 \cdot 2HCl \cdot 0.25H_2O$	C;H,N,Cl[p]	90 (12.5)	1/6	153 (6.25)	0
VII-15	2,3-benzo	H	80	320-325	$C_{26}H_{24}N_8 \cdot 2HCl$	C,H,N,Cl	91 (12.5)	0	136 (6.25)	1/10

[a-e] See footnotes *c-g* in Table VI. [f] N: calcd, 21.8; found, 21.0. Cl: calcd, 20.6; found, 19.4. [g] N: calcd, 22.2; found, 21.2. [h] ^1H NMR shows the presence of C_3H_7OH. [j] N: calcd, 22.1; found, 19.9; Cl: calcd, 14.0; found, 12.8. [k] N: calcd, 22.1; found, 21.5. [l] Cl: calcd, 14.1; found, 15.2. [m] N: calcd, 21.7; found, 23.4. Cl: calcd, 13.7; found, 15.4. [n] N: calcd, 20.1; found, 19.2. Cl: calcd, 12.8; found, 12.2. [o] N: calcd, 19.8; found, 18.2. Cl: calcd, 18.8; found, 20.3. [p] H: calcd, 5.1; found, 5.9. N: calcd, 21.3; found, 19.8. Cl: calcd, 20.3; found, 13.5.

50 and 6.25 mg/kg, respectively). It was reported to be active vs. L-1210 leukemia,[32] to intercalate into DNA,[33] and to inhibit both DNA synthesis and DNA-dependent RNA polymerase activity.[33] In two limited clinical trials[34,35] it caused myelosuppression, blushing, and urticaria, but it did appear to give brief favorable responses in three patients with squamous cell carcinoma, melanoma, and histiocytic sarcoma, respectively. It proved to be a potent UV photosensitizer; acute phototoxicity after exposure to sunlight was a severe problem, except with dark-skinned patients.

(32) S. K. Carter, *Cancer Chemother. Rep.*, 1(1, Part 3), 153 (1968).
(33) P. P. Saunders and G. F. Saunders, *Mol. Pharmacol.*, **6**, 335 (1970).
(34) E. Frei, J. K. Luce, and T. L. Loo, *Cancer Chemother. Rep.*, **55**(1, Part 1), 91 (1971).
(35) W. L. Wilson, A. J. Weiss, and N. C. Andrews, *Cancer Chemother. Rep.*, **55**(5, Part 1) 525 (1971).

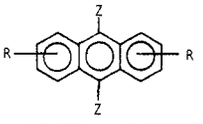
Table VIII. 9,10-Anthracenedicarboxaldehyde Bis(thiosemicarbazones)



No	R	Method	% Yield	Mp, °C	Formula	Analyses[a]	Median% Increase in Life Span (Optimum Doses mg/kg) P-388 Leukemia[b]	"Cures"[c]	B-16 Melanoma[d]	"Cures"[e]
VIII-1	$NH(CH_2)_3N(CH_3)_2$	Y	73	221-223	$C_{28}H_{38}N_8S_2 \cdot 2HCl \cdot 1/2H_2O$	C,H,N,S,Cl	100 (6.25)	0	77 (6.00)	0
VIII-2	$NH(CH_2)_2OCH_3$	Y	81	228-230 (dec)	$C_{24}H_{28}N_6O_2S_2$	C,H,N,S	0		NT	
VIII-3	NH-C6H4-N(CH3)2	Y	92	295-298 (dec)	$C_{34}H_{34}N_8S_2 \cdot 1/4H_2O$	C,H,N,S	0		NT	
VIII-4	$NH(CH_2)_2N(CH_3)_2$	Y	62	289-290 (dec)	$C_{26}H_{34}N_8S_2 \cdot 2HBr$	C,H,N,S;Br[f]	106 (6.25)	0	47 (3.00)	0
VIII-5	$NH(CH_2)_5N(CH_3)_2$	Y	91	189-191 (dec)	$C_{32}H_{46}N_8S_2 \cdot 2HCl$	C,H,N,S,Cl	48 (25.0)	0	97 (6.00)	0
VIII-6	$NH(CH_2)_7N(CH_3)_2$	Y	12	202-204	$C_{36}H_{54}N_8S_2 \cdot HCl \cdot H_2O$	C,H,N;S,Cl[g]	39 (6.25)	0	0	
VIII-7	$NH(CH_2)_2N(C_2H_5)_2$	Y	20	188-190	$C_{30}H_{42}N_8S_2 \cdot 2HCl \cdot H_2O$	C,N,S,Cl;H[h]	95 (6.25)	0	NT	
VIII-8	$NH(CH_2)_2N(i\text{-}C_3H_7)_2$	Y	20	201-203 (dec)	$C_{34}H_{50}N_8S_2 \cdot 2HCl \cdot 1.5H_2O$	C,H,N,S,Cl	53 (100)	0	NT	
VIII-9	$NH(CH_2)_2N(i\text{-}C_4H_9)_2$	Y	44	182-184	$C_{38}H_{58}N_8S_2 \cdot 2HCl$	C,H,N,S,Cl	33 (25.0)	0	NT	
VIII-10	$NH(CH_2)_2$-morpholino	Y	70	249-251 (dec)	$C_{32}H_{42}N_8O_2S_2 \cdot 2HCl \cdot 1\frac{1}{2}H_2O$	C,H,N,S,Cl	58 (50.0)	0	0	
VIII-11	$NH(CH_2)_2$-morpholino	Y	98	235-237 (dec)	$C_{30}H_{38}N_8O_2S_2 \cdot 2HCl \cdot 1/2H_2O$	C,H,N,S,Cl	80 (50.0)	0	103 (12.0)	1/10
VIII-12	$NH(CH_2)_3$-morpholino	Y	35	273-275 (dec)	$C_{32}H_{42}N_8O_2S_2 \cdot 2HCl \cdot 1/2H_2O$	C,H,N,S,Cl	53 (100)	0	75 (50.0)	0
VIII-13	N-C5H4-NCH3	Y	30	201-203	$C_{28}H_{34}N_8S_2 \cdot 2HCl \cdot H_2O$	C,H,N,S,Cl	61 (200)	0	NT	

