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Original article

Design, synthesis and cytotoxic properties of novel 1-[4-(2-alkylaminoethoxy)phenylcarbonyl]-3,5-bis(arylidene)-4-piperidones and related compounds

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Abstract

The 3,5-bis(arylidene)-4-piperidones **1** contain the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore which is considered to interact at a complementary binding site in susceptible neoplasms. The hypothesis was formulated that the presence of an acyl group attached to the piperidyl nitrogen atom in series **1** may interact with an additional binding site thereby enhancing cytotoxic potencies. This concept led to the synthesis of various *N*-acyl-3,5-bis(arylidene)-4-piperidones **3**–**7** many of which displayed significant cytotoxicity towards a variety of cancer cell lines. A comparison of the potencies between the compounds in series **1** and the related nonquaternary analogues **3**–**6** revealed that in approximately half of the comparisons made, the *N*-acyl analogues had increased potencies.

Keywords: *N*-Acyl-4-piperidones; Structure–cytotoxicity relationships; α,β-Unsaturated ketones; Apoptosis

1. Introduction

One of the principal aims of these laboratories is to discover novel cytotoxic and anticancer agents. In many cases, the design of the target compounds has incorporated one or more conjugated unsaturated keto moieties into the molecules. These groups have a greater and, on occasions, exclusive affinity for thiols in contrast to amino and hydroxy groups [1–3]. Since thiols are absent in nucleic acids, the genotoxic effects associated with a number of currently available anticancer drugs [4] may be absent in these enones. Initial studies incorporated one α,β-unsaturated keto function into the molecules but subsequently two of these groups have been utilized thereby creating bifunctional alkylating agents for the following reason. On a number of occasions, chemosensitization is greater in tumours than in normal cells [5,6]; when this occurs, the tumours are more vulnerable to a subsequent chemical insult. In this manner, greater toxicity for malignant cells may occur. Hence the potential for two sequential electrophilic attacks with cellular thiols was incorporated into the design of the candidate cytotoxins. It is likely that enones exert their bioactivities by interacting with a number of different molecular targets. Since there are a variety of dysregulated processes occurring in neoplasia, such pleiotropy may be a distinct advantage. In fact, the benefits of promiscuous binding of a ligand at multiple sites have recently been articulated [7].

Recently the cytotoxic properties of 3,5-bis(arylmethylene)-4-piperidones **1a–c** and related compounds towards

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human Molt 4/C8 T-lymphocytes and other cell lines were described [8]. In addition, the hydrochloride salt of **1a** was well tolerated in mice whereby five daily doses up to and including 240 mg/kg did not cause mortalities [9]. This compound lowered hepatic glutathione concentrations in mice [9] confirming, at least in part, that this compound acted as a thiol alkylator. The development of series **1** seemed therefore to be judicious and the following approach was considered. The 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore has been incorporated not only into piperidine rings but also into alicyclic compounds with retention of cytotoxic properties [10,11] and thus a reasonable hypothesis is that this group interacts with cellular constituents at a specific binding site referred to subsequently as site A (Fig. 1). However, alignment of the 1,5-diaryl-3-oxo-1,4-pentadienyl group at site A may be influenced by the nature of the group on the heterocyclic nitrogen atom. This group could lead to increases in cytotoxic potencies due to either additional binding with cellular constituents at an auxiliary binding site (site B, Fig. 1) or alternatively by facilitating the interaction of the cytotoxin at site A. On the other hand, the group on the nitrogen atom may lead to a reduction in potency due to repulsion between this group and site B thereby preventing interaction between the 1,5-diaryl-3-oxo-1,4-pentadienyl moiety and site A.

In order to evaluate this hypothesis, various model compounds were designed based on the following considerations. One of the potential problems with **1a** which has a basic centre is that under physiological conditions, the molecule exists as an equilibrium between the ionized molecule and the free base. The charged molecules may be unable to penetrate cell membranes and exert a cytotoxic effect. Hence *N*-acylation was considered a route to follow rendering the nitrogen atom in **1a** nonbasic. An aryl ring in the *N*-acyl function was chosen since it may allow van der Waal bonding with site B and hence the synthesis and bioevaluation of **2a** was suggested. The cytotoxicity of the initial compounds was monitored using the Molt 4/C8 assay and as Fig. 2 reveals, there was no difference in potency between **1a** and **2a**.

In order to explore whether alkoxy or aminoalkoxy groups placed in ring C would interact at a complementary area at a binding site, **2b** and **3a** were examined for cytotoxic potencies. The result indicated in Fig. 2 reveals that while **2b** had statistically indistinguishable IC_{50} figures in the Molt 4/C8 screen from **1a** and **2a**, the presence of the dimethylaminoethoxy group found in **3a** led to increased potencies. Thus **3a** became the lead molecule in this study and development was planned in two

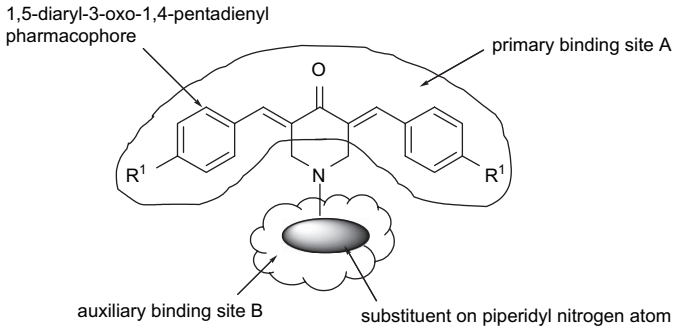
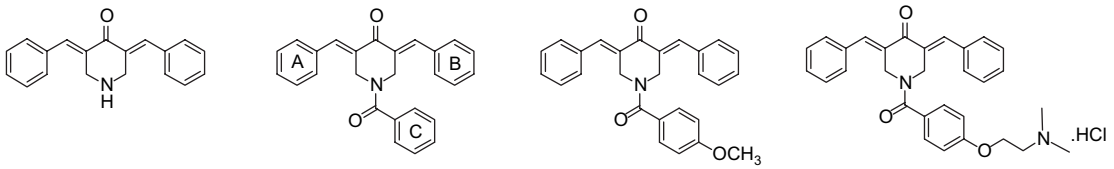


Fig. 1. Design of potential cytotoxins to interact at binding sites A and B.

directions. First, various substituents were placed in rings A and B which should influence the rate and extent of thiolation at the adjacent olefinic linkages. Second, the terminal basic centre of **2a** was altered in order to determine whether the pK_a and size of these groups would afford some idea of the structural requirements at a binding site. Such considerations led to the decision to prepare series **3**–**7**. With a view to identifying any general trends, all compounds were planned to be evaluated not only in the Molt 4/C8 screen but also against human CEM T-lymphocytes and murine L1210 cells.

