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Original article

Structure–activity studies on the anti-proliferation activity of ajoene analogues in WHCO1 oesophageal cancer cells

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ABSTRACT

The organosulfur compound ajoene derived from the rearrangement of allicin found in crushed garlic can inhibit the proliferation of tumour cells by inducing G_2/M cell cycle arrest and apoptosis. We report on the application of a concise four-step synthesis (Hunter et al., 2008 [1]) that allows access to ajoene analogues with the end allyl groups substituted. A library of twelve such derivatives tested for their anti-proliferation activity against WHCO1 oesophageal cancer cells has identified a derivative containing *p*-methoxybenzyl (PMB)-substituted end groups that is twelve times more active than *Z*-ajoene, with an IC_{50} of 2.1 μM (Kaschula et al., 2011 [2]). Structure–activity studies involving modification of the sulfoxide and vinyl disulfide groups of this lead have revealed that the disulfide is the ajoene pharmacophore responsible for inhibiting WHCO1 cell growth, inducing G_2/M cell cycle arrest and apoptosis by caspase-3 activation, and that the vinyl group serves to enhance the anti-proliferation activity a further eightfold. Reaction of the lead with cysteine in refluxing THF as a model reaction for ajoene's mechanism of action based on a thiol/disulfide exchange reveals that the allylic sulfur of the vinyl disulfide is the site of thiol attack in the exchange.

1. Introduction

Ajoene was first isolated as an *E*/*Z*-mixture by Block and Apitz-Castro in 1984 [3] as a rearrangement product of the thiosulfinate compound allicin, which is an organosulfur compound produced from freshly-crushed garlic. Its structure was established to be an allyl sulfoxide containing an unusual vinyl disulfide group, the latter rarely seen in natural product structures. The authors [3,4] suggested that the key step in ajoene's biosynthetic pathway involves the addition of allylsulfenic acid to an *S*-thioallylated vinylthionium ion formed from the self-condensation of allicin (Scheme 1).

Ajoene is not found in freshly crushed garlic but is found in treated or aged preparations, with increased concentrations in heated preparations [3,5]. The *E*-isomer is more abundant in fresh extracts [5] with the *Z*-isomer predominating in heated extracts (i.e. when crushed garlic is heated in oil) [4,5]. The *E*-isomer is more predominant in aged preparations due to its increased chemical stability over its *Z*-isomer. In Block and Apitz-Castro's work [4], the focus of ajoene's biological activity centred on its anti-thrombotic activity [6,7], but in subsequent years ajoene has demonstrated a range of other biological activities including anti-microbial [8], anti-obesity [9,10], anti-fungal [11–14], and anti-cancer [15–17] activities, with the latter of particular interest in the current work. Ajoene has been shown to inhibit tumour cell proliferation by inducing apoptosis in a number of cell lines, with the IC_{50} ranging from 5 to 40 μM [1,18–20]. *In vivo*, ajoene has been shown to inhibit the growth of skin carcinoma in both rodents and humans [21,22], and to inhibit tumour cell growth in mouse xenografts by 2–3-fold [18,19].

Several groups have uncovered aspects of mode of action and molecular targets of ajoene, but a comprehensive picture has yet to emerge. Ajoene and the related garlic organosulfur compounds diallyl disulfide and diallyl trisulfide have been shown to induce cell-cycle arrest in the G_2/M phase of the cell cycle [23,24]. Apoptosis has also been demonstrated to proceed via the

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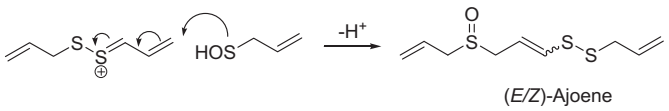


Scheme 1. The key step in ajoene biosynthesis.

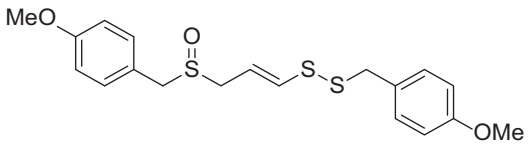


Fig. 1. The chemical structure of the ajoene analogue *E*/*Z*-**4l**.

mitochondrial-dependent caspase cascade with involvement of mitochondrial membrane permeabilization (MMP), release of cytochrome c, caspase 3 activation and cleavage of the anti-apoptotic protein Bcl-2 [19,24–30]. It has been proposed [2,31,32] that the anti-proliferation activity of garlic organosulfur compounds may be tied to the chemical reactivity of the disulfide bond, which may thiolate cysteine residues in proteins. In support of this hypothesis, the related garlic compound diallyl trisulfide has been found to oxidatively modify β-tubulin in a cell-free environment to form mixed disulfides at cysteine residues Cys-12β and Cys-354β [33]. In another cell-free model, ajoene was found to act as a covalent inhibitor and substrate of glutathione reductase through mixed disulfide formation with Cys58 in the active site of the enzyme [34].

