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Original article

Cytotoxic 1,3-diarylidene-2-tetralones and related compounds

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Abstract

A number of 1,3-arylidene-2-tetralones **1**, **2** and **4** were synthesised and demonstrated cytotoxic activity towards murine P388 and L1210 cells as well as human Molt 4/C8 and CEM T-lymphocytes. In general, the related 1-arylidene-2-tetralones **3** possessed lower potencies in these screens than the compounds in series **1** and **4**. Approximately, half of the compounds were evaluated against a panel of human tumour cell lines. In this screen, most of the enones were more cytotoxic than the established anticancer agent melphalan and some demonstrated selective toxicity towards leukemic and colon cancer cells. The modes of action of representative compounds include interfering with the biosyntheses of nucleic acids and proteins as well as altering redox potentials. The compounds were well tolerated when administered intraperiteonally to mice. Thus these novel enones are promising prototypic molecules due to their potent cytotoxic properties and lack of significant murine toxicity.

Keywords: 2-Tetralones; Cytotoxicity; Murine toxicity; Redox potentials

1. Introduction

Previous studies have revealed the promising cytotoxic properties of various 2,6-bis(arylidene)cyclohexanones [1] and 3,5-bis(arylidene)-4-piperidones [2]. Both groups of compounds contain the 1,5-diaryl-1,4-dien-3-oxo group. These compounds were designed as thiol alkylators, since α,β-unsaturated ketones have an affinity for thiols in contrast to amino and hydroxy groups [3] which are found in nucleic acids. Hence enones may lack genotoxic properties. In addition, a number of cytotoxics such as tamoxifen [4] and various combretastatins [5] are stilbenes and the incorporation of this structural motif into the compounds under consideration was planned. Such observations led to the decision to incorporate the 1,5-diaryl-1,4-dien-3-oxo and stilbene moieties into series **1** and **2** (Figs. 1 and 2).

There was however a further reason for preparing series **1**. A number of studies have shown that lowering the concentrations of chemoprotectant thiols in cells prior to the administration of anticancer drugs is more deleterious to cancer cells than normal tissue [6,7]. The compounds in series **1** and **2** have two olefinic centres in different electronic and steric environments permitting the sequential attack of thiols. In other words, an initial thiol alkylation could occur followed by a subsequent interaction with thiols at the second methine group of **1** and **2**. If such a process is important in eliciting cytotoxic activity, the monoarylidene compounds **3** should have significantly less than half of the potencies

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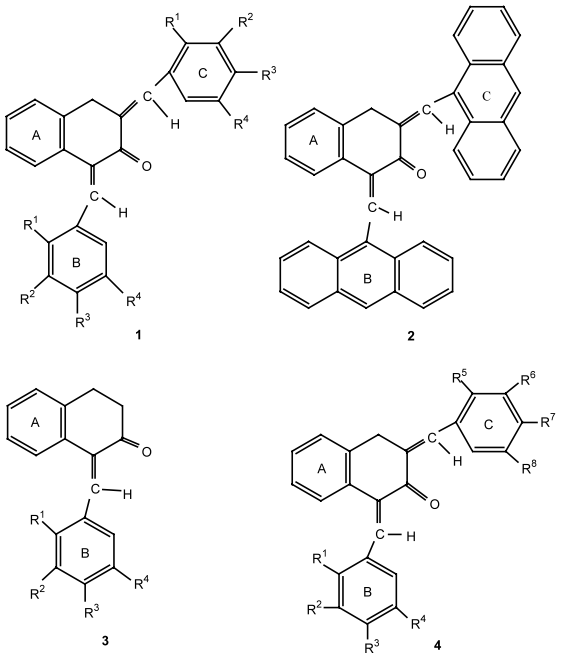

Fig. 1. The structures of the compounds in series **1**–**4**. The aryl substitution patterns are indicated in Table 1.

of the analogues in series **1**. Furthermore, in order to exacerbate the magnitude of the differences between the fractional positive charges on the olefinic carbon atoms, series **4** was proposed in which the substituents in rings B and C of each compound were divergent.

The choice of substituents in series **1** was predicated by the following considerations. A variety of groups were placed in the aryl rings with varying σ and π values; in fact, substituents in all four quadrants of a Craig plot for *para* substituents [8] were employed. In addition to a consideration of the σ and π values of the aryl substituents, groups of varying sizes were inserted into rings B and C in series **1**. The bioevaluations of the tetralones **1a**–**c**,**g**,**j** permit a Topliss analysis [9] which may indicate the structure of further analogues with increased potencies. Compound **1k** possesses a 3,4,5-trimethoxyphenyl group which is present in various combretastatins [5] and some naturally occurring antineoplastic agents such as podophyllotoxin [10]. In the case of the 3,5-bis(arylidene)-4-piperidones, the highest potencies were found in the compound possessing a 4-nitro substituent [2] which may have been due to reduction to one or more toxic species [11]. Consequently, in **1p**–**r**, a nitro group was placed in different positions of the aryl rings. Various nitrophenols cause uncoupling of oxidative phosphorylation [12] and hence the aryl substitution pattern of **1s** was suggested.

Compound **2** was designed in order to evaluate the effect on potency of attaching groups at the *ortho* and *meta* positions of rings B and C of **1a**. The choice of substituents in ring B of series **3** was made for similar reasons as those indicated for the enones **1**.

The evaluation of representative compounds for anticonvulsant activity and neurotoxicity was also planned. Compounds which penetrate the central nervous system (CNS) could be of value in treating tumours of the CNS; conversely, such compounds may have unwanted side effects.

In summary, the aims of the present investigation were to prepare a series of bis and mono arylidene 2-tetralones as candidate antineoplastic agents coupled to an evaluation of their murine toxicity.

2. Chemistry

Acid catalysed condensation was undertaken between the appropriate aryl aldehyde and either 2-tetralone

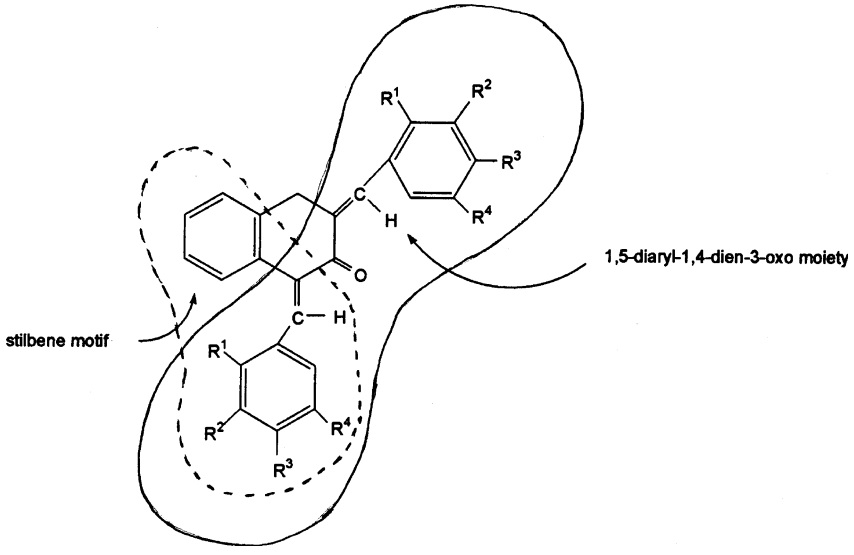

Fig. 2. The 1,5-diaryl-1,4-dien-3-oxo and stilbene portions of series **1** and **4**.

