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Original article

Cytotoxic *N*-[4-(3-aryl-3-oxo-1-propenyl)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidones and related compounds

Jonathan R. Dimmock [a,*], Amitabh Jha [a], Gordon A. Zello [a], J. Wilson Quail [b], Eliud O. Oloo [b], Kurt H. Nienaber [c], Earl S. Kowalczyk [c], Theresa M. Allen [d], Cheryl L. Santos [d], Erik De Clercq [e], Jan Balzarini [e], Elias K. Manavathu [f], James P. Stables [g]

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

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Abstract

A series of 4-carboxychalcones **1** were prepared and coupled to 3,5-bis(phenylmethylene)-4-piperidone (**2**) giving rise to a novel series of *N*-[4-(3-aryl-3-oxo-1-propenyl)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidones (**3**). Molecular simplification of the amides **3** led to the formation of the corresponding *N*-(3-aryl-1-oxo-2-propenyl)-3,5-bis(phenylmethylene)-4-piperidones (**4**). A cytotoxic evaluation of the compounds in series **1**–**4** utilized murine P388 and L1210 cells as well as human Molt 4/C8 and CEM T-lymphocytes. In general, the compounds displayed significant toxicity; the IC_{50} values of 54% of the enones were less than 10 μM when all four screens were considered and less than 1 μM for all members of series **3** in the P388 assay. Various correlations were established between the potencies of the compounds in series **1**, **3** and **4** and the Hammett σ, Hansch π and molecular refractivity constants of the aryl substituents. Several torsion angles and interatomic distances of five representative compounds in series **3** and **4** were determined by X-ray crystallography, some of which contributed to the observed bioactivity. The marked cytotoxicity and lack of murine toxicity of most of the compounds described in this study, as well as their selective toxicity towards different tumour cell lines, revealed that development of the enones **2**–**4** as novel candidate antineoplastic agents should be pursued.

Keywords: 4-Piperidones; Cytotoxicity; Murine toxicity; Structure–activity relationships

1. Introduction

The principal aim of this laboratory is the preparation of conjugated styryl ketones and related compounds as candidate cytotoxic and anticancer agents [1,2]. These compounds are α,β-unsaturated ketones which possess a marked affinity towards thiols both in vitro [3,4] and in vivo [5,6]. In addition, these enones display a capacity for preferential electrophilic attack towards thiols rather than amino or hydroxy groups [7–10]. Since these latter two functions are found in nucleic acids, conjugated enones may lack the genotoxic properties associated with currently used anticancer alkylating agents [11]. The objectives of the present study included the syntheses of some novel compounds designed as thiol alkylators and to evaluate their cytotoxicity. In addition, should significant potencies be demonstrated, investigations would be undertaken in order to seek correlations between the structures of the compounds and the magnitude of their bioactivities. Such information would be of value in a subsequent project aimed at preparing analogues with increased potency and selectivity for neoplastic rather than the corresponding normal cells.

* Correspondence and reprints
E-mail address: dimmock@skyway.usask.ca (J.R. Dimmock).

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The specific aims of the present investigation were as follows. First, the preparation of a series of prototypic chalcones **1** was planned. Recently the antineoplastic properties of various 1,3-diaryl-2-propen-1-ones or chalcones have been reviewed [12] and the cytotoxicity of different series of chalcones has been described [2]. A carboxy group was attached to ring A in order that facile conjugation procedures in vivo would lead to the formation of water soluble glucuronides of promising compounds that would expedite excretion of the molecules. In this way amelioration of mammalian toxicity and reduction or prevention of adsorption in lipoidal tissues of the body may take place. Furthermore the aryl carboxy group was chosen in order that molecular hybridization could occur vide infra.

A second goal of this study was as follows. The marked cytotoxicity of 3,5-bis(phenylmethylene)-4-piperidone (**2**) has been described recently [13]; these data are presented in Table 1. A previous study revealed that the hydrochloride salt of **2** was well tolerated in mice since no lethalities were noted after multiple daily doses of 240 mg kg^{-1} were administered [6]. Compound **2** has greater potency towards two human T-lymphocytes than the established anticancer alkylating agent melphalan and has ca. 27% of the activity of melphalan towards two murine cell lines (Table 1). N-Acylation of **2** with acryloyl chloride led to the corresponding amide which possessed greater toxicity than **2** towards P388 cells and equal activity as **2** in the three other screens [13]. In general, when analogues of **2** possessing different aryl substituents were converted into the corresponding N-acryloyl amides, increases in cytotoxicity were also noted [13]. Hence, one of the reasons for preparing series **3** was to afford an insight into the structural requirements for cytotoxicity of the amidic group attached to the piperidyl nitrogen atom of **2**. Furthermore, if the compounds in series **1** were cytotoxic, the joining of these molecules to **2** would lead to molecular hybridization, i.e. the linking of bioactive molecules via covalent bond formation [14]. This approach may lead to

Table 1
Potencies of **1**–**4** and melphalan towards murine P388 and L1210 leukemic cells and human Molt 4/C8 and CEM T-lymphocytes

Compound	IC_{50} (μM) [a]				SR [b]
	P388 cells	L1210 cells	Molt 4/C8 cells	CEM cells	
1a	25.6±1.1	127±45	11.5±3.7	13.1±0.8	11.0
1b	21.8±2.1	43.8±1.6	9.08±0.95	8.89±0.17	4.93
1c	9.33±1.6	37.6±4.9	5.70±0.75	7.81±0.39	6.60
1d	20.0±0.9	46.4±2.3	11.3±0.6	11.7±3.7	4.11
1e	6.65±0.4	36.6±4.0	7.78±1.13	9.10±0.03	5.50
1f	14.1±0.6	56.4±12.7	13.5±6.9	15.5±0.1	4.18
1g	5.21±0.4	17.0±6.6	8.24±1.05	8.55±0.01	3.26
1h	32.6±2.7	185±55	40.0±3.5	42.5±1.5	5.68
1i	21.3±0.2	109±36	54.0±14.0	54.8±13.1	5.12
2 [c]	0.77±0.02	7.96±0.11	1.67±0.15	1.70±0.02	10.3
3a	0.623±0.05	5.56±4.88	1.94±0.26	1.58±0.26	8.93
3b	0.755±0.04	94.1±33.7	42.3±2.0	32.3±3.8	125
3c	0.312±0.02	32.7±10.0	9.71±2.39	6.35±3.13	105
3d	0.371±0.02	44.2±3.9	32.1±8.8	23.0±16.7	119
3e	0.919±0.03	57.1±18.0	29.2±18.9	30.0±21.0	62.1
3f	0.477±0.01	9.79±0.30	4.56±3.20	4.60±1.81	20.5
3g	0.399±0.1	9.67±0.89	1.67±0.20	1.92±0.40	24.2
3h	0.221±0.01	>20 [d]	>20 [d]	>20 [d]	>91
3i	0.250±0.01	5.34±2.41	1.60±0.06	1.61±0.10	21.4
4a	1.25±0.06	38.4±1.2	33.1±18.0	27.2±15.3	30.7
4b	2.63±0.02	60.6±13.5	27.6±10.7	32.4±2.2	23.0
4c	2.08±0.08	39.8±2.8	66.2±16.5	63.0±7.1	31.8
4d	1.74±0.04	8.47±0.37	7.50±2.08	6.67±0.58	4.87
4e	1.92±0.2	9.94±3.62	3.61±2.79	3.45±2.69	5.18
4f	0.803±0.03	8.96±0.68	1.89±0.11	2.01±0.18	11.2
4g	0.648±0.02	24.4±9.5	9.05±0.29	8.44±0.06	37.7
4h	1.16±0.1	9.90±0.28	1.97±0.10	2.01±0.11	8.54
4i	11.6±1.5	112±37	39.3±3.7	40.8±0.4	9.66
Melphalan [e]	0.220±0.01	2.13±0.03	3.24±0.79	2.47±0.30	14.7

