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Original article

Design, synthesis and antiproliferative activity of some 3-benzylidene-2,3-dihydro-1-benzopyran-4-ones which display selective toxicity for malignant cells

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Abstract

A series of 3-benzylidene-4-chromanones **1a–l** were prepared and their cytotoxicity towards human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 lymphoid leukemia cells were compared to the previously generated biodata in these three assays for the isosteric 2-benzylidene-1-tetralones **2a–l**. Over 40% of the compounds in series **1** were more potent than their counterparts in series **2**, while equipotency was noted in one-third of the comparisons made. In general the IC_{50} values of **1a–l** towards the human T-lymphocytes were in the low micromolar range. Molecular modelling revealed differences in shapes of representative molecules in series **1** and **2** which may contribute to the variation in cytotoxic potencies. Most of the compounds in series **1** displayed greater potencies towards HSC-2, HSC-3, HSC-4 and HL-60 neoplasms than HGF, HPC, and HPLF normal cells and were well tolerated in mice.

Keywords: 3-Benzylidene-4-chromanones; α,β-Unsaturated ketones; Molecular modelling; Cytotoxicity; Murine toxicity

1. Introduction

The major focus in our laboratories is the study of antineoplastic α,β-unsaturated ketones [1,2]. The interest is due to a number of factors including the observation that conjugated enones possess a marked affinity for thiols in contrast to hydroxy and amino groups [3,4]. Thus interactions of conjugated unsaturated ketones with the latter two groups in nucleic acids should be absent and hence the genotoxic properties associated with certain alkylating agents [5] may be avoided. Since there are a number of dysregulated processes in carcinogenesis, the identification of thiol alkylators which react with multiple molecular targets may lead to clinically useful anticancer drugs. Of particular interest is the discovery of novel cytotoxins which have two important properties, namely a greater toxicity to cancer cells than normal cells as well as being well tolerated *in vivo*.

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Currently there is a considerable interest in the bioactivities of chalcones [6,7]. These compounds are able to adopt a range of conformations and several years ago, a series of 2-benzylidene-1-tetralones were prepared which may be regarded as rigid analogs of chalcones [8,9]. These alicyclic compounds displayed a wide range of potencies towards three neoplastic or transformed cell lines, namely human Molt 4/C8 and CEM T-lymphocytes and murine L1210 leukemia cells. A number of these 2-benzylidene-1-tetralones are well tolerated in mice, i.e., doses up to and including 300 mg/kg did not produce mortalities although some neurotoxicity was observed [9].

The initial aim of the present study was to prepare a number of prototypic molecules in which the 4-methylene group of the 2-benzylidene-1-tetralones was replaced by oxygen to give a series of 3-benzylidene-2,3-dihydro-1-benzopyran-4-ones **1a–l** referred to subsequently as 3-benzylidene-4-chromanones (Scheme 1). This molecular modification was undertaken for the following reasons. The size of the alicyclic ring B in series **2–4** (Fig. 1) influences the relative positions of the aryl rings A and C [8,9] and these topographical differences were considered to contribute to the variation in cytotoxic properties. Hence if the shapes of the molecules **1** and the 2-benzylidene-1-tetralones **2** differ, variation in cytotoxic potencies may result which could provide valuable insights into the structural features controlling cytotoxic potencies. Second, the inclusion of the ether oxygen atom in ring B of **1** may enhance the formation of hydrogen bonding between this atom and a complementary group on a receptor. Third, the hydrophobicity of series **1** would be anticipated to be much lower than the analogs in series **2**. Fourth, the torsion angle θ between ring C and the adjacent olefinic group may vary between series **1** and **2** when the same aryl substituents are present in ring C.

Thus the initial aim was to prepare **1a–l** and compare the cytotoxicity of these compounds with those analogs in series **2** which possessed the same aryl substituents. In addition, if the potencies of **1a–l** were promising (IC_{50} values in the low micromolar range), an investigation was planned to evaluate whether preferential toxicity to malignant rather than normal cells would be discerned and whether the compounds would be well tolerated *in vivo*. From these biodata, a decision should be possible regarding whether future experimentation should be undertaken on some or all of the compounds in series **1** such as mode of action studies and *in vivo* evaluations.

2. Chemistry

The 4-chromanones **1a–l** were synthesized from 2,3-dihydro-1-benzopyran-4-one and the appropriate aryl aldehyde as indicated in Scheme 1. In the case of the enones in series **2–4** and related compounds, ^{1}H NMR spectroscopy revealed that the olefinic hydrogen atoms of the *E* isomers absorb in the range of 7.2–8.0 ppm [8–12] while X-ray crystallography of representative molecules in series **2–4** confirmed the *E* stereochemistry [8,9]. In the case of **1a–l**, ^{1}H NMR spectroscopy indicated that the compounds are isomerically pure and the olefinic protons absorb in the region of 7.8–8.0 ppm, confirming that these compounds are the *E* isomers.