[a-e] See footnotes *c* to *g* in Table VI. [f] Br: calcd, 23.4; found, 22.8. [g] S: calcd, 8.5; found, 9.2. Cl: calcd, 9.4; found, 8.8. [h] H: calcd, 6.9; found, 6.4.

Table IX. Miscellaneous



No.	R	Z	Method	% Yield	Mp, °C	Formula	Analyses	Median % Increase in Life Span (Optimal Dose, mg/kg) P-388 Leukemia	"Cures"	B-16 Melanoma	"Cures"
IX-1	2-chloro	CH=NNH-(2-imidazolinyl)	H	30	210-214	$C_{24}H_{25}N_8Cl \cdot 2HCl \cdot H_2O$	C,H,N,Cl	127 (6.25)	1/6	194 (3)	6/10
IX-2	2-chloro	CH=NNH-C(=NH)N(CH3)2	H	80	230 (dec)	$C_{22}H_{25}N_8Cl \cdot 2HI$	C,H,N	82 (12.5)	0	39 (6)	0
IX-3	H	CH_2CH_2CH=NNH-(2-imidazolinyl)	Z	See Experimental Section				30 (50)	0	0 (50)	0
IX-4	H	CH=CHCH=NNH-(2-imidazolinyl)	AA	See Experimental Section				36 (200)	0	46 (25)	0

Our syntheses and evaluations of other polycyclic aromatic systems with bis-basic substitution will be reported separately.

Experimental Section

Melting points were taken on a Fischer-Johns block or a Mel-Temp apparatus and are uncorrected. Solids were pressed

Table X. Comparative Antitumor Activity of VI-1 and Selected Analogues

No.	P-388 Leukemia (1,5,9)[a]			B-16 Melanoma (1-9)[a]			L-1210 Leukemia (1,5,9)[a]			Colon 26 (1,5,9)[a]			Advanced P-388 Leukemia (4,8,12)[a]			Advanced B-16 Melanoma (5,9,13)[a]			Advanced L-1210 Leukemia (+2)[f]			Madison Lung 109 (s.c.) (1, 5, 9)[f]	
	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 30 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 60 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 30 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 60 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 30 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 30 (days)	Optimal Dose (mg/kg)	ILS[b] (%)	S/T[c] 30 (days)	% Tumor Inhibition at non-lethal Dose	ILS[b] (%)
VI-1	6.2-12.5	137[d]	27/139	6.2	98-122	2/20	12.5	62[d]	0/17	6.2	114-150	6/20	12.5	76	0/6	12.5	54	2/10	100	48	0/6	20	<25
VI-15	25	157[4]	6/24	3.1-12.5	144-145	8/20	25.0	62	0/5	12.5	129[d]	2/26	25.0	108	1/6	25.0	62	5/10	50	50	0/6	89[e]	53
VII-2	6.2-12.5	90-100	2/12	6.2	206	4/10	3.1	37	0/6	6.2	100	1/10				12.5	32	0/10					
VII-7	6.2-12.5	125-145	1/12	6.2	172	4/10	6.2	42	0/5	≥12.5	≥63	0/10				6.2	35	3/10					
VI-17	6.2	103[d]	3/18	1.5-3.1	116-129	2/20	6.2	100	0/6	6.2	167	1/10				6.2	41	2/10					
VI-7	12.5	100-120	0/12	6.2	>248	5/10													12.5	28	0/6		
VI-8	100.0	205	3/6	25.0	143	4/10				25.0	153	0/8	25.0	88	0/6				200.0	29	0/6	31	<25
IX-1	6.2	127	1/6	3.1	194	6/10				3.1	175	1/8	12.5	80	0/6				6.2	28	0/6	16	<25
VI-25	25.0	25-91	0/12	12.5	100-167	6/20				12.5	109	0/8	25.0	56	0/6					<25			

[a] Day(s) of ip treatment relative to tumor inoculation. [b] Percent increase in life span. [c] Number of survivors/no treated. [d] Average value of three or more tests. [e] Confirmed active in a second test. [f] VI-1 and all analogues tested were inactive when tested against advanced L-1210 (treatment days 5, 9, and 13), advanced L-1210 (treatment day +2 iv), systemic L-1210 (tumor inoculated iv), and adriamycin-resistant P-388 leukemia (treatment days 1, 5, and 9, ip).

