Sequential Cytotoxicity: A Theory Evaluated Using Novel 2-[4-(3-Aryl-2-propenoyloxy)phenylmethylene]cyclohexanones and Related Compounds

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Five series of novel compounds were synthesized in order to evaluate the theory of sequential cytotoxicity which seeks to exploit the view that various cancer cells are particularly susceptible to successive attacks by cytotoxic agents. The compounds prepared were various 2-[4-(3-aryl-2-propenoyloxy)phenylmethylene]cyclohexanones **1** and the related Mannich bases **2**. In addition the analogues **3**−**5** lacking an olefinic bond in the ester group were also synthesized, which were predicted to be less cytotoxic than the compounds of series **1** and **2**. The atomic charges at the potential sites for interaction with cellular constituents were determined by molecular modeling calculations. The biodata obtained from murine and human neoplastic cells revealed that the predictions made regarding the viability of the theory were fulfilled in approximately two-thirds of the cases indicating that further investigation of this hypothesis is warranted. In addition, the significant potencies of some of the Mannich bases toward human tumor cell lines, in particular coupled to their selective toxicity toward human leukemic and colon cancer cells, confirms their usefulness in serving as lead molecules for further development. A preliminary investigation into the mode of action of representative compounds revealed their ability to induce apoptosis and inhibit the biosyntheses of ribonucleic acid and proteins.

Introduction

The major emphasis in the present investigation was the design of various groups of compounds as candidate cytotoxics in order to examine a theory entitled sequential cytotoxicity. In addition, the aspiration was made that novel prototypic molecules for further development as candidate cytotoxic and anticancer agents would emerge from this study. The theory of sequential cytotoxicity may be defined as the proposal that the successive release of two or more cytotoxic agents will cause greater toxicity to malignant tissue than to normal cells.[1] The arguments leading to the proposal of this theory have been presented at length,[1] some of which are presented in this report vide infra.

The reasons for the design of the compounds prepared in this study and their utility in evaluating this theory are as follows. Antineoplastic agents that interact with thiols (which are not found in nucleic acids) may be devoid of the mutagenic and carcinogenic properties of certain anticancer drugs which interact with the hydroxy and amino groups of DNA and RNA.[2] In regard to the theory of sequential cytotoxicity, on occasions the lowering of cellular thiols prior to the administration of various anticancer agents enhanced the toxicity of these drugs toward tumors relative to normal cells.[3,4] Thus chemosensitization of malignant cells took place. In addition, lonidamine, which is an inhibitor of cellular energy metabolism, potentiated the activity of a number of antineoplastic agents.[5] Some of the thiol alkylators prepared in this report are Mannich bases, representatives of which inhibit respiration in isolated mitochondria.[6] On the basis of these arguments, sequential thiol alkylation may lead to compounds with preferential toxicity to certain malignant tissues.

To examine this hypothesis, compounds developed from 2-arylmethylenecyclohexanones were considered since previous reports from this laboratory revealed the significant cytotoxicity of some of these compounds.[7,8] Thus the preparation of the enones in series **1**−**5** as indicated in Scheme 1 was proposed. These compounds have different numbers of electrophilic centers. In addition, the rates and extent of thiolation would be expected to vary in those compounds having multiple alkylation sites. The reasons for preparing each cluster of α,β-unsaturated ketones are as follows. Compound **1a** possesses two potential sites for thiolation which were designated the α and β locations (Chart 1). Initial attack will be predicted to be at the β site, since at this portion of the molecule there will be electron-attracting influences on the olefinic carbon atom by (i) the aryl ring B, (ii) the adjacent ester group, and (iii), to a lesser extent, the aryl ring A with an electron-withdrawing group at the para position. On the other hand, the electron-attracting influences at the α site are (i) the

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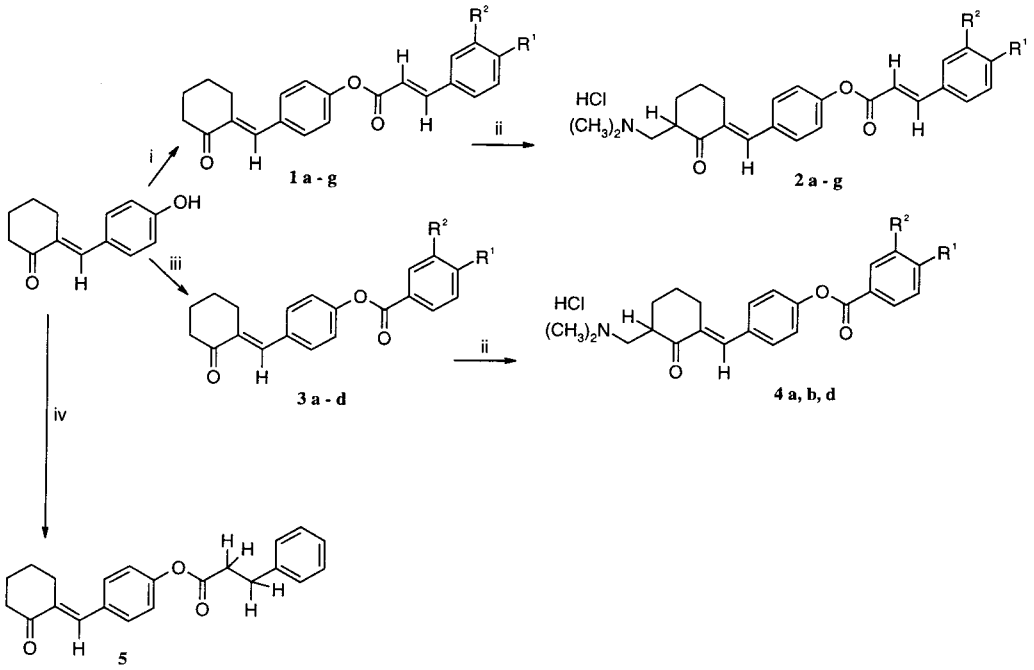
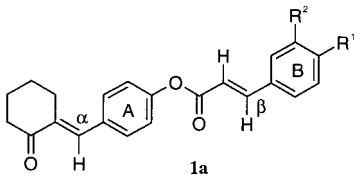
Scheme 1[a]



[a] Reagents: (i) $ClCOCH{=}CHArR^1,R^2$; (ii) $(CH_3)_2{}^+N{=}CH_2{}^-Cl$; (iii) $ClCOArR^1,R^2$; (iv) $ClCOCH_2CH_2C_6H_5$. The letters **a**−**g** indicate the following aryl substitution pattern: **a**, $R^1 = R^2 = H$; **b**, $R^1 = Cl$, $R^2 = H$; **c**, $R^1 = CH_3$, $R^2 = H$; **d**, $R^1 = OCH_3$, $R^2 = H$; **e**, $R^1 = F$; $R^2 = H$; **f**, $R^1 = Br$, $R^2 = H$; **g**, $R^1 = R^2 = Cl$.

