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E,E,E-1-(4-Arylamino-4-oxo-2-butenoyl)-3,5-bis(arylidene)-4-piperidones: A topographical study of some novel potent cytotoxins

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Abstract—A series of *E,E,E*-3,5-bis(arylidene)-1-(4-arylamino-4-oxo-2-butenoyl)-4-piperidones **4** (phenylidene) and **5** (4-nitrophenylidene) were prepared in order to explore the structural features of the *N*-acyl group which affects the cytotoxic potency. Evaluation toward human Molt 4/C8 and CEM T-lymphocytes revealed that many of the IC_{50} figures were submicromolar and lower than melphalan. Marked inhibitory potencies toward murine leukemia L1210 cells were also noted. When evaluated against a panel of human tumor cell lines, three representative compounds in series **4** displayed selective toxicity to leukemia and colon cancer cell lines and were significantly more potent than the reference drug melphalan. Molecular modeling of representative compounds in both series **4** and the analogs, in which the configuration of the olefinic double bond was changed from *E* to *Z* (series **3**), revealed that the torsion angles of the arylidene aryl rings and locations of the terminal arylaminocarbonyl groups may have contributed to the greater cytotoxic properties displayed in **3**. Compounds **4c** (3,4-dichlorophenylamino), **d** (4-methylphenylamino) and **5c** (3,4-dichlorophenylamino), **d** (4-methylphenylamino) inhibited the activity of human *N*-myristoyltransferase by approximately 50% at concentrations of 50–100 μM. The compounds in series **4** and **5** were well tolerated in a short-term toxicity study in mice.

1. Introduction

Various series of acyclic Mannich bases of conjugated styryl ketones have been prepared as candidate cytotoxic and anticancer agents.[1] These compounds were designed as thiol alkylators with little or no capacity for interaction with amino or hydroxy groups which are found in nucleic acids.[2,3] Hence such molecules should be bereft of the genotoxic properties of many alkylating agents used in cancer chemotherapy today.[4] In addition, their novelty of structure suggested that cross-resistance to other anticancer drugs, especially those acting by alkylation, may be absent. The observation of the sensitivity of human MCF-7 and rat Mat B cells which were resistant to melphalan and other clinically used alkylating agents to several acyclic Mannich bases of α,β-unsaturated ketones[5] supports this contention. However, many acyclic Mannich bases are toxic to mice.[6,7] These compounds are flexible molecules capable of assuming a wide variety of conformations, some of which may have contributed to the antineoplastic properties, while others initiated a sequence of unwanted biotoxic reactions.

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The hypothesis was advanced that the formation of rigid analogs may lock important functional groups into specific spatial relationships which would lead to the desired bioactivity without the accomplishment of host toxicity. Such considerations led to the decision to attach styryl groups to the cyclic Mannich base, 4-piperidone. One of the initial compounds prepared was **1a** (as the hydrochloride salt) which possessed an IC_{50} value toward P388 MRI cells of 16.0 pM, and mice tolerated five daily doses of 240 mg/kg of this compound when administered intraperitoneally.[8] Furthermore, **1a** hydrochloride lowered hepatic thiol concentrations when administered to mice,[8] confirming that its mode of action, at least in part, was by thiol alkylation.

Based on these observations of the lead molecule **1a** hydrochloride, various substituents were placed in the aryl rings leading to the synthesis of **1b**–**f** (Fig. 1) which were evaluated toward human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemic cells.[9] The average IC_{50} values of **1a**–**f** toward these three cell lines are 3.78, 21.2, 63.3, 14.4, 15.2, and 232 μM, respectively. The thiol-alkylating capacity of the compounds in series **1** is considered to be due to the 1,5-diaryl-1,4-pentadien-3-oxo pharmacophore which could interact at a complementary site referred to subsequently as binding site A, as indicated in Figure 2. The decision was made to determine whether additional interactions of ligands related to **1** with one or more auxiliary binding sites could take place. The initial studies concentrated on the area in the vicinity of the piperidyl nitrogen atom as indicated in Figure 2, which was designated auxiliary binding site B. The aim was to attach different groups onto the heterocyclic nitrogen atom which would allow interaction with site B, determine the relative location of these groups, and attempt to correlate these data with cytotoxicity.

The attachment of an acryloyl group to the piperidyl nitrogen atom, thereby creating a further site of alkylation in area B, was accomplished leading to **2a**–**f** (Fig. 1). The average IC_{50} values of these compounds toward Molt 4/C8, CEM, and L1210 cells are 3.86, 0.96, 1.21, 1.70, 0.28, and 5.58 μM, respectively,[9] revealing, on average, a 26-fold increase in potencies of the compounds in series **2** compared to the precursor amines **1**. The modes of action of two representative 4-piperidones **1c** and **2c** were shown to include the induction of apoptosis and inhibition of the biosyntheses of RNA and proteins.[9] Greater potency was displayed by **2c** than **1c** in these assays which may have contributed

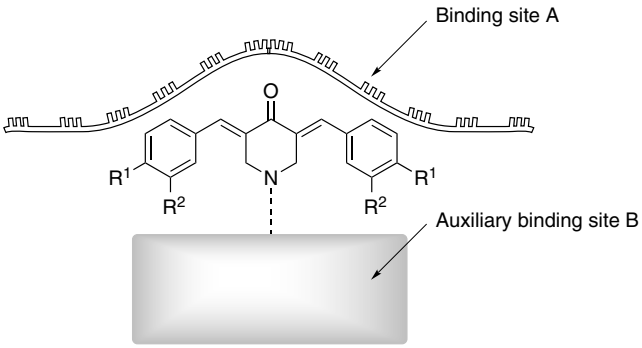

Figure 2. Proposed interactions of 3,5-bis(arylidene)-4-piperidones and related *N*-substituted analogs at a locus of bioactivity.

to the higher potency of **2c** toward Molt 4/C8, CEM, and L1210 cells.

In the light of this positive development, the replacement of one of the terminal hydrogen atoms of the acryloyl group by an arylaminocarbonyl function leading to **3a** (Fig. 3) was undertaken for two reasons. First, the σ^* values of the hydrogen atom and phenylcarbamoyl group are 0.49 and 1.56, respectively,[10] indicating that the adjacent olefinic double bond should be more electrophilic in **3** than **2**. Second, the 1,4-dioxo-2-butenyl group (COCH=CHCO) would impart a rigidity to much of the *N*-acyl function thereby allowing a good estimate of its topography. Cytotoxic evaluation of **3a** would enable an estimate of the location of groups in the auxiliary binding site B which interact with the phenylaminocarbonyl function in **3a**. The IC_{50} values of **3a** toward Molt 4/C8, CEM, and L1210 cells were 0.95, 0.74, and 2.07 μM, respectively,[11] in contrast to figures of 1.42, 1.48, and 8.69, respectively, for **2a**. This

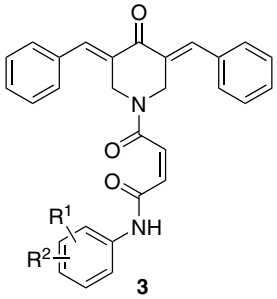

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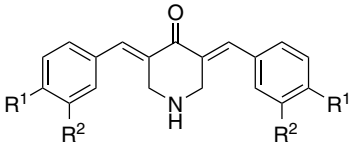
Figure 3. Structures of the compounds in series **3**. The nature of the substituents R^1 and R^2 is given in Scheme 1.



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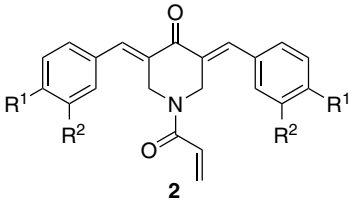

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Figure 1. Structures of series **1** and **2**. The substituents in the aryl rings were as follows: **a**, $R^1 = R^2 = H$; **b**, $R^1 = Cl$, $R^2 = H$; **c**, $R^1 = R^2 = Cl$; **d**, $R^1 = F$, $R^2 = H$; **e**, $R^1 = NO_2$, $R_2 = H$; **f**, $R^1 = OCH_3$, $R_2 = H$.

encouraging observation led to the decision to prepare further analogs of **3a**, namely **3b**–**g**,**i**.[11] The IC_{50} values of **3a**–**g**,**i** toward Molt 4/C8 and CEM cells were within the range of 0.4–1.7 μM, while figures of 1.9–5.0 μM were noted in the L1210 test.[11] Hydrolysis of **3a**–**g**,**i** to **1** and the corresponding *N*-arylmaleamic acids was considered unlikely since the compounds in series **3** were more potent than **1** in general and the corresponding *N*-arylmaleamic acids exhibited extremely low potencies (the IC_{50} values of approximately two-thirds of the compounds were in excess of 500 μM). A further noteworthy feature of these compounds was that **3b**,**d**–**h** were administered intraperitoneally to mice using doses up to and including 300 mg/kg and after 4 h no fatalities were noted.[11]

The aims of the present investigation were as follows. First, compounds **3a**–**g**,**i** are clearly a novel group of cytotoxic agents and the *N*-acyl side chain in this series contributed significantly to the observed bioactivity. The question posed is whether the functional groups on the side chain of **3** were in the optimal positions for cytotoxicity. Consequently the decision was made to alter the stereochemistry of the side chain olefinic double bond from *Z* to *E* leading to **4a**–**g**,**i** and to compare the relative locations of various groups in both series of compounds. It was aimed that a perception of the interactions of the compounds at strategic complementary locations on site B would lead to guidelines for designing further analogs. Second, a previous study revealed that the introduction of a 4-nitro group into the aryl ring of **2a** leading to **2e** increased potency considerably; the average IC_{50} values against Molt 4/C8, CEM, and L1210 cells of **2a** and **2e** were 3.86 and 0.28 μM, respectively, reflecting a 14-fold difference in potencies.[9] Hence a comparison between each of the compounds in series **4** with the 4-nitro analogs **5a**–**i** was contemplated.