[a] The IC_{50} values indicate the concentration of compound required to inhibit cell growth by 50%.
[b] Selectivity Ratio, i.e. or the ratio of the highest to the lowest IC_{50} values for each compound.
[c] Reprinted in part from J. Med. Chem. 44 (2001) 586. Copyright 2001 American Chemical Society.
[d] Insolubility at concentrations higher than 20 μM was noted.
[e] Reprinted in part from Eur. J. Med. Chem. 35 (2000) 967. Copyright 2000 Éditions scientifiques et médicales Elsevier.

increased cytotoxicity if the binding sites of the chalcones **1** and compound **2** are contiguous and various portions of the molecules **3** are appropriately positioned to elicit bioactivity.

A third aspect of this study was the examination of whether correlations existed between the cytotoxicities and the electronic, hydrophobic and steric properties of the aryl substituents in the series of prepared compounds. The establishment of such relationships would permit the expansion of clusters of compounds on a rational basis. In addition, murine toxicity was evaluated in tandem with the cytotoxicity evaluations in order to guide the direction of further studies with this type of compounds.

In summary, the objectives of the investigation were to synthesize different series of prototypic molecules and evaluate their cytotoxicity and murine toxicity with a view to providing data enabling the design of further candidate cytotoxic molecules.

2. Chemistry

The preparation of the compounds in series **1** and **3** are outlined in Fig. 1. A Claisen-Schmidt condensation between 4-carboxybenzaldehyde and various 1-aryl-1-ethanones led to the formation of **1**. Conversion of the enones **1** into the corresponding acid chlorides, followed by coupling with 3,5-bis(phenylmethylene)-4-piperidone (**2**), led to the desired compounds **3**. The compounds in series **4**, which are discussed subsequently, were prepared by the route presented in Fig. 2. A Doebner modification of a Knoevenagel reaction led to the formation of the appropriate 3-aryl-2-propenoic acids, which were converted into the corresponding acid chlorides and condensed with **2** leading to the isolation of the *N*-(3-aryl-1-oxo-2-propenyl)amides (**4**).

^{1}H-NMR spectroscopy revealed that the olefinic double bonds of **1** and also in the N-acyl groups of **3** and **4** adopted the E configuration. A previous X-ray crystallographic study had revealed that the 3,5-phenylmethylene groups of the hydrochloride salt of **2** also possessed the E stereochemistry [10]. Various torsion angles and interatomic distances of **3d** and **4c**,**e**,**f**,**h** were determined by X-ray crystallography and are presented in Table 3.

3. Biological evaluations

The compounds in series **1**, **3** and **4** were examined for cytotoxic activity using murine P388 and L1210 cells as well as human Molt 4/C8 and CEM T-lymphocytes. These data are presented in Table 1. The enones **2**, **3a**, **3b** and **4a** were assessed against a panel of 53 ± 2 human tumour cell lines and these results are summarized in Table 4. Compounds **1a**, **e**, **2**, **3a** and **3e** were examined for antifungal activity using three isolates of *Aspergillus fumigatus* and one of *Candida albicans*. All compounds were evaluated for murine toxicity using doses of 30, 100 and 300 mg kg^{-1} as well as for possible penetration of the central nervous system (CNS) using the maximum electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) anticonvulsant screens.

4. Results and discussion

The compounds in series **1**–**3** were evaluated for cytotoxicity against murine P388 and L1210 cells, since these cell lines have been claimed to be predictors of clinically useful anticancer drugs [16]. The Molt 4/C8 and CEM T-lymphocytes were utilized in order to observe whether activity towards human cell lines would be demonstrated.

Initially, comparisons of the cytotoxicities of the compounds in series **1**–**3** towards each cell line were made. The average IC_{50} values of the compounds in series **1** and **3** in the P388 screen were 17.4 and 0.481 μM, respectively, i.e. overall the compounds in series **3**

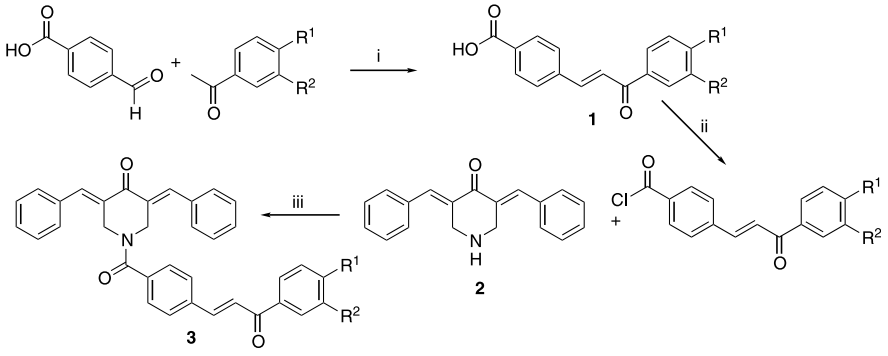

Fig. 1. Synthesis of series **1** and **3**. The reagents used were as follows, namely: (i) sodium hydroxide; (ii) thionyl chloride, dimethylformamide; (iii) triethylamine. The aryl substitution pattern was as follows: **a**: $R^1 = R^2 = H$; **b**: $R^1 = Cl$, $R^2 = H$; **c**: $R^1 = R^2 = Cl$; **d**: $R^1 = CH_3$, $R^2 = H$; **e**: $R^1 = R^2 = CH_3$; **f**: $R^1 = OCH_3$, $R^2 = H$, **g**: $R^1 = R^2 = OCH_3$; **h**: $R^1 = NO_2$, $R^2 = H$; **i**: $R^1 = OH$; $R^2 = H$.