leading to series **1** and **2** or a monoarylidene-2-tetralone which gave rise to series **4**. The enones **3** were synthesised by the Knoevenagel method. ^{1}H-NMR spectroscopy revealed the stereohomogeneity of the molecules. X-ray crystallography of **1b**,**c**,**g**,**j** and **3h** indicated that the configuration of the olefinic bonds was E and the assumption was made that this stereochemistry was displayed in the analogues of these compounds which is consistent with studies involving 2,6-bis(arylidene)cyclohexanones [1,13], a 2-arylidenecyclohexanone [14] and 3,5-bis(arylidene)-4-piperidones [2]. The redox potentials of **1a**,**k**,**n**,**p**,**q**,**r**,**t**, **3a**,**g** and **4b** were determined; these data are portrayed in Table 4.

3. Bioevaluations

All of the compounds in series **1**–**4** were evaluated for cytotoxic activity against murine P388 and L1210 leukemic cells as well as human Molt 4/C8 and CEM T-lymphocytes. These data are presented in Tables 1 and 2. Approximately, half of the compounds were examined for their effect on a panel of human tumour cell lines and the results obtained are portrayed in Table 7. The inhibitory effects of **1a**,**p**,**r**, **3a**,**g** on DNA, RNA and protein biosyntheses in L1210 cells was undertaken and the results are presented in Table 8. In addition, **1a**,**p**, **3a**,**g** were evaluated for DNA binding properties while the antifungal properties of **1b**, **3b** and **3f** were assessed against *Aspergillus fumigatus* and *Candida albicans*. Approximately, two-thirds of the compounds were examined for anticonvulsant activity and neurotoxicity in mice.

4. Results and discussion

All of the compounds in series **1**–**4** were assessed against P388, L1210, Molt 4/C8 and CEM cells; these data are portrayed in Tables 1 and 2. Approximately, 40–50% of the compounds had one-fifth or more of the potency of the established antineoplastic alkylating agent melphalan. In particular, the following compounds possessed equal or greater cytotoxicity than melphalan (cell line in parentheses), namely **1q** (P388), **1d**–**f**,**h**,**k**,**p**, **4a**–**c** (L1210), **1a**,**b**,**d**–**f**,**h**,**i**,**k**,**l**,**o**,**p**, **4a**–**c** (Molt 4/C8), and **1a**,**b**,**d**–**f**,**h**,**i**,**k**,**l**,**p**, **4a**–**c** (CEM). These comparisons revealed that a greater number of compounds displayed equal or superior activity to melphalan in the T-lymphocyte screens rather than towards murine cell lines. In addition, the average IC_{50} figures towards all four cell lines were computed. The most potent compounds (average IC_{50} value in μM in parentheses) were **1e** (1.17), **1h** (1.28), **1p** (1.46), **4a** (2.01), **4b** (1.53) and **4c** (1.70) which compare favourably with melphalan (2.02). The conclusion to be drawn from this overview is that the general structures **1** and **4** are clearly promising novel molecular templates.

Some comments will be made regarding the potencies of the 1,3-bis(arylidene)-2-tetralones **1** and **4** in relation to the 2,6-bis(arylidene)cyclohexanones and 3,5-bis(arylidene)-4-piperidones mentioned earlier. In the first place, this study has provided further evidence that the 1,5-diaryl-1,4-dien-3-oxo-group is an important toxophore thereby providing a justification for its incorporation into candidate cytotoxic and anticancer agents. Secondly, in the case of the 2,6-bis(arylidene)cyclohexanones, in general different substituents were placed in each of the two aryl rings. Hence a comparison of the IC_{50} values of these compounds with the enones **1** was not possible. However, the average IC_{50} values of 13 2,6-bis(arylidene)cyclohexanones in the four screens was 27.0 (4.29–283) μM while for the compounds **1a**–**l**,**o**–**t** and **4a**–**c** the figure was 9.81 (1.17–47.0) μM. (The esters **1m** and **1n** were omitted from consideration due to a lack of precise IC_{50} figures in some of the screens.) Hence one may tentatively draw the conclusion that the presence of ring A in series **1** and **4** may contribute to cytotoxicity by creating van der Waals' bonds at a binding site. The average IC_{50} of seven 3,5-bis(arylidene)-4-piperidones was 60.1 (3.03–174) μM [2]. The same aryl substitution was present in **1a**–**d**,**j**,**r**,**t** for which the median IC_{50} value was 17.9 (2.11–47) μM. The increase in potencies of the analogues in series **1** compared to the 4-piperidones may be due to the presence of ring A in **1** as well as the absence of a basic centre in the tetralones.

The next phase of the investigation was directed to evaluating whether cytotoxicity was correlated with one or more physicochemical properties of the aryl substituents in series **1** and **3**. Linear and semilogarithmic plots were obtained between the Hammett σ and/or Taft σ^* values, the Hansch π figures and molar refractivity (MR) constants, which reflect the electronic, hydrophobic and steric properties of the aryl groups, respectively, and the IC_{50} concentrations generated for each cell line. Compounds **1m**,**n** and **3f** were omitted from this evaluation since specific IC_{50} concentrations were not available in each test. Negative correlations were established between the σ/σ^* constants and cytotoxicity in the P388 screen for the compounds in series **1** when both linear ($P < 0.001$) and semilogarithmic ($P < 0.001$) plots were made. No other correlations were noted in the other screens for both series **1** and **3**, suggesting that, in general, one single physicochemical parameter is unlikely to contribute significantly to bioactivity.

During the investigation, the cytotoxicity of **2** was determined. The data in Table 1 reveal that the potency of this compound was substantially lower than **1a** suggesting that the attachment of bulky groups at the *ortho* and *meta* positions of rings B and C was

Table 1
Cytotoxicity of the compounds in series **1**–**3** against murine P388 and L1210 cells as well as Molt 4/C8 and CEM T-lymphocytes

