g, 0.010 mol) was then added and the reaction was stirred at 20 °C for 45 h. The reaction mixture was washed with 10% NaOH solution and the organic layer dried (Na_2SO_4). After filtration, the solution was concentrated under reduced pressure and the residue placed on a dry silica gel column. Elution with 90:10 $CHCl_3$/EtOAc afforded the desired compound **9** (R_f 0.35) as a white powder: yield 1.15 g (23%).

Method D. To a stirring mixture of *N*-(*tert*-butoxycarbonyl)glycine (7.01 g, 0.04 mol), Et_3N (4.05 g, 0.04 mol), and 250 mL of dry THF in an ice bath under an argon atmosphere was added pivaloyl chloride (4.9 mL, 4.81 g, 0.04 mol) at one time. After the mixture was stirred for 0.3 h, Et_3N (2.02 g, 0.02 mol) was added followed by the dropwise addition of 1 (8.07 g, 0.02 mol) in 100 mL of dry THF, maintaining the reaction temperature at <5 °C. The ice bath was removed and the reaction stirred at 20 °C for 15 h. Since TLC [Quantum MQ6F plates, EtOAc/$CHCl_3$ (1:1), UV visualization] indicated that 1 was still present, the reaction mixture was cooled in an ice bath and 7.01 g (0.04 mol) of *N*-(*tert*-butoxycarbonyl)glycine and 7.08 g (0.06 mol) of Et_3N were added. Pivaloyl chloride (4.80 g, 0.04 mol) was then added dropwise, maintaining the reaction temperature at <5 °C. After this addition, the ice bath was removed and the reaction was stirred at 20 °C for 18 h. The reaction mixture was filtered and the filtrate concentrated under reduced pressure. The residue was taken up in EtOAc and washed sequentially with 10% NaOH solution, H_2O, and saturated NaCl solution. The organic layer was concentrated under reduced pressure and the residue chromatographed on dry silica gel column (1.3 m × 50 mm flat diameter) using EtOAc/$CHCl_3$ (1:1) as developer. The compound at R_f 0.60 was collected and rewashed with 10% NaOH solution to remove residual pivaloyl chloride. The organic layer was concentrated under high vacuum, where it foamed and crystallized affording 6.04 g of yellow solid, **14**.

Method E. Compound **9** (20.0 g, 0.34 mol) and 50 mL of glacial HOAc were stirred at 20 °C under an argon atmosphere. To this solution was added 50 mL of 4 N HBr/HOAc and the reaction was stirred for 1.25 h. The yellow reaction mixture was poured slowly into anhydrous Et_2O. The precipitate was washed with anhydrous Et_2O and the solvent decanted. The residue was dissolved in water, frozen, and lyophilized to produce a fluffy, off-white solid **16**: yield 15.21 g. To a solution of 13.13 g (0.024 mol) of **16** in 200 mL of H_2O was added 50 mL of 10% NaOH solution. The solution was extracted with EtOAc, and the combined organic layers were washed with a saturated NaCl solution and dried (Na_2SO_4). After filtration, the filtrate was concentrated to afford a yellow oil, which was dissolved in anhydrous Et_2O and poured slowly into an Et_2O–HCl solution (25 mL of 6 M HCl/dioxane to 600 mL of anhydrous Et_2O). The precipitate was washed with anhydrous Et_2O, dissolved in H_2O, frozen, and lyophilized. After 90 h, a light yellow powder (7.85 g) of the desired HCl salt, **17**, was harvested as a monohydrate.

Method F. Compound **9** (1.0 g, 1.68 mmol), 10% Pd/C (300 mg), and 50 mL of glacial HOAc were mixed and placed on a Parr hydrogenator. The mixture was shaken under 15 psi of H_2 for 16 h at 20 °C and then filtered through a Celite filter pad. The filtrate was made basic with a 10% NaOH solution and extracted with EtOAc. The organic phase was washed with saturated NaCl solution, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The red residue was dissolved in Et_2O and then slowly poured into Et_2O–HCl (35 mL of 6 M HCl/dioxane in 250 mL of anhydrous Et_2O). The precipitate was washed three times with 600-mL portions of anhydrous Et_2O. Yield of the desired salt, **17**, was 0.33 g.

