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Novel Disulfides with Antitumour Efficacy and Specificity

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Some disulfides have previously been shown to possess antifungal and/or antileukaemic activity. Importantly, this cytotoxicity can be selective. We have previously shown that a subset of these compounds does not block the proliferative potential of normal, non-transformed cells. Based on these results and proposed mechanisms of action, a new set of structurally modified organosulfur compounds, including α-substituted disulfides and a thiosulfonate ester, have been prepared and evaluated for their potential as antileukaemic agents. Compounds were screened for antiproliferative activity against a panel of human cells derived from acute lymphocytic and acute myelogenous leukaemia, as well as non-transformed cells. We have identified five new disulfides and a thiosulfonate that can trigger tumour cells to undergo cell death by an apoptotic mechanism in a sensitive and specific manner.

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Introduction

Triggering the apoptotic pathway is a desirable cell-death pathway to target with anticancer agents.[1,2] Apoptosis is a highly regulated molecular mechanism that is characterized by cell shrinkage, DNA fragmentation, membrane blebbing, and separation of the cell into apoptotic bodies.[3] Unlike other forms of cell death, such as necrosis, apoptosis does not trigger an inflammatory response or result in damage to the surrounding tissue. Although anticancer agents currently in use may induce apoptosis, both malignant and healthy non-transformed cells are affected. Consequently, the development of novel therapeutic agents with greater specificity for malignant cells is critical. To this end, we have prepared low molecular weight compounds and screened them for their ability to specifically induce transformed cells to undergo apoptosis.

In pursuit of such novel apoptotic agents, naturally-occurring organosulfur compounds (OSCs) have been of great interest.[4–12] We began structure–activity work by modifying the structure of dysoxysulfone **1** (Scheme 1), which was originally isolated from the Fijian medicinal plant *Dysoxylum richii* and shown to possess both antifungal and anticancer activity.[13,14]

In an effort to identify compounds with superior specificity and potency, we previously synthesized seven structural relatives of dysoxysulfone and screened them for antifungal and antitumour activity. Initial antileukaemic testing showed a correlation between disulfide functionality and anticancer activity. In particular, α-sulfone disulfides **2** (Scheme 2) induced apoptosis in both non-transformed and leukaemia cells.

More interestingly, structure–activity analysis revealed disulfides **3** and **4** (Scheme 3) as promising antileukaemic agents that were not cytotoxic to non-transformed cells.[15]

Two modes of behaviour were proposed to rationalize the biological activity displayed (see Schemes 4 and 5). For the

$CH_3SO_2CH_2SCH_2SSCH_2SO_2CH_3$

1

Scheme 1.

RSO_2CH_2SSR'

2

Scheme 2.

$CH_3SSCH_2CO_2CH_3$ **3** $CH_3SO_2CH_2CH_2SSCH_3$ **4**

Scheme 3.

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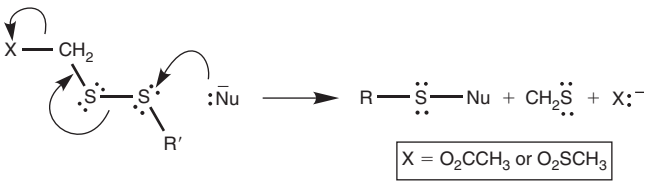
present study, we exploited both Schemes 4 and 5 and the results from our previous antileukaemic study[15] to design a second-generation set of potentially selective antileukaemic disulfides. Six disulfides and a thiosulfonate were prepared and screened for their ability to trigger apoptosis in a tumour-specific manner (see Scheme 6). Six of these compounds induced apoptosis with specificity for leukaemic cells.

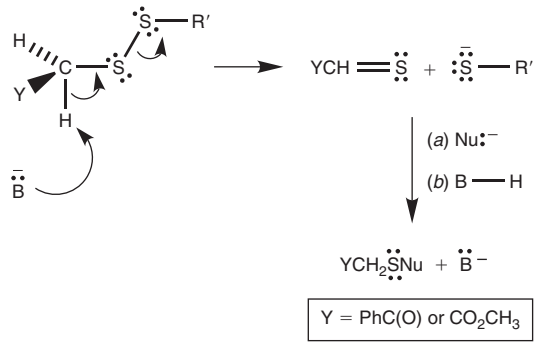
Results and Discussion

Structural Design

In the current study, we evaluated the apoptotic activity of seven OSCs (see Scheme 6). Scheme 6 includes a representative example of each of our newly established, biologically active disulfide types, namely an α-sulfone disulfide **5**, an α-ester disulfide **7** (ester group is attached to the disulfide framework by a C—O bond), and an aryl methyl disulfide **11**. Each of these disulfides exploits a specifically different manner in which it activates the disulfide linkage toward nucleophilic attack (see Schemes 4 and 5[16,17]). The α-sulfone disulfide **5** features a relatively long, unbranched alkyl substituent. Unbranched C_5–C_9 alkyl substituents or phenyl rings serve to optimize the balance between hydrophilicity and hydrophobicity, which in turn enhances pharmacological activity.[18]



Scheme 4. Proposed chemical rationale for biological activity of disulfides in which the α-carbon bears a leaving group.



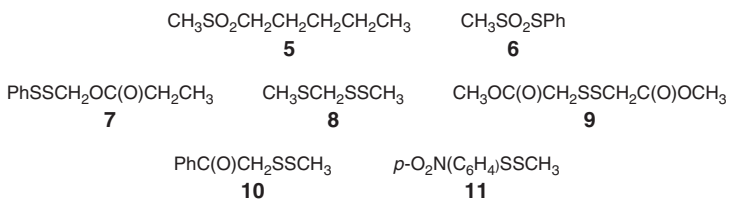
Scheme 5. Proposed rationale for biological activity of disulfides in which α-protons are acidified but the α-carbon does not bear a leaving group.

In light of the results from our previous study,[15] we chose to examine a pair of structures, namely **6** and **8**, in which the S—S bond was less aggressively activated for nucleophilic attack. In particular, S—R in **8** is less nucleofugal than SO_2R in **2**, while the thiosulfonate **6** presents a more sterically congested sulfenyl sulfur atom than that of **2**. In this way, we hoped to maintain the antileukaemic properties of the disulfides while significantly diminishing their toxicity to normal cells.