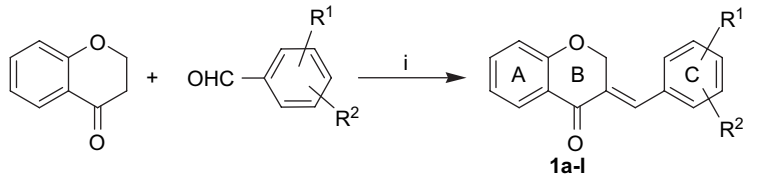
3. Bioevaluations

All the compounds were evaluated against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemic cells. These data are presented in Table 1. In addition, the cytotoxicity of **1a–l** was examined using human HSC-2, HSC-3 and HSC-4 squamous cell carcinomas and human HL-60 promyelocytic leukemia cells. Three non-malignant human cells were also employed viz the HGF gingival fibroblast, HPC pulp cells and the HPLF peridontal ligament fibroblast. The results are summarized in Table 3. In order to obtain some idea of the *in vivo* toxicity of **1a–l**, doses of 30, 100 and 300 mg/kg were injected intraperitoneally into mice and the animals were observed at the end of 0.5 and 4 h for survival and signs of impaired functioning. In addition, the animals were examined for neurotoxicity using the rotorod test [13].

4. Results and discussion

The evaluation of the 4-chromanones **1a–l** against human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 cells is presented in Table 1. In the case of the Molt 4/C8 and CEM screens, in general the IC_{50} values are in the low micromolar range and the nature of the aryl substituents has little effect on cytotoxic potencies. On the other hand, L1210 cells are considerably more resistant to these compounds and the IC_{50} values vary considerably. Since **1l** is an outlier in the CEM assay and no IC_{50} is available for this compound, comparisons were made between the cytotoxic potencies of **1a–k** in these three screens. The average IC_{50} values of **1a–k** in the Molt 4/C8, CEM and L1210 screens are 8.08, 9.78 and 103 μM, respectively, revealing their potential towards human transformed cells.

A comparison was made between the potencies of **1a–l** and the corresponding 1-tetralones **2a–l** in which the aryl substituents were identical, i.e., the IC_{50} values of **1a** and **2a** in the Molt 4/C8 assay were compared, then in the CEM assay and



Scheme 1. Synthesis of 3-benzylidene-4-chromanones **1a–l**. The R^1 and R^2 substituents are presented in Table 1 and i = piperidine.

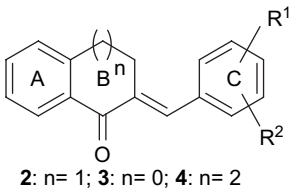


Fig. 1. Structures of some 2-benzylidenebenzocycloalkanones **2**–**4**.

finally, in the L1210 assay. Relative potency (RP) values for each compound were computed which are the quotients of the IC_{50} values of the 1-tetralones and the corresponding 4-chromanones. These data are presented in Table 1 while a summary of the comparisons of the potencies between **1a**–**l** and **2a**–**l** is provided in Table 2. Two conclusions drawn by considering the effects on cytotoxic potencies by replacing the 4-methylene group of series **2** by oxygen to form **1a**–**l** are as follows. First, increases in potencies were noted in 42% of the compounds in series **1** while equipotency was observed in one-third of the remaining comparisons. In particular, huge increases in potencies were noted towards Molt 4/C8 cells by **1b**,**e**,**k** and towards CEM cells by **1e**,**k**, as compared to their counterparts in series **2**. Second, the changes in cytotoxic potencies between the analogs in series **1** and **2** were highly dependent on the cell line under consideration. Thus in general the human T-lymphocytes are more susceptible to the 4-chromanones than the analogs in series **2**. On the other hand, the 1-tetralones rather than **1a**–**l** have greater cytotoxic potencies in the L1210 assay.