Chart 1



keto group of the cyclohexane ring and (ii) the aryl ring A to which is attached an electron-withdrawing ester function. In addition, steric impedance to electrophilic attack of cellular thiols would be expected to be greater at the α site. The choice of an ester group was made since on hydrolysis a phenol and carboxylic acid will be liberated, both of which should be readily excreted either per se or as polar metabolites. Hence the soft drug principle[9] will be exemplified whereby the compound can exert its cytotoxic effect after which facile excretion may take place thereby reducing unwanted toxicity.

Molecular modification of **1a** was planned as follows. First, the placement of various substituents in aryl ring B leading to compounds **1b**−**g** would permit alterations in the fractional positive charge at the *β* site which may correlate with variations in cytotoxicity. It is conceivable that small changes in the polarity at the α site may also occur. Aryl substituents with both positive and negative Hammett *σ* constants were therefore chosen. Second, the conversion of the compounds in series **1** into the corresponding Mannich bases **2** was predicated on the basis not only of their potential to sensitize tumor cells to chemical insults vide supra but also of the observation that *β*-amino ketones may undergo deamination[10] which would lead to a third alkylation site. Third, to evaluate further the theory of sequential cytotoxicity, a null hypothesis[11] was considered. In the case of series **3** and compound **5** multiple alkylation is precluded. Similarly the compounds in series **4** have less capacity for thiolation than the analogues **2**.

In summary, therefore, a comparison of the cytotoxicities of the compounds in series **1**−**5** may shed some light on the viability or otherwise of the theory of sequential cytotoxicity. In addition, one or more compounds with significant antineoplastic activity may emerge from this investigation serving as templates for future molecular modifications.

Chemistry

The compounds in series **1**−**5** were prepared according to the procedure indicated in Scheme 1. The stereochemistry of the chiral axis adjacent to the cyclohexane ring was assigned the *E* configuration based on ^{1}H NMR spectroscopic and X-ray crystallographic considerations. Thus the methine protons, when separated from the absorptions of the aryl hydrogen atoms, were noted at 7.4−7.8 ppm in the ^{1}H NMR spectra. Previous studies revealed that the methine protons of the *E* isomers of a series of related 2-arylmethylene-1-cycloheptanones absorbed in the region of 7.1−7.8 ppm.[12] In addition, while the methine proton of the *E* isomer of 2-phenylmethylene-6,6-diphenylcyclohexanone appeared in this region, the corresponding absorption of the *Z* isomer was located at 6.3 ppm.[13] Furthermore, X-ray crystallography of a representative compound (**1b**) revealed the *E* configuration at the chiral axis which was in accord with previous observations from this laboratory pertaining to various 2-arylmethylenecycloalkanones[14,15] and related 3,5-bis(arylidene)-4-piperidones and related compounds.[16] The double bond present in the 2-propenoyl esters **1** and **2** was shown from the coupling constants of the olefinic protons of the ^{1}H NMR spectra to have the *E* configuration. The X-ray crystallographic data generated for a representative compound (**1b**) supported this conclusion.

Table 1. Atomic Charges on the α and β Carbon Atoms of the Compounds in Series **1**−**5**

	charge densities[a]	
compd	α carbon atom	β carbon atom
1a−**g**	0.024 (0.023−0.025)	0.052 (0.047−0.057)
2a−**g**	0.073 (0.070−0.074)	0.062 (0.057−0.068)
3a−**d**	0.024 (0.023−0.025)	
4a,b,d	0.073 (0.072−0.073)	
5	0.023	

[a] For the first four entries, the average value for the series of compounds is presented while the range of charge densities is given in parentheses.

Table 2. Cytotoxicity of **1**−**5** Against Murine P388 and L1210 Cells and Human Molt 4/C8 and CEM T-Lymphocytes

	IC_{50} (μM)[a]			
compd	P388 cells	L1210 cells	Molt 4/C8 cells	CEM cells
1a	20.3 ± 1.21	30.7 ± 0.7	30.7 ± 1.1	26.0 ± 21.0
1b	>50	64.3 ± 8.1	78.6 ± 27.3	63.4 ± 32.1
1c	>50	202 ± 11	156 ± 55	230 ± 148
1d	9.03 ± 0.83	10.2 ± 0.3	2.06 ± 1.69	8.78 ± 2.15
1e	>50	30.9 ± 0.5	26.6 ± 8.8	14.4 ± 6.31
1f	>50	127 ± 38	180 ± 10	131 ± 82
1g	>50	64.8 ± 1.6	47.3 ± 3.4	63.8 ± 22.6
2a	4.02 ± 0.12	32.8 ± 3.8	39.5 ± 1.6	42.8 ± 3.0
2b	1.61 ± 0.24	1.74 ± 0.01	2.65 ± 1.60	3.56 ± 2.48
2c	1.29 ± 0.03	2.43 ± 0.95	3.39 ± 2.00	7.02 ± 2.60
2d	2.41 ± 0.05			
2e	4.55 ± 0.02	1.84 ± 0.06	3.78 ± 1.72	6.00 ± 4.52
2f	4.03 ± 0.53	1.78 ± 0.08	3.06 ± 2.21	4.44 ± 2.68
2g	4.18 ± 0.43	3.31 ± 1.84	4.57 ± 2.59	5.82 ± 3.38
3a	>50			
3b	25.50 ± 2.10	3.33 ± 10.1	38.0 ± 4.4	41.0 ± 7.4
3c	>50	44.8 ± 0.7	25.6 ± 4.9	22.7 ± 14.0
3d	>50	43.4 ± 1.3	34.0 ± 3.0	36.1 ± 10.9
4a	0.180 ± 0.02	23.4 ± 15.3	24.1 ± 18.2	29.4 ± 6.8
4b	0.425 ± 0.04	5.61 ± 2.75	5.11 ± 2.67	6.15 ± 0.92
4d	0.332 ± 0.06	2.87 ± 1.64	2.36 ± 1.23	5.07 ± 3.98
5	21.90 ± 0.97	52.4 ± 1.6	31.1 ± 3.3	34.4 ± 2.9
melphalan	0.22 ± 0.01	2.13 ± 0.03	3.24 ± 0.79	2.47 ± 0.30
5-fluorouracil	0.49 (0.01)	0.28 ± 0.14	23 ± 3.0	8.9 ± 0.43

[a] Concentration of compound required to inhibit 50% of the growth of the neoplasm.

Molecular modeling was undertaken for the compounds **1**−**5**, and the atomic charges at the α and β carbon atoms were determined using a HyperChem program. The specific values generated are given in the Experimental Section, while the average values for the unsaturated ketones in series **1**−**4** as well as for **5** are presented in Table 1. In addition the torsion angles between the olefinic centers and the adjacent aryl rings were measured for **1a, 2a, 3a, 4a**, and **5**.

Cytotoxic and Biochemical Evaluations

The α,β-unsaturated ketones **1**−**5** were examined against murine P388 leukemic cells in vitro, while most of the compounds were also evaluated for cytotoxicity toward murine L1210 leukemic cells as well as human Molt 4/C8 and CEM T-lymphocytes. These results are presented in Table 2. Selected compounds were assessed against a panel of human tumor cell lines, and the overall cytotoxicity, as well as the potencies toward the leukemic and colon cells, are given in Table 4. Three compounds were also examined in the hollow fiber assay (Table 5), while the results of determining the apoptotic indices and effects on RNA and protein syntheses in human Jurkat leukemia cells are summarized in Table 6.