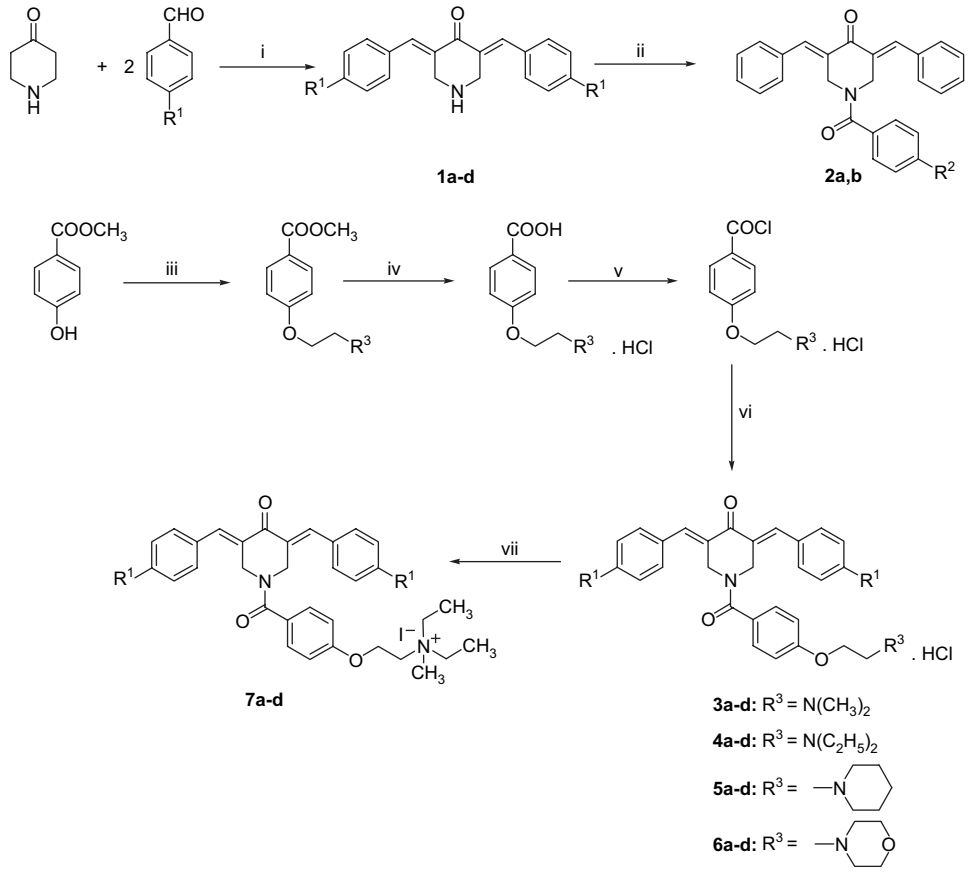
2. Chemistry

The synthetic chemical routes employed in producing series **1**–**7** are portrayed in Scheme 1. The compounds in series **1** were prepared by acid-catalyzed condensation between various aryl aldehydes and 4-piperidone. The reaction of **1a** with different aroyl chlorides led to the isolation of **2a**,**b**. Alkylation of methyl 4-hydroxybenzoate with 2-dimethylaminoethyl hydrochloride gave rise to the corresponding ether which upon hydrolysis produced 4-(2-dimethylaminoethyloxy)benzoic acid isolated as the hydrochloride salt. This compound was converted to the corresponding acid chloride which condensed with **1a**–**d** leading to the formation of **3a**–**d**, respectively. A similar synthetic pathway was employed using related *N*-(2-chloroethyl)amine hydrochlorides in place of 2-dimethylaminoethyl hydrochloride leading to the formation of **4a**–**d**, **5a**–**d** and **6a**–**d**. Reaction of the free bases derived from **4a**–**d** with methyl iodide gave rise to the corresponding quaternary ammonium salts **7a**–**d**. In addition, the preparation of 4-(2-diethylaminoethoxy)benzoic acid **8**, which is structurally related to the compounds in series **4**, was synthesized by the procedure indicated in Scheme 2.



1a (1.67 ± 0.15) **2a** (1.70 ± 0.07) **2b** (2.39 ± 0.73) **3a** (0.58 ± 0.09)

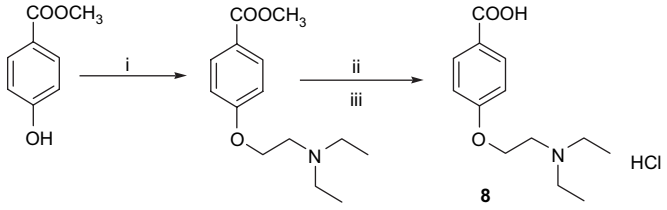
Fig. 2. Structures of **1a**, **2a**, **2b** and **3a** with IC_{50} values in μM towards Molt 4/C8 lymphocytes in parentheses.



Scheme 1. Synthesis of the compounds **1**–**7**. The substituents R^1 in series **1**, **3**–**7** were as follows: **a** = R^1 = H; **b**: R^1 = Cl; **c**: R^1 = NO_2; **d**: R^1 = CH_3. The nature of the R^2 moieties in series **2** were **a**: R^2 = H; **b**: R^2 = OCH_3. The following reagents were used in the synthetic routes *viz* i = HCl/CH_3COOH; ii = $ClCOC_6H_4R^2$; iii = $ClCH_2CH_2R^3$.HCl/K_2CO_3; iv = NaOH/HCl; v = $SOCl_2$; vi = **1a**–**d**; vii = CH_3I/K_2CO_3.

3. Bioevaluations

All the compounds in series **1**–**7** were evaluated against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells. In addition, in order to assess the possible contribution of the *N*-acyl-group *per se* to cytotoxicity, compound **8** was included in these assays. These data are presented in Table 1. The cytotoxic potencies of various compounds towards a panel of approximately 49 human tumour cell lines are given in Table 2. A TUNEL assay of a representative compound **6b** revealed that at a concentration of 20 μM, apoptosis occurred in human HepG2 liver cancer cells.



Scheme 2. Synthesis of compound **8**. The reagents used in preparing this compound were as follows: i = $ClCH_2CH_2N(C_2H_5)_2$. HCl/K_2CO_3/KI; ii = NaOH; iii = HCl.

4. Results and discussion

The evaluation of the compounds in series **1**–**8** towards human Molt 4/C8 and CEM T-lymphocytes was undertaken in order to determine whether the compounds were cytotoxic to human transformed cells. In addition, a number of clinically useful anticancer drugs inhibit the growth of murine L1210 cells [12] and consequently assays using this cell line were also undertaken. The data are presented in Table 1 which is so formatted that variation in potencies within different series, between series and in relation to melphalan may be rapidly perceived. Furthermore this presentation reveals the sensitivity of the different cell lines to the compounds.

The following comments will be made in reference to first the potencies of the molecules, second whether the data support the concept of a supplementary binding site B and third whether the nature of either the basic group in series **3**–**7** or the aryl substituents in rings A and B influence cytotoxic potencies.