Several physicochemical determinations were undertaken with the goal of finding reasons for the disparity in cytotoxic potencies between the compounds in series **1** and **2**. First, molecular models of **1a**–**l** and **2a**–**l** were built. An important way whereby the compounds in both series **1** and **2** exert their bioactivity is considered to be the reaction of the enone moiety with cellular thiols [14]. Hence two compounds in both series were chosen, namely **1a**,**b** and **2a**,**b**, since as the data in Table 1 reveal, there were statistically significant differences in potencies between **1a** and **2a** as well as **1b** and **2b** in five of the six comparisons made. The root mean square values of the overlap of the O=C–C=C atoms of **1a/2a** and **1b/2b** are 0.9633 and 0.4926, respectively, indicating that the shapes of the enone groups are very similar and therefore unlikely to account for the differences in cytotoxic potencies. However, these overlaps revealed the marked differences in the spatial orientations of aryl ring C in **1a/2a** and **1b/2b** which is illustrated for **1b** and **2b** in Fig. 2. Hence the torsion angles between ring C and the adjacent olefinic group were measured in both series of compounds and designated θ_1 and θ_2, respectively; these data are presented in Table 1. The deviation from coplanarity of ring C and the olefinic group was attributed to nonbonded interactions between one of the *ortho*-protons of ring C and the equatorial hydrogen atom in ring B located beta to the carbonyl group. The huge differences in θ values between **1a**–**l** and **2a**–**l** indicate that ring C in series **1** adopts a markedly different topography than is displayed by the 1-tetralones **2** which may contribute significantly to the variation in

Table 1
Cytotoxicity of **1a**–**l** towards human Molt 4/C8 and CEM T-lymphocytes as well as murine L1210 leukemia cells

Compound	R^1	R^2	Molt 4/C8		CEM		L1210		Torsion angle[c]		clog *P*[d]	TPSA[d]
			IC_{50} (μM)[a]	RP[b]	IC_{50} (μM)[a]	RP[b]	IC_{50} (μM)[a]	RP[b]	θ_1	θ_2		
1a	2-Cl	H	7.70 ± 0.25	3.84*	8.87 ± 1.01	0.99	38.8 ± 1.8	0.77*	59.4	119.8	4.46	26.31
1b	4-Cl	H	8.33 ± 0.17	>60.0*	8.24 ± 0.57	5.85*	242 ± 37	0.29*	50.0	131.1	4.46	26.31
1c	2-Br	H	7.63 ± 0.078	4.82*	11.6 ± 4.2	2.19	36.3 ± 2.9	0.92	62.0	116.1	4.61	26.31
1d	3-Br	H	5.22 ± 3.53	4.06*	4.81 ± 3.78	1.83	80.3 ± 36.3	1.97	50.5	130.6	4.61	26.31
1e	4-Br	H	8.11 ± 0.08	>61.7*	8.43 ± 0.61	>59.3*	254 ± 41	0.80	50.1	131.1	4.61	26.31
1f	2-OCH_3	H	7.75 ± 0.44	4.04*	7.92 ± 0.18	1.59*	46.9 ± 6.6	0.86	54.5	130.0	3.67	35.54
1g	3-OCH_3	H	7.72 ± 0.32	1.57*	8.05 ± 0.18	1.09*	148 ± 52	0.41*	50.6	130.5	3.67	35.54
1h	4-OCH_3	H	13.8 ± 6.9	0.68	25.5 ± 19.0	0.35	67.3 ± 23.4	0.65	49.6	131.6	3.67	35.54
1i	2-OCH_3	4-OCH_3	6.83 ± 0.25	0.25*	7.87 ± 0.71	0.24*	18.1 ± 2.9	0.44*	54.1	126.9	3.76	44.77
1j	3-CH_3	H	7.61 ± 0.42	4.70*	7.94 ± 0.10	1.19*	124 ± 35	0.59	50.4	130.7	4.25	26.31
1k	4-CH_3	H	8.21 ± 0.71	>60.9*	8.30 ± 0.44	55.4*	71.4 ± 28.3	2.26	49.9	131.2	4.25	26.31
1l	4-$N(CH_3)_2$	H	12.0 ± 1.7	0.18*	>100[e]	<0.02*	91.0 ± 0.8	0.19*	49.2	132.2	3.91	29.54
Melphalan	–	–	3.24 ± 0.79	–	2.47 ± 0.30	–	2.13 ± 0.03	–	–	–	–	–

[a] The IC_{50} value is the concentration of compound required to inhibit the growth of the cells by 50%.
[b] The letters RP refer to relative potency, i.e., the ratio between the IC_{50} values of the 1-tetralone in series **2** and 1-chromanone in series **1** which have the same aryl substituents. An asterisk (*) indicates a statistically significant difference in potencies taking standard deviations into account.
[c] The θ_1 and θ_2 values refer to the torsion angles between the arylidene aryl rings and the adjacent olefinic linkages in series **1** and **2**, respectively.
[d] The letters clog *P* and TPSA refer to the calculated log *P* and topological polar surface area of the molecules in series **1**, respectively.
[e] Insolubility of **1l** was noted at 500 μM while 28% inhibition was observed at 100 μM.