with KBr for IR spectral determinations on a Perkin-Elmer Model 21 or a Nicolet Model 7199-FT spectrophotometer. NMR spectra were obtained with a Varian Model HA-100 spectrophotometer; chemical shifts (δ) are reported in parts per million relative to Me_4Si. A Cary Model 14 spectrophotometer was used for UV spectral determinations. The structures of all new compounds were confirmed by IR and NMR spectra and by elemental analyses that were within 0.4% of theoretical values, except where specified otherwise. Compounds in Table VI for which hydrazine precursors were not commercially available and references for preparation of those precursors are as follows: **1–3** and **8**, ref 36; **17**, **21**, **23**, and **24**, ref 37; **15**, ref 38; **20**, ref 39a. 9,10-Anthracenedicarboxaldehyde was purchased from Eastman Kodak Co. The other dialdehydes were prepared as described in the following paragraphs. Tumors were propagated and used for antitumor testing in accordance with test protocols described by the National Cancer Institute.[39b]

Method A. *cis*-9,10-Dihydro-9,10-ethanoanthracene-11,12-diol Cyclic Carbonate (I-1). A mixture of 8.9 g (0.05 mol) of anthracene and 43 g (0.5 mol) of vinylene carbonate was refluxed under N_2 for 8 h. The solution was cooled, treated with 1 vol of methanol, and cooled overnight. The colorless crystals were collected by filtration, washed with methanol, and dried to yield 12.6 g (95%) of product, mp 259–260 °C (lit.[7] mp 259–259.6 °C). The mother liquor was saved for recovery of the excess vinylene carbonate.

Method B. *cis*-9,10-Dihydro-9,10-ethanoanthracene-11,12-diol (II-1). A mixture of 5.6 g (0.021 mol) of the anthracene cyclic carbonate (I-1), 4.9 g of potassium hydroxide in 6.4 mL of water, and 53 mL of ethanol was stirred at 70–75 °C for 2 h. The resulting two-layer system was filtered to remove suspended matter, diluted with 2 vol of water, and cooled. The colorless crystals which formed were removed, washed well with water, and dried to yield 4.5 g (89%) of product, mp 202–204 °C (lit.[7] mp 201.9–202.7 °C).

Method C. *cis*-9,10-Dihydro-9,10-anthracenedicarboxaldehyde (III-1). A solution of the cyclic diol (II-1; 2.38 g, 0.01 mol) in 40 mL of glacial acetic acid at room temperature was treated portionwise with 4.8 g (0.011 mol) of lead tetraacetate while stirring. After 10 min, a colorless solid had formed in a yellow solution, and all the lead tetraacetate had been consumed as shown by a starch–iodide test strip. After the mixture was cooled at 15 °C, the colorless solid was collected by filtration, washed with glacial acetic acid and water, and then dried to yield 1.5 g (61%) of product: mp 144–146 °C; IR (KBr) 5.85 (aliphatic CHO), 3.5 μm (tert H); NMR ($CDCl_3$) δ 9.45 (s, aldehydic, 2), 5.00 (s, tert aliphatic, 2), 7.45 (m, aromatics, 8).

Method D. *cis*-9,10-Dihydro-2-methyl-9,10-anthracenedicarboxaldehyde (III-4). A suspension of 2.5 g (0.01 mol) of the cyclic diol (II-3) in 100 mL of water and 1 mL of ethanol was treated with 2.14 g (0.01 mol) of sodium periodate and stirred for 2 h at room temperature. The insoluble material was filtered off, washed well with water, and dried to leave 2.4 g of a pale yellow solid: mp 125–126 °C; IR (KBr) 5.82 μm (aliphatic CHO); NMR ($CDCl_3$) δ 9.40 (s, aliphatic CHO, 2), 4.90 (s, tert aliphatic, 2), 2.42 (s, methyl, 3), 7.33 (d, aromatics, 7).

Method E. 1,5-Dichloro-9,10-dihydro-9,10-anthracenedicarboxaldehyde (III-12).[40] To a stirred mixture of 4.31 g (0.0898

(36) A. S. Tomcufcik, R. G. Wilkinson, and R. G. Child, U.S. Patent 3 931 152 (1976). Monohydrochlorides can be converted to dihydrochlorides by solution in ethanol and addition of excess ethanolic HCl. Monohydriodides can be converted to dihydrochlorides by (a) solution in a minimum amount of water and addition of excess concentrated HCl and ethanol or (b) by treatment with excess Dowex 2-X8 and acidification of the filtrate with excess concentrated HCl.

(37) W. G. Finnegan, R. A. Henry, and E. Lieber, *J. Org. Chem.*, 18, 779 (1953).

(38) G. W. Kirsten and G. B. L. Smith, *J. Am. Chem. Soc.*, **58**, 800 (1936).

