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E,*E*-2-Benzylidene-6-(nitrobenzylidene)cyclohexanones: Syntheses, cytotoxicity and an examination of some of their electronic, steric, and hydrophobic properties

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Abstract—Three series of structurally isomeric 2-benzylidene-6-(nitrobenzylidene) cyclohexanones **1**–**3** were prepared and evaluated against human Molt/C8 and CEM T-lymphocytes as well as murine L1210 cells. The IC_{50} values of the majority of compounds are less than 10 μM and in some assays, the figures for **1d** and **1e** are submicromolar. Correlations were discerned between cytotoxic potencies and the atomic charges on one of the olefinic carbon atoms, the torsion angles between an aryl ring, and the adjacent unsaturated group as well as log *P* values. Three representative compounds were examined for their effect on respiration in rat liver mitochondria.

1. Introduction

The principal interest in our laboratory is the syntheses of a variety of conjugated styryl ketones as candidate antineoplastic agents. These compounds are thiol alkylators having little or no capacity to interact with amino or hydroxy groups[1,2]; since these latter groups, but not thiols, are found in nucleic acids, enones may be devoid of the genotoxic problems displayed by a number of anticancer drugs.[3] Recently molecules have been designed to enable successive alkylation of thiols to occur since on occasion sequential reactions with cellular constituents have been claimed to be more detrimental to malignant cells than the corresponding normal tissues.[4] These considerations led to the decision to prepare a number of compounds which contain the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore (ArCH=CR–CO–CR=CHAr)[5,6] thereby allowing stepwise alkylation of cellular thiols. Recently a small number of 2,6-bis(benzylidene) cyclohexanones were prepared in which the substituents in each of the aryl rings differed in their electronic properties.[7,8] In these molecules, the charges on the olefinic carbon atoms are predicted to be divergent thereby enhancing sequential reactions.

The objectives of the present study were twofold. First, an evaluation was planned of the hypotheses that cytotoxic potencies were correlated with both the charge densities and the steric environment of the olefinic carbon atoms. Second, the original series consisted of a small group of compounds which possessed widely differing potencies in the Molt 4/C8, CEM, and L1210 bioassays.[8] Hence expansion of the cluster of compounds was indicated in order to draw meaningful conclusions pertaining to those structural features which contribute to cytotoxicity.

In ring A of series **1**, the strongly electron-attracting 2-nitro group was proposed which should cause the olefinic carbon atoms, designated C^A and C^B as indicated in Figure 1, to be electron deficient thereby enhancing thiol alkylation. Substituents with varying Hammett sigma (σ) values were considered for insertion onto ring B, including the 3,4,5-trimethoxy group due to our recent disclosure of the cytotoxicity of compounds containing the 3-(3,4,5-trimethoxyphenyl)-2-propenoyl substituent.[9] In addition, the rate of electrophilic attack with

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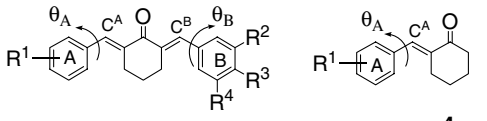


1-3 **4**

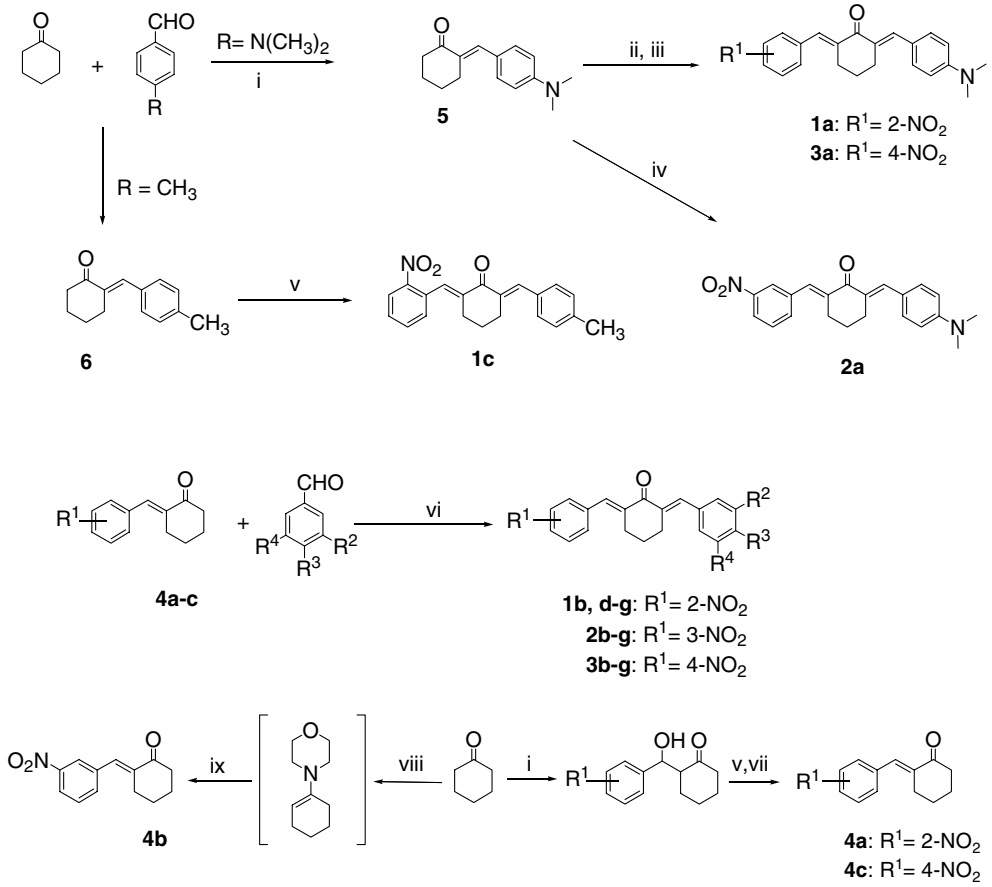
1a-g: R^1= 2-NO_2 **4a**: R^1= 2-NO_2
2a-g: R^1= 3-NO_2 **4b**: R^1= 3-NO_2
3a-g: R^1= 4-NO_2 **4c**: R^1= 4-NO_2

Figure 1. General structures of series **1**–**4**. The R^2, R^3, and R^4 substituents in series **1**–**3** are as follows, namely **a**: $R^2 = R^4$ = H, $R^3 = N(CH_3)_2$; **b**: $R^2 = R^4$ = H, $R^3 = OCH_3$; **c**: $R^2 = R^4$ = H, $R^3 = CH_3$; **d**: $R^2 = R^3 = R^4 = OCH_3$; **e**: $R^2 = R^3 = R^4$ = H; **f**: $R^2 = R^4$ = H, R^3 = F; **g**: $R^2 = R^4$ = H, R^3 = Cl.

thiols will be influenced by the topography of the molecules in the environment of the C^A and C^B atoms. Hence the determination of the torsion angles θ_A and θ_B created between the aryl rings A and B with the adjacent olefinic carbon atoms was suggested. Such considerations led to the decision to prepare series **1**. In order for these hypotheses to be examined further, the placement of the nitro group in other locations of ring A was planned leading to series **2** and **3**. In addition, to assist in the understanding of those structural features in series **1**–**3** which contribute to cytotoxic potencies, the monobenzylidene analogs **4a–c** were also proposed. The general structures of these compounds are presented in Figure 1.