Table 2
Comparison of the potencies of **1a–l** with **2a–l** when the aryl substituents are identical

Assay	Greater potency in		Equal potency between **1a–l** and **2a–l**
	1a–l	**2a–l**	
Molt 4/C8	9	2	1
CEM	6	2	4
L1210	0	5	7
Total	15	9	12
%	42	25	33

potencies observed. For example, the shapes of many of the compounds in series **1** may enhance interaction at a binding site in the Molt 4/C8 cells compared to the 1-tetralones whereas the reverse phenomenon is likely in the L1210 assay. Second, the replacement of the 4-methylene group (CH_2) in series **2** by an oxygen atom will affect the hydrophobicity and polarity of the molecules. Hence the clog *P* and topological polar surface area (TPSA) figures for **1a–l** were calculated and are listed in Table 1. The clog *P* figures of **2a–l** are 0.41 higher than the analogs in series **1** while the TPSA values of **1a–l** are 9.23 Å^2 greater than the related 4-tetralones **2**. Hence, depending on the cell line under consideration, both of these physicochemical parameters likely contribute to the disparities in cytotoxic potencies. In order to seek correlations between cytotoxic properties and the θ_1, clog *P* and TPSA values, linear, semilogarithmic and logarithmic plots were constructed between each of these values and the IC_{50} data in the L1210 screen (the similarity of the potencies of **1a–l** in the Molt 4/C8 and CEM assays suggested that the nature of the nuclear substituents had little effect on the extent of cytotoxicity). A negative correlation was noted between the L1210 IC_{50} figures and the θ_1 values ($p < 0.05$). Thus the placement of two bulky substituents in the *ortho* position of ring C may lead to compounds possessing increased cytotoxic potencies.

In order to examine further the potential of the 3-benzylidene-4-chromanones as candidate cytotoxins, two additional studies were planned, namely the evaluation of the ability of these compounds to demonstrate greater lethality to neoplasms than normal cells and secondly to evaluate their toxicity *in vivo*.

Compounds **1a–l** were evaluated against four human neoplastic cell lines and three human normal cells and the results are presented in Table 3. With the exception of **1e** and **1l**, the IC_{50} values of the 4-chromanones towards malignant cells are in the range of 5–28 μM. The average CC_{50} figures generated using HSC-2, HSC-3, HSC-4 and HL-60 cells reveal that the values for **1a–l** are lower than is displayed by melphalan. In particular **1d** and **1j** are 13 and 9 times more potent, respectively, than the reference drug and are lead molecules. In regard to the ability to display greater toxicity to malignant cells, selectivity index (SI) figures were calculated revealing that in each case, **1a–l** were less toxic to normal cells than to the neoplasms. A SI figure of five was arbitrarily chosen to demonstrate noteworthy selectivity which was observed for all of the compounds except for **1a**,**c**,**h**. A comparison was made between the cytotoxic potencies towards malignant cells and SI values of **1b**,**e**,**h**,**k**,**l** which possess a single substituent in the 4 position of ring C. The most promising compounds **1b** and **1k** bear chloro and methyl substituents, respectively. Hence expansion of this cluster of compounds should consider the placement of single or multiple fluoro, iodo and alkyl groups onto the benzylidene aryl ring.

An estimate of the toxicity of **1a–l** in mice was made. The animals were injected intraperitoneally using doses of 30, 100 and 300 mg/kg of each compound and the animals were observed after 0.5 and 4 h for deaths and signs of neurotoxicity. Doses up to and including 300 mg/kg of all compounds did not cause any mortalities. This result compares favourably with a number of alkylating anticancer drugs, e.g., the LD_{50} values of melphalan, chlorambucil and lomustine in mice after intraperitoneal injection are 4.0, 34.0 and 53.0 mg/kg, respectively [15]. Using a dose of 300 mg/kg, minimal neurotoxicity was observed for **1a**,**e**,**g**,**k** (in 1/4 animals) and **1i** (2/4 mice) after 0.5 h while after 4 h, neurotoxicity was only displayed by **1e** and **1k** in one of two mice. One may conclude that the 3-benzylidene-4-chromanones are well tolerated *in vivo*.

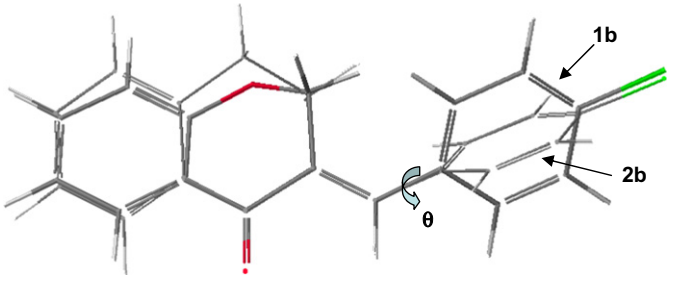


Fig. 2. Molecular modelling of **1b** (thick lines) and **2b** (thin lines) in which the enone O=C–C=C atoms are overlapped.