Our initial study[15] established that ester disulfide **3** (Scheme 3) showed significant antileukaemic activity without any substantial accompanying activity against normal cells. Hence, the α,α′-diester disulfide **9** was targeted for study. Furthermore, as Scheme 5 rationalizes the antitumour activity of **3**, and ketones are better carbon acids than esters, the α-ketodisulfide **10** was prepared to test for specific activity as an antileukaemic agent in vitro.

Growth Inhibitory Effect of OSCs

To assess the potential tumour-specific antiproliferative activity of the OSCs (six disulfides and a thiosulfonate, Scheme 6), a panel of leukaemic cell lines was screened to assess each line's sensitivity to each sulfur compound. Two cell lines derived from acute lymphocytic leukaemia (ALL), KK and B1, acute myelogenous leukaemia (AML), OCI-AML-3 (hereafter referred to as AML-3), and NB-4, as well as a non-transformed human diploid fibroblast cell line WI38 were exposed to increasing concentrations of OSCs and assayed for proliferation using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay measures the ability of cellular mitochondrial dehydrogenase to convert the yellow MTT substrate into a purple formazan product. The resultant light absorbency values were normalized to untreated controls. Exposure to each OSC resulted in a dose-dependent decrease in proliferation in all leukaemic cell lines (Table 1). In contrast, the ability of each OSC to decrease proliferation of the normal diploid cell line was less robust, with the exception of compound **11**. The MTT_{50} values (the dose required to reduce proliferation to 50%) suggest that compounds **5**, **6**, **7**, **8**, **9**, and **10** possess antiproliferative activity that is specific for leukaemic cell lines, while compound **11** is a general cytotoxic agent (see Table 1). Compounds **9** and **10** appear to be more specific for three of the four leukaemic cell lines (AML-3, NB-4, B1). Furthermore, compounds **5**, **6**, and **7** show greater potency in comparison



Scheme 6.

to compounds **8**, **9**, and **10**; this demonstrates that the choice of α-substituent does affect disulfide activity.

OSC-Induced Changes in Cell-Cycle Profile and Apoptosis

To determine whether the decrease in proliferation was the result of apoptosis, and to further evaluate tumour specificity, leukaemic and non-transformed fibroblast cells were exposed to OSCs at two different concentrations for 48 h. The cellular DNA content was then measured by staining the cells with propidium iodide (PI), a DNA intercalating dye. The amount of DNA within the cell was then analyzed by flow cytometry. By this approach (referred to as fixed PI), the percentage of cells in a given phase of the cell cycle can be evaluated. Briefly, the cell cycle can be divided into four sequential phases: G_1, the cell prepares to duplicate DNA; S, DNA is duplicated; G_2, the cell prepares to divide; and M, cell division occurs. Cell cycle checkpoints, which exist before replication of the DNA, during the G_1 phase, and before separation of the chromosomes during the G_2 phase govern whether the cell will progress through the cycle, arrest in cell cycle, or undergo apoptosis.[19] In addition, fixed PI permits measurement of cells that are undergoing apoptosis following exposure to a compound, by quantifying the percentage of cells in the pre-G_1 region of the profile.[20]

Treatment of leukaemic cell lines with the seven compounds (**5**–**11**) resulted in an increase in cells with a DNA content less than G_1 (Table 2). Treatment of the WI38 non-transformed fibroblast cells with six of the compounds (**5**–**10**) resulted in minimal pre-G_1 regions of 1–5% (Fig. 1*a*, data not shown). However, compound **11**, previously noted in the MTT assay as a general cytotoxin, caused a considerable percentage of the non-transformed cells to undergo apoptosis. Interestingly, exposure of the WI38 cells to OSCs **5**–**10** resulted in a change in the cell-cycle profile, with an accumulation of cells in the G_2 phase. This suggests that OSCs can trigger a G_2 cell-cycle arrest in non-transformed cells (Fig. 1*a*, data not shown). Clearly, five of the OSCs (**5**–**7**, **9**, **10**) are considerably more cytotoxic to ALL and AML cell lines than non-transformed fibroblast cells; this suggests that these OSCs possess tumour-specific activity (Fig. 1*b*). Interestingly, a robust accumulation of cells in the pre-G_1 region was not as evident with compound **8**, yet the

Table 1. The dose (μM) required to reduce the MTT activity to 50% (MTT_{50}) of the non-transformed diploid cell line (WI38), the AML cell lines (AML-3, NB-4), and the ALL cell lines (KK, B1)

The MTT_{50} value was determined by exposing the cells to a wide concentration range (0–200 μM) of OSCs for 48 h and conducting MTT assays. Results shown are the mean MTT_{50} for three independent experiments performed with three replicates in each experiment

Compound tested	Cell line				
	WI38	AML-3	NB-4	B1	KK
5	59.6 ± 11.2	11.3 ± 2.8	12.3 ± 1.9	10.3 ± 2.1	13.3 ± 3.5
6	57.4 ± 18.3	13.6 ± 5.8	25.3 ± 5.1	16.4 ± 10.1	12.8 ± 6.5
7	65.0 ± 9.3	16.1 ± 3.7	16.8 ± 5.5	16.6 ± 4.6	24.2 ± 5.1
8	>200	97.7 ± 20.0	71.6 ± 10.6	97.7 ± 35.2	118.6 ± 17.4
9	155.9 ± 27.4	62.2 ± 7.6	76.8 ± 17.4	77.8 ± 10.2	130.9 ± 29.8
10	144.7 ± 26.7	71.9 ± 19.4	38.9 ± 9.5	99.9 ± 36.1	155.4 ± 23.5
11	10.1 ± 1.9	13.9 ± 3.5	14.5 ± 3.1	17.0 ± 5.9	10.0 ± 2.2

Table 2. Percentage of apoptotic (pre-G_1 as determined by fixed PI) leukaemic and fibroblast cells after 48 h exposure to OSCs (mean ± standard error of mean)