2. Results

The compounds in series **1**–**4** were prepared by the synthetic routes outlined in Scheme 1. The majority of the bis(benzylidene)cyclohexanones were prepared by condensation of **4a**, **4b**, or **4c** with various aryl aldehydes under acidic conditions. However, attempts to use this procedure in the preparation of **1a**, **1c**, **2a**, and **3a** led to the formation of dark polymeric material from which no products were obtained. Under basic conditions, 2-(4-dimethylaminobenzylidene) cyclohexanone **5** and the related 4-methyl analog **6** reacted with the appropriate nitrobenzaldehyde to afford **1c** and **2a**. However under these conditions, reaction of **5** with both 2-nitrobenzaldehyde and 4-nitroarylaldehydes led to the formation of multiple products but under acidic conditions, both **1a** and **3a** were formed. Initial attempts to prepare **4a–c** from cyclohexanone and the relevant nitrobenzaldehyde under acidic conditions led to isolation of the corresponding 2,6-bis(nitrobenzylidene)cyclohexanones. In the presence of sodium hydroxide solution, the 2-nitro and



Scheme 1. Synthetic chemical routes in the preparation of the compounds in series **1**–**4**. Reagents: (i) NaOH; (ii) HCl/2-$NO_2C_6H_4CHO$; (iii) HCl/4-$NO_2C_6H_4CHO$; (iv) NaOH/3-$NO_2C_6H_4CHO$; (v) NaOH/2–$NO_2C_6H_4CHO$; (vi) HCl; (vii) NaOH/4-$NO_2C_6H_4CHO$; (viii) morpholine/4-$CH_3C_6H_4SO_2OH$; (ix) 3-$NO_2C_6H_4CHO$.

4-nitro benzaldehydes condensed with cyclohexanone to produce the intermediate aldols which were dehydrated by acid to give **4a** and **4c**, respectively. Under basic conditions, the 3-nitroaldehyde gave only 2,6-bis(3-nitrobenzylidene)cyclohexanone. Hence **4b** was prepared via the enamine route as illustrated in Scheme 1. ^{1}H NMR spectroscopy revealed that each of the products in series **1**–**4** was isomerically pure. The absorptions of the olefinic protons were in the region of 7.47–7.97 ppm which is characteristic of *E* isomers, since compounds possessing the *Z* configuration absorb at higher fields.[10] Furthermore, X-ray crystallography revealed that the olefinic double bonds adopted the *E* configuration in **3c**[11] and **3f**[12] as well as a related 2,6-bis(benzylidene)cyclohexanone.[13] The assumption was made therefore that the olefinic bonds in series **1**–**4** adopted the *E* configuration. Models of the compounds in series **1**–**4** were built and the charge densities of the C^A and C^B atoms as well as the torsion angles θ_A and θ_B were determined and are presented in Table 2. In addition, the log *P* values of all of the compounds were obtained and are portrayed in Table 2.

All the compounds in series **1**–**4** were evaluated against human Molt 4/C8 and CEM T-lymphocytes and murine L1210 lymphoid leukemia cells. These data are summarized in Table 1. The effect of representative compounds on respiration in mitochondria isolated from rat liver cells is presented in Figure 2.

Table 1. Cytotoxic properties of compounds **1–4**

Compound	IC_{50}[a] (μM)		
	Molt 4/C8	CEM	L1210
1a	122 ± 6	168 ± 36	164± 27
1b	8.90±0.20	7.45±0.08	42.4±1.3
1c	7.52±0.45	6.09±2.12	7.77±0.45
1d	0.702±0.22	0.402±0.033	1.52±0.29
1e	1.48±0.11	0.925±0.056	4.84±0.40
1f	1.87±0.06	1.51±0.04	8.40±0.13
1g	3.86±1.00	1.75±0.14	9.38±0.47
2a	10.9±0.8	11.7±0.8	156±134
2b	7.98±0.54	8.22±0.12	29.5±9.8
2c	9.53±1.23	10.1±0.6	41.8±3.7
2d	44.0± 2.7	45.2±7.5	42.2±3.3
2e	1.70±0.42	2.29±0.75	9.44±1.07
2f	5.12±2.31	5.05±3.02	16.3±0.3
2g	2.02±0.28	1.75±0.00	9.16±0.87
3a	>500	>500	>500
3b[b]	300±54	250±6	240±8
3c	8.44±0.49	8.53±0.31	8.16±0.35
3d[b]	6.42±1.07	4.61±3.89	6.97±1.80
3e	8.35±0.95	9.32±0.20	9.80±0.18
3f	17.1±4.6	18.6±6.9	26.8±2.8
3g[b]	9.12±0.28	8.18±0.20	9.41±0.97
4a	33.3±3.1	36.4±1.3	23.8±12.4
4b	8.28±0.69	8.12±0.92	50.1±10.4
4c	13.9±1.0	19.3±1.5	46.5±9.1
Melphalan[b]	3.24 ±0.79	2.47 ± 0.30	2.13±0.03

[a] The IC_{50} values indicate the concentrations of compounds required to inhibit the growth of the cells by 50%.

[b] The data were previously reported in Ref. 8 [copyright (2006) by Elsevier].

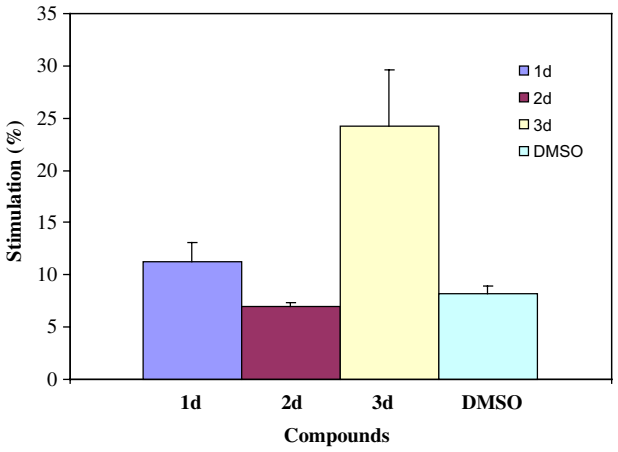


Figure 2. The effect of **1d**, **2d**, **3d**, and solvent control (dimethylsulfoxide 4 μL) on respiration in rat liver mitochondria. The figures for **1d**, **2d**, and **3d** are different from each other and the solvent control taking standard deviations into account.