Method G. A solution of 1.50 g (0.00268 mol) of **14**, 25 mL of dioxane, and 25 mL of 6 M HCl/dioxane was stirred at 20 °C for 2.75 h and then concentrated using high vacuum. The residue was poured into anhydrous Et_2O and the precipitate was washed two times with anhydrous Et_2O (2 × 600 mL). The solid residue was dissolved in H_2O, frozen, and lyophilized. After 45 h, the lyophilization afforded a white fluffy powder (1.16 g) of the desired salt, **17**, as the trihemihydrate.

Antihypertensive Activity. The antihypertensive activity of the compounds was determined by Pharmakon Laboratories, Scranton, PA, under the direction of Richard J. Matthews. The experimental procedure for these determinations is reported in the accompanying paper.[1]

Some Short-Chain N^6-Substituted Adenosine Analogues with Antitumor Properties[1]

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The compounds N^6-allyl-, N^6-isopropyl-, N^6-propargyl-, and N^6-(2-methylallyl)adenosine were prepared by reacting 6-chloropurine riboside with an excess of the corresponding amines in ethanol, in the presence of two acid acceptors resulting in virtually quantitative yields. The compounds showed biological activity in a number of in vitro and in vivo tumor cell systems. Very good increases in life spans of mice bearing mammary carcinoma were obtained by treatment with the N^6-allyl, N^6-isopropyl, and N^6-propargyl analogues, respectively. In rats, the N^6-allyl analogue slowed the rate of transplantable mammary tumor growth by one-fourth. The short-chain adenosine analogues are more active in the treatment of animal carcinomas than in the leukemia or sarcoma tumor cell systems.

A number of N^6-substituted adenosine analogues containing five or more carbon atoms in the substituent chain[2-4] including one with a nitrogen mustard moiety[5] were synthesized and examined in recent years, and a significant proportion of these were found to possess antitumor activity in a variety of in vitro systems. Several of the compounds also had moderate activity against murine L-1210 leukemia in vivo, with an increase in life span of 41–50% over controls. An interesting finding was that these compounds had no cytotoxic activity against a leukocyte cell line in vitro which had originated from normal cells, while against certain cultures of tumor cell

(1) Presented in part before the Division of Medicinal Chemistry of the American Chemical Society–Japan Chemical Society Congress in April, 1979. See M. H. Fleysher, R. J. Bernacki, and E. Mihich, "Abstracts of Papers", ACS/CSJ Chemical Congress, Honolulu, HI, Apr 1–6, 1979, American Chemical Society: Washington, D.C., 1979, Abstr MEDI 29.

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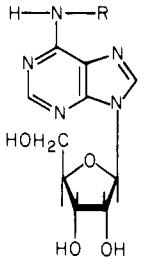
Table I. Growth Inhibition in Several Cell Lines (in Vitro)

		molecular concn for 50% growth inhibn (ID_{50}), M × 10^{-6}							
no.	N^6-Ado substit	sarcoma S-180 cells	leukemia L-1210 cells	carcinoma TA-3 cells	Hela cells	human bladder carcinoma cells	human Burkitt lymphoma cells	human fibroblasts	mouse fibroblasts
1	N^6-allyl	20.0	12.0	3.0		7.5	2.3	2.7	14.0
2	N^6-isopropyl	30.0	19.0	13.0		11.0	27.0	6.5	20.0
3	N^6-propargyl		1.0	2.5	3.5	3.8	8.3	2.8	3.1
4	N^6-(2-methylallyl)		19.0	18.0		9.0	11.0	1.5	25.0
	N^6-benzyl[a]	45.0	8.5	14.0			11.0		

[a] For comparison.

lines of human origin[3] they were toxic at micromolar concentration. These compounds had no myelotoxic effects in vivo[6] at the doses used.