A molecular target of interest in these laboratories is human *N*-myristoyltransferase (hNMT) which catalyzes the covalent attachment of a myristoyl group to the amino portion of terminal glycine residues of various proteins. This enzyme is present in higher concentrations in various tumors than the corresponding normal cells.[12] Hence perturbation of this enzyme may be more deleterious to malignant tissues and the examination of representative compounds on the activity of hNMT was planned. Finally an estimate of the murine toxicity of the compounds in series **4** and **5** was considered an important aspect in considering the overall potential of these molecules for future development.

2. Chemistry

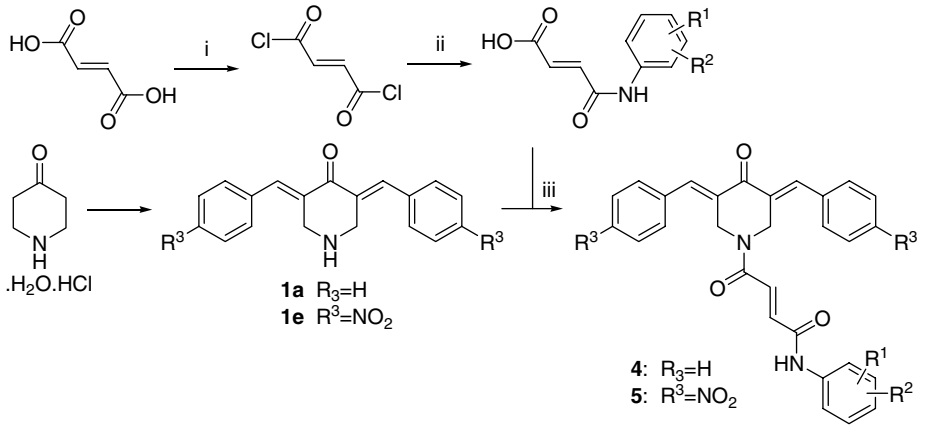
3,5-Bis(arylidene)-4-piperidones **1a**,**e** were synthesized utilizing a Claisen-Schmidt condensation of 4-piperidone monohydrate hydrochloride and appropriate aromatic aldehydes by passing dry HCl gas through an acetic acid solution as described previously.[9] *N*-aryl fumaramic acids were prepared from fumaryl chloride and various substituted aromatic amines as described earlier.[22] The compounds in series **4** and **5** were prepared from appropriate 3,5-bisarylidene-4-piperidone and *N*-aryl fumaramic acids by the route indicated in Scheme 1.[22] The ^1H NMR spectra of the compounds in series **4** and **5** revealed them to be isomerically pure and that the olefinic double bonds possessed the *E* stereochemistry. The X-ray crystallography of a number of 3,5-bis(arylidene)-4-piperidones had previously confirmed them being the *E* isomers.[8,9] In the case of fumaroyl double bond, the *J* value in the range of 14.8–15.1 Hz for the coupling of olefinic Hs clearly indicated the *E* stereochemistry. The shapes of **3d**,**e**,**i** were compared with those of **4d**,**e**,**i** using molecular modeling techniques. In this procedure, various interatomic distances as well as bond and torsion angles were measured and these data are presented in Table 3.

3. Results and discussion

All of the compounds in series **4** and **5** were evaluated against human Molt 4/C8 and CEM T-lymphocytes and murine leukemia L1210 cells. The results are summarized in Table 1. In addition, **4b**,**cf** were examined against a panel of human tumor cell lines and these data are displayed in Table 4. The 4-piperidones **4c**,**d**,**c**,**d** were examined for inhibitory activity of hNMT. All of the compounds in series **4** and **5** were evaluated against five strains of *Aspergillus fumigatus* and in short-term survival and neurotoxicity assays in mice.

The 4-piperidones **4a**–**i** and **5a**–**i** were evaluated against human Molt 4/C8 and CEM T-lymphocytes. These cell lines were chosen in order to obtain an appreciation of the toxicity of these compounds toward human transformed cells. In addition, the L1210 cytotoxicity assay was performed since a number of clinically useful anticancer drugs are effective toward these cells.[13] From the data in Table 1, the following observations were made. First, in general, the compounds displayed noteworthy toxicity to both T-lymphocytes, the IC_{50} values being submicromolar in 61% of the assays conducted while, with the exception of **4g**, the remaining compounds had IC_{50} figures of 1–8 μM. The L1210 cells were more refractory to these compounds and only **5i** possessed a submicromolar IC_{50} value, although 82% of the other compounds had IC_{50} figures of less than 10 μM. Second, the potencies of **4a**–**i** and **5a**–**i** were compared to melphalan. In series **4**, two-thirds of the compounds had lower IC_{50} values than melphalan when the Molt 4/C8 and CEM assays were considered. In fact, **4a** and **c** were six and seven times as potent as melphalan in the Molt 4/C8 and CEM tests, respectively. All of the compounds **5a**–**i** were more potent than melphalan toward both Molt 4/C8 and CEM cells. In these assays the lowest IC_{50} value was displayed by **5i** having 15 and 11 times the potency of melphalan, respectively, and bearing in mind it possessed the greatest potency in the L1210 screen (three times the potency of melphalan), **5i** is clearly a useful lead molecule.

A number of comparisons were made between the IC_{50} values of various 3,5-bis(arylidene)-4-piperidones in order to ascertain the effect on potencies of (i) *N*-acylation



Scheme 1. Synthesis of series **4** and **5**. The reagents used were as follows: (i) $SOCl_2$; (ii) $_2NArR^1R^2$; and (iii) $ClCO_2CH_3$, $N(C_2H_5)_3$. The substituents in the distal ring were as follows viz. **a**, $R^1 = R^2 = H$; **b**, R^1 = 4-Cl, $R^2 = H$; **c**, R^1 = 3-Cl, R^2 = 4-Cl; **d**, R^1 = 4-CH_3, $R^2 = H$; **e**, R^1 = 3-CH_3, R^2 = 4-CH_3; **f**, R^1 = 4-OCH_3, $R^2 = H$; **g**, R^1 = 4-NO_2, $R^2 = H$; **h**, R^1 = 4-$COCH_3$, $R^2 = H$; **i**, R^1 = 2–CH_3, R^2 = 6-CH_3.

Table 1. Cytotoxic properties and comparative potencies of **4a–i** and **5a–i** toward human Molt 4/C8 and CEM T-lymphocytes and murine L1210 leukemic cells

Compound	Molt 4/C8 cells		CEM cells		L1210 cells	
	IC_{50} (μM)	Comparative potency[a]	IC_{50} (μM)	Comparative potency[a]	IC_{50}(μM)	Comparative potency[a]
4a	0.52 ± 0.21	1.83	1.1 ± 0.8	0.67	3.6 ± 1.9	0.58
4b	1.8 ± 0.2	0.31*	0.84 ± 0.02	1.57*	7.8 ± 0.3	0.30*
4c	1.7 ± 0.0	0.62	0.35 ± 0.02	3.97	7.5 ± 0.6	0.25*
4d	0.66 ± 0.20	2.05	1.1 ± 0.2	1.33	5.8 ± 2.1	0.45*
4e	7.9 ± 0.7	0.22	7.1 ± 1.5	0.22	31 ± 10	0.10*
4f	1.1 ± 0.1	0.76	0.51 ± 0.36	1.55	6.8 ± 1.0	0.38*
4g	31 ± 0	0.03	40 ± 0	0.02	40 ± 4	0.06*
4h	1.4 ± 0.3	—	1.7 ± 0.1	—	5.5 ± 1.1	—
4i	6.6 ± 1.0	0.06	7.4 ± 1.8	0.09	22 ± 15	0.23*
5a	0.36 ± 0.11	1.44	0.37 ± 0.10	2.97	1.8 ± 0.0	2.00
5b	1.2 ± 1.0	1.50	0.81 ± 0.35	1.04	3.6 ± 1.2	2.17
5c	0.60 ± 0.21	2.83	0.38 ± 0.05	0.92	1.7 ± 0.4	4.41*
5d	0.70 ± 0.30	0.94	0.89 ± 0.27	1.24	4.4 ± 0.8	1.32
5e	0.66 ± 0.43	12.0	0.70 ± 0.41	10.1	4.9 ± 0.6	6.33*
5f	0.94 ± 0.76	1.17	0.76 ± 0.44	0.67	2.9 ± 1.5	2.35*
5g	0.43 ± 0.07	72.1	0.41 ± 0.05	97.6	1.0 ± 0.5	40.0*
5h	0.39 ± 0.03	3.59	0.36 ± 0.04	4.72	1.4 ± 0.7	3.93*
5i	0.21 ± 0.07	31.4	0.23 ± 0.07	32.2	0.69 ± 0.26	31.9*
Melphalan	3.24 ± 0.79	—	2.47 ± 0.03	—	2.13 ± 0.03	—