(39) (a) D. F. Percival and R. M. Herbst, *J. Org. Chem.*, **22**, 925 (1957). (b) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

mol) of sodium hydride (50% oil dispersion) and 11.08 g (0.040 mol) of 1,5-dichloroanthraquinone in 250 mL of dry dimethyl sulfoxide at room temperature in the dark and under nitrogen was added dropwise a solution of 18.3 g (0.898 mol) of trimethylsulfonium iodide in 140 mL of dry dimethyl sulfoxide over a period of 25 min. The reaction mixture was stirred for an additional 2 h and filtered through a sintered glass funnel. The filtrate was poured into 700 mL of ice–water and allowed to stand for 20 min. The solid was collected by filtration and washed with water and methanol. The pale yellow solid was dried overnight under reduced pressure at room temperature over phosphorus pentoxide and gave 10.5 g (85%) of 1′,5′-dichloridispiro[oxirane-2,9′(10′*H*)-anthracene-10′,2″-oxirane]: mp 172–178 °C; NMR ($CDCl_3$) δ 2.96 (d, J = 7.0 Hz, CH_2O, 2 H), 3.52 (d, J = 7.0 Hz, CH_2O, 2 H), 7.12 (m, aromatic, 6 H). Anal. Calcd for $C_{16}H_{10}Cl_2O_2$ (M_r 305): C, 63.0; H, 3.3; Cl, 23.2. Found: C, 62.9; H, 3.6; Cl, 22.5.

A solution of 2.9 g (0.0095 mol) of the above dioxirane and 0.2 mL of boron trifluoride etherate in 200 mL of dry toluene was stirred for 6 h and filtered. The solid was washed with two 10-mL portions of a cold 1:1 solution of saturated sodium bicarbonate and water to give 1.6 g of crude product, mp 185–188 °C. Recrystallization from 60 mL of ethyl acetate after treatment with activated carbon gave 1.0 g of white product, mp 175–186 °C. The toluene filtrate was washed in the same fashion as was the solid, dried over anhydrous sodium sulfate, evaporated to dryness under reduced pressure, and gave 1.3 g of crude product, mp 157–174 °C. The total yield of crude product was 2.9 g (100%). This yellow product was recrystallized from 70 mL of ethyl acetate to give 0.8 g of yellow analytically pure product.

Method F. 9,10-Anthracenedicarboxaldehyde (IV-1). A solution of 2.38 g (0.01 mol) of the cyclic diol (II-1) in 50 mL of glacial acetic acid at 30–35 °C was stirred and treated portionwise with lead tetraacetate until a blue color persisted on starch–iodide test paper [approximately 8 g (0.018 mol) was added]. The reaction was stirred at 30–35 °C for 2 h. The yellow-orange crystalline solid was collected by filtration, washed with water, and dried, leaving 2.0 g (85%) of product. Recrystallization of the product from methylene chloride gave 1.5 g of orange needles, mp 245–246 °C (lit.[6] mp 244–245 °C).

Method G. 1-Chloro-2-methyl-9,10-anthracenedicarboxaldehyde (IV-11). A solution of 2.15 g (0.0084 mols) of 1-chloro-2-methylanthraquinone dissolved in 100 mL of dry Me_2SO containing 0.9 g (0.019 mol) of NaH (50% in oil) was treated at room temperature under N_2 all at once with 3.9 g (0.019 mol) of trimethylsulfonium iodide. After stirring in the dark for 5 h, the purple solution was filtered into 200 mL of ice–water. The yellow colloidal solution was extracted with three portions of ether, dried over sodium sulfate, filtered, and concentrated to dryness, leaving 2.4 g of 1′-chloro-2′-methyldispiro[oxirane-2,9′(10′*H*)-anthracene10′,2″-oxirane] as a yellow oil: NMR ($CDCl_3$) δ 2.57 (s, 3 CH_3), 3.13 (d, 4 CH_2), 7.32 (d, 6, aromatic).

A mixture of the above 2.4 g (0.0084 mol) of the yellow oil and 3.4 g of lithium bromide in 150 mL of acetonitrile was stirred in the dark at 60 °C for 16 h and then concentrated to half volume under vacuum. The resulting dark gummy yellow solid was removed, washed with water, and dried to yield 1.3 g of a yellow solid: IR (KBr) 6.0 (aromatic aldehyde), 2.9 μm (methylene). The filtrate and washings, after concentration to dryness, washing, and drying gave an additional 1.1 g of yellow solid. The products were combined and dried under vacuum to give 2.4 g of 1-chloro-2-methyl-10(9)-(hydroxymethyl)-9(10)-anthracenecarboxaldehyde.

A solution of the above 2.4 g of yellow solid in 40 mL of Me_2SO and 22 mL of diethylamine was treated all at once with a solution of 8 g of pyridine–sulfur trioxide complex in 40 mL of Me_2SO, stirred for 45 min, and then treated slowly with 400 mL of cold water. The yellow granular solid was collected, washed with water, and dried to yield 2.1 g of a yellow solid. Recrystallization from methylene chloride–methanol gave 0.57 g of the title compound as an orange crystalline solid: NMR ($CDCl_3$) δ 2.52 (s, 3, CH_3), 7.4–8.6 (m, 6, aromatic), 11.2 (d, 2 CHO); IR (KBr) 5.95 μm (very strong, CHO).

Method H. 9,10-Dihydro-9,10-anthracenedicarboxaldehyde Bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] Dihydrochloride (V-1). A solution of 2.36 g (0.01 mol) of dihydrodialdehyde III-1 in 200 mL of 1-propanol was treated with 3.46 g (0.02 mol) of 2-hydrazinoimidazoline dihydrochloride and boiled on a hot plate over the course of 1.5 h while concentrating to 100 mL. On standing overnight at 4 °C there was formed 0.55 g of a pale yellow solid, mp 250–255 °C. The mother liquor and washings on standing gave an additional 0.55 g. The two fractions were combined and recrystallized from water to give 0.6 g of colorless flakes, mp 258–262°.