Compound tested	Dose	Cell line				
		WI38	AML-3	NB-4	B1	KK
11	25 μM	30.8 ± 7.1	47.0 ± 3.9	47.0 ± 7.1	53.5 ± 20.2	66.0 ± 13.9
	50 μM	33.4 ± 3.1	35.8 ± 7.7	62.7 ± 10.3	39.9 ± 10.3	45.4 ± 10.0
5	25 μM	0.5 ± 0.8	21.2 ± 0.2	32.4 ± 5.5	22.9 ± 5.5	10.4 ± 2.3
	50 μM	1.3 ± 0.2	53.6 ± 17.1	58.3 ± 5.2	37.4 ± 4.3	28.7 ± 7.5
6	25 μM	0.5 ± 0.1	11.6 ± 3.3	15.2 ± 1.2	7.5 ± 1.6	19.9 ± 8.6
	50 μM	1.3 ± 0.3	45.6 ± 10.2	36.5 ± 3.3	27.2 ± 2.2	25.1 ± 5.2
7	25 μM	0.4 ± 0.2	14.4 ± 7.5	25.2 ± 8.0	23.3 ± 5.3	36.6 ± 12.4
	50 μM	1.7 ± 0.3	29.0 ± 7.9	64.6 ± 5.8	49.6 ± 5.9	39.2 ± 5.8
8	50 μM	1.6 ± 0.5	10.1 ± 2.8	27.2 ± 3.0	14.5 ± 4.0	7.9 ± 1.3
	100 μM	5.3 ± 1.5	10.2 ± 2.6	34.0 ± 1.9	8.7 ± 1.8	6.9 ± 0.6
9	50 μM	0.7 ± 0.3	2.6 ± 0.7	10.0 ± 1.3	8.8 ± 6.7	36.9 ± 15.4
	100 μM	1.9 ± 0.7	22.4 ± 10.6	26.8 ± 3.2	26.9 ± 11.3	37.8 ± 17.6
10	50 μM	1.2 ± 0.2	9.9 ± 0.7	23.8 ± 1.3	6.8 ± 0.6	27.8 ± 19.7
	100 μM	2.2 ± 0.4	47.3 ± 18.1	40.2 ± 2.1	33.1 ± 4.3	38.8 ± 14.6

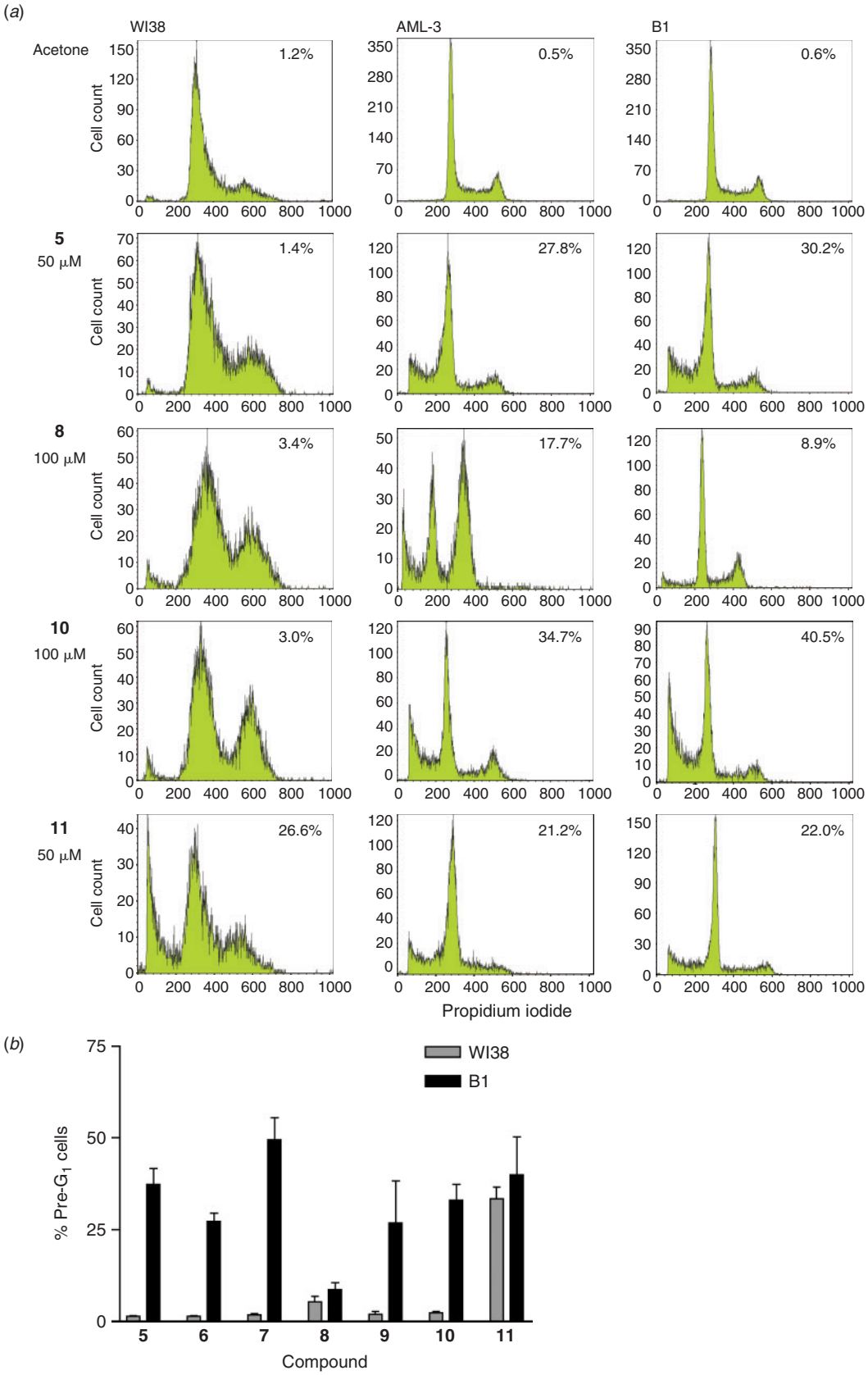


Fig. 1. OSCs alter leukaemic and WI38 cell-cycle profiles. Cells were exposed to acetone (solvent control) and two concentrations of OSCs for 48 h then stained with propidium iodide and analyzed by flow cytometry: (*a*) representative cell-cycle profiles in which the percentage of cells stained in the pre-G_1 region (apoptotic) is displayed in the upper right corner of each histogram; (*b*) a histogram comparing the average percentage of cells stained in the pre-G_1 region (apoptotic) after exposure of the non-transformed diploid cell line WI38 and a representative leukaemic cell line B1 to OSCs **5**–**7** and **11** at 50 μM, and OSCs **8**–**10** at 100 μM. The results shown are an average calculated from three independent experiments ± standard error of the mean.