The potencies of the compounds in series **1**–**7** were compared to the values generated from melphalan which is an anticancer drug exerting its action by alkylating cellular constituents [13]. Compounds **1a**,**d**, **2a**, **3a**–**d**, **4a**,**c**,**d**, **5a**,**c**,**d** and **6c**,**d** were more potent than melphalan in both the Molt 4/C8 and CEM assays. In particular, the IC_{50} values of the

Table 1
Potencies of the compounds in series **1**–**8** towards human Molt 4/C8 and CEM T-lymphocytes and murine L1210 cells[a]

	IC_{50} (μM)[b]					
Compound	Molt 4/C8 cells	SD	CEM cells	SD	L1210 cells	SD
1a[c]	1.67	0.15	1.70	0.02	7.96	0.11
1b[c]	13.4	4.0	8.63	0.48	41.4	0.3
1c[c]	8.28	0.75	4.47	2.28	32.9	4.2
1d	1.69	0.09	1.69	0.00	8.47	0.14
2a	1.70	0.07	1.64	0.03	12.0	0.0
2b	2.39	0.73	5.00	3.33	37.8	3.7
3a	0.58	0.09	1.24	0.57	10.2	0.8
3b	1.44	0.57	1.09	0.67	7.18	1.30
3c	0.314	0.035	0.319	0.013	5.19	0.71
3d	1.39	0.21	0.884	0.532	7.14	1.33
4a	1.28	0.15	1.65	0.15	8.23	0.43
4b	18.3	14.9	12.3	5.9	47.3	4.7
4c	0.31	0.01	0.34	0.01	3.63	0.20
4d	1.32	0.45	1.33	0.30	2.28	0.08
5a	1.48	0.12	1.16	0.64	8.40	0.22
5b	34.0	19.5	9.98	2.99	103	47
5c	0.330	0.011	0.315	0.002	4.74	2.47
5d	0.91	0.45	1.14	0.73	5.14	1.64
6a	8.42	0.16	6.84	0.09	222	13
6b	18.8	10.1	8.26	1.00	81.4	54.6
6c	0.343	0.013	0.379	0.032	6.34	1.08
6d	1.72	0.21	1.36	0.13	21.8	9.6
7a	124	82	110	12	160	30
7b	55.8	20.6	71.2	43.6	729	230
7c	40.2	2.9	39.3	5.2	65.0	0.9
7d	12.4	2.6	23.7	4.3	18.7	1.5
8	> 500	n/a	> 500	n/a	> 500	n/a
Melphalan[c]	3.24	0.56	2.47	0.21	2.13	0.02

[a] The background colors reflect the potencies expressed as IC_{50} values, namely black (<1 μM), dark grey (1–10 μM), grey (11–99 μM) and light grey (>100 μM).
[b] The IC_{50} value is the concentration of a compound which is required to inhibit the growth of the cells by 50%.
[c] Data reproduced from J. Med. Chem. **2001**, 44, 586. Copyright American Chemical Society.

4-nitro analogues **3c**, **4c**, **5c** and **6c** (all in the submicromolar range) towards both cell lines were, on average, nine times lower than the reference drug. The data, therefore, provide ample evidence for pursuing these novel antineoplastic agents.

The second phase of evaluating the biodata involved comparing the potencies of the compounds in series **1** with those obtained for the *N*-acyl analogues **2**–**7**. The aim was to determine whether a favourable alignment of the *N*-acyl group occurred or not. Compounds **1a**, **2a** and **2b** were equipotent in both the Molt 4/C8 and CEM assays indicating that the *N*-aroyl groups of **2a**,**b** had no effect on potencies. However, both **2a** and **2b** had greater IC_{50} values than **1a** in the L1210 screen, implying that the aroyl group in **2a** and **2b** exerted a dystherapeutic effect.

The promising effect of **3a** in the Molt 4/C8 screen predicated that a more thorough study should be conducted on analogues of this compound whereby variation of the basic group could occur as well as the placement of different substituents in the arylidene aryl rings A and B (Fig. 2). The basic groups studied varied in size and electronic properties while the aryl substituents were chosen from three of the four quadrants of a Craig scatter diagram [14]. The first analysis examined the effect on cytotoxic potencies of converting **1a**–**d** into the amides bearing the same aryl substituent in series **3**–**6**, *e.g.*, the potency of **1a** was compared with **3a**, then **4a**, **5a** and **6a** in each of the three cell lines. The potencies of the amides in series **3**–**6** compared to the analogues in series **1** were either increased, equipotent or diminished in 48%, 35% and 17%, respectively, of the comparisons made. The increases in potencies were most noticeable in **3c**, **4c**, **5c** and **6c** which contained a 4-nitro group. In all cases, a comparison of the cytotoxicity of the quaternary ammonium salts **7a**–**d** with the data generated for **1a**–**d** revealed reductions in cytotoxic potencies. While a number of quaternary ammonium compounds possess antineoplastic properties [15], the positively charged nitrogen atom in series **7** may have impeded, although not prevented, the penetration of the molecules via the cell membranes to one or more sites of action inside the cell. Thus, in general, potency was either increased or retained when the 4-piperidones **1a**–**d** were converted into the nonquaternary amides in series **3**–**6**. As mentioned previously, the compounds prepared in this study likely interact with different molecular targets. Hence the structural requirements of ligands for alignment at various binding sites will vary. However, the data obtained in this study suggest that for some of the molecular targets, an auxiliary binding site exists with which the 2-alkylaminoethoxyphenylcarbonyl group of **3**–**6** interacts.

A further point to consider in assessing the biodata presented in Table 1 is as follows. In the case of those amides in series **3**–**6** which were more potent than the analogues in series **1**, the possibility exists that the *N*-acyl group *per se* possesses cytotoxic properties. In addition, if hydrolysis of the amides occurs, a cytotoxic carboxylic acid could be liberated. In order to assess this issue, 4-(2-diethylaminoethyloxy)benzoic acid hydrochloride **8** was prepared. The IC_{50} values of **8** in the Molt 4/C8, CEM and L1210 bioassays are presented in Table 1 and its lack of potent cytotoxic properties suggests that the *N*-acyl groups in **3**–**6** exert an anchoring effect

Table 2
Cytotoxicity of representative compounds towards human tumour cell lines

Compound	All cell lines		Colon cancer cells, IC_{50} (μM)						Leukemic cells, IC_{50} (μM)			
	IC_{50} (μM)	S.I.[a]	COLO 205	HCT-116	HCT-15	KM12	SW-620	HCC-2998	K-562	RPMI-8226	HL-60 (TB)	SR
1a[b]	1.62	93.3	1.55	0.182	0.631	0.794	0.575	2.00	0.490	0.275	0.363	0.209
3b	1.91	141	1.70	6.03	0.295	1.74	0.209	0.263	0.331	0.098	1.41	0.126
3d	1.15	39.8	1.02	1.38	0.912	0.776	0.562	0.229	1.82	0.224	1.32	0.479
4a	<1.38	>2455	2.63	<0.01	1.17	1.74	1.35	–	0.043	<0.01	–	–
5b	>11.5	>66.1	15.8	47.9	49.0	19.1	3.55	1.58	4.37	0.759	20.4	2.29
5d	1.38	93.3	6.92	1.58	1.07	0.148	0.339	–	0.589	0.123	0.933	–
6a	>2.63	>457	22.9	0.339	1.45	0.129	0.191	–	0.158	0.123	0.562	–
6b	<0.562	>2188	–	7.25	2.19	2.29	1.45	<0.005	6.76	0.132	5.50	–
6d	<0.708	>1738	1.15	2.45	0.832	1.12	1.02	–	0.275	0.115	1.10	0.245
7a	>41.7	>4.47	20.4	>50.1	>50.1	43.7	30.2	28.2	43.7	17.4	>50.1	38.0
7d	7.76	166	8.91	14.1	9.33	9.33	10.5	6.17	1.35	1.26	11.8	22.9
5-Fluorouracil	>29.5	>4365	3.39	2.04	6.61	7.94	18.6	5.75	126	3.55	85.1	60.3
Melphalan	26.9	118	66.1	30.2	36.3	43.7	38.9	41.7	43.7	66.1	2.04	1.86

[a] The letters S.I. refer to the selectivity index, *i.e.*, the ratio of the IC_{50} figures for the least and most sensitive cell lines to the compound.
[b] The hydrochloride salt of **1a** was used in this assay.

thereby enhancing the critical interactions of the 1,5-diaryl-3-oxo-1,4-pentadienyl group with cellular constituents. In addition, hydrolysis of the amides **4a–d** to a cytotoxic carboxylic acid does not appear to take place.