Table 3. Evaluation of the Theory of Sequential Cytotoxicity Using the Biodata for Compounds in Series **1**−**5**

		observation[a]			
entry	prediction	P388 cells	L1210 cells	Molt 4/C8 cells	CEM/O cells
1	**1a**−**d** > **3a**−**d**	+	−	−	−
2	**1a** > **5**	+	+	+	+
3	**2a** > **5**	+	+	−	−
4	**2a**−**d** > **3a**−**d**	+	+	+	+
5	**2a,b,d** > **4a,b,d**	−	− − −	− − −	− − −
6	**2a**−**g** > **1a**−**g**	+	+	+	+
7	**3a** = **5**	−	N/A	N/A	N/A
8	**4a,b,d** > **3a,b,d**	+	+	+	+

[a] The designations + and − refer to verification and negation of the hypothesis, respectively. Comparisons of most of the biodata for **2a,b,d** with **4a,b,d** did not reveal in which group greater activity resided; these observations are recorded as − − −. The letters NA indicate that due to some of the cytotoxicity data for **3a** being not available, no comparisons of bioactivity could be made.

Table 4. Evaluation of Various Compounds Against a Panel of Human Tumor Cell Lines

	all cell lines	leukemic cells		colon cancer cells	
compd	MG MID (μM)[a]	MG MID (μM)[a]	SI[b]	MG MID (μM)[a]	SI[b]
1a	36.3	8.32	4.4	29.1	1.3
1b	91.2	60.5	1.5	81.0	1.1
1d	49.7	9.36	5.3	25.4	2.0
1g	91.2	53.0	1.7	100	0.9
2a	2.41	1.06	2.3	2.21	1.1
2b	3.58	0.864	4.1	2.30	1.6
2g	3.57	0.83	4.3	2.38	1.5
4b	1.66	0.307	5.4	1.21	1.4
melphalan	23.5	4.65	5.1	44.6	0.5
5-fluorouracil	32.6	27.6	1.2	7.90	4.1
helenalin	1.45	0.620	2.3	1.42	1.0

[a] The letters MG MID refer to the mean graph midpoint values. This term is explained in the Discussion section. [b] SI refers to the selectivity index, i.e., the ratio between the MG MID value for all cell lines and the MG MID value for either leukemic or colon cancer cells.

Table 5. Evaluation of Various Compounds in the Hollow Fiber Assay[a]

compd	ip score	sc score	net cell kill
2a	12	14	+
2b	0	6	−
4b	2	10	−

[a] Different tumor cell lines in hollow fibers were implanted in mice by the intraperitoneal (ip) and subcutaneous (sc) routes, while the compounds were administered intraperitoneally to the animals using two different doses (see Experimental Section for further details). A total of 12 tumor cell lines were used, and a score of 2 was given when a 50% reduction or more of the viability of the cancer cells was noted. A maximum score is therefore 96 (2 × 12 cell lines × 2 routes of implantation × 2 doses). Net cell kill indicates whether cytotoxicity has occurred (+) or is absent (−) at either implant site for one or more cell lines.

Discussion

The theory of sequential cytotoxicity requires that candidate antineoplastic agents possess at least two sites which react with cellular constituents at different rates. To confirm that this situation prevails in the compounds under review, the atomic charges at the α and β carbon atoms were determined (Table 1). In the case of series **1**, electrophilicity at the β carbon atom was greater than at the α site, as predicted. When considering the α carbon atoms, the atomic charges in

Table 6. Cytotoxicity, Apoptotic Indices, and Inhibition of RNA and Protein Syntheses of **1f**, **2b**,**f**, and **4b** Using Human Jurkat T Cells[a]

				% inhib of syntheses	
compd	IC_{50} (μM)	IC_{80} (μM)	apoptotic index	RNA (SD)	protein (SD)
1f	19.8	31.7	23.3	77.5 (7.3)	69.1 (3.5)
2b	2.53	4.04	87.0	78.0 (1.9)	37.1 (7.4)
2f	4.47	7.63	59.2	84.5 (1.9)	46.6 (2.4)
4b	3.50	5.60	73.3	88.4 (4.4)	62.0 (0.6)
melphalan	2.20[b]	3.52[b]	63.2[b]	85.1[b] (4.7)	17.3 (4.9)
actinomycin D	0.0045[b]	0.0072[b]	35.5	90.5 (2.2)	49.2 (1.5)
cycloheximide	0.93[b]	1.54[b]	38.3	93.3[b] (3.6)	71.9[b] (3.5)

[a] The IC_{50} and IC_{80} values were obtained after exposure of the cells to the compounds for 48 h. Apoptotic indices and inhibition of RNA and protein syntheses were noted after 24, 8 and 8 h, respectively. These latter three determinations were made using the 80% inhibitory concentrations (IC_{80}) of the compounds. [b] Reprinted in part from *J. Med. Chem.* **1999**, *42*, 1363. Copyright 1999 American Chemical Society.

series **2** were approximately 3 times the values obtained for series **1** due to the electron-withdrawing influence of the quadrivalent nitrogen atom in the Mannich bases **2**. There was a small differential in the α and β atomic charges in series **2**. Similar atomic charges were noted at the α sites between the compounds in series **1, 3**, and **5** as well as between the Mannich bases **2** and **4**. Thus the compounds prepared permit an evaluation of the hypothesis. Furthermore in order to evaluate the prediction that steric impedance to nucleophilic attack is greater at the α site than at the β location vide supra, the torsion angles (θ) between the olefinic axis and the adjacent aryl ring were measured for the unsubstituted compounds **1a, 2a, 3a, 4a**, and **5**. The average θ value at the α site for these five compounds was 53.8° (53.6−54.0°), while at the β site of **1a** and **2a** the θ value was 23.3° in both cases. These data support the notion that initial attack in series **1** will occur at the β site.