In the case of analogues containing a short carbon chain (e.g., an alkyl chain of 3 carbon atoms) in the N^6 moiety, earlier work indicated activity against a subline of sarcoma S-180 cells in vitro.[2] Later trials with L-1210 leukemia in mice demonstrated that the allyl and isopropyl analogues were very toxic at the dosage of 100 mg/kg but sharp reduction of the dosage to about 10 mg/kg improved responses in vivo. Accordingly, two additional analogues have been synthesized to extend this series of compounds. The present study is limited to an improved synthesis and the antitumor effects of those analogues were the N^6 substituent consists of a short three-carbon chain or a simple modification of it. The compounds are N^6-allyl- (1), N^6-isopropyl- (2), N^6-propargyl (3), and N^6-(2-methylallyl)adenosine (4).



R

adenosine = H
1, allyl = $-CH_2CH=CH_2$
2, isopropyl = $-CH(CH_3)_2$
3, propargyl = $-CH_2C\equiv CH$
4, 2-(methylallyl) = $-CH_2C(CH_3)=CH_2$

Synthesis. Synthesis of the N^6-allyl and the N^6-isopropyl analogues was described previously.[2] At that time the synthesis consisted of reacting 6-chloropurine riboside with an excess of the appropriate amine, with or without an additional acid acceptor such as $CaCO_3$ or Et_3N, by refluxing in anhydrous EtOH, and 60–65% yields were obtained. The addition of either acceptor had little effect upon the yields. In the present work the same procedure was used except that *two* acid acceptors, $CaCO_3$ and Et_3N, were employed simultaneously in the reaction. This ensured the completion of the reaction with nearly theoretical yields.

Two of the compounds, the N^6-allyl and the N^6-propargyl analogues were also prepared via the N-1 quaternization reaction, by reacting adenosine with the appropriate bromide in dimethylformamide, followed by isolation of the N^1-quaternary adduct, and subsequent conversion by the Dimroth rearrangement to the N^6 analogue with aqueous NH_4OH.[2,3]

Table II. Growth Inhibition of Human Breast Tumor Cells (in Vitro)

	molecular concn for 50% growth inhibn (ID_{50}), M × 10^{-6}	
compd	SW-613 human breast adenocarcinoma cells (insensitive to estrogen)	MCF-7 human breast carcinoma cells (sensitive to estrogen)
N^6-allyladenosine (1)	50	1.4
N^6-isopropyladenosine (2)	150	6.3
N^6-2-(methylallyl)adenosine (4)	4.0	0.4
N^6-propargyladenosine (3)		1.6
N^6-benzyladenosine[a]	4.0	22.0

[a] For comparison.

Biological Activity and Discussion. Compounds 1–4 demonstrated different activity against various cell lines in vitro (Table I). In general, the propargyl analogue was the most active compared against a variety of cell lines, including L-1210 leukemia where it had an ID_{50} of 1 μM. In the case of human breast tumors grown in culture (Table II), the MCF-7 cells (sensitive to estrogen) were more sensitive to the adenosine analogues than the SW-613 cells (insensitive to estrogen). The results suggest that these analogues may be useful for the treatment of estrogen receptor positive, human breast carcinoma.

Studies with these analogues in vivo demonstrated considerable toxicity, necessitating sharply reduced dosages or fractionated regimens to be effective; for example (Table III in the widely used L1210 mouse leukemia system, a dosage of 6 to 12 mkd [(mg/kg)/day] divided into two doses per day compared to 100 or more mkd for the more lipophylic benzyl analogue produced similar increases in life span. Under these conditions a 30% ILS (increased life span) was obtained for the allyl and 37% ILS for the isopropyl analogue. Benzyladenosine at a much larger dose of 125 mkd had up to 49% ILS. However, against carcinoma TA-3 (in A/S female mice) and against Taper hepatoma (in Ha/ICR female Swiss mice) these four analogues had no antitumor activity at doses up to 50 mkd with toxicity developing at the higher dose levels.

An extensive in vivo study was done with fast and slow growing breast tumors, which were developed in this department.[7] The fast growing mammary tumor (MT-F)

(6) R. Catane, J. H. Kaufman, F. A. Nime, J. T. Evans, and A. Mittelman, *Cancer Treat. Rep.*, 62(9), 1371 (1978).