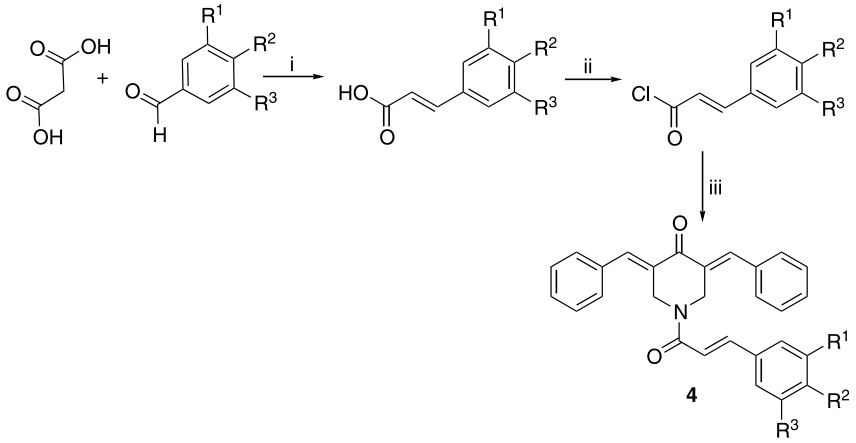

Fig. 2. Synthesis of series **4**. The reagents used were as follows: (i) pyridine, piperidine; (ii) thionyl chloride, dimethylformamide; (iii) **2**, triethylamine. The aryl substitution pattern was as follows: **a**: $R^1 = R^2 = R^3 = H$; **b**: $R^1 = R^3 = H$, $R^2 = Cl$; **c**: $R^1 = R^2 = Cl$, $R^3 = H$; **d**: $R^1 = R^3 = H$, $R^2 = CH_3$; **e** = $R^1 = R^3 = H$, $R^2 = OCH_3$; **f**: $R^1 = R^2 = OCH_3$, $R^3 = H$; **g**: $R^1 = R^2 = R^3 = OCH_3$; **h**: $R^1 = R^2 = OCH_2O$, $R^3 = H$; **i**: $R^1 = R^3 = H$, $R^2 = NO_2$.

were 36 times more potent than the analogues in series **1** in this assay. All of the compounds in series **3** were more potent than **2**, except for **3b** and **3e**. The enone **3h** displayed the same cytotoxicity against P388 cells as melphalan, while the remaining compounds in series **3** had 24–88% of the potency of this established alkylating drug. In the case of the L1210, Molt 4/C8 and CEM tests, the IC_{50} value for **3h** was not determined due to its insolubility when concentrations in excess of 20 μM were employed. Therefore, when the potencies for the compounds in series **1** were compared with series **3** in these three tests, compound **3h** as well as its analogue in series **1**, namely **1h**, were omitted from consideration. In contrast to the P388 screen, the differences between the cytotoxicities of **1a**–**g**, **i** and **3a**–**g**, **i** in the L1210, Molt 4/C8 and CEM tests were much smaller. Thus the average IC_{50} values of **1a**–**g**, **i** in the L1210, Molt 4/C8 and CEM screens were 59.2, 15.1 and 16.2 μM, respectively, while for **3a**–**g**, **i** the comparable values were 32.3, 15.4 and 12.7 μM, respectively. All of the compounds in series **1** had lower potencies than melphalan. On the other hand, **3a**,**g**,**i** were more potent than this reference drug in both the Molt 4/C8 and CEM tests and **3f** had activity equal to melphalan towards Molt 4/C8 cells.

An important feature of promising cytotoxic agents is the ability to display differential cytotoxicity towards various cell lines [17]. In other words, compounds are identified which possess markedly divergent potencies towards various unrelated cell lines in contrast to those molecules which exert similar biocidal or biostatic effects to different cells. One noteworthy feature of this differential cytotoxicity is that it opens up the possibility that compounds displaying differential cell growth inhibition may exert a selective toxicity for malignant cells in contrast to the corresponding normal tissue. For example, in the case of melphalan, there is a 14.7-fold difference between the potencies to P388 and Molt 4/C8 cells. Thus in order to evaluate whether such properties were displayed in the compounds in series **1**–**3**, the selectivity ratio (SR) values of each compound were computed, SR being the ratio of the highest and

Table 2
Summary of the comparisons of potencies between different compounds in series **1**–**4** [a]

Series compared	Relationship evaluated	Percentage correlations			
		P388	L1210	Molt 4/C8	CEM
1, **3**	3 > 1	100	33	33	44
	1 > **3**	0	22	56	22
	Equal potency	0	44	11	33
2, **3**	**3** > **2**	78	11	0	0
	2 > **3**	11	78	56	67
	Equal potency	11	11	44	33
3a–**d**,**f**–**h**,**4a**–**f**,**i**	**3a**–**d**,**f**–**h** > **4a**–**f**,**I**	100	14	29	29
	4a–**f**,**i** > **3a**–**d**,**f**–**h**	0	29	43	14
	Equal potency	0	57	29	57

[a] In the case of compounds in series **1**, **3** and **4**, comparisons of potencies were made with analogues possessing identical aryl substituents.

lowest IC_{50} values observed in the four tests. These data are presented in Table 1. A SR value of 10 was arbitrarily chosen as an indication of promising selectivity and the following compounds met this criterion, namely **1a**, **2** and **3b**–**i**. Thus the molecular hybridization of each molecule in series **1** with those in series **2**, yielding **3a**–**i**, led to increases in the SR values with the exception of the transformation of **1a** into **3a**. The SR values of **3b**–**i** were greater than the value obtained for melphalan and the enormous differences in cytotoxicity displayed by **3b**–**e** and **3h** are particularly noteworthy.

The question of whether series **3**, formed by molecular hybridization between the compounds in series **1** and the 4-piperidone **2**, would display greater potencies than the compounds in both series **1** and **2** was addressed. Comparisons were made between the IC_{50} values of each of the compounds in series **1** with the analogue in series **3** in the same screen, e.g. the IC_{50} value of **1a** was compared with **3a**, **1b** with **3b** and so forth. In a similar manner, the potency of **2** was contrasted with each of the compounds in series **3**. The data are summarized in Table 2. The results indicate that the relative cytotoxicities were dependent on the cell line. Thus, when the P388 screen was considered, the compounds in series **3** were more potent than the analogues in series **1** and with compound **2** in 100 and 78%, respectively, of the comparisons made. On the other hand, comparisons undertaken using L1210, Molt 4/C8 and CEM cells revealed that the compounds in series **3** had greater potencies than **1** in less than half of the cases. When the IC_{50} values of the compounds in series **3** were compared with those in **2** in the L1210, Molt 4/C8 and CEM tests, greater activity of the compounds in series **3** was found in 11% of the cases when L1210 cells were considered and none in the assays using human T-lymphocytes. Thus in the P388 screen, it is conceivable that the binding sites for the chalcones **1** and compound **2** are in close proximity and the topography of the molecules **3** enables the alignment of both portions of the compounds to contribute to cytotoxicity. In the case of the three other cell lines, the bulky N-acyl group may impede alignment of the majority of the compounds in series **3** at the binding site of the 4-piperidone **2**, thereby diminishing potencies.

In order to gain further information on the structural features of the N-acyl group that influence cytotoxicity, the synthesis of **4a**–**i** was carried out. The decision was based on the following considerations. First, the process of molecular simplification [18] was utilized whereby one of the aryl rings and a carbonyl function in the N-acyl groups of **3** were excised. Second, the aryl substituents in **4a**–**f**,**i** were made identical to those found in **3a**–**d**, **f**–**h** so that comparisons of the potencies of these compounds could be made. Third, the excellent bioactivity displayed by **3g** suggested that, in addition to synthesizing **4f**, the close structural analogues **4g** and **4h** should also be prepared.