Method J. 9,10-Anthracenedicarboxaldehyde Bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] Dihydrochloride (VI-1). A mixture of 2.34 g (10 mmol) of 9,10-anthracenedicarboxaldehyde, 3.5 g (20 mmol) of 2-hydrazino-1-imidazoline dihydrochloride, and 100 mL of ethanol was heated under reflux for 2 h and then cooled and filtered to give 4.7 g (99%) of orange product: IR (KBr) 6.04 μm; UV (H_2O) λ_{max} 260 nm (ε 72 700), 415 (16 300); UV peaks were less sharp and lower if glassware was not previously specially washed to remove all traces of alkali; mass spectrum, m/e 228 [Ar$(CN)_2$]; ^{1}H NMR (D_2O) δ 3.75 (s, 8 H, NCH_2CH_2N), 7.18 (q, 4 H, Ar), 7.66 (q, 4 H, Ar), 8.15 (s, 2 H, CH=).

Method K. 1,1′-[9,10-Anthrylenebis(methylidynenitrilo)]bis[1-(2-hydroxyethyl)guanidine] Dihydrochloride (VI-19). A solution of 11.6 g (50 mmol) of *S*-methylthiosemicarbazide hydriodide and 3.2 g (50 mmol) of ethanolamine in 50 mL of ethanol was heated under reflux for 3 h. The solution was cooled and treated with 15 mL of 8 N ethanolic HCl, cooled again, and treated with 25 mL of ether to give a thick gum. The supernatant solution was decanted, and the residue was dissolved in 50 mL of hot ethanol, treated with 1 mL of water, and cooled to give 350 mg of a solid, which was collected by filtration and discarded. The filtrate was treated with more ethanolic HCl to give a viscous gum, and the supernatant solution was decanted. The residue (4.8 g) [crude 3-(2-hydroxyethyl)-1-aminoguanidine dihydrochloride] was dissolved in 65 mL of ethanol, treated with 1.9 g (8 mmol) of 9,10-anthracenedicarboxaldehyde, and heated under reflux for 2.5 h. The solution was filtered hot and cooled overnight to give 1.7 g of product. Recrystallization from 15 mL of dimethylformamide gave 1.0 g of solid, which was slurried in 6 mL of 2-methoxyethanol and filtered to give 450 mg of orange product.

Method M. 1,1′-[9,10-Anthrylenebis(methylidynenitrilo)]diguanidine Dihydrochloride (VI-14). A mixture of 3.5 g (15 mmol) of 9,10-anthracenedicarboxaldehyde, 4.1 g (30 mmol) of aminoguanidine bicarbonate, 5.4 mL of 8 N ethanolic HCl, and 100 mL of ethanol was heated under reflux for 2 h. The mixture was cooled and the product was collected to give 5.0 g (80%) of the product.

Method N. 1,1′-[9,10-Anthrylenebis(methylidynenitrilo)]bis(2,3-dimethylguanidine) Dihydriodide (VI-18). A mixture of 3.5 g (15 mmol) of 9,10-anthracenedicarboxaldehyde, 6.9 g (30 mmol) of 1-amino-2,3-dimethylguanidine hydriodide, 13 mL of 3.4 N ethanolic HI, and 100 mL of ethanol was heated under reflux for 2 h. The mixture stood at room temperature overnight, and the solid was collected to give 8.8 g of product. Recrystallization from 450 mL of water gave 6.1 g (61%) of product.

Method P. 9,10-Anthracenedicarboxaldehyde Bis(dimethylhydrazone) (VI-34). A mixture of 4.7 g (20 mmol) of 9,10-anthracenedicarboxaldehyde, 2.4 g of 1,1-dimethylhydrazine, 2 drops of acetic acid, and 200 mL of ethanol was stirred and heated under reflux for 2 h. The resulting solution was filtered hot and then cooled to give 4.4 g (70%) of the product.

Method Q. *N,N*″-(9,10-Anthrylenedimethylidyne)bis(*N′,N′*-dimethylethylenediamine) (VI-36). A mixture of 4.7 g (20 mmol) of 9,10-anthracenedicarboxaldehyde, 5.3 g (50 mmol) of *N,N*-dimethylethylenediamine, and 100 mL of toluene was stirred and heated under reflux while collecting byproduct water in a Dean–Stark trap. After 30 min no more water was being formed. The solution was filtered, then concentrated to 25 mL, and cooled to give a solid mixture. Washing with petroleum ether (bp 35–60 °C) gave 5.2 g (70%) of yellow product.

(40) Attempts to oxidize dihydrodicarboxaldehyde III-12 or its bishydrazone V-8 to their aromatic counterparts with the usual oxidizing agents failed, perhaps due to the steric effect of the bulky chlorine atoms in positions 1 and 5.