5. Conclusions

This study revealed that a number of 3-benzylidene-4-chromanones **1a–l** have IC_{50} values in the low micromolar range towards human Molt 4/C8 and CEM T-lymphocytes. In general, compounds **1a–l** were more potent in these assays than the related 2-benzylidene-1-tetralones **2a–l**. The difference in potencies between the compounds in series **1** and **2** was attributed, at least in part, to the varied topography of the molecules especially in relation to the benzylidene aryl rings. In particular, a negative correlation was noted between the IC_{50} figures of **1a–l** and the θ_1 values in the L1210 screen. Furthermore, the lower lipophilicity and greater TPSA values in **1a–l** may have influenced the biodata obtained. The 4-chromanones **1a–l** displayed greater cytotoxicity to a number of malignant cells than to various normal cells and were well tolerated in mice. The information gathered indicates that a further study with these compounds is clearly warranted. Thus in the future, probing of the ways whereby cytotoxicity is mediated, molecular modifications to improve potencies and *in vivo* anticancer evaluations should be undertaken.

Table 3
Cytotoxic properties of **1a–l** towards HSC-2, HSC-3, HSC-4 and HL-60 neoplasms and HGF, HPC and HPLF normal cells

Compound	CC_{50}[a] (μM)									SI[b]
	Human tumour cells					Human normal cells				
	HSC-2	HSC-3	HSC-4	HL-60	Average	HGF	HPC	HPLF	Average	
1a	26 ± 8	23 ± 6	10 ± 1	20 ± 2	19.8	76 ± 1	64 ± 12	64 ± 30	68.0	3.43
1b	16 ± 7	28 ± 5	10 ± 0	12 ± 2	16.5	>400	284 ± 18	245 ± 85	>310	>18.8
1c	21 ± 4	16 ± 2	7 ± 2	15 ± 3	14.8	71 ± 4	51 ± 14	54 ± 27	58.7	3.97
1d	7 ± 2	9 ± 3	2 ± 1	3 ± 2	5.25	40 ± 6	21 ± 5	30 ± 20	30.3	5.77
1e	31 ± 10	103 ± 39	14 ± 3	28 ± 11	44.0	>400	>400	>304	>368	>8.36
1f	17 ± 3	19 ± 4	7 ± 1	7 ± 2	12.5	71 ± 5	64 ± 8	61 ± 30	65.3	5.22
1g	13 ± 2	15 ± 1	5 ± 0	7 ± 2	10.0	66 ± 4	42 ± 4	45 ± 24	51.0	5.10
1h	16 ± 3	17 ± 1	9 ± 1	12 ± 5	13.5	81 ± 12	39 ± 2	60 ± 16	60.0	4.44
1i	16 ± 6	23 ± 3	16 ± 2	11 ± 7	16.5	243 ± 19	136 ± 26	123 ± 48	167	10.1
1j	9 ± 2	14 ± 1	3 ± 0	4 ± 1	7.50	66 ± 5	36 ± 1	59 ± 39	53.7	7.16
1k	11 ± 2	14 ± 2	7 ± 1	8 ± 2	10.0	68 ± 3	35 ± 1	58 ± 34	53.4	5.34
1l	45 ± 17	134 ± 87	31 ± 15	25 ± 15	58.8	>400	>400	>400	>400	>6.80
Melphalan[c]	28	121	115	2.9	66.7	>200	>200	>200	>200	>3.00

[a] The CC_{50} value corresponds to the concentration of the compound required to kill 50% of the cells.
[b] The letters SI indicate the Selectivity Index, i.e., the ratio of the average CC_{50} value towards normal cells to the average CC_{50} figure for tumour cells.
[c] Solubility considerations precluded using concentrations higher than 200 μM. The determinations for melphalan were made in duplicate and the differences between the two assays were within 5%.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Boetius apparatus and are uncorrected. Elemental analyses were undertaken on **1a–l** at the Department of Organic Chemistry, Eötvös Lorand University, Budapest, Hungary and were within 0.3% of the calculated values. ^{1}H NMR spectra were determined in deuterochloroform using a Perkin Elmer R12 (60 MHz) instrument for **1c–e,g,h,j,k** and a Bruker AM 500 FT (500 MHZ) for all compounds. Infrared spectra were recorded as potassium bromide discs using a Nicolet Impact 400 FTIR spectrophotometer. Column chromatography utilized Merck Kieselgel 60 with an eluting solvent of toluene. The TLC of the compounds used Merck silica gel 60 F_{254} alumina sheets and developing solvents of both toluene and toluene/ethanol (4:1). The R_f values of **1a–l** were determined by TLC using silica gel and an eluent of toluene/methanol (4:1).