3. Discussion

The bioevaluations of **1a–g**, **2a–g**, **3a–g**, and **4a–c** toward three cell lines are presented in Table 1. The IC_{50} values of **1d** and **1e** are submicromolar in some of the bioassays and 58% of the IC_{50} values were less than 10 μM. In view of these promising results, various studies were initiated to seek correlations between cytotoxic potencies and different physicochemical and biochemical parameters of these molecules with the aim of obtaining guidelines for expansion of this project.

Table 2. Some physicochemical properties of compounds **1–4**

Compound	Atomic charges[a]		Torsion angles[b]		log *P*
	q_A	q_B	θ_A	θ_B	
1a	−0.086	−0.032	76.79	−51.12	4.80
1b	−0.081	−0.041	76.76	−51.27	4.76
1c	−0.082	−0.047	−76.83	51.58	5.15
1d	−0.092	−0.053	76.90	−51.53	4.33
1e	−0.133	−0.055	76.59	−51.86	4.70
1f	−0.130	−0.058	−76.79	51.55	4.86
1g	−0.130	−0.061	−76.84	51.72	5.38
2a	−0.093	−0.028	−51.30	50.72	5.01
2b	−0.089	−0.042	−51.28	51.12	4.96
2c	−0.083	−0.045	−51.27	51.42	5.35
2d	−0.079	−0.053	−51.32	51.83	4.53
2e	−0.087	−0.053	−51.27	51.56	4.90
2f	−0.085	−0.056	−51.25	51.36	5.07
2g	−0.084	−0.059	−51.24	51.58	5.58
3a	−0.092	−0.022	−51.14	50.98	5.03
3b	−0.096	−0.039	−51.11	51.09	4.98
3c	−0.089	−0.043	−51.14	51.41	5.38
3d	−0.086	−0.053	51.17	−51.86	4.56
3e	−0.095	−0.050	−51.14	51.56	4.93
3f	−0.092	−0.053	−51.14	51.37	5.09
3g	−0.092	−0.056	−51.16	51.57	5.61
4a	−0.092	—	−69.54	—	2.92
4b	−0.083	—	−51.50	—	3.12
4c	−0.090	—	−50.68		3.14

[a] The atomic charges in esu are the electron densities on the carbon atoms designated C^A and C^B in Figure 1.

[b] θ_A and θ_B refer to the torsion angles which are illustrated in Figure 1.

Interactions with cellular thiols are believed to occur at the olefinic carbon atoms designated C^A and C^B. The atomic charges on these atoms in the compounds **1–4** are presented in Table 2. The nitro group in ring A is the most electron-attracting substituent having a Taft σ^* value of 0.97[14] (series **1** and **4a**) and Hammett σ values of 0.71 (series **2** and **4b**) and 0.78 (series **3** and **4c**).[15] The σ constants for the ring B substituents in **a–g** are −0.83, −0.27, −0.17, −0.03, 0.00, 0.06, and 0.23, respectively, [16] and are arranged in sequence with **a** bearing the most electron-repelling group and **g** the most electron-attracting substituent. For each compound in series **1–3** , the electron densities are lower on the C^B rather than the C^A atoms. Thus the polarization of the π electrons in the conjugated 1,5-diaryl-3-oxo-1,4-pentadienyl group is toward the nitro substituents, causing the C^B atom to have lower electron densities than C^A. Hence thiol alkylation is predicted to take place initially at C^B and subsequently at C^A. In order to examine whether cytotoxic potencies are correlated with the electron densities on the C^A and C^B atoms, linear plots were made between these values and the IC_{50} figures of **1a–g** in each of the three bioassays.This experiment was repeated with **2a–g** and finally with **3a–g**. Positive correlations ($p < 0.05$) were noted when considering the atomic charges on the C_B atoms except for the Molt 4/C8 and CEM biodata for series **2**. No correlations were noted between the IC_{50} figures and the charges on the C_A atom ($p > 0.05$). This evaluation was repeated except that the IC_{50} values were plotted against the σ constants in ring B. Negative correlations ($p < 0.01$) were obtained in all cases except for **2a–g** in the Molt 4/C8 and CEM tests ($p > 0.05$). Thus potency increases (IC_{50} values diminish) as the electron densities on the C^B atom are decreased (positive correlation) and the σ constants are elevated (negative correlation). This observation may be rationalized by considering that attack of cellular thiols at C^B will be enhanced by a reduction in the atomic charges on the C^B atoms. Thus in the future, compounds may be designed having substituents in ring B which have large positive sigma values.

Consideration was given to the possibility that the steric environment at the olefinic carbon atoms influences the extent of thiol alkylation and hence cytotoxic potencies. Thiolation is believed to occur initially at C^B and the θ_B values recorded in Table 2 reveal that they are virtually constant. Thus the average θ_B values for series **1**, **2**, and **3** are 51.5°, 51.4°, and 51.4°, respectively, and there are very small variations in these torsion angles within each series. Hence the differences in cytotoxic potencies are unlikely to be influenced by the torsion angles θ_B. The average θ_A angles in series **1**, **2**, and **3** are 76.8°, 51.3°, and 51.1°, respectively, and minimal variation of these torsion angles was noted within each series. Since the cytotoxic potencies of the compounds in series **1** are greater than the analogs in series **2** and **3** vide infra, these torsion angles may influence the magnitude of the cytostatic effect. Hence in the future, groups with larger molecular refractivity values than nitro group should be placed in the 2 position of ring A or two ortho substituents should be employed which may lead to more potent analogs. The insertion of a second arylidene ring onto **4a–c** leading to series **1–3**, respectively, caused only minimal changes in the C^A and θ_A values as the data in Table 2 indicate.

The biodata in Table 1 were examined further with a view to discerning those structural features which influence cytotoxic potencies. First, the optimal position of the nitro group in ring A was considered. A point system of 3 (highest potency), 2, and 1 (lowest potency) was used in comparing the IC_{50} values of compounds having the same substituents in ring B. Thus in the Molt 4/C8 assay, the IC_{50} figures of **1a**, **2a**, and **3a** were compared, then **1b**, **2b**, and **3b** and so forth. Standard deviations were taken into account and when the IC_{50} values were statistically indistinguishable, equal points were allocated bearing in mind that for each comparison of three compounds, a total of six points were invariably awarded. Use of this methodology indicated that the figures for series **1**, **2**, and **3** are 16.5, 16.5, and 9, respectively (Molt 4/C8 assay), 19.5, 13.5, and 9, respectively (CEM test) and 18, 13, and 11, respectively (L1210 screen). Hence the optimal position of the nitro group in ring A in terms of potency is the 2 position.