Table 3. Percentage of TUNEL-positive cells after 48 h exposure to OSCs (mean ± standard error of mean)

Compound	Dose	Cell line			
		AML-3	NB-4	B1	KK
11	25 μM	76.9 ± 1.0	33.8 ± 1.3	74.5 ± 0.2	67.1 ± 0.2
	50 μM	63.0 ± 10.4	51.7 ± 8.2	70.5 ± 3.6	62.8 ± 7.8
5	25 μM	86.3 ± 3.3	57.9 ± 22.1	26.1 ± 4.6	29.7 ± 2.1
	50 μM	77.1 ± 3.1	73.1 ± 3.8	50.6 ± 5.4	40.5 ± 8.8
6	25 μM	55.2 ± 38.8	9.4 ± 2.5	39.2 ± 2.9	29.7 ± 1.6
	50 μM	84.8 ± 0.2	24.9 ± 6.6	75.4 ± 0.7	74.5 ± 6.5
7	25 μM	12.4 ± 3.8	12.4 ± 3.8	22.1 ± 4.3	31.8 ± 7.1
	50 μM	50.1 ± 12.4	42.4 ± 13.8	60.4 ± 8.1	45.9 ± 7.5
8	50 μM	14.0 ± 6.0	12.0 ± 2.7	20.7 ± 9.0	20.8 ± 8.3
	100 μM	26.6 ± 7.3	38.6 ± 10.5	33.7 ± 3.9	33.7 ± 3.9
9	50 μM	11.0 ± 1.0	6.8 ± 0.5	46.1 ± 5.7	30.1 ± 20.9
	100 μM	91.4 ± 0.6	28.6 ± 2.8	45.5 ± 28.2	47.3 ± 8.8
10	50 μM	16.1 ± 5.5	21.7 ± 7.7	25.9 ± 6.7	31.4 ± 12.8
	100 μM	48.2 ± 17.4	37.9 ± 13.6	54.6 ± 5.0	49.5 ± 17.3

MTT results clearly showed that compound **8** was as potent as the other five compounds in reducing proliferation exclusively in leukaemia cells. Indeed, this result is reminiscent of our previous analysis of compounds **3** and **4**. In particular, it showed that apoptosis could not be readily detected using a fixed PI approach as OSCs **3** and **4** were found to induce cells to undergo apoptosis from the G_2/M phase of the cell cycle.[15] Hence, we required a specific method that would readily detect cells undergoing apoptosis in the G_2/M phase of the cell cycle.

Apoptosis Detection Through DNA Fragmentation

We employed the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) assay to evaluate whether the OSCs induced apoptosis in the leukaemia cell lines. This assay allows characterization of drug-induced apoptosis on a single-cell basis by directly measuring DNA fragmentation.[21] ALL and AML cells were incubated in the presence of each OSC for 48 h, fixed, and then the presence of fragmented DNA was evaluated by labelling the 3′ ends of the fragmented DNA with a biotin-dUTP/fluorescein isothiocyanate (FITC)-avidin system. Flow cytometry was then applied to quantify the percentage of FITC-labelled cells.

Using this approach, we confirmed that the OSCs examined in this study induced the leukaemic cells to undergo apoptosis. The cells were exposed to two concentrations of each compound in order to evaluate the dose response. The percentage of positive staining cells increased with an increase in concentration of the sulfur compound and the results were consistent across a panel of four leukaemic cell lines (see Table 3, Fig. 2).

The cells were counterstained with PI during the TUNEL approach in order to identify the phase of the cell cycle in which apoptosis occurred. Indeed, as proposed, OSC **8** appears to target the G_2/M phase of the cell cycle. Therefore, although not fully detectable by fixed PI as was the case for the two lead compounds **3** and **4**,[15] OSC **8** does actually induce apoptosis in leukaemic cells.

Conclusions

Chemistry

Our initial report[15] disclosed that a pair of α-sulfonyl disulfides, one a methyl disulfide and one a phenyl disulfide, were too toxic to normal cells. The current results show that α-sulfonyl pentyl disulfide **5** has significantly diminished toxicity towards non-transformed cells and is a competitive antileukaemic in comparison to the compounds examined herein (see Table 1). It is now clear that appropriately substituted α-sulfone disulfides have significant promise as antileukaemic agents. Results for **8** (see Table 1) support our contention that less aggressively activated α-substituted disulfides, which may behave in accordance with Scheme 1, are significantly more selective antileukaemics than the α-sulfone disulfides examined in our earlier study.[15]

Tables 2 and 3 show results for compound **5** and several other promising disulfides, including **6** and **7**, which can be correlated with antileukaemic efficacy using the Scheme 1 mechanism. The encouraging results (Tables 2 and 3) for compounds **9** and **10** offer support for the notion that the mechanism in Scheme 5 may serve as a basis for the development of a second class of α-substituted disulfide antileukaemics.

Biology

Our novel disulfides show antiproliferative and apoptotic activity with specificity for leukaemic cell lines. The compounds reduced the viability of the leukaemic cell lines in a dose-dependent manner. From the MTT_{50} values, compound **11** was determined to be a general cytotoxin, while compounds **5**–**10** showed specificity for transformed cell lines.

