(B) **Compound 12.** Compound 12 was prepared by the method for compound 2, parts B–D, but with 2-(bromomethyl)-benzofuran. A white solid was obtained, mp 112–113 °C.

***N*-[3-(2-Benzthiazolylmethoxy)phenyl]-2-oxopyrrolidine-4-carboxylic Acid Methyl Ester (18). (A) *N*-(3-Hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic Acid.** A mixture of 3-aminophenol (10.9 g, 0.1 mol) and itaconic acid (13.0 g, 0.1 mol) was heated to 120–130 °C for 5 min. After cooling, a solid formed, giving 21.5 g (97% yield) of product, mp 214–215 °C.

(B) *N*-(3-Hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic Acid Methyl Ester. A solution of *N*-(3-hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic acid (21.0 g, 94.9 mmol) in methanol with *p*-toluenesulfonic acid (0.1 g) was heated to reflux. The reaction mixture was refluxed for 4 days while water was removed with a Soxhlet extractor filled with 3A molecular sieves. The mixture was cooled and a solid formed, which was filtered and dried, giving 9.5 g (42% yield) of product, mp 178–180 °C.

(C) Compound 18. A mixture of 2-(chloromethyl)benzthiazole (see compound 2, part A) (3.12 g, 17 mmol), *N*-(3-hydroxyphenyl)-2-oxopyrrolidine-4-carboxylic acid methyl ester (4.0 g, 17 mmol), cesium carbonate (5.3 g, 17 mmol), sodium carbonate (1.8 g), potassium iodide (0.1 g), and acetone (200 mL) was heated at reflux overnight. The mixture was filtered and the resulting solution concentrated to an oil. The oil was triturated with ether, forming a solid, which was filtered and dried to give 4.0 g (62% yield), mp 99–102 °C.

Compounds 19 and 21 were prepared by following the procedures used in the preparation of compound 18 and employing 1-methyl-2-(chloromethyl)benzimidazole, 2-(chloromethyl)benzoxazole and 2-(chloromethyl)quinoline.

***N*-[3-(2-Benzthiazolylmethoxy)phenyl]pyrrolidine-2,5-dione (22). (A) 4-[(3-Hydroxyphenyl)amino]-4-oxobutanoic Acid Methyl Ester.** To an ice-cold solution of 3-aminophenol (21.8 g, 0.2 mol) and triethylamine (21.3 g, 0.2 mol) in THF (250 ml) was added a solution of 3-carbomethoxypropionyl chloride (30.1 g, 0.2 mol) in THF. The reaction mixture was allowed to warm to room temperature and was filtered through a pad of Celite and silica gel. The solvent was removed in vacuo to give a solid. Recrystallization from ethyl acetate gave 39.9 g (88% yield) of product, mp 144–146 °C.

(B) Compound 22. A mixture of 4-[(3-hydroxyphenyl)-amino]-4-oxobutanoic acid methyl ester (1.45 g, 6.5 mmol), 2-(chloromethyl)benzthiazole (1.20 g, 6.5 mmol) (see Experimental Section for compound 2, part A), cesium carbonate (1.0 g), sodium carbonate (0.7 g), potassium iodide (5 mg), and acetone (60 ml) was heated at reflux for 2 h. The reaction mixture was filtered through a pad of Celite and silica gel, and the solvent was removed in vacuo. Recrystallization from acetone gave 1.34 g (56% yield) of product, mp 175–176 °C.

Compounds 23 and 24 were prepared by following the procedure used in the preparation of compound 22 and employing *N*-methyl-2-(chloromethyl)benzimidazole and 2-(chloromethyl)-quinoline.

Biological Test Procedures. Experimental detail for the rat PMN 5-LO and the GP LTD_4- and OA-induced bronchospasm model are provided in ref 1.