[a] In the case of **4a–g,i**, the comparative potencies are the ratios of the IC_{50} values of the compounds in series **3** divided by the figures for the analogs in series **4**. For **5a–i**, the comparative potencies are the ratios of the potencies of the compounds in series **4** divided by the IC_{50} values of the analogs in series **5**. Comparisons were made between compounds bearing the same aryl substituents. Figures superscripted with an asterisk indicate significant differences in comparative potencies taking the standard deviation figures into account.

of **1a,e** giving rise to series **3–5**, (ii) changing the configuration of the olefinic group in the *N*-acyl side chain of series **3** and **4**, and (iii) insertion of a 4-nitro group into the arylidene aryl rings of series **4** giving rise to **5**. A summary of these comparisons is presented in Table 2. The following conclusions were drawn. First, *N*-acylation of **1a** leading to **3a–g,i** and **4a–g,i** was accompanied by increase in cytotoxic potencies in 58% of the compounds made. Conversion of **1e** into **5a–i** invariably led to increased potencies (100%). These data suggest that in general the hypothesis of the presence of an auxiliary binding site B is valid. Second, assuming that the compounds in series **3** and **4** align initially at binding site A, the 2-arylaminocarbonyl group (ArNHCO) in these two series will occupy different positions on the auxiliary site depending on whether the configuration of the olefinic double bond in the *N*-acyl group has the *Z*(series **3**) or E (series **4**) configuration. The preferred stereochemistry in terms of potency is *Z*, since in nearly 60% of the comparisons greater potencies were demonstrated by **3a–g,i** compared to **4a–g,i**. Third, the introduction of a 4-nitro group into the arylidene aryl rings of **4** leading to **5** was accompanied by increased potencies in 59% of the comparisons made and equal potencies in the remaining cases. A number of tumors are hypoxic compared to the corresponding non-malignant cells[14] and hence reduction of the nitro group to biotoxic species[14] may have contributed to the increased cytotoxicity of series **5** compared to the analogs in series **4**. Alternatively, the 4-nitro substituents in the arylidene aryl rings in series **5** ($\sigma_p = 0.78$)[15] would be expected to diminish the electron densities on the adjacent olefinic carbon atoms

Table 2. Comparisons of potencies of various compounds in the Molt 4/C8, CEM, and L1210 assays[a]

	Comparison		Cell line			Total
			Molt 4/C8	CEM	L1210	
1	**1a** and **3a–g,i**	Greater potency in **1a**	0	0	0	0 (0%)
		Greater potency in **3a–g,i**	4	7	8	19 (79%)
		Equal potencies	4	1	0	5 (21%)
2	**1a** and **4a–g,i**	Greater potency in **1a**	3	3	2	8 (33%)
		Greater potency in **4a–g,i**	3	4	2	9 (38%)
		Equal potencies	2	1	4	7 (29%)
3	**1e** and **5a–i**	Greater potency in **1e**	0	0	0	0 (0%)
		Greater potency in **5a–i**	9	9	9	27 (100%)
		Equal potencies	0	0	0	0 (0%)
4	**3a–g,i** and **4a–g,i**	Greater potency in **3a–g,i**	4	3	7	14 (58%)
		Greater potency in **4a–g,i**	1	3	0	4 (17%)
		Equal potencies	3	2	1	6 (25%)
5	**4a–i** and **5a–i**	Greater potency in **4a–i**	0	0	0	0 (0%)
		Greater potency in **5a–i**	5	4	7	16 (59%)
		Equal potencies	4	5	2	11 (41%)

[a] In making these comparisons, standard deviations were taken into account.

which may therefore make them more susceptible to nucleophilic attack by cellular constituents than the analogs in series **4** ($\sigma_p = 0.00$).[15]

The difference in potencies between the compounds in series **3** and **4** was addressed by considering the topography of both groups of molecules. There are at least two major effects which could have produced the variations in cytotoxicity. First, differences in the shapes of the 1,5-diphenyl-1,4-pentadien-3-oxo group in series **3** and **4** could affect potencies. Second, various portions of the *N*-acyl groups would act at different locations on the auxiliary site B. Molecular modeling was employed in order to address both of these issues and models of three representative compounds in both series, namely **3d**,**e**,**i** and **4d**,**e**,**i**, were constructed. In addition to the goal of discerning some of the reasons for the disparity in potencies between series **3** and **4**, it was aimed that modeling would provide some guidelines for designing future clusters of compounds which would shed light on the structural requirements of ligands for increased interactions at site B. The results indicated very little differences in the shapes of the models of the compounds in each series, for example, the data for **3d**,**e**,**i** were virtually identical, and hence only the average figures are presented in Table 3.

Two determinations were made in order to examine whether the different *N*-acyl groups in **3** and **4** impacted upon the shapes of the 1,5-diphenyl-3-oxo-1,4-pentadienyl group. In the first place, variations in the torsion angles θ_1 and θ_2 (see Fig. 4a) were noted. The larger θ_1 values for **3d**,**e**,**i** were due to interactions between the methyl and/or hydrogen atoms in ring C with some of the protons in ring A. The greater θ_2 figures of **4d**,**e**,**i** than **3d**,**e**,**i** were attributed to non-bonding interactions between the protons of ring B and one of the olefinic hydrogen atoms of the *N*-acyl group. These different θ values may have contributed to the variations in potency between the compounds in series **3** and **4**. Amplification of this project should include the placement of one and two *ortho* substituents of varying sizes in rings A and B in order to investigate whether a correlation between the θ values and cytotoxic potencies exists. Second, the six atoms comprising the α, α^x, β, β^x-diolefinic keto group [C═C─C(═O)─C═C] of **3d** were overlapped with **4d**, **3e** with **4e**, and **3i** with **4i**. The root mean square figures for these comparisons were 0.396, 0.013, and 0.015 Å, respectively, indicating that in general a very minimal effect of the *N*-acyl group on the topography of the diolefinic keto group was observed.

Next, the location of the *N*-acyl groups in relation to the binding site A was considered. The reactions of the compounds in series **3** and **4** with cellular constituents

Table 3. Various torsion angles (θ_1, θ_2, °), interatomic distances (d_1–d_{12}, Å), and bond angles (ψ_1–ψ_6, °) in **3d**,**e**,**i** and **4d**,**e**,**i** determined by molecular modeling

Measurement	Average values		
	3d,**e**,**i**	**4d**,**e**,**i**	Δ%[a]
θ_1	71.5	67.2	6.4
θ_2	70.6	80.1	14
d_1	4.427	4.296	3.1
d_2	5.641	5.573	1.2
d_3	6.037	6.282	4.1
d_4	5.558	7.646	38
d_5	4.963	8.463	71
d_6	5.201	11.202	115
d_7	1.581	1.666	5.4
d_8	2.190	2.284	4.3
d_9	3.222	3.377	4.8
d_{10}	3.895	4.328	11
d_{11}	4.052	5.736	42
d_{12}	5.176	7.761	50
ψ_1	56.5	57.0	0.9
ψ_2	48.2	49.6	2.9
ψ_3	45.7	54.6	20
ψ_4	50.0	50.2	0.4
ψ_5	63.4	56.3	13
ψ_6	85.5	54.1	58

[a] The Δ% figures are the differences between the average values in series **3** and **4** expressed as a percentage.

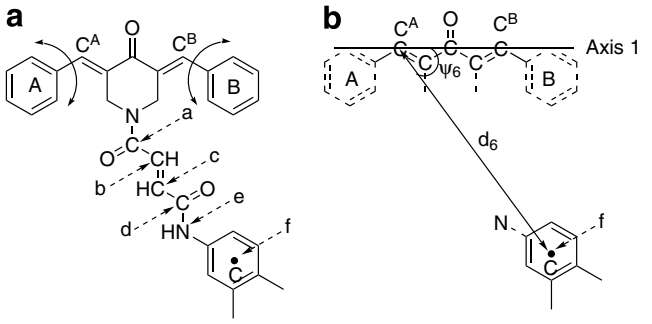


Figure 4. Molecular modeling studies. (a) Designation of the portions of the *N*-acyl groups **a**–**f** and torsion angles θ_1 and θ_2 of a representative compound **4e**. (b) Determination of the location of ring C in relation to carbon atoms C^A and C^B in **4e**.

were predicted to include nucleophilic attack at the olefinic carbon atoms designated C^A and C^B (Fig. 4a). Hence, the differences in the positions of the *N*-acyl groups of **3d**,**e**,**i** and **4d**,**e**,**i** were determined in relation to these olefinic carbon atoms as follows. The distances d_1–d_6 were measured between C^A and the atoms **a**–**e** and the center of aryl ring C which was designated **f** (Fig. 4a). Axis 1 was constructed between carbon atoms C^A and C^B and the angles ψ_1–ψ_6 were made between this axis and **a**–**f** (Fig. 4b). The distances d_7–d_{12} reflect the extent that each of the atoms or group **a**–**f** was either above or below a plane consisting of the C^A and C^B atoms and the proton attached to C^A. The data for θ_1, θ_2, d_1–d_{12}, and ψ_1–ψ_6 are presented in Table 3.