Compound	R^1	R^2	R^3	R^4	IC_{50} (μM)			
					P388	L1210	Molt 4/C8	CEM
1a	H	H	H	H	1.79±0.2	6.23±0.10	5.38±2.70	2.44±0.59
1b	H	H	Cl	H	1.20±0.03	2.49±0.24	2.45±0.62	2.30±0.08
1c	H	Cl	Cl	H	0.849±0.05	41.4±1.3	47.0±0.9	50.1±0.9
1d	H	H	F	H	1.04±0.04	4.40±2.72	1.71±0.06	1.74±0.18
1e	H	F	H	F	0.281±0.03	1.67±0.15	1.44±0.05	1.30±0.16
1f	H	H	Br	H	0.548±0.01	3.86±2.04	3.70±1.94	4.67±3.04
1g	H	H	CH_3	H	4.00±0.2	45.0±1.2	46.2±7.6	40.0±4.0
1h	H	H	CF_3	H	0.471±0.02	1.26±0.40	1.70±0.03	1.69±0.06
1i	H	H	OH	H	3.55±0.34	7.87±0.01	1.63±0.09	2.00±0.06
1j	H	H	OCH_3	H	3.85±0.08	38.4±6.1	8.76±1.69	9.59±0.28
1k	H	OCH_3	OCH_3	OCH_3	0.693±0.03	4.88±3.21	1.64±0.09	1.65±0.03
1l	H	H	$OCOCH_3$	H	4.58±0.32	8.56±0.13	1.98±0.01	3.15±1.09
1m	H	H	$OCOCH{=}CHC_6H_5$	H	>50±1.8	>500	>500	>500
1n	H	H	$COOCH_3$	H	1.48±0.23	431±98	>500	>500
1o	H	H	$COOC_3H_5$	H	0.732±0.04	6.05±0.49	4.77±1.88	6.20±0.36
1p	NO_2	H	H	H	0.98±0.06	1.85±0.03	1.62±0.15	1.38±0.07
1q	H	NO_2	H	H	0.187±0.03	14.5±9.1	6.66±1.65	7.15±1.27
1r	H	H	NO_2	H	0.508±0.04	40.9±5.78	16.6±6.37	23.3±5.81
1s	H	NO_2	OH	H	1.02±0.06	12.1±1.3	17.0±8.9	18.6±5.7
1t	H	H	$N(CH_3)_2$	H	3.79±0.25	61.8±23.6	50.2±23.3	72.2±19.0
2	–	–	–	–	>50±1.3	323±4	>500	349±31
3a	H	H	H	H	14.6±0.92	44.4±2.2	39.0±1.5	39.2±5.6
3b	H	H	Cl	H	7.00±0.93	39.6±1.3	31.3±2.1	25.4±8.8
3c	H	Cl	Cl	H	3.57±0.38	38.8±0.7	14.2±2.3	25.4±12.7
3d	H	H	CH_3	H	7.15±1.12	45.9±1.0	36.0±0.6	43.8±2.3
3e	H	H	OCH_3	H	7.49±0.34	50.0±1.8	32.9±6.5	18.7±3.0
3f	H	H	$OCOCH{=}CHC_6H_5$	H	17.6±0.9	436±90	>500	401±48
3g	NO_2	H	H	H	1.93±0.43	6.42±0.36	7.12±1.17	5.95±0.06
3h	H	H	NO_2	H	12.3±0.6	39.4±0.0	34.0±5.9	25.5±14.8
3i	H	H	$N(CH_3)_2$	H	7.03±0.07	52.3±3.3	41.2±1.8	30.4±3.0
3j	H	H	COOH	H	19.3±0.3	67.4±3.9	64.4±15.4	50.6±4.6
3k	H	OCH_3	OCH_3	OCH_3	4.79±0.4	27.7±12.7	7.80±0.13	8.21±0.24
Melphalan [a]	–	–	–	–	0.22±0.01	2.13±0.03	3.24±0.79	2.47±0.30

[a] These data are reproduced from Ref. [1], p. 970.

disadvantageous. Attention was therefore directed to the influence of *para* substituents in rings B and C.

The possibility exists that the aryl ring B (in series **1** and **3**) and also ring C in series **1** align at complementary areas on binding sites. Thus the presence of substituents in these rings could increase potencies compared to the unsubstituted compound **1a** by interacting with cellular constituents at an auxiliary binding site adjacent to the locus of action with the aryl rings B and C. Alternatively, the aryl substituents could impede alignment of other parts of the molecule with a binding site. In order to explore this possibility, two approaches were used involving those compounds possessing a single *para* substituent. In the first place, linear and logarithmic plots between the IC_{50} values of **1b**,**d**,**f**–**j**,**l**,**o**,**r**,**t** as well as **3b**,**d**,**e**,**h**–**j** for each cell line and the MR figures of the R^3 substituents were made in each series (**1m**,**n**, **3f** were eliminated from consideration since specific IC_{50} values were not available in each screen). No correlations ($P > 0.1$) were observed. Second, the IC_{50} values of each of

Table 2
Evaluation of the cytotoxicity of **4a**–**c** against murine P388 and L1210 cells and human Molt 4/C8 and CEM T-lymphocytes

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50} (μM)			
									P388	L1210	Molt 4/C8	CEM
4a	H	H	NO_2	H	H	H	Cl	H	0.454±0.02	4.18±2.16	1.72±0.03	1.70±0.01
4b	H	H	NO_2	H	H	OCH_3	OCH_3	OCH_3	1.06±0.2	1.94±0.12	1.56±0.01	1.54±0.08
4c	H	OCH_3	OCH_3	OCH_3	H	H	NO_2	H	0.383±0.03	3.31±2.21	1.55±0.08	1.55±0.20

the compounds **1b**,**d**,**f**–**j**,**l**–**o**,**r**,**t** and **3b**,**d**–**f**,**h**–**j** in the four cytotoxicity assays were compared with the IC_{50} figures of the unsubstituted compounds, namely **1a** and **3a**, respectively. In the case of the thirteen analogs in series **1**, compounds with a single *para* substituent were more potent than **1a** in 6, 3, 4 and 1 cases when the P388, L1210, Molt 4/C8 and CEM data, respectively, were considered, i.e. overall in 27% of the comparisons made. When the seven enones in series **3** were examined, greater activity was found in 5, 2, 3 and 2 compounds when comparisons with **3a** were made in the four screens, i.e. overall in 39% of the comparisons made. The conclusion to be drawn from these evaluations is that, in general, when only topological features of the molecules are considered, substitution in the *para* location of rings B and C decreases cytotoxic potencies. This observation suggests that sites of steric inhibition may be adjacent to the *para* position of the aryl rings.

An examination was made of the theory that compounds possessing two sites for thiol alkylation should have an enhanced capacity to kill malignant cells compared to analogues causing a single chemical attack with critical biomacromolecules. In other words, the 2-tetralones in series **1** (two alkylation sites) should be more than twice as potent as the related compounds in series **3** (one alkylation site). Comparisons were therefore made between the potencies of the following pairs of compounds which had the same aryl substituents, namely **1a/3a**, **1b/3b**, **1c/3c**, **1g/3d**, **1j/3e**, **1k/3k**, **1p/3g**, **1r/3h** and **1t/3i** in each screen. No comparisons were made between **1m** and **3f** since specific IC_{50} concentrations were not available in every assay. The potencies of the compounds in series **1** were more than twice those of the analogs in series **3** in 5/9, 4/9, 5/9 and 4/9 of the cases when data in the P388, L1210, Molt 4/C8 and CEM screens, respectively, were considered. Overall the theory was validated in 50% of the comparisons although greater potencies were displayed by the analogs in series **1** than **3** in 69% of the cases. These observations suggest that the mode of action of the compounds in series **1** is not due exclusively to sequential interaction with cellular thiols although this pathway may be followed in some cases.