In order to obtain guidelines for expanding this study, reviews of the biodata summarized in Table 1 for the compounds in series **3–7** were made in order to find the terminal group of the *N*-acyl side chain and the aryl substituent which were optimal in terms of cytotoxic potencies. In regard to the five terminal groups of the *N*-acyl side chains in **3–7**, a scale of 5 (lowest IC_{50} values), 4, 3, 2 and 1 (lowest potency) was employed which is described in greater detail in Section 6. The total scores for the four analogues in the three bioassays in each of the series **3–7** were 49, 45, 44, 28.5 and 13.5, respectively. The disparity in potencies between the series of compounds could have been due to variations in the sizes and electronic properties of the terminal group of the *N*-acyl side chain. Consequently linear, semilogarithmic and logarithmic plots were made between the potency scores in **3–6** and both the solvent accessible surface area (SASA) figures and the pK_a values of the basic groups. In addition, plots were made between the potency scores of series **3–7** and the SASA figures of the protonated basic groups of series **3–6** plus the quaternary ammonium function in **7**. However, no correlations were found ($p > 0.1$).

The relative contributions of the arylidene aryl substituents in series **3–7** were examined using a sliding scale of 4, 3, 2 and 1. The combined scores for the unsubstituted, 4-chloro, 4-nitro and 4-methyl compounds in the Molt 4/C8, CEM and L1210 tests were 29.5, 22, 52 and 46.5, respectively. In order to determine whether the electronic, hydrophobic or steric properties of the aryl substituents were responsible for the marked influences of the substituents on potencies, linear and semilogarithmic plots between the potency scores and the Hammett sigma, Hansch pi and molar refractivity values of the aryl substituents were made but no correlations were found ($p > 0.1$). The conclusions to be drawn from evaluating the contributions of the terminal group of the *N*-acyl side chain and aryl substituents are that future developments should involve the placement of strongly basic groups at the end of the 4-ethoxyphenylcarbonyl chain and inserting nitro or methyl substituents in the arylidene aryl rings.

The data in Table 1 revealed that the human transformed cells (Molt 4/C8 and CEM T-lymphocytes) were particularly vulnerable to many of the compounds prepared in this study. Hence an evaluation of selected compounds against a substantially greater number of human malignant cell lines was undertaken. The results of this determination are summarized in Table 2. In this assay, an average of 49 cell lines from nine different neoplastic diseases, namely leukemia, melanoma, non-small cell lung, colon, central nervous system, ovarian, renal, prostate and breast cancers were employed [16].

The average IC_{50} values against all cell lines were lower than that of melphalan except for **7a**. In particular, the data for **6b** and **6d** were particularly noteworthy since in the case of both compounds, the IC_{50} values against some of the cell lines were lower than the minimum concentration used, *viz*. $10^{-8.3}$ M. Hence **6b** and **6d** possessed in excess of 48 and 38 times, respectively, the potency of melphalan.

An important feature of novel antineoplastic agents is the display of greater toxicity for malignant cells rather than the corresponding normal tissues. A differentiation in cytotoxicity towards the cell lines would reveal that the compound displayed selective toxicity in contrast to being a general biocidal agent; such selectivity may translate into a preferential lethality for tumour cells rather than related non-malignant cells. Accordingly, a selective index (SI) figure for each compound was calculated being the ratios of the IC_{50} values between the least potent and most sensitive cell lines. A figure of 100 was arbitrarily chosen as significant since it approximated to the data for melphalan. The amides **3b**, **4a**, **6a**,**b**,**d** and **7d** met this criterion and, in particular, the high selectivity of **4a**, **6b** and **6d** indicated the utility of these three molecules as lead compounds.

A review of the mean graphs [17] revealed that in general the IC_{50} values of the compounds were lower towards colon

and leukemic neoplasms than the other cell lines. The selective toxicity for these groups of cancers is revealed in Table 2 since the IC_{50} figures of many of the colon and leukemic cell lines were lower than the values for all cell lines. An effective drug against colon tumours is 5-fluorouracil [18]. The data in Table 2 reveal that the IC_{50} figures for the compounds prepared in this study towards the six colon cancer cell lines indicated in Table 2 were lower than that of 5-fluorouracil in 67% of the cases. Melphalan is used in combination chemotherapy to treat chronic leukemias [19] and in 80% of the comparisons made for the K-562, RPMI-8226, HL-60 (TB) and SR cell lines, the compounds in series **1**, **3**–**7** had lower IC_{50} figures than melphalan. A general conclusion to be drawn from the evaluation of representative compounds against a panel of approximately 49 human tumour cell lines is that their potencies are substantially greater than certain clinically used drugs and that they have a particular toxicity towards colon cancer and leukemic cells.

The possible way in which these compounds exert their cytotoxic action was addressed. In a previous study, a cytotoxic piperidine bearing pendant conjugated arylvinylketo and arylvinyl groups was found to cause apoptosis in human Jurkat T leukemic cells [20]. Furthermore a number of anticancer drugs induce apoptosis [21]. Hence a representative compound was evaluated for apoptosis-inducing properties. A TUNEL assay revealed that at a concentration of 20 μM, **6b** induced apoptosis in human HepG2 liver cancer cells, indicating that, at least in part, this mechanism of action accounts for the cytotoxicity of **6b** and likely for the related analogs as well.

5. Conclusions

The 1-[4-(2-alkylaminoethoxy)phenylcarbonyl]-3,5-bis(arylidene)-4-piperidones are a novel group of cytotoxins. Many of these compounds exhibit significantly greater potencies than two established anticancer drugs in different bioassays. The attachment of the *N*-acyl group to the 3,5-bis(arylidene)-4-piperidones **1** leading to series **3**–**6** was accompanied by increases in potencies in approximately half of the comparisons made with the analogues in series **1**. This observation suggests that in the case of various members of series **3**–**6**, alignment of the *N*-acyl groups with auxiliary binding sites takes place reinforcing the interaction of the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore with a primary binding site.

6. Experimental protocols

6.1. Chemistry

Melting points are uncorrected. Elemental analyses (C, H, N) were undertaken on **1d** and **2**–**7** by the Microanalytical Laboratory, Department of Chemistry, University of Alberta and were within 0.4% of the calculated values. Compounds **3b**–**d**, **4a**, **5a**,**d**, **6a**,**c**,**d** and **7a**,**d** were isolated as the hemihydrates, **7b** as the monohydrate and **5c** was obtained with 1.5 mol of water of crystallization. The ^{1}H NMR spectra (500 MHz) of all of the compounds in series **1**–**8** and the ^{13}C NMR spectra (125 MHz) of representative compounds were determined in deuterated solvents using a Bruker AM 500 FT NMR machine.