Virtually all of the compounds were examined against two murine and two human tumor cell lines, and the results are presented in Table 2. The murine cell lines were chosen based on the claim of their being predictors of clinically useful anticancer agents,[17] while activity against the Molt 4/C8 and CEM T-lymphocytes would indicate the sensitivity of certain human cells to these compounds. To evaluate the theory of sequential cytotoxicity, comparisons of potencies were made between different groups of compounds by comparing the IC_{50} values of analogues having the same aryl substituents. Thus, for example, when evaluating the prediction that **2a**−**g** > **1a**−**g** would support the hypothesis under consideration, the potency of **2a** was compared with **1a, 2b** with **1b**, and so on. Verification of the theory was recorded if the expectations were fulfilled in the majority of the comparisons made, and vice versa for negation of the hypothesis. The results are presented in Table 3. Support for the theory of sequential cytotoxicity was noted in 19 of the 29 comparisons made (66%). The values generated indicated the verification of the theory was somewhat higher for the murine cell lines (73%) than for the human T-lymphocytes (57%). Some comments on the anticipated and generated cytotoxicity will be made (the number of the prediction indicated in Table 3 is in parentheses). First the Mannich bases **2** and **4** were more potent than the analogous α,β-unsaturated ketones in series **1** and **2** (entries 4, 6, 8) when comparisons were made using all four cell lines. This observation suggests that the gradual formation of an additional alkylating group after initial damage to the cells has occurred may well lead to potent antineoplastic agents. Further support for this viewpoint is as follows. The Mannich bases **4a,b,d** have one initial site for thiolation and one further center after deamination has occurred. On the other hand, while **1a,b,d** are potentially bis-alkylators, their cytotoxicity in all four screens is lower than **4a,b,d**. Second, the prediction **1a** > **5** was fulfilled in all four screens (entry 2). However **2a** being substantially less cytotoxic than the related Mannich bases in series **2** may explain why the anticipated greater activity of **2a** than **5** was noted in only one-half of the comparisons made (entry 3). Third, a review of entries 1 and 5 in Table 3 indicated that in general the esters **3** and **4** were more cytotoxic than the vinylogues **1** and **2**. It is conceivable that the 2-(4-aroyloxyphenylmethylene) substituent in **3** and **4** is better accommodated at a vulnerable site on a biological macromolecule than the related group in series **1** and **2** which has an additional olefinic linkage.

To evaluate further the cytotoxicity data in reference to the theory of sequential cytotoxicity, the biodata for three series of compounds containing one (**3**), two (**1**), and three (**2**) electrophilic centers were subjected to a statistical analysis. These three groups of compounds were chosen having the largest number of entries in Table 2. A one-way analysis of variance followed by post hoc analysis with a least-significant difference test[18] was used. In the P388 screen, the potencies of compounds in series **2** were greater than the potencies of compounds in series **1** and **3** ($p = 0.00$). For L1210, Molt 4/C8, and CEM cells, compounds **2** with three electophilic centers were more active than analogues in series **1** ($p = 0.02$, 0.03, and 0.05, respectively). No other significant differences were noted: i.e., $p > 0.05$. Thus the evidence revealed that compounds with the greatest number of electrophilic centers, i.e., series **2,** were more potent than series **1** and **3** which had fewer sites for thiol attack. In general therefore, the biodata generated revealed that the theory of sequential cytotoxicity is worthy of further consideration.

In view of the significant potencies of a number of compounds prepared in this study, linear plots were constructed between the Hammett σ values of the aryl substituents of **1a**−**g, 2a**−**g, 3b**−**d**, and **4a,b,d** and the IC_{50} values of these compounds in each of the L1210, Molt 4/C8, and CEM screens. In addition, similar plots were generated with **2a**−**g** and **4a,b,d** using P388 cells. No correlations were noted ($p > 0.1$) suggesting that cytotoxicity is mediated by factors other than solely electronic considerations.

The remainder of this report is devoted to further investigations of some of these lead molecules. The data in Table 2 revealed that the greatest cytotoxicity was noted among the Mannich bases **2** and **4**. The average IC_{50} values for the four cell lines employed for **2a**−**c,e**−**g, 4a,b,d**, and melphalan (percentage potency of the compound compared to melphalan in parentheses) were as follows: **2a**, 29.78 (7); **2b**, 2.39 (85); **2c**, 3.53 (57); **2e**, 4.04 (50); **2f**, 3.33 (61); **2g**, 4.46 (45); **4a**, 19.27 (11); **4b**, 4.32 (47); **4d**, 2.66 (76); melphalan, 2.02 (100) μM. Thus with the exception of the unsubstituted compounds

2a and **4a**, compounds with potencies approaching that of the reference drug melphalan were obtained in series **2** and **4**.

Four Mannich bases (**2a,b,g** and **4b**) were evaluated against a panel of 55 ± 5 human tumor cell lines. In addition four related α,β-unsaturated ketones (**1a,b,d,g**) were included in this screen, which were predicted to be substantially less cytotoxic than **2a,b,g** and **4b**. In this assay, determination of the bioactivity of the compounds toward cell lines from the neoplastic diseases leukemia, melanoma, non-small-cell lung, colon, central nervous system, ovarian, renal, prostate, and breast cancers[19] was undertaken. The concentrations of compounds employed in this assay were generally log 10^{-4} to log 10^{-8} M, and the amounts required to inhibit 50% of the growth of each tumor were noted. On occasion 50% of the growth of a cell line was not inhibited at the maximum concentration utilized, e.g., log 10^{-4} M, although this number was incorporated into the average value for all cell lines. Hence the term mean graph midpoint (MG MID) was used since the numbers generated were not invariably IC_{50} values. The number of cell lines for which actual IC_{50} values were obtained is given in the Experimental Section. In general, when the MG MID values were less than 40 μM, IC_{50} values were generated for most of the cell lines. On the other hand, any MG MID value of 95−100 signifies that few or none of the cell lines were inhibited by 50% at the highest concentration of compound utilized. The MG MID values of various compounds are presented in Table 4.

The data in Table 4 revealed a similar cytotoxicity pattern as the results obtained using the P388, L1210, Molt 4/C8, and CEM cell lines. Thus a comparison of the MG MID values between **2a,b,g** and **1a,b,g** indicated greater cytotoxicity of the Mannich bases **2** as predicted by the hypothesis assuming that deamination occurred liberating a third olefinic center. On the other hand, **4b** was approximately twice as potent as its vinylogue **2b** in opposition to the theory under consideration.

Two other features pertaining to the data in Table 4 will be mentioned. First, while **1a,b,d,g** were less active than melphalan, **2a,b,g** and **4b** possessed 10, 7, 7, and 14 times, respectively, the potency of this established alkylating agent, suggesting their utilization as prototypic molecules for analogue development. Second, a review of the mean graphs[20] revealed that various compounds displayed selective toxicity toward leukemic and colon cancer cells. A comparison between the MG MID values of each compound toward all neoplasms and either leukemic or colon cancer cells gave rise to selectivity index (SI) values which are indicated in Table 4. The arbitrary assumption was made that a SI value of 1.5 was a noteworthy indication of selectivity for a group of tumors: i.e., the particular tumors were 50% more sensitive to the compound when compared to all cell lines. The criterion for leukemic cells was met in all eight compounds. In fact in the case of **1d** and **4b**, the selectivity was greater than for the reference drug melphalan which is used clinically in treating leukemia.[21] When bioevaluation toward colon cancer cells was reviewed, comparisons were made with 5-fluorouracil which is a drug used in treating colon cancer.[22] On average **2a,b,g** and **4b** possessed 4 times the potency of 5-fluorouracil toward colon cancers and **1d,2b,g** had SI values of 1.5 or more. The remarkable selectivity of **1d** against both leukemic and colon cancer cells is noteworthy. In addition, since the compounds prepared in this study are considered to interact with cellular thiols, the data for helenalin, which is an established thiol alkylator,[23] was obtained and incorporated into Table 4. Helenalin was highly cytotoxic toward the panel of human tumor cells, and in concert with the results for the enones described herein it displayed selective toxicity toward leukemic cells.