The structures of **3e** and **4e** determined by molecular modeling are displayed in Figure 5 which reveals clearly the considerable differences in the topography of parts of the *N*-acyl side chains. The data in Table 3 were generated to quantify these differences in order to find where the larger divergences existed. The d_1–d_3, d_7–d_9, ψ_1, ψ_2, and ψ_4 figures for **3d**,**e**,**i** and **4d**,**e**,**i** revealed little variations while somewhat surprisingly the ψ_3, but not d_3 or d_9, values differed by 20%. Thus, in general, the locations of atoms **a**–**c** are unlikely to contribute markedly to the potency differences between series **3** and **4**. On the other hand, the d_4–d_6, d_{10}–d_{12}, ψ_3, ψ_5, and ψ_6 figures indicate wide disparities in the locations of the terminal arylaminocarbonyl group atoms (**d**–**f**). Based on the observations made, future groups of compounds should be designed from at least three different vantage points. First, the atoms **a**–**e** and ring C may be retained but oriented in different locations. Such modifications of series **3** could include the placement of alkyl groups of varying sizes on one or both of the olefinic carbon atoms as well as the nitrogen atom, substitution of one or both of the amidic oxygen atoms by sulfur, and the introduction of large groups in ring C which could increase non-bonded interactions between rings A and C. Second, the introduction of spacers between different portions of the *N*-acyl groups in series **3** should be undertaken such as the insertion of polymethylene chains between atom **e** and ring C. The information obtained from both of these approaches may be valuable in determining the nature and sizes of the *N*-acyl groups which confer high potencies on the compounds. Third, the placement in rings A and B of one or more nitro groups in various locations as well as substituting these rings with other strongly electron-withdrawing groups such as the trifluoromethyl and cyano functions should be undertaken.

Experiments were performed to examine whether the cytotoxicity in the Molt 4/C8, CEM, and L1210 tests was correlated with the electronic, hydrophobic, and steric properties of the aryl substituents in ring C of the series **4** and **5**. Accordingly, linear and semilogarithmic plots were made between the Hammet sigma, Hansch pi, and molar refractivity values of the aryl substituents in **4** and **5**. No significant correlations were noted, that is, $p > 0.05$. However a trend toward significance was noted in the linear plots between the sigma values and cytotoxicity in series **4** using CEM cells ($p = 0.146$, positive correlation) and in series **5** in the L1210 test ($p = 0.075$, negative correlation). These data suggest that variation in cytotoxicity is most likely caused principally by the shapes of the *N*-acyl groups rather than changes in the substituents in ring C.

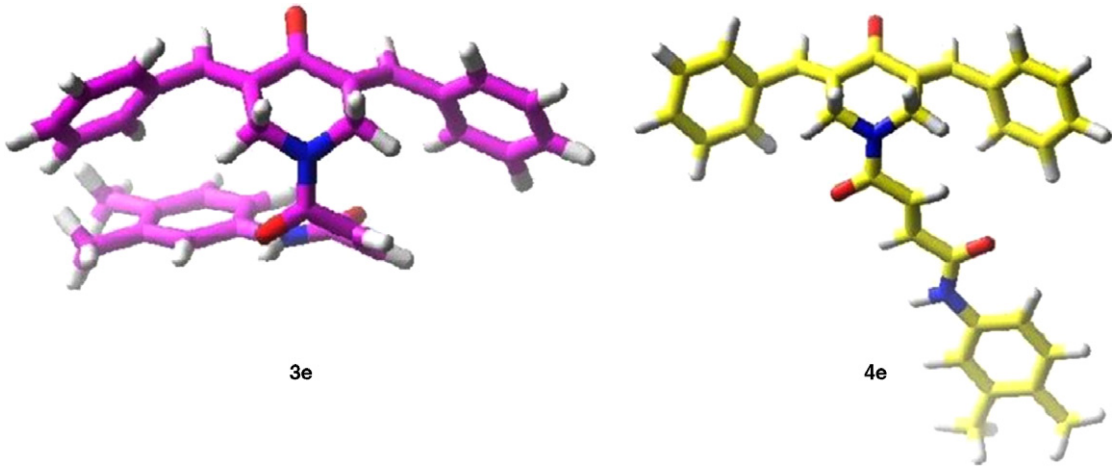


Figure 5. Molecular Models of **3e** and **4e**.

In order to further explore the cytotoxic potential of the compounds prepared in this study, three representative compounds **4b**,**c**,**f** were evaluated against 56 ± 1 human tumor cell lines representing nine different neoplastic conditions, namely leukemia, melanoma, non-small cell lung, colon, central nervous system, ovarian, renal, prostate, and breast cancers. The data generated are summarized in Table 4. In the case of **4b** and 5-fluorouracil, the IC_{50} figures toward three cell lines were in excess of the highest concentrations utilized and hence the term GI_{50}[16] rather than IC_{50} was used.

The results documented in Table 4 reveal that when all cell lines are considered, **4b**,**c**,**f** are significantly more cytotoxic than melphalan; in particular **4c** had 20 times the potency of the reference drug. An important feature of a candidate cytotoxin is selective toxicity for certain cells rather than their being indiscriminately biotoxic. The SI figures in regard to all cell lines reveal that there was a wide differential sensitivity of the human tumors to **4b**,**d**,**f**. A review of the mean graphs[16] revealed that **4b**,**c**,**f** exerted greater toxicity to leukemic and colon cancer cell lines than the other neoplastic diseases. Melphalan is used in combination chemotherapy to treat chronic leukemias[17] and 5-fluorouracil is effective toward various colon cancers.[18] The results in Table 4 reveal that **4b**,**c**,**f** are far more potent than these established drugs, for example, **4f** has 38 times the potency of melphalan toward leukemic cells and is eight times more cytotoxic than 5-fluorouracil to colon cancers. The demonstration that the compounds possess significant potencies and preferential cytotoxicity for certain tumor cells coupled with excellent tolerance to mice vide infra suggests that 1-(4-arylamino-4-oxo-2-butenoyl)-3,5-bis(arylidene)-4-piperidones are candidate anticancer agents which may display selective toxicity for various malignancies compared to the corresponding normal tissues.

Efforts were made with a view to discern the mode(s) of action of the compounds prepared in this study. First, the possibility of the 4-piperidones **4** and **5** exerting their activity on hNMT was considered for the following reasons. The mercapto group of the 192-cysteine portion of hNMT is believed to be part of the active site of the enzyme[19] and hence the possibility exists of it reacting with thiol-alkylators. Furthermore certain Mannich bases of conjugated enones inhibited hNMT when cAMP-dependent protein kinase derived peptide was used as the substrate.[20] The activity of hNMT was reduced by approximately 50% by **4c** and **5c** (100 μM) and **4d** and **5d** (50 μM). However, inhibition of this enzyme is unlikely to contribute to the cytotoxicity displayed since the IC_{50} values of **4c**,**d**, and **5c**,**d** presented in Table 1 are in the 0.35–7.5 μM range indicating that other molecular targets contributed to the overall cytotoxicity of the compounds in series **4** and **5**.

Second, the COMPARE program is a computerized algorithm which aims at identifying the similarity or dissimilarity between the mean graphs of candidate anticancer agents and cytotoxins whose mode(s) of action are known.[21] It uses the data generated by the panel of human tumor cell lines vide supra. The correlation coefficients for **4b**, **c**, and **f** using the GI_{50} data were highest toward various tyrosine kinases (*r* values of 0.41–0.56). Thus an additional method whereby the compounds described in this study exert their bioactivity is likely by inhibiting various tyrosine kinases.

Finally two investigations were launched with a view to determining whether evidence could be provided that the compounds in series **4** and **5** displayed selective toxicity for malignant cells. Compounds **4a**–**i** and **5a**–**i** were examined for both in vitro antifungal properties as well as short-term survival and neurotoxicity in mice. None of the compounds exerted significant antifungal properties toward five strains of *A. fumigatus* since the MIC values were in excess of 50 mg/L which is more than 200 times the MIC figure of an established antifungal drug voriconazole. Second, 4 h after administering doses up to and including 300 mg/kg of **4a**–**i** and **5a**–**i** to mice, there were no fatalities, while, in general, only minimal neurotoxicity was caused by approximately half of the compounds. These in vitro and in vivo experiments support the contention that, despite marked potencies of **4a**–**i** and **5a**–**i** to neoplastic cells, they are not general biocidal agents and may have a greater tendency for harming tumorous cells than normal tissues.