In 2008 we published a letter [1] on the first synthetic route to access end-substituted ajoenes (see Scheme 2 for a general formula), which demonstrated that terminal end substitution (sulfoxide end, R^1) of ajoene results in retention, and in some cases a modest improvement, in the anti-proliferation activity against transformed CT-1 fibroblast cells compared to the parent natural product. We now provide a full account of activity against the WHCO1 cell-line involving seven of the previously published analogues together with five new analogues in which both R^1 and R^2 are substituted. This study has resulted in identification of our most active analogue *E*/*Z*-**4l** to date substituted with a *p*-methoxybenzyl (PMB)-group at both terminal positions (Fig. 1). This analogue is more active than *Z*-ajoene at inhibiting cancer-cell proliferation of cultured prostrate, breast, cervical and oesophageal cancer cells, and more specifically displays a twelve-fold improvement against the WHCO1 oesophageal cancer cell-line [2]. We also present data on five new analogues of *E*/*Z*-**4l** in which the central vinyl disulfide/sulfoxide core is varied. The findings in this paper have enabled the generation of a structure–activity model for the anti-proliferation activity of ajoene in WHCO1 cancer cells.

2. Results and discussion

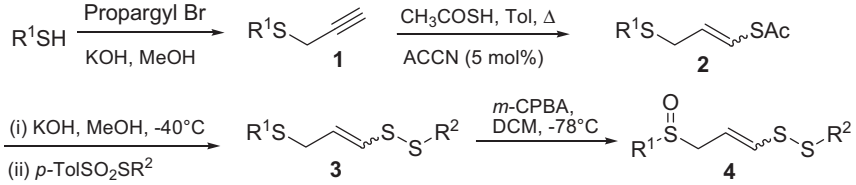
2.1. Synthesis aspects

2.1.1. Part 1: synthesis of terminally-substituted ajoene analogues

The only known synthesis of ajoene to date is the biomimetic rearrangement of allicin according to Block [3,4,35], by heating it in aqueous acetone. Apart from our experience of obtaining low yields of the natural product using this route, the biomimetic synthesis demands that an *S*-allyl group (or substituted allyl) is present in the substituted thiosulfinate starting material, which results in an obligatory allyl group at the disulfide end of the molecule. Thus, it was clear that a new synthesis needed to be developed for accessing derivatives of type **4** shown in Scheme 2 with variable end-groups. Consideration of the development of such a synthesis suggested the possibility of using cross-coupling methodology [36] for accessing a stereo-defined protected substituted vinyl sulfide; this had the attraction of accessing individual *E*/*Z*-stereoisomers of the targets. However, ultimately it was decided to base the key step of our synthesis [1] on a reaction studied in the 1960s by Kampmeier [37] involving regioselective radical addition of thiolacetic acid to 1-hexyne at its terminus to form a vinyl thioacetate. Kampmeier showed that the *Z*-product was kinetically favoured, and application of this finding to our system resulted in the development [1,38] of a moderately *Z*-stereoselective four-step synthesis of the ajoene core structure in which the end groups could be varied, and one that was amenable to production of derivatives for biological evaluation. A general overview of it is depicted in Scheme 1.

Thus, a thiol R^1SH obtained commercially or via reaction of R^1Br with thiourea was propargylated under standard conditions to form propargylic thioether **1**. This underwent regioselective radical addition with thiolacetic acid in refluxing toluene using ACCN as radical initiator to give an *E*/*Z*-mixture of stereoisomeric vinyl thioacetates **2** in around 60% after chromatography (see Table 1). Low temperature hydroxide-promoted cleavage of the thioester followed by sulfenylation with an appropriate *S*-alkylated thio-*p*-toluenesulfonate afforded vinyl disulfide **3** in high yield after chromatography. Finally, chemoselective sulfide oxidation with *m*-CPBA (1.1 equiv) in DCM at low temperature gave substituted ajoene **4** in yields of around 60–70% after chromatography. In some cases the polarities of the isomeric *E*- and *Z*-sulfoxide products **4** was sufficiently different such that they could be separated chromatographically. The overall yield for the sequence was generally not lower than about 30%, allowing production of sufficient material of a range of derivatives for biological evaluation purposes. Unfortunately, the ajoene parent (R^1, R^2 = allyl) couldn't be accessed by this route, possibly as a result of cyclization of the β-vinyl radical onto the *S*-allyl group (R^1 = allyl in **2** of Scheme 2) via a 5-*exo*- or 6-*endo*-*trig* process [1].