Table III. Activity against Mouse Leukemia, L-1210 (DBA/2 Ha Mice)

compd	dose, (mg/kg)/day	mean life span, days	% ILS	mean life span, days (fractionated dose)[a]	% ILS
N^6-allyladenosine (1)	control	7.7		7.7	
	1.56	8.3	8.0		
	3.12	7.6			
	6.25	8.3	8.0	10.0	30.0
N^6-isopropyladenosine (2)	control	7.5		7.5	
	6.25	7.3		8.6	14.7
	12.50	8.8	17.4	10.3	37.4
N^6-propargyladenosine (3)	control	7.5			
	6.25	8.2			
	12.5	9.0	20.0		
N^6-benzyladenosine[b]	control	6.9			
	125	9.7-10.3	41-49		
N^6-(2-methylallyl)adenosine					

[a] Dose divided in two halves and administered in two daily injections. [b] For comparison. [c] NA = no activity indicated.

Table IV. Activity against Slow Growing Spontaneous Mammary Tumors in the DBA/2 Ha-DD Mouse

compd	dose, (mg/kg)/day (mkd)	treatment	mean life span, days	day of tumor half growth	% increased life span (ILS)
N^6-allyladenosine (1)	control		39.6	16	
	50 × 5	once on 5 consec days	72.3	16[a]	82.5
N^6-isopropyladenosine (2)	control		39.6	16	
	12.5 × 5	once on 5 consec days	49.7	17	25.5
	25.0 × 5		63.3	24	60.0
	control	5 doses, 2× weekly	33.6	17	
	6.25 × 5		54.8	17[a]	63.1
melphalan plus addition of N^6-allyladenosine	control		33.6	12	
	3.8 mkd × 5 melphalan	5 consec days, 2× weekly	46.6	26	38.7
	+50 mkd N^6-allyl		48.0	30.0	42.8
or of N^6-isopropyladenosine (2)	+25 mkd N^6-isopropyl	2× weekly	23.6		
N^6-(2-methylallyl)adenosine (4)	control		25.2	14	
	12.5	once daily on 5 consec days	30.0	15	19.0
N^6-propargyladenosine (3)	control		34.4	17	
	6.25	once daily on 5 consec days	50.0	18	60.0
N^6-benzyladenosine[a]	control		35.0	17	
	100.00	twice weekly, 5 doses	38.8	20	10.9
	control		49.8	18	
	50.00	2× weekly for 7 weeks	62.6	21	26.0

[a] See Discussion. [b] For comparison.

and the slower growing variety (MT-S) are distinctly different from each other in that mice bearing MT-F survive about 31 days and those bearing MT-S about 40 days. These differences have been ascribed to changes in antigencity and oncogenic potential.[7] A study of cell population kinetics[8] attributed the differences in growth rates of the tumor to prolongation of the cell cycle. In this study the best results were obtained with the slow growing mammary tumors (MT-S) in the DBA-2 Ha DD mice (Table IV). The N^6-allyl analogue was the most effective; however, good results were also obtained with the N^6-isopropyl and the N^6-propargyl analogues. Combination therapy involving mephalan with the N^6-allyl analogue resulted in no increase in life span above that of melaphalan alone, while its combination with N^6-isopropyladenosine resulted in host toxicity. the N^6-(2-methylallyl)analogue was only weakly active, as was the relatively liphophylic N^6-benzyl analogue.[3]

The fast growing MT-F tumors were not affected by treatment with compounds 1–4 and appear to be completely resistant. A major point of difference between the MT-F cells tumor and their slower growing counterpart, the MT-S tumor, lies in their morphology. Micrographs[8] of the MT-F and MT-S tumor tissue under the same magnification reveal a densely packed tumor cell structure

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Table V. Synthesis of Compounds 2-4

N^6-adenosine analogue	yield, %	mp, °C	UV ($\epsilon \times 10^{-3}$)	NMR pattern	crystn solvent	other variables
N^6-isopropyl (2)	95.0	157	similar to 1	similar to 1	MeCN-EtOH	evap condensation to dryness and recrystallize
N^6-propargyl (3)	99.0	169	similar to 1	similar to 1	hot EtOH	evap and crystallized product[a]
N^6-(2-methylallyl) (4)	99.0	142	similar to 1	similar to 1	EtOH-H_2O	evap and crystallize by adding water, then recrystallize

