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2-(3-Aryl-2-propenoyl)-3-methylquinoxaline-1,4-dioxides: A novel cluster of tumor-specific cytotoxins which reverse multidrug resistance

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ABSTRACT

A series of 2-(3-aryl-2-propenoyl)-3-methylquinoxaline-1,4-dioxides **3a–l** were prepared by condensation of various aryl aldehydes with 2-acetyl-3-methylquinoxaline-1,4-dioxide **2**. These compounds inhibit the growth of human Molt 4/C8 and CEM T-lymphocytes and the IC_{50} values are mainly in the 5–30 μM range. The quinoxaline 1,4-dioxide **3j** inhibited the growth of 58 human tumor cell lines, particularly leukemic and breast cancer neoplasms. All of the compounds **3a–l** reversed the multidrug resistance (MDR) properties of murine L-5178Y leukemic cells which were transfected with the human MDR1 gene. The MDR-reversing effect may be due to the conjugated π-electron system forming a weak electron charge transfer complex with the P-glycoprotein-mediated efflux pump. The compounds in series **2** and **3** were assessed against HL-60, HSC-2, HSC-3 and HSC-4 malignant cells as well as HGF, HPC and HPLF normal cell lines which revealed that the majority of the compounds displayed a greater toxicity to neoplastic than normal cells. Various ways in which the project may be expanded are presented.

1. Introduction

A major interest in our laboratory is the design and synthesis of conjugated arylidene ketones as antineoplastic agents.[1,2] This decision is based on the avidity of α,β-unsaturated ketones for thiols and not amino or hydroxyl groups.[3,4] Hence interactions with nucleic acids (which contain amino and hydroxyl functionalities, but not mercapto groups) should be avoided and the problems of genotoxicity associated with a number of anticancer drugs[5] should be absent. Hence the present study is a continuation of the quest to find novel antineoplastic agents which are structurally divergent from drugs used today. Thus the problem of cross-resistance may be absent and such prototypic molecules may be able to treat drug-resistant tumors.

In addition, the discovery in our laboratories of the multidrug resistance (MDR) revertant properties of compounds containing the 3-aryl-2-propenoyl pharmacophore has recently been reported.[6,7] Hence the attachment of this structural moiety to other scaffolds may lead to additional series of compounds which reverse MDR.

The aim of the present investigation was to prepare a series of 2-(3-aryl-2-propenoyl)-3-methylquinoxaline 1,4-dioxides **3a–l** in order to explore the hypothesis that such compounds represent a novel class of cytotoxic agents which reverse MDR. Such compounds may be regarded as dual agents which exert their antineoplastic effect and are not effluxed rapidly from malignant cells. The decision was made to attach the 3-aryl-2-propenoyl group to a quinoxaline 1,4-dioxide scaffold and the reasons for the choice of this heterocycle are as follows. (1) A number of neoplasms have hypoxic regions[8] which may permit reduction of the N-oxides to the corresponding tertiary amines. Various tumors are more acidic than the corresponding normal cells[9] and in such cases there will be a higher percentage of protonated amines among the tumors. The quadrivalent nitrogen atoms will exert a strongly electron-attracting influence on the 3-aryl-2-propenoyl group thereby increasing the fractional positive charge on the β carbon atom of the olefinic linkage and thus increasing the thiol-alkylating properties of the molecules. If this process of reduction followed by protonation occurs preferentially in tumor cells, then the rate and extent of interactions with cellular constituents will be greater in neoplasms and selective toxicity to tumors takes place. (2)

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Studies have revealed that certain malignant cells are more sensitive to sequential chemical attacks than the corresponding normal cells.[10,11] In other words, greater chemosensitization may occur with neoplasms than non-malignant cells. Hence the successive reduction of the two N-oxides thereby permitting an alteration in the rate of thiol alkylation may lead to preferential cytotoxicity towards neoplasms. In the heterocycle chosen, the two nitrogen atoms are in different molecular environments and in order to exacerbate this differential, the electron-releasing methyl group was placed in the 3 position of the heterocycle.

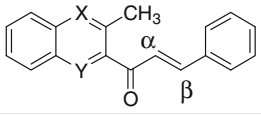
The last aspect of the design of series **3** involved the choice of the substituents in ring A which may enable an understanding of which physicochemical parameters influence any cytotoxic and MDR-revertant properties that may be observed. The differing electronic, hydrophobic and steric effects of aryl substituents may be measured using the Hammett sigma, Hansch pi and molecular refractivity (MR) constants, respectively. The groups in ring A have divergent σ and π values and are present in all four quadrants of the Craig plot.[12] The MR figures varied from 2.98 to 23.61.

A preliminary communication revealed that **3a–j**,**l** are cytotoxic to two murine neoplasms, namely B16 melanoma and L1210 leukemia cell lines.[13] This report describes the design and syntheses of **3a–l** and their evaluation against human cell lines and also as candidate MDR revertants.

2. Results

The compounds in series **2** and **3** were synthesized using the procedure outlined in Scheme 1. The electron densities on the olefinic carbon atoms of **3a** and related compounds were undertaken and the results are portrayed in Table 1. The N-oxides **2** and **3a–l** were examined for cytostatic properties using human Molt 4/C8 and CEM T-lymphocytes and these data are presented in Table 2. Correlations were sought between the magnitude of the cytostatic and MDR-revertant properties of **3a–l** with first, the σ, π and MR constants of the R^1–R^3 atoms or groups and second, the torsion angle between the olefinic moiety and the adjacent aryl ring. The growth-inhibitory properties of three representative compounds, namely **3b**,**c**,**j**, were assessed against a panel of 59 human tumor cell lines from nine different neoplastic conditions using a concentration of 10 μM. The greatest potency was displayed by **3j** as illustrated in Figure 1. This compound was evaluated further using concentrations of 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} molar and the marked inhibitory potency towards some leukemic and breast cancer cell proliferation is presented in Figure 2. The MDR-revertant properties of **2** and **3a–l** were examined using murine L-5178 lymphoma cells transfected with the human MDR1 gene and the results are given in Table 2. All of the compounds in series **2** and **3** were assayed against HL-60 promyelocytic leukemic cells and HSC-2, HSC-3 and HSC-4 squamous cell carcinomas. In addition, these molecules were evaluated using non-malignant HGF gingival