6.1.1. Synthesis of *1a–d*

Compounds **1a**–**c** were prepared by a literature procedure [8]. The same procedure was used to prepare **1d** which was purified by recrystallization from chloroform–ethanol to give the desired product.

*6.1.1.1. 3,5-Bis(4-methylphenylmethylene)-4-piperidone (**1d**).* M.p. 180–181 °C. Yield: 79%. ^{1}H NMR ($CDCl_3$): 2.40 (s, 6H), 4.16 (s, 4H), 7.24 (d, 2H, $J = 7.75$ Hz), 7.31 (d, 2H, $J = 7.85$ Hz), 7.80 (s, 2H). Found C, 82.87; H, 6.94; N, 4.60%. Anal. ($C_{26}H_{21}NO_2$) requires C, 83.13; H, 6.98; N, 4.62%.

6.1.2. General procedure for the synthesis of *2a,b*

A solution of the aroyl chloride (0.015 mol) in 1,2-dichloroethane (50 ml) was added over 0.5 h to a solution of **1a** (0.01 mol) and triethylamine (0.03 mol) in 1,2-dichloroethane (30 ml) at ~5–6 °C. After stirring at room temperature for 6 h, the solvent was removed *in vacuo*. An aqueous solution of potassium carbonate (10% w/v, 50 ml) was added to the residue and the resultant slurry was stirred for 4 h at room temperature. The precipitate was collected, washed with water and dried. The products were purified by crystallization from chloroform–methanol.

*6.1.2.1. 1-Benzoyl-3,5-bis(phenylmethylene)-4-piperidone (**2a**).* M.p. 165–166 °C. Yield: 85%. ^{1}H NMR ($CDCl_3$): 4.72 (br s, 2H), 5.04 (br s, 2H), 7.06 (t, 2H), 7.22 (d, 4H), 7.38 (br s, 9H), 7.92 (s, 2H). Found C, 82.02; H, 5.49; N, 3.56%. Anal. ($C_{26}H_{21}NO_2$) requires C, 82.30; H, 5.58; N, 3.69%.

*6.1.2.2. 1-(4-Methoxybenzoyl)-3,5-bis(phenylmethylene)-4-piperidone (**2b**).* M.p. 177–178 °C. Yield: 72%. ^{1}H NMR ($CDCl_3$): 3.70 (s, 3H), 4.87 (br s, 4H), 6.51 (d, 2H, $J = 8.62$ Hz), 7.17 (d, 2H, $J = 8.69$ Hz), 7.40 (br s, 10H), 8.07 (s, 2H). Found C, 79.19; H, 5.72; N, 3.25%. Anal. ($C_{27}H_{23}NO_3$) requires C, 79.19; H, 5.66; N, 3.42%.

6.1.3. General procedure for the synthesis of series *3–6*

The general method employed for the preparation of the compounds in series **3**–**6** is exemplified by the synthesis of **4a**. A mixture of **8** *vide infra* (4.10 g, 0.015 mol), thionyl chloride (23.8 g, 2 mol) and dimethylformamide (0.01 ml) was heated under reflux for 3 h. Evaporation *in vacuo* led to the isolation of the corresponding acid chloride which was dissolved in 1,2-dichloroethane (50 ml) and added over a period of 0.5 h to a solution of **1a** (2.75 g, 0.01 mol) and triethylamine (3.03 g, 0.03 mol) in 1,2-dichloroethane (30 ml) at ~5–6 °C. After stirring at room temperature for 6 h, the solvent was removed *in vacuo*. The solid obtained was suspended in a solution of potassium carbonate (10% w/v, 50 ml) and stirred at room temperature for 4 h. The precipitate was collected, washed with water and dried to give

1-[4-(2-diethylaminoethyloxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone, *i.e.*, the free base corresponding to **4a**. The hydrochloride salt was prepared by dissolving the free base in a mixture of isopropanol and chloroform (3:2; 50 ml) and after the addition of activated charcoal (0.5 g), the mixture was stirred at room temperature for 1 h. The solution was acidified with dry hydrogen chloride gas and the mixture was stirred at room temperature for 1 h. The solvent was removed *in vacuo* to yield a viscous oil to which was added acetone (50 ml) and the mixture was heated under reflux for 0.5 h. On cooling to room temperature, the precipitate was collected and recrystallized from acetone to give **4a**. The analogs **4b–d** were prepared using the same methodology except that isopropanol was used as the recrystallization solvent for **4b** and **4c**. Series **3**, **5** and **6** were obtained in a similar manner from methyl 4-hydroxybenzoate and the appropriate 2-chloro-*N*-substituted-ethylamine hydrochloride. The solvent used in recrystallizing **3a–d**, **5a**,**b** and **6a–d** was isopropanol except that diethyl ether–methanol was used to purify **5c**, while **5d** was recrystallized from acetone.

*6.1.3.1. [4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis (phenylmethylene)-4-piperidone hydrochloride (**3a**).* M.p. 244–245 °C. Yield: 76%. ^{1}H NMR (DMSO-d_6): 2.83 (s, 6H), 3.46 (t, 2H), 4.22 (t, 2H), 4.80 (br s, 4H), 6.55 (d, 2H, $J = 8.55$ Hz), 7.08 (d, 2H, $J = 8.65$ Hz), 7.37 (br s, 10H), 7.76 (s, 2H), 11.08 (br s, 1H). Found C, 71.80; H, 5.90; N, 5.50%. Anal. ($C_{30}H_{31}ClN_2O_3$) requires C, 71.63; H, 6.21; N, 5.57%.

*6.1.3.2. 3,5-Bis(4-chlorophenylmethylene)-1-[4-(2-dimethylaminoethoxy)phenylcarbonyl]-4-piperidone hydrochloride hemihydrate (**3b**).* M.p. 250–251 °C. Yield: 71%. ^{1}H NMR (DMSO-d_6): 2.92 (s, 6H), 3.47 (t, 2H), 4.22 (t, 2H), 4.81 (br s, 4H), 6.58 (d, 2H, $J = 7.61$ Hz), 7.14 (d, 2H, $J = 7.65$ Hz), 7.39 (m, 8H), 7.82 (d, 2H, $J = 8.75$ Hz), 12.84 (br s, 1H). Found C, 62.45; H, 5.15; N, 4.86%. Anal. ($C_{30}H_{29}Cl_3N_2O_3 \cdot 0.5H_2O$) requires C, 62.02; H, 5.03; N, 4.82%.

*6.1.3.3. 1-[4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)4-piperidone hydrochloride hemihydrate (**3c**).* M.p. 215–217 °C. Yield: 62%. ^{1}H NMR (DMSO-d_6): 2.78 (s, 6H), 3.42 (t, 2H), 4.21 (t, 2H), 4.85 (br s, 4H), 6.70 (d, 2H, $J = 8.28$ Hz), 7.15 (d, 2H, $J = 8.25$ Hz), 7.72 (m, 4H), 7.82 (s, 2H), 8.27 (br s, 4H), 11.08 (br s, 1H). Found C, 59.45; H, 4.93; N, 9.08%. Anal. ($C_{30}H_{29}ClN_4O_7 \cdot 0.5H_2O$) requires C, 59.85; H, 4.85; N, 9.30%.