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Chemical Differentiating Agents. Differentiation of HL-60 Cells by Hexamethylenebis[acetamide] Analogues

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Hexamethylenebis[acetamide] (HMBA) is an agent in clinical trial that induces differentiation of certain types of tumor cells to nonmalignant phenotypes. In an attempt to discover a more potent compound, a number of bis-functionalized amides, imides, and hydrazine derivatives of HMBA were prepared and evaluated in vitro with the HL-60 human promyelocytic leukemia cell line. Among the compounds evaluated, the 5,5-dimethylhydantoin derivative is almost 10 times more potent than HMBA in inducing differentiation. The bis-imide, diacetyl-HMBA, is both more potent and effective than its parent compound. Six of the 16 compounds evaluated cause at least 20% differentiation. An inverse relationship between the degree of differentiation and the percentage of viable cells is described for HMBA and its analogues.

Compounds that induce cancer cells to differentiate to a less malignant phenotype provide an attractive area for the development of new anticancer drugs. Reduced toxicity relative to conventional chemotherapeutic agents is a distinct possibility since the mechanism of antitumor action is not based primarily on cytotoxicity.

The number of compounds that influence cell differentiation and growth characteristics continues to increase. These materials, which include simple organic molecules as well as proteins,[1-3] are thought to influence gene expression. An important aid in the ability to search for agents that induce terminal differentiation in malignant cells occurred when it was discovered that a virus-induced murine erythroleukemia cell line (MELC), when treated with Me_2SO, expressed many of the features common to terminally differentiated erythroid cells.[4,5] The development of the human HL-60 myeloid leukemia cell line in 1977 provided another important in vitro differentiation system.[6-8] While there are a number of cell lines now

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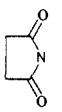
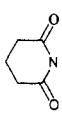
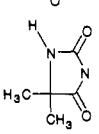
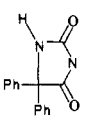
Table I. HMBA Analogues ($R(CH_2)_6R$)

no.	R	yield, %	mp, °C	formula[a]
2	$CH_3CON(CH_3)$	53	138[b]	$C_{12}H_{24}N_2O_2 \cdot 0.25H_2O$[c]
4		41	160[b]	$C_{16}H_{28}N_2O_2 \cdot 0.5H_2O$[c]
8	PhCONH	64	159–160	$C_{20}H_{24}N_2O_2$
9	$(CH_3CO)_2N$	68	58	$C_{14}H_{24}N_2O_4$
10	$CH_3CONHCO$	52	180–181	$C_{12}H_{20}N_2O_4$
11		43	115	$C_{14}H_{20}N_2O_4$
12		78	102–103	$C_{16}H_{24}N_2O_4$
13		42	145–146	$C_{16}H_{26}N_4O_4$
14		74	261–263	$C_{36}H_{34}N_4O_4$
15	$CH_3CONHNHCO$	74	242–243	$C_{12}H_{22}N_4O_4$
16	$CH_3CONHNH$-CONH	78	204	$C_{12}H_{24}N_6O_4$
17	p-CH_3PhSO_2NH	49	150–151	$C_{20}H_{28}N_2O_4S_2$[d]

[a] Correct C, H, N analyses (±0.4% of theory). [b] Bp (°C at 0.1 torr). [c] Correct analysis also for oxygen. [d] Correct analysis also for sulfur.

known to differentiate in the presence of small molecules, the MELC and HL-60 lines have been used most often.[1]

With Me_2SO as a lead compound, many organic materials with various degrees of effectiveness have been studied as differentiation inducers.[1,8,9] DMF and *N*-methylacetamide causes MELC and HL-60 cells to differentiate, but with optimum concentrations of ca. 150 and 50 mM, respectively,[9] they are not potent enough to be clinically practical if similar concentrations are required in vivo. However, these amides are still considerably more potent than Me_2SO (180–280 mM).[7,9]

A discovery of potential clinical importance occurred when Marks and co-workers found that placing two amide functions in the same molecule increased compound potency.[10-12] Both activity and potency are maximized in the polymethylenebis[acetamide] series with five or six methylene groups[10] and a well-designed subsequent study answered numerous questions regarding basic structure–activity relationships in this series.[11] More recently, studies with dicarboxylic acid amides[13] and diamine analogues with different acyl groups[14] have been reported, but none of the compounds tested appear to be superior to HMBA.