We have previously synthesized seven analogues of **4** in which only R^2 was varied (Table 1, compounds **4a**–**g**) [1]. We now report the synthesis of a further 5 analogues which probe both the R^1 and R^2 terminal positions (Table 1, compounds **4h**–**l**). All compounds have been fully characterised by ^1H and ^{13}C NMR spectroscopy (see Experimental section), and the *E*/*Z*-isomer ratios in compounds **3** and **4** were established by virtue of J_{cis}/J_{trans} vinyl couplings in the



Scheme 2. Synthesis of doubly-end substituted ajoenes of type **4**.

Table 1
Yields and *E*/*Z* ratios for the synthesis of compounds **1a**–**g**, **2a**–**g**, **3a**–**l** and **4a**–**l**.

R^1	R^2	R^1S **1**		R^1S SAc **2**		R^1S S S R^2 **3**		O R^1 S S S R^2 **4**	
		Cmpd no	Yield % (*E*/*Z*)	Cmpd no	Yield % (*E*/*Z*)	Cmpd no	Yield % (*E*/*Z*)	Cmpd no	Yield % (*E*/*Z*)
						3a	97 (4:5)	**4a**	82 (2:3)
		1a	80	**2a**	73 (1:2)	**3h**	76 (1:1)	**4h**	68 (3:2)
	CH_3					**3i**	76 (2:3)	**4i**	68 (2:3)
		1b	57	**2b**	73 (1:2)	**3b**	89 (1:2)	**4b**	78 (1:2)
OTBS		**1c**	70	**2c**	31 (1:1)	**3c**	89 (1:2)	**4c**[a]	86[b] (2:3)
OPMB		**1d**	57	**2d**	64 (2:3)	**3d**	89 (2:3)	**4d**	92 (1:2)
O N O		**1e**	69	**2e**	66 (2:3)	**3e**	67 (1:1)	**4e**	64 (1:2)
		1f	98	**2f**	53 (1:2)	**3f**	86 (3:4)	**4f**	65 (5:6)
OMe						**3g**	84 (2:3)	**4g**	85 (1:2)
OMe		**1g**				**3j**	88 (2:3)	**4j**	71 (2:3)
OMe	F		66	**2g**	55 (2:3)	**3k**	70 (3:4)	**4k**	74 (3:4)
OMe	OMe					**3l**	83 (1:1)	**4l**	78 (3:2)

[a] Isolated as the free alcohol via silyl deprotection with HF in acetonitrile.
[b] Yield refers to the *m*-CPBA oxidation step on the silyl ether.

^{1}H NMR spectra. Similarly, IR spectroscopy revealed a thioacetate carbonyl stretching absorption for the vinyl thioesters **2** at around 1700 cm^{-1}. All vinyl thioacetates **2a**–**g** were isolated by column chromatography as a mixture of stereoisomers and as oils that couldn't be separated, but which all gave correct high resolution mass spectrometry data. *E*/*Z*-Ratios revealed a preference on average of about 2:1 in favour of the *Z*-isomer, which was pleasing in view of the report that *Z*-ajoene is more active than its *E*-isomer as an anti-thrombotic agent [4], and that some studies in cancer have also focused primarily on the *Z*-isomer [19,30]. Such stereoselectivity indicates that the rate of reduction of the intermediate *Z*-vinylthioacetate radical by thiolacetic acid is higher than that of the *E*-radical in the hydrogen-quenching propagation step, presumably owing to less steric hindrance between the attached thioacetate group and the incoming thiolacetic acid (Table 1 summarises the results).

With a range of vinyl thioacetates in hand, we set about incorporating the vinyl disulfide moiety. Such a functionality was first synthesized by Brandsma [39] via sulfenylation of a 1-alkenethiolate prepared in situ by cleavage of a 1-alkenyl alkyl sulfide with Li/NH_3, and this methodology was subsequently exploited by Block [40] in his synthesis of cepaene natural products from onion. In our case, it was found that low temperature thioacetate cleavage with KOH to the enethiolate was successful at around −40 °C. Reaction with the appropriately substituted *S*-allyl *p*-toluenesulfonylthioate was carried out in each case (**2a**–**g**) resulting in a high-yielding conversion to the vinyl disulfide products **3a**–**l**. Interestingly, and importantly from the bioactivity discussion that follows, in most cases the starting *E*/*Z*-ratio in **2a**–**g** was retained or only marginally increased in favour of the *E*-isomer in **3a**–**g** indicating that enethiolate–thioaldehyde tautomerism with stereoinversion is slow at −40 °C. Other sulfenylating agents could be easily accessed, so derivatives **3h**–**l** were also prepared in which R^2 was varied, (see Table 1). Once again *cis*/*trans*-isomerization was minimal. All vinyl disulfides **3a**–**l** were characterised as their *E*/*Z*-mixtures, and with the aid of HSQC and COSY NMR spectroscopy, isomers could be fully assigned (see Experimental section). Completion of the synthesis was achieved by chemoselective oxidation of the sulfide sulfur of each derivative **3a**–**l** using *m*-CPBA at low temperature in dichloromethane. Following chromatography, target ajoenes **4a**–**l** were obtained in an acceptable yield of around the 70% mark, and in some cases the *E*/*Z*-isomers could be separated by column chromatography (**4a**, **4c**, **4d**, **4e**, **4h**, **4i**) or crystallized (**4j**–**l**) as a mixture of isomers (by ^{1}H NMR). The *E*- and *Z*-isomers of **4c** containing a terminal primary hydroxyl group could be obtained via desilylation (HF/CH_3CN) of the TBS derivative. All compounds were fully characterised by a range of spectroscopic and analytical methods. The oxidation resulted in slight fluctuations either way of the *E*/*Z*-stereochemistry ratios going from **3** to **4**, which was ascribed to the experimental conditions and the isolation and purification steps. Evidence for the site of oxidation came from the downfield shifts in the sulfoxide α-methylene hydrogens and α-carbons in the ^{1}H NMR and ^{13}C NMR spectra respectively. Furthermore, the α-sulfoxide hydrogens of **4** allylic to the vinyl disulfide double bond always resonated as diastereotopic ABXX′ double double doublets, and these could be identified for each isomer.