*6.1.3.4. 1-[4-(2-Dimethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenylmethylene)-4-piperidone hydrochloride hemihydrate (**3d**).* M.p. 224–226 °C. Yield: 64%. ^{1}H NMR ($CDCl_3$): 2.40 (s, 6H), 2.90 (t, 2H), 3.43 (t, 2H), 4.42 (t, 2H), 4.87 (br s, 4H), 6.58 (d, 2H, $J = 8.10$ Hz), 7.23 (m, 10H), 7.88 (s, 2H), 13.08 (br s, 1H). Found C, 71.38; H, 6.75; N, 5.21%. Anal. ($C_{32}H_{35}ClN_2O_3 \cdot 0.5H_2O$) requires C, 71.16; H, 6.53; N, 5.18%.

*6.1.3.5. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (**4a**).* M.p. 184–185 °C. Yield: 72%. ^{1}H NMR ($CDCl_3$): 1.45 (t, 6H, $2 \times CH_3$), 3.22 (m, 4H, $2 \times NCH_2CH_3$), 3.40 (t, 2H, OCH_2CH_2N), 4.41 (t, 2H, OCH_2CH_2N), 4.85 (br s, 4H, $2 \times$ piperidyl NCH_2), 6.50 (d, 2H, aryl H, $J = 8.1$ Hz), 7.17 (d, 2H, aryl H, $J = 9.05$ Hz), 7.44 (br s, 10H, aryl H), 7.92 (s, 2H, =CH), 12.70 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): 187.41 (*C*O), 170.14(−*C*O−N−), 158.77, 134.88, 132.21, 130.77, 130.08, 129.70, 129.25, 127.99, 114.19, 63.05 (OCH_2), 50.95 (OCH_2-CH_2-N), 47.39 (NCH_2), 8.89 (CH_3). Found C, 71.22; H, 6.41; N, 4.79%. Anal. ($C_{32}H_{35}ClN_2O_3 \cdot 0.5H_2O$) requires C, 71.16; H, 6.71; N, 5.18%.

*6.1.3.6. 3,5-Bis(4-chlorophenylmethylene)-1-[4-(2-diethylaminoethoxy)phenylcarbonyl]-4-piperidone hydrochloride (**4b**).* M.p. 210–211 °C. Yield: 68%. ^{1}H NMR ($CDCl_3$): 1.45 (t, 6H), 3.22 (br s, 4H), 3.41 (t, 2H), 4.45 (t, 2H), 4.81 (br s, 4H), 6.57 (d, 2H, $J = 8.34$ Hz), 7.17 (d, 2H, $J = 8.30$ Hz), 7.31 (m, 4H), 7.41 (d, 2H, $J = 6.54$ Hz), 7.84 (s, 2H), 12.70 (br s, 1H). Found C, 64.28; H, 5.26; N, 4.98%. Anal. ($C_{32}H_{33}Cl_3N_2O_3$) requires C, 64.06; H, 5.54; N, 4.67%.

*6.1.3.7. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone hydrochloride (**4c**).* M.p. 197–198 °C. Yield: 61%. ^{1}H NMR ($CDCl_3$): 1.46 (t, 6H), 3.19 (br s, 2H), 3.26 (br s, 2H), 4.45 (t, 2H), 4.45 (t, 2H), 4.82 (br s, 4H), 6.60 (d, 2H, $J = 8.25$ Hz), 7.14 (d, 2H, $J = 8.25$ Hz), 7.51 (br s, 4H), 7.89 (s, 2H), 8.26 (d, 2H, $J = 6.35$ Hz), 12.57 (br s, 1H). Found C, 62.06; H, 5.38; N, 9.19%. Anal. ($C_{32}H_{33}ClN_4O_7$) requires C, 61.88; H, 5.36; N, 9.02%.

*6.1.3.8. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenylmethylene)-4-piperidone hydrochloride (**4d**).* M.p. 204–205 °C. Yield: 62%. ^{1}H NMR ($CDCl_3$): 1.45 (t, 6H), 2.40 (s, 6H), 3.22 (m, 4H), 3.40 (t, 2H), 4.46 (t, 2H), 4.87 (br s, 4H), 6.57 (d, 2H, $J = 8.53$ Hz), 7.23 (d, 2H, $J = 8.37$ Hz), 7.32 (m, 4H), 7.88 (s, 2H), 12.62 (br s, 1H). Found C, 73.34; H, 6.86; N, 5.36%. Anal. ($C_{34}H_{39}ClN_2O_3$) requires C, 73.04; H, 7.03; N, 5.01%.

*6.1.3.9. 1-[4-{2-(1-Piperidinyl)ethoxy}phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (**5a**).* M.p. 214–215 °C. Yield: 52%. ^{1}H NMR ($CDCl_3$): 1.43 (t, 1H), 1.88 (t, 3H), 2.28 (q, 2H), 2.76 (t, 2H), 3.34 (br s, 2H), 3.60 (t, 2H), 4.44 (br s, 2H), 4.86 (br s, 4H), 6.50 (d, 2H, $J = 7.75$ Hz), 7.16 (d, 2H, $J = 7.2$ Hz), 7.38 (br s, 10H), 7.90 (s, 2H), 12.61 (br s, 1H). Found C, 71.48; H, 6.38; N, 5.04%. Anal. ($C_{33}H_{35}ClN_2O_3 \cdot 0.5H_2O$) requires C, 71.79; H, 6.38; N, 5.07%.

*6.1.3.10. 3,5-Bis(4-chlorophenylmethylene)-1-[4-{2-(1-piperidinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride (**5b**).* M.p. 257–258 °C. Yield: 75%. ^{1}H NMR ($CDCl_3$): 1.44 (q, 1H), 1.92 (t, 3H), 2.32 (q, 2H), 2.80 (q, 2H), 3.38 (br s, 2H), 3.63 (d, 2H, $J = 10.23$ Hz), 4.54 (br s, 2H), 4.80 (br s, 4H), 6.57 (d, 2H, $J = 7.84$ Hz), 7.17 (d, 2H, $J = 7.99$ Hz), 7.34 (m, 8H), 7.90 (s, 2H), 12.68 (br s, 1H). Found C, 64.35; H, 5.39; N, 4.45%. Anal. ($C_{33}H_{33}Cl_3N_2O_3$) requires C, 64.77; H, 5.44; N, 4.58%.

*6.1.3.11. 3,5-Bis(4-nitrophenylmethylene)-1-[4-{2-(1-piperidinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride 1.5 hydrate (**5c**).* M.p. 224–226 °C. Yield: 64%. ^{1}H NMR (DMSO-d_6): 1.42 (t, 1H), 1.81 (t, 3H), 2.04 (q, 2H), 2.86 (q, 2H), 3.36 (d, 2H), 3.48 (m, 2H), 4.32 (br s, 2H), 4.75 (br s, 4H), 6.56 (d, 2H, $J = 8.25$ Hz), 7.07 (d, 2H, $J = 8.10$ Hz), 7.60 (m, 4H), 7.78 (s, 2H), 8.16 (br s, 4H), 11.53 (s, 1H). Found C, 71.38; H, 6.75; N, 5.21%. Anal. ($C_{32}H_{35}ClN_2O_3 \cdot 1.5H_2O$) requires C, 71.16; H, 6.53; N, 5.18%.