6.1.1. Synthesis of *1a–l*

The compounds were prepared by a literature procedure [16] which involved condensing 4-chromanone with various aryl aldehyes using piperidine as the catalyst. The compounds were purified by column chromatography followed by recrystallization from methanol.

*6.1.1.1. 3-(2-Chlorobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1a**).* M.p. 100–103 °C. Yield: 58%. R_f: 0.88. IR: 1675 (C=O) cm^{-1}. ^{1}H NMR (500 MHz): 5.21 (s, 2H, OCH_2), 6.99 (d, 1H, Ar-H, $J = 8.34$ Hz), 7.13 (m, 2H, Ar-H), 7.37 (m, 2H, Ar-H), 7.52 (q, 2H, Ar-H), 7.98 (s, 1H, =CH), 8.07 (d, 1H, Ar-H, $J = 7.82$ Hz). Found: C, 70.71; H, 4.23%. Anal. ($C_{16}H_{11}ClO_2$) requires C, 70.99; H, 4.10%.

*6.1.1.2. 3-(4-Chlorobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1b**).* M.p.169–171 °C (lit.[16] m.p. 172–173 °C). Yield: 73%. R_f: 0.85. IR: 1672 (C=O) cm^{-1}. ^{1}H NMR (500 MHz): δ: 5.34 (s, 2H, OCH_2), 7.00 (d, 1H, Ar-H, $J = 8.27$ Hz), 7.11 (t, 1H, Ar-H), 7.27 (d, 2H, Ar-H, $J = 8.77$ Hz), 7.46 (d, 2H, Ar-H, $J = 8.22$ Hz), 7.53 (t, 1H, Ar-H), 7.84 (s, 1H, =CH), 8.05 (d, 1H, Ar-H, $J = 7.62$ Hz). Found: C, 70.82; H, 4.28%. Anal. ($C_{16}H_{11}ClO_2$) requires C, 70.99; H, 4.10%.

*6.1.1.3. 3-(2-Bromobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1c**).* M.p.114–117 °C. Yield: 59%. R_f: 0.82. IR: 1676 (C=O) cm^{-1}. ^{1}H NMR (500 MHz): δ: 5.18 (s, 2H, OCH_2), 7.02 (d, 1H, Ar-H, $J = 8.30$ Hz), 7.12(q, 2H, Ar-H), 7.28(t, 1H, Ar-H), 7.39 (t, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.69(d, 1H, Ar-H, $J = 8.02$ Hz), 7.91(s, 1H, =CH), 8.06(dd, 1H, Ar-H). Found: C, 61.15; H, 3.56%. Anal. ($C_{16}H_{11}$, BrO_2) requires C, 60.98; H, 3.52%.

*6.1.1.4. 3-(3-Bromobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1d**).* M.p. 131–134 °C. Yield: 64%. R_f: 0.84. IR: 1672 (C=O) cm^{-1}. ^{1}H NMR (500 MHz): δ: 5.39 (s, 2H, OCH_2), 7.01 (d, 1H, Ar-H, $J = 8.30$ Hz), 7.12 (t, 1H, Ar-H), 7.27 (d, 1H, Ar-H, $J = 7.79$ Hz), 7.36 (t, 1H, Ar-H), 7.48 (s, 1H, Ar-H), 7.55 (m, 2H, Ar-H), 7.81 (s, 1H, =CH), 8.05 (d, 1H, Ar-H, $J = 7.84$ Hz). Found: C, 60.77; H, 3.64%. Anal. ($C_{16}H_{11}BrO_2$) requires C, 60.98; H, 3.52%.

*6.1.1.5. 3-(4-Bromobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1e**).* M.p. 169–173 °C (lit [16] m.p. 173–174 °C). Yield: 75%. R_f: 0.88. IR: 1673 (C=O) cm^{-1}. ^{1}H NMR (500 MHz): δ: 5.32 (s, 2H, OCH_2), 6.99 (d, 1H, Ar-H, $J = 8.30$ Hz), 7.10

(t, 1H, Ar-H), 7.19 (d, 2H, Ar-H, $J = 8.35$ Hz), 7.52 (m, 1H, Ar-H), 7.60 (d, 2H, Ar-H), 7.80 (s, 1H, =CH), 8.04 (dd, 1H, Ar-H). Found: C, 60.83; H, 3.58%. Anal. ($C_{16}H_{11}BrO_2$) requires C, 60.98; H, 3.52%.