The data in Table 4 revealed that the in vivo evaluation of some of these compounds, particularly the Mannich bases, was warranted. The hollow fiber assay[24] was employed whereby different tumor cell lines were placed in polyvinylidene fluoride fibers and inserted intraperitoneally (ip) or subcutaneously (sc) into mice. In the present investigation **2a,b,4b** were administered intraperitoneally, and the results are summarized in Table 5. Activity is denoted when a compound achieved one of the following criteria for activity: namely a total ip + sc score of more than 20, a sc value of greater than 8, and a net cell kill in one or more cell lines. Thus **2a** fulfilled all three criteria, while **4b** possessed a sc score of 10, revealing that both compounds are promising candidates for further in vivo experimentation. In addition, greater activity at the more remote implant site (sc) relative to ip implantation is characteristic of a compound being converted into a more potent product in vivo.[25] It is of interest to note that the sc scores of all three compounds were higher than the ip results suggesting that the Mannich bases were converted into more toxic species in vivo.

Finally in view of the promising cytotoxicity displayed by various compounds described in this study, a preliminary investigation into the possible mode(s) of action of representative compounds was undertaken. A number of anticancer agents induce apoptosis[26] and/or inhibit the syntheses of ribonucleic acid (RNA) and proteins.[27] The compounds chosen were **1f**, possessing very low cytotoxicity, **2f**, which is a potent analogue of **1f**, **4b**, which displayed the highest activity against a panel of human tumor cell lines, and its vinylogue **2b**. A recent investigation from this laboratory revealed that human Jurkat T cells were more sensitive to an α,β-unsaturated ketone than other cell lines,[28] and hence this neoplasm was used in the present study. The results of examining the modes of action of **1f, 2b,f, 4b**, and melphalan as well as actinomycin D and cycloheximide, which are potent inhibitors of RNA and protein, respectively, are summarized in Table 6.

A review of the IC_{50} values for **1b, 2b,f** and **4b** presented in Table 2 revealed the far greater activity of **2b,f** and **4b** than of **1f**. The same observation was made when Jurkat T cells were employed as revealed by the data in Table 6. To determine whether apoptosis occurred, concentrations of compounds were used which caused the death of the majority of the cells, and hence IC_{80} values were generated. An apoptotic index [(the number of cells with apoptotic nuclei/the number of cells counted) × 100] was calculated for each compound. Apoptosis was induced by all four compounds but to a much greater extent by the more potent compounds **2b,f**

and **4b** than **1f**. All of the compounds markedly inhibited RNA synthesis, while, in general, protein synthesis was inhibited to a lesser extent. No correlations between cytotoxicity and inhibition of the syntheses of either RNA or protein were noted. One may conclude that the compounds in this study likely exert their cytotoxicity at least in part by inducing apoptosis and interfering with the biosynthesis of important macromolecules.

Conclusions

This study describing the bioactivities of a selected number of novel prototypic molecules revealed that the theory of sequential cytotoxicity was fulfilled in approximately two-thirds of the evaluations made. Hence this hypothesis is worthy of further consideration with an expanded series of analogues as well as with other groups of compounds. In particular various Mannich bases displayed potent cytotoxicities toward murine cells, human T-lymphocytes, and a panel of human tumor cell lines, showed selective toxicity to human leukemic and colon cells, and demonstrated activity in vivo. These molecules may therefore be considered prototypic molecules for further development as candidate cytotoxic and anticancer agents.

Experimental Section

Melting points are uncorrected. Acetonitrile, used in the synthesis of the Mannich bases, was dried over molecular sieves. Cinnamoyl chloride used in the preparation of **1a**, the aroyl chlorides required for the synthesis of **3a**−**d** and 3-phenylpropionyl chloride required for the preparation of **5** were obtained from Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada. Elemental analyses were undertaken on **1a**−**g**, **3a**−**d**, **5** (C,H) and **2a**−**g**, **4a,b,d** (C,H,N) and were within 0.4% of the calculated values. The Mannich bases **2f,g** were obtained as the hemihydrates and **4b** as the monohydrate. ^{1}H NMR spectroscopy was carried out using a Bruker AMX 500 FT NMR machine. A Nonius CAD-4 diffractometer was used for the collection of X-ray crystallographic data. Thin-layer chromatography (TLC) was employed to monitor the progress of the reactions and to determine the homogeneity of the reaction products using silica gel 60 F_{254} precoated TLC plastic sheets and a solvent system of hexane:ethyl acetate (7:3); observations were made under ultraviolet light and/or in an iodine chamber.

Synthesis of 2-(4-Hydroxyphenylmethylene)cyclohexanone. A solution of 4-hydroxybenzaldehyde (0.10 mol) in aqueous sodium hydroxide solution (4.1% w/v, 150 mL) was added slowly to a mixture of cyclohexanone (0.48 mol) in aqueous sodium hydroxide solution (4.1% w/v, 50 mL). The reaction mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 N) and extracted with chloroform (100 mL). The organic extract was washed with water, dried (anhydrous magnesium sulfate) and evaporation of the solvent gave an oil which was extracted while hot with petroleum ether (bp 45−60 °C). Upon cooling a yellow precipitate was obtained and collected. Distillation of the product using a Kugelrohr apparatus gave 2-(4-hydroxyphenylmethylene)cyclohexanone: bp 136−138 °C/10^{-1} mbar; mp 180−181 °C (lit.[29] mp 172 °C); 40% yield.

Synthesis of 3-Aryl-2-propenoyl Chlorides. The 3-aryl-2-propenoyl chlorides required in the synthesis of **1b**−**g** were obtained as follows. The appropriate 3-aryl-2-propenoic acid (0.02 mol), prepared by a literature procedure,[30] was mixed with thionyl chloride (15 mL) using a condenser to which was attached a calcium chloride guard tube. After heating under reflux for 4 h, the mixture was evaporated under reduced pressure to produce an oily residue which was dissolved in petroleum ether (bp 35−45 °C). Removal of the organic solvent gave the acid chloride which was used immediately in subsequent reactions.