*6.1.3.12. 3,5-Bis(4-methylphenylmethylene)-1-[4-{1-piperidinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride hemihydrate (**5d**).* M.p. 105 °C (dec). Yield: 65%. ^{1}H NMR ($CDCl_3$): 1.42 (q, 1H), 1.89 (t, 3H), 2.22 (q, 2H), 2.80 (br s, 2H), 3.40 (br s, 2H), 3.65 (d, 2H, $J = 9.56$ Hz), 4.48 (br s, 2H), 4.88 (br s, 4H), 5.44 (br s, 2H), 6.56 (d, 2H, $J = 7.91$ Hz), 7.23 (m, 10H), 7.99 (s, 2H), 11.80 (br s, 1H). Found C, 71.38; H, 6.75; N, 5.21%. Anal. ($C_{32}H_{35}ClN_2O_3 \cdot 0.5H_2O$) requires C, 71.16; H, 6.53; N, 5.18%.

*6.1.3.13. 1-[4-{2-(4-Morpholinyl)ethoxy}phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone hydrochloride hemihydrate (**6a**).* M.p. 208–209 °C. Yield: 73%. ^{1}H NMR ($CDCl_3$): 3.05 (q, 2H), 3.40 (br s, 2H), 3.53 (d, 2H, $J = 12.02$ Hz), 4.02 (dd, 2H), 4.30 (t, 2H), 4.47 (br s, 2H), 4.86 (br s, 4H), 6.51 (d, 2H, $J = 8.56$ Hz), 7.19 (d, 2H, $J = 8.50$ Hz), 7.44 (br s, 10H), 7.92 (s, 2H), 13.69 (br s, 1H). Found C, 69.54; H, 5.95; N, 4.96%. Anal. ($C_{32}H_{33}ClN_2O_4 \cdot 0.5H_2O$) requires C, 69.36; H, 6.00; N, 5.05%.

*6.1.3.14. 3,5-Bis(4-chlorophenylmethylene)-1-[4-{2-(4-morpholinyl)ethoxy}phenylcarbonyl-4-piperidone hydrochloride (**6b**).* M.p. 240–241 °C. Yield: 74%. ^{1}H NMR ($CDCl_3$): 3.06 (br s, 2H), 3.40 (br s, 2H), 3.55 (t, 2H), 4.04 (d, 2H, $J = 11.97$ Hz), 4.29 (t, 2H), 4.30 (t, 2H), 4.50 (br s, 2H), 4.81 (br s, 4H), 6.59 (d, 2H, $J = 7.30$ Hz), 7.14 (d, 2H, $J = 7.47$ Hz), 7.51 (m, 4H), 7.90 (s, 2H), 8.26 (br s, 4H), 13.59 (s, 1H). Found C, 62.39; H, 4.94; N, 4.47%. Anal. ($C_{32}H_{31}Cl_3N_2O_4$) requires C, 62.60; H, 5.09; N, 4.56%.

*6.1.3.15. 1-[4-{2-(4-Morpholinyl)ethoxy}phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone hydrochloride hemihydrate (**6c**).* M.p. 224–226 °C. Yield: 64%. ^{1}H NMR ($CDCl_3$): 3.05 (q, 2H), 3.40 (br s, 2H), 3.53 (d, 2H, $J = 12.02$ Hz), 4.02 (dd, 2H), 4.30 (t, 2H), 4.47 (br s, 2H), 4.86 (br s, 4H), 6.51 (d, 2H, $J = 8.56$ Hz), 7.19 (d, 2H, $J = 8.50$ Hz), 7.44 (br s, 10H), 7.92 (s, 2H), 13.59 (br s, 1H). Found C, 59.52; H, 4.72; N, 8.51%. Anal. ($C_{32}H_{31}ClN_4O_8 \cdot 0.5H_2O$) requires C, 59.67; H, 4.85; N, 8.69%.

*6.1.3.16. 3,5-Bis(4-methylphenylmethylene)-1-[4-{2-(4-morpholinyl)ethoxy}phenylcarbonyl]-4-piperidone hydrochloride hemihydrate (**6d**).* M.p. 235–236 °C. Yield: 66%. ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H), 3.03 (m, 2H), 3.40 (br s, 2H), 3.54 (d, 2H, $J = 11.98$ Hz), 4.02 (dd, 2H), 4.29 (t, 2H), 4.50 (br s, 2H), 4.87 (s, 4H), 6.58 (d, 2H, $J = 8.63$ Hz), 7.24 (m, 10H), 7.88 (s, 2H), 13.68 (br s, 1H). Found C, 70.39; H, 6.69; N, 4.79%. Anal. ($C_{34}H_{37}ClN_2O_4 \cdot 0.5H_2O$) requires C, 70.15; H, 6.57; N, 4.81%.

*6.1.4. General procedure for the synthesis of **7a–d***

Activated charcoal (0.5 g) was added to a solution of the free base of **4a** *vide supra* (4.95 g, 0.0l mol) in acetone (25 ml) and the mixture was stirred at room temperature for 1 h. The suspension was filtered via celite and the celite bed was washed with acetone (10 ml). Methyl iodide (2.13 g, 0.015 mol) was added to the combined filtrates and the mixture was stirred at room temperature for 4–5 h. The resultant precipitate was collected, dried and recrystallized from diethyl ether/methanol to give the desired products.

*6.1.4.1. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(phenylmethylene)-4-piperidone methoiodide (**7a**).* M.p. 161–162 °C. Yield: 85%. ^{1}H NMR ($CDCl_3$): 1.45 (t, 6H, $2 \times CH_3$), 3.34 (s, 3H, N–CH_3), 3.70 (m, 4H, $2 \times NCH_2CH_3$), 4.17 (br s, 2H, OCH_2CH_2N), 4.39 (br s, 2H, OCH_2CH_2N), 4.86 (br s, 4H, piperidyl H), 6.56 (d, 2H, aryl H, $J = 8.58$ Hz), 7.19 (d, 2H, aryl H, $J = 8.03$ Hz), 7.39 (br s, 12H, aryl H), 7.91 (s, 2H, olefinic H). Found C, 61.11; H, 5.64; N, 4.23%. Anal. ($C_{33}H_{37}IN_2O_3 \cdot 0.5H_2O$) requires C, 61.39; H, 5.77; N, 4.33%.

*6.1.4.2. 3,5-Bis(4-chlorophenylmethylene)-1-[4-(2-diethylaminoethoxy)phenylcarbonyl]-4-piperidone methoiodide (**7b**).* M.p. 220–223 °C. Yield: 69%. ^{1}H NMR (DMSO-d_6): 1.32 (t, 6H), 3.07 (d, 3H, $J = 7.99$ Hz), 3.45 (t, 4H), 3.74 (t, 2H), 4.30 (br s, 1H), 4.39 (br s, 1H), 4.61 (br s, 4H), 6.60 (d, 1H, $J = 8.22$ Hz), 7.11 (d, 1H, $J = 8.35$ Hz), 7.23 (d, 1H, $J = 8.36$ Hz), 7.71 (br s, 1H), 7.89 (s, 2H). Found C, 54.44; H, 4.79; N, 3.75%. Anal. ($C_{33}H_{35}Cl_2IN_2O_3 \cdot 0.5H_2O$) requires C, 54.78; H, 4.87; N, 3.92%.