Method R. *N,N''*-(9,10-Anthrylenedimethylidyne)bis-(*N',N'*-dimethylglycine hydrazide) Dihydrochloride (VI-37). A mixture of 4.7 g (20 mmol) 9,10-anthracenedicarboxaldehyde, 6.2 g (40 mmol) of *N,N*-dimethylglycine hydrazide and 200 mL of ethanol was stirred and heated under reflux for 2 h. When cool, the mixture was filtered to give 9.5 g of orange solid. A turbid solution of this material in 400 mL of hot methanol was filtered through Celite, concentrated to 140 mL, cooled, and treated with 150 mL of ether. The resulting gum solidified upon standing overnight. This solid was collected, washed with acetone, redissolved in 200 mL of methanol, and chromatographed on silica gel, eluting with methanol until the major orange band had eluted. Another orange band remained on the column. The eluate on standing deposited a small amount (0.25 g) of solid, which was discarded. Evaporation of the filtrate gave 5.8 g of solid, which was dissolved in 150 mL of hot methanol, filtered, concentrated to 50 mL, seeded, then gradually diluted with 50 mL of ether as crystallization proceeded to give 5.7 g of orange product.

Method U. 1,1'-[9,10-Anthrylenebis(methylidynenitrilo)]bis(1-methylguanidine) Dihydrobromide (VI-16). A mixture of 2.3 g (10 mmol) of 9,10-anthracenedicarboxaldehyde, 3.4 g (20 mmol) of 1-amino-1-methylguanidine dihydrobromide, 1.3 mL of 7.3 N ethanolic HBr, and 200 mL of ethanol was heated under reflux for 17 h. The suspension was filtered hot to give 4.5 g (84%) of yellow product.

Method V. 1,1'-[9,10-Anthrylenebis(methylidynenitrilo)]bis[3-(pyrid-4-ylmethyl)guanidine] Tetrahydrochloride (VI-27). A mixture of 3.5 g (15 mmol) of 9,10-anthracenedicarboxaldehyde, 7.4 g of 1-amino-2-(pyrid-4-ylmethyl)guanidine dihydrochloride, 150 mL of ethanol, and 10 mL of water was heated under reflux for 4 h. The mixture was cooled to room temperature and filtered to give 9.5 g (93%) of product.

Method X. 9,10-Anthracenedicarboxaldehyde Bis[(4,5-dihydro-1*H*-imidazol-2-yl)methylhydrazone] Dihydriodide (VI-4). A solution of 1.0 g (2.1 mmol) of VI-1 in 30 mL of water was treated with 0.45 g (4.2 mmol) of Na_2CO_3 to give 0.77 g of the crystalline free base of VI-1, mp 307–308 °C dec. A mixture of 500 mg of this free base, 16 mL of dimethylformamide, and 6 mL of methyl iodide was stirred at room temperature for 2.75 h. The solid dissolved and a new crystalline product separated: yield 700 mg (81%). Mixture melting point, IR spectra, and TLC showed this material to be identical with a sample of the compound made by method N.

Method Y. 9,10-Anthracenedicarboxaldehyde Bis[4-[2-(dimethylamino)ethyl]-3-thiosemicarbazone] Dihydrobromide (VIII-4). A stirred solution of methyl dithiocarbazinate (12.2 g, 0.1 mol), 15 mL of dimethylaminoethylamine, and 50 mL of H_2O was heated under reflux for 0.75 h. After cooling to room temperature, the solution was evaporated under reduced pressure to yield an oily residue. This residue was dissolved in 100 mL of benzene/methanol (4:1) and then filtered through a basic alumina pad. The pad was washed with 750 mL of benzene/methanol solution, and the combined filtrate and washes was evaporated to yield a colorless solid, which, when crystallized from 75 mL of benzene, gave 8.10 g (50%) of 4-[2-(dimethylamino)-ethyl]-3-thiosemicarbazide, mp 109.5–111 °C.

A solution of 2.1 g (8.96 mmol) of 9,10-anthracenedicarboxaldehyde, 3.5 g (21.6 mmol) of 4-[2-(dimethylamino)ethyl]-3-thiosemicarbazide, and 12 mL of 33% HBr/CH_3CO_2H in 200 mL of 95% ethanol was heated under reflux for 3.0 h, and the resulting precipitate was isolated as an orange-red solid. Recrystallization of the crude material from ethanol/H_2O/ether yielded 2.6 g of product.

Method Z. 9,10-Anthracenedipropionaldehyde Bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] Dihydrochloride (IX-3). A solution of 1.0 g (3.4 mmol) of 9,10-bis(3-hydroxypropyl)anthracene,[41] 1.54 g (7.46 mmol) of dicyclohexylcarbodiimide, and 4 drops of 100% H_3PO_4 in 10 mL of anhydrous Me_2SO was stirred at ambient temperature for 18 h. The solution then was diluted with 50 mL of toluene and filtered, and the filtrate was successively washed well with saturated LiCl solution and saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to yield a yellow-colored solid residue. This residue was triturated with cold ether, and the residual solid was isolated and dried to yield 0.58 g (58%) of anthracene-9,10-bispropionaldehyde. This material (1.98 mmol) was heated under reflux in 50 mL of ethanol with 0.69 g (4.0 mmol) of 2-hydrazino-2-imidazoline dihydrochloride for 4.0 h and then allowed to cool to room temperature. The solvent was evaporated under reduced pressure to yield a light-yellow solid residue, which, when crystallized from CH_3OH/H_2O/ether, gave 0.6 g (57%) of IX-3, mp 263–265 °C. IR and NMR spectral data were consistent with structures of the final product and the intermediate dialdehyde. Anal. Calcd for $C_{26}H_{30}N_8 \cdot 2HCl \cdot 2.25H_2O$ (M_r 568): C, 55.0; H, 6.5; N, 19.8; Cl, 12.5; H_2O, 7.2. Found: C, 55.2; H, 6.0; N, 19.1; Cl, 12.0; H_2O, 7.4.