In order to assess whether the compounds in series **1**–**3**, which permit sequential alkylation to occur, have increased cytotoxic potencies vis-à-vis the analogs in which this process will not occur (series **4**), the IC_{50} values of **1a–g**, **2a–g**, and **3a–g** were compared with those generated for **4a**, **4b**, and **4c**, respectively. The results are summarized in Table 3. In general, structural modification of **4a**, **4b**, and **4c** into series **1**, **2**, and **3**, respectively, was accompanied by increases in potencies in all three bioassays except conversion of **4b** into **2a–g** did not lead to compounds with lower IC_{50} values toward CEM cells.

The rate and extent of the ability of compounds to penetrate the cell membranes of neoplastic and transformed cells is dependent on a number of structural features including the lipophilicity of the molecules. The log *P* values of the compounds were calculated and are presented in Table 1. The average log *P* values for **1a–g**, **2a–g**, and **3a–g** are 4.86, 5.06, and 5.08, respectively, and hence the lower lipophilicity of the compounds in series **1** may have contributed to the greater potencies than the analogs in series **2** and **3**. The generally lower IC_{50} potencies displayed by the compounds in series **1–3** than **4a–c** also reflect a negative correlation with the log *P* values.

Table 3. Comparison between the potencies of the bisalkylators **1a–g**, **2a–g**, and **3a–g** with the monobenzylidene analogs **4a**, **4b**, and **4c**, respectively

Bioassay	Comparison of potencies[a]								
	1a–g	**4a**	Equal	**2a–g**	**4b**	Equal	**3a–g**	**4c**	Equal
Molt 4/C8	86	14	0	43	29	29	57	29	14
CEM	86	14	0	29	43	29	57	29	14
L1210	71	29	0	57	0	43	71	29	0
Total	81	19	0	43	24	33	62	29	9

[a] The figures represent the percentage of compounds displaying greater potency or were equipotent. The standard deviations of the IC_{50} figures were taken into account when making these comparisons.

Various compounds which are thiol reagents such as *N*-ethylmaleimide and mersalyl interact with different mercapto groups in mitochondria.[17] Furthermore, a Mannich base of a conjugated styryl ketone inhibited respiration in rat liver mitochondria and the mode of action, at least in part, was based on competition with the conjugated unsaturated ketone coenzyme Q_{10}.[18] Thus the decision was made to determine whether representative compounds interfered with respiration in mitochondria isolated from rat liver cells, and if so whether the magnitude of this effect correlated with cytotoxic potencies. Three related compounds, namely, **1d**, **2d**, and **3d**, were chosen since they possessed markedly different potencies having average IC_{50} figures of 0.88, 43.8, and 6.00 μM, respectively, in the three cytotoxicity screens. A concentration of 10 μM of each compound was chosen which is in excess of the IC_{50} values of **1d** and **3d** and substantially below that of **2d**. The data in Figure 2 reveal that **1d** and **3d** stimulated respiration. However, the magnitude of the stimulatory effect was negatively correlated with cytotoxic potencies. The least potent of these three compounds, namely **2d**, had virtually no effect on respiration. Increasing the concentration of **2d** to 100 μM revealed no statistically significant difference in stimulation of respiration from the solvent control (data not shown). Nevertheless if the causes for the relative cytotoxic potencies observed in this study are multifactorial, the differences in the effects on mitochondrial function may have exerted some contributions to the disparity of IC_{50} values.

4. Conclusions

A number of novel cytotoxic agents have been prepared, many of which display good potencies toward Molt 4/C8, CEM, and L1210 cells. The highest potencies were displayed by the compounds in series **1** and in particular **1d** and **1e** are lead molecules having submicromolar IC_{50} values in some of the assays. Factors which influence cytotoxic potencies in series **1**–**3** include the atomic charges on the C^B atoms, the torsion angle θ_A, and log *P* values. Another factor which may have contributed to the variation in IC_{50} values is the differences in the effects on respiration in rat liver mitochondria. A number of guidelines for amplifying this project have been proposed.

5. Experimental

5.1. Synthesis of compounds

Melting points in Celsius degrees were determined on a Gallenkamp apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a Bruker AMX 500 FT machine while elemental analyses were obtained using an Elementer analyzer.

5.1.1. Syntheses of 1a, 2a, and 3a. 2-(4-Dimethylaminobenzylidene)cyclohexanone **5** was prepared by a reported procedure[19] and crystallized from ethanol at 5–6 °C to give the desired product in 45% yield, mp 130 °C [lit.[19]] 127–127.5 °C]. ^{1}H NMR ($CDCl_3$): 1.79 (p, 2H), 1.91 (p, 2H), 2.52 (t, 2H), 2.88 (t, 2H), 3.03 (s, 6H, 2× NCH_3), 6.71 (d, 2H, Ar–H, J = 8.85 Hz), 7.41 (d, 2H, J = 8.83 Hz), 7.55 (s, 1H, =CH).

Dry hydrogen chloride was passed into a solution of **5** (0.005 mol) and 2-nitrobenzaldehyde (0.005 mol) in acetic acid (15 mL) and the mixture stirred at room temperature overnight. Acetic acid was removed in vacuo and the residue triturated with potassium carbonate solution (10% w/v, 20 mL) and extracted with chloroform. The organic extract was washed with water and dried. Evaporation of the solvent gave a semisolid which was purified by chromatography using a column of silica gel 60 (70–230 mesh) and an eluting solvent of 10–30% ethyl acetate in hexane to produce **1a**, mp 152 °C in 41% yield. ^{1}H NMR ($CDCl_3$): δ 1.79 (p, 2H), 2.60 (t, 2H), 2.96 (t, 2H), 3.05 (s, 6H, 2× $N(CH_3)_2$), 6.73 (d, 2H, Ar–H, J = 8.80 Hz), 7.38 (d, 1H, Ar–H, J = 7.60 Hz), 7.49 (m, 3H, Ar–H), 7.64 (t, 1H, Ar–H), 7.84 (s, 1H, =CH), 7.95 (s, 1H, =CH), 8.13 (d, 1H, Ar–H, J = 8.20 Hz). Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.62; H, 5.98; N, 7.53%.