Series **4**, in which the groups in rings B and C are different, was prepared in order to examine the hypothesis that substantial divergences between the electronic properties of the substituents in rings B and C were greater contributors to cytotoxicity than the magnitude of the σ/σ^* values of the aryl substituents. The electronic nature of the R^1–R^4 substituents in ring B and R^5–R^8 groups in ring C for **4a**–**c** as well as the relevant data for the related compounds **1b**, **1r** and **1k** are presented in Table 3. If the potencies of **4a**–**c** were greater than **1b** and **1k**, the result could be attributed to two indistinguishable features, namely the greater magnitude of the σ/σ^* figures of the aryl substituents in **4a**–**c** as well as the greater divergence of the σ/σ^* values in rings B and C in **4a**–**c**. Hence comparisons were made only between the IC_{50} values of **4a**–**c** ($\Sigma\ \sigma/\sigma^* = 1.70$ or 2.00) with **1r** ($\Sigma\ \sigma/\sigma^* = 2.54$) in the four cytotoxicity screens. Of the comparisons made, **4a** > **1r**, **4b** > **1r** and **4c** > **1r** using the L1210, Molt 4/C8 and CEM cell lines while in the P388 screen, **4a** = **1r**, **1r** > **4b** and **4c** > **1r**. Thus the hypothesis was validated in 10 of the 12 comparisons made (83%). Bearing in mind the very high potencies of **4a**–**c**, the expansion of this series of compounds in which the differences between the electronic properties of the aryl substituents are exaggerated still further is clearly warranted.

Table 3
The σ and σ^* values of different atoms and groups in rings B and C of **4a**–**c** and **1b**,**r**,**k**

Compound	Combined σ and σ^* values		
	Ring B [a]	Ring C [b]	Total
4a	1.27	0.73	2.00
4b	1.27	0.43	1.70
4c	0.43	1.27	1.70
1b	0.73	0.73	1.46
1r	1.27	1.27	2.54
1k	0.43	0.43	0.86

[a] The figures refer to the R^1–R^4 atoms and groups.
[b] These values are for the R^5–R^8 atoms and groups for **4a**–**c** and R^1–R^4 atoms and groups in **1b**,**r**,**k**.

Analyses of the data for **1a**–**c**,**g**,**j** and **3a**–**e** using a potency order table [9] did not reveal any parameter dependencies. The application of the decision tree approach [15] using the available data for **1a**–**c**,**g** as well as **3a**–**c** suggested that the synthesis of further compounds in series **1** and **3** should possess strongly electron-attracting substituents in the aryl rings in order for potency to be increased.

The insertion of two additional methoxy groups to **1j** leading to **1k** was accompanied by an approximately sixfold increase in cytotoxicity in each of the four cell lines suggesting the importance of the 3,4,5-trimethoxyphenyl motif in these clusters of compounds. In fact **1k** was more potent than melphalan when the two human T-lymphocytes were considered. The three structurally isomeric mononitro compounds **1p**–**r** displayed good activity towards P388 cells. However, only the 2-nitro analogue **1p** was markedly toxic to the other three cell lines. In general, the order of activity was **1p** > **1q** > **1r**. Insertion of a hydroxy group into **1q** to give a potential mitochondrial poison **1s** was accompanied by a reduction in cytotoxicity in three of the four cell lines.

Previous studies have revealed correlations between the redox potentials of different chalcones and cytotoxicity [16]. In the present case, compounds **1a**,**k**,**n**,**p**,**q**,**r**,**t**, **3a**,**g** and **4b** were chosen which have a wide range of IC_{50} values in the four screens. Their redox potentials are

Table 4
Redox potentials of **1a**,**k**,**n**,**p**,**q**,**r**,**t**, **3a**,**g** and **4b**

Compound	Aryl substituents	Redox potential (mV)
1a	H	1759
1k	3,4,5-$(OCH_3)_3$	1573
1n	4-$COOCH_3$	1764
1p	2-NO_2	1860
1q	3-NO_2	1834
1r	4-NO_2	1780
1t	4-$N(CH_3)_2$	1930
3a	H	1845
3g	2-NO_2	1950
4b	4-NO_2/3,4,5-$(OCH_3)_3$	1518

portrayed in Table 4. A comparison between the unsaturated ketones in series **1** and **3** possessing the same aryl substituents, namely **1a** and **3a**, as well as **1p** and **3g**, indicated that the compound in series **1** had greater reducing properties and higher potencies. In order to evaluate the generality of a possible cytotoxicity/redox potential relationship, linear and logarithmic plots were made between the IC_{50} values of **1a**,**k**,**p**,**q**,**r**,**t**, **3a**,**g** and **4b** for each cell line and the redox potentials. A positive correlation was noted in the case of Molt 4/C8 cells ($P = < 0.1$ in both the linear and logarithmic plots) and a positive trend was noted with CEM cells ($P =$ 0.105 and 0.106 in the linear and logarithmic plots, respectively). No other correlations were noted. These data indicate that the role of this physicochemical parameter in mediating cytotoxicity is dependent upon the cell line under consideration.

The structures of **1b**,**c**,**g**,**j** and **3h** were determined by X-ray crystallography. In all cases the E stereochemistry pertaining to the olefinic double bonds was noted. In the present study, the lack of coplanarity between the planes of rings B and C with the adjacent olefinic groups as determined by X-ray crystallography were found and designated θ_1 and θ_2, respectively. An ORTEP diagram [17] of a representative compound **1g** is presented in Fig. 3; the θ_1 and θ_2 values are the torsion angles C1–C11–C12–C13 and C3–C18–C19–C20, respectively. The average figures for θ_1 and θ_2 in **1b**,**c**,**g**,**j** were 40.7 and 25.3, respectively. The larger θ_1 values may have been due to the non-bonded interactions between the protons on carbon atoms 9 and 13. The θ_1 value of **3h** is similar in magnitude to the figures obtained in the 1,3-diarylidene-2-tetralones **1b**,**c**,**g**,**j** indicating that the 3-arylidene group has little or no effect upon the topography of the 1-arylidene substituent. The negative signs in Table 5 indicate that the torsion angles are in an anticlockwise direction. The enone **1c** possesses an unsymmetrical aryl substitution pattern and the X-ray crystallographic data revealed that the chloro atoms are located on carbon atoms 15, 16, 22 and 23.

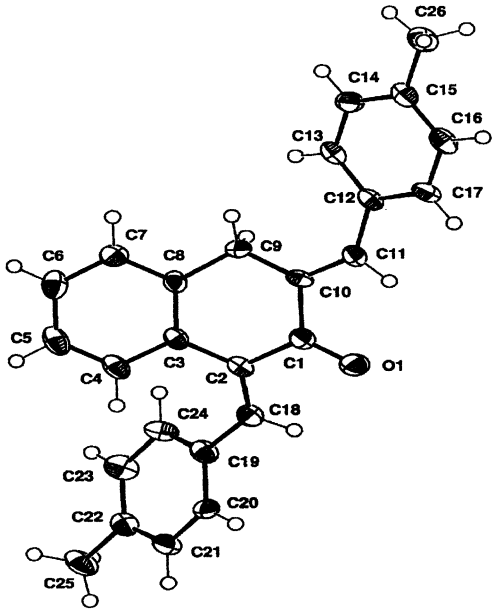

Fig. 3. ORTEP diagram of **1g**.