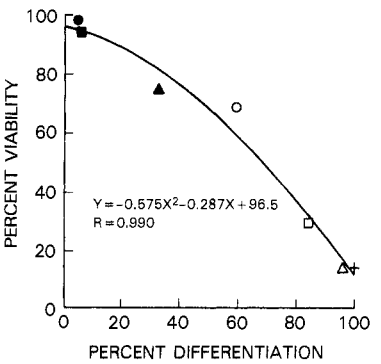


Figure 1. Relationsip between HL-60 cell differentiation and viability as a function of HMBA concentration. Values in parentheses are the number of determinations. (●) 0.3 mM (1), (■) 1.0 mM (15), (▲) 2.0 mM (2), (○) 3.0 mM (11), (□) 4.0 mM (5), (△) 5.0 mM (3), (+) 6.0 mM (2).

HMBA presently is undergoing clinical trials based on differentiation as a mechanism of antitumor action.[15] In vitro studies in the MELC system show that 5-day exposure to 5 mM HMBA gives optimum results.[10] However, studies with rats and dogs indicate that these conditions might be difficult to maintain in vivo without significant toxicity.[16-18] For this reason, the present investigation was undertaken in an attempt to find analogues with a greater therapeutic index than HMBA.

Results and Discussion

The materials investigated fell into three classes of polymethylene bis-functionalized compounds, i.e., amides, imides, and hydrazine derivatives (Table I). In almost all cases, penta- or hexamethylene analogues were prepared since earlier studies had established that these provided optimum spacer distances.[10,11] While the chemistry was relatively straightforward, a wide variety of synthetic procedures were required to synthesize the desired compounds.

A measurement of the percentage of differentiated cells (% D) is commonly used in the HL-60 system for assessing the relative activities of various compounds. This measurement is most meaningful when the cytotoxicity is low. However, under cytotoxic conditions a portion of an apparent increase in % D can be the result of an enrichment of the preexisting population of differentiated cells, especially if the cytotoxicity is directed particularly against growing, nondifferentiated cells.[19] This is because % D in the HL-60 system is determined by dividing the number of viable differentiated cells by the total number of viable cells. To our knowledge there is no unequivocal method to corrrect quantitatively for this possible enrichment. However, induction of differentiation in culture would be indicated if there is an increase in the viable-cell concentration as well as a net increase in the concentration of mature cells that is greater than what could have occurred in the control culture at the same cell density.[19] The higher the percent viability (% V), the better the % D value will

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Table II. Biological Data

compd	concn, mM	total cells (10^{-5}/mL)	% D[a]	% V[b]
control		12.0 ± 3.9 (18)[c]	4	96
1 (HMBA)	2.0	6.9 ± 0.35 (2)	32	74
	3.0	5.2 ± 1.40 (11)	59	68
	4.0	3.9 ± 0.62 (5)	84	29
Me_2SO	38	9.7 (1)	6	97
	64	9.8 ± 1.1 (2)	9	96
	77	9.3 (1)	6	94
2	1.0	11.2 (1)	10	90
	2.0	7.0 (1)	74	57
	2.5	5.1 (1)	93	32
	3.0	4.2 (2)	94	30
4	1.0	8.3 (1)	12	87
	2.0	7.6 (1)	40	65
	2.5	6.8 (1)	72	62
	3.0	3.7 (1)	87	25
5	3.0	6.8 ± 0.48 (2)	40	72
	4.0	6.9 ± 0.26 (2)	71	56
6	1.0	7.0 ± 3.2 (2)	24	85
	1.5	10.7 (1)	13	78
	2.0	9.4 (1)	12	82
9	2.0	5.3 ± 2.2 (2)	65	71
	2.5	4.8 (1)	76	51
	3.0	3.0 ± 0.65 (3)	80	45
13	0.1	8.7 ± 2.9 (2)	9	96
	0.3	4.8 ± 1.4 (4)	26	82
	0.4	4.2 ± 1.35 (2)	45	53
	0.5	3.1 ± 0.92 (2)	76	43