4. Conclusion

In conclusion, this study has revealed the pronounced cytostatic properties of various *E*,*E*,*E*-1-(4-arylamino-4-oxo-2-butenoyl)-4-piperidones toward a number of different malignant cell lines. The location of the terminal arylaminocarbonyl portion of the *N*-acyl group has

Table 4. Evaluation of **4b**,**c**,**f** and reference compounds against a panel of human tumor cell lines

Compound	All cell lines		Leukemic cells		Colon cancer cells	
	GI_{50}[a] (μM)	SI[b]	GI_{50}[a] (μM)	SI[b]	GI_{50}[a] (μM)	SI[b]
4b	3.72	>525	0.589	6.32	1.15	3.24
4c	1.23	195	0.447	2.75	0.646	1.90
4f	2.04	1950	0.105	19.4	0.759	2.69
Melphalan	24.5	178	3.98	6.16	50.1	0.49
5-Fluorouracil	29.5	>4365	52.5	0.56	5.75	5.13

[a] GI_{50} refers to the compound concentrations required to inhibit the growth of the cells by 50%.

[b] SI refers to the selectivity index. The SI figures for all cell lines were obtained by dividing the GI_{50} values of the least and most sensitive cells. The SI data for the leukemic and cancer cell lines were generated by dividing the GI_{50} values for all cell lines by the GI_{50} figures of the leukemic and colon cancer cells, respectively.

a marked influence on the potencies of the compounds. Guidelines for the amplification of this novel area of antineoplastic agents were given. The major mode of action of these compounds is unlikely to be an inhibition of hNMT. However, there may be a likelihood of interactions with tyrosine kinases. A preliminary toxicological study indicated that the compounds do not cause deaths of mice within a short-time frame, which enhances their potential as candidate anticancer agents.

5. Experimental

5.1. Instrumentations and general materials

All melting points are uncorrected. Compound **4b** is reported in the literature and the melting point matches with the one recorded previously.[22] Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of Alberta, Edmonton, AB, Canada. The ^{1}H NMR spectra were recorded on a Bruker AV300 FT NMR spectrometer (300 MHz). TLC was undertaken using precoated silica gel aluminum-backed sheets with fluorescent indicator (SiO_2-60, F-254) and a solvent system of 5% methanol in chloroform. All chemicals and reagents were purchased from Aldrich Chemical Co. and were used without further purification.

5.1.1. General procedure for the synthesis of *E*,*E*,*E*-1-(4-arylamino-4-oxo-2-butenoyl)-3,5-bis(arylidene)-4-piperidones (4a–i) and (5a–i). Literature procedures were used to synthesize 3,5-bis(phenylmethylene)-4-piperidone,[9] 3,5-bis(4-nitrophenylmethylene)-4-piperidone[9] and *N*-aryl-fumaramic acids.[22] For the subsequent amide bond formation step, a slightly modified version of the procedure reported earlier[22] was utilized. The appropriate *N*-aryl-fumaramic acid (3.0 mmol) and triethylamine (3.6 mmol) were dissolved in dry tetrahydrofuran (30 mL) under nitrogen and the mixture was stirred for 10 min. at room temperature. Under anhydrous conditions, methyl chloroformate (3.0 mmol) in dry tetrahydrofuran (20 mL) was added dropwise in 10 min to the ice-cold solution of the above mixture. The resulting solution was stirred for 0.5 h at room temperature. The solution was again cooled to 0 °C and the appropriate 3,5-bis(arylmethylene)-4-piperidone (3.0 mmol, fine powder) was added in one portion. The resulting solution was stirred for 17 h at room temperature. The contents were evaporated under vacuum to dryness. Water was added and the residue was triturated. The solid product was filtered and washed with cold water. The dried solid was digested in methanol (30 mL; for **4a**–**i**) or acetone (30 mL; for **5a**–**i**) at 50 °C to remove side products and unreacted starting materials. It was then filtered, washed with cold methanol (30 mL), and dried to yield pure products.

5.1.1.1. *E*,*E*,*E*-1-(4-phenylamino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4- piperidone (4a). It was obtained as a yellow solid in 78% yield, mp 220–222 °C; IR (KBr): 3269 (NH), 1679 (ketonic CO), 1643 (amidic CO), 1615 (amidic CO), 1551, 1442, 1331, 1286, 1262, 1169, 977, 955, 761, and 693 cm^{-1}; UV (CH_3OH): 324 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.99 (4H, br s, piperidyl H), 6.93 and 7.06 (1H each, d, J = 14.8 Hz, *E*-vinylic H), 7.20–7.80 (15H, m, aryl H), 7.71 and 7.75 (1H each, s, arylidene H) and 10.36 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.34, 47.03, 119.72, 124.22, 129.13, 129.22, 129.60, 130.03, 130.87, 132.51, 134.33, 134.64, 135.69, 136.84, 138.97, 162.02, 164.05 and 186.09; ESI-MS: Calcd for $C_{29}H_{24}N_2O_3$ $[M]^+$ 448.1, observed $[M]^+$ 448.1. Anal. Calcd for $C_{29}H_{24}N_2O_3$: C, 77.66; H, 5.39; N, 6.25. Found: C, 77.39; H, 5.49; N, 6.27.

5.1.1.2. *E*,*E*,*E*-1-(4-chlorophenylamino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4b). It was obtained as a yellow solid in 52% yield, mp 231–234 °C (literature[22] mp 231–235 °C); the spectral analysis was found identical with the reported literature.[22]

5.1.1.3. *E*,*E*,*E*-1-(4-(3,4-dichlorophenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4c). It was obtained as a yellow solid in 44% yield, mp 222–224 °C; IR (KBr): 3255 (NH), 1686 (ketonic CO), 1642 (amidic CO), 1613 (CO), 1529, 1475, 1446, 1388, 1295, 1171, 989, 762 and 692 cm^{-1}; UV ($CHCl_3$): 326 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.96 and 4.99 (4H, 2 br s, piperidyl H), 6.86 and 7.24 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.46–7.76 (13H, m, aryl H), 7.97 and 7.98 (1H each, s, arylidene H) and 10.64 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.87, 47.51, 120.31, 121.45, 126.25, 129.73, 130.55, 131.00, 131.38, 131.61, 131.92, 133.00, 134.84, 135.30, 137.47, 139.52, 162.95, 164.41 and 186.59; ESI-MS: Calcd for $C_{29}H_{23}Cl_2N_2O_3$ $[M+H]^+$ 517.1, observed $[M+H]^+$ 517.1. Anal. Calcd for $C_{29}H_{22}Cl_2N_2O_3$: C, 66.17; H, 4.40; N, 5.32. Found: C, 66.23; H, 4.25; N, 5.14.

5.1.1.4. *E*,*E*,*E*-1-(4-(4-methylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4d). It was obtained as a yellow solid in 81% yield, mp 223–224 °C; IR (KBr): 3270 (NH), 1679 (ketonic CO), 1641 (amidic CO), 1614 (amidic CO), 1542, 1449, 1332, 981, 771 and 691 cm^{-1}; UV ($CHCl_3$): 329 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.24 (3H, s, CH_3), 4.96 and 4.99 (2H each, br s, piperidyl H), 6.92 and 7.14 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.47–7.53 (14H, m, aryl H), 7.58 and 7.60 (2H, s, arylidene H) and 10.30 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 21.34, 43.83, 47.58, 120.18, 128.80, 129.77, 130.06, 130.10, 130.58, 130.71, 131.37, 131.44, 132.58, 133.03, 133.79, 134.25, 134.83, 135.15, 135.64, 136.37, 137.00, 137.31, 137.47, 139.40, 162.65, 164.54 and 186.61; ESI-MS: Calcd for $C_{30}H_{26}N_2O_3$ $[M]^+$ 462.2, observed $[M]^+$ 462.2. Anal. Calcd for $C_{30}H_{26}N_2O_3$: C, 77.90; H, 5.67; N, 6.06. Found: C, 77.47; H, 5.61; N, 5.80.

5.1.1.5. *E*,*E*,*E*-1-(4-(3,4-dimethylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4e). It was obtained as a yellow solid in 82% yield, mp 217–218 °C; IR (KBr): 3264 (NH), 1678 (ketonic CO), 1639 (amidic CO), 1617 (amidic CO), 1542, 1445, 1329, 1295, 1195, 990, 763 and 691 cm^{-1}; UV ($CHCl_3$): 331 nm; ^{1}H

NMR (300 MHz, DMSO-d_6): δ 2.15 and 2.16 (6H, 2s, 3H each, C-3′CH_3 and C-4′CH_3), 4.96 and 4.99 (2H each, br s, piperidyl H), 6.90 and 7.05 (1H, d, J = 15.0 Hz, *E*-vinylic H), 7.12–7.65 (13H, m, aryl H), 7.71 and 7.79 (2H, s, arylidene H) and 10.25 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 19.68, 20.46, 43.82, 47.59, 117.74, 120.18, 128.80, 129.19, 129.69, 129.78, 130.50, 131.37, 131.44, 132.63, 133.04, 134.25, 134.83, 135.15, 136.47, 137.23, 137.46, 162.24, 164.53 and 186.59; ESI-MS: Calcd for $C_{31}H_{28}N_2O_3$ $[M]^+$ 476.2, observed $[M]^+$ 476.2. Anal. Calcd for $C_{31}H_{28}N_2O_3$: C, 78.13; H, 5.92; N, 5.88. Found: C, 77.67; H, 5.73; N, 5.73.