Aqueous sodium hydroxide solution (20% w/v, 1 mL) was added to a solution of **5** (0.005 mol) and 3-nitrobenzaldehyde (0.005 mol) in ethanol (15 mL) at 8–10 °C. The solution was stirred at room temperature for 0.5 h. The resultant precipitate was collected, washed with water (3× 15 mL), dried and crystallized from chloroform/ethanol (1:9) to give **2a**, mp 169 °C in 68% yield. ^{1}H NMR ($CDCl_3$): δ 1.86 (p, 2H), 2.93 (t, 2H), 3.01 (t, 2H), 3.07 (s, 6H, 2× $N(CH_3)_2$), 6.75 (d, 2H, Ar–H, J = 8.84 Hz), 7.50 (d, 2H, Ar–H, J = 8.82Hz), 7.60 (t, 1H, Ar–H), 7.76 (d, 1H, Ar–H, J = 7.7 Hz), 7.79 (s, 1H, =CH), 7.84 (s, 1H, =CH), 8.20 (d, 1H, Ar–H, J = 8.18 Hz), 8.32 (s, 1H, Ar–H). Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.87; H, 6.0; N, 7.46%.

Dry hydrogen chloride was passed into a solution of **5** (0.005 mol) and 4-nitrobenzaldehyde (0.005 mol) in acetic acid (15 mL) and the mixture was stirred overnight at room temperature. The precipitate was collected, washed with diethyl ether (2× 10 mL), and potassium carbonate solution (10% w/v, 30 mL). The solid obtained was washed with water (3× 10 mL), dried and crystallized from chloroform/ethanol (1:9) to give **3a**, mp 91–92 °C in 66% yield. ^{1}H NMR ($CDCl_3$): δ 1.86 (p, 2H), 2.91 (t, 2H), 3.0 (t, 2H), 3.07 (s, 6H, 2× $N(CH_3)_2$), 6.75 (d, 2H, Ar–H, J = 8.84 Hz), 7.50 (d, 2H, Ar–H, J = 8.84 Hz), 7.60 (d, 2H, Ar–H, J = 8.63 Hz), 7.79 (s, 1H, =CH), 7.84 (s, 1H, =CH), 8.27 (d, 2H, Ar–H, J = 8.65 Hz). Anal. Calcd for $C_{22}H_{22}N_2O_3$: C, 72.91; H, 6.12; N, 7.73. Found: C, 72.94; H, 6.13; N, 7.52%.

5.1.2. Synthesis of 1c. 2-(4-Methylbenzylidene)cyclohexanone **6** was prepared by a literature procedure[20] and crystallized from methanol to give **6**, mp 71 °C [lit.[20] mp 60 °C] in 40% yield. ^{1}H NMR ($CDCl_3$): 1.78 (p, 2H), 1.92 (m, 2H), 2.38 (s, 3H, CH_3), 2.53 (t, 2H), 2.86 (t, 2H), 7.21 (d, 2H, Ar–H, J = 7.90 Hz), 7.32 (d, 2H, Ar–H, J = 7.96 Hz), 7.50 (s, 1H, =CH).

Aqueous sodium hydroxide solution (20% w/v, 1 mL) was added to a solution of **6** (0.005 mol) and 2-nitrobenzaldehyde (0.005 mol) in ethanol (15 mL) at 8–10 °C. The solution was stirred at room temperature for 0.5 h. The reaction mixture was acidified with dilute hydrochloric acid and extracted with chloroform. Evaporation of the organic solvent gave a viscous oil which was purified by chromatography using a column of silica gel 60 (70–230 mesh) and an eluting solvent of ethyl acetate/hexane (1:9) to give **1c**, mp 120 °C in 30% yield. ^{1}H NMR($CDCl_3$): δ 1.78 (p, 2H), 2.40 (s, 3H, CH_3), 2.62 (p, 2H), 2.94 (t, 2H), 7.24 (d, 2H, Ar–H, J = 7.94 Hz), 7.39 (t, 3H, Ar–H), 7.52 (t, 1H, Ar–H), 7.65 (t, 1H, Ar–H), 7.83 (s, 1H, =CH), 7.96 (s, 1H, =CH), 8.14 (d, 1H, Ar–H). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.42; H, 5.71; N, 4.27%.

5.1.3. Synthesis of 1b, d–g, 2b–g, and 3b–g. The enones **1b**, **d–g**, **2b–g**, and **3b–g** were prepared by the following general procedure. Dry hydrogen chloride was passed into a solution of **4a** , **4b**, or **4c** vide infra (0.005 mol) and the appropriate aryl aldehyde (0.006 mol) in ether (40 mL) and methanol (4 mL). The reaction mixture was stirred at room temperature for 24 h and the resultant solid was collected and crystallized from chloroform/methanol (1:3).

5.1.3.1. *E*,*E*-2-(4-Methoxybenzylidene)-6-(2-nitrobenzylidene)cyclohexanone (1b). Mp 157 °C; yield 80%. ^{1}H NMR ($CDCl_3$): δ 1.79 (p, 2H), 2.61 (t, 2H), 2.94 (t, 2H), 3.86 (s, 3H, OCH_3), 6.98 (d, 2H, Ar–H, J = 8.3 Hz), 7.38 (d, 1H, Ar–H, J = 7.65 Hz), 7.48 (d, 2H, Ar–H, J = 8.45 Hz), 7.52 (t, 1H, Ar–H), 7.65 (t, 1H, Ar–H), 7.82 (s, 1H, =CH), 7.96 (s, 1H, =CH), 8.14 (d, 1H, Ar–H, J = 8.20 Hz). Anal. Calcd for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N, 4.01. Found: C, 71.89; H, 5.53; N, 3.90%.

5.1.3.2. *E*,*E*-2-(3,4,5-Trimethoxybenzylidene)-6-(2-nitrobenzylidene)cyclohexanone (1d). Mp 159 °C; yield 94%. ^{1}H NMR ($CDCl_3$): δ 1.80 (p, 2H), 2.62 (t, 2H), 2.97 (t, 2H), 3.91 (s, 9H, 3× OCH_3), 6.73 (s, 2H, Ar–H), 7.39 (d, 1H, Ar–H, J = 7.60 Hz), 7.52 (t, 1H, Ar–H), 7.66 (t, 1H, Ar–H), 7.77 (s, 1H, =CH), 7.96 (s, 1H, =CH), 8.14 (d, 1H, Ar–H, J = 8.15 Hz). Anal. Calcd for $C_{23}H_{23}NO_6$: C, 67.47; H, 5.66; N, 3.42. Found: C, 67.60; H, 5.61; N, 3.58%.