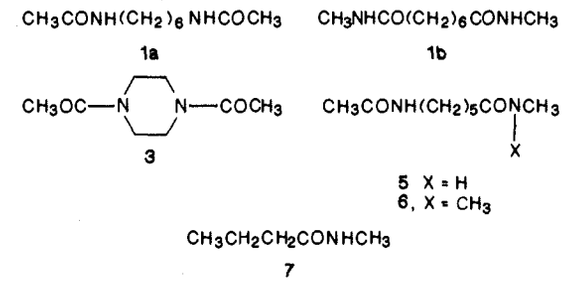
[a] Percent differentiation; average value for multiple experiments. [b] Percent viability; average value for multiple experiments. [c] Number of experiments in parentheses.

be as a true measure of differentiation. The lower the % V, the greater will be the possibility that enrichment will influence the results. Therefore, viability determinations are critical for an accurate assessment of inducers of differentiation that are also cytotoxic. The relationship between differentiation and viability at various concentrations of HMBA is shown in Figure 1. There is an inverse nonlinear relationship between the % D and the % V. The nature of this curve indicates that at lower doses of HMBA there is relatively more differentiation than cytotoxicity. At higher concentrations of HMBA the reverse is the case.

Since simple N-alkylation of amides often enhanced effectiveness or potency in the MELC system,[12] dimethyl-HMBA (**2**) was prepared. This compound was slightly more potent and effective than HMBA (Table II). While diacetylpiperazine (**3**) was inactive, compound **4**, the 1-piperidone analogue of HMBA, was at least as effective as HMBA. Piperidone and its *N*-methyl derivative had been shown previously to be active but not as effective as HMBA in the MELC system.[12] Other simple cyclic ureas also are known to be effective MELC differentiating agents.[20]

Earlier work[11] had shown that there was not much difference in differentiation-inducing activity between diamides based on acylated 1,6-diaminohexane, e.g., HMBA (**1a**), and diamides based on adipic acid (**1b**). Pentamethylene analogues possessing the molecular characteristics of both compounds in the same molecule were prepared. Both were active, but the *N*-methyl compound (**5**) proved more effective than the *N,N*-dimethyl derivative (**6**). *N*-Methylbutyramide (**7**), which is essentially one-half of the **1b** molecule, did not induce differentiation. The aromatic benzamide (**8**) was also inactive.

Several bis-imides were prepared to determine whether this group could replace the HMBA amide function. The



cyclic succinimide (**11**) and glutarimide (**12**) analogues were inactive, but the acyclic *N,N'*-diacetyl-HMBA (**9**) was superior to the parent compound in terms of both potency and effectiveness. Whether **9** is merely a more effective prodrug form of **1a** is unknown at present. When the *N*-methyl groups in the adipic acid amide **1b** were replaced by acyl groups (**10**), no activity was observed. Although **1b** was not tested in our HL-60 system, activity could be expected based on the activity correlation usually observed between MELC and HL-60. Incorporation of hydantoin groups in place of the amides produced an active compound in the case of the 5,5-dimethyl analogue (**13**). This compound is almost 10 times more potent than HMBA. The phenytoin analogue (**14**) is very insoluble and this may have limited its activity.

In limited studies with hydrazine containing molecules, the acetyl hydrazide (**15**) and semicarbazide (**16**) analogues were ineffective. Because of solubility problems, further analogues were not synthesized. The bis-tosyl derivative of hexamethylenediamine **17** also was not effective.