The results obtained from evaluating the compounds in series **4** against P388, L1210, Molt 4/C8 and CEM cells are presented in Table 1. The data show that the P388 cells were the most susceptible to the compounds; a similar result was noted with the enones **3**. In order to determine the influence of the N-acyl group present in **3** and **4** upon cytotoxicity, the IC_{50} values of **4a**–**f**,**i** were compared with those of the analogues **3a**–**d**,**f**–**h**. The results, which are summarized in Table 2, revealed a dependence upon the cell line. Thus, the *N*-(3-aryl-1-oxo-2-propenyl)piperidines **4a**–**f**,**i** had lower IC_{50} values than the analogues in series **3** in the majority of the comparisons made in L1210 and Molt 4/C8 cells. On the other hand, the enones **3** were invariably more cytotoxic towards P388 cells and on average had lower IC_{50} values in the CEM screen. The general conclusion to be drawn from the data in this study is that there is substantial tolerance to variations in the acyl group attached to the nitrogen atom of **2**.

Two other features of the biodata pertaining to series **4** were noted as follows. First, the SR values of **4a**–**c**,**f**,**g** indicate substantial variations in potencies which, with the exception of **4f**, were greater than the SR value of melphalan. Nevertheless, the enones **4a**–**f**,**i** had lower SR values than the analogues **3a**–**d**,**f**–**h** with the exception of **4a** which was higher than **3a**. Second, comparison of the potencies of **4f** and **3g**, which both contain the 3,4-dimethoxyphenyl group, revealed that the compounds were equiactive, except that **3g** possessed twice the potency of **4f** in the P388 screen. A comparison of the cytotoxicities between **4f** and both of the closely related congeners **4g** and **4h** was made. In the P388 screen, **4f** was less cytotoxic than **4g** but more potent than **4h**. Evaluation in the three other screens revealed that **4f** was more active than **4g** and equipotent with **4h**. Thus, molecular modification of the 3,4-dimethoxy group of **4f** led to retention in cytotoxic properties and in the case of **4g**, a SR value of ca. 38 was achieved which surpassed other compounds in series **4** and also **3g**.

The evidence obtained from the four cytotoxicity assays revealed that the compounds in both **3** and **4** represent novel series of antineoplastic agents, many of which are promising lead molecules. The importance of the electronic, hydrophobic and steric properties of the aryl substituents in conferring cytotoxicity was examined with a view to establishing guidelines for the subsequent development of the project. Compound **3h** was not included in this analysis since specific IC_{50} values were not available in three of the four screens. In addition, **4h** was not included in the plots involving steric constants due to the apparent unavailability of the molecular refractivity (MR) value of the methylenedioxy substituent. Linear and semilogarithmic plots were

made between the IC_{50} values in each cell line with the Hammett σ, Hansch π and MR values of the nuclear groups. In addition, plots between the SR values in series **1**, **3** and **4** and the σ, π and MR values of the aryl groups were made in order to determine whether one or more physicochemical constants influence the disparities in toxicities between the cell lines. The P values obtained as either <0.01, <0.05 or <0.1 are listed in Section 6 of this report.

In the case of series **3**, positive correlations were noted between the Hansch π values and the IC_{50} values in the L1210, Molt 4/C8 and CEM assays as well as in the SR values. Positive correlations were also found with the π constants in series **4** and the cytotoxicity towards Molt 4/C8 and CEM cells. In addition, positive correlations were obtained between the σ values of the enones **4** and the IC_{50} values in the P388, L1210, Molt 4/C8 and CEM tests. The compounds in series **1** displayed a negative correlation between the Hansch π values in the L1210, Molt 4/C8 and CEM assays and also negative relationships between the MR values in the P388 and L1210 cells. No other correlations ($P > 0.1$) were noted.

In general, the relationships were specific for each group of compounds. The following guidelines pertaining to amplification of these series of compounds emerged from this analysis. For the development of series **3**, increased lipophilicity is suggested. This approach should also increase the SRs. Amplification of series **4** should include a major emphasis in preparing compounds with strongly electron-withdrawing groups in the aryl ring which should be lipophilic. The data from the chalcone series **1** indicate that aryl substitution with small hydrophilic groups should be prepared in order to increase potency.

In order to seek correlations between cytotoxicity and the shapes of representative molecules, X-ray crystallography was employed. Suitable crystals of **3d** and **4c**,**e**,**f**,**h** were obtained. The numbering of various atoms in these compounds are indicated in Fig. 3. The numbering of the atoms 1–21 is equivalent in all five compounds and hence most of these numbers are omitted from the structures of **4c**,**e**,**f**,**h** for the sake of clarity. If the enones interact with cellular mercaptans, then the steric environment of the olefinic carbon atoms at which thiolation occurs likely influences bioactivity. Furthermore, the aryl rings of the compounds in series **3** and **4** may form van der Waals bonds with similar groups at a binding site and hence the spatial orientations of the aryl rings in these compounds would be predicted to influence potency. Thus the torsion angles θ_1–θ_5 in **3d** and θ_1, θ_2 and θ_6 in **4c**,**e**,**f**,**h** were measured. Furthermore, variations in potencies between these five compounds could be due to differences in the relative locations of the sites of thiolation which are believed to be carbon atoms 7, 14 and 28 in **3d** and 7, 14 and 23 in the case of **4c**,**e**,**f**,**h**. In order to examine this hypothesis, two different measurements were made. First, the distances between the three sites of thiolation were obtained and designated d_1–d_3 as indicated in Fig. 3. Second, the elevation or depression of the C14 atom and either the C28 (**3d**) or C23 (**4c**,**e**,**f**,**h**) atoms in relation to the plane defined by the sp^2 bonds of the C7 atom were measured and designated d_4 and d_5, respectively. The results are portrayed in Table 3.

The reported θ_1 and θ_2 values of **2** as the hydrochloride salt are 12.4 and $-12.9°$ [15]. The lack of coplanarity of the aryl rings with the adjacent olefinic linkages in this compound was attributed, at least in part, to non-bonded interactions between the equatorial protons at positions 2 and 6 ($C2H_e$ and $C6H_e$) with the ortho hydrogen atoms attached to atoms 13 and 20 [6]. In the case of **3d**, C_2H_e–C13H, C12H–C23H, C12H–C24H and C13H–C23H non-bonded interactions were absent. However, the molecule was orientated to avoid such interactions between rings A and D (Fig. 3) which led to large θ_1–θ_3 values. The θ_4 and θ_5 values reveal a lack of coplanarity of rings A and B with the adjacent olefinic and carbonyl groups, respectively. Non-bonded interactions between the $C2H_e$ and C13H atoms were noted in **4c**,**e**,**f**,**h** but C12H–C22H, C12H–C25H and C13H–C22H interactions were absent. The lack of coplanarity of ring E with the adjacent unsaturated linkage in **4c**,**e**,**f**,**h** (θ_6) was less than the θ_3–θ_5 values in **3d**. In general, $\theta_2 > \theta_1$, which was attributed to the steric effect of the N-acyl group. The results in Table 3 reveal that the distance d_1 was virtually identical in **3d** and **4c**,**e**,**f**,**h**. On the other hand, while the larger d_2 and d_3 values of **3d** compared to **4c**,**e**,**f**,**h** were predictable, the variation among **4c**,**e**,**f**,**h** of the d_2 and d_3 values was surprising. The d_4 distances revealed that, in general, the C7 and C14 atoms lay in the same plane. On the other hand, in the case of **4c**,**e**,**f**,**h**, the d_5 values indicated that the C23 atoms were ca. 3.9 Å out of the plane of the C7 atom.