5.1.3.3. *E*,*E*-2-(Benzylidene)-6-(2-nitrobenzylidene)-cyclohexanone (1e). Mp 116 °C; yield 47%. ^{1}H NMR ($CDCl_3$): δ 1.78 (p, 2H), 2.63 (t, 2H), 2.95 (t, 2H), 7.40 (m, 5H, Ar–H), 6.79 (d, 2H, Ar–H, J = 7.6 Hz), 7.52 (t, 1H, Ar–H), 7.65 (t, 1H, Ar–H), 7.85 (s, 1H, =CH), 7.97 (s, 1H, =CH), 8.15 (d, 1H, Ar–H, J = 8.20 Hz). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22, H, 5.37; N 4.39. Found: C, 74.82; H, 5.26; N, 4.04%.

5.1.3.4. *E*,*E*-2-(4-Fluorobenzylidene)-6-(2-nitrobenzylidene)cyclohexanone (1f). Mp 136 °C; yield 52%. ^{1}H NMR ($CDCl_3$): δ 1.79 (p, 2H), 2.63 (t, 2H), 2.91 (t, 2H), 7.12 (t, 2H, Ar–H), 7.39 (d, 1H, Ar–H, J = 7.65 Hz), 7.47 (q, 2H, Ar–H), 7.52 (t, 1H, Ar–H), 7.65 (t, 1H, Ar–H), 7.80 (s, 1H, =CH), 7.97 (s, 1H, =CH), 8.15 (d, 1H, Ar–H, J = 8.20 Hz). Anal. Calcd for $C_{20}H_{16}FNO_3$: C, 71.21; H, 4.78; N 4.15. Found: C, 70.93; H, 4.79; N 3.88%.

5.1.3.5. *E*,*E*-2-(4-Chlorobenzylidene)-6-(2-nitrobenzylidene)cyclohexanone (1g). Mp 149 °C; yield 41%. ^{1}H NMR ($CDCl_3$): δ 1.79 (p, 2H), 2.63 (t, 2H), 2.84 (t, 2H), 7.40 (m, 5H, Ar–H), 7.53 (t, 1H, Ar–H), 7.66 (t, 1H, Ar–H), 7.77 (s, 1H, =CH), 7.97 (s, 1H, =CH), 8.15 (d, 1H, Ar–H, J = 8.2 Hz). Anal. Calcd for $C_{20}H_{16}ClNO_3$: C, 67.90; H, 4.56; N, 3.96. Found: C, 67.70; H 4.61; N 3.78%.

5.1.3.6. *E*,*E*-2-(4-Methoxybenzylidene)-6-(3-nitrobenzylidene)cyclohexanone (2b). Mp 114 °C; yield 68%. ^{1}H NMR ($CDCl_3$): δ 1.85 (p, 2H), 2.94 (t, 2H), 2.98 (t, 2H), 6.96 (d, 2H, Ar–H, J = 8.50 Hz), 7.49 (d, 2H, Ar–H, J = 8.45 Hz), 7.60 (t, 1H, Ar–H), 7.75 (d, 1H, Ar–H, J = 7.7 Hz), 7.79 (s, 1H, =CH), 7.81 (s, 1H, =CH), 8.19 (d, 1H, Ar–H, J = 8.10 Hz), 8.32 (s, 1H, Ar–H). Anal. Calcd for $C_{21}H_{19}NO_4$: C, 72.19; H, 5.48; N 4.01. Found: C, 71.91; H, 5.46; N, 3.90%.

5.1.3.7. *E*,*E*-2-(4-Methylbenzylidene)-6-(3-nitrobenzylidene)cyclohexanone (2c). Mp 156 °C; yield 60%. ^{1}H NMR ($CDCl_3$): δ 1.84 (p, 2H), 2.94 (t, 2H), 2.98 (t, 2H), 7.25 (t, 2H, Ar–H), 7.41 (d, 2H, Ar–H, J = 7.85 Hz), 7.60 (t, 1H, Ar–H), 7.76 (d, 2H, Ar–H, J = 7.65 Hz), 7.81 (s, 1H, =CH), 7.82 (s, 1H, =CH), 8.20 (d, 1H, Ar–H, J = 8.20 Hz), 8.32 (s, 1H, Ar–H). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.58; H, 5.88; N, 3.92%.

5.1.3.8. *E*,*E*-2-(3,4,5-Trimethoxybenzylidene)-6-(3-nitrobenzylidene)cyclohexanone (2d). Mp 172 °C; yield 82%. ^{1}H NMR ($CDCl_3$): δ 1.86 (p, 2H), 2.95 (t, 2H), 3.00 (t, 2H), 3.91 (s, 9H, 3× OCH_3), 6.74 (s, 1H, Ar–H), 7.61 (t, 1H), 7.76 (d, 2H, Ar–H, =CH, J = 7.70 Hz), 7.80 (s, 1H, =CH), 8.21 (d, 1H, Ar–H, J = 8.15 Hz), 8.29 (s, 1H, Ar–H). Anal. Calcd for $C_{23}H_{23}NO_6$: C, 67.47; H, 5.66; N, 3.42. Found: C, 67.29; H, 5.57; N, 3.30%.

5.1.3.9. *E*,*E*-2-(Benzylidene)-6-(3-nitrobenzylidene)-cyclohexanone (2e). Mp 120 °C; yield 22%. ^{1}H NMR ($CDCl_3$): δ 1.85 (p, 2H), 2.97 (m, 4H), 7.38 (t, 1H, Ar–H), 7.44 (m, 2H, Ar–H), 7.49 (d, 2H, Ar–H, J = 7.65 Hz), 7.61 (t, 1H, Ar–H), 7.76 (d, 1H, Ar–H, J = 7.65 Hz), 7.80 (s, 1H, =CH), 7.84 (s, 1H, =CH), 8.21 (d, 1H, Ar–H, J = 8.25 Hz), 8.33 (s, 1H, Ar–H). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22, H 5.37; N, 4.39. Found: C, 75.28; H, 5.44; N, 4.28%.

5.1.3.10. *E*,*E*-2-(4-Fluorobenzylidene)-6-(3-nitrobenzylidene)cyclohexanone (2f). Mp 126 °C; yield 63%. ^{1}H NMR ($CDCl_3$): δ 1.85 (p, 2H), 2.81 (t, 2H), 7.13 (t, 2H, Ar–H), 7.48 (dd, 2H, Ar–H), 7.61 (t, 1H, Ar–H), 7.76 (d, 1H, Ar–H, J = 7.65 Hz), 7.80 (s, 2H, =CH), 8.21 (d, 1H, Ar–H, J = 8.15 Hz), 8.32 (s, 1H, Ar–H). Anal. Calcd for $C_{20}H_{16}FNO_3$: C, 71.21; H, 4.78; N, 4.15. Found: C, 70.97; H, 4.70; N, 4.08%.