While a differentiating agent without cytotoxicity would be ideal, it is conceivable that an agent possessing both differentiating and cytotoxic properties still might be useful if some selective cytotoxicity were observed for tumor cells. For this reason, compounds **3–6** and **8–17** were evaluated in vivo against murine intraperitoneal P388 leukemia under the standard NCI protocol.[21] In general, neither significant toxicity nor antitumor activity was observed at doses of 50–400 mg/kg with a day 1–5 treatment schedule.

Among the new HMBA analogues evaluated here, the hexamethylene bis-amide family shows effectiveness with several analogues (**2,4,5**) possessing differentiating activity similar to that of HMBA. Diacetyl-HMBA (**9**) is slightly more potent and somewhat more effective than HMBA. It is of interest that incorporation into Figure 1 of the data for the six HMBA analogues listed in Table II results in essentially all data points clustering close to the line drawn in Figure 1. This indicates that none of these compounds has an advantage based on a greater differentiation inducing activity to toxicity ratio. However, the goal of finding an active HMBA analogue more potent than the parent compound was achieved with the dimethylhydantoin analogue (**13**). This compound is 10 times more potent than HMBA with approximately equivalent differentiating activity.

Experimental Section

Biology. Differentiation studies were conducted as previously described with the HL-60 human myeloid leukemia cell line.[22] In this study, minimal compound activity is defined as a % D value of 20% or greater. While all compounds synthesized were evaluated, only those meeting this minimum criterion are de-

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scribed in Table II. Differentiation was assessed by counting the cells that reduced nitro blue tetrazolium (NBT) to its black formazan form.[23] This reaction is dependent on the production of superoxide anion as a reducing agent and is characteristic of differentiated but not undifferentiated HL-60 cells. Formazan production is also dependent on cell viability since only living cells are capable of superoxide production. Total cell numbers were counted with a Coulter counter and the percentage of the total cells that were viable was determined by trypan blue exclusion. The initial cell concentration was 2×10^5/mL, and cells were counted on day 4. Test compounds were generally insoluble in water and were dissolved in ethanol or Me_2SO prior to addition to the cell suspension. Final concentrations of Me_2SO in the test system did not exceed 77 mM. This concentration had no effect on cell differentiation (Table II).

Chemistry. Commericially available reagents were purchased from Aldrich Chemical Co. Compound **3** was obtained from the Drug Synthesis and Chemistry Branch, National Cancer Institute. Thomas-Hoover melting points and Kugelrohr boiling points are uncorrected. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN. ^1H NMR data ($CDCl_3$) were obtained for each compound on a Varian T-60 instrument. Since the spectra of most compounds had many similarities, individual data are not presented. The absorptions of internal methylene, acyl methyl, nitrogen-attached methyl, and nitrogen-attached methylene groups generally appeared at ca. δ 1.4 (broad), 2.0 (singlet), 2.9 (doublet), and 3.2 (broad multiplet), respectively, relative to tetramethylsilane. The electron-impact mass spectrum of **7** was obtained with a VG Analytical 7070E mass spectrometer.

***N,N'*-Dimethyl-*N,N'*-hexamethylenebis[acetamide] (2).** To a 50% oil suspension of sodium hydride (3.36 g, 70 mmol) in dry THF (60 mL) under nitrogen was added *N*-methylacetamide (5.0 g, 69 mmol) in dry THF (10 mL), and the mixture was refluxed for 5 h. 1,6-Dibromohexane (6.0 g, 24 mmol) was added and the resulting mixture refluxed for additional 2 h. Cold water (200 mL) was added and the aqueous phase extracted with chloroform (3 × 100 mL). The organic layer was washed with water and dried ($MgSO_4$) and the solvent evaporated in vacuo to afford an oil. Kugelrohr distillation of this material afforded 2.5 g of a pure oil (53%), bp 138 °C (0.1 torr).