Table 1
Atomic charges on the olefinic atoms of **3a** and related compounds

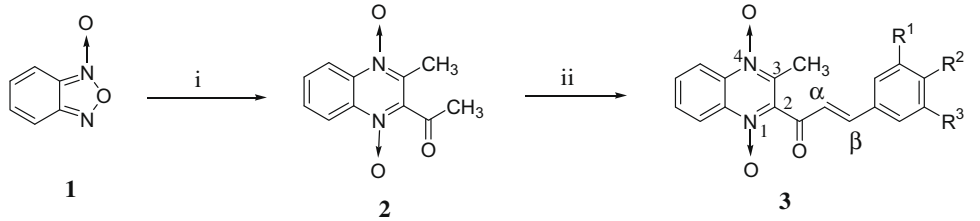


X	Y	Atomic charges (esu)	
		α atom	β atom
N→O	N→O	−0.231	−0.024
N	N	−0.230	−0.035
(+) NH	N	−0.284	0.046
N	(+)NH	−0.333	0.081
(+)NH	(+)NH	−0.377	0.153

Table 2
Evaluation of **2** and **3a–l** against human Molt 4/C8 and CEM T-lymphocytes and as candidate MDR revertants

Compound	Aryl substituents	IC_{50}[a] (μM)		FAR values[b]	
		Molt 4/C8	CEM	20 μM	200 μM
2	–	44.6 ± 24.0	87.4 ± 54.6	1.06	1.04
3a	H	6.76 ± 1.5	5.92 ± 0.3	10.9	36.8
3b	4-OCH_3	25.8 ± 5.4	27.6 ± 18.6	4.25	31.7
3c	3,4-$(OCH_3)_2$	18.5 ± 10.0	19.2 ± 16.5	5.01	23
3d	3,4,5-$(OCH_3)_3$	5.51 ± 0.7	5.22 ± 0.1	8.15	41.9
3e	3,4-OCH_2O	24.2 ± 0.2	28.1 ± 10.6	1.58	3.67
3f	4-CH_3	8.40 ± 0.8	22.4 ± 13.7	9.79	27.2
3g	4-Cl	7.75 ± 0.1	16.0 ± 12.3	2.63	5.97
3h	3,4-Cl_2	10.1 ± 3.2	15.4 ± 0.6	4.42	11.9
3i	4-Br	17.1 ± 1.0	11.6 ± 7.5	2.03	2.81
3j	4-F	6.40 ± 0.4	10.0 ± 2.7	4.81	25.8
3k	4-NO_2	261 ± 67	164 ± 12	1.28	2.25
3l	3-NO_2	16.3 ± 6.4	15.8 ± 6.0	1.89	3.75
Melphalan[c]	–	3.24 ± 0.8	2.47 ± 0.3	–	–

[a] The IC_{50} figures are the concentrations required to inhibit the growth of the tumor cells by 50%.
[b] The fluorescence activity ratio (FAR) values are the ratios of the fluorescent intensities of rhodamine 123 in treated and untreated murine L-5178Y tumor cells which are transfected with the human MDR1 gene. The FAR value of a reference compound verapamil is 7.86 using a concentration of 5.2 μM.
[c] The data were previously reported in Ref. 31 [copyright (2006) by Elsevier].



	R^1	R^2	R^3		R^1	R^2	R^3
a:	H	H	H	**g:**	H	Cl	H
b:	H	OCH_3	H	**h:**	Cl	Cl	H
c:	OCH_3	OCH_3	H	**i:**	H	Br	H
d:	OCH_3	OCH_3	OCH_3	**j:**	H	F	H
e:	OCH_2O		H	**k:**	H	NO_2	H
f:	H	CH_3	H	**l:**	NO_2	H	H

Scheme 1. The reagents used in the syntheses of **2** and **3** are as follows, namely (i) $CH_3COCH_2COCH_3/N(C_2H_5)_3$ and (ii) aryl aldehyde/NaOH.

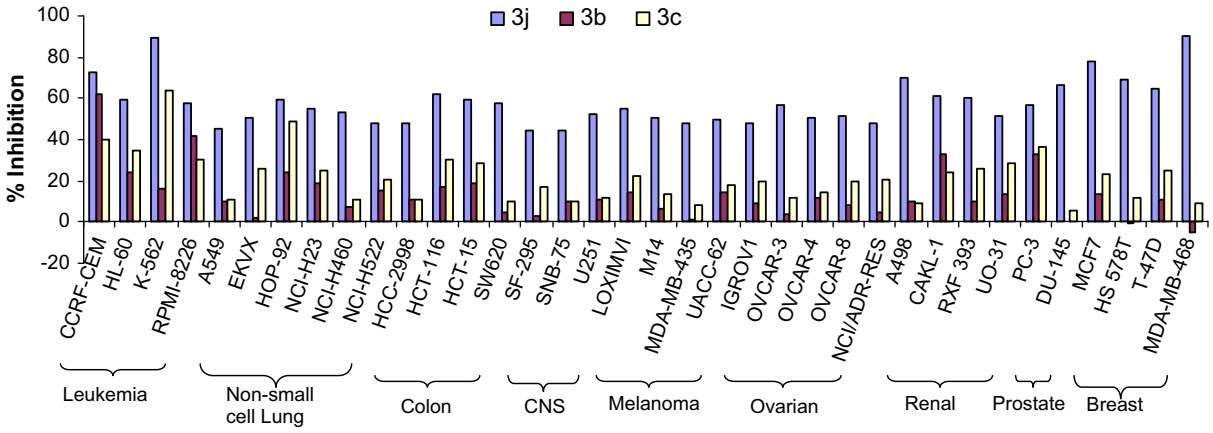


Figure 1. The growth-inhibiting properties of 10 μM of **3b**,**c**,**j** on a number of human tumor cell lines.