5.1.3.11. *E*,*E*-2-(4-Chlorobenzylidene)-6-(3-nitrobenzylidene)cyclohexanone (2g). Mp 138 °C; yield 68%. ^{1}H NMR ($CDCl_3$): δ 1.99 (p, 2H), 2.96 (q, 4H), 7.42 (m, 4H), 7.62 (t, 1H), 7.77 (d, 2H, Ar–H & =CH, J = 8.80 Hz), 7.81 (s, 1H, =CH), 7.84 (s, 1H, =CH), 8.16 (d, 1H, Ar–H, J = 8.16 Hz), 8.34 (s, 1H, Ar–H). Anal. Calcd for $C_{22}H_{16}ClNO_3$: C, 67.90; H, 4.56; N, 3.96. Found: C, 67.33; H, 4.58; N, 3.84%.

5.1.4. Compounds 3b–g. The synthesis of **3b**, **d**, and **g** has been reported previously.[8]

5.1.4.1. *E*,*E*-2-(4-Methylbenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (3c). Mp 136 °C; yield 54%. ^{1}H NMR ($CDCl_3$): δ 1.84 (p, 2H), 2.41 (s, 3H, CH_3), 2.92 (t, 2H), 2.98 (t, 2H), 7.25 (d, 2H, Ar–H, J = 7.8Hz), 7.41 (d, 2H, Ar–H, J = 7.80 Hz), 7.60 (d, 2H, Ar–H, J = 8.35 Hz), 7.79 (s, 1H, =CH), 7.82 (s, 1H, =CH), 8.27 (d, 2H, Ar–H, J = 8.40 Hz). Anal. Calcd for $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 75.45; H, 5.75; N, 4.13%.

5.1.4.2. *E*,*E*-2-(Benzylidene)-6-(4-nitrobenzylidene)cyclohexanone (3e). Mp 141 °C; yield 40%. ^{1}H NMR ($CDCl_3$): δ 1.84 (p, 2H), 2.93 (t, 2H), 2.98 (t, 2H), 7.38 (t, 1H, Ar–H), 7.44 (t, 1H, Ar–H), 7.49 (d, 2H, Ar–H, J = 7.75 Hz), 7.60 (d, 2H, Ar–H, J = 8.30 Hz), 7.80 (s, 1H, =CH), 7.84 (s, 1H, =CH), 8.27 (d, 2H, Ar–H, J = 8.40 Hz). Anal. Calcd for $C_{20}H_{17}NO_3$: C, 75.22; H, 5.37; N, 4.39. Found: C, 75.02; H, 5.29; N, 4.19%.

5.1.4.3. *E*,*E*-2-(4-Fluorobenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (3f). Mp 167 °C; yield 33%. ^{1}H NMR ($CDCl_3$): δ 1.85 (p, 2H), 2.94 (t, 4H), 7.13 (t, 2H), 7.48 (t, 2H), 7.60 (d, 2H), 7.79 (s, 2H, =CH), 8.27 (d, 2H, Ar–H, J = 8.5 Hz) Anal. Calcd for $C_{20}H_{16}FNO_3$: C, 71.21; H, 4.78; N, 4.18. Found: C, 70.88; H, 4.70; N, 4.10%.

5.1.5. Synthesis of 4a. A solution of sodium hydroxide (0.6 g, 0.015 mol) in water (5 mL) was added dropwise to a mixture of cyclohexanone (3.0 g, 0.02 mol) and 2-nitrobenzaldehyde (5.85 g, 0.056 mol) at room temperature for 0.25 h and the stirring was continued for 4 h. The solid was collected, dried, and recrystallized from chloroform/methanol to yield 2-(α-hydroxy-2-nitrobenzyl) cyclohexanone (**7**), mp 126 °C in 28% yield. ^{1}H NMR ($CDCl_3$): δ 1.71 (m, 4H), 2.13 (m, 1H), 2.45 (m, 2H), 2.82 (m, 1H), 4.18 (d, 1H), 5.46 (t, 1H), 7.44 (t, 1H), 7.65 (t, 1H), 7.78 (d, 1H, J = 7.9 Hz), 7.86 (d, 1H, J = 8.15 Hz).

Hydrochloric acid (2 mL) was added to a solution of **7** (10.5 g, 0.045 mol) in ethanol (30 mL) and the reaction mixture was heated at 40–45 °C for 4 h. The solvent was removed in vacuo at 40–45 °C and water (100 mL) was added to the residue. The solid was collected and dried to give **4a**, mp 92 °C in a yield of 64%. ^{1}H NMR ($CDCl_3$): δ 1.75 (p, 2H), 1.95 (p, 2H), 2.54 (t, 2H), 2.58 (t, 2H), 2.58 (t, 2H), 7.33 (d, 1H, Ar–H, J = 7.63 Hz), 7.51 (t, 1H, Ar–H), 7.60 (s, 1H, =CH), 7.64 (t, 2H, Ar–H), 8.12 (d, 1H, Ar–H, J = 8.21Hz). Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.40; H, 5.49; N, 6.29%.

5.1.6. Synthesis of 4b. A solution of cyclohexanone (4.9 g, 0.05 mol), morpholine (4.75 g, 0.055 mol), 4-toluenesulfonic acid (0.02 g) in toluene (50 mL) was heated under reflux using a Dean-Stark apparatus until the stoichiometric amount of water separated (~8 h). 3-Nitrobenzaldehyde (6.8 g, 0.045 mol) was added to the reaction mixture and heating under reflux was continued for 12 h. Water (25 mL) was added to the reaction mixture which was heated at 50–55 °C for ~1 h. The organic phase was separated, washed with hydrochloric acid (5%, 20 mL) and water (3× 50 mL), and dried. Toluene was removed in vacuo at 50–55 °C to give a viscous oil which was purified by chromatography using a column of silica gel 60 (70–230 mesh) and an eluting solvent of ethyl acetate/hexane (1:9) to give **4b**, mp 51–52 °C in 45% yield. ^{1}H NMR($CDCl_3$): 1.82 (p, 2H), 1.98 (p, 2H), 2.59 (t, 2H), 2.86 (t, 2H), 7.52 (s, 1H, =CH), 7.58 (t, 1H, Ar–H), 7.69 (d, 1H, Ar–H, J = 7.6 Hz), 8.19 (d, 1H, Ar–H, J = 8.15 Hz), 8.25 (s, 1H, Ar–H). Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.30; H, 5.48; N, 6.41%.

5.1.7. Synthesis of 4c. This compound was prepared by a literature procedure[21] to give **4c**, mp 119 °C [lit.[21]] mp 118–120 °C] in 72% yield with respect to 4-nitrobenzaldehyde. ^{1}H NMR($CDCl_3$): 1.82 (p, 2H), 1.98 (p, 2H), 2.59 (t, 2H), 2.83 (m, 2H), 7.47 (s, 1H), 7.53 (d, 2H), 8.25 (d, 2H).

5.2. Molecular modeling

Models of the compounds in series **1**–**4** were built using a BioMedCache program.[22] The lowest energy conformers were generated using the CONFLEX program and optimized by mechanics using augmented MM2 parameters.

5.3. Determination of log *P* values

The log *P* values for enones **1**–**4** were generated with the JME molecular editor.[23]

5.4. Cytotoxicity assays

A literature procedure was employed to examine the cytotoxicity of **1a–g** , **2a–g** , **3a–g** , and **4a–c** toward human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells.[24] In brief, different concentrations of compounds were incubated with the cells in RPMI 1640 medium at 37 °C for 72 h (Molt 4/C8 and CEM T-lymphocytes) or 48 h (L1210 cells). The correct IC_{50} values for **4c** are presented in Table 1 which replaces the figures quoted previously.[25]

5.5. Evaluation of 1d, 2d, and 3d on respiration in rat liver mitochondria

Rats were anesthetized with isoflurane and decapitated. A previously reported procedure was employed to isolate mitochondria from the liver.[26] The consumption

of oxygen by mitochondria was determined by polarography using a literature methodology.[27]

5.6. Statistical analyses

The linear, semilogarithmic and logarithmic plots were constructed using a statistical software package.[28]

References and notes

1. Mutus, B.; Wagner, J. D.; Talpas, C. J.; Dimmock, J. R.; Phillips, O. A.; Reid, R. S. *Anal. Biochem.* **1989**, *177*, 237–243.
2. Baluja, G.; Municio, A. M.; Vega, S. *Chem. Ind.* **1964**, 2053–2054.
3. 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*, 988–991.
4. Dimmock, J. R.; Sidhu, K. K.; Chen, M.; Reid, R. S.; Allen, T. M.; Kao, G. Y.; Truitt, G. A. *Eur. J. Med. Chem.* **1993**, *28*, 313–322.
5. Dimmock, J. R.; Padmanilayam, M. P.; Zello, G. A.; Nienaber, K. H.; Allen, T. M.; Santos, C. L.; De Clercq, E.; Balzarini, J.; Manavathu, E. K.; Stables, J. P. *Eur. J. Med. Chem.* **2003**, *38*, 169–177.
6. Dimmock, J. R.; Padmanilayam, M. P.; Zello, G. A.; Quail, J. W.; Oloo, E. O.; Prisciak, J. S.; Kraatz, H.-B.; Cherkasov, A.; Lee, J. S.; Allen, T. M.; Santos, C. L.; Manavathu, E. K.; De Clercq, E.; Balzarini, J.; Stables, J. P. *Eur. J. Med. Chem.* **2002**, *37*, 813–824.
7. Dimmock, J. R.; Das, U.; Gul, H. I.; Kawase, M.; Sakagami, H.; Baráth, Z.; Ocsovsky, I.; Molnár, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1633–1636.
8. Das, U.; Gul, H. I.; Alcorn, J.; Shrivastav, A.; George, T.; Sharma, R. K.; Nienaber, K. H.; De Clercq, E.; Balzarini, J.; Kawase, M.; Kan, N.; Tanaka, T.; Tani, S.; Werbovetz, K. A.; Yakovich, A. J.; Manavathu, E. K.; Stables, J. P.; Dimmock, J. R. *Eur. J. Med. Chem.* **2006**, *41*, 577–585.
9. Das, U.; Kawase, M.; Sakagami, H.; Ideo, A.; Shamada, J.; Molnár, J.; Baráth, Z.; Bata, Z.; Dimmock, J. R. *Bioorg. Med. Chem.* **2007**, *15*, 3373–3380.
10. Hassner, A.; Mead, T. C. *Tetrahedron* **1964**, *20*, 2201–2210.
11. Quail, J. W.; Doroudi, A.; Pati, H. N.; Das, U.; Dimmock, J. R. *Acta Cryst.* **2005**, *E61*, 1795–1797.
12. Quail, J. W.; Doroudi, A.; Pati, H. N.; Das, U.; Dimmock, J. R. *Acta Cryst.* **2005**, *E61*, 1774–1776.
13. Quail, J. W.; Das, U.; Dimmock, J. R. *Acta Cryst.* **2005**, *E61*, 1150–1152.
14. Taft, R. W., Jr. In *Steric Effects in Medicinal Chemistry*; Newman, M. S., Ed.; John Wiley and Sons, Inc.: New York, 1956; p 591.
15. Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979, p 49.
16. Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979, pp 49–50.
17. Bryla, J. In *Inhibition of Mitochondrial Function*; Erecińska, M., Wilson, D. F., Eds.; Pergamon Press: Oxford, 1981; pp 243–246.
18. Hamon, N. W.; Bassendowski, D. L.; Wright, D. E.; Dimmock, J. R.; Noble, L. M. *J. Pharm. Sci.* **1978**, *67*, 1539–1542.
19. Shriner, R. L.; Teeters, W. O. *J. Am. Chem. Soc.* **1938**, *60*, 936–939.
20. Colonge, J.; Sibeud, J. *J. Bull. Soc. Chim. France* **1952**, 786–789.
21. Vieweg, H.; Wagner, G. *Pharmazie* **1979**, *34*, 785–788.
22. BioMedCache 6.1 Windows, BioMedCache, Fujitsu America; Inc., 2003.
23. JME Molecular editor, April 2006 Version; http://www.molinspiration.com.
24. Baraldi, P. B.; Nunez, M. Del. C.; Tabrizi, M. A.; De Clercq, E.; Balzarini, J.; Bermejo, J.; Estévez, F.; Romagnoli, R. *J. Med. Chem.* **2004**, *47*, 2877–2886.
25. Dimmock, J. R.; Chamankhah, M.; Das, U.; Zello, G. A.; Quail, J. W.; Yang, J.; Nienaber, K. H.; Sharma, R. K.; Selvakumar, P.; Balzarini, J.; De Clercq, E.; Stables, J. P. *J. Enzyme Inhib. Med. Chem.* **2004**, *19*, 1–10.
26. Kowaltowski, A. J.; Castilho, R. F.; Grijalba, M. T.; Bechara, E. J.; Vercesi, A. E. *J. Biol. Chem.* **1996**, *271*, 2929–2934.
27. Estabrook, R. W. *Methods Enzymol.* **1967**, *X*, 41–47.
28. Statistical Package for Social Sciences (2005), SPSS for Windows, Release 14.0.0 (SPSS Inc., Chicago).