Linear and logarithmic plots were made between the IC_{50} values in all four screens with the magnitude of, first, the θ_1 and θ_2 values of **2**, **3d**, **4c**,**e**,**f**,**h**, second, the θ_6 values of **4c**,**e**,**f**,**h** and finally, the distances d_2–d_5 of **3d**, **4c**,**e**,**f**,**h**. Correlations were only noted using the biodata obtained from the P388 screen. Cytotoxicity was negatively correlated with the θ_6 values of **4c**,**e**,**f**,**h** as well as the d_2 and d_3 distances of these compounds plus **3d**. The P values are listed in Section 6. No other correlations ($P > 0.1$) were noted. On the basis of these results, amplifications of these two series of compounds in the following ways may lead to novel compounds with increased potencies in the P388 screen. First, the absence of ortho and meta substituents in ring E in series **4** would diminish the magnitude of the θ_6 values [19]. Second, the shortening of the d_2 and d_3 distances may be accomplished by joining rings A or E to either ring D or the piperidine ring with a short linker group.

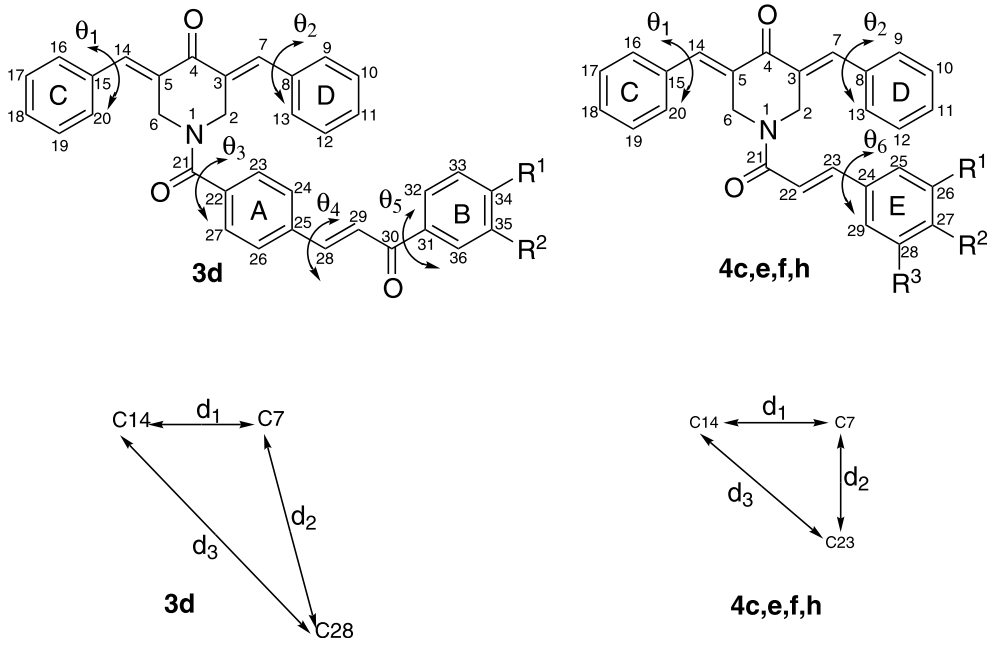

Fig. 3. The numbers assigned to some of the atoms, the torsion angles (θ_1–θ_6) and interatomic distances (d_1–d_3) of the X-ray crystallographic structures of **3d** and **4c**,**e**,**f**,**h** are indicated.

The enones **2**, **3a**, **b** and **4a**, as well as two reference drugs, were evaluated for cytotoxicity towards a number of human tumour cell lines representing different groups of neoplasms [20]. In general, the compounds were screened using a concentration range of 10^{-8}–10^{-4} M. The average cytotoxicity towards all cell lines was expressed in terms of the mean graph midpoint (MG MID) values rather than IC_{50} values since, if a compound did not inhibit the growth of the tumour cells by 50% at the highest concentration utilized namely 10^{-4} M, the value of 10^{-4} M was still included in determining the average cytotoxicity towards all cell lines. In the case of **2**, **3a**, **4a** and melphalan, however, the MG MID and IC_{50} values are the same. The data generated are portrayed in Table 4.

The order of cytotoxicity of **3a**,**b** and **4a** towards human tumour cell lines was **3a** > **4a** > **3b**. Both **3a** and **4a** were ca. 1.6 and 1.1 times, respectively, more potent than melphalan. Furthermore there is an interest in this laboratory in discovering novel compounds which have marked potencies towards colon cancers and leukemias [21]. Hence the MG MID values of **3a**,**b** and **4a** towards these groups of tumours were calculated and compared to the data generated for 5-fluorouracil and melphalan, which are used clinically to combat colon tumours and leukemia, respectively [22,23]. The data in Table 4 reveal that both **3a** and **4a** were more cytotoxic than either of the reference drugs for these two groups of tumours. In addition, a selectivity index (SI) was calculated for each of the compounds in Table 4, i.e. the average MD MID values obtained for all cell lines was divided by the MG MID values of either colon cancer or leukemic cells. A 50% increase in selectivity was arbitrarily chosen as a promising indicator of selectivity. The enones **3a** and **4a** met this criterion when both colon cancer and leukemic cells were considered and these compounds had similar SI values as melphalan. Furthermore, the SR data for **3a** and **4a** are noteworthy. The evaluation using a large number of human tumour cell lines revealed that **3a** and **4a** are very useful prototypic cytotoxic molecules.

The data presented so far revealed that the compounds exerted differential cytotoxicity towards various

Table 3
The torsion angles θ_1–θ_6 (°) and distances d_1–d_5 (Å) in **3d** and **4c**,**e**,**f**,**h** determined by X-ray crystallography [a]

Compound	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6	d_1	d_2	d_3	d_4	d_5
3d	24.4	−39.9	48.5	−159.5	−12.3	–	4.86	6.72	10.1	0.21	−3.90
4c	22.3	−29.4	–	–	–	172.9	4.85	4.76	6.33	0.06	4.05
4e	25.4	−41.6	–	–	–	−170.7	4.85	5.26	7.01	−0.70	−3.69
4f	26.4	−23.8	–	–	–	178.0	4.87	5.39	6.65	−0.012	4.04
4h	0.2	−36.8	–	–	–	176.3	4.88	5.23	7.18	0.094	−3.78

[a] The positive and negative signs of the θ_1–θ_6 figures refer to clockwise and anticlockwise rotations, respectively. The positive and negative signs of the d_4 and d_5 distances refer to the locations of various atoms as either above or below the plane of the C7 atom, respectively.

cell lines. A broader approach was instigated in order to confirm that these compounds are not general biocidal agents. First, the enones **1a**,**e**, **2** (as the hydrochloride salt) and **3a**,**e**, which have widely divergent IC_{50} values towards the four cell lines portrayed in Table 1, were examined against three isolates of *A. fumigatus* and one of *C. albicans*. The minimal inhibitory concentration (MIC) values of the five enones towards the four fungal pathogens were in excess of 32 μg mL^{-1} and hence they possess no significant activity towards these fungi. This observation provides further evidence of the selective tumour toxicity displayed by these compounds.