2.1.2. Part 2: synthesis of E/Z-4l analogues

Four structural analogues of *E*/*Z*-**4l** were synthesized (see Schemes 3 and 4 for structures) including a vinyl sulfide (removal of one S of the S–S functionality) as well as a saturated disulfide (removal of the double bond), while keeping everything else constant. To this end, derivative **6**, with the vinyl disulfide of *E*/*Z*-**4l** replaced by a vinyl sulfide, could be prepared from vinyl thioacetate **2g** (Table 1) in 80 % yield and as a 3:2 *Z*:*E* mixture of stereoisomers, using a similar sequence to the one used to obtain *E*/*Z*-**4l** except with alkylation of the enethiolate with PMB chloride to generate **5**. Subsequent selective oxidation of the more nucleophilic sulfide sulfur of **5** furnished derivative **6** in 82 % yield (*Z*:*E* = 3:2) containing a vinyl sulfide/sulfoxide combination rather than the vinyl disulfide/sulfoxide found in *E*/*Z*-**4l** (Scheme 3).

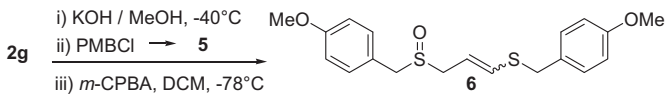
Both compounds **5** and **6** are new compounds that were comprehensively characterised as an *E*/*Z*-isomeric mixture in the case of **5** and as crystalline, individual stereoisomers for **6** that gave correct microanalyses. The site-selectivity of oxidation was established as before by observation of downfield shifts in the NMR spectra for the sulfoxide α-hydrogens and carbons originating from the sulfide sulfur in **5**. For compounds **7** and **8** (Scheme 4) containing a saturated disulfide instead of a vinyl disulfide grouping, hydrogenation of *E*/*Z*-**3l** or *E*/*Z*-**4l** failed to afford the desired product. Thus, a novel route for synthesis of **7** was developed, which involved two successive alkylations of 1,3-propane-dithiol in a one-pot sequence using: i) PMBCl; ii) *S*-PMB *p*-toluenesulfonylthioate, to afford **7** in high overall yield (85%). Subsequent oxidation of the more nucleophilic sulfur of **7** gave sulfoxide/disulfide **8** after column chromatography lacking the double bond of the ajoene derivative *E*/*Z*-**4l**. As before, NMR provided comprehensive support for the site of oxidation as stated (see Experimental section), Scheme 4.

2.2. Biological activity

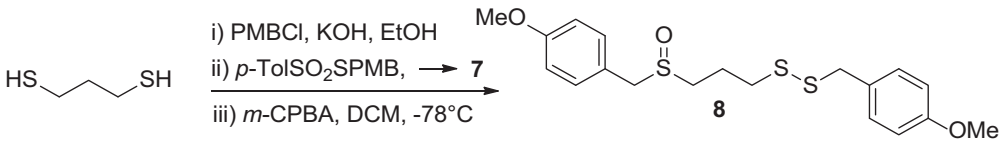
2.2.1. Effect of R1 and R2 substitution

The ajoene analogues **4a**–**l** were all tested for their ability to inhibit the proliferation of WHCO1 oesophageal cancer cells by the MTT cell proliferation assay. Strength of inhibition is reported as an IC_{50}, defined as the concentration found to inhibit 50 % of cell proliferation after 48 h. Some of the isomeric mixtures of **4** were separable chromatographically and were hence tested as pure geometric isomers (**4a**, **4c**, **4d**, **4e**, **4h**, **4i**), whereas the others (**4b**, **4f**, **4g**, **4j**, **4k**, **4l**) were inseparable and were tested as *E*/*Z* mixtures (see Table 2 for anti-proliferation activities reported as an average and standard deviation of three independent determinations).