5.1.1.6. *E*,*E*,*E*-1-(4-(4-methoxyphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4f). It was obtained as a yellow solid in 81% yield, mp 180–182 °C; IR (KBr): 3333 (NH), 1677 (ketonic CO), 1646 (amidic CO), 1609 (amidic CO), 1540, 1511, 1445, 1288, 1241, 1171, 1033, 832, 765 and 694 cm^{-1}; UV ($CHCl_3$): 326 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.69 (3H, s, OCH_3), 4.96 (4H, br s, piperidyl H), 6.85 and 6.89 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.17–7.55 (14 H, m, aryl H), 7.72 (2H, s, arylidene H) and 10.20 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.83, 47.56, 56.03, 114.81, 121.68, 129.56, 129.74, 130.54, 131.40, 132.67, 133.02, 134.81, 135.13, 136.43, 137.35, 162.05, 164.58, and 186.59; ESI-MS: Calcd for $C_{30}H_{26}N_2O_4$ $[M]^+$ 478.2, observed $[M]^+$ 478.2. Anal. Calcd for $C_{30}H_{26}N_2O_4$: C, 75.30; H, 5.48; N, 5.85. Found: C, 74.86; H, 5.52; N, 6.16.

5.1.1.7. *E*,*E*,*E*-1-(4-(4-nitrophenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4g). It was obtained as a light yellow solid in 67% yield, mp 287–290 °C; IR (KBr): 3302 (NH), 1697 (ketonic CO), 1651 (amidic CO), 1603 (amidic CO), 1552, 1505, 1446, 1333, 1288, 1167, 966, 753 and 692 cm^{-1}; UV ($CHCl_3$): 330 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.97 and 4.99 (2H each, br s, piperidyl H), 6.90 and 7.28 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.36–7.84 (14H, m, aryl H), 8.21 and 8.24 (1H each, s, arylidene H) and 10.91 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.82, 47.58, 120.08, 125.87, 129.18, 129.76, 130.60, 131.30, 131.42, 131.63, 133.01, 134.61, 135.10, 136.42, 137.53, 143.50, 145.55, 163.35, 164.39 and 186.63; ESI-MS: Calcd for $C_{29}H_{24}N_3O_5$ $[M+H]^+$ 494.2, observed $[M+H]^+$ 494.2. Anal. Calcd for $C_{29}H_{23}N_3O_5$: C, 70.58; H, 4.70; N, 8.51. Found: C, 70.31; H, 4.91; N, 8.13.

5.1.1.8. *E*,*E*,*E*-1-(4-(4-acetylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4h). It was obtained as a yellow solid in 84% yield, mp 242–244 °C, IR (KBr): 3323 (NH), 1673 (ketonic CO), 1651 (ketonic CO), 1630 (amidic CO), 1602 (amidic CO), 1533, 1444, 1350, 1269, 1174, 961 and 690 cm^{-1}; UV ($CHCl_3$): 321 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.50 (3H, s, $COCH_3$), 4.97 and 4.99 (2H each, br s, piperidyl H), 6.93 and 7.24 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.50–7.74 (14H, m, aryl H), 7.91 and 7.94 (1H each, s, arylidene H) and 10.68 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 27.27, 43.87, 47.56, 119.60, 129.74, 130.31, 130.54, 130.97, 131.38, 133.02, 133.10, 135.58, 137.44, 143.72, 163.03, 164.48, 186.61 and 197.34; ESI-MS: Calcd for $C_{31}H_{26}N_2O_4$ $[M]^+$ 490.2, observed $[M]^+$ 490.2. Anal. Calcd for $C_{31}H_{26}N_2O_4$: C, 75.90; H, 5.34; N, 5.71. Found: C, 75.62; H, 5.34; N, 5.61.

5.1.1.9. *E*,*E*,*E*-1-(4-(2,6-dimethylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(phenylidene)-4-piperidone (4i). It was obtained as a yellow solid in 66% yield, mp 237–239 °C; IR (KBr): 3279 (NH), 1672 (ketonic CO), 1634 (amidic CO), 1609 (amidic CO), 1521, 1443, 1287, 1174, 983, 765 and 695 cm^{-1}; UV ($CHCl_3$): 332 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.13 (6H, s, C-2′CH_3 and C-6′CH_3), 4.97 and 5.00 (2H each, br s, piperidyl H), 6.95 and 7.19 (1H each, d, J = 15.1 Hz, *E*-vinylic H), 7.28–7.54 (13H, m, aryl H), 7.58 and 7.72 (2H, s, arylidene H) and 9.75 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 18.89, 43.84, 47.63, 127.56, 128.55, 129.77, 130.02, 130.61, 131.42, 132.94, 133.03, 134.78, 135.14, 135.24, 135.35, 135.71, 137.33, 137.53, 162.54, 164.75 and 186.57; ESI-MS: Calcd for $C_{31}H_{28}N_2O_3$ $[M]^+$ 476.2, observed $[M]^+$ 476.2. Anal. Calcd for $C_{31}H_{28}N_2O_3$: C, 78.13; H, 5.92; N, 5.88. Found: C, 77.90; H, 6.02; N, 5.87.

5.1.1.10. *E*,*E*,*E*-1-(4-phenylamino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5a). It was obtained as a yellow solid in 81% yield, mp 210–211 °C; IR (KBr): 3308 (NH), 1687 (ketonic CO), 1646 (amidic CO), 1621 (amidic CO), 1518, 1441, 1344, 1261, 1171, 973, 855 and 760 cm^{-1}; UV ($CHCl_3$): 332 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.96 and 5.01 (2H each, br s, piperidyl H), 6.88 and 7.17 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.04–8.36 (15H, m, arylidene H and aryl H) and 10.33 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.84, 47.30, 120.14, 124.62, 129.63, 130.20, 132.36, 135.08, 135.93, 136.15, 139.44, 141.31, 148.28, 162.45, 164.93 and 186.63; ESI-MS: Calcd for $C_{29}H_{22}N_4O_7$ $[M]^+$ 538.1, observed $[M]^+$ 538.1. Anal. Calcd for $C_{29}H_{22}N_4O_7$: C, 64.14; H, 4.17; N, 10.32. Found: C, 63.79; H, 3.87; N, 10.01.

5.1.1.11. *E*,*E*,*E*-1-(4-(4-chlorophenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5b). It was obtained as a yellow solid in 78% yield, mp 225–226 °C; IR (KBr): 3301 (NH), 1686 (ketonic CO), 1647 (amidic CO), 1617 (amidic CO), 1519, 1490, 1343, 1261, 1090, 971, 835 and 714 cm^{-1}; UV ($CHCl_3$): 330 nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.96 and 5.00 (2H each, br s, piperidyl H), 6.84 and 7.18 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.34–8.33 (14H, m, arylidene H and aryl H) and 10.47 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.31, 46.80, 121.15, 124.08, 127.84, 129.03, 130.04, 131.83, 134.53, 134.79, 135.20, 135.39, 137.86, 140.97, 147.75, 162.05, 164.35 and 186.10; ESI-MS: Calcd for $C_{29}H_{22}ClN_4O_7$ $[M+H]^+$ 573.1, observed $[M+H]^+$ 573.1. Anal. Calcd for $C_{29}H_{21}ClN_4O_7$: C, 60.79; H, 3.69; N, 9.78. Found: C, 60.31; H, 3.59; N, 9.41.

5.1.1.12. *E*,*E*,*E*-1-(4-(3,4-dichlorophenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5c). It was obtained as a yellow solid in 76% yield, mp

218–219 °C; IR (KBr): 3300 (NH), 1687 (ketonic CO), 1646 (amidic CO), 1612 (amidic CO), 1520, 1461, 1344, 1262, 1172, 967 and 871 cm^{-1}; UV ($CHCl_3$): 328 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 4.86 and 4.99 (2H each, br s, piperidyl H), 6.79 and 7.18 (1H, d, J = 15.1 Hz, *E*-vinylic H), 7.45–8.33 (13H, m, arylidene H and aryl H) and 10.61 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.30, 47.30, 120.17, 121.30, 124.67, 126.26, 131.26, 131.64, 131.98, 132.40, 135.05, 135.84, 136.00, 139.44, 148.28, 162.84, 164.84 and 186.69; ESI-MS: Calcd for $C_{29}H_{20}Cl_2N_4O_7$ $[M]^+$ 606.1, observed $[M]^+$ 606.1. Anal. Calcd for $C_{29}H_{20}Cl_2N_4O_7$: C, 56.51; H, 3.43; N, 9.08. Found: C, 56.21; H, 3.10; N, 8.62.