Method AA. 9,10-Anthracenediacrolein Bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone] Dihydrochloride (IX-4). To a stirred solution of 9.86 g (44.0 mmol) of triethyl phosphonoacetate in 200 mL of anhydrous DMF at room temperature was added 2.12 g (~44.0 mmol) of NaH (50% suspension in mineral oil), and the resultant mixture was stirred for 1.0 h. Anthracene-9,10-dicarboxaldehyde (4.69 g, 20.0 mmol) was added to the reaction solution, and the resultant solution was stirred vigorously at room temperature for 18.0 h. The solvent was removed under reduced pressure, the residue was dissolved in 100 mL of CH_2Cl_2, and the solution was washed well successively with saturated solutions of LiCl, $NaHCO_3$, and NaCl, then dried ($MgSO_4$), and filtered through a Magnesol pad. The filtrate was evaporated under reduced pressure to yield 6.9 g (92%) of yellow solid. IR and ^1H NMR spectral data were consistent with the expected structure, diethyl 9,10-anthracenediacrylate. Crystallization of this crude diester from aqueous ethanol gave orange-yellow crystals, mp 148.5–150.5 °C. Anal. ($C_{24}H_{22}O_4$) C, H.

To a stirred solution of 1.85 g (4.94 mmol) of the above diester in 25 mL of toluene under a N_2 atmosphere at 0 °C was added, by syringe, 30 mL (~33.0 mmol) of diisobutylaluminum hydride (20% in hexanes). The resultant reaction solution was stirred at room temperature for 7 h. The solution then was poured onto 100 g of cracked ice, the mixture was left to melt, and the organic layer was separated, dried (Na_2SO_4), and evaporated to yield a solid residue. This residue was crystallized from toluene at 0 °C to yield 0.95 g (66%) of 9,10-hydroxypropen-1-yl)anthracene, mp 164–166 °C or mp 167–169 °C after recrystallization from toluene. Anal. ($C_{20}H_{14}O_2$) C, H.

A mixture of 436.0 mg (1.5 mmol) of the above dicarbinol and 2.6 g of MnO_2 (Sterling-Winthrop) in 30 mL of benzene was heated under reflux for 20 h. The mixture then was filtered, and the cake was washed well with CH_2Cl_2. The combined filtrate and wash was evaporated under reduced pressure to yield an orange solid (275 mg), which, when twice crystallized from toluene, gave 125 mg (29%) of 9,10-anthracenediacrolein, mp 247–249 °C. Anal. ($C_{20}H_{14}O_2$) C, H.

A solution of 0.5 g (1.75 mmol) of the above 9,10-anthracenediacrolein, 0.67 g (3.84 mmole) of 2-hydrazino-2-imidazoline dihydrochloride, and 2 mL of concentrated HCl in 100 mL of 95% ethanol was heated under reflux for 5.0 h. The reaction solution was evaporated under reduced pressure, and the residue was crystallized from ethanol/ether to yield 0.55 g (60%) of orange-red crystals, mp 291–293 °C dec. IR and ^1H NMR spectral data were consistent with the expected structure of the bishydrazone IX-4. Anal. Calcd for $C_{26}H_{26}N_8 \cdot 2HCl \cdot 2.25H_2O$ (M_r 564): C, 55.3; H, 5.8; N, 19.9; Cl, 12.6; H_2O, 7.2. Found: C, 55.6; H, 5.3; N, 19.2; Cl, 12.1; H_2O, 7.3.

9-Anthraldehyde (4,5-Dihydro-1*H*-imidazol-2-yl)-hydrazone. A mixture of 6.2 g (0.03 mmol) of 9-anthraldehyde, 5.2 g (0.03 mol) of 2-hydrazino-1-imidazoline dihydrochloride, 2.4 g of anhydrous sodium acetate, and 100 mL of 1-butanol was stirred and heated until it boiled and then for just 1 min more, then filtered, and cooled to about 50 °C. The crystalline product was collected and washed with acetone to give 7.5 g (74%) of product as golden yellow prisms, mp 171–174 °C. Anal. ($C_{18}H_{14}N_4 \cdot HCl \cdot 0.75H_2O$) C, H, N, Cl.

1-Amino-2-(pyrid-4-ylmethyl)guanidine Dihydrochloride. A mixture of 46.6 g (0.2 mol) of *S*-methylthiosemicarbazide hydriodide, 43.0 g (0.4 mol) of 4-(aminomethyl)pyridine, and 200 mL of ethanol was heated under reflux for 2.5 h and cooled overnight to obtain 40 g of crystalline product. This was re-

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

crystallized from 380 mL of ethanol to give 29 g of product, which was dissolved in 300 mL of hot ethanol and filtered and the filtrate was treated with 65 mL of concentrated hydrochloric acid to give 20 g of crystalline product, mp 247–251 °C. This was suspended in 350 mL of ethanol and the resulting mixture was heated to boiling and water was added just to solution. This was filtered, the filtrate was treated with 30 mL of concentrated HCl, and the solution was cooled quickly to give 13.7 g (29%) of product, mp 248–252 °C. Anal. ($C_7H_{11}N_5 \cdot 2HCl$) C, H, N, Cl.