For ajoene analogues in which at least one of the allyl groups of ajoene was varied as propyl and/or methyl **4a**, **4h**, **4i**, and for which *E*- and *Z*-isomers could be separated, retention of activity was observed against WHCO1 cell proliferation regardless of whether one or both allyl groups was substituted, complementing our previous findings on transformed CT-1 cells [1], and implying that the ajoene pharmacophore does not reside within the terminal end allyl groups. This finding contrasts with that of Sundaram et al. [41,42] in which the related garlic compound diallyl disulfide (DADS) was found to be ineffective at inhibiting growth of human colon, skin and lung cancer cells when the terminal allyl groups were substituted for propyl. It is unlikely that this discrepancy is cell-line specific as **4a** was also previously found to be fully active against CT-1 cells [1]. It would therefore appear that different mechanisms govern the anti-proliferation activity of ajoene and DADS in cancer cells. Furthermore, and in agreement with previous findings on different cancer cell lines [1,2], *Z*-ajoene and the *Z*-analogues were found to be marginally more active at inhibiting



Scheme 3. Synthesis of disulfides **5** and **6**.



Scheme 4. Synthesis of disulfides **7** and **8**.

cell proliferation than *E*-ajoene and the corresponding *E*-analogues. This data supports the hypothesis that stereospecific protein–drug interactions appear to play a minor role in ajoene's anti-proliferation activity in cancer cells.

In our previous study [1], we identified that *p*-methoxybenzyl substitution at R^1 (R^2 = allyl) (**4g**) produced the most active analogue at inhibiting the proliferation of transformed CT1 cells and with increased lipophilicity appearing to play a role. In the

Table 2
Effect of R^1 and R^2 substitution on WHCO1 cell proliferation.

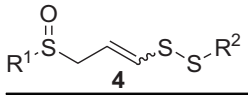
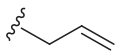
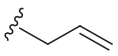
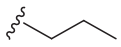
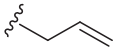
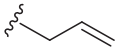
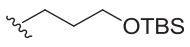
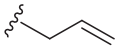
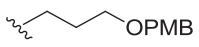
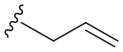
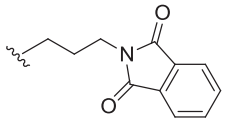
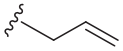
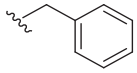
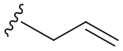
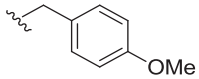
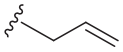
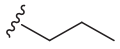
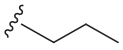
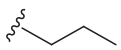
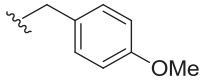
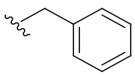
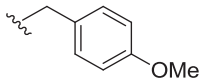
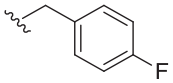
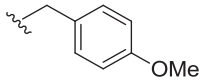
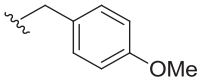
Cmpd no.	Isomer	 4		$IC_{50} \pm SD$ (μM)
		R^1	R^2	
Ajoene	*Z* *E*			25.0 ± 2.8 [2] 39.0 ± 7.8 [2]
4a	*Z* *E*			23.0 ± 4.2 37.0 ± 4.5
4b	*EZ*			23.0 ± 6.7
4c	*Z* *E*			38.0 ± 5.9 27.0 ± 5.6
4d	*Z* *E*			21.0 ± 0.6 18.0 ± 5.7
4e	*Z* *E*			33.0 ± 1.4 68.0 ± 15
4f	*EZ*			8.9 ± 1.2
4g	*EZ*			7.4 ± 0.7
4h	*Z* *E*			18.0 ± 4.1 24.0 ± 2.8
4i	*Z* *E*		CH_3	26.0 ± 7.5 28.0 ± 7.2
4j	*EZ*			3.1 ± 1.1
4k	*EZ*			16 ± 1.1
4l	*EZ*			2.1 ± 0.4 [2]

Table 3
Anti-proliferation activity of *E*/*Z*-**4l**, cisplatin and 5-fluorouracil on WHCO1 cells.