The fixed PI and TUNEL results indicate that growth inhibitory effects are associated with an induction of apoptosis. The cell-cycle profiles suggest that leukaemic cell lines undergo apoptosis, while the non-transformed WI38 fibroblast cell line undergoes cell cycle arrest in response

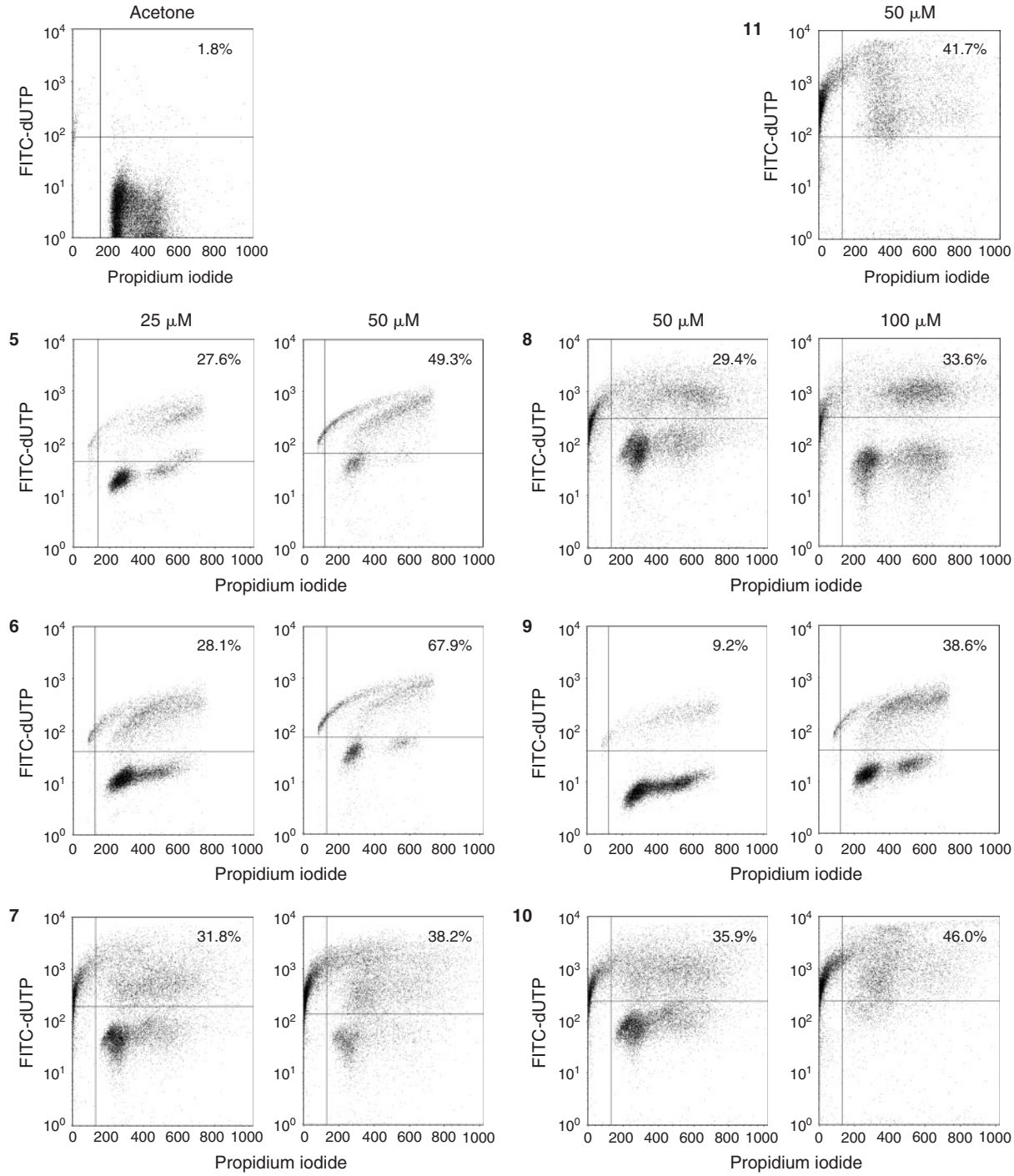


Fig. 2. OSCs induce apoptosis in leukaemic cell lines. TUNEL staining of representative leukaemic KK cells. The cells were exposed to acetone (solvent control), OSC **11** at 50 μM (positive control), and to each of the other OSCs **5**–**7** at 25 and 50 μM, and **8**–**10** at 50 and 100 μM, and analyzed by flow cytometry. The percentage of cells stained TUNEL positive (apoptotic) is displayed in the top right quadrant of each profile. Results shown are representative of three independent experiments.

to compounds **5**–**10**. Increased sensitivity of the transformed cells to disulfide-induced apoptosis in comparison to the non-transformed cell line WI38 is consistent with the concept that cells which harbour genetic abnormalities that deregulate cell-cycle checkpoints are sensitized to undergo apoptosis when exposed to various cytotoxic or cytostatic agents.[22] Therefore, the OSCs may provoke the leukaemic cell lines to undergo apoptosis as a consequence of deregulated cell-cycle checkpoints in the transformed cells.

Although the biological mechanism of the apoptosis induction by these OSCs remains unknown, compound **8** appears to induce apoptosis from the G_2/M phase of the cell cycle. However, it is not known whether growth arrest of cells in the G_2/M phase of the cell cycle actually occurs and

apoptosis is simply a consequence of the arrest in the G_2/M phase. Indeed, this profile is similar to that of OSCs **3** and **4** reported previously.[15] The induction of G_2/M phase arrest appears to be a characteristic of other disulfides under investigation for antitumour activity, such as ajoene, diallyl disulfide, and imidazolyl disulfides.[23–26] Exposure of the cells to the six remaining compounds revealed that death occurred from all phases of the cell cycle. It will be illuminating to elucidate the mechanisms of disulfide activity and compare them with those of other cell-cycle inhibitors. Furthermore, disulfides such as ajoene and diallyl disulfide or dithiosulfinates have been associated with the induction of reactive oxygen species, inhibition of metabolic enzymes, DNA modification, and disruption of signalling pathways.[27–36] It will be of interest to compare and contrast the mechanism of apoptosis induced by the disulfides in this study.