5.1.1.13. *E*,*E*,*E*-1-(4-(4-methylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5d). It was obtained as a yellow solid in 80% yield, mp 223–224 °C; IR (KBr): 3305 (NH), 1685 (ketonic CO), 1644 (amidic CO), 1619 (amidic CO), 1516, 1448, 1342, 1262, 1172, 971 and 825 cm^{-1}; UV ($CHCl_3$): 332 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 2.30 (3H, s, CH_3), 4.96 and 5.00 (2H each, br s, piperidyl H), 6.87 and 7.15 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.10–8.33 (14H, m, arylidene H and aryl H) and 10.28 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 21.35, 43.30, 47.30, 120.08, 123.90, 124.66, 129.93, 130.06, 132.42, 133.82, 135.06, 135.35, 135.92, 136.30, 136.94, 141.25, 148.26, 162.21, 164.90 and 186.68; ESI-MS: Calcd for $C_{30}H_{24}N_4O_7$ $[M]^+$ 552.2, observed $[M]^+$ 552.2. Anal. Calcd for $C_{30}H_{24}N_4O_7$: C, 64.68; H, 4.43; N, 10.05. Found: C, 64.66; H, 4.45; N, 9.72.

5.1.1.14. *E*,*E*,*E*-1-(4-(3,4-dimethylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5e). It was obtained as a yellow solid in 81% yield, mp 227–229 °C, IR (KBr): 3305 (NH), 1685 (ketonic CO), 1643 (amidic CO), 1617 (amidic CO), 1517, 1447, 1262, 1343, 1175, 969 and 855 cm^{-1}; UV ($CHCl_3$): 334 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 2.02 and 2.03 (6H, 2s, 3H each, C-3′CH_3 and C-4′CH_3), 4.96 and 5.00 (2H each, br s, piperidyl H), 6.85 and 7.11 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.02–8.59 (13H, m, arylidene H and aryl H) and 10.16 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 19.69, 20.45, 43.81, 47.30, 117.63, 121.26, 124.66, 129.87, 130.49, 132.40, 132.64, 135.06, 135.39, 135.98, 136.29, 137.18, 137.33, 141.27, 141.52, 148.28, 162.12, 164.95 and 186.67; ESI-MS: Calcd for $C_{31}H_{26}N_4O_7$ $[M]^+$ 566.2, observed $[M]^+$ 566.2. Anal. Calcd for $C_{31}H_{26}N_4O_7$: C, 65.72; H, 4.63; N, 9.89. Found: C, 65.34; H, 4.58; N, 9.56.

5.1.1.15. *E*,*E*,*E*-1-(4-(4-methoxyphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenyl-idene)-4-piperidone (5f). It was obtained as a yellow solid in 83% yield, mp 223–225 °C; IR (KBr): 3303 (NH), 1682 (ketonic CO), 1647 (amidic CO), 1621 (amidic CO), 1518, 1344, 1261, 1170 and 834 cm^{-1}; UV ($CHCl_3$): 336 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 3.70 (3H, s, OCH_3), 4.97 (4H, br s, piperidyl H), 6.84 and 7.13 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.47–8.29 (14H, m, arylidene H and aryl H) and 10.18 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.05, 46.80, 55.51, 114.28, 121.05, 124.11, 129.12, 131.85, 134.59, 135.42, 135.86, 140.96, 147.75, 155.99, 161.42, 164.42 and 186.11; ESI-MS: Calcd for $C_{30}H_{24}N_4O_8$ $[M]^+$ 568.2, observed $[M]^+$ 568.2. Anal. Calcd for $C_{30}H_{24}N_4O_8$: C, 63.38; H, 4.25; N, 9.85. Found: C, 62.93; H, 3.93; N, 9.69.

5.1.1.16. *E*,*E*,*E*-1-(4-(4-nitrophenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5g). It was obtained as a yellow solid in 60% yield, mp 253–254 °C, IR (KBr): 3449 (NH), 1687 (ketonic CO), 1653 (amidic CO), 1618 (amidic CO), 1515, 1344, 1257, 1168 and 853 cm^{-1}; UV ($CHCl_3$): 329 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 4.96 (4H, br s, piperidyl H), 6.84 and 7.22 (1H, d, J = 15.0 Hz, *E*-vinylic H), 7.78–8.27 (14H, m, arylidene H and aryl H) and 10.84 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 43.73, 47.17, 119.85, 124.49, 125.68, 131.62, 132.25, 134.86, 135.30, 135.76, 141.37, 143.39, 145.36, 148.15, 163.14, 164.64 and 186.52; ESI-MS: Cald for $C_{29}H_{21}N_5O_9$ $[M]^+$ 583.1, observed $[M]^+$ 583.1. Anal. Calcd for $C_{29}H_{21}N_5O_9$: C, 59.69; H, 3.63; N, 12.00. Found: C, 59.47; H, 3.56; N, 11.56.

5.1.1.17. *E*,*E*,*E*-1-(4-(4-acetylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenylidene)-4-piperidone (5h). It was obtained as a yellow solid in 72% yield, mp 216–218 °C, IR (KBr): 3279 (NH), 1698 (ketonic CO), 1674 (ketonic CO), 1647 (amidic CO), 1615 (amidic CO), 1595, 1518, 1344, 1262, 1172, 964 and 854 cm^{-1}; UV ($CHCl_3$): 329 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 2.51 (3H, s, $COCH_3$), 4.96 (4H, br s, piperidyl H), 6.87 and 7.19 (1H each, d, J = 15.0 Hz, *E*-vinylic H), 7.75–8.27 (14H, m, arylidene H and aryl H) and 10.60 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 27.14, 43.65, 47.18, 119.39, 124.33, 124.49, 130.17, 130.93, 132.24, 132.99, 134.95, 135.30, 135.42, 135.78, 141.19, 143.54, 148.15, 162.82, 164.70, 186.51 and 197.22; ESI-MS: Calcd for $C_{31}H_{24}N_4O_8$ $[M]^+$ 580.2, observed $[M]^+$ 580.2. Anal. Calcd for $C_{31}H_{24}N_4O_8$: C, 64.13; H, 4.17; N, 9.65. Found: C, 63.75; H, 4.19; N, 9.67.

5.1.1.18. *E*,*E*,*E*-1-(4-(2,6-dimethylphenyl)amino-4-oxo-2-butenoyl)-3,5-bis(4-nitrophenyl-idene)-4-piperidone (5i). It was obtained as a yellow solid in 81% yield, mp 239–242 °C; IR (KBr): 3309 (NH), 1678 (ketonic CO), 1643 (amidic CO), 1616 (amidic CO), 1594, 1519, 1441, 1345, 1295, 1264, 1179, 986 and 853 cm^{-1}; UV ($CHCl_3$): 335 nm; ^1H NMR (300 MHz, DMSO-d_6): δ 2.00 (6H, s, C-2′CH_3 and C-6′CH_3), 4.97 and 5.01 (2H each, br s, piperidyl H), 6.94 and 7.17 (1H, d, J = 15.1 Hz, *E*-vinylic H), 7.04–8.29 (13H, m, arylidene H and aryl H) and 9.67 (1H, br s, CONH); ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 18.67, 43.68, 47.34, 124.35, 124.51, 127.43, 128.43, 129.82, 132.29, 135.09, 135.40, 135.51, 135.76, 141.21, 148.13, 162.34, 164.87 and 186.39; ESI-MS: Calcd for $C_{31}H_{26}N_4O_7$ $[M]^+$ 566.2, observed $[M]^+$ 566.2. Anal. Calcd for $C_{31}H_{26}N_4O_7$: C, 65.72; H, 4.63; N, 9.89. Found: C, 65.93; H, 4.57; N, 9.97.

5.2. Molecular modeling

The models of **3d**,**e**,**i** and **4d**,**e**,**i** were built using MacroModel 8.0.[23] The conformations of the molecules were minimized following a Monte Carlo search of 1000 iterations. The detection of non-bonded interactions between the hydrogen atoms of the aryl rings A and B and the substituents of the distal ring of **3d**,**e**,**i** and **4d**,**e**,**i** was undertaken. Distances between atoms of less than 3 Å were considered to reflect such interactions and were noted in the following cases. The distance between one of the meta hydrogen atoms in ring A of **3e** and two of the protons of the 3-methyl group in the distal aryl ring were 2.686 and 2.839 Å. The distances between one of the meta hydrogen atoms in ring B of **4i** and one of the protons of the 2-methyl group in the distal aryl ring was 2.689 Å.