[a] Notes on compound 3: During the progress of this work this compound was disclosed on a paper by S. P. Dutta et al.[9] The synthesis was similar, except that no auxiliary catalysts were employed and the yield reported was 70%. Compound 3 was also prepared in this way via the N^1-adenosine quaternization reaction with propargyl bromide at 35-40 °C for 3 days to obtain an indicated (TLC) yield of 50% and an isolated yield of 46% of a satisfactory product. The condensation time for compound 3 is 10 h and must not be exceeded to avoid decomposition and lowering of yield and purity. Compounds 1 to 4 are characterized by an improved water solubility (3-4 mg/mL) as compared with about 1 mg/mL for the more lipophilic analogues condensing five or more carbon atoms in the side chain.

for MT-F, whereas in the case of MT-S there are large areas of extracellular space, including glandular areas. Human breast tumor cells have normally a loosely structured morphology[14] and perhaps the SMT-S may be a good murine in vivo model for human disease. These animal studies indicate that these purine adenosine analogues, especially the N^6-allyl and possibly the N^6-propargyl analogues, may be effective chemotherapeutic agents for the treatment of the human breast carcinoma. The in vivo results reflect the good activity these agents demonstrated in vitro with the human MCF-7 human breast cell line. It appears therefore that these purine analogues may be effective for the treatment of estrogen receptor positive human breast carcinoma.

Some work was done with rats bearing mammary tumors. Wistar-Furth rats bearing MTW-9B mammary tumors were injected (ip) with N^6-allyladenosine. It was found that the controls were growing at four times the rate as the tumors in the treated animals.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp melting point apparatus and are not corrected. UV spectra were obtained with a Cary Model 14 recording spectrophotometer and thin-layer chromatography (TLC) was done with a E. M. Reagents (E. Merck) precoated TLC sheet with silica gel 60 F 254, aluminum backed, and the spots detected by ultraviolet light. The solvent systems employed were ethyl acetate-MeOH (3:1) and $CHCl_3$-MeOH (3:1). The NMR spectra were obtained with a Varian XL-100 spectrophotometer. The 6-chloropurine riboside was purchased from Sigma Chemical Co. and the amines from Aldrich Chemcial Co. All evaporations were carried out in vacuo with a rotary evaporator. Where analyses are indicated by symbols of the elements, analytical results obtained are within 0.4% of the theoretical values.

N^6-Allyladenosine (1). Allylamine (3.59 g, 63.0 mmol), $CaCO_3$ (6.3 g, 63.0 mmol), triethylamine (6.3 g, 63.0 mmol) and 6-chloropurine riboside (6.3 g, 20.9 mmol) were added to 200 mL of absolute EtOH, and the mixture was stirred and refluxed for 20 h until no residual 6-chloropurine riboside remained (TLC). The hot reaction was filtered to remove the insoluble calcium salts, which were washed with hot EtOH, and washings were added to the filtrate. The combined solutions were evaporated to dryness, employing several additions of anhydrous EtOH during the evaporation to assist removal of all the volatile amine residues. The dry crystalline residue was recrystallized from water (charcoal) containing a trace of EtOH and dried in vacuo over P_2O_5: yield (three crops) 6.23 g (97%); mp 167 °C (lit.[2] mp 166-167 °C).

The analysis and UV absorption maxima were the same as reported earlier:[2] NMR (Me_2SO-d_6-D_2O) δ 8.39, 8.25 (2 s, 2 H, H-2 and H-8), 8.09 (t, 1 H, N-4), 5.99 (m superimposed with anomeric proton, 1 H, H_a), 5.92 (d, $J_{1',2'}$ = 6.29 Hz, 1 H, H-1'), 5.19 (q, 1 H, H_b), 5.10 (q, 1 H, H_b), 4.63 (t, 2 H (CH_2 attached to NH), 4.29-4.13 (m, 2 H, sugar 2'-3' protons), 4.02 (d, 1 H, 4' proton), 3.69 (t 2 H, 5' and 5'' protons of sugar).