1,5-Bis(2-oxo-1-piperidinyl)hexane (4). To a 50% oil suspension of sodium hydride (3.16 g, 65 mmol) in dry DMF (60 mL) under nitrogen was added 6.52 g (65 mmol) of δ-valerolactam. The mixture was stirred overnight. 1,6-Dibromohexane (4.0 g, 16.3 mmol) was added and the mixture stirred for an additional 6 h. Water (200 mL) was added and the aqueous phase extracted with chloroform. The organic layer was washed with water and dried ($MgSO_4$) and solvent removed in vacuo. Fractional distillation afforded 1.9 g (41%) of pure material as an oil, bp 160 °C (0.1 torr).

General Procedure for 5–7. 6-(Acetylamino)-*N*-methylhexanamide (5). Methyl 6-acetamidohexanoate was prepared as a low-melting solid by sequential treatment of 6-acetamidohexanoic acid with thionyl chloride and methanol. This compound (5.0 g, 27 mmol) was dissolved in an excess of 40% aqueous methylamine solution and the resulting mixture stirred for 16 h at room temperature. The reaction mixture was saturated with sodium chloride and extracted several times with chloroform. The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo to afford a crude residue. Recrystallization from THF afforded 4.5 g (90%) of pure product, mp 99–100 °C. Anal. C, H, N.

6-(Acetylamino)-*N,N*-dimethylhexanamide (6). This compound was prepared as described for **5**. It was obtained in 76% yield as an oil, bp 168 °C (2 torr). Anal. C, H, N, O.

***N*-Methylbutanamide (7).** This compound was prepared by the reaction of aqueous methylamine solution with ethyl butyrate in ethanol to give an oil: bp 78–80 °C (0.5 torr [lit.[24] bp 110–111 °C (15 torr)]; MS, m/z (relative intensity), 101 ($M^{+\cdot}$; 9), 100 (3), 73 (100), 58 (94), 43 (69), 42 (12) [lit.[25] m/z 101 (11), 100 (3), 73 (97), 58 (100), 43 (75), 42 (13)].

General Procedure for *N,N'*-1,6-Hexanediylbis[benzamide] (8) and *N,N'*-1,6-Hexanediylbis[4-benzenesulfonamide] (17). To a cooled (0–10 °C) solution of the corresponding acyl or sulfonyl chloride in dry THF (1:3) was added 1,6-hexanediamine in dry THF. A white precipitate formed. After addition was complete, the reaction mixture was stirred for additional 20 min at room temperature. Sodium hydroxide (10% in water) was slowly added until the pH of the solution was neutral. Water was added and a copious precipitate formed. The aqueous slurry was extracted with chloroform. The organic phase was washed with water and dried ($MgSO_4$) and the solvent removed in vacuo to afford a white solid. Recrystallization from ethanol gave a pure product.

***N,N,N',N'*-Tetraacetylhexamethylenediamine (9).** The general procedure of Mariella and Brown[26] was used. A mixture of hexamethylenebisacetamide (7.0 g, 35 mmol), anhydrous sodium acetate (4.0 g, 48 mmol), and acetic anhydride (80 mL) was refluxed for 20 h. Excess acetic anhydride was removed in vacuo and water (60 mL) added to the residue. The aqueous phase was extracted with chloroform (3 × 100 mL). The organic layer was washed with water, dried ($MgSO_4$), and then passed through a short silica gel column ($CHCl_3$). After solvent removal, 6.8 g (68%) of pure compound was isolated.

***N,N'*-Diacetyloctanediamide (10).** The general procedure of Thompson[27] was used. To a cooled solution (−78 °C) of suberoyl chloride (6.33 g, 30 mmol) in dry chloroform (150 mL) under nitrogen was added dry pyridine (5.0 mL, 60 mmol), and the resulting white suspension was stirred for 1 h. A solution of acetamide (3.54 g, 60 mmol) in dry chloroform was added to the cold solution, and the resulting mixture was stirred for 20 h at room temperature. The initially clear solution formed a white precipitate. The precipitate was broken up by addition of methanol and filtered in vacuo. The resulting white powder is recrystallized from hot ethanol to give 4.0 g (52%) of pure product.