The functional screen approach employed in this study revealed that selected disulfides have potent apoptotic activity, and that changes to the regions that flank the disulfide moiety modulate the toxicity and specificity of each compound toward transformed cells. Clearly, structural relatives of compounds **5**–**10** have the potential to be exploited further to gain increased sensitivity in inducing apoptosis while maintaining specificity for transformed cells. Experimental evidence provided in this study suggests that disulfides have therapeutic and clinical potential as novel anticancer agents.

Experimental

Biology

Cell Culture and Cell Lines

The human ALL cell lines KK and B1, as well as the human AML cell lines AML-3 and NB-4 were derived from patient samples as described previously.[37] The human fibroblast cell line WI38 was purchased from American Type Culture Collection (Manassas, VA). All cell lines were assayed as asynchronously growing cells and maintained in α-minimal essential medium (α-MEM; Princess Margaret Hospital Media Services) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO) and 1% penicillin/streptomycin at 37°C under 5% CO_2. The WI38 cell line was plated subconfluently 24 h before treatment.

Biological Testing: General Sample Preparation

Approximately 20 mg of each compound was dissolved in ACS grade acetone (5 mL, Sigma). Stock solutions of the compounds were stored in the dark at 4°C. Compounds were diluted, as indicated, immediately before each experiment.

MTT Assays

As described previously,[15] leukaemic and WI38 fibroblast cells were seeded at a density of 2.7×10^5 and 6.7×10^4 cells mL^{-1}, respectively, in 96-well plates (Falcon, Mississauga, ON). The cells were exposed to an acetone control as well as a dose range of each compound in a total volume of 150 μL and assayed in triplicate. After a 48 h incubation under 5% CO_2 at 37°C, 40 μL of a 5 mg mL^{-1} solution of MTT substrate (Sigma) in Dulbecco's phosphate-buffered saline (D-PBS) was added to each well. The resultant violet formazan precipitate went into solution overnight after addition of 80 μL of a 0.01 M HCl and 10% sodium dodecyl sulfate (SDS; Sigma) solution and a further 4 h of incubation at 37°C under 5% CO_2. The plates were then analyzed using the BioRad Benchmark Microplate Reader (BioRad Laboratories, Hercules, CA) at 570 nm to determine the optical density of the samples. MTT data was analyzed by the Chou–Talalay method[38,39] using *Prism 3.0* (GraphPad Software Inc., San Diego, CA). A dose–response curve for each compound was repeated, independently, three times.

Fixed PI Staining

Approximately 1×10^6 leukaemia cells and 3.5×10^5 WI38 cells were seeded for PI staining in a six-well dish (Falcon). Cells were then exposed for 48 h to an acetone control and two concentrations of each compound: **5**–**7**, **11** (25 and 50 μM), and **8**–**10** (50 and 100 μM). The cells were subsequently harvested, washed, and fixed with 80% ethanol. Cells were stained with 50 μg mL^{-1} PI as described previously[37] and approximately 2.0×10^4 cells were analyzed using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). Cell-cycle parameters were measured with Cellquest software (Becton Dickinson, San Jose, CA). Each sample was repeated, independently, three times.

TUNEL

Approximately 1×10^6 leukaemia cells were seeded into a six-well dish (Falcon). The cells were then exposed to an acetone control and two concentrations of each compound: **5**–**7**, **11** (25 and 50 μM), and **8**–**10** (50 and 100 μM). After 48 h, cells were harvested and fixed with 4% formaldehyde for 15 min. The cells were washed in cold D-PBS and stored at −20°C in 70% ethanol. Samples were labelled with FITC using the TUNEL method as described previously.[15] Briefly, about 1×10^6 treated cells were incubated in labelling mixture that contained biotin-dUTP (Roche) and 12.5 U TdT enzyme (Boehringer Mannheim) for 45 min at 37°C. Samples were washed and spun at 1000 rpm for 5 min. The cell pellet was resuspended in 200 μL of 1 : 1000 fluorescein isothiocyanate (FITC)-conjugated avidin (Sigma) in 4 × SSC, 5% skim milk powder, and 0.05% Tween-20 (Sigma) for 1 h. Cells were then washed and stained with 10 μg mL^{-1} PI for 30 min. Approximately 2×10^4 cells were analyzed for FITC-positive cells using the FACScalibur flow cytometer (Becton Dickinson, San Jose, CA). Samples were analyzed using *Cellquest* software (Becton Dickinson, San Jose, CA). Each sample was repeated, independently, three times.

Chemistry

General

Details have been provided earlier.[16]

Thiosulfonate and Disulfide Syntheses

Some OSCs (see Scheme 6) were prepared as described earlier, namely the sulfone disulfide **5**,[40] the thiosulfonate **6**,[41] the nitrophenyl disulfide **11**,[17] the diester disulfide **9**,[42] and the α-ester disulfide **7**.[43]

*Preparation of 2,3,5-Trithiahexane **8***

Sodium metal (0.02 g, 0.87 mmol) was dissolved in methanol (2 mL) and methanethiol (20 mL) was bubbled slowly through the solution. The solvent was evaporated and the residue was dried under vacuum before being dissolved in DMSO (10 mL). A portion (1 mL) of the resultant solution was added to a solution of dimethyl disulfide (12 mL) and 2,4,5,7-tetrathiaoctane[44] (2.0 g, 10.7 mmol). The reaction mixture was stirred at ambient temperature for 8 days. Hydrochloric acid (70 mL, 2.5% v/v) was added and the resultant mixture was extracted with diethyl ether (3 × 50 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was rectified at reduced pressure to afford unchanged dimethyl disulfide (3.52 g) and 2,3,5-trithiahexane **8** (2.3 g, 78%), bp 75–85°C/18 Torr. Isolated compound **8** [δ_C (68 MHz, $CDCl_3$) 65.1, 73.4, 94.2] was identical to previously described material.[44]