Synthesis of 1a−g, 3a−d, and 5. The esters **1a**−**g** were prepared as follows. A solution of the appropriate 3-aryl-2-propenoyl chloride (0.01 mol) in pyridine (0.5 mL) was added to a solution of 1-(4-hydroxyphenylmethylene)cyclohexanone (0.01 mol) in dry acetonitrile (40 mL) and the mixture was heated under reflux for 5−6 h. Moisture was excluded by using a calcium chloride guard tube on the reflux condenser. On cooling, the precipitates were collected, washed successively with hydrochloric acid (1 N), aqueous sodium carbonate solution (10% w/v) and water and recrystallized from chloroform−diethyl ether (**1a**), chloroform−acetonitrile (**1b**−**f**) or chloroform (**1g**). The melting points (°C) and yields (%) were as follows: **1a**: 129−131, 74; **1b**: 174−176, 75; **1c**: 154−157, 65; **1d**: 143−145, 60; **1e**: 147−149, 75; **1f**: 175−177, 80; **1g**: 164−166, 72. The ^{1}H NMR spectrum of a representative compound **1c** was as follows: δ ($CDCl_3$) 1.75−1.79 (m, 2H, 4-CH_2), 1.91−2.04 (m, 2H, 5-CH_2), 2.39 (m, 3H, CH_3), 2.54 (t, 2H, 3-CH_2); 2.82−2.86 (m, 2H, 6-CH_2), 6.58 (d, 1H, OCOC*H*═CH, J = 16.0 Hz), 7.18−7.25 (m, 4H, aryl H of ring B), 7.42−7.50 (m, 5H, CH and aryl H of ring A), 7.85 (d, 1H, OCOCH═C*H*, J = 16.0 Hz).

Compounds **3a**−**d** were prepared in a similar manner as **1a**−**g** except that the time of heating under reflux was 7−8 h after which time the reaction mixture was poured into water (150 mL). The precipitates were collected after 1−2 h, washed as described previously and purified from water−methanol (**3a**) or water−acetonitrile (**3b**−**d**). The melting points (°C) and yields (%) were as follows: **3a**: 108−109, 64; **3b**: 121−123, 70; **3c**: 119−120, 55, **3d**: 103−105, 68. The ^{1}H NMR spectrum of a representative compound **3d** was as follows: δ ($CDCl_3$) 1.74−1.79 (m, 2H, 4-CH_2), 1.90−1.95 (m, 2H, 5-CH_2), 2.53 (t, 2H, 6-CH_2, J = 6.7 Hz), 2.82−2.85 (m, 2H, 3-CH_2), 3.86 (s, 3H, OCH_3), 6.97 (d, 2H, 2 and 5 H of aryl ring B, J = 8.7 Hz), 7.22 (d, 2H, 2 and 6 H of aryl ring A, J = 8.5 Hz), 7.23 (d, 2H, 2 and 5 H of aryl ring A, J = 8.5 Hz), 7.48 (s, 1H, ═CH), 8.14 (d, 2H, 2 and 6 H of aryl ring B, J = 8.7 Hz).

The enone **5** was prepared in a similar manner as **1a**−**g** except that the time of heating under reflux was 4 h. The crude product was recrystallized from acetonitrile to give **5**: mp 97−99 °C; 80% yield.

Synthesis of 2a−g and 4a,b,d. The Mannich bases **2a**−**g** were prepared as follows. A solution of the precursor ester in series **1** (0.005 mol) in dry acetonitrile (40 mL) was prepared, if necessary by warming, to which was added *N*,*N*-dimethylmethyleneammonium chloride (0.01 mol), which was synthesized by a literature procedure.[31] The mixture was stirred at room temperature for 2−3 h and the resultant precipitate was collected, washed with warm acetonitrile and recrystallized from acetonitrile (**2a,d**) or chloroform (**2b,c,e**−**g**). The melting points and yields were as follows: **2a**: 120−122, 48; **2b**: 165−167, 60; **2c**: 169−170, 50; **2d**: 171−173, 62; **2e**: 165−167, 60; **2f**: 170−171, 60; **2g**: 145−147, 52. The ^{1}H NMR spectrum of a representative compound **2e** was as follows: δ ($CDCl_3$) 1.73−1.89 (m, 2H, 4-CH_2), 1.94−2.02 (m, 2H, 5-CH_2), 2.61−2.72 (m, 2H, 3-CH_2), 2.71 [d, 3H, N(CH_3), J = 4.6 Hz], 2.85 [d, 3H, N(CH_3), J = 4.5 Hz], 3.00−3.10 [m, 2H, C*H*$_2$N(CH_3)$_2$], 3.62−3.70 (m, 1H, 6-CH), 6.54 (d, 1H, OCOC*H*═CH, J = 16.0 Hz), 7.10−7.57 (m, 8H, aryl H), 7.44 (s, 1H, CH═), 7.82 (d, 1H, OCOCH═C*H*, J = 16.0 Hz).

The Mannich bases **4a,b,d** were prepared in a similar manner as **2a**−**g** except 25 mL of acetonitrile was employed and the times of stirring at room temperature were 3−6 h. The products were recrystallized from methanol (**4a**) or acetonitrile (**4b,d**). The melting points and yields were as follows: **4a**: 171−173, 70; **4b**: 160−161, 75; **4d**: 154−155, 65. The ^{1}H NMR spectrum of a representative compound **4b** was as follows: δ ($CDCl_3$) 1.75−1.86 (m, 2H, 4-CH_2) 1.98−2.00 (m, 2H, 5-CH_2), 2.63−2.71 (m, 2H, 3-CH_2), 2.79 [d, 3H, N(CH_3), J = 4.8 Hz], 2.85 [d, 3H, N(CH_3), J = 4.7 Hz], 3.02−3.10 [m, 2H, C*H*$_2$N(CH_3)$_2$], 3.64−3.69 (m, 1H, 6-CH), 7.23−7.25 (m, 2H, 3 and 5 H of aryl ring B), 7.42−7.47 (m, 5H, CH═, 2 and 6 aryl H of ring A, 3 and 5 aryl H of ring B), 8.11−8.13 (d, 2H, 2 and 6 aryl H of ring B).

X-ray Crystallography of 1b. Compound **1b** was recrystallized from ethanol−2-propanol by vapor diffusion. An ω scan was used for data collection and the structure was solved by direct methods using NRCVAX[32] and refined using SHELXL 97.[33] Atomic scattering factors were taken from the literature.[34] All non-hydrogen atoms were found on the E-map and were refined anistotropically. Hydrogen atom positions were calculated and not refined.

Molecular Modeling. The structures of the compounds in series **1**−**5** were built using the MacroModel version 4.5 program.[35,36] A conformational search was undertaken using the Monte Carlo method and MM2 force-field parameters in order to obtain minimum energy conformations. The torsion angles θ were obtained from these molecules. The θ values formed between the olefinic group and the adjacent aryl ring A in **1a, 2a, 3a, 4a** and **5** were −53.7°, −53.7°, 53.8°, 54.0° and −53.6°, respectively. The θ values obtained between the ester olefinic group and aryl ring B were −23.3° and 23.3°, respectively. The signs of the torsion angles are positive or negative if the rotations are anticlockwise or clockwise, respectively. A HyperChem molecular modeling program[37] was utilized to obtain the atomic charges on the α and β carbon atoms. The atomic charges at the α and β sites (**1, 2**) and at the α sites (**3**−**5**) are as follows: **1a**: 0.024, 0.052; **1b**: 0.024, 0.051; **1c**: 0.025, 0.054; **1d**: 0.025, 0.057; **1e**: 0.024, 0.053; **1f**: 0.025, 0.051; **1g**: 0.023, 0.047; **2a**: 0.070, 0.057; **2b**: 0.073, 0.061; **2c**: 0.074, 0.065; **2d**: 0.074, 0.068; **2e**: 0.073, 0.064; **2f**: 0.074, 0.062; **2g**: 0.073, 0.058; **3a**: 0.023; **3b**: 0.024; **3c**: 0.025; **3d**: 0.025; **4a**: 0.073; **4b**: 0.072; **4d**: 0.073; **5**: 0.023.