A second and more extensive investigation was undertaken in order to determine whether the compounds prepared in this study had the potential to spare normal cells yet have lethal properties towards certain tumours. All of the compounds were administered intraperitoneally into mice using doses of 30, 100 and 300 mg kg^{-1} and the animals were observed after 0.5 and 4 h. Neurotoxicity and other pathological symptoms were displayed by some of the compounds (Section 6). The only mortality was after 4 h in one out of two mice that received 300 mg kg^{-1} of **1a**. In addition, no murine toxicity was displayed by **1c**,**e**,**h**, **2**, **3a**–**e**,**g**–**i** and **4a**,**c**,**h**,**i**. Thus, in general, the compounds in series **3** possessed less murine toxicity than the chalcones **1** and amides **4**. Anticonvulsant activity was demonstrated by **1i** and **3d** in the MES screen and by **1a**,**b**,**g**,**i** in the scPTZ test, indicating that some CNS penetration occurred with these compounds and possibly with related analogues. Oral administration of three representative compounds, namely **1b**, **3d** and **4h** to rats did not reveal any overt toxicity. Each of these three compounds displayed marginal activity in the anticonvulsant screens indicative of CNS penetration.

The conclusions to be drawn from the antifungal, toxicity and anticonvulsant screens are that the compounds described herein, while possessing marked cytotoxic properties for tumour cells, displayed minimal toxicity to other tissues. In particular, the virtual absence of murine toxicity displayed by series **3** is a further indicator of this group of novel enones should be considered to be lead molecules.

5. Conclusions

This study has revealed that *N*-[4-(3-aryl-3-oxo-1-propenyl)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidones (**3**) are novel cytotoxic agents. In particular, significant potency towards P388 cells was demonstrated with the IC_{50} values of all compounds in this series being less than 1 μM. The SR values of various members of series **3** are noteworthy. In general, the compounds in series **3** were more potent than the analogues **1** and **4** but less cytotoxic than the precursor 4-piperidone **2**. The absence of both antifungal activity of representative enones and marked murine toxicity in virtually all of the compounds, coupled to the variation in potencies towards different tumours, suggest that those molecules are selectively toxic to certain cells. Various guidelines for amplification of the different series of compounds have been made including those derived from structure–activity relationships using the various physicochemical constants of the aryl substituents as well as X-ray crystallography. The evidence suggests that development of the compounds in series **2**–**4** as candidate antineoplastic agents is warranted.

6. Experimental

6.1. Chemistry

M.p. are in Celsius degrees and are uncorrected. Elemental analyses, undertaken by K. Thoms, Department of Chemistry, University of Saskatchewan, were performed on **1a**–**i**, **3a**–**i** and **4a**–**i** and were within 0.4%

Table 4
Evaluation of **2**, **3a**, **3b**, **4a** and reference compounds against various human tumour cell lines

Compound	All cell lines	Colon cancer cells		Leukemic cells		SR [c]
	MG MID [a] (μM)	MG MID [a] (μM)	SI [b]	MG MID [a] (μM)	SI [b]	
2	1.62	0.658	2.46	0.455	3.56	93
3a	5.01	3.19	1.57	1.79	2.80	85
3b	53.7	60.8	0.88	27.9	1.93	14
4a	7.25	4.09	1.77	2.35	3.09	62
Melphalan	7.94	7.87	1.01	2.61	3.04	87
5-Fluorouracil	29.5	6.47	4.56	3.63	8.13	> 6456

[a] The letters MG MID refer to the mean graph midpoint which is discussed in the text.
[b] The letters SI refer to the selectivity index, i.e. the average MG MID values of all cell lines divided by the average MG MID figures for either the colon cancer or leukemic cells.
[c] The letters SR refer to the selectivity ratio which was obtained by dividing the IC_{50} figures of the compound towards the least susceptible and most susceptible cell lines.

of the calculated values. ^{1}H-NMR spectra were determined on all compounds using a Bruker AM 500 FT-NMR instrument (500 MHz). TLC was carried out using silica gel 60 F_{254}-precoated plastic sheets and an eluting solvent of $CHCl_3$:CH_3OH (9:1).

*6.1.1. Synthesis of **1a–i***

4-Carboxybenzaldehyde (0.03 mol) was added to a solution of NaOH (0.05 mol) in water (25 mL) and EtOH (10 mL) and the resultant mixture was stirred for 0.25 h with ice bath cooling. The appropriate 1-aryl-1-ethanone (0.03 mol) was added and the solution stirred at room temperature (r.t.) for 12 h. The reaction mixture was cooled using an ice bath and acidified with HCl (20% w/v). The precipitate was collected, washed with cold water, dried and purified by recrystallization from MeOH or digested in this solvent. The m.p. and percentage yields of the compounds in series **1** were as follows: **1a**: 220–221 (lit. [24] m.p. 209–211), 68; **1b**: 266–267 (lit. [25] m.p. 267), 67; **1c**: 259–260, 65; **1d**: 242–243, 54; **1e**: 258, 63; **1f**: 224–226, 68; **1g**: 208 (lit. [24] m.p. 208–209), 69; **1h**: 274, 71; **1i**: 282–283 (lit. [26] m.p. 300–301), 56. The ^{1}H-NMR spectrum of a representative compound **1c** was as follows: δ (dmso-d_6): 7.80 (d, 1H, CH=C*H*CO, $J = 15.6$ Hz), 7.84 (d, 1H, C6′H, $J = 8.4$ Hz, 7.98 (d, 2H, C3H, C5H, $J = 8.3$ Hz), 8.03 (d, 2H, C2H, C6H, $J = 8.3$ Hz), 8.07 (d, 1H, C*H*=CHCO, $J = 15.6$ Hz), 8.12 (m, 1H, C5′H), 8.43 (d, 1H, C2′H, $J = 1.8$ Hz), 13.15 (bs, 1H, COOH).