Compound name	$IC_{50} \pm SD$ (μM)
E/*Z*-**4l**	2.1 ± 0.4 [2]
Cisplatin	9.2 ± 0.1
5-Fluorouracil	7.9 ± 0.6

current study, a similar trend was also observed in WHCO1 cells (see Table 2 for the IC_{50}s). We therefore selected *p*-methoxybenzyl as our active lead for the R^1 position, and turned our attention towards optimizing R^2. To this end, a small library of analogues (**4j**–**4l**) was prepared in which R^2 was varied as benzyl (**4j**), *p*-fluorobenzyl (**4k**) and *p*-methoxybenzyl (*E*/*Z*-**4l**). The geometrical isomers of these analogues were all inseparable and were hence tested as *E*/*Z* mixtures, which revealed a trend of OMe > H ≫ F (Table 2) suggesting that electron-rich end groups may favour increased activity. In such a way, we identified *E*/*Z*-**4l** as our most active analogue, which as an *E*/*Z*-mixture returned a twelvefold increase in activity (2.1 μM) relative to the more active parent isomer, *Z*-ajoene (25 μM) [2]. This IC_{50} falls within the *in vitro* clinical range where two other oesophageal cancer chemotherapeutics cisplatin and 5-fluorouracil were found to have an IC_{50} of 9.2 μM and 7.9 μM respectively on the same cell line (see Table 3).

To further confirm the MTT data, WHCO1 cells were treated with *E*/*Z*-**4l**, *Z*-ajoene and *E*-ajoene, and cell growth, adhesion and spreading were monitored as a function of time by Roche xCelligence (Fig. 2). When compared to the control cells (pink), 10 μM of *Z*-ajoene (green) or 10 μM of *E*-ajoene (blue) was found to partially inhibit WHCO1 cell-growth, followed by recovery within two days. Conversely, treatment with 10 μM *E*/*Z*-**4l** (red) caused immediate inhibition of cell growth with very little recovery up to 5 days. (For interpretation of the references to colour in this paragraph, the reader is referred to the web version of this article.)

2.3. Structure–activity analysis of the pharmacophore

2.3.1. Importance of the disulfide in E/Z-4l anti-proliferation activity

Having established that the R^1 and R^2 end groups are not critical for anti-proliferation activity in WHCO1 cells but appear to play a modulation role, the focus of the structure–activity analysis was next directed at the vinyl disulfide and sulfoxide functionalities of the most active analogue *E*/*Z*-**4l**. Analogues **5**–**8** were then tested for WHCO1 anti-proliferation activity by (i) the MTT assay (Table 4), and (ii) xCelligence (Fig. 3).

Analogues lacking the disulfide bond (*E*/*Z*-**5**, *Z*-**6** and *E*-**6**) were found to be inactive both by the MTT assay and xCelligence (Fig. 3), strongly implying that the pharmacophore resides within the disulfide. This agrees with reports on the two garlic sulfides diallyl sulfide (DAS) and S-allyl cysteine, which are also both inactive at inhibiting the growth of colon, lung and skin cancer cells [42–44], although DAS is reported to be active at inducing apoptosis in human colon 320 DM cancer cells [45]. Removal of only the vinyl group (analogue **8**) still rendered the analogue active but caused an eightfold reduction in activity as measured by the MTT assay, which was confirmed by xCelligence, supporting the hypothesis that the vinyl group serves to activate the disulfide pharmacophore. Analogue *E*/*Z*-**3l** containing an intact vinyl disulfide pharmacophore but lacking the sulfoxide was in fact found to be threefold more active than *E*/*Z*-**4l**, returning a nanomolar IC_{50} (700 nM) by the MTT assay but with a reduced activity when measured by xCelligence. Interestingly, analogue **7**, which contains the disulfide pharmacophore but lacks both the vinyl and sulfoxide groups, was found to be inactive by both methods implying some synergy between these two groups.

2.3.2. The importance of the disulfide in E/Z-4l cell-cycle arrest and apoptosis

Ajoene and the garlic related organosulfides are reported to inhibit cancer cell growth by inducing G_2/M cell cycle arrest and apoptosis through caspase-3 activation [19,24,46]. We were therefore interested in assessing whether *E*/*Z*-**4l** and analogues of