1,1'-(1,6-Hexanediyl)bis[2,5-pyrrolidinedione] (11). A succinimide solution (8.1 g, 81.7 mmol) in dry DMF (20 mL) was slowly added to a cooled (0 °C) slurry of 50% sodium hydride in oil (3.92 g, 81.7 mmol) in dry DMF (50 mL) under nitrogen. After the addition was complete, the mixture was refluxed for 20 min. 1,6-Dibromohexane (5.0 g, 20 mmol) was added and the resulting mixture stirred for an addition 3 h. The reaction was quenched with cold water and the aqueous phase extracted with chloroform (4 × 60 mL). The organic layer was washed with water and dried ($MgSO_4$) and the solvent evaporated in vacuo. Prepurification of the product by passage through a silica gel column ($CHCl_3$) was followed by crystallization of the purer material from ethanol to afford 2.5 g (43%) of pure product.

1,1'-(1,6-Hexanediyl)bis[2,6-piperidinedione] (12). A mixture of glutaric anhydride (7.84 g, 68.7 mmol) and 1,6-hexanediamine (4.0 g, 34.4 mmol) in xylene was refluxed for 2 days while the water formed was removed with a Dean–Stark apparatus. Removal of the xylene in vacuo, followed by passage of the resulting residue through a silica gel column ($CHCl_3$), afforded a material, which was recrystallized from ethanol/ether to give a pure product (8.5 g, 78%).

General Procedure for 3,3'-(1,6-Hexanediyl)bis[5,5-dimethyl-2,4-imidazolinedione] (13) and the 5,5-Diphenyl Analogue (14). To a solution of 5,5-dimethylhydantoin in absolute ethanol was added an equimolar amount of potassium hydroxide. The mixture was stirred until a homogenous solution was obtained. 1,6-Dibromohexane (0.25 molar equiv) was added in one portion, and the mixture was refluxed for 20 h. Water was added (3 volumes) and the aqueous phase extracted with chloroform. The organic layer was washed with water and dried ($MgSO_4$) and the solvent removed in vacuo to afford pure **13**.

In the diphenylhydantoin case (**14**), the product crystallized directly from the reaction mixture. This was washed with water and recrystallized from acetone.

***N,N'*-Diacetamidooctanediamide (15).** To a well-stirred, ambient solution of acetylhydrazine (3.0 g, 40 mmol) under nitrogen was added suberoyl chloride (2.0 g, 9 mmol). After the addition was complete, the white suspension formed was stirred

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for 20 min, sodium hydroxide solution (0.9 g in 5 mL of water) was slowly added, and the mixture was stirred for an additional 10 min. The precipitate was filtered and washed consecutively with cold water and ethanol. Recrystallization from methanol afforded 2.0 g (74%) of pure product.

***N,N'*-[1,6-Hexanediylbis[(aminocarbonyl)amino]]bis-[acetamide] (16).** To a cooled (0 °C) solution of acetylhydrazine (7.04 g, 95 mmol) in dry THF was added 1,6-diisocyanatohexane (4.0 g, 24 mmol) in dry THF. The product precipitated instantly. Filtration followed by washing with dry THF and ether afforded pure product (5.9 g, 78%).