Preparation of Phenacyl Thiolacetate

A solution of thiolacetic acid (0.56 g, 7.3 mmol) in dry pyridine (4 mL) was added to a solution of phenacyl chloride (1.0 g, 6.5 mmol) and the resultant reaction was maintained at 83°C for 1.5 h. Chloroform (200 mL) was added to the orange reaction mixture, which was

washed with 5% v/v hydrochloric acid (150 mL) and extracted with 2.5% w/v aqueous sodium hydroxide (100 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent was evaporated. The crude product was purified by chromatography on silica gel (5 g) employing light petroleum (400 mL) as eluent. The solvent was evaporated and the residue was rectified at reduced pressure to afford phenacyl thiolacetate (0.96 g, 75%), bp 137–139°C/2.4 Torr. ν_{max}/cm^{-1} 1700, 1680. δ_H (270 MHz, $CDCl_3$; Me_4Si) 2.38 (3H, s, CH_3), 4.39 (2H, s, CH_2), 7.46 (2H, t, ArH), 7.58 (1H, t, ArH), 7.97 (2H, d, ArH). δ_C (68 MHz; $CDCl_3$) 30.2, 36.6, 128.4, 128.7, 133.7, 135.5, 193.1, 194.1. *m/z* (GLC/MS) 194 (0.5%, $M^{+\bullet}$), 152 (7.4), 105 (100).

Preparation of Diphenacyl Disulfide

Potassium carbonate (19.0 g) was added to methanol (160 mL) and the resultant mixture was cooled in an ice-water bath for 1 h before phenacyl thiolacetate (4.2 g, 21.4 mmol) was added. The cold reaction was then stirred for 0.5 h. Diethyl ether (205 mL) and water (200 mL) were added and the ice-water bath was maintained for a further 0.5 h. Iodine (3.59 g) was then added in small portions over 30 min followed by an aqueous solution of saturated sodium thiosulfate (16 mL) and diethyl ether (85 mL). The organic layer was separated, washed with water (2 × 200 mL), dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by chromatography on silica gel (400 g) employing chloroform (100 mL fractions) as eluent. Fractions 16–29 were concentrated and combined. Purified diphenacyl disulfide was recrystallized twice from benzene/methanol to afford a granular solid (0.18 g, 3%), mp 68–72°C (Found: C 63.2, H 4.6. $C_{16}H_{14}O_2S_2$ requires C 63.5, H 4.7%). ν_{max}/cm^{-1} 1680. δ_H (270 MHz, $CDCl_3$; Me_4Si) 4.20 (2H, s, CH_2), 7.49 (2H, t, ArH), 7.59 (1H, t, ArH), 7.94 (2H, d, ArH). δ_C (68 MHz, $CDCl_3$) 45.4, 128.7, 128.8, 133.7, 135.4, 194.3. *m/z* (GLC/MS) 105 (100%), 77 (52).

*Preparation of Phenacyl Methyl Disulfide **10***

Sodium metal (0.019 g, 0.82 mmol) was dissolved in methanol (10 mL) and methanethiol (20 mL) was slowly bubbled into the reaction mixture. The solvent was evaporated and the residue was dried under vacuum. DMSO (10 mL) was added and the mixture was stirred at ambient temperature for 0.5 h. A portion (1 mL) was then added to a solution of diphenacyl disulfide (1.68 g, 5.5 mmol) in dimethyl disulfide (12 mL) and the resultant reaction mixture was stirred at ambient temperature for 8 days. Hydrochloric acid (70 mL, 2.5% v/v) was added and the resultant mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The crude product was purified by chromatography on silica gel (200 g) employing 3 : 7 chloroform/light petroleum (100 mL fractions) as eluent. Fractions 12–19 were combined and concentrated under reduced pressure. The purified disulfide was distilled at reduced pressure to afford phenacyl methyl disulfide **10** (1.12 g, 51%), bp 145–150°C/1.7 Torr. ν_{max}/cm^{-1} 1690. δ_H (270 MHz, $CDCl_3$; Me_4Si) 2.37 (3H, s, $SSCH_3$), 4.09 (2H, s, CH_2), 7.47 (2H, t, ArH), 7.59 (1H, t, ArH), 7.97 (2H, d, ArH). δ_C (68 MHz, $CDCl_3$) 22.4, 43.7, 128.1, 128.2, 132.9, 134.7, 193.9. *m/z* (GLC/MS) 198 (18%, $M^{+\bullet}$), 153 (3), 105 (100), 77 (43).

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References

[1] J. C. Reed, *Trends Mol. Med.* **2001**, *7*, 314. doi:10.1016/S1471-4914(01)02026-3

[2] L. Z. Penn, *Curr. Opin. Investig. Drugs* **2001**, *5*, 684.

[3] M. Zornig, A. Hueber, W. Baum, G. Evan, *Biochim. Biophys. Acta* **2001**, *1551*, F1.

[4] F. R. G. Braunschweig, *Cancer Lett.* **1990**, *53*, 103. doi:10.1016/0304-3835(90)90201-8

[5] K. Sakomoto, L. D. Lawson, J. A. Milner, *Nutr. Cancer* **1997**, *29*, 152.

[6] S. G. Sundaram, J. A. Milner, *Biochim. Biophys. Acta* **1996**, *1315*, 15.

[7] G. Sigounas, J. L. Hooker, W. Li, A. Anagnostou, M. Steiner, *Nutr. Cancer* **1997**, *28*, 153.

[8] G. Sigounas, J. Hooker, A. Anagnostou, M. Steiner, *Nutr. Cancer* **1997**, *27*, 186.

[9] H. Nakagawa, K. Tsuta, K. Kiuchi, H. Senzaki, K. Tanaka, K. Hioki, A. Tsubura, *Carcinogenesis* **2001**, *22*, 891. doi:10.1093/CARCIN/22.6.891

[10] S. G. Sundaram, J. A. Milner, *Carcinogenesis* **1996**, *17*, 669.

[11] V. M. Dirsch, A. L. Gerbes, A. M. Vollmar, *Mol. Pharmacol.* **1998**, *53*, 402.