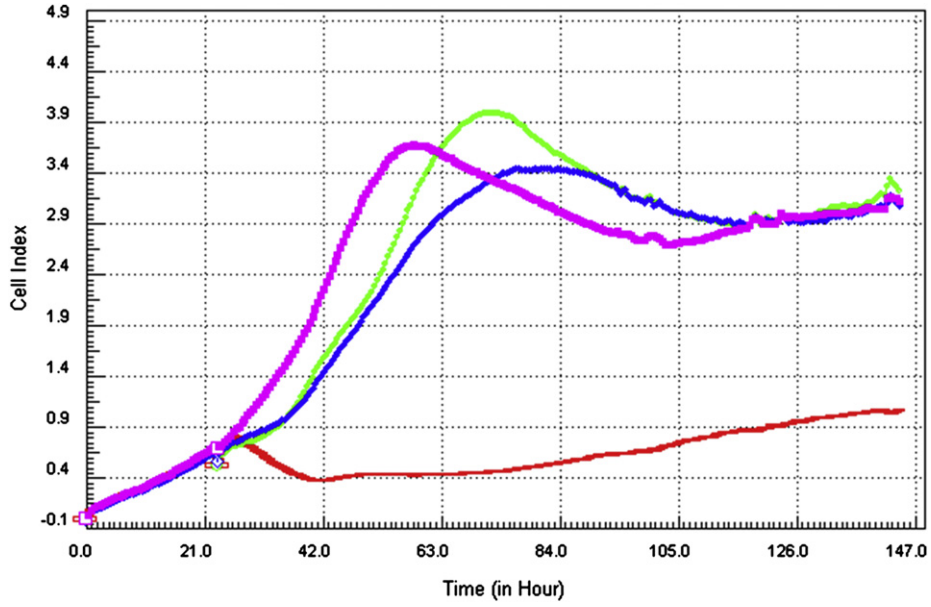
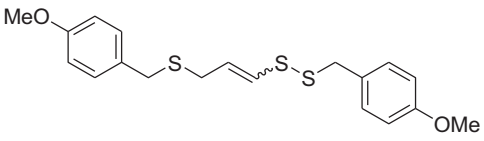
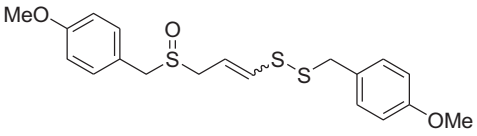
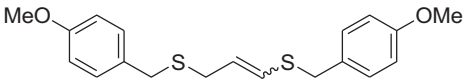
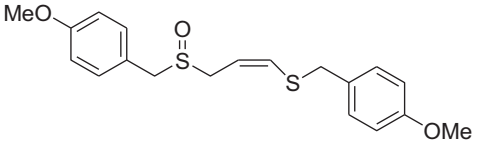
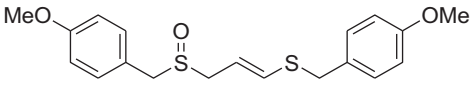
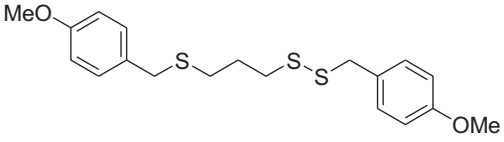
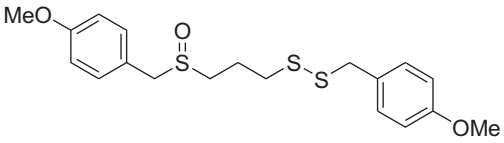


Fig. 2. WHCO1 cell growth monitored by Roche xCelligence as a function of time. 24 h after plating, WHCO1 cells (2500 cells per well) were treated with *Z*-ajoene (10 μM, green), *E*-ajoene (10 μM, blue) or *E*/*Z*-**4l** (10 μM, red) in 0.1% DMSO and control cells received 0.1% DMSO alone (pink). Cell growth was then monitored continuously for a further 5 days. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Structure–activity pharmacophore analysis of *E*/*Z*-**4l** regarding inhibition of WHCO1 cell proliferation.

Cmpd no.	Chemical structure	WHCO1 $IC_{50} \pm SD$ (μM)
E/*Z*-**3l**		0.7 ± 0.3
E/*Z*-**4l**		2.1 ± 0.4 [2]
E/*Z*-**5**		>200.0
Z-**6**		>200.0
E-**6**		>200.0
7		>200.0
8		16 ± 3.7

E/*Z*-**4l** that lack the disulfide are also active at inducing cell-cycle arrest or apoptosis. Thus WHCO1 cells were treated either with 10 μM of *E*/*Z*-**4l**, *E*/*Z*-**3l** or the sulfides *E*-**6** and *Z*-**6** for 16 h and analysed by flow cytometry (Fig. 4). The compounds were also assayed for their ability to induce apoptosis in WHCO1 cells, which was quantified using the Roche Cell Death Elisa Assay Kit (Fig. 5). This assay is a photometric enzyme-immunoassay which quantitates histone-associated DNA fragments (mono- and oligonucleosomes) in the cytoplasm after treatment.