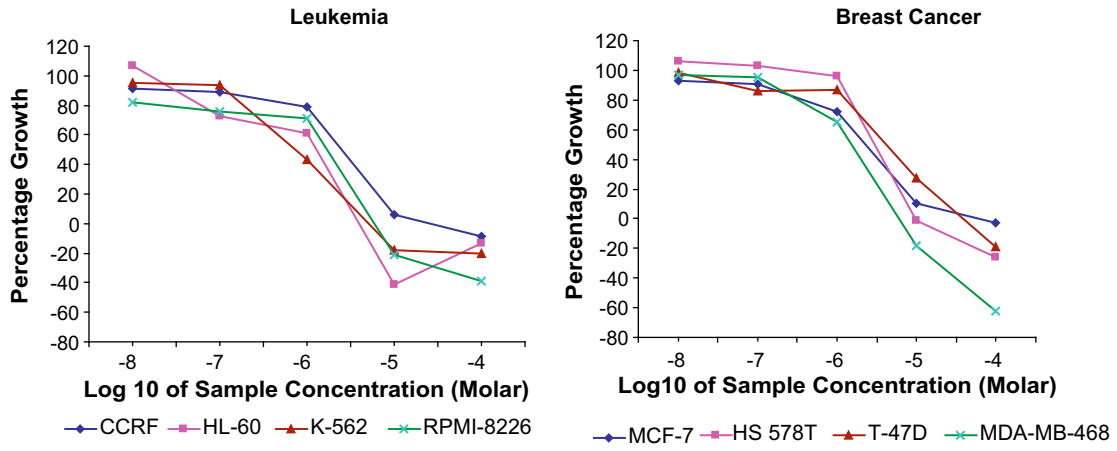


Figure 2. Dose response curves of **3j** towards some leukemic and breast cancer cell lines.

Table 3
A comparison of the cytotoxic effects of **2** and **3a–l** on malignant and normal cells

Compound	Human normal cells, CC_{50} (μM)[a]				Human malignant cells, CC_{50}[a] (μM)								Ave SI
	HGF	HPC	HPLF	Ave	HL-60	SI[b]	HSC-2	SI[b]	HSC-3	SI[b]	HSC-4	SI[b]	
2	>400	352 ± 14	>400	384	26 ± 7.5	14.8	196 ± 4.5	2.0	146 ± 7.5	2.6	134 ± 36	2.9	5.6
3a	32 ± 3.7	36 ± 1.8	35 ± 4.3	34.3	4.1 ± 0.5	8.4	8.4 ± 0.3	4.1	9.7 ± 0.3	3.5	9.6 ± 1.6	3.6	4.9
3b	52 ± 16	92 ± 11	93 ± 23	79.0	16 ± 1.7	4.9	20 ± 0.2	4.0	23 ± 0.1	3.4	11 ± 4.9	7.2	4.9
3c	117 ± 5.0	142 ± 1.5	156 ± 7.0	138	14 ± 1.5	9.9	25 ± 0.5	5.5	31 ± 1.5	4.5	38 ± 1.5	3.6	5.9
3d	35 ± 1.6	31 ± 2.5	33 ± 1.9	33.0	1.9 ± 0.1	17.4	7.8 ± 0.8	4.2	6.6 ± 0.3	5.0	6.5 ± 0.3	5.1	7.9
3e	75 ± 5.5	79 ± 7.3	72 ± 3.6	75.3	13 ± 1.9	5.8	23 ± 2.8	3.3	25 ± 6.0	3.0	28 ± 5.5	2.7	3.7
3f	32 ± 2.5	40 ± 15	23 ± 1.2	31.7	4.1 ± 0.8	7.7	8.4 ± 0.6	3.8	14 ± 1.9	2.3	11 ± 2.6	2.9	4.2
3g	27 ± 2.7	21 ± 0.1	12 ± 0.3	20.0	11 ± 1.1	1.8	13 ± 0.3	1.5	38 ± 8.9	0.5	19 ± 1.7	1.1	1.2
3h	29 ± 13	13 ± 3.0	5.7 ± 0.1	15.9	27 ± 4.1	0.6	17 ± 4.1	0.9	84 ± 9.1	0.2	15 ± 2.6	1.1	0.7
3i	53 ± 11	43 ± 3.4	26 ± 3.6	40.7	23 ± 3.4	1.8	19 ± 1.4	2.1	56 ± 16	0.7	36 ± 7.4	1.1	1.4
3j	31 ± 1.0	48 ± 6.5	20 ± 8.6	33.0	4.6 ± 0.2	7.2	6.2 ± 0.1	5.3	7.7 ± 0.2	4.3	8.1 ± 0.1	4.1	5.2
3k	57 ± 13	>400	91 ± 26	>183	262 ± 39	~0.7	55 ± 4.7	~3.3	>400	~0.5	73 ± 20	~2.5	~1.8
3l	47 ± 14	46 ± 8.4	18 ± 1.2	37.0	62 ± 6.8	0.6	34 ± 4.0	1.1	122 ± 14	0.3	54 ± 3.9	0.7	0.7

[a] The CC_{50} figure is the concentration of the compound required to reduce the number of viable cells by 50%. The highest concentration used is 400 μM.
[b] The letters SI indicate the selectivity index. This figure is the quotient of the average CC_{50} value of the three normal cell lines and the CC_{50} figure of a malignant cell line.

fibroblasts, HPC pulp cells and HPLF periodontal ligament fibroblasts. The biodata generated is portrayed in Table 3.

3. Discussion

The synthesis of the desired compounds **3a–l** is presented in Scheme 1. Benzofurazan-1-oxide **1**, which was prepared by hypochlorite oxidation of 2-nitroaniline according to a literature procedure[14], reacted with acetylacetone to yield 2-acetyl-3-methylquinoxaline-1,4-dioxide **2**. A number of aryl aldehydes were condensed with **2** leading to **3a–l**. ^{1}H NMR spectroscopy revealed that the olefinic double bond in **3a–l** possesses the *E*-configuration.