[12] S. G. Sundaram, J. A. Milner, *J. Nutr.* **1996**, *126*, 1355.

[13] E. Block, R. DeOrazio, M. Thiruvazhi, *J. Org. Chem.* **1994**, *59*, 2273.

[14] M. K. Jogia, R. J. Andersen, E. K. Mantus, J. Clardy, *Tetrahedron Lett.* **1989**, *30*, 4919. doi:10.1016/S0040-4039(01)80542-6

[15] W. W. Wong, S. MacDonald, R. F. Langler, L. Z. Penn, *Anticancer Res.* **2000**, *20*, 1367.

[16] F. J. Baerlocher, R. F. Langler, M. U. Frederiksen, N. M. Georges, R. D. Witherell, *Aust. J. Chem.* **1999**, *52*, 167. doi:10.1071/C98141

[17] F. J. Baerlocher, M. O. Baerlocher, R. F. Langler, S. L. MacQuarrie, M. E. Marchand, *Aust. J. Chem.* **2000**, *53*, 1. doi:10.1071/CH99139

[18] R. B. Silverman, *The Organic Chemistry of Drug Design and Drug Action* **1992**, p. 16 (Academic Press: New York, NY).

[19] M. Malumbres, M. Barbacid, *Nat. Rev. Cancer* **2001**, *1*, 222. doi:10.1038/35106065

[20] I. Nicoletti, G. Migliorati, M. C. Pagliacci, F. Grignani, C. Riccardi, *J. Immunol. Methods* **1991**, *139*, 271. doi:10.1016/0022-1759(91)90198-O

[21] S. Nagata, *Exp. Cell Res.* **2000**, *256*, 12. doi:10.1006/EXCR.2000.4834

[22] G. I. Evan, A. H. Wyllie, C. S. Gilbert, T. D. Littlewood, H. Land, M. Brooks, C. M. Waters, L. Z. Penn, D. C. Hancock, *Cell* **1992**, *69*, 119. doi:10.1016/0092-8674(92)90123-T

[23] L. M. Knowles, J. A. Milner, *Nutr. Cancer* **1998**, *30*, 169.

[24] D. J. Frantz, B. G. Hughes, D. R. Nelson, B. K. Murray, M. J. Christensen, *Nutr. Cancer* **2000**, *38*, 255. doi:10.1207/S15327914NC382_15

[25] M. Li, J. R. Ciu, Y. Ye, J. M. Min, L. H. Zhang, K. Wang, M. Gares, J. Cros, M. Wright, J. Leung-Tack, *Carcinogenesis* **2002**, *23*, 573. doi:10.1093/CARCIN/23.4.573

[26] A. Vogt, K. Tamura, S. Watson, J. S. Lazo, *J. Pharmacol. Exp. Ther.* **2000**, *294*, 1070.

[27] M. Focke, A. Feld, K. Lichtentaler, *FEBS Lett.* **1990**, *261*, 106. doi:10.1016/0014-5793(90)80647-2

[28] R. Gebhardt, H. Beck, *Lipids* **1996**, *12*, 1269.

[29] S. Lee, S. Park, J. W. Oh, C. Yang, *Planta Med.* **1998**, *64*, 303.

[30] H. Nakagawa, K. Tsuta, K. Kiuchi, H. Senzaki, K. Tanaka, K. Hioki, A. Tsubura, *Carcinogenesis* **2001**, *22*, 891. doi:10.1093/CARCIN/22.6.891

[31] V. Robert, B. Mouille, C. Mayeur, M. Michaud, F. Blachier, *Carcinogenesis* **2001**, *22*, 1155. doi:10.1093/CARCIN/22.8.1155

[32] V. M. Dirsh, D. S. Antisperger, H. Hentze, A. M. Vollmar, *Leukemia* **2002**, *16*, 74. doi:10.1038/SJ.LEU.2402337

[33] V. M. Dirsch, A. L. Gerbes, A. M. Vollmar, *Mol. Pharmacol.* **1998**, *53*, 402.

[34] G. Filomeni, K. Aquilano, G. Rotilio, M. R. Ciriolo, *Cancer Res.* **2003**, *63*, 5940.

[35] D. S. Antisperger, V. M. Dirsch, D. Ferreira, J. L. Su, M. L. Kuo, A. M. Vollmar, *Oncogene* **2003**, *22*, 582. doi: 10.1038/SJ.ONC.1206161
[36] L. M. Knowles, J. A. Milner, *J. Nutr.* **2003**, *133*, 2901.
[37] T. C. Chou, P. Talalay, *Adv. Enzyme Reg.* **1984**, *22*, 27. doi:10.1016/0065-2571(84)90007-4
[38] *The Median-Effect Principle and the Combination Index for Quantitation of Synergism and Antagonism* (Ed. T. C. Chou) **1991**, p. 61 (Academic Press: San Diego, CA).
[39] J. Dimitroulakos, D. Nohynek, K. L. Backway, D. W. Hedley, H. Yeger, M. H. Freedman, M. D. Minden, L. Z. Penn, *Blood* **1999**, *93*, 1308.
[40] F. J. Baerlocher, M. O. Baerlocher, R. F. Langler, S. L. MacQuarrie, P. E. O'Connor, *Sulfur Lett.* **2002**, *25*, 135. doi:10.1080/02786110213980
[41] F. J. Baerlocher, M. O. Baerlocher, C. L. Chaulk, R. F. Langler, S. L. MacQuarrie, *Aust. J. Chem.* **2000**, *53*, 399. doi:10.1071/CH00030
[42] F. J. Baerlocher, M. O. Baerlocher, K. D. Guckert, R. F. Langler, S. L. MacQuarrie, P. E. O'Connor, G. C. Y. Sung, *Aust. J. Chem.* **2001**, *54*, 397. doi:10.1071/CH01046
[43] F. J. Baerlocher, M. O. Baerlocher, C. L. Chaulk, R. F. Langler, E. M. O'Brien, *Sulfur Lett.* **2000**, *24*, 101.
[44] P. Dubs, R. Stuessi, *Helv. Chim. Acta* **1978**, *61*, 2351.