Table 5
Torsion angles θ_1 and θ_2 (esd) of **1b**,**c**,**g**,**j** and **3h** determined by X-ray crystallography and some relative potency scores

Compound	θ_1 (°)	θ_2 (°)	Relative potency score [a]
1b	−44.1 (3)	−46.1 (3)	15
1c	41.5 (7)	−13.8 (7)	8.5
1g	43.7 (7)	−10.1 (8)	7
1j	−33.4 (7)	31.3 (8)	9.5
3h	−42.5 (12)	–	–

[a] The relative potency scores were obtained by allocating 4, 3, 2 and 1 points for the relative potencies for the four compounds in each screen, i.e. 4 for the most potent analogue, 3 for the next most cytotoxic compound and so forth. Standard deviations of the IC_{50} figures were taken into consideration. The total points available in each screen were invariably 10.

A number of investigators have described correlations between bioactivity and the torsion angles between aryl rings and adjacent unsaturated groups [18–20]. In the present investigation, the relative potencies of **1b**,**c**,**g**,**j** in each cell line were computed, allocating a score of 4 to the most potent compound, 3 to the next in terms of potency, then 2 and finally 1 to the compound displaying the lowest bioactivity. A maximum of 10 points was available for the four compounds when each cell line was considered. The standard deviations of the IC_{50} values were taken into consideration in calculating the relative potency scores. Thus if the IC_{50} figures of the two least potent compounds in a screen were indistinguishable statistically, each compound would be allocated a score of 1.5 since three points were available for the two least cytotoxic analogues. The data in Table 5 indicate that the order of potencies of the four compounds was **1b** > **1j** > **1c** > **1g**. It is of interest to

note the relative potencies of **1b**,**c**,**g**,**j** was positively correlated with the θ_2 figures while no correlation between cytotoxicity and θ_1 values was detected. The relative potency scores revealed the marked superiority of the cytotoxic properties of **1b** compared to the three analogues **1c**,**g**,**j**. The figures in Table 5 reveal that the orientation of both aryl rings in **1b**,**c**,**g**,**j** were similar only in the case of **1b** which may be a significant contributor to its potency. For example, potent cytotoxicity may be dependent on the specific orientations of both aryl rings in series **1** fitting into clefts on a binding site. Thus expansion of this series of compounds in which *ortho* substituents are placed in the aryl rings may lead to molecules having the same orientations and potent cytotoxicity.

A further experiment was conducted with small groups of compounds in order to obtain an estimate of the electron densities at the predicted sites of thiolation, namely the olefinic carbon atoms adjacent to rings B and C, and were designated the C_B and C_C atoms, respectively. Both PM3 and Mulliken charges were obtained and the data are presented in Table 6.

The following conclusions may be drawn from these results. First, differences between the electron densities on the C_B and C_C atoms exist in these compounds. In series **1** and **4**, thiolation would likely occur at the olefinic carbon atom bearing the lower electron density which was either the C_B and C_C atoms depending on the compound using PM3 calculations while the Mulliken charges indicated the C_C atoms in most cases. Second, the differences in atomic charges between the C_B and C_C atoms are generally greater in the compounds in series **4** than **1** as predicted. Third, the differences in electron densities of the C_B atoms in **1** and **3** were noted when the same aryl substituents were present in ring B. The disparities between **1b** and **3b**, **1k** and **3k** and **1r** and **3h** were 0.029, 0.024 and 0.036 esu, respectively (PM3 charges) and 0.041, 0.029 and 0.046 esu, respectively (Mulliken calculations). These data indicate that the 3-arylidene group in series **1** exerts an electron-attracting influence on the C_B atoms. Finally, there were no correlations between the atomic charges and the σ/σ^* values which may have been due to the strong resonance effects present in these molecules. This observation may explain, at least in part, the lack of positive correlations between the σ/σ^* figures and cytotoxicity vide supra.

In view of the promising cytotoxic activity of many of these compounds in these four screens, representative enones were evaluated against a panel of 55–60 human tumour cell lines from nine different neoplastic diseases, namely leukemia and melanoma, as well as non-small cell lung, colon, CNS, ovarian, renal, prostate and breast cancers. The number of cell lines in each subpanel of a specific type of cancer varied ranging from two in the case of prostate cancer to nine when non-small cell lung tumours were used. These results are portrayed in Table 7. The data revealed that without exception, the compounds were more potent than the reference drug melphalan when all cell lines are considered. Of particular note is the observation that **4b** possesses 40 times the potency of this clinically useful drug. The average IC_{50} values of the compounds in series **1**, **3** and **4** were 4.93, 7.06 and 1.59 μM, respectively, which are substantially lower than the figures for the two reference drugs. Although not all compounds in each of the series **1**, **3** and **4** were evaluated against the human tumour cell lines, the average IC_{50} figures suggest that the order of potencies were **4** > **1** > **3** which is a similar observation to the data presented in Table 1.

Previous studies from this laboratory revealed the selective toxicity of a variety of enones towards leukemic and/or colon cancer cell lines [1,21]. In the present study, a selectivity index (SI) figure was calculated for each compound whereby the IC_{50} values towards either leukemic or colon cancer cells were compared with the potencies towards all cell lines. Compounds displaying

Table 6
Atomic charges at the olefinic carbon atoms of **1a**,**b**,**k**,**r**, **3b**,**h**,**k** and **4a**–**c**

Compound	PM3 charges			Mulliken charges			σ/σ^* Values [a]	
	C_B	C_C	C_C–C_B	C_B	C_C	C_C–C_B	Ring B	Ring C
1a	−0.020	−0.014	−0.006	−0.105	−0.095	−0.010	0.49	0.49
1b	−0.006	−0.019	0.013	−0.106	−0.096	−0.010	0.73	0.73
1k	−0.012	−0.028	0.016	−0.114	−0.097	−0.017	0.43	0.43
1r	−0.032	−0.038	0.006	−0.115	−0.103	−0.012	1.27	1.27
3b	−0.035	–	–	−0.147	–	–	0.73	–
3h	−0.068	–	–	−0.161	–	–	1.27	–
3k	−0.036	–	–	−0.143	–	–	0.43	–
4a	−0.042	−0.006	−0.036	−0.120	−0.090	−0.030	1.27	0.73
4b	−0.051	−0.020	−0.031	−0.133	−0.097	−0.036	1.27	0.43
4c	−0.011	−0.053	0.042	−0.103	−0.114	0.011	0.43	1.27

[a] The σ/σ^* values are for the substituents R^1–R^4 in ring B in series **1**, **3** and **4** and for ring C, these figures are of the R^1–R^4 groups in series **1** and the R^5–R^8 substituents in series **4**.