In order to evaluate the hypothesis that reduction followed by protonation of the compounds in series **3** would lead to increases in their avidity for cellular thiols vide supra, the atomic charges on the olefinic carbon atoms of a representative compound **3a**

and related molecules were measured. As may be observed from Table 1, protonation depletes the electron densities on the β carbon atoms which are the loci of reactions with cellular thiols. These observations support the design of the compounds that reduction and subsequent protonation of the compounds in series **3** may increase thiol alkylation preferentially in neoplastic cells compared to the corresponding normal tissues.

All of the compounds in series **3** as well as the analog bereft of an alkylating function namely **2** were evaluated for their inhibitory activity against human Molt 4/C8 and CEM T-lymphocyte proliferation. The data are presented in Table 2. The IC_{50} values of the following compounds are less than 10 μM (percentage of the potency of the reference drug melphalan in parentheses) namely **3a** (48), **3d** (59), **3f** (39), **3g** (42) and **3j** (51) in the Molt 4/C8 assay and **3a** (42) and **3d** (47) in the CEM screen which are clearly lead molecules. The IC_{50} figures are less than 30 μM for the remaining compounds in both tests with the exception of the outlier **3k**. A comparison between the IC_{50} values of **2** and **3a–j,l** revealed that **3a**,**d**,**f–j**,**l** (Molt 4/C8 assay) and **3a**,**d**,**g–j**,**l** (CEM screen) are more potent than **2** while the remaining compounds in series **3** are equipotent with **2**. Hence the attachment of the alkylating group to the heterocycle led to increases in antiproliferative potencies in general while some contribution to the antineoplastic properties displayed is made by the scaffold to which the alkylating moiety has been attached.

In order to obtain guidelines for expansion of this project, a QSAR study as well as molecular modeling were undertaken. The variation in cytotoxic potencies in series **3** may be governed by the electronic, hydrophobic and steric properties of the aryl substituents. Accordingly linear and semilogarithmic plots were made between the IC_{50} values of **3a–l** generated in the Molt 4/C8 screen and the Hammett σ values, then the Hansch π figures and finally the molecular refractivity (MR) constants. Logarithmic plots were also made between the MR values and the IC_{50} figures, since all MR constants (but not all σ and π values) are positive. Then the same approach was used in evaluating series **3** against CEM cells. No correlations ($p > 0.05$) were noted although a trend towards a positive correlation was observed between the σ values and the IC_{50} figures in the Molt 4/C8 screen ($p = 0.09$) and the CEM bioassay ($p = 0.12$). Thus development of these compounds should consider introducing electron-releasing substituents in the arylidene aryl ring in order to increase cytotoxic potencies. The statistical analyses were repeated omitting the data for the outlier **3k** but no correlations or trends towards significance were observed ($p > 0.2$).

A further consideration pertaining to physicochemical parameters which may influence cytotoxic potencies of the test compounds is the torsion angles between the arylidene aryl ring and the adjacent enone group. A number of studies revealed that the magnitude of bioactivity is dependent on whether an aryl ring is coplanar or not with an adjacent unsaturated group.[15] In some instances coplanarity is favored while on other occasions a lack of coplanarity is required for bioactivity. Hence models of **3a–l** were built and the torsion angle θ was determined; specific values are given in the experimental section. Linear, semilogarithmic and logarithmic plots were constructed between the θ figures and the IC_{50} values in the Molt 4/C8 and CEM screens. A positive trend was observed in the Molt 4/C8 assay ($p = 0.11$) and the CEM test ($p = 0.10$) which suggests that a lowering of the interplanar angle may lead to compounds with greater cytotoxicity. Thus replacement of one or both of the ortho protons of **3a** by fluorine (the MR value of fluorine is 0.92 compared to 1.03 for hydrogen)[16] should be considered. No other correlations or trends towards significance were observed ($p > 0.2$).

In order to ascertain further the potential of the 2-(3-aryl-2-propenoyl)-3-methyl quinoxaline 1,4-dioxides **3** as novel candidate cytotoxins, three representative compounds **3b,c,f** were evaluated against 59 human tumor cell lines originating from the following neoplastic conditions, namely leukemia and melanoma as well as non-small cell lung, colon, central nervous system, ovarian, renal, prostate and breast cancers.[17] A concentration of 10 μM was employed and the data of the effect of **3b,c,j** on a number of these cell lines in Figure 1 reveals clearly that **3j** is much more inhibitory than **3b** and **3c**. The greater growth-inhibiting properties of the 4-fluoro analog **3j** than **3b** and **3c**, which contain 4-methoxy and 3,4-dimethoxy aryl substituents, respectively, may be due, at least in part, to the positive σ and π values and lower MR figure of the aryl substituent in **3j**. The quinoxaline 1,4-dioxide **3j** was examined further using concentrations of 10^{-4}–10^{-8} molar. The average IC_{50} figure of 3.98 μM towards 58 human cancer cell lines is seven times lower than the inhibitory value for melphalan.[18] A review of the mean graphs[19] revealed that significant potencies are displayed towards various leukemic and breast cancer cells lines as illustrated in Figure 2. Another noteworthy feature of **3j** is the observation that the IC_{50} values vary considerably towards the different malignant cell lines over a 22-fold range, that is, the IC_{50} figure of the most sensitive cell line is 22 times lower than for the most refractory neoplasm. It is conceivable that this difference in sensitivity may translate into a preferential toxicity for tumor cells than the corresponding normal tissues. These data reinforce the decision to pursue these compounds in further studies.