*6.1.1.6. 3-(2-Methoxybenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1f**).* M.p. 105–107 °C (lit [17] m.p. 106–107 °C). Yield: 56%. R_f: 0.77. IR: 1677 (C=O) cm^{-1}. ^1H NMR (500 MHz): 3.88 (s, 3H, CH_3), 5.38 (s, 2H, OCH_2), 6.97(d, 2H, Ar-H, $J = 8.32$ Hz), 7.02 (t, 1H, Ar-H), 7.08 (t, 2H, Ar-H), 7.41 (t, 1H, Ar-H), 7.49 (t, 1H, Ar-H), 8.03 (s, 1H, =CH), 8.05 (dd, 1H, Ar-H). Found: C, 76.54; H, 5.35%. Anal. ($C_{17}H_{14}O_3$) requires C, 76.68; H, 5.30%.

*6.1.1.7. 3-(3-Methoxybenzylidine)-2,3-dihydro-1-benzopyran-4-one (**1g**).* M.p. 91–92 °C. Yield: 63%. R_f: 0.76. IR: 1673 (C=O) cm^{-1}. ^1H NMR (500 MHz); δ: 3.86 (s, 3H, OCH_3), 5.37 (s, 2H, OCH_2), 6.86 (s, 1H, Ar-H), 6.91(d, 1H, Ar-H, $J = 7.55$ Hz), 6.99 (m, 2H, Ar-H), 7.08 (t, 1H, Ar-H), 7.38 (t, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.08 (t, 1H, Ar-H), 7.86(s, 1H, =CH), 8.04 (dd, 1H, Ar-H). Found: C, 76.72; H, 5.51%. Anal. ($C_{17}C_{14}O_3$) requires C, 76.68; H, 5.30%.

*6.1.1.8. 3-(4-Methoxybenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1h**).* M.p. 133–135 °C (lit [16] m.p. 131–132 °C). Yield: 72%. R_f: 0.75. IR: 1665 (C=O) cm^{-1}. ^1H NMR (500 MHz): δ: 3.88 (s, 3H, OCH_3), 5.40 (s, 2H, OCH_2), 6.99 (q, 3H, Ar-H), 7.08 (t, 1H, Ar-H), 7.30(d, 2H, Ar-H), 7.50 (t, 1H, Ar-H), 7.86 (s, 1H, =CH), 8.02 (dd, 1H, Ar-H). Found: C, 76.47; H, 5.33%. Anal. ($C_{17}H_{14}O_3$) requires C, 76.68; H, 5.30%.

*6.1.1.9. 3-(2,4-Dimethoxybenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1i**).* M.p. 133–135 °C. Yield: 72%. R_f: 0.67. IR: 1663 (C=O) cm^{-1}. ^1H NMR (500 MHz): δ: 3.87 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.28 (s, 2H, OCH_2), 6.53 (s, 1H, Ar-H), 6.55 (d, 1H, Ar-H, $J = 8.41$ Hz), 6.97 (d, 1H, Ar-H, $J = 8.27$ Hz), 7.02 (d, 1H, Ar-H, $J = 8.38$ Hz), 7.08 (t, 1H, Ar-H, $J = 8.38$ Hz), 7.49 (t, 1H, Ar-H), 8.02 (s, 1H, =CH), 8.05 (d, 1H, Ar-H, $J = 7.77$ Hz). Found: C, 72.79; H, 5.57%. Anal. ($C_{18}H_{16}O_4$) requires C, 72.96; H, 5.44%.

*6.1.1.10. 3-(3-Methylbenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1j**).* M.p. 68–71 °C. Yield: 74%. R_f: 0.80. IR: 1670 (C=O) cm^{-1}. ^1H NMR (500 MHz): δ: 2.42 (s, 3H, CH_3), 5.37 (s, 2H, OCH_2), 6.98 (d, 1H, Ar-H, $J = 8.31$ Hz), 7.10 (q, 3H, Ar-H), 7.24(d, 1H, Ar-H, $J = 7.55$ Hz), 7.36 (t, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.87(s, 1H, =CH), 8.04 (dd, 1H, Ar-H). Found: C, 81.45; H, 5.78%. Anal. ($C_{17}H_{14}O_2$) requires C, 81.58; H, 5.64%.