Table 7
Evaluation of various compounds against a panel of human tumour cells lines [a]

Compound	All cell lines	Leukemic cells		Colon cancer cells	
	IC_{50} (μM)	IC_{50} (μM)	SI [b]	IC_{50} (μM)	SI [b]
1a	2.24	1.58	1.4	1.90	1.2
1b	2.09	2.20	1.0	1.83	1.1
1c	10.23	2.30	4.5	8.91	1.2
1d	2.75	2.37	1.2	2.29	1.2
1f	2.69	1.91	1.4	2.37	1.1
1i	2.95	1.45	2.0	2.17	1.4
1j	14.79	6.22	2.4	7.43	2.0
1l	3.47	1.14	3.0	3.32	1.1
1p	1.35	0.528	2.6	1.10	1.2
1r	2.34	1.90	1.2	1.92	1.2
1s	9.33	4.49	2.1	8.43	1.1
3c	11.75	6.37	1.8	12.88	0.9
3g	5.25	3.91	1.3	4.41	1.2
3k	4.17	1.94	2.2	3.48	1.2
4a	2.45	1.01	2.4	2.10	1.2
4b	0.589	0.301	2.0	0.372	1.6
4c	1.74	0.482	3.6	1.17	1.5
Melphalan	23.5	4.65	5.1	44.6	0.5
5-Fluorouracil	32.6	27.6	1.2	7.90	4.1

[a] A total of 55–60 cell lines were used in this screen from nine different groups of neoplasms namely leukemia, melanoma and non-small cell lung, colon, CNS, ovarian, renal, prostate and breast cancers. The data for melphalan and 5-fluorouracil were taken from Ref. [1], p. 971.
[b] SI, selectivity index, i.e. the IC_{50} values for all cell lines divided by the IC_{50} figures of either leukemic or colon cancer cells.

selectivity for one or more groups of cancers may exert a preferential antineoplastic activity towards malignant rather than normal cells. These data are also presented in Table 7 and so are the relevant data for melphalan, which is used clinically in treating leukemia [22], and 5-fluorouracil which is used in the treatment of carcinoma of the colon [23]. A SI value of 1.5 was arbitrarily chosen to be an indicator of promising selectivity.

The evaluation against leukemic cells revealed that all of the compounds were more potent than melphalan except for **1j** and **3c**. In particular, **1p**, **4b** and **4c** had IC_{50} values of less than 1 μM and these three compounds were, on average, 11 times more potent than melphalan. No less than 65% of the compounds had SI values of greater than 1.5 when assessed against leukemic cells and maximum selectivity was displayed by **1c** and **4c**. The compounds prepared in this study were more cytotoxic to colon cancers than 5-fluorouracil except for **1c**, **1s** and **3c**. Maximum activity was displayed by **4b** having 21 times the potency of 5-fluorouracil. This compound, as well as **1j** and **4c**, had SI values of 1.5 or greater.

The next stage of the investigation sought to explore whether the modes of action of these compounds could be attributed, at least in part, to interference with the biosynthesis of DNA, RNA and proteins as well as binding to DNA. Initially possible inhibiting effects on macromolecular biosyntheses were considered. Five representative compounds were chosen having IC_{50} values of ca. 2 (**1p**), 6 (**1a**, **3g**) and 43 (**1r**, **3a**) μM in the L1210 screen. The effects of 2-tetralones on macromolecular biosyntheses in L1210 cells are presented in Table 8. Linear and logarithmic plots between the extent of inhibition of each of the macromolecular syntheses and the IC_{50} figures obtained in the L1210 screen were undertaken. Using linear plots, positive correlations were noted between the IC_{50} values obtained in the L1210 screen and the inhibition of the biosyntheses of DNA ($P < 0.1$), RNA ($P < 0.05$) and proteins ($P < 0.05$). The logarithmic plots gave similar results except that a P value of < 0.05 in the DNA assay was obtained. These results afford strong evidence that the mode of action of the compounds prepared in this study includes interference with the synthesis of cellular macromolecules.

An ethidium displacement assay revealed that compounds **1a**,**p** and **3a**,**g** did not bind to calf thymus DNA and it is unlikely therefore that DNA binding contributes to the cytotoxicity of these 2-tetralones.

Finally, the question was addressed as to whether these compounds were likely to be general biocidal agents or to display some selectivity towards neoplastic cells. Two approaches were utilised in this regard, namely the evaluation of representative compounds against certain fungi as well as toxicity studies in mice.

Compounds **1b**, **3b** and **3f** have substantially divergent cytotoxicity towards various neoplastic cells as the data in Table 1 reveal. At the highest concentration utilised, namely 500 μM, fungicidal activity was absent. Thus the possibility that the compounds prepared in this study

Table 8
Inhibition of **1a**,**p**,**r**, **3a**,**g** on DNA, RNA and protein biosyntheses in murine L1210 cells

Compound	IC_{50} (μM)			
	DNA synthesis	RNA synthesis	Protein synthesis	L1210 proliferation
1a	13.6	17.5	13.2	6.23
1p	13.8	15.5	12.4	1.85
1r	333	300	102	40.9
3a	169	283	83.5	44.4
3g	55.5	58.5	19.4	6.42

have low toxicities suggested a more extensive in vivo study. Compounds **1a**,**c**–**h**,**j**,**k**,**o**–**q**,**s**,**t**, **2**, **3d**,**e**,**g**,**h**,**k**, **4b** and **4c** were injected intraperitoneally into mice using doses of up to and including 300 mg kg^{-1}. The animals were observed 0.5 and 4 h after administration of the compounds for pathological symptoms including neurotoxicity. Furthermore, in order to explore the possibility of CNS penetration by these 2-tetralones, these compounds were examined in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) screens for possible protection against seizures. Any activity noted in either screen implies CNS penetration which could be beneficial in suggesting a utility of these compounds in treating CNS tumours; conversely, this property may be detrimental in causing unwanted toxicity. No mortalities were noted and marginal neurotoxicity was noted only with **1f**,**h**,**o**,**s**. None of the compounds were active in the MES screen and only **1q** afforded protection in some of the mice at the maximum dose of 300 mg kg^{-1} in the scPTZ test. In order to evaluate the tolerability of these compounds in vivo, **1a**,**d**,**p**,**q**, **3g**,**h** were administered orally to rats using doses of 30 mg kg^{-1} or higher. Observations were made in general over the 0.25–4 h time span. No deaths of the animals were reported and toxicity was undetected. However, in contrast to the mouse intraperitoneal screen, the dienones **1a**,**d**,**p**,**q** gave protection in the MES screen suggesting that CNS penetration had occurred. The conclusion to be drawn is that, under the experimental conditions utilised, these compounds were well tolerated in vivo.