A major problem in cancer chemotherapy is MDR. This phenomenon can arise by different biochemical mechanisms including the overexpression of P-glycoprotein (P-gp) which acts as a drug efflux pump. The compounds in series **2** and **3** were assayed for MDR-revertant properties using murine L-5178 lymphoma cells transfected with the MDRI gene.[20] These cells have greater amounts of P-gp than the parental cells. The concentrations of rhodamine 123 in treated and untreated transfected and parental cells were measured and the ratios of the fluorescence intensities are referred to as fluorescence activity ratio (FAR) values. A FAR value of greater than 1 indicates that reversal of MDR has taken place.

Initial experiments were conducted using a concentration of 20 μM which, in general, is slightly in excess of the IC_{50} values of the compounds towards Molt 4/C8 and CEM cells. These data are presented in Table 2. The results indicate that all of the compounds in series **3** have MDR-revertant properties in contrast to **2**. Of particular note is the efficacy of **3a**,**c**,**d**,**f** which possess FAR values greater than 5. The experiment was repeated using a tenfold increase in compound concentration for two reasons. First, the data may identify potent MDR-revertants. Second, the FAR values may rise as the concentration increases. Alternatively, hormesis may occur, that is, MDR reversal is greater at the lower concentration; a situation which has been observed previously.[21] The data in Table 1 indicate that **3a–d**,**f**,**j** are potent MDR-revertants having FAR values in excess of 20. In the case of the other compounds in series **3**, the FAR values increase as the concentration is raised while even when 200 μM of **2** was used, no reversal of MDR was observed. These results afford further evidence that the 3-aryl-2-propenoyl group is an important pharmacophore in counteracting drug resistance.

In order to seek correlations between the physicochemical properties of the aryl substituents and MDR reversal potencies, linear and semilogarithmic plots were constructed between the σ, π and MR constants of the groups in ring A and the FAR values. Logarithmic plots were also made when the MR values were used. In addition, linear, semilogarithmic and logarithmic plots were constructed between the torsion angles θ and the FAR values. When the data for 20 μM of **3a–l** was considered, a trend towards a negative correlation was noted with the σ values ($p = 0.10$) as well as a negative correlation between the θ figures and the FAR values ($p = 0.05$). When the concentration was increased to

200 μM, a negative correlation was noted between the FAR figures and the σ constants ($p < 0.05$) and a negative trend towards significance found when the θ values were considered ($p = 0.07$). When these analyses were repeated using **3a–j,l**, that is, omitting the data for the outlier **3k**, neither correlations of $p < 0.05$ nor trends to significance of $p < 0.1$ were noted. One may conclude therefore that, in general terms, MDR-revertant properties are increased by employing electron-releasing aryl substituents and reducing the torsion angles θ.

The statistical analyses suggest that both the cytotoxic potencies and MDR-revertant properties of **3a–l** are influenced by the σ and θ figures but not by the π and MR physicochemical parameters. The possibility exists therefore that the IC_{50} and FAR values are negatively correlated. In order to examine this hypothesis, linear, semilogarithmic and logarithmic plots were constructed between the IC_{50} values in the Molt 4/C8 and CEM screens with the FAR figures generated when concentrations of 20 and 200 μM were used. Negative correlations ($p < 0.05$) were noted between the IC_{50} figures of **3a–l** in the Molt 4/C8 bioassay and the FAR values using 20 and 200 μM concentrations and in the CEM screen when 20 μM of compound was used. A trend towards significance ($p = 0.06$) was observed when plots were made between the IC_{50} figures in the CEM screen and the FAR values when 200 μM of **3a–l** was employed. The experiments were repeated omitting the outlier **3k** which revealed a negative correlation between the Molt 4/C8 biodata and the FAR values using 20 μM of the compounds ($p < 0.05$). No other correlations of $p < 0.05$ nor trends to a correlation of $p < 0.1$ were noted. The fact that there is a correlation between cytotoxic and MDR-revertant properties suggests that the increased intracellular retention of candidate cytotoxins contributes to a rise in cytotoxic potencies. This observation enhances the potential for development of these prototypic molecules in series **3** insofar as by exploiting the correlations noted, further compounds may be designed with the likelihood of increased antineoplastic potencies and MDR-revertant properties.

The data presented indicate that the 2-(3-aryl-2-propenoyl)-3-methylquinoxaline 1,4-dioxides **3** are a novel group of cytotoxins and possess MDR-revertant properties which may contribute to the potencies observed. The crucial issue to be addressed is whether these compounds display greater cytotoxicity for neoplasms than normal cells as suggested by the reduction–protonation hypothesis; a theory supported by the atomic charges portrayed in Table 1. Accordingly all of the compounds in series **2** and **3** were evaluated against malignant HL-60, HSC-2, HSC-3 and HSC-4 cell lines as well as HGF, HPC and HPLF normal cells. These data are presented in Table 3.

In vivo, the malignant cells of a neoplastic condition will be surrounded by different types of normal cells. Hence in order to assess whether the quinoxaline N-oxides **2** and **3** have greater toxicity for malignancies, the CC_{50} values of each compound towards a cancer cell line were compared to the average CC_{50} figure of the three normal cells. These calculations led to the selectivity index (SI) values presented in Table 3. The data reveal that in general the compounds display greater toxicity to the cancers than the normal cells. Thus 81% of the SI figures are greater than 1. This selectivity is dependent on the cell line since the average SI values for series **3** towards HL-60, HSC-2, HSC-3 and HSC-4 cells are 5.6, 3.3, ~2.4 and 3.0, respectively. In order to identify lead molecules, an average SI figure of 4 was chosen arbitrarily as an indication of noteworthy selective toxicity to neoplasms and this criterion was achieved by **2,3a–d,f,g**. In terms of cytotoxicity, the low CC_{50} values of **3a,d,f,g** to the neoplastic cell lines are apparent. Once again the useful properties of **3j** are noted; in this case in terms of both cytotoxic potencies and selective toxicity which confirms it to be a prototypic molecule for analog development. As noted previously, **2** and **3k** have low cytotoxic potencies.