Cytotoxicity Evaluations. Literature procedures were followed when examining the compounds against murine P388 D1 cells[38] as well as murine L1210 and human Molt 4/C8 and CEM T-lymphocytes[39] and human tumor cell lines.[19] The highest concentration of compound employed was log 10^{-4} M except for **2b**, melphalan and 5-fluorouracil in which cases the maximum concentrations used were log $10^{-4.3}$, log $10^{-3.6}$ and log $10^{-2.6}$ M, respectively. The number of cell lines in the human tumor assay whose growth was inhibited by 50% or more at the maximum concentration of compound used per total number of cell lines employed was as follows: **1a**: 47/53; **1b**: 6/53; **1d**: 25/60; **1g**: 6/54; **2a**: 53/53, 54/54; **2b**: 60/60, 52/52; **2g**: 60/60, 52/52; **4b**: 52/52; melphalan: 55/55, 59/59, 57/57; 5-fluorouracil: 44/50, 54/57, 56/58; helenalin: 51/51. The respective ranges of the MG MID values for all cell lines, leukemic cells and colon cancer cells when the assays were conducted in duplicate (**1d, 2a,b,g**) or triplicate (melphalan and 5-fluorouracil) were as follows: **1d**: 46.8−52.5, 7.91−10.8, 23.2−27.6; **2a**: 2.00−2.82, 0.801−1.31, 1.64−2.78; **2b**: 2.14−5.01, 0.648−1.08, 1.28−3.31; **2g**: 3.24−3.89, 0.417−1.24, 2.31−2.44; melphalan: 19.1−26.9, 2.82−7.16, 41.7−49.9; 5-fluorouracil: 12.0−56.2, 8.05−52.9, 3.09−14.8. The average MG MID values are presented in Table 4.

Hollow Fiber Assay. The experimental procedure using the hollow fiber assay has been described previously.[24,40] In the present case, tumor cell lines form the following tissues were employed: colon (SW-620, COLO 205), breast (MDA-MB-231, MDA-MB-435), non-small-cell lung (NCI-H23, NCI-H522), melanoma (LOX, UACC-62), ovarian (OVCAR 3, OVCAR 5) and central nervous system (SF-295, U251). Intraperitoneal injections of **2a** (12 and 18 mg/kg), **2b** (25 and 37.5 mg/kg) and **4b** (50 and 75 mg/kg) were made daily for 4 days and the fibers collected 1 day after the last treatment. The anticancer effects were calculated by determining the viable cell mass using the formazan dye (MTT) conversion assay.[41] Net cell kill was observed with the COLO 205 and MDA-MB-231 cell lines. Data for melphalan and 5-fluorouracil were not available.

Determination of Cytotoxicity, Apoptotic Indices, Inhibition of RNA, and Protein Synthesis of Various Compounds in Jurkat T Cells. The cytotoxicity and apoptotic indices were determined by a literature procedure[28] except that in place of trypan blue, nigrosin was used in the cytotoxicity experiments and a solution of ethidium bromide (0.1% w/v) and acridine orange (0.1% w/v) was employed in the apoptosis determinations. The inhibition of RNA and protein synthesis was undertaken by a previously published methodology.[42]

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Supporting Information Available: Details of the X-ray crystallographic structure of **1b** including the atomic coordinates and equivalent isotropic displacement parameters, anisotropic displacement parameters, hydrogen coordinates and isotropic displacement parameters, and certain bond lengths and bond angles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dimmock, J. R.; Sidhu, K. K.; Chen, M.; Reid, R. S.; Allen, T. M.; Kao, G. Y.; Truitt, G. A. Evaluation of some Mannich bases of cycloalkanones and related compounds for cytotoxic activity. *Eur. J. Med. Chem*. **1993**, *28*, 313−322.

(2) Benvenuto, J. A.; Connor, T. A.; Monteith, D. K.; Laidlaw, J. L.; Adams, S. C.; Matney, T. S.; Theiss, J. C. Degradation and inactivation of antitumor drugs. *J. Pharm. Sci*. **1993**, *82*, 988−991.

(3) Mitchell, J. B.; Russo, A. The role of glutathione in radiation and drug induced cytotoxicity. *Br. J. Cancer* **1987**, *55*, 96−104.

(4) Tsutsui, K.; Komuro, C.; Ono, K.; Nishidai, T.; Shibamoto, Y.; Takahashi, M.; Abe, M. Chemosensitization by buthionine sulfoximine in vivo. *Int. J. Radiat. Oncol. Biol. Phys*. **1986**, *12*, 1183−1186.

(5) Teicher, B. A.; Herman, T. S.; Holden, S. A.; Epelbaum, R.; Liu, S. D.; Frei, E., III. Lonidamine as a Modulator of Alkylating Agent Activity In Vitro and In Vivo. *Cancer Res*. **1991**, *51*, 780−784.

(6) Dimmock, J. R.; Hamon, N. W.; De Gooijer, C. A.; Grant, G. F.; Jonnalagadda, S. S.; Hancock, D. S. Effect of some 4-alkyl-5-dimethylamino-1-phenyl-1-penten-3-one hydrochlorides and related compounds on respiration in mouse liver mitochondria. *Pharmazie* **1989**, *44*, 560−562.

(7) Dimmock, J. R.; Sidhu, K. K.; Chen, M.; Li, J.; Quail, J. W.; Allen, T. M.; Kao, G. Y. Synthesis and cytotoxic evaluation of some cyclic arylidene ketones and related oximes, oxime esters and analogues. *J. Pharm. Sci*. **1994**, *83*, 852−858.

(8) Dimmock, J. R.; Hamon, N. W.; Hindmarsh, K. W.; Sellar, A. P.; Turner, W. A.; Rank, G. H.; Robertson, A. J. Evaluation of 2-benzylidenecylohexanones and 2,6-bis(benzylidene)cyclohexanones for antitumor and cytotoxic activity and as inhibitors of mitochondrial function in yeasts: metabolism studies of (*E*)-2-benzylidenecyclohexanone. *J. Pharm. Sci*. **1976**, *65*, 538−543.

(9) Bodor, N. In *Advances in Drug Research*; Testa, B., Ed.; Academic Press: London, 1984; Vol. 13, pp 255−330.

(10) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry Part B: Reactions and Synthesis*; Plenum Press: New York, 1977; pp 44−46.

(11) Beveridge, W. I. B. *Seeds of Discovery*; Heinemann Educational Books: London, 1980; p 62.