5. Conclusions

This study has revealed that a number of 1,3-diarylidene-2-tetralones possess significant cytotoxicities towards various murine and human cancer cell lines. In particular, the compounds in series **4**, and especially **4b**, were more potent that melphalan in most of the assays utilised. The bioevaluation of these compounds revealed that their design to undergo sequential reactions with cellular constituents warrants further investigation. In addition, the demonstration of selective toxicity by a number of the compounds towards leukemic and colon cancer cells is particularly noteworthy. The mode of action of these compounds includes interference with macromolecular biosyntheses and probably cellular redox reactions. The observation that the compounds are well tolerated by mice enhances the need to investigate these molecules further.

6. Experimental

6.1. Chemistry

M.p.s are unconnected and are quoted in °C. Elemental analyses were undertaken on **1a**–**o**, **2** and **3a**–**f**,**j**,**k** (C, H) and **1p**–**t**, **3g**–**i** and **4a**–**c** (C, H, N) by K. Thoms, Department of Chemistry, University of Saskatchewan and are within 0.4% of the calculated values. ^{1}H-NMR spectra were determined on all compounds in series **1**–**4** using a Bruker AM 500 FT NMR machine (500 MHz). An Enraf–Nonius CAD-4 diffractometer was used for the collection of X-ray crystallographic data. The recrystallisation solvents were $CHCl_3$–MeOH (A), $CHCl_3$–MeOH (1:9, B), ether–MeOH (C), MeCN (D), $CHCl_3$ (E), dioxane (F) and $CHCl_3$–MeOH (2:8, G).

6.1.1. Synthesis of series *1* and *2*

The compounds in series **1** and **2** were prepared by the following general method. Hydrogen chloride gas was passed into a solution of 2-tetralone (10 mM) and the appropriate aryl aldehyde (21 mM) in Et_2O (20 mL) for 0.25 h and the resultant mixture was stirred at room temperature (r.t.) for 12 h. The precipitate was removed by filtration, washed with Et_2O (2×25 mL) and recrystallised to give the desired product.

The m.p.s (°C), yields (%) and recrystallisation solvents, respectively, were as follows: **1a**: 156–157, 65, A; **1b**: 166, 65, A; **1c**: 202, 59, A; **1d**: 166–167, 70, B; **1e**: 175–177, 65, B; **1f**: 185–186, 72, B; **1g**: 197–198, 69, A; **1h**: 137–139, 71, B; **1i**: 210–212, 70, C; **1j**: 194–195, 72, A; **1k**: 139, 70, B; **1l**: 155–156, 90, D; **1m**: 189–190, 85, A; **1n**: 206–208, 55, E; **1o**: 150–152, 49, B; **1p**: 185, 30, A; **1q**: 222–224, 61, E; **1r**: 229–230, 68, E; **1s**: 202–204,

51, F; **1t**: 200, 79, E; **2**: 271–273, 63, E. The ^{1}H-NMR spectrum ($CDCl_3$) of a representative compound **1i** was as follows: 4.20 (s, 2H, CH_2), 6.74–6.76 (d, 2H, $J = 8.6$ Hz, aryl H), 6.88–6.89 (d, 2H, $J = 8.6$ Hz, aryl H), 7.05–7.06 (m, 1H, methine H), 7.18 (m, 2H, aryl H), 7.24–7.25 (d, 1H, $J = 7.4$ Hz, aryl H), 7.36–7.38 (d, 2H, $J = 8.6$ Hz, aryl H), 7.45–7.50 (m, 3H, aryl H), 7.70 (s, 1H, methine H).

6.1.2. Syntheses of series 3

A mixture of 2-tetralone (10 mM), the appropriate aryl aldehyde (10 mM), AcOH (50 mg), piperidine (50 mg), 4A molecular sieves (2.5 g) and $C_6H_5CH_3$ (30 mL) was stirred at r.t. for 24 h. The molecular sieves, which were removed by filtration, were washed with EtOAc (25 mL). The combined organic extracts were stirred with an aqueous solution of sodium metabisulphite (5% w/v, 25 mL) for 0.25 h. The separated organic phase was washed with water (25 mL) and brine (25 mL) and dried (anhydrous Mg_2SO_4). The solvents were removed to produce a residue which was purified by passage through a column of silica gel (60–200 mesh) using solvents of C_6H_{14} and subsequently a mixture of EtOAc in C_6H_{14} (2% v/v). The products obtained were further purified by recrystallisation.

The m.p.s (°C), yields (%) and recrystallisation solvents, respectively, were as follows: **3a**: 116–117, 75, A; **3b**: 138, 62, A; **3c**: 106–107, 35, A; **3d**: 96–97, 84, A; **3e**: 83–84, 64, A; **3f**: 179, 53, A; **3g**: 124, 68, A; **3h**: 154–155, 65, A; **3i**: 110–111, 38, A; **3j**: 231–233, 40, A; **3k**: 143, 65, G. The ^{1}H-NMR spectrum ($CDCl_3$) of a representative compound **3e** was as follows: 2.58–2.59 (t, 2H, $J = 6.7$ Hz, 4-CH_2), 2.99–3.02 (t, 2H, $J = 6.6$ Hz, 3-CH_2), 3.79 (s, 3H, OCH_3), 6.76–6.78 (d, 2H, $J = 9.7$ Hz, aryl H), 7.04 (m, 1H, aryl H), 7.18 (bs, 1H, aryl H), 7.39–7.40 (m, 1H, aryl H), 7.41–7.42 (d, 1H, aryl H), 7.42–7.43 (d, 2H, $J = 9.5$ Hz, aryl H), 7.62 (s, 1H, methine CH).

6.1.3. Synthesis of series 4

A stream of dry hydrogen chloride gas was passed through a solution of the appropriate 1-arylmethylene-2-tetralone (2.5 mM) and aryl aldehyde (2.8 mM) in $CHCl_3$ (10 mL) for 10 min. After stirring at r.t. for 12 h, $CHCl_3$ and hydrogen chloride were removed by evaporation. The resultant residue was purified using a chromatography column of silica gel (60–200 mesh) and eluants of EtOAc in C_6H_{14} (2% v/v increasing to 3% v/v) to yield products which were further purified by recrystallisation.

The m.p.s (°C), yields (%) and recrystallisation solvents, respectively, were as follows: **4a**: 193–194, 55, B; **4b**: 185–186, 52, B; **4c**: 176–178, 68, B. The ^{1}H-NMR spectrum ($CDCl_3$) of a representative compound **4a** was as follows: 4.15 (s, 2H, 4-CH_2), 7.05 (t, 1H, aryl H), 7.18 (d, 1H, aryl H), 7.25 (d, 2H, $J = 8.1$ Hz, aryl H), 7.42–7.44 (2d, 4H, aryl H), 7.58 (s, 1H, methine H), 7.60–7.61 (d, 2H, $J = 8.8$ Hz, aryl H), 7.81 (s, 1H, methine H), 8.13–8.15 (d, 2H, $J = 8.8$ Hz, aryl H).