Several preparations of the N^6-allyl analogue were also made via the adenosine N-1 quaternization reaction described previously.[2] The yields averaged 45% and the properties of the product were the same as given (see Table V).[2]

Growth Inhibition Studies (in Vitro) (Table I). Sarcoma S-180 Cells. The compounds were added in aqueous solution to cultures of S-180 cells grown as monolayers in T-15 flasks in Eagle's[10] medium containing 5% horse serum. The cells were grown for 6 days involving four changes of the medium and the control cultures increased by 10- to 15-fold. The quantity of cells was estimated by the crystal violet method of Grady et al.[11] and by protein determinations.[11,3]

Leukemia L-1210 Cells. The cells were maintained in suspension culture in RPMI medium 1640, supplemented with 10% fetal calf serum which has been inactivated at 57 °C for 30 min. The drug concentration inhibitory cell growth by 50% was determined as described[4] and expressed as ID_{50} value.

Carcinoma TA3 Cells. This cell line (mouse adenocarcinoma TA-3) was grown in RPMI-1640 medium supplemented with 10% fetal calf serum. The test system consisted of tube culture (12 × 75 mm) incubated with 5×10^4 cells per mL of medium, supplemented with 1 mL of medium containing the compound, and incubated at 37 °C in an upright position. All concentrations were tested in triplicate, growth was monitored after 3 days by counting the cell number with a Coulter counter, and the ID_{50} values were calculated as described.[4]

Hela Cells. This cell line was grown in RPMI-1640 medium supplemented with 10% fetal serum. The test consisted of tube cultures (13 × 100 mm tubes) in which 5×10^4 cells per mL of medium were supplemented with the drug in 1 mL of medium and incubated for 4 days. The quantity of cells was estimated by the Lowry protein assay.[12]

Bladder Carcinoma, Lymphoma, and Human and Mouse Fibroblasts. The additional group of cell lines used in this study were (a) human transitional bladder carcinoma cells (RT4), obtained from Dr. J. Fogh of the Sloan Kettering Institute; (b) Burkitt's lymphoma cells (DAUDi), obtained from Dr. Fogh; (c) human diploid foreskin fibroblasts (BG-9), isolated at R.P.M.I. in the laboratory of Dr. J. S. Horoszewicz;[13] and (d) mouse fibroblast NCTC clone-929, obtained from the American Type Culture Collection. All of these cells were grown in media RPMI-1640 [Gibco supplemented with 15% heat-inactivated fetal calf serum (KC Biological)] and 0.2 mM L-glutamine (Gibco). Cells were seeded in FB-24 TC trays (Lambes) in 1 mL of culture medium. Initial inoculum of cells were as follows: RT4 cells, 7×10^4 cells/mL; Daudi cells, 2.4×10^5 cells/mL; BG-9 P-26, 2×10^4 cells/mL; and L-929, 5×10^3 cells/mL.

The cells were incubated at 37 °C in 7.5% CO_2 and incubated for 2 days or in the case of Daudi cells overnight. Stock solutions of the drugs were prepared in culture medium and filtered thru a 0.22-μm Sweeny filter, and dilutions of the four drugs were added to duplicate wells of the four cell lines above. Medium without drugs added to duplicate wells served as the control. The cells were incubated for an additional 5 days, and in the case of the Daudi cells an additional 5 days (120 h). The cells in each well were trypsinized and counted by means of a Coulter counter

(14) R. W. McDivitt, F. W. Stewart, and J. W. Berg, "Tumors of the Breast. An Atlas of Tumor Pathology", Armed Forces Institute of Pathology, Washington, D.C., 1968.

(Model ZBI). Since the Daudi cells were grown in suspension culture, they were counted without trypsinization. The average number of cells/mL (in duplicate) for each drug was plotted against the concentration and response curves obtained. The concentration at 50% inhibition is the (ID_{50}) cell growth concentration. Results of this group of cells are given in Table I.