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5-Quinone Derivatives of 2'-Deoxyuridine 5'-Phosphate: Inhibition and Inactivation of Thymidylate Synthase, Antitumor Cell, and Antiviral Studies

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Both photochemical aromatic substitution and palladium(0)-catalyzed biaryl coupling reactions have been employed in the synthesis of 5-substituted 2'-deoxyuridines. The former procedure was useful in the preparation of the 3,4-dimethyl-2,5-dimethoxyphenyl derivative **12a** and the 3,4,6-trimethyl-2,5-dimethoxyphenyl derivative **12b**. The latter reaction was efficient in the preparation of the 2-(3-methyl-1,4-dimethoxynaphthyl) derivative **14**. These compounds and their nucleotides (**20a-c**) were converted to the corresponding quinone nucleosides **19a-c** and nucleotides **6-8** by an oxidative demethylation reaction using ceric ammonium nitrate and silver(II) oxide, respectively. The kinetics and products of the reaction of the quinone nucleosides **19a,b** with methyl thioglycolate showed rapid addition to the quinone ring in the trisubstituted derivative **19a** and somewhat slower redox reactions with the tetrasubstituted quinones **19b** and **19c**. All six nucleotides had high affinity for the title enzyme from *Lactobacillus casei* with K_i values ranging from 0.59 to 3.6 μM; the most effective compounds were the dimethyl quinone **6** and the naphthoquinone **8**. Somewhat higher inhibitory constants were observed with the quinones against the L1210 enzyme. The dimethyl quinone nucleotide **6** showed time-dependent inactivation (k_{inact} = 0.015 s^{-1}) against the *L. casei* enzyme, a rate saturation effect, and substrate protection in accord with the kinetic expression for an active-site-directed alkylating agent. The apparent second-order rate of this reaction (2.5×10^4 M^{-1} s^{-1}) is one-twentieth the rate ($k_{cat.}$) of the normal enzymatic reaction leading to product. None of the compound exhibited sufficient activity in the antitumor cell or antiviral assays to warrant further study.

The inhibition of thymidylate synthase is recognized as a viable approach to the control of cancer and holds promise for the development of agents for the treatment of DNA viral infections.[1] This enzyme catalyzes a unique two-step reductive alkylation reaction to form a new carbon–carbon bond in the conversion of 2'-deoxyuridine 5'-phosphate to thymidine 5'-phosphate, a vital precursor for DNA synthesis. The acknowledged mechanism for the first step of the enzymatic reaction,[2] cysteine thiol addition to carbon 6 of the substrate followed by reaction of the carbanion generated at carbon 5 with the electrophilic cofactor, 5,10-methylenetetrahydrofolic acid, can be utilized in the development of mechanism-based inhibitors for this enzyme.

Studies in these laboratories have been directed to the development of pseudosubstrates for the enzyme that have three primary chemical features: (1) high affinity, (2) enhanced nucleophilic reactivity, and (3) the potential for the formation of a chemically reactive intermediate. 5-Nitro-2'-deoxyuridine 5'-phosphate was one such agent that fulfilled the first two requirements.[3] More recently attempts to incorporate the third feature, generation of a chemically reactive intermediate in the enzymatic reaction, entailed the synthesis of 5-*p*-benzoquinonyl-2'-deoxyuridine 5'-phosphate (**1**).[4] This substrate analogue had high affinity (K_i = 2.0 μM), rapidly inactivated the enzyme (k_{inact} = 0.065 s^{-1}), displayed substrate protection in accord with the kinetic equation (eq 1), and was not reversible on prolonged dialysis with 2-mercaptoethanol solution. One potential mechanism for the reaction (pathway a, Scheme I) initially proposed is the addition of the enzyme nucleophile (Cys-198 thiol anion) to carbon 6 of **1** to give the unstable intermediate **2**, which has a high probability for rearrangement to the stable covalent enzyme-inhibitor product **3**. If the reaction followed this pathway, the product, **3**, with the enzyme covalently bonded to an sp^2 carbon, should be stable.

Recognizing the fact that quinones also are thiol reagents leads to a second mechanism (pathway b, Scheme I) for enzyme inactivation wherein the enzymatic thiol anion adds directly to the quinone ring to generate **5**. The result would be the same and the usual features used to verify mechanism-based inactivation would be observed. How-

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