4. Conclusions

A number of 2-(2-aryl-2-propenyl)-3-methylquinoxaline-1,4-dioxides **3a–l** have been prepared. The atomic charges on the olefinic carbon atoms of **3a** and related compounds support the hypothesis that a reduction–protonation process in tumors may lead to compounds demonstrating preferential toxicity to malignant cells. The compounds inhibit the growth of human Molt 4/C8 and CEM cells in the low micromolar range and in particular the potencies displayed by **3a,d,j** establish them to be lead molecules. One of these three compounds, namely **3j**, displays excellent growth-inhibitory properties towards a range of human neoplasms, especially leukemic and breast cancer cell lines. All of the molecules in series **3** reverse MDR in murine L-5178Y cells and this observation provides further evidence that the 3-aryl-2-propenoyl group is a MDR-revertant pharmacophore. The huge FAR values of **3a,b,d** of over 30 are particularly impressive. The physicochemical properties which enhance the magnitude of the cytotoxic and MDR-revertant properties are the insertion of electron-releasing substituents in the arylidene aryl ring and the lowering of the torsion angle θ. These guidelines should be useful in the development of this novel cluster of cytotoxins which reverse MDR. Finally the demonstration that the majority of the compounds in series **2** and **3** display greater toxicity to neoplasms than normal cells provides further evidence of the importance of developing these novel cytotoxins.

5. Experimental

5.1. Chemistry

Melting points are in degrees Celsius and were recorded using a Gallenkamp apparatus and are uncorrected. The melting points of **3a**,**b**,**f**,**g**,**i**,**k**,**l** recorded in the literature are of non-hydrated materials in contrast to the compounds prepared in this study. ^{1}H NMR spectra were determined on a Bruker AMX 500 FT machine while combustion analyses were obtained using an Elementer analyzer.

5.1.1. 2-Acetyl-3-methylquinoxaline-1,4-dioxide 2

A mixture of benzofurazan-1-oxide **1** (0.05 mol), acetylacetone (0.55 mol), triethylamine (5 mL) and ethanol (20 mL) was stirred at room temperature for 24 h. The resultant precipitate was collected by filtration, dried and recrystallized from ethanol to give **2**, mp 154 °C (lit.[22] mp 152 °C) in 78% yield. ^{1}H NMR ($CDCl_3$): δ 2.56 (s, 3H, CH_3), 2.75 (s, 3H, $COCH_3$), 7.89(m, 2H, Ar-H), 8.58(dd, 1H, Ar-H), 8.65(dd, 1H, Ar-H).

5.1.2. 2-(3-Aryl-2-propenyl)-3-methylquinoxaline-1,4-dioxides 3a–l

A mixture of 2-acetyl-3-methylquinoxaline-1,4-dioxide **2** (0.003 mol) and an aryl aldehyde (0.0035 mol) in methanolic sodium hydroxide solution (5% w/v, 10 mL) was stirred at 5–10 °C for 5–10 min. The solid which deposited was collected, washed with water and recrystallized from chloroform–ethanol to provide the compounds in series **3**.

5.1.2.1. 3-Methyl-2-(3-phenyl-2-propenoyl)-quinoxaline-1,4-dioxide (3a). Yield: 62%, mp 172–173 °C (lit.[22] mp 154 °C). ^{1}H NMR ($CDCl_3$): δ 2.65 (s, 3H, CH_3), 7.16 (d, 1H, =CH, J = 16.15 Hz), 7.45 (m, 3H, Ar-H), 7.59 (m, 3H, =CH and Ar-H), 7.90 (m, 2H, Ar-H), 8.60 (d, 1H, Ar-H, J = 8.60 Hz), 8.70 (d, 1H, Ar-H, J = 8.4 Hz). Anal. Calcd for $C_{18}H_{14}N_2O_3.0.5{\cdot}H_2O$: C, 68.55; H, 4.47; N, 8.88. Found: C, 68.56; H, 4.58; N, 9.10.

5.1.2.2. 2-[3-(4-Methoxyphenyl)-2-propenoyl]-3-methyl-quinoxaline-1,4-dioxide (3b). Yield: 71%, mp 169–170 °C (lit.[23] mp

186–187 °C). ^{1}H NMR($CDCl_3$): δ 2.59 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 6.93 (d, 2H, Ar-H, J = 8.74 Hz), 7.02 (d, 1H, =CH, J = 16.08 Hz), 7.53 (t, 3H, =CH and Ar-H), 7.90 (m, 2H, Ar-H), 8.62 (d, 1H, Ar-H, J = 8.36 Hz), 8.70 (d, 1H, Ar-H, J = 8.44 Hz). Anal. Calcd for $C_{19}H_{16}N_2O_4{\cdot}0.25H_2O$: C, 66.99; H, 4.73; N, 8.22. Found: C, 66.65; H, 4.76; N, 8.20.

5.1.2.3. 2-[3-(3,4-Dimethoxyphenyl)-2-propenoyl]-3-methylquinoxaline-1,4-dioxide (3c). Yield: 68%, mp 179–180 °C. ^{1}H NMR ($CDCl_3$): δ 2.63 (s, 3H, CH_3), 3.96 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 6.89 (d, 1H, Ar-H, J = 8.37 Hz), 7.05 (d, 1H, =CH, J = 16.10 Hz), 7.10 (s,1H, Ar-H), 7.18 (dd, 1H, Ar-H), 7.51 (d, 1H, =CH, J = 16.07 Hz), 7.90 (m, 2H, Ar-H), 8.63 (d, 1H, Ar-H, J = 8.36 Hz), 8.69 (d, 1H, Ar-H, J = 8.60 Hz). Anal. Calcd for $C_{20}H_{18}N_2O_5{\cdot}0.25H_2O$: C, 64.76; H, 4.89; N, 7.55. Found: C, 64.37; H, 4.91; N, 7.50.