*6.1.1.11. 3-(4-Methylbenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1k**).* M.p. 114–117 °C (lit [16] m.p. 115–116 °C). Yield: 67%. R_f: 0.83. IR: 1668 (C=O) cm^{-1}. ^1H NMR (500 MHz): δ: 2.42 (s, 3H, CH_3), 5.38 (s, 2H, OCH_2), 6.98 (d, 1H, Ar-H, $J = 8.29$ Hz), 7.09 (t, 1H, Ar-H), 7.23 (d, 2H, Ar-H, $J = 8.08$ Hz), 7.28 (d, 2H, Ar-H, $J = 7.97$ Hz), 7.50 (m, 1H, Ar-H), 7.88 (s, 1H, =CH), 8.04 (dd, 1H, Ar-H). Found: C, 81.34; H, 5.51%. Anal. ($C_{17}H_{14}O_2$) requires C, 81.58; H, 5.64%.

*6.1.1.12. 3-(4-Dimethylaminobenzylidene)-2,3-dihydro-1-benzopyran-4-one (**1l**).* M.p. 150–153 °C (lit [16] m.p. 148–149 °C). Yield: 67%. R_f: 0.67. IR: 1659 (C=O) cm^{-1}. ^1H NMR (500 MHz): δ: 3.08 (s, 6H, $2 \times N-CH_3$), 5.47 (s, 2H, OCH_2), 6.75 (d, 2H, Ar-H, $J = 8.60$ Hz), 6.98 (d, 1H, Ar-H, $J = 8.26$ Hz), 7.08 (t, 1H, Ar-H), 7.29 (d, 2H, Ar-H, $J = 7.55$ Hz), 7.48 (t, 1H, Ar-H), 7.86 (s, 1H, =CH), 8.04 (d, 1H, Ar-H, $J = 7.83$ Hz). Found: C, 77.27; H, 6.04%. Anal. ($C_{18}H_{17}NO_2$) requires C, 77.40; H, 6.13%.

6.1.2. Molecular modelling

The models of **1a–l** and **2a–l** were built using BioMedCache 6.1 for Windows [18]. The lowest energy conformations were obtained from optimized geometry calculations in MOPAC using AM1 parameters. The TPSA data were also derived from this modelling programme.

6.1.3. clog P determinations

The clog *P* calculations were made using a software package [19].

6.1.4. Statistical evaluations

Linear, semilogarithmic and logarithmic plots were made between the θ_1, clog *P* and TPSA values of **1a–l** and the IC_{50} figures of these compounds in the L1210 assay using a commercial software package [20]. The Pearson coefficients and significance when the θ_1 values of **1a–l** were plotted against IC_{50} figures were as follows, namely −0.527, 0.079 (linear), −0.637, 0.026 (semilogarithmic) and −0.646, 0.023 (logarithmic).

6.1.5. Cytotoxicity screens

The methodology for evaluating **1a–l** in the Molt 4/C8, CEM and L1210 assays has been described previously [21]. In brief, cells were incubated at 37 °C in RPMI 1640 medium for 72 h using Molt 4/C8 and CEM cells and for 48 h in the L1210 assay.

The methodology for evaluating **1a–l** and melphalan against HSC-2, HSC-3, HSC-4, HGF, HPC and HPLF cells has been described previously [22]. In brief, the cells were incubated in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum and cell viability was assessed by the MTT method. A similar process was followed using HL-60 cells except they were cultured in RPMI 1640 medium containing 10% fetal bovine serum and the cytotoxicity was evaluated using the trypan blue exclusion procedure. The time of incubation was 24 h.

*6.1.6. Evaluation of **1a–l** for murine toxicity and neurotoxicity*

These experiments were conducted by the National Institute of Neurological Disorders and Stroke according to their protocols [23]. In brief, doses of 30, 100 and 300 mg of **1a–l** were injected intraperitoneally into mice and death of

the animals were observed at the end of 0.5 and 4 h. Neurotoxicity was measured by the rotorod test [13]. The following compounds demonstrated neurotoxicity (dose in mg/kg, time in h, number of mice displaying neurotoxicity/total number of animals), namely **1a** (100, 0.5, 2/8; 100, 4, 2/4; 300, 0.5, 1/4), **1b** (100, 0.5, 1/8), **1c** (100, 0.5, 1/8; 100, 4, 1/4), **1e** (100, 0.5, 2/8; 300, 0.5, 1/4); 300, 4, 1/2), **1f** (100, 0.5, 2/8), **1g** (30, 0.5, 1/4); 300, 0.5, 1/4), **1h** (100, 0.5, 1/8), **1i** (300, 0.5, 2/4), **1k** (30, 0.5, 1/4; 100, 0.5, 1/8; 300, 0.5, 1/4; 300, 4, 1/2) and **1l** (100, 4, 1/4). Four hours after mice received a dose of 300 mg/kg of **1k**, there was a loss of righting reflex in one of two animals.

The animals were housed, fed and handled using the procedures outlined in the National Research Council publication ''Guide for the Care and Use of Laboratory Animals'' and euthanized following the policies of the Institute of Laboratory Resources.

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