*6.1.2. Synthesis of **3a–i***

Thionyl chloride (0.007 mol) was added to a solution of the appropriate 4-carboxychalcone (0.003 mol) in CH_2Cl_2 (40 mL) which contained a catalytic quantity of dimethylformamide (0.04 mL). The solution was heated under reflux for 6 h. The formation of the acid chloride was monitored by adding small quantities of the reaction mixture to MeOH and noting the appearance of the methyl ester by TLC. The solvents were removed in vacuo and the residue was dissolved in dry $CHCl_3$ (30 mL). A solution of 3,5-bis(phenylmethylene)-4-piperidone hydrochloride (0.003 mol), prepared by a literature method [13], in dry $CHCl_3$ (20 mL) and Et_3N (0.006 mol) was added to a solution of the acid chloride under ice-cold, dry conditions over the course of 10 min. For the synthesis of **3i**, the addition procedure was reversed insofar as the acid chloride was added dropwise to a solution of **2** over 0.5 h. The resultant mixture was stirred at r.t. for 4 h; the reaction being monitored by TLC. The solvents were evaporated and to the residue was added MeOH (ca. 20 mL per gram of product) and the suspension was heated at 60 °C for 4 h. The precipitate was collected by filtration, washed with cold MeOH and dried. Compounds **3a**,**e**,**g** were subsequently recrystallized from $CHCl_3$ and **3c** was digested with $CHCl_3$. The amides **3a** and **3g** crystallized with one quarter molecule of water, **3d**,**e** and **3i** with one half molecule of water and **3h** with one molecule of water of crystallization. The m.p. and percentage yields of these compounds were as follows: **3a**: 190–192, 65; **3b**: 220–222, 67; **3c**: 195–196, 61; **3d**: 223, 56; **3e**: 240–242, 69; **3f**: 186–187, 62; **3g**: 172–174, 71; **3h**: 255–260, 67; **3i**: 240–243, 45. The ^{1}H-NMR spectrum of a representative compound **3g** is as follows: δ (dmso-d_6): 3.85 (s, 3H, 3′OCH_3), 3.87 (s, 3H, 4′OCH_3), 4.68 (bs, 2H, NC*H* × 2), 5.01 (bs, 2H, NC*H* × 2), 7.11–7.91 (m, 2IH, aryl and olefinic H).

*6.1.3. Synthesis of **4a–i***

The 3-aryl-2-propenoyl chlorides required in the synthesis of **4a–i** were obtained as follows. 3-Phenyl-2-propenoyl chloride was purchased from the Aldrich Chemical Company. The remaining acid chlorides were synthesized by the following method. A mixture of the appropriate 3-aryl-2-propenoic acid (0.003 mol), prepared by a literature procedure [27], thionyl chloride (0.007 mol), *N*,*N*-dimethylformamide (0.04 mL), and dry dichloroethane (40 mL) was heated under reflux for 6 h. Evaporation in vacuo led to the isolation of the acid chloride which was dissolved in dichloroethane (30 mL). To this solution **2** (0.003 mol) was added and the reaction mixture was cooled in an ice-bath for 20 min. A solution of Et_3N (0.006 mol) in dichloroethane (20 mL) was added dropwise over 0.5 h at ice-bath temperature. The source of cooling was removed and the reaction mixture gradually rose to r.t. The mixture was stirred for 6 h after the addition of Et_3N. After evaporation in vacuo, HCl (2% w/v, 30 mL) was added and the suspension stirred for 1 h at r.t. The precipitate was collected, washed with water (3 × 50 mL) and MeOH (30 mL) and dried. The compounds were recrystallized from a mixture of CH_2Cl_2 and MeOH (3:2). The amides **4b**,**d**,**g** crystallized with one quarter mole of water and **4f** was obtained as the monohydrate. The m.p. and percentage yields were as follows: **4a**: 197 (lit. [28] 194–196), 77; **4b**: 181, 78; **4c**: 200–201, 77; **4d**: 188, 75; **4e**: 199, 69; **4f**: 194–195, 78; **4g**: 191–193, 79; **4h**: 178, 74; **4i**: 237–238, 78. The ^{1}H-NMR spectrum of a representative compound **4b** is as follows: δ ($CDCl_3$): 4.80 (bs, 2H, N–CH_2), 4.98 (bs, 2H, N–CH_2), 6.27 (d, 1H, COC*H*, $J = 15.4$ Hz), 6.90 (d, 2H, C3H, C5H of N-acyl ring, $J = 8.4$ Hz), 7.17 (d, 2H, C2H, C6H of N-acyl ring, $J = 8.4$ Hz), 7.35–7.55 (m, 11H, 2 × C_6H_5, COCH=C*H*), 7.84 (s, 2H, 2 × CH=).

6.1.4. Statistical analyses

The σ and π constants of the aryl substituents in **1a–i**, **3a–g**,**i** and **4a–i** were culled from a reference source [29]. The MR figures at positions 3, 4 and 5 of aryl ring B of **1a–i**, **3a–g**,**i** and ring E in **4a–g**,**i** were taken from the literature [29]. The linear and semilogarithmic plots were generated using a commercial software package [30].

The following correlations (i.e. Pearson's correlation) were noted [physicochemical constant, assay, linear (l) or semilogarithmic (sl) plots, P value] namely **3**: π, L1210, l, <0.1; π, L1210, sl, <0.05; π, Molt 4/C8, sl, <0.05; π, CEM, sl, <0.05; π, SR, l and sl, <0.05; **4**: π, Molt 4/C8, l, <0.1; π, CEM, l, <0.1; σ, P388, l and sl, <0.05; σ, L1210, l and sl, <0.01; σ, Molt 4/C8, l and sl, <0.01; σ, CEM, l and sl, <0.01; **1**: π, L1210, sl, <0.1; π, Molt 4/C8, l, <0.05; π, Molt 4/C8, sl, <0.01; π, CEM, l and sl, <0.05; MR, P388, l, <0.05; MR, P388, sl, <0.01 and MR, L1210, sl, <0.05.

The correlations obtained between the IC_{50} values in the P388 screen and certain torsion angles and interatomic distances obtained by X-ray crystallography were as follows [physicochemical measurements, linear (l) or logarithmic (ln) plots, P value]: θ_6, l, <0.1; θ_6, ln, <0.1, d_2, l, <0.1; d_2, ln, <0.05 and d_3, ln, <0.1.

6.1.5. X-ray crystallography

X-ray crystallographic data were collected for **3d** and **4c**,**e**,**f**,**h** using an Enraf–Nonius CAD-4 diffractometer with ω scans. Data were processed using XTAL-3.6 [31]. The structures were solved using NRCVAX [32], while SHELXL [33] was used for refinement. Atomic scattering factors and anomalous dispersion corrections were obtained from the literature [34]. Non-hydrogen atoms were found in E-maps and were refined anisotropically. Hydrogen atoms were placed on atoms by geometry using a riding model and assigned temperature factors derived from the attached atoms. The torsion angles θ_1–θ_6 for **3d** and **4c**,**e**,**f**,**h** were obtained using the following atoms: θ_1: C5–C14–C15–C20; θ_2: C3–C7–C8–C13; θ_3: O2–C21–C22–C27; θ_4: C26–C25–C28–C29: θ_5: O3–C30–C31–C36; θ_6: C22–C23–C24–C29. The interatomic distances H_e(C2)–H(C13) for **4c**,**e**,**f**,**h** were 2.133, 2.247, 2.028 and 2.182 Å, respectively, while the H_e(C2)–H(C13), H(C13)–H(C23), H(C12)–H(C23) and H(C12)–H(C24) distances for **3d** were 2.133, 2.247, 2.028 and 2.182 Å and the H(C13)–H(C22), H(C12)–H(C25) and H(C12)–H(C22) distances for **4c**,**e**,**f**,**h** were in excess of 2.4 Å. There are very short H_e(C2)–H(C22) distances in **4c**,**e**,**f**,**h**, namely 1.76, 1.90, 1.93 and 1.96 Å, respectively. These distances are less than the sum of the hydrogen atom van der Waals radii (2.2 Å). Since these hydrogen atoms were placed by the geometry of the non-hydrogen atoms and the data are not of sufficient quality to refine the hydrogen atoms, it is likely that their true positions are somewhat displaced due to the collision of the hydrogen atoms, thus resulting in some strain in the structures.