In agreement with the MTT and xCelligence cell proliferation data, the two active compounds that both contain a disulfide (*E*/*Z*-**4l** and *E*/*Z*-**3l**) were found to induce apoptosis and G_2/M cell cycle arrest in WHCO1 cells after 16 h treatment. This data supports the hypothesis that the disulfide but not the sulfoxide is critical for activity. As a representative disulfide for further analysis, active analogue *E*/*Z*-**4l** was assayed for its ability to activate caspase 3 enzyme activity in treated WHCO1 cells using the Ac-DEVD-AFC fluorogenic substrate for caspase 3. Treatment with *E*/*Z*-**4l** (25 μM) for 24 h was found to induce a threefold increase in caspase 3 enzyme activity relative to untreated or DMSO treated cells suggesting, similar to ajoene, that *E*/*Z*-**4l** inhibits WHCO1 cell proliferation by inducing apoptosis through the activation of caspase-3 (Fig. 6). Both sulfide isomers (*E*-**6** and *Z*-**6**) lacking the disulfide functionality were each found to be inactive at inducing cell-cycle arrest and apoptosis in WHCO1 cells at the same molar concentration (Figs. 4 and 5 respectively), although significantly more cells were found in the S-phase relative to the untreated DMSO control. Taken together, these findings support the hypothesis that the ajoene pharmacophore responsible for anti-proliferation activity, G_2/M cell cycle arrest and induction of apoptosis in WHCO1 oesophageal cancer cells is the disulfide functional group.

2.3.3. Reaction of E/Z-4l with N-Boc-L-cysteine ethyl ester

The vinyl disulfide's enhanced activity compared to that of the saturated disulfide suggests some type of chemical activation of this grouping, in which reaction with a thiol group of a host protein residue seems to be a likely possibility. Indeed, the study by Krauth-Siegel [34] and co-workers dating back just over ten years revealed via an X-ray structure that ajoene reacts with glutathione reductase (GR) to afford allylSOCH$_2$CH]CHSS(Cys$_{58}$)-GR formed between the

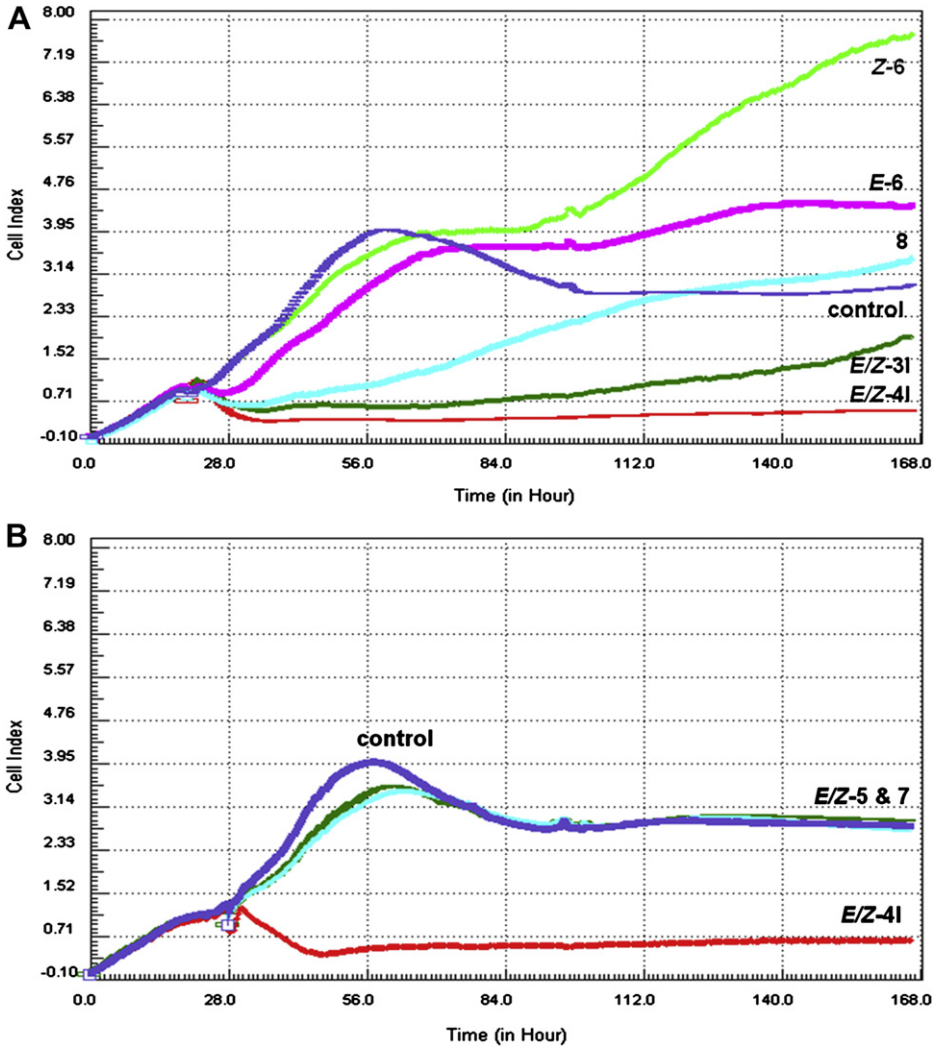