(12) Dimmock, J. R.; Kandepu, N. M.; Nazarali, A. J.; Kowalchuk, T. P.; Motaganahalli, N.; Quail, J. W.; Mykytiuk, P. A.; Audette, G. F.; Prasad, L.; Perjesi, P.; Allen, T. M.; Santos, C. L.; Szydlowski, J.; DeClercq, E.; Balzarini, J. Conformational and quantitative structure−activity relationship study of cytotoxic 2-arylidenebenzocyloalkanones. *J. Med. Chem*. **1999**, *42*, 1358−1366.

(13) Hassner, A.; Mead, T. C. The stereochemistry of 2-benzalcyclohexanones and 2-benzalcyclopentanones. *Tetrahedron* **1964**, *20*, 2201−2210.

(14) Dimmock, J. R.; Arora, V. K.; Quail, J. W.; Pugazhenthi, U.; Allen, T. M.; Kao, G. Y.; De Clercq, E. Cytotoxic evaluation of some 3,5-diarylidene-4-piperidones and various related quaternary ammonium compounds and analogues. *J. Pharm. Sci*. **1994**, *83*, 1124−1130.

(15) Jia, Z.; Quail, J. W.; Dimmock, J. R. Structure of 2-(4-dimethylaminobenzylidene)cyclohexanone. *Acta Crystallogr*. **1990**, *C46*, 2467−2468.

(16) Dimmock, J. R.; Arora, V. K.; Wonko, S. L.; Hamon, N. W.; Quail, J. W., Jia, Z; Warrington, R. C.; Fang, W. D.; Lee, J. S. 3,5-bis-Benzylidene-4-piperidones and related compounds with high activity toward P388 leukemia cells. *Drug Des. Deliv.* **1990**, *6*, 183−194.
(17) Goldin, A.; Schepartz, S. A.; Venditti, J. M., DeVita, V. T., Jr. In *Methods in Cancer Research*; DeVita, V. T., Jr., Busch, H., Eds.; Academic Press: New York, 1979; Vol. XVI, Part A, p 194.
(18) *SPSS Professional Statistics 9.0.1*; SPSS Inc.: Chicago, 1999.
(19) Boyd, M. R.; Paull, K. D. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(20) Grever, M. R.; Schepartz, S. A.; Chabner, B. A. The National Cancer Institute: cancer drug discovery and development program. *Semin. Oncol.* **1992**, *19*, 622−638.
(21) Greenspan, E. M.; Bruckner, H. W. Aspects of clinical pharmacology. In *Clinical Cancer Chemotherapy*; Greenspan, E. M., Ed.; Raven Press: New York, 1975; p 37.
(22) Remers, W. A. Antineoplastic agents. In *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 10th ed.; Delgado, J. N., Remers, W. A., Eds.; Lippincott-Raven: Philadelphia, 1998; p 367.
(23) Hall, I. H.; Lee, K.-H.; Mar, E. C.; Starnes, C. O.; Waddell, T. G. Antitumor agents 21. A proposed mechanism of inhibition of cancer growth by tenulin and helenalin and related cyclopentenones. *J. Med. Chem.* **1977**, *20*, 333−337.
(24) Hollingshead, M.; Alley, M.; Abbott, B.; Mayo, J.; Gever, M. Short-term in vivo cultivation of human tumor cell lines for assessing potential chemotherapeutic agents. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, 429.
(25) Argade, A. B.; Devraj, R.; Vroman, J. A.; Haugwitz, R. D.; Hollingshead, M.; Cushman, M. Design and synthesis of brefeldin A sulfide derivatives as prodrug candidates with enhanced aqueous solubilities. *J. Med. Chem.* **1998**, *41*, 3337−3346.
(26) Tsurusawa, M.; Salki, K.; Fujimoto, T. Differential induction of apoptosis on human lymphoblastic leukemia Nalm-6 and Molt-4 cells by various antitumor drugs. *Int. J. Hematol.* **1997**, *66*, 79−88.
(27) Foye, W. O.; Sengupta, S. K. Cancer chemotherapy. In *Principles of Medicinal Chemistry*, 4th ed.; Foye, W. O., Lemke, T. L., Williams, D. A., Eds.; Williams and Wilkins: Media, PA, 1995, p 829.
(28) Vashishtha, S. C.; Nazarali, A. J.; Dimmock, J. R. Application of fluorescence microscopy to measure apoptosis in Jurkat T cells after treatment with a new investigational anticancer agent (N.C. 1213). *Cell. Mol. Neurobiol.* **1998**, *18*, 437−445.
(29) Iyer, R. N.; Gopalchari, R. Synthesis of potential antifertility agents: Part XV. Synthesis of oxiranes, pyrazolines, cyclopropanes, bicycloenones and arylidenecycloalkanones. *Indian J. Chem, Sect. B* **1976**, *14B*, 194−198.
(30) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman Scientific and Technical: Harlow, U.K., 1989; p 1038.
(31) Böhme, H.; Hartke, K. α-Halogenated amines. VII. The cleavage of aminals and α-dialkylamino ethers with carboxylic acid halides. *Chem. Ber.* **1960**, *93*, 1305−1309.
(32) Gabe, E. J.; LePage, Y.; Charland, J. P.; Lee, F. L.; White, P. S. An interactive program system for structure analyses. *J. Appl. Crystallogr.* **1989**, *22*, 384−387.
(33) Sheldrick, G. M. *Program for the refinement of crystal structures*; University of Göttingen: Germany, 1997.
(34) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, 1974; Vol. IV.
(35) *MacroModel*, version 4.5; Department of Chemistry, Columbia University: New York, 1994.
(36) Mohamadi, F.; Richards, N. G. J.; Guide, W. C.; Liskamp, M. L.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel − An integrated software system for modeling organic and bioorganic molecules using molecular mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.
(37) *HyperChem*, release 5.0 for Windows; Hypercube Inc.: Waterloo, Ontario, Canada, 1996.
(38) Phillips, O. A.; Nelson, L. A., Knaus, E. E.; Allen, T. M.; Fathi-Afshar, R. Synthesis and cytotoxic activity of pyridylthio, pyridylsulfinyl and pyridosulfonyl methyl acrylates. *Drug Des. Deliv.* **1989**, *4*, 121−127.
(39) Balzarini, J.; DeClercq, E.; Mertes, M. P.; Shugar, D.; Torrence, P. F. 5-Substituted 2-deoxyuridines: correlation between inhibition of tumor cell growth and inhibition of thymidine kinase and thymidylate synthetase. *Biochem. Pharmacol.* **1982**, *31*, 3673−3682.
(40) Plowman, J.; Dykes, D. J.; Hollingshead, M.; Simpson-Herren, L.; Alley, M. C. In *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Teicher, B., Ed.; Humana Press: Totowa, NJ, 1997; pp 101−125.
(41) Alley, M. C.; Pacula-Cox, C. M.; Hursey, M. L.; Rubinstein, L. R.; Boyd, M. R. Morphometric and colorimetric analyses of human tumor cell line growth and drug sensitivity in soft agar culture. *Cancer Res.* **1991**, *51*, 1247−1256.
(42) Martin, S. J.; Lennon, S. V.; Bonham, A. M.; Cotter, T. G. Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA and protein synthesis. *J. Immunol.* **1990**, *145*, 1859−1867.

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