*6.1.4.3. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-nitrophenylmethylene)-4-piperidone methoiodide (**7c**).* M.p. 193–195 °C (dec). Yield: 74%. ^{1}H NMR (DMSO-d_6): 1.24 (t, 6H), 3.00 (s, 3H), 3.38 (q, 4H), 3.64 (t, 2H), 4.27 (t, 2H), 4.40 (br s, 4H), 6.70 (d, 2H, $J = 8.41$ Hz), 7.16 (d, 2H, $J = 8.39$ Hz), 7.74 (br s, 4H), 7.83 (s, 2H), 8.27 (br s, 4H). Found C, 54.41; H, 4.71; N, 7.60%. Anal. ($C_{33}H_{35}IN_4O_7$) requires C, 54.55; H, 4.86; N, 7.71%.

*6.1.4.4. 1-[4-(2-Diethylaminoethoxy)phenylcarbonyl]-3,5-bis(4-methylphenylmethylene)-4-piperidone methoiodide (**7d**).* M.p. 212–213 °C. Yield: 72%. ^{1}H NMR (DMSO-d_6): 1.24 (t, 6H), 2.34 (s, 6H), 3.01 (s, 3H), 3.39 (q, 4H), 3.68 (t, 2H), 4.33 (t, 2H), 4.87 (br s, 4H), 6.75 (d, 2H, $J = 8.34$ Hz), 7.22 (d, 2H, $J = 8.30$ Hz), 7.28 (m, 8H), 7.72 (s, 2H). Found C, 62.65; H, 6.25; N, 4.10%. Anal. ($C_{35}H_{41}IN_2O_3 \cdot 0.5H_2O$) requires C, 62.40; H, 6.13; N, 4.15%.

*6.1.5. Synthesis of **8***

A mixture of methyl 4-hydroxybenzoate (15.21 g, 0.1 mol), anhydrous potassium carbonate (34.55 g, 0.25 mol), 2-chloro-*N*,*N*-diethylamine hydrochloride (25.81 g, 0.15 mol), potassium iodide (0.166 g, 0.001 mol) and acetone (75 ml) was heated under reflux for 8–9 h. On cooling, the solid was removed by filtration and the solvent was evaporated. The residue was dissolved in toluene (75 ml) and the solution was washed with sodium hydroxide solution (2% w/v, 30 ml) and deionized water (2 × 30 ml). Removal of the solvent afforded methyl 4-(2-diethylaminoethyloxy)benzoate as an oil which was dissolved in ethanol (50 ml) and added to a solution of sodium hydroxide (8.0 g, 0.2 mol) in water (50 ml). The mixture was heated under reflux for 2 h. The ethanol was removed *in vacuo* and the aqueous solution was acidified with hydrochloric acid (12 N) at 5–6 °C. The solid was collected, triturated with water (previously cooled to 5–6 °C), filtered and dried at 55–60 °C *in vacuo* and recrystallized from water to give **8**.

*6.1.5.1. 4-(2-Diethylaminoethoxy)benzoic acid hydrochloride (**8**).* M.p. 170–172 °C [lit. [22], m.p. 171–174 °C]. Yield: 73%. ^{1}H NMR (D_2O): 1.17–1.20 (t, 6H, 2 × CH_3), 3.13–3.24 (m, 4H, 2 × CH_2), 3.47–3.48 (t, 2H, OCH_2CH_2N), 4.26–4.28 (t, 2H, OCH_2CH_2N), 6.89–6.91 (d, 2H, aryl H), 7.80–7.82 (d, 2H, aryl H).

6.1.6. Statistical evaluations

The SASA figures of the dimethylamino, diethylamino, piperidino and morpholino groups (free bases and protonated species) as well as the diethylmethylammonium substituent were obtained from MacroModel 7.1 [23], while the pK_a values of the basic groups in series **3–6** were culled from the literature [24]. The Hammett sigma, Hansch pi and molar refractivity values of the hydro, 4-chloro, 4-nitro and 4-methyl substituents were taken from a reference source [25].

6.2. Cytotoxic evaluations

The compounds in series **1–8** and melphalan were evaluated against Molt 4/C8, CEM and L1210 cells by a literature procedure [26]. In these assays, at least three different concentrations of compounds were incubated with the neoplastic cells at 37° for 48 h after which time inhibition of growth was determined.

Selected 4-piperidones were examined for antineoplastic properties towards a panel of 49 (38–59) human tumour cell lines using a literature method [16]. In addition, both 5-fluorouracil and melphalan were evaluated towards 57 cell lines. In this assay, compounds were incubated for 48 h using five different concentrations at series tenfold dilutions of $10^{-8.3}$ M–$10^{-4.3}$ M except for **1a**, **4a** (10^{-8} M–10^{-4} M), 5-fluorouracil ($10^{-6.6}$ M–$10^{-2.6}$ M) and melphalan ($10^{-7.6}$ M–$10^{-3.6}$ M). The MG MID figures for **1a**, **3b**,**d**, **5d**, **7d** and melphalan were IC_{50} values. In the following cases, the number of cell lines compared to the total number of cell lines for which an IC_{50} figure were not obtained were as follows [maximum or minimum concentrations (molar) in parentheses], namely **4a**: 2/53 ($<10^{-8}$), **5b**: 2/50 ($>10^{-4.3}$), **6a**: 3/48 ($>10^{-4.3}$), **6b**: 11/38 ($<10^{-8.3}$), **6d**: 3/44 ($<10^{-8.3}$), **7a**: 36/52 ($>10^{-4.3}$) and 5-fluorouracil 3/57 ($>10^{-2.6}$).

*6.3. Rankings of potencies of the compounds in series **3–7***

The first investigation involved comparing the IC_{50} values of those compounds in series **3–7** which had the same substituents in the arylidene aryl ring. The scores for the compounds in each of the Molt 4/C8, CEM and L1210 cell lines were obtained as follows. The compound having the highest potency was assigned a value of 5, the compound with the next lowest IC_{50} figures was given a rating of 4 and so forth; the amide with lowest potency was given a figure of 1. The standard deviations for each IC_{50} value were taken into consideration. The scores available for equipotent compounds were divided equally and a total of 15 points were awarded for each of the comparisons. The second analysis was designed to find the optimal substitution pattern in the arylidene aryl rings in the three cell lines. Thus in each of the series **3–7**, the most potent compound was given a rating of 4, the second most potent analog 3 and so forth. A total of 10 points were used in each comparison. Standard deviations were taken into consideration and the scores for compounds having the same potencies were divided equally.

6.4. Apoptosis assay

HepG2 cells obtained from the ATCC were grown in Dulbecco's modified eagle medium supplemented with a 1% antibiotic solution containing penicillin G sodium, streptomycin sulfate and amphoterin B (GIBCO, Burlington, Canada) as well as 10% fetal calf serum. An environment of humidified air containing 5% carbon dioxide was maintained at 37 °C. The HepG2 cells were cultured in chambered slides using 20 μM of **6b** while control experiments with the cells in the absence and presence of dimethylsulfoxide were undertaken. The cells were fixed and permeabilized after 48 h and apoptotic cells were detected by the TUNEL assay. This assay employed the Apoptosis Detection System, Fluorescein (Promega, Madison) and followed the protocol of the manufacturer. Apoptosis cells having fluorescently labelled DNA were visualized under a fluorescent microscope. The percentage of apoptotic cells was 29.25 ± 7.6.

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