1-Amino-2-(pyrid-2-ylmethyl)guanidine Dihydrochloride. A mixture of 46.6 g (0.2 mol) of *S*-methylthiosemicarbazide hydriodide, 43.0 g (0.4 mol) of 2-(aminomethyl)pyridine, and 200 mL of ethanol was heated under reflux for 2.5 h, then cooled, and treated with 85 mL of concentrated HCl followed by cooling to give 65 g of crystalline solid. A solution of this solid in 500 mL of boiling ethanol and 50 mL of water was filtered, the filtrate was treated with 25 mL of concentrated HCl, and the solution was cooled to give 30 g of crystalline product, mp 210–213 °C. This solid was dissolved in a hot solution of 400 mL of ethanol and 15 mL of water, treated with 20 mL of concentrated HCl, and cooled quickly to obtain 24.5 g (54%) of crystalline product, mp 213–216 °C. Anal. ($C_7H_{11}N_5 \cdot 2HCl$) C, H, N, Cl.

1-Amino-2-(pyrid-3-ylmethyl)guanidine Trihydrochloride. A mixture of 46.6 g (0.2 mol) of *S*-methylthiosemicarbazide hydriodide and 43.0 g (0.4 mol) of 3-(aminomethyl)pyridine in 200 mL of ethanol was heated under reflux for 2.5 h and then cooled overnight to give 37 g of crystalline product. This solid was dissolved in 400 mL of hot ethanol, the solution was filtered, and the filtrate was treated with 50 mL of concentrated HCl and then cooled to give a gummy oil, which crystallized: yield 29.5 g. A solution of this material in 400 mL of boiling ethanol and 15 mL of water was cooled to about 50 °C and treated with 20 mL of concentrated HCl to give 17.5 g of product. The process was repeated to give 8.9 g of product. All of these materials had unsatisfactory melting points and/or analyses. This material was dissolved in 10 mL of water and treated with 20 mL of concentrated HCl and then with 55 mL of ethanol to obtain 2.5 of crystalline product, mp 250–255 °C. Anal. ($C_7H_{11}N_5 \cdot 3HCl$) C, H, N, Cl.

Acknowledgment. We thank L. M. Brancone and associates for microanalyses, W. Fulmor, George Morton, and Dr. William Gore for spectral data and interpretations, Drs. A. S. Tomcufcik, R. G. Wilkinson, and Roger Addor for gifts of intermediates, and Sandra E. Chillous for technical assistance.

Inhibition by 5-(Substituted-benzyl)-2,4-diaminopyrimidines of Murine Tumor (L5178Y) Cell Cultures Sensitive to and Resistant to Methotrexate.[1] Further Evidence for the Sensitivity of Resistant Cells to Hydrophobic Drugs

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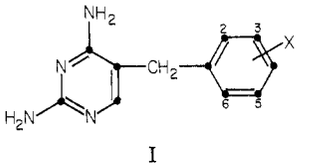
Forty-three 5-(substituted-benzyl)-2,4-diaminopyrimidines have been studied as inhibitors of murine tumor cell cultures (L5178Y). Two types of cells were used—one resistant to methotrexate and one sensitive to methotrexate. The formulation of quantitative structure–activity relationships showed that the methotrexate-resistant cells are more sensitive to the more hydrophobic congeners. π_0 for the sensitive cells is about 1.4, while π_0 for the methotrexate-resistant cells is above 3. These results are similar to those found for 2,4-diaminotriazines (Selassie, C. D.; Guo, Z. R.; Hansch, C.; Khwaja, T. A.; Pentecost, S. *J. Med. Chem.* **1982**, *25*, 157).

The great success of the inhibitors of dihydrofolate reductase (DHFR) as antibacterials (trimethoprim, tetroxoprim) and antitumor agents [methotrexate (MTX), Baker's antifols] is a fascinating chapter in medicinal chemistry. Although the general mechanism of action of the antifols is understood,[3] the details of how these compounds achieve their success are still unclear. Why is trimethoprim so selective to bacterial enzyme as compared to human reductase? Although MTX shows little, if any, selectivity for DHFR from different sources, why is it so remarkably effective in the treatment of a variety of cancers,[4-8] as well as other diseases?[9] We believe that much improved and more selective drugs for many diseases can be discovered by gaining a clearer understanding of the details of how ligands interact with various DHFR's. For this reason we have been systematically studying the inhibitory action of two classes of drugs (I and II) on purified DHFR.[10-13] The quantitative structure–activity relationships (QSAR) formulated from these investigations provide ideas for the synthesis of new analogues.

While the inhibition constants one finds for isolated DHFR are a good measure of the intrinsic activity of an inhibitor, we cannot yet predict with much assurance how



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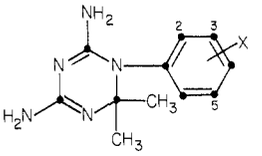

II

such inhibitors will behave in cell cultures or, especially, in animals. In order to gain some general knowledge of

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(1) This research was supported by Grants CA-11110 (C.H.) and CA-14089 (T.K.) from the National Cancer Institute.
(2) Visiting Professor from Beijing Medical College, Beijing, China.
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