6.1.4. X-ray crystallographic determinations of 1b,c,g,j and 3h

Suitable crystals for X-ray crystallography were obtained by vapour diffusion. A mixture of $CHCl_3$ and MeOH (3:7) was used for **1b**,**c** and **1j** while a solution of $CHCl_3$ and MeOH (1:4) and MeOH were used for **1g** and **3h**, respectively. Data were collected using ω scans. Structures were solved using NRCVAX [24] and refined using SHELXL [25]. Atomic scattering factors and anomolous dispersion corrections were taken from the literature [26]. Hydrogen atoms were placed by geometry and are not refined. Specific details of the X-ray crystallographic data of these compounds may be obtained from the authors.

6.1.5. Measurement of redox potentials

The redox potentials of **1a**,**k**,**n**,**p**,**q**,**r**,**t**, **3a**,**g** and **4b** were determined by a previously reported methodology [2]. In brief, solutions of the compounds in C_3H_6O (0.1 M) were prepared and the determinations utilised a glassy carbon working electrode and a Pt wire counter electrode.

6.1.6. Charge density calculations

The geometry of each of the molecules **1a**,**b**,**k**,**r**, **3b**,**h**,**k** and **4a**–**c** were optimised using the semiempirical PM3 computational methodology. All charges from the Mulliken populations were calculated by the Hartree–Fock approach using the 6–31** basis. Hyperchem 6.0 software was used for geometry optimisations and charge calculations.

6.1.7. Statistical analyses

The σ values were taken from the literature [27] and the σ^* figures were obtained from a reference source [28]. The aryl π and the MR values have been described previously [29]. When performing the statistical analysis using the σ/σ^*, π and MR values, all of the R^1–R^4 substituents in series **1** and **3** were taken into consideration. For example, the electronic effect of these groups in **3b** was calculated to be 0.73 ($R^1 = H = 0.49$, $R^2 = R^4 = H = 0$, $R^3 = Cl = 0.24$). Similarly, the MR value of this compound was 9.12 ($R^1 = R^2 = R^4 = H = 1.03 \times 3$, $R^3 = Cl = 6.03$). In the case of series **1**, the figures of each physicochemical constant of the R^1–R^4 group were doubled prior to undertaking the statistical evaluation.

6.2. Screening

6.2.1. Cytotoxicity evaluations

The evaluation of the compounds described in the study against P388D1 cells was undertaken by a

reported procedure [30], while the screening against L1210, Molt 4/C8 and CEM cells used a method which has been described previously [31].

The cytotoxicity of various compounds was examined towards human tumour cell lines using a literature procedure [32]. This screen utilised a concentration range of 10^{-4}–10^{-8} M and 58 (range of 55–60) cell lines. The compounds inhibited the growth of all of the cell lines by more than 50% with the following exceptions (number of cell lines in which the IC_{50} of the compound was greater than 10^{-4} M/total number of cell lines), namely **1c** (2/58), **1j** (1/58) and **4c** (3/55). The figures listed in Table 7 are IC_{50} concentrations for leukemic and colon cancer cell lines.

6.2.2. DNA binding studies

Compounds **1a**,**p**, **3a** and **3g** were evaluated for any binding properties by an ethidium displacement assay [33]. In brief, excess of the compound was incubated with 50 μg mL^{-1} of calf thymus DNA at 20 °C for 24 h in tromethamine hydrochloride buffer, pH 8.0. Aliquots of the DNA were removed and assessed for their ability to bind ethidium.

6.2.3. Antifungal evaluation

The enones **1b**, **3b** and **3f** were evaluated against three isolates of *A. fumigatus* (ATCC 208995, ATCC 208996 and ATCC 208997) and one isolate of *C. albicans* (ATCC 90028) using the broth dilution method which has been described previously [34].

6.2.4. Effects of various compounds on macromolecular biosyntheses

In each well of a 96-well microtiter plate were placed L1210 cells ($\sim 2.5 \times 10^5$), different concentrations of **1a**,**p**,**r**, **3a** or **3g** and 0.25 μCi of [methyl-^{3}H]deoxythymidine, [5-^{3}H]uridine or [3,4-^{3}H]leucine. After 20 h incubation at 37 °C, the trichloroacetic acid-insoluble fractions of the cell cultures were analysed for radioactivity using a liquid scintillation counter.

6.2.5. Evaluations in the murine toxicity, MES and scPTZ screens

Compounds **1a**,**c**–**h**,**j**,**k**,**o**–**q**,**s**,**t**, **2**, **3d**,**e**,**g**,**h**,**k**, **4b** and **4c** were examined in the initial murine toxicity, MES and scPTZ screens [35]. In brief, doses of 30, 100 and 300 mg kg^{-1} of each compound were injected intraperitoneally into mice and the animals were observed at the end of 0.5 and 4 h. Neurotoxicity was measured by the rotorod procedure [36] and was observed with the following compounds (number of animals displaying neurotoxicity/total number of animals used, time of observation in h, dose in mg kg^{-1}): **1f** (1/4, 0.5, 300), **1h** (1/4, 0.5, 300), **1o** (1/8, 0.5, 100; 2/4, 0.5, 300; 1/4, 4, 100; 1/2, 4, 300) and **1s** (2/4, 0.5, 300). None of the compounds afforded protection in the MES screen. The 2-tetralone **1q** protected 2/5 mice in the scPTZ test using a dose of 300 mg kg^{-1} at the end of 0.5 h. In addition, **1g** caused toxic extension in the scPTZ screen at the end of 0.5 h when a dose of 30 mg kg^{-1} was used.

Six compounds were administered per os to rats using doses of 30 (**1a**,**p**, **3g**,**h**), 30 and 125 (**1d**) and 50 mg kg^{-1} (**1q**). Observations for toxicity were made at the end of 0.25, 0.5, 1, 2 and 4 h for **1a**,**d** (30 mg kg^{-1} dose),**p**,**q** (although no observation was made at the end of 4 h), **3g** and **3h**. In the case of **1d** (125 mg kg^{-1} dose), evaluations were made after 2, 4, 6 and 8 h after administration of the compound. In this test, evidence of toxicity was noted when ataxia, abnormal gait and stance occurred. None of the compounds displayed toxicity. Evaluation of **1a**,**d**,**p**,**q**, **3g**,**h** in the oral MES screen was also undertaken using a dose of 30 mg kg^{-1} and the animals were observed at the end of 0.25, 0.5, 1, 2 and 4 h. Protection was afforded by the following compounds (number of rats protected out of four, time in h): **1a** (1, 1; 2, 2), **1d** (2, 0.25; 1, 0.5; 1, 1; 3, 2; 1, 4), **1p** (1, 0.5; 1, 0.5; 1, 1; 3, 2; 4, 4) and **1q** (1, 0.25; 1, 0.5; 1, 4). Elevation of the dose of **1d** to 100 mg kg^{-1} led to protection of 1, 2, 1 and 2 rats out of four at the end of 1, 2, 4 and 6 h, respectively. An oral dose of 50 mg kg^{-1} of **1q** administered to rats afforded protection in the scPTZ screen in one of four rats, while no activity was observed at the end of 0.5, 1 and 2 h.

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