5.3. Statistical analyses using σ, σ^*, π, and MR values

The σ, π, and MR values were taken from the literature,[24] while the σ^* figure was obtained from published data.[25] The MR value of the hydrogen atom is 1.03 and not 0.00. Hence for the unsubstituted compounds **4a** and **5a**, the MR figure is 2.06 while 1.03 was added to the MR values of monosubstituted compounds. The trends toward significance were found between the sigma values in the CEM screen (linear plot, $p = 0.146$, $r = 0.526$) in series **4** and in the L1210 assay (linear plot, $p = 0.075$, $r = -0.621$ and semilogarithmic plot, $p = 0.119$, $r = -0.557$) in series **5**. The plots were generated using a commercial software package.[26]

5.4. Biological evaluations

5.4.1. Cytotoxicity evaluations. The Molt 4/C8, CEM, and L1210 assays were conducted using a literature methodology.[27] The human tumor cell line screen was undertaken by a reported procedure[16] using 55 (**4b**, **f**, melphalan) or 57 (**4c**, 5-fluorouracil) cell lines. The concentrations of compounds used were 10^{-4}–10^{-8} M (**4b**,**c**,**f**), $10^{-3.6}$–$10^{-7.6}$ M (melphalan) or $10^{-2.6}$–$10^{-6.6}$ M (5-fluorouracil). IC_{50} figures were generated in all cases except for one non-small cell lung, one central nervous system, and one breast cancer in the assay of **4b** and also one non-small cell lung, one renal, and one breast tumor in the case of 5-fluorouracil.

5.4.2. Human *N*-myristoyltransferase assay. The evaluation of **4c**,**d** and **5c**,**d** against hNMT was performed by a literature procedure.[20] Briefly, *Escherichia coli* DH5α with recombinant pT-7.hNMT was grown to stationary phase in LB medium at 37 °C to produce hNMT which was purified by a reported method. The determination was undertaken using cAMP-dependent protein kinase derived peptide, which was obtained from Research Genetics, Huntsville, AL, USA. Evaluations were determined in duplicate using concentrations which did not lead to precipitation, namely 50 μM (**4d** and **5d**) and 100 μM (**4c** and **5c**). The percentage activities for **4c**,**d**, and **5c**,**d** were 52.1 ± 5.8, 52.4 ± 1.3, 48.9 ± 4.9, and 44.2 ± 5.0, respectively.

5.4.3. Antifungal evaluation. Compounds **4a**–**i**, **5a**–**i** and voriconazole were evaluated against five strains of *A. fumigatus* (F69827, M44251, ATCC 280995, ATCC 280996, and ATCC 280997) using a reported methodology.[28] The MIC values of all of the 4-piperidones were in excess of 50 mg/L, while the MIC figure of voriconazole was equal to or less than 0.25 mg/L.

5.4.4. Toxicity evaluations. Compounds **4a**–**i** and **5a**–**i** were examined for short-term survival and neurotoxicity by the National Institute of Neurological Disorders and Stroke according to their protocols.[29] In brief, mice were injected intraperitoneally with doses of 30, 100, and 300 mg/kg of each compound and the animals were observed at the end of 0.5 and 4 h. No mortalities were noted. The following compounds demonstrated neurotoxicity in the rotorod method[30] (number of animals demonstrating neurotoxicity, time of test in h, dose of compound in mg/kg), namely: **4b** (1/4, 4, 100; 1/2, 4, 300), **4c** (1/8, 0.5, 100), **4d** (1/8, 0.5, 100; 2/4, 0.5, 300; 2/2, 4, 300), **4e** (1/8, 0.5, 100), **4h** (1/4, 0.5, 30; 2/8, 0.5, 100); **4i** (1/8, 0.5, 100), **5e** (1/4, 0.5, 30; 1/4, 0.5, 300; 1/4, 4, 100), **5f** (2/4, 0.5, 300;1/4, 4, 100; 1/2, 4, 300), **5g** (1/8, 0.5, 100; 2/4, 4, 100), and **5h** (1/8, 0.5, 100). Mice were fed, handled, and housed in accordance with the procedures outlined in the National Research Council publication 'Guide for the Care and Use of Laboratory Animals'. The animals were euthanized using the guidelines of the Institute of Laboratory Resources.

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References and notes

1. Dimmock, J. R.; Kumar, P. *Curr. Med. Chem.* **1997**, *4*, 1.
2. Dimmock, J. R.; Raghavan, S. K.; Logan, B. M.; Bigham, G. E. *Eur. J. Med. Chem.* **1983**, *18*, 248.
3. Mutus, B.; Wagner, J. D.; Talpas, C. J.; Dimmock, J. R.; Phillips, O. A.; Reid, R. S. *Anal. Biochem.* **1989**, *177*, 237.
4. Benvenuto, J. A.; Connor, T. H.; Monteith, D. K.; Laidlaw, J. A.; Adams, S. C.; Matney, T. S.; Theiss, J. C. *J. Pharm. Sci.* **1993**, *82*, 988.
5. Dimmock, J. R.; Kumar, P.; Quail, J. W.; Pugazhenthi, U.; Yang, J.; Chen, M.; Reid, R. S.; Allen, T. M.; Kao, G. Y.; Cole, S. P. C.; Batist, G.; Balzarini, J.; De Clercq, E. *Eur. J. Med. Chem.* **1995**, *30*, 209.
6. Rousseaux, C. G.; Townsend, H. G.; Phillips, O. A.; Dimmock, J. R. *Fund. Appl. Toxicol.* **1990**, *14*, 318.

7. Dimmock, J. R.; Taylor, W. G. *J. Pharm. Sci.* **1975**, *64*, 241.
8. 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. *Drug Des. Deliv.* **1990**, *6*, 183.
9. Dimmock, J. R.; Padmanilayam, M. P.; Puthucode, R. N.; Nazarali, A. J.; Motaganahalli, N. L.; Zello, G. A.; Quail, J. W.; Oloo, E. E.; Kraatz, H.-B.; Prisciak, J. S.; Allen, T. M.; Santos, C. L.; Balzarini, J.; De Clercq, E.; Manavathu, E. K. *J. Med. Chem.* **2001**, *44*, 586.
10. Perrin, D. D.; Dempsey, B.; Serjeant, E. J. In *pKa Prediction of Organic Acids and Bases*; Chapman and Hall: London, 1981; pp 110–123.
11. Dimmock, J. R.; Jha, A.; Zello, G. A.; Sharma, R. K.; Shrivastav, A.; Selvakumar, P.; Allen, T. M.; Santos, C. L.; Balzarini, J.; De Clercq, E.; Manavathu, E. K.; Stables, J. P. *J. Enz. Inhib. Med. Chem.* **2003**, *18*, 325.
12. Selvakumar, P.; Lakshmikuttayamma, A.; Shrivastav, A.; Das, S. B.; Dimmock, J. R.; Sharma, R. K. *Prog. Lipid Res.* **2007**, *46*, 1.
13. Suffness, M.; Douros, T. In *Methods of Cancer Research, Volume XVI, Part A*; De Vita, V. T., Jr., Busch, H., Eds.; Academic Press: New York, 1979; Vol. XVI, p 84.
14. Lloyd, A. W.; Smith, H. J. In *Smith and Williams' Introduction to the Principles of Drug Design and Action*; Smith, H. J., Ed., 3rd ed.; Harwood Academic Publishers: Amsterdam, 1998; p 255.
15. Perrin, D. D.; Dempsey, B.; Serjeant, E. P. In *pKa Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981; p 110.
16. Boyd, M. R.; Paull, K. D. *Drug. Dev. Res.* **1995**, *34*, 91.
17. Althaus, B. In *Textbook of Therapeutics, Drug and Disease Management*; Herfindal, E. T., Gourley, D. R., Eds., 7th ed.; Lippincott Williams and Wilkins: Philadelphia, 2000; p 1724.
18. Remers, W. A. In *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*; Delgado, J. N., Remers, W. A., Eds., 10th ed.; Lippincott-Raven Publishers: Philadelphia, 1998; p 367.
19. Peseckis, S. M.; Resh, M. D. *J. Biol. Chem* **1994**, *269*, 30888–30892.
20. King, M. J.; Sharma, R. K. *Anal. Biochem.* **1991**, *199*, 149–153.
21. Paull, K. P.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. *J. Natl. Cancer Inst.* **1989**, *81*, 1088–1092.
22. Jha, A.; Dimmock, J. R. *Synth. Commun.* **2003**, *33*, 1211–1233.
23. MacroModel, Version 8.0, Department of Chemistry, Columbia University, New York, April 2002.
24. Hansch, C.; Leo, A. J. In *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979; pp 49–50.
25. Perrin, D. D.; Dempsey, B.; Serjeant, E. P. In *pKa Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981; p 111.
26. Statistical Package for the Social Sciences, SPSS 12.0 for Windows, Release 12.0.0, SPSS Inc., Chicago, IL, 2003.
27. Balzarini, J.; De Clercq, E.; Mertes, M. P.; Shugar, D.; Torrence, P. F. *Biochem. Pharmacol.* **1982**, *31*, 3673–3682.
28. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: approved standard M38-A. National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.
29. Stables, J. P.; Kupferberg, H. J. In *Molecular and Cellular Targets for Antiepileptic Drugs*; Avanzini, G., Tanganelli, F., Avoil, M., Eds.; John Libbey and Company Ltd: London, 1997; pp 191–198.
30. Dunham, M. S.; Miya, T. A. *J. Am. Pharm. Ass. Sci. Ed.* **1957**, *46*, 208–209.