5.1.2.4. 3-Methyl-2-[3-(3,4,5-trimethoxyphenyl)-2-propenoyl]-quinoxaline-1,4-dioxide (3d). Yield: 67%, mp 180–182 °C. ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 3.88 (s, 6H, 2 × OCH_3), 3.91 (s, 3H, OCH_3), 6.81 (s, 2H, Ar-H), 7.04 (d, 1H, =CH, J = 16.10 Hz), 7.48 (d, 1H, =CH, J = 15.98 Hz), 7.93 (m, 2H, Ar-H), 8.62 (d, 1H, Ar-H, J = 7.73 Hz), 8.70 (d, 1H, Ar-H, J = 8.01 Hz). Anal. Calcd for $C_{21}H_{20}N_2O_6{\cdot}0.25H_2O$: C, 62.91; H, 5.02; N, 6.98. Found: C, 62.62; H, 5.04; N, 7.03.

5.1.2.5. 2-[3-(3,4-Methylenedioxyphenyl)-2-propenoyl]-3-methyl-quinoxaline-1,4-dioxide (3e). Yield: 72%, mp 185–186 °C. ^{1}H NMR ($CDCl_3$): δ 2.63 (s, 3H, CH_3), 6.05 (s, 2H, O–CH_2–O), 6.83 (d, 1H, Ar-H, J = 8.05 Hz), 6.97 (d, 1H, =CH, J = 16.05 Hz), 7.07 (dd, 1H, Ar-H), 7.11 (d, 1H, Ar-H, J = 1.47 Hz), 7.50 (d, 1H, =CH, J = 16.04 Hz), 7.89 (m, 2H, Ar-H), 8.61 (d, 1H, Ar-H, J = 8.37 Hz), 8.68 (d, 1H, Ar-H, J = 8.33 Hz). Anal. Calcd for $C_{19}H_{14}N_2O_5{\cdot}0.25H_2O$: C, 64.31; H, 3.97; N, 7.89. Found: C, 63.86; H, 4.03; N, 7.97.

5.1.2.6. 3-Methyl-2-[3-(4-methylphenyl)-2-propenoyl]-quinoxaline-1,4-dioxide (3f). Yield: 46%, mp 189–191 °C (lit.[24] mp 174–178 °C). ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H, CH_3), 2.59(s, 3H, CH_3), 7.11 (d, 1H, =CH, J = 16.15 Hz), 7.23 (d, 2H, Ar-H, J = 7.97 Hz), 7.48 (d, 2H, Ar-H, J = 8.09 Hz), 7.56 (d, 1H, =CH, J = 16.16 Hz), 7.90 (m, 2H, Ar-H), 8.62 (d, 1H, Ar-H, J = 8.32 Hz), 8.70 (d, 1H, Ar-H, J = 8.32 Hz). Anal. Calcd for $C_{19}H_{16}N_2O_3{\cdot}0.25H_2O$: C, 70.25; H, 4.96; N, 8.62. Found: C, 70.13; H, 5.04; N, 8.31.

5.1.2.7. 2-[3-(4-Chlorophenyl)-2-propenoyl]-3-methyl-quinoxaline-1,4-dioxide (3g). Yield: 72%, mp 196–197 °C (lit.[22] mp 190 °C). ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 7.13 (d, 1H, =CH, J = 16.10 Hz), 7.40 (d, 2H, Ar-H, J = 8.51 Hz), 7.53 (d, 2H, Ar-H, J = 8.50 Hz), 7.58 (d, 1H, =CH, J = 16.15 Hz), 7.92 (m, 2H, Ar-H), 8.61 (dd, 1H, Ar-H), 8.70 (dd, 1H, Ar-H). Anal. Calcd for $C_{18}H_{13}ClN_2O_3{\cdot}0.25H_2O$: C, 62.61; H, 3.79; N, 8.11. Found: C, 62.31; H, 3.86; N, 8.08.

5.1.2.8. 2-[3-(3,4-Dichlorophenyl)-2-propenoyl]-3-methylquinoxaline-1,4-dioxide (3h). Yield: 69%, mp 211–212 °C. ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 7.15 (d, 1H, =CH, J = 16.08 Hz), 7.44 (dd, 1H, Ar-H), 7.51 (d, 1H, Ar-H, J = 8.33 Hz), 7.57 (d, 1H, =CH, J = 16.12 Hz), 7.69 (d, 1H, Ar-H, J = 1.88 Hz), 7.93 (m, 2H, Ar-H), 8.60 (d, 1H, J = 8.31 Hz), 8.70 (d, 1H, Ar-H, J = 8.22 Hz). Anal. Calcd for $C_{18}H_{12}C_{l2}N_2O_3{\cdot}0.25H_2O$: C, 56.93; H, 3.18; N, 7.37. Found: C, 56.74; H, 3.23; N, 7.25.

5.1.2.9. 2-[3-(4-Bromophenyl)-2-propenoyl]-3-methylquinoxaline-1,4-dioxide (3i). Yield: 54%, mp 209–210 °C (lit.[22] mp 212 °C). ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 7.15 (d, 1H, =CH, J = 16.10 Hz), 7.46 (d, 2H, Ar-H, J = 8.47 Hz), 7.57 (m, 3H, =CH and Ar-H), 7.92 (m, 2H, Ar-H), 8.61 (d, 1H, Ar-H, J = 8.35 Hz), 8.70 (d, 1H, Ar-H, J = 8.05 Hz). Anal. Calcd for $C_{18}H_{13}Br\ N_2O_3{\cdot}0.25H_2O$: C, 55.47; H, 3.36; N, 7.18. Found: C, 55.26; H, 3.33; N, 7.10.

5.1.2.10. 2-[3-(4-Fluorophenyl)-2-propenoyl]-3-methylquinoxaline-1,4-dioxide (3j). Yield: 66%, mp 195–197 °C. ^{1}H NMR ($CDCl_3$): δ 2.59 (s, 3H, CH_3), 7.11 (m, 3H, =CH and Ar-H), 7.57 (m, 3H, =CH and Ar-H), 7.89 (m, 2H, Ar-H), 8.61 (d, 1H, Ar-H, J = 8.23 Hz), 8.69 (d, 1H, Ar-H, J = 8.60 Hz). Anal. Calcd for $C_{18}H_{13}FN_2O_3{\cdot}0.25H_2O$: C, 65.74; H, 3.98; N, 8.51. Found: C, 65.58; H, 4.02; N, 8.47.