Growth Inhibition of Human Mammary Cells (in Vitro) (Table II). (a) Human mammary SW-613 cells (adenocarcinomas) were supplied by Dr. E. M. Jensen, of the E.G. & G/Mason Research Institute, Rockville, MD. The cloning efficiency of these cells in soft agar was 9%, and they were not responsive to estrogens. Their tumorgenicity in nude mice was excellent. For test purposes, 5×10^6 SW-613 cells were grown as a monolayer in 35-mm petri dishes in a medium containing RPMI 1640, 10% fetal calf serum, and neomycin. The analogues were tested as described previously.[15]

(b) Human mammary MCF-7 cells (Scirrhous carcinoma of the breast) were supplied by Dr. E. M. Jensen. These epithelial cells grow in monolayers and are sensitive to estrogens. For test purposes, 5×10^6 MCF-7 cells were grown as a monolayer in Eagle's minimal essential medium (MEM) with Hanks' balanced salt solution (BSS), 10% calf serum, 10 μg/mL insulin, 100 units/mL penicillin, and 100 μg/mL streptomycin and incubated in culture in the same manner as in the preceding tumor cells. The controls had a doubling time of 36 h. The workup and assay were done in the same manner as in the case of the SW-613 cells.[15]

In Vivo Tumor Cells Systems. Mouse Leukemia, L-1210 (DBA/2 Ha Mice). Female DBA/Ha mice, 6–8 weeks old (19–20 g), were obtained from the RPMI breeding colony. Each animal was injected ip with 10^6 cells of leukemia L-1210 and treated with the drug, once daily (or twice daily if fractionated or divided doses are used) for 5 consecutive days starting the day after inoculation. The drugs were in aqueous solution, or in the case of sparing solubility as N^6-benzyladenosine, in aqueous dispersion containing Tween 80. Survival time was noted for each animal. Tests were run in groups of five mice on three separate occasions. Results are given in Table III.

Tests with *carcinoma TA3 and Taper hepatoma* were carried out using A/ST female mice and HA/ICR Swiss female mice, respectively, in the same manner as in the preceding test, but no activity was obtained here which resulted in increased life spans (ILS).

Wistar–Furth Rats Bearing TW-98 Mammary Tumors. Two groups of ten rats each were injected (ip) with 24 mkd of N^6-allyladenosine five times per week for 3 weeks. Tumors were measured throughout. After termination of the experiment, the tumors were excised and weighed. Upon comparison with the controls, the latter were found to be growing at four times the rate as those of the treated animals.

Tumors Derived from Spontaneous Mammary Tumors of the DBA/2 HaDD Mouse (Table IV). The isolation and establishment of these tumor lines are discussed above. These tumors are not responsive to steroid drugs (estrogens). Tumor fragments, approximately 1 mm in diameter, were transplanted by standard trocar technique subcutaneously in the milk gland line into groups of five female mice. On day 3, the tumor was measured by calipers and the mice were weighed. Normally the drug was injected on 5 successive days. In the case of slow growing tumors, the first injection of the drug was given on day 6 and was continued for 5 successive days, although this regimen was varied as noted below. The tumors were measured twice a week, normally ending with the death of the mouse. The mortality was checked daily, 7 days a week. If the drug was given twice a day, it was done in the morning and late afternoon. The data for this study are given in Table IV. In some cases the tumor half-growth of the treated mice equals that of the control, and yet the treated animals have increased survival time. This is due to an increase in growth rate right after treatment is stopped, which slows down after half-growth rate is reached.

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On the Rational Selection of Test Series. 1. Principal Component Method Combined with Multidimensional Mapping

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A method for the rational selection of optimal test series with high data variance and low collinearities is presented (PCMM method). The method combines the technique of multidimensional mapping originally introduced by Wootton and colleagues with the principal component method, and it is superior to other selection methods with respect to its collinearity decreasing power. Two examples of the application of PCMM are given, and the results are compared with corresponding results from other selection techniques.

Quantitative structure–activity relationships (QSAR) have developed into an important tool to rationalize drug design. The evaluation of meaningful QSAR and their effective application in the process of lead optimization requires that, in a first step, a test series with optimal properties is selected. This is not an easy task, especially for a batch situation (synthesis quicker than biological testing[1]) where this selection has to be performed prior to any experimental work. An optimal test series must provide a maximum of information with the smallest possible number of analogues, and this supposition can only be fulfilled if all physicochemical and structural properties governing biological potency are varied systematically and independently from each other over a sufficiently large range. That means that the analogues comprising the test series must be selected in such a way that the variances of hydrophobic, electronic, and steric molecule parameters are maximized and, at the same time, collinearities between these parameters are minimized. The analogues must, furthermore, span a sufficiently large part of the parameter space with sufficiently large and about equal Euclidean distances between them within this space. This is not only

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