6.2. Screening

6.2.1. Cytotoxicity evaluations

The evaluation of the compounds **1**, **3** and **4** against P388D1 cells was undertaken using a literature methodology [35] and the L1210, Molt 4/C8 and CEM tests utilized a previously reported method [36]. The piperidones **2**, **3a**,**b**, **4a** as well as melphalan and 5-fluorouracil were evaluated against 55 (52–59) human tumour cell lines by a reported procedure [20]. Evaluation was made using cells from the following neoplastic conditions, namely leukemia, melanoma and non-small cell lung, colon, CNS, ovarian, renal, prostate and breast tumours, with the exception of **2** and **4a** which were not screened against prostate and breast neoplasms but were evaluated using small cell lung tumour cell lines. In the case of **2**, **3a**, **4a** and melphalan, all of the $\log_{10}$ MG MID values were IC_{50} values. However, specific values were obtained for 34/55 and 51/57 cell lines for **3b** and 5-fluorouracil, respectively. In the evaluation of **3b**, the MG MID values of four of the seven colon cancer cell lines were greater than 10^{-4}; specific MG MID values were obtained against this panel of tumours in the case of 5-fluorouracil and towards leukemic cells for both compounds.

6.2.2. Antifungal evaluations

Compounds **1a**, **e**, **2**, **3a** and **3e** were examined for antifungal activity using three isolates of *A. fumigatus* (ATCC 208995, 208996 and 208997) and one isolate of *C. albicans* (ATCC 90028) by the broth microdilution test [37]. The MIC values of all five compounds was in excess of 32 μg mL^{-1}. In this assay, a reference drug voriconazole possessed a MIC value of 0.25 μg mL^{-1}.

6.2.3. Toxicity, MES and scPTZ screens

Various compounds were administered to mice by the intraperitoneal route and examined for neurotoxicity [38] as well as anticonvulsant properties in the MES and scPTZ tests [39].

Compounds **1a**–**i**, **2**, **3a**–**i** and **4a**–**i** were examined in the murine toxicity, MES and scPTZ screens. The animals were observed 0.5 and 4 h after receiving doses of 30, 100 and 300 mg kg^{-1} of the enones. Neurotoxicity was noted with the following compounds (dose in mg kg^{-1}, time in h, number of animals displaying toxicity), namely **1a**: 300, 4, 2/2; **1b**: 300, 0.5, 2/4; 100, 4, 1/4; 300, 4, 2/2; **1d**: 100, 0.5, 1/8; 300, 0.5, 1/4; 100, 4, 2/4; 300, 4, 2/2; **1f**: 100, 0.5, 1/8; 300, 0.5, 1/4; **1g**: 300, 0.5, 1/4; **1i**: 100, 0.5, 1/8; 300, 0.5, 2/4; 300, 4, 1/2; **3f**: 100, 0.5, 2/8; 300, 0.5, 3/4; 100, 4, 1/4; 300, 4, 1/2; **4b**: 100, 0.5, 2/8; 300, 0.5, 2/4; 300, 4, 1/2; **4d**: 100, 0.5, 3/8; 300, 0.5, 1/4; **4e**: 100, 0.5, 3/8; 300, 0.5, 1/4; 100, 4, 1/4; 300, 4, 2/2; **4f**: 100, 0.5, 2/8; 300, 0.5, 3/4; 300, 4, 1/2; **4g**: 300, 0.5, 2/4. In addition, mice receiving a dose of 300 mg kg^{-1} of **1a**,**b**,**d**,**g** were unable to grasp a rotorod after 0.5 (**1g**) or 4 (**1a**,**b**,**d**) h after administration of the compounds. Death was noted in one of two mice 4 h after administration of 300 mg kg^{-1} of **1a**. In the MES screen, a dose of 300 mg kg^{-1} of **1i** and **3d** afforded protection after 0.5 and 4 h, respectively. Protection in

the scPTZ test was displayed by the following compounds (dose in mg kg^{-1}, time in h, number of animals protected), namely **1a**: 300, 0.5, 3/5; **1b**: 300, 0.5, 4/5; **1g**: 300, 0.5, 1/1; **1i**: 300, 4, 1/1; **5c**: 300, 0.5, 1/5. Tonic extensions were noted in the scPTZ screen by **1b** (300 mg kg^{-1} dose after 4 h) and **4d** (100 mg kg^{-1} dose after 0.5 h). In the same test, myoclonic jerks were noted 4 h after administration of a 300 mg kg^{-1} dose of **1i**. No other toxicities or protection against convulsions were noted in these murine screens.

Compounds **1f**, **3d** and **4h** were administered to rats per os using doses of 50, 30 and 300 mg kg^{-1}, respectively, and evaluated for neurotoxicity by observing the animals for overt evidence of ataxia as well as abnormal gait and stance. Evaluations in the MES and scPTZ screens were undertaken by a literature procedure [39]. Observations were made 0.25, 0.5, 1, 2 and 4 h after administration of the compounds except in the case of **4h**, where the 1 and 2 h observations were omitted. No pathological symptoms were noted in the toxicity screen. In addition, **3d** and **4h** were examined in the MES screen and **1f** and **4h** in the scPTZ test using the same doses and observation times as were employed in the toxicity screen. In the MES test, **3d** afforded protection in 1/4 rats after 0.5, 1 and 4 h and in 3/4 animals after 2 h, while **4d** displayed anticonvulsant activity in 1/2 rats after 4 h. Evaluation in the scPTZ screen revealed that **1f** protected 1/4 animals against seizures after 0.5 and 1 h and **4h** after 0.25 and 0.5 h in 1/2 rats. At other time intervals in the MES and scPTZ screens, no protection was noted. In the case of **4h**, one of the two animals in the scPTZ screen died after 0.25 h. A dose of 100 mg kg^{-1} of **4h** was administered intraperitoneally to rats and observations were made in the toxicity and MES screens after 0.25, 0.5, 1, 2 and 4 h. No toxicity was observed and 1/2 rats was protected in the MES test after 0.25 h only. The Anticonvulsant Screening Project of the National Institute of Neurological Disorders and Stroke requires that all rats and mice are housed, fed and handled in a manner consistent with the recommendations in the National Research Council Publication, 'Guide for the Care and Use of Laboratory Animals'. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals.

7. Supplementary material

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 192106, 192105, 192103, 192104 and 192102 for compounds **3d**, **4c**, **4e**, **4f**, **4h**, respectively. Specific details of the X-ray crystallographic data for **3d** and **4c**,**e**,**f**,**h** are available from the authors on request.

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