5.1.2.11. 3-Methyl-2-[4-nitrophenyl)-2-propenoyl]quinoxaline-1,4-dioxide (3k). Yield: 42%, mp 222–224 °C (lit.[22] mp 232 °C). ^{1}H NMR ($CDCl_3$): δ 2.50 (s, 3H, CH_3), 7.43 (d, 1H, =CH, J = 16.52 Hz), 7.97 (m, 5H, =CH and Ar-H), 8.27 (d, 2H, Ar-H, J = 8.59 Hz), 8.44 (d, 1H, Ar-H, J = 8.25 Hz), 8.55 (d, 1H, Ar-H, J = 8.48 Hz). Anal. Calcd for $C_{18}H_{13}N_3O_5{\cdot}0.25H_2O$: C, 60.75; H, 3.68; N, 11.80. Found: C, 60.54; H, 3.52; N, 11.75.

5.1.2.12. 3-Methyl-2-[3-(3-nitrophenyl)-2-propenoyl]quinoxaline-1,4-dioxide (3l). Yield: 58%, mp 206–208 °C (lit.[24] mp 220–222 °C). ^{1}H NMR ($CDCl_3$): δ 2.70 (s, 3H, CH_3), 7.30 (d, 1H, =CH, J = 16.18 Hz), 7.63 (t, 1H, Ar-H), 7.51 (d, 1H, =CH, J = 16.09 Hz), 7.94 (m, 3H, Ar-H), 8.30 (d, 1H, Ar-H, J = 8.18 Hz), 8.46 (s, 1H, Ar-H), 8.61 (d, 1H, Ar-H, J = 7.63 Hz), 8.71 (d, 1H, Ar-H, J = 7.79 Hz). Anal. Calcd for $C_{18}H_{13}N_3O_5{\cdot}0.25H_2O$: C, 60.75; H, 3.68; N, 11.80. Found: C, 60.45; H, 3.74; N, 11.69.

5.1.3. Statistical analyses

The Hammett σ, Hansch π and MR values were taken from the literature[25] with the exception of the 3,4-methylenedioxy sigma value which was obtained from another source.[26] The MR value of hydrogen is 1.03 not 0.00. Hence the figures of 1.03, 2.06 (2 × 1.03) and 3.09 (3 × 1.03) were added to the MR figures of the aryl substituents in the case of the disubstituted, monosubstituted and unsubstituted analogs, respectively. The linear, semilogarithmic and logarithmic plots were made using a commercial software package.[27] The following correlations or trends to significance were noted pertaining to the biodata for **3a–l** using linear (l), semilogarithmic (sl) and logarithmic (log) plots viz. IC_{50} (Molt 4/C8) versus σ: p = 0.09 (l); IC_{50} (CEM) versus σ: p = 0.12 (l); IC_{50} (Molt 4/C8) versus θ: p = 0.11 (l); IC_{50} (CEM) versus θ: p = 0.10 (l); FAR (20 μM) versus σ: p = 0.10 (sl); FAR (20 μM) versus θ: p = 0.05 (sl) and p = 0.06 (log); FAR (200 μM) versus σ: p = 0.05 (l) and p = 0.04 (sl); FAR (200 μM) versus θ: p = 0.11 (l) and p = 0.07 (sl) and p = 0.09 (log); FAR (20 μM) versus IC_{50} (Molt 4/8): p = 0.05 (sl) and p = 0.01 (log); FAR (20 μM) versus IC_{50} (CEM): p = 0.07 (sl) and p = 0.03 (log); FAR (200 μM) versus Molt 4/C8: p = 0.09 (sl) and p = 0.04 (log); FAR (200 μM) versus CEM: p = 0.07 (sl) and p = 0.06 (log). When plots were created using **3a–j**, **l**, the following trend towards significance was observed, namely FAR (200 μM) versus σ: p = 0.12 (l).

5.1.4. Molecular modeling

Models of **3a–l** were built using BioMedCache 6.1 for Windows[28] and the lowest energy conformations were found using optimized geometry calculations in MOPAC and AM1 parameters. The torsion angles θ determined for the compounds in series **3** are as follows, namely **3a**: 14.0; **3b**: 13.0; **3c**: 14.9; **3d**: 16.4; **3e**: 22.1; **3f**: 15.0; **3g**: 13.5; **3h**: 17.1; **3i**: 14.3; **3j**: 17.4; **3k**: 20.9; **3l**: 18.9.

5.2. Cytostatic and cytotoxic assays

The methodology for evaluating the inhibitory activity of **2** and **3a–l** towards Molt 4/C8 and CEM cell proliferation has been described previously[29] and was based on the determination of the tumor cell number by a Coulter counter after an incubation period

of three days of the CEM and Molt4/C8 tumor cells in the presence or absence of serial dilutions of the test compound. The assessment of **3b**,**c**,**j** towards human tumors followed a literature procedure.[17]

The evaluation of the cytotoxicity of **2** and **3a–l** against near confluent HL-60, HSC-2, HSC-3, HSC-4, HGF, HPC and HPLF cells utilized a method setting the incubation time of 48 h which has been described previously.[2,30] Human oral normal cells (HGF, HPC, HPLF) were prepared from the periodontal tissues, according to the guideline of the Intramural Board of Ethic Committee (No. A0808), after obtaining the informed consents from the patients. The biodata for a reference compound melphalan has been determined before using the following cell lines (CC_{50} figure in μM in parentheses), namely HGF (>200), HPC (>200), HPLF (>200), HL-60 (6.0), HSC-2 (35) and HSC-4 (81). [2] The CC_{50} figure of melphalan towards HSC-3 cells is 115 μM.

5.3. Evaluation of 2 and 3a–l as MDR revertants

The assay utilized has been described previously[20] while a brief summary of this methodology has been published very recently.[21]

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