Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 169–177
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Cytotoxic analogues of 2,6-bis(arylidene)cyclohexanones

Jonathan R. Dimmock [a,*], Maniyan P. Padmanilayam [a], Gordon A. Zello [a], Kurt H. Nienaber [b], Theresa M. Allen [c], Cheryl L. Santos [c], Erik De Clercq [d], Jan Balzarini [d], Elias K. Manavathu [e], James P. Stables [f]

[a] College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, Sask., Canada S7N 5C9
[b] Department of Biochemistry, University of Saskatchewan, 107 Wiggins Road, Saskatoon, Sask., Canada S7N 5E5
[c] Department of Pharmacology, University of Alberta, Edmonton, Alta, Canada T6G 2H7
[d] Rega Institute for Medical Research, Katholieke Universiteit Leuwen, 3000 Leuwen, Belgium
[e] Department of Medicine, Wayne State University, Detroit, MI 48201-1908, USA
[f] National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892-9020, USA

Received 18 July 2002; received in revised form 18 November 2002; accepted 20 November 2002

Abstract

A series of 2,6-bis(arylidene)cycloalkanones (**1**) and related compounds containing one or two substituents at the four position of the cyclohexyl ring were prepared and shown to display cytotoxic activity towards murine P388 and L1210 cells as well as human Molt 4/C8 and CEM T-lymphocytes. In some of the series of compounds, positive correlations were noted between the potencies of the enones and the magnitude of the Hammett σ values of the aryl substituents. Four representative compounds were cytotoxic to a number of human tumours in vitro, particularly towards colon cancer and leukemic cells. A noteworthy feature of the compounds prepared in this study is that, in general, they were well tolerated when administered to rodents. A number of lead molecules emerged from this investigation as well as guidelines for future expansion of these series of compounds.

Keywords: α,β-Unsaturated ketones; Cytotoxicity; Structure–activity relationships; Bioscreens

1. Introduction

A number of years ago the noteworthy cytotoxicity of 2,6-bis(phenylmethylene)cyclohexanone (**1a**) was disclosed [1]. The IC_{50} figure of this compound is 36.5 nM towards murine P388/MRI leukemic cells revealing it possessed 25 times the potency of a reference drug carmustine towards this cell line. This unsaturated ketone did not bind to a synthetic DNA, namely poly[dAT] and, as described in this study, **1a** did not induce mortality in mice which received 300 mg kg^{-1} of this compound (Figs. 1 and 2).

The objective of the present investigation was to develop structure–activity relationships from the lead compound in the following ways. First, the placement of various groups in the aryl rings of **1a** was proposed leading to **1b**–**g**. The chosen aryl substituents had different electronic, hydrophobic and steric properties. In fact, the groups in **1b**–**e**, **g** are found in three of the four quadrants of a Craig plot for para substituents [2] which indicate the divergence of the σ_p and π values of the chosen substituents. Second, many α,β-unsaturated ketones exert their bioactivity, at least in part, by reaction with cellular thiols [3]. In the present study, the decision was made to explore the possibility of the existence of an additional site of action with cellular constituents that are distinct from the olefinic carbon atoms Thus at position four of the cyclohexane ring various groups were placed which had different steric, electronic and hydrophobic properties, namely the bromo, *t*-butyl, carboethoxymethylene, dimethylenedioxy and acetoxy functions that gave rise to series **2**–**6**, respectively. Where available, certain substituent constants of the group at position four of the cycloaliphatic ring supported the divergence of their physicochemical properties. Thus the solvent accessible surface

* Corresponding author.
E-mail address: dimmock@skyway.usask.ca (J.R. Dimmock).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(02)01444-7

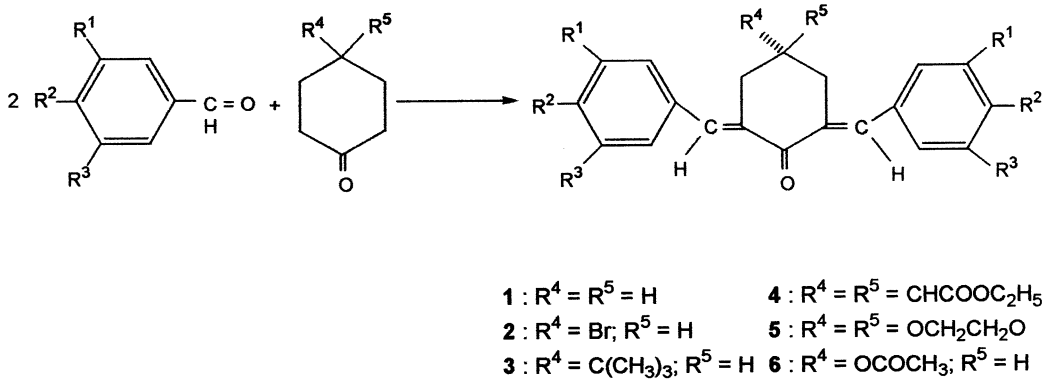

Fig. 1. Synthesis of the compounds in series **1**–**6**. The nature of the substituents R^1–R^3 are indicated in Table 1.

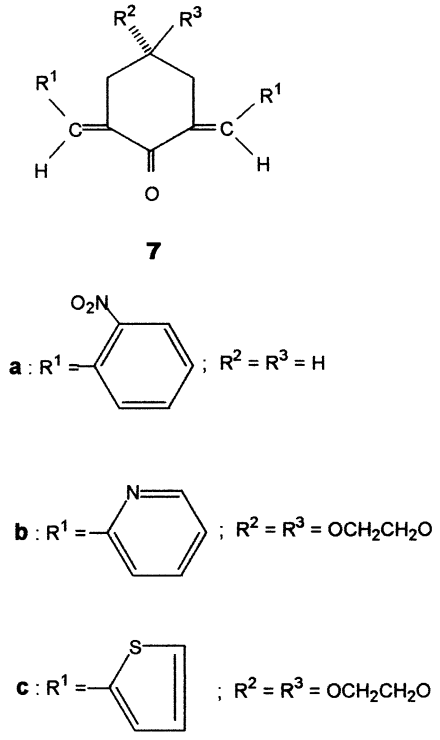

Fig. 2. Structures of compounds in series **7**.

area (SASA) values of all of the substituents at position four of the cyclohexane ring in series **1**–**6** were 46.1, 88.9, 169.4, 167.3, 129.4 and 145.7 $Å^2$, respectively [4]. Furthermore various physicochemical constants are available for the hydrogen, bromo, *t*-butyl and acetoxy groups. For example, the field ($\mathscr{F}$) effects for these four substituents are 0.00, 0.44, −0.07 and 0.41, respectively, while the fragment constants (*f*) are 0.23, 0.20, 2.22 and −0.72, respectively, and the molar refractivity (MR) values are 1.03, 8.80, 19.62 and 11.85, respectively [5].

In addition, compounds **7a**–**c** were also synthesized in order to compare their cytotoxicities with those of certain related congeners. Thus the structural isomer of **1g**, which displayed excellent potency in the P388 screen vide infra, namely **7a**, was prepared. In addition, **7b**,**c** were synthesized with a view to comparing their potencies with **5a**, which possesses IC_{50} values lower than melphalan when evaluated against the two human T-lymphocytes vide infra. Previous work with certain Mannich bases of conjugated arylidene ketones revealed that these compounds demonstrated not only cytotoxicity [6] but also possessed antifungal properties [7]. However mice receiving either a single dose [8] or multiple doses [6] of various members of this class of compounds displayed toxicity including lethality. Hence the evaluation of representative compounds for antimycotic activity as well as toxicity to mice was planned.

2. Chemistry

Cyclohexanone, 4-*t*-butylcyclohexanone and 1,4-cyclohexanedione mono-ethylene ketal, which were used in the synthesis of the compounds in series **1**, **3** and **5**, respectively, were obtained from commercial sources. The cycloaliphatic ketones required in the synthesis of series **2**, **4** and **6** were prepared as follows. Reaction of 1,4-cyclohexanediol with hydrobromic acid led to the isolation of 4-bromocyclohexanol, which on treatment with chromic oxide produced 4-bromocyclohexanone, which was used in the synthesis of series **2**. Reaction of ethyl bromoacetate with triphenylphosphine and subsequent treatment with sodium hydroxide led to the corresponding Wittig reagent which was condensed with 1,4-cyclohexanedione to give 4-oxycyclohexylidene acetic acid ethyl ester from which series **4** was prepared. Chromic acid oxidation of 1,4-cyclohexanediol led to the formation of 4-hydroxycyclohexanone, which was acylated with acetic anhydride to give 4-acetoxycyclohexanone, used in the preparation of series **6**. Each of these six cyclic ketones was condensed with the appropriate aryl aldehydes to give rise to the enones **1**–**7**. ^{1}H-NMR spectroscopy revealed the compounds to be isomerically pure. Previous X-ray crystallographic studies of **1a** [1] and related compounds [9–11] revealed that the olefinic double bonds adopted the *E* config-

uration. Hence the compounds prepared in the present investigation were considered to have the same stereochemistry.

3. Bioevaluations

The α,β-unsaturated ketones **1**–**7** were evaluated for cytotoxicity towards murine P388 and L1210 leukemic cells and human Molt 4/C8 and CEM T-lymphocytes. These data are presented in Table 1. In addition, four representative compounds, namely **2b**, **4c**, **5a** and **5f**, were examined against a panel of human tumours and

Table 2
Evaluation of **2b**, **4c**, **5a** and **5f** against human tumour cell lines

Compound	MG MID [a] (μM)		
	All cell lines	Colon cancer cells	Leukemic cells
2b	20.4	13.1	7.94
4c	8.71	5.55	4.59
5a	4.68	2.53	2.85
5f	19.5	13.5	6.68
Melphalan	19.1	42.9	3.55
5-Fluorouracil	29.5	5.82	52.9

[a] The letters MG MID refer to the mean graph midpoint values which are explained in the text.

Table 1
Cytotoxicity of the compounds in series **1**–**7** and melphalan against murine P388 and L1210 cells and human Molt 4/C8 and CEM T-lymphocytes

Compound	Aryl substituents [a]			IC_{50} (μM)			
	R^1	R^2	R^3	P388 cells	L1210 cells	Molt 4/C8 cells	CEM cells
1a				4.82±0.3	18.3±4.03	8.84±0.48	8.75±0.11
1b		Cl		3.91±0.8	9.60±0.71	8.89±0.13	9.52±1.80
1c		CH_3		>50±1.2	248±14.1	303±18.4	262±13.4
1d		OCH_3		>50±2.3	236±14.8	246±19.8	196±12.7
1e		F		4.06±0.3	20.5±12.9	9.25±1.06	10.6±2.74
1f	Cl	Cl		1.48±0.03	12.5±0.71	9.37±0.46	8.91±0.035
1g		NO_2		0.639±0.04	8.46±1.18	8.20±0.88	8.21±0.09
2a				0.873±0.06	5.68±0.61	2.14±0.26	4.67±2.34
2b		Cl		0.731±0.1	14.8±9.46	5.99±0.66	2.79±1.16
2c		CH_3		3.85±0.1	312±9	≥500	225±6
2d		OCH_3		2.67±0.3	11.6±1.06	7.89±0.92	7.66±0.62
2e		F		1.04±0.02	6.30±1.55	1.82±0.084	1.76±0.078
3a				5.42±0.07	9.05±0.10	7.86±0.23	7.66±0.8
3b		Cl		2.19±0.06	10.6±1.26	8.43±0.21	9.32±0.20
3c		CH_3		21.0±1.7	171±49.5	121±9.89	113±7.07
3d		OCH_3		10.4±0.5	222±35	234±85	184±48
3e		F		2.66±0.08	241±29.6	172±20.5	186±0.0
3f	Cl	Cl		2.45±0.03	38.4±5.72	33.2±2.19	41.0±3.68
3g		NO_2		0.241±0.05	1.50±0.38	0.32±0.0042	0.34±0.035
4a				1.06±0.08	9.48±0.29	29.2±16.7	6.94±0.96
4b		Cl		2.74±0.2	26.6±10.8	40.2±13.1	15.3±4.8
4c		CH_3		0.786±0.03	8.44±0.11	10.8±2.05	8.88±0.49
4d		OCH_3		1.45±0.06	43.2±1.1	38.7±4.7	36.1±2.3
4e		F		22.3±1.4	39.4±3.5	41.4±14.8	17.6±6.1
4f	Cl	Cl		2.80±0.1	19.9±8.3	41.1±1.8	35.7±0.9
5a				3.94±0.13	12.8±2.3	1.86±0.26	2.31±0.06
5b		Cl		12.6±0.3	402±67	≥500	≥500
5c		CH_3		>50	>500	>500	>500
5d		OCH_3		7.71±0.2	>500	>500	>500
5e		F		5.47±0.4	15.9±1.8	81.2±53.4	46.8±26.2
5f	Cl	Cl		1.38±0.2	13.6±0.8	8.52±0.16	8.75±0.72
5g		NO_2		0.981±0.2	40.1±0.9	7.94±0.81	8.99±0.13
5h	OCH_3	OCH_3	OCH_3	0.835±0.1	2.64±0.26	1.42±0.03	1.62±0.01
6a				2.26±0.3	14.2±2.82	2.88±0.59	7.15±0.014
6b		Cl		0.598±0.01	8.96±0.80	1.96±0.23	2.08±0.21
6c		CH_3		3.72±0.09	13.3±4.60	8.20±0.35	9.63±2.16
6d		OCH_3		3.72±0.08	41.1±1.3	10.5±1.7	9.75±2.04
7a	–	–	–	0.510±0.02	2.01±0.40	1.51±0.01	1.50±0.08
7b	–	–	–	0.208±0.01	0.835±0.049	0.344±0.011	0.321±0.028
7c	–	–	–	31.4±5.0	>500	>500	>500
Melphalan	–	–	–	0.22±0.01	2.13±0.03	3.24±0.79	2.47±0.79

[a] For the sake of clarity, only non-hydrogen atoms are indicated.

the results are portrayed in Table 2. Six representative enones **1b**, **c**, **3b**, **c**, **6a** and **6b** were screened against three isolates of *Aspergillus fumigatus* and one of *Candida albicans*. Most of the compounds were evaluated for murine toxicity.

4. Results and discussion

All of the compounds in series **1**–**7** were evaluated against two murine cell lines, namely P388 and L1210 neoplasms, as well as human Molt 4/C8 and CEM T-lymphocytes. These data are presented in Table 1. The compounds in series **1**–**7** which display cytotoxicity are believed to do so by alkylation of various cellular nucleophiles. Hence melphalan was included as the reference compound in this screen since it is an established anticancer alkylating agent.

Previous studies from these laboratories revealed that in general P388 cells are more sensitive to conjugated styryl ketones than L1210, Molt 4/C8 and CEM cells [12]. Hence the maximum concentration of compounds used in the P388 test was 50 μM, whereas 500 μM was the upper limit in the three other screens. Comparisons between the IC_{50} values of the compounds in the P388 screen were made with the other three cell lines except for **1c**, **d** and **5c**, since no IC_{50} figures were available in the P388 screen for these three compounds. The lowest IC_{50} values were invariably found in the P388 screen except in the cases of **4e** and **5a**. The percentage of compounds with IC_{50} values less than 10 μM in the P388, L1210, Molt 4/C8 and CEM screens were 80, 30, 53 and 58, respectively, revealing that an average of 55% of these molecules display moderate potencies towards these cell lines. In order to identify those compounds with the greatest cytotoxicity towards all four cell lines, the average IC_{50} values of the most potent compounds were determined. The following enones were identified as lead molecules based on their average IC_{50} values in μM and their potency compared to the average IC_{50} value of melphalan, i.e. 2.02 μM, given, respectively, in parentheses: **7b** (0.43, 4.7), **3g** (0.58, 3.5), **7a** (1.39, 1.5), **5h** (1.63, 1.2), **2e** (2.73, 0.7), **2a** (3.34, 0.6) and **6b** (3.40, 0.6). Notably the three most potent compounds, namely **7b**, **3g** and **7a**, have strongly electron-attracting groups directly attached to the olefinic carbon atoms. These molecular features would be expected to expedite attack with cellular thiols.

A comparison of the cytotoxicities of **1g** and **5a** with the closely related analogues in series **7** was undertaken. First, changing the location of the nitro group from the 4 position in **1g** to the ortho location as is present in **7a** led to increased cytotoxicity in all four screens. Second, replacement of the phenylmethylene group of **5a** by a 2-pyridyl function led to **7b** which possessed greater potencies in all four tests. On the other hand, the 2-thienyl analogue **7c** had lower potency (or possibly complete inactivity) in these assays. Thus the 2-nitrophenylmethylene and 2-pyridylmethylene groups may be cytotoxic pharmacophores and future synthetic chemical strategies should incorporate these groups into candidate cytotoxic and anticancer agents.

A further analysis of the biodata was undertaken in order to determine whether correlations existed between certain physicochemical constants of the aryl substituents and cytotoxicity. Thus linear, and semilogarithmic plots were made between the σ, π and MR values of the R^1–R^3 groups in each of the series **1**–**6** with the IC_{50} figures in each screen. The P values noted as <0.1, <0.05 and <0.01 are summarized in Section 6. The following relationships were established. First, negative correlations were observed between the Hammett σ values and the IC_{50} figures in series **1** and **3** (P388, L1210, Molt 4/C8 and CEM screens), **2** (P388 test) and **6** (P388, Molt 4/C8 and CEM screens). Second, a negative correlation was obtained between the MR values and P388 cells in series **1**. On the other hand, the MR values positively correlated with the IC_{50} figures of series **2** and **4** in the Molt 4/C8 and CEM screens, respectively. Third, positive correlations were found between the SR values and the σ constants in series **1** as well as the π values in series **2**. No other correlations were noted.

The value of establishing these relationships in drug design includes the following considerations. The negative correlations with the σ values in series **1**, **2**, **3** and **6** indicated that potency increased due to a rise in the magnitude of the Hammett values. This observation is consistent with the theory that these compounds exert their bioactivity, at least in part, by thiolation. Thus by increasing the fractional positive charge on the olefinic carbon atoms attached at positions two and six of the cyclohexyl ring, the compounds had an enhanced electrophilicity for cellular thiols which led to greater cytotoxicity. Future expansion of these groups of compounds should incorporate multiple strongly electron-attracting substituents into the aryl rings. The sizes and hydrophobicity of the aryl substituents are less of a determinant factor in influencing cytotoxicity.

The data in Table 1 reveal that when the 2,6-bis(arylidene) groups are constant, cytotoxicity varied depending on the nature of the R^4 and R^5 substituents. The issue of whether the topography of the groups at position 4 was correlated with cytotoxicity was addressed using the data of the unsubstituted compounds **1a**, **2a**, **3a**, **4a**, **5a** and **6a**. SASA figures are available for all of the R^4 and R^5 groups of these compounds vide supra and linear and semilogarithmic plots were constructed between these data and the IC_{50} values in each of the cytotoxicity screens. No correlations ($P>0.1$) were observed. In addition, molecular modeling of **1a**, **2a**, **3a**, **4a**, **5a** and **6a** revealed that when carbon atoms 1, 2 and 6 of the cyclohexyl ring were superimposed, the

rest of the molecules overlapped completely except when the R^4 and R^5 groups were not hydrogen atoms. Thus variation in the nature of the R^4 and R^5 groups do not alter the relative positions of the rest of the molecules. These observations reinforce the earlier conclusion that the electron densities on the arylidene methine carbon atoms are likely the most dominant effect influencing cytotoxicity.

In order to examine further the potential of 4-substituted 2,6-bis(arylidene)cyclohexanones as cytotoxic agents, four compounds were evaluated by the National Cancer Institute, USA against a panel of approximately 56 human tumour cell lines from nine different types of neoplasms, namely leukemia, melanoma and non-small cell lung, colon, central nervous system, ovarian, renal, prostate and breast cancers [13]. The concentration range used in these experiments is normally 10^{-4} to 10^{-8} M. The amount of compound required to inhibit the growth of various cell lines by 50% is noted. On occasions, a 50% reduction of the growth of the cells was not achieved when the maximum concentration of a compound was employed, i.e. 10^{-4} M; however this figure of 10^{-4} M was used in calculating the average cytotoxicity towards all cell lines. Hence the term mean graph midpoint (MG MID) rather than IC_{50} was employed.

The data in Table 2 reveal that the enones **2b**, **4c**, **5a** and **5f** have 0.9, 2.2, 4.1 and 1.0 times the potencies of melphalan, respectively, when cytotoxicity to all cell lines was considered. An interest in these laboratories is the discovery of new groups of compounds with potent activities towards colon cancers [14] and leukemia [15]. Thus, the MG MID values of **2b**, **4c**, **5a** and **5f** towards colon cancer and leukemic cell lines were calculated and these data are presented in Table 2. These data are presented in Table 2 along with the related information for 5-fluorouracil, which is used in treating colorectal cancers [16], and the antileukemic agent melphalan [17]. The potencies of **4c** and **5a** were 1.1 and 2.3 times, respectively, that of 5-fluorouracil towards colon cancer cells and they possessed 0.8 and 1.3 times, respectively, the antileukemic activity of melphalan. Taking into consideration the potencies towards all cell lines and, in particular, colon and leukemic cells, **4c** and **5a** are clearly useful lead molecules.

A final phase of the investigation sought to explore further the therapeutic potential of representative compounds. Accordingly evaluation for antifungal activity and murine toxicity was undertaken.

Previous studies revealed that 5-dimethylamino-1-phenyl-1-penten-3-one hydrochloride, which contains an α,β-unsaturated keto group, displayed antifungal activity [18] due, in part at least, to interference with mitochondrial function [19]. More recently the antifungal activity of an enone was associated with its interference with H^+-ATPase [20]. The minimum inhibitory concentration (MIC) figures of **1b**, **c**, **3b**, **c**, **6a** and **6b** towards three isolates of *A. fumigatus* and one of *C. albicans* were in excess of 32 μg mL^{-1} whereas the MIC value of a reference antifungal agent voriconazole was 0.25 μg mL^{-1} towards all four isolates. These representative compounds are clearly bereft of marked potencies towards these fungi. It is possible therefore that the compounds in series **1**–**7** lack antifungal properties.

A number of alkylating agents used in cancer chemotherapy have marked mammalian toxicity [21]. Previous work directed to determining murine toxicity of various compounds containing the α,β-unsaturated group involved the intraperitoneal administration of single doses of 30, 100 and 300 mg kg^{-1} to mice and observations were made after 0.5 and 4 h [12,22]. A number of the compounds examined under these conditions proved to be lethal within these time frames and other evidences of toxicity including neurotoxicity and respiratory depression were observed. In the present investigation, **1a**–**g**, **2a**,**c**–**e**, **3a**–**g**, **4a**,**b**,**d**–**f**, **5a**–**h**, **6a**,**b** and **7a**–**c** were examined in a similar fashion. No deaths of the animals occurred.

Neurotoxicity, as determined by the rotorod test [23], was absent in two-thirds of the compounds evaluated and respiratory depression was not observed. In addition, the enones **1a**,**g**, **5a**,**d** and **7a** were administered orally to rats and at the doses utilized did not display any overt toxic symptoms. In summary, the animal experiments were encouraging insofar as at the maximum doses employed, the compounds were non-lethal and in general were well tolerated.

5. Conclusions

A number of 2,6-bis(arylidene)cyclohexanones **1** and related analogues **2**–**7** have been prepared. In general, these compounds displayed moderate potencies towards P388, L1210, Molt 4/C8 and CEM cells. Four compounds possessed greater or equal potencies as melphalan when examined against a panel of human tumour cell lines. Representative compounds did not display significant antifungal activity nor marked murine toxicity. Guidelines for the future expansion of these compounds included the following considerations. First, positive correlations were noted between the Hammett σ values of the aryl substituents and potencies in a number of cases. Second, various cytotoxic molecules were found serving as prototypes for systematic molecular modification. Thus from the P388, L1210, Molt 4/C8 and CEM assays, **2a**,**e**, **3g**, **5h**, **6b**, **7a** and **7b** were identified as lead molecules. The enones **2b**, **4c**, **5a** and **5f** displayed moderate potencies towards a panel of human tumour cell lines. In addition, future work should be directed to ascertaining the mode(s) of action of various

molecules and undertaking in vivo experiments for anticancer activity.

6. Experimental protocols

6.1. Chemistry

Melting points are uncorrected and are in degrees Celsius. Elemental analyses were carried out for **1a**–**f**, **2a**–**e**, **3a**–**f**, **4a**–**f**, **5a**–**f**, **6a**–**d**, **7c** and 4–bromo-2,6-bis(3,4-dichlorophenylmethylene)cyclohexanone (C, H) and **1g**, **3g**, **5g**, **7a** and **7b** (C, H, N) by Mr. K. Thoms, Department of Chemistry, University of Saskatchewan. The results obtained were within 0.4% of the calculated values except for **2b** (calculated for H: 3.58%; found: 3.11%). ^{1}H-NMR spectra were determined on all compounds using a Bruker AM 500 FT-NMR instrument (500 MHz).

6.1.1. Synthesis of series *1* and *7a*

Compounds **1a**–**g** and **7a**, which have been described previously, were prepared by a literature procedure [24] in yields of 61–85%. All compounds were recrystallized from chloroform: methanol (2:8). The melting points were in accord with those described in the literature. The ^{1}H-NMR spectrum of a representative compound **1d** was as follows: δ ($CDCl_3$): 1.77–1.79 (m, 2H, 4-CH_2), 2.88–2.91 (m, 4H, 3-CH_2, 5-CH_2), 3.82 (s, 6H, OCH_3), 6.91 (d, 4H, $J = 9.70$ Hz, aryl H), 7.43 (d, 4H, $J = 8.77$ Hz, aryl H), 7.74 (s, 2H, =CH).

6.1.2. Synthesis of series *2*

A mixture of 4-bromocyclohexanone (1.0 g, 5.65 mmol), which was prepared in 68% yield by a literature method [25], the appropriate aryl aldehyde (11.87 mmol) and hydrochloric acid (37% w/v, 0.5 g) was stirred at room temperature for 16 h. Methanol (24 mL) was added and the precipitate was collected and recrystallized from chloroform–methanol (3:7) except for **2a**, which was recrystallized from methanol. The melting points and percentage yields were as follows: **2a**: 157–158, 40; **2b**: 192–193, 35; **2c**: 192–194, 33; **2d**: 182 (dec.), 30; **2e**: 177–179, 45. The 3,4-dichloro analogue (**2**, $R^1 = R^2 = Cl$, $R^3 = H$), mp 189–190, was prepared in 32% yield and purified by recrystallization from chloroform–methanol (3:7). The ^{1}H-NMR spectrum of a representative compound **2c** was as follows: δ ($CDCl_3$): 2.35 (s, 6H, CH_3), 3.32–3.40 (m, 2H, 3-CH_2), 3.46–3.54 (m, 2H, 5-CH_2), 4.42-4.48 (m, 1H, 4-CH), 7.23 (d, 4H, $J = 8.92$ Hz, aryl H), 7.32 (d, 4H, $J = 8.03$ Hz), 7.89 (s, 2H, =CH).

6.1.3. Synthesis of series *3*

A mixture of 4-*t*-butylcyclohexanone (1.54 g, 10 mmol), the appropriate aryl aldehyde (21 mmol) and hydrochloric acid (37% w/v, 0.5 g) was stirred at room temperature for 16 h. Methanol was added and the precipitate was collected and recrystallized from chloroform–methanol (15:85), except that chloroform–methanol (1:9) was used in the case of **3b**. The melting points and percentage yields were as follows: **3a**: 144 (lit. [26] mp 146), 85; **3b**: 172–173, 80; **3c**: 155 (lit. [26] m.p. 164–166, 76); **3d**: 173–175, 71; **3e**: 173–174, 72; **3f**: 170–172, 73; **3g**: 208–209, 68. The ^{1}H-NMR spectrum of a representative compound **3d** was as follows: δ ($CDCl_3$): 0.95 (s, 9H, *t*-C_4H_9), 1.46 (m, 1H, 4-CH), 2.15 (m, 2H, 3-CH_2), 3.15 (dd, 2H, 5-CH_2), 3.83 (s, 6H, OCH_3), 6.93 (d, 4H, $J = 8.74$ Hz, aryl H, 7.43 (d, 4H, $J = 8.71$ Hz, aryl H), 7.71 (s, 2H, =CH).

6.1.4. Synthesis of series *4*

Triphenylcarbethoxymethylenephosphorane (15.55 g, 44.68 mmol) which had been prepared by a literature method [27] was added to a solution of 1,4-cyclohexanedione (5.0 g, 44.64 mmol) in benzene (100 mL). The mixture was heated under reflux for 12 h. The residue obtained after removal of the solvent in vacuo was chromatographed on silica gel (60–100 mesh) using a solvent of ethyl acetate in hexane (5:95) to give ethyl 4-oxocyclohexylideneacetate [28] in 71% yield. ^{1}H-NMR: δ ($CDCl_3$): 1.26 (t, 3H, CH_3), 2.42–2.50 (m, 4H, 2-CH_2, 6-CH_2), 2.60–2.66 (m, 2H, 3-CH_2), 3.12–3.20 (m, 2H, 5-CH_2), 4.12–4.18 (q, 2H, C$H_2$$CH_3$), 5.86 (s, 1H, =CH). This ketone was reacted with the appropriate aldehyde in the same manner as used in the preparation of series **2** to give the crude esters which were purified by recrystallization from methanol (**4a**), chloroform: methanol (15:85) (**4b**,**d**–**f**) or chloroform–methanol (2:8) (**4c**). The melting points and percentage yields were as follows: **4a**: 107–109, 60; **4b** 122, 58; **4c**: 121–122, 51; **4d**: 152–153, 61; **4e**: 112–114, 50; **4f**: 162–164, 56. The ^{1}H-NMR spectrum of a representative compound **4d** was as follows: δ ($CDCl_3$): 1.26 (t, 3H, CH_3), 3.65 (s, 2H, 3-CH_2), 3.85 (s, 6H, OCH_3), 4.20 (q, 2H, C$H_2$$CH_3$), 4.35 (s, 2H, 5-$CH_2$), 5.86 (s, 1H, C*H* $COOC_2H_5$), 6.95 (d, 4H, $J = 8.97$ Hz, aryl H), 7.37 (d, 4H, $J = 8.64$ Hz, aryl H), 7.69 (s, 1H, C*H*-aryl), 7.75 (s, 1H, C*H*-aryl).

6.1.5. Synthesis of series *5*

A solution of sodium hydroxide (0.1 g, 2.5 mmol) in water (1 mL) was added to a solution of 1,4-cyclohexanedione mono-ethylene ketal (0.7 g, 4.49 mmol) and the appropriate aryl aldehyde (9.43 mmol) in ethanol (95% v/v, 20 mL) at 0–5 °C. After the reaction mixture had reached room temperature, it was stirred for 24 h. Water (20 mL) was subsequently added and the precipitate was collected, washed with ethanol (95% v/v, 5 mL) and recrystallized from ethanol (95% v/v). The melting points and percentage yields were as follows: **5a**: 165–166, 62; **5b**: 210–212, 67; **5c**: 238–240, 65; **5d**: 224–226, 68; **5e**: 208–210, 61; **5f**: 206–208, 52; **5g**: 244–246,

53; **5h**: 162–164, 52. The ^{1}H-NMR spectrum of a representative compound **5g** was as follows: δ ($CDCl_3$): 3.10 (s, 4H, 3-CH_2, 5-CH_2), 3.90 (s, 4H, OCH_2CH_2O), 7.55 (d, 4H, $J = 8.61$ Hz, aryl H), 7.86 (s, 2H, =CH), 8.25 (d, 4H, $J = 8.61$ Hz, aryl H).

6.1.6. Synthesis of series 6

4-Hydroxycyclohexanone (4.0 g, 35.1 mmol), prepared by a literature method [29], was dissolved in chloroform (40 mL). The solution was cooled to 0–5 °C to which was added pyridine (8.32 g, 105.3 mmol) and acetic anhydride (7.16 g, 70.2 mmol). The mixture was stirred at room temperature for 12 h and diluted with water (50 mL). The organic phase was separated, washed with hydrochloric acid (7.3% w/v, 50 mL) and aqueous sodium bicarbonate solution (10% w/v, 50 mL), and evaporation of the solvent afforded 4-acetoxycyclohexanone (4.4 g, ~80%), which was used without further purification.

A mixture of 4-acetoxycyclohexanone (1.0 g, 6.41 mmol), the appropriate aryl aldehyde (13.50 mmol) and hydrochloric acid (36.5% w/v, 0.5 g) was stirred at room temperature for 20 h. Methanol (20 mL) was added and the precipitate was collected and recrystallized from methanol (**6a**–**c**) or a mixture of chloroform and methanol (**6d**). The melting points and percentage yields were as follows: **6a**: 164–165 (lit. [30], m.p. 165 °C, 35; **6b**: 181–182, 40; **6c**: 174–175, 40; **6d**: 168–169, 36. The ^{1}H-NMR spectrum of a representative compound **6c** was as follows: δ ($CDCl_3$): 1.90 (s, 3H, $COCH_3$), 2.35 (s, 6H, aryl CH_3), 3.08–3.18 (m, 4H, 3-CH_2, 5-CH_2), 5.14–5.22 (m, 1H, 4-CH), 7.20 (d, 4H, $J = 7.94$ Hz, aryl H), 7.33 (d, 4H, $J = 8.01$ Hz, aryl H), 7.86 (s, 2H, =CH).

6.1.7. Synthesis of 7b and 7c

A solution of sodium hydroxide (0.1 g, 0.56 mmol) in water (1 mL) was added to a solution of 1,4-cyclohexanedione mono-ethylene ketal (0.7 g, 4.49 mmol) and 2-pyridinecarboxaldehyde (1.0 g, 9.5 mmol) in ethanol (95% v/v, 15 mL) at 0–5 °C. The mixture was stirred at room temperature for 48 h, diluted with water (25 mL) and the precipitate was collected and recrystallized from methanol–chloroform to give **7b**, m.p. 164–165 °C in 41% yield. The 2-thienyl analogue **7c**, m.p. 180–181 °C was prepared in a similar manner in 68% yield. The ^{1}H-NMR spectrum of a representative compound **7b** was as follows: δ ($CDCl_3$): 3.62 (s, 4H, 3-CH_2, 5-CH_2), 3.96 (s, 4H, OCH_2CH_2O), 7.16–7.17 (m, 2H, heteroaryl H), 7.42 (d, 2H, heteroaryl H), 7.67 (m, 4H, heteroaryl H), 8.67 (s, 2H, =CH).

6.1.8. Statistical analyses

The σ, π and MR values used in the statistical analyses were taken from the literature [31]. The individual physicochemical constants for the R^1, R^2 and R^3 groups were added and multiplied by two since two aryl rings are present in series **1**–**6**. The linear and semilogarithmic plots were generated using a commercial software package [32]. The following correlations were noted [physicochemical constant, cytotoxicity screen, linear (l) or semilogarithmic (sl) plots, [P value] namely **1**: σ, P388, l, < 0.01; **1**: σ, P388, sl, < 0.01; **1**: σ, L1210, l, < 0.1; **1**: σ, L1210, sl, < 0.05; **1**: σ, Molt 4/C8, l, < 0.1; **1**: σ, Molt 4/C8, sl, < 0.1; **1**: σ, CEM, l, < 0.1; **1**: σ, CEM, sl, < 0.1; **1**: MR, P388, l, < 0.1; **2**: σ, P388, l, < 0.1; **2**: σ, P388, sl, < 0.05; **2**: MR, Molt 4/C8, l, < 0.01; **2**: MR, Molt 4/C8, sl, < 0.01; **3**: σ, P388, l, < 0.1; **3**: σ, P388, sl, < 0.01; **3**: σ, L1210, l, < 0.1; **3**: σ, L1210, sl, < 0.1; **3**: σ, Molt 4/C8, l, < 0.1; **3**: σ, Molt 4/C8, sl, < 0.05; **3**: σ, CEM, sl, < 0.1; **4**: MR, CEM, l, < 0.1; **6**: σ, P388, l, < 0.05; **6**: σ, P388, sl, < 0.05; **6**: σ, Molt 4/C8, l, < 0.1; **6**: σ, Molt 4/C8, sl, < 0.05; **6:** σ, CEM, l, < 0.05; **6**: σ, CEM, sl, < 0.1.

6.1.9. Molecular modeling studies

Molecular modeling was undertaken using the MacroModel version 4.5 programme [4] for the calculation of the SASA figures, the minimization of the structures of **1a**, **2a**, **3a**, **4a**, **5a** and **6a** and the overlap of these six molecules.

6.2. Bioevaluations

6.2.1. Cytotoxicity determinations

The compounds were evaluated against murine P388D1 cells using a reported procedure [33]. In brief, solutions of at least three different concentrations of compounds in suitable solvents were incubated at 37 °C with the neoplastic cells and the percentage survival noted after 48 h. Control experiments in which the solvents were added to the leukemic cells were also incubated at 37 °C for 48 h. All tests and control experiments were carried out in triplicate at each concentration of the compounds. A similar procedure was employed for the L1210, Molt 4/C8 and CEM assays [34].

Compounds **2b**, **4c**, **5a**, **5f**, melphalan and 5-fluorouracil were evaluated against 56 (53–59) human tumour cell lines using a previously described methodology [13]. In brief, compounds are evaluated using a minimum of five concentrations at tenfold dilutions. The time of the drug exposure to the cells was 48h and a protein assay using sulphorhodamine B was used to measure cell protein content and to estimate cell viability or growth when compared to controls. IC_{50} values were obtained for all cell lines except one ovarian tumour (**2b**), one breast tumour (**5a**), two non-small cell lung, one prostate and one breast neoplasms (**5b**) as well as one non-small cell lung, one renal and one breast cancers in the case of 5-fluorouracil. The number of cell lines in the colon and leukemic subpanels were seven and six,

respectively, except five leukemic cell lines were used in evaluating **5a**.

6.2.2. Antifungal evaluations

The enones **1b**, **c**, **3b**, **c**, **6a** and **6b** were evaluated against three isolates of *A. fumigatus* (ATCC 208995-208997) and one isolate of *C. albicans* (ATCC 90028) using the broth microdilution method [35]. In this assay, the MIC of a reference compound voriconazole was 0.25 $\mu g\ mL^{-1}$.

6.2.3. Toxicity evaluations

Compounds were evaluated for toxicity in mice or rats by a reported methodology [36].

Doses of 30,100 and 300 mg/kg of **1a-g**, **2a**, **c**–**e**, **3a**–**g**, **4a**, **b**, **d**–**f**, **5a**–**h**, **6a**, **b** and **7a**–**c** were injected intraperitoneally into mice and the animals observed after 0.5 and 4 h. After 0.5 h, neurotoxicity was observed by the following compounds (in parentheses are the number of animals displaying neurotoxicity/number of animals in the experiment and the dose in mg/kg): **2a** (2/8, 100; 1/4, 300), **2c** (4/8, 100; 4/4, 300); **3a** (1/4, 30; 1/8, 100; 3/4, 300), **4a** (2/8, 100; 3/4, 300); **5b** (1/8, 100; 2/4, 300); **5c** (1/4, 300); **5e** (1/8, 100; 1/4, 300), **5f** (1/8, 100), **5g** (1/8, 100; 1/4, 300), **7a** (1/8, 100; 1/4, 300), **7b** (2/8, 100; 1/4, 300) and **7c** (1/8, 100; 2/4, 300). After 4 h, neurotoxicity was observed in the following cases, namely **2a** (1/2, 300), **2c** (1/4, 100), **3a** (2/2, 300), **5b** (1/2, 300), **5g** (1/4, 100) and **7b** (1/4, 100).

Evaluation for neurotoxicity after oral dosing to rats was also undertaken using **1a**, **g**, **7a** (30 mg/kg) and **5a**, **d** (50 mg/kg). The animals were observed after 0.25, 0.5, 1, 2 and 4 h, except in the case of **5d**, where the 0.25 and 4 h times were omitted. No toxicity was observed. The Anticonvulsant Screening Program of the National Institute of Neurological Disorders and Stroke, USA requires that all mice and rats be housed, fed and handled in a manner consistent with the recommendations of the National Research Council Publication 'Guide for the Care and Use of Laboratory Animals'. All animals are euthanized in accordance with the policies of the Institute of Laboratory Resources dealing with the humane care of laboratory animals.

Acknowledgements

The following organizations are thanked for their financial support of this study, namely Purdue Neuroscience Company, USA (J. R. D.), National Cancer Institute of Canada (T. M. A.), Flemish Fonds Voor Geneeskundig Weterschappelijk Onderzoek (E. D. C., J. B.) and the National Institute for Neurological Disorders and Stroke (J. P. S.). The National Cancer Institute, USA, is thanked for the evaluations using a panel of human tumour cell lines.

References

[1] J.R. Dimmock, V.K. Arora, S.L. Wonko, N.W. Hamon, J.W. Quail, Z. Jia, R.C. Warrington, W.D. Fang, J.S. Lee, Drug Des. Deliv. 6 (1990) 183–194.
[2] P.N. Craig, J. Med. Chem. 14 (1971) 680–684.
[3] S.N. Pandeya, J.R. Dimmock, An Introduction to Drug Design, New Age International (P) Ltd. Publishers, New Delhi, 1997, p. 94.
[4] MacroModel, version 4.5, Department of Chemistry, Columbia University, NY, August 1994.
[5] C. Hansch, A.J. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979, pp. 52–53.
[6] J.R. Dimmock, W.G. Taylor, J. Pharm. Sci. 64 (1975) 241–249.
[7] J.R. Dimmock, C.B. Nyathi, P.J. Smith, J. Pharm. Sci. 67 (1978) 1543–1546.
[8] C.G. Rousseaux, H.G. Townsend, O.A. Phillips, J.R. Dimmock, Fundam. Appl. Toxicol. 14 (1990) 318–326.
[9] J.R. Dimmock, P. Kumar, A.J. Nazarali, N.L. Motaganahalli, T.P. Kowalchuk, M.A. Beazely, J.W. Quail, E.O. Oloo, T.M. Allen, J. Szydlowski, E. De Clercq, J. Balzarini, Eur. J. Med. Chem. 35 (2000) 967–977.
[10] J.R. Dimmock, M.P. Padmanilayam, R.N. Puthucode, A.J. Nazarali, N.L. Motaganahalli, G.A. Zello, J.W. Quail, E.O. Oloo, H.-B. Kraatz, J.S. Prisciak, T.M. Allen, C.L. Santos, J. Balzarini, E. De Clercq, E.K. Manavathu, J. Med. Chem. 44 (2001) 586–593.
[11] J.R. Dimmock, N.M. Kandepu, A.J. Nazarali, T.P. Kowalchuk, N. Motaganahalli, J.W. Quail, P.A. Mykytiuk, G.F. Audette, L. Prasad, P. Perjési, T.M. Allen, C.L. Santos, J. Szydlowski, E. De Clercq, J. Balzarini, J. Med. Chem. 42 (1999) 1358–1366.
[12] J.R. Dimmock, A. Jha, P. Kumar, G.A. Zello, J.W. Quail, E.O. Oloo, J.J. Oucharek, M.K. Pasha, D. Seitz, R.K. Sharma, T.M. Allen, C.L. Santos, E.K. Manavathu, E. De Clercq, J. Balzarini, J.P. Stables, Eur. J. Med. Chem. 37 (2002) 35–44.
[13] M.R. Boyd, K.D. Paull, Drug Dev. Res. 34 (1995) 91–109.
[14] J.R. Dimmock, O.A. Phillips, S.L. Wonko, R.A. Hickie, R.G. Tuer, S.J. Ambrose, R.S. Reid, B. Mutus, C.J. Talpas, Eur. J. Med. Chem. 24 (1989) 217–226.
[15] J.R. Dimmock, S.C. Vashishtha, S.A. Patil, N. Udupa, S.B. Dinesh, P.U. Devi, R. Kamath, Pharmazie 53 (1998) 702–706.
[16] W.O. Foye, S.K. Sengupta, in: W.O. Foye, T.L. Lemke, D.A. Williams (Eds.), Principles of Medicinal Chemistry, 4th ed., Williams and Wilkins, Baltimore, MD, 1995, p. 833.
[17] E.M. Greenspan, H.W. Bruckner, in: E.M. Greenspan (Ed.), Clinical Cancer Chemotherapy, Raven Press, New York, 1975, p. 37.
[18] J.R. Dimmock, A.M. Qureshi, L.M. Noble, P.J. Smith, H.A. Baker, J. Pharm. Sci. 65 (1976) 38–43.
[19] J.R. Dimmock, N.W. Hamon, K.W. Hindmarsh, D.G. Mills, L.E. Negrave, G.H. Rank, A.J. Robertson, J. Pharm. Sci. 65 (1976) 482–488.
[20] E.K. Manavathu, J.R. Dimmock, S.C. Vashishtha, P.H. Chandrasekar, J. Antimicrob. Chemother. 47 (2001) 491–494.
[21] K.M. Duncan, G. Ogawa, U. Clibon, in: E.T. Herfindal, D.R. Gourley (Eds.), Textbook of Therapeutics, 7th ed., Lippincott Williams and Wilkins, Philadelphia, PA, 2000, pp. 1699–1710.
[22] J.R. Dimmock, S.S. Jonnalagadda, O.A. Phillips, E. Erciyas, K. Shyam, H.A. Semple, J. Pharm. Sci. 81 (1992) 436–440.
[23] M.S. Dunham, T.A. Miya, J. Am. Pharm. Assoc. Sci. Ed. 46 (1957) 208–209.
[24] P.J. Smith, J.R. Dimmock, W.A. Turner, Can. J. Chem. 51 (1973) 1458–1470.
[25] P.S. Engel, A. Kitamura, D.E. Keys, J. Org. Chem. 52 (1987) 5015–5021.

[26] H. Frey, Synthesis (1992) 387–90.
[27] D.B. Denney, S.T. Ross, J. Org. Chem. 27 (1962) 998–1000.
[28] A. Srikishna, P.P. Kumar, R. Viswajanani, Tetrahedron Lett. 37 (1996) 1683–1686.
[29] M. Haslanger, R.G. Lawton, Synth. Commun. 4 (1974) 155–159.
[30] J.B. Aldersley, G.N. Burkhardt, A.E. Gillam, N.C. Hindley, J. Chem. Soc. (1940) 10–6.
[31] C. Hansch, A.J. Leo, Substituted Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979, p. 47, 49.
[32] Statistical Package for Social Sciences, SPSS for Windows, Standard Version, Release 10.0.5, SPSS Inc., Chicago, IL, 1999.
[33] O.A. Phillips, L.A. Nelson, E.E. Knaus, T.M. Allen, R. Fathi-Afshar, Drug Des. Deliv. 4 (1989) 121–127.
[34] J. Balzarini, E. De Clercq, M.P. Mertes, D. Shugar, P.F. Torrence, Biochem. Pharmacol. 31 (1982) 3673–3682.
[35] National Committee for Clinical Laboratory Standards, Publications M38-P and M27-A, NCCLS, Wayne, PA, 1997.
[36] J.P. Stables, H.J. Kupferberg, in: G.A. Vanzini, P. Tanganelli, M. Avoli (Eds.), Molecular and Cellular Targets for Antiepileptic Drugs, John Libbey and Company Ltd, London, 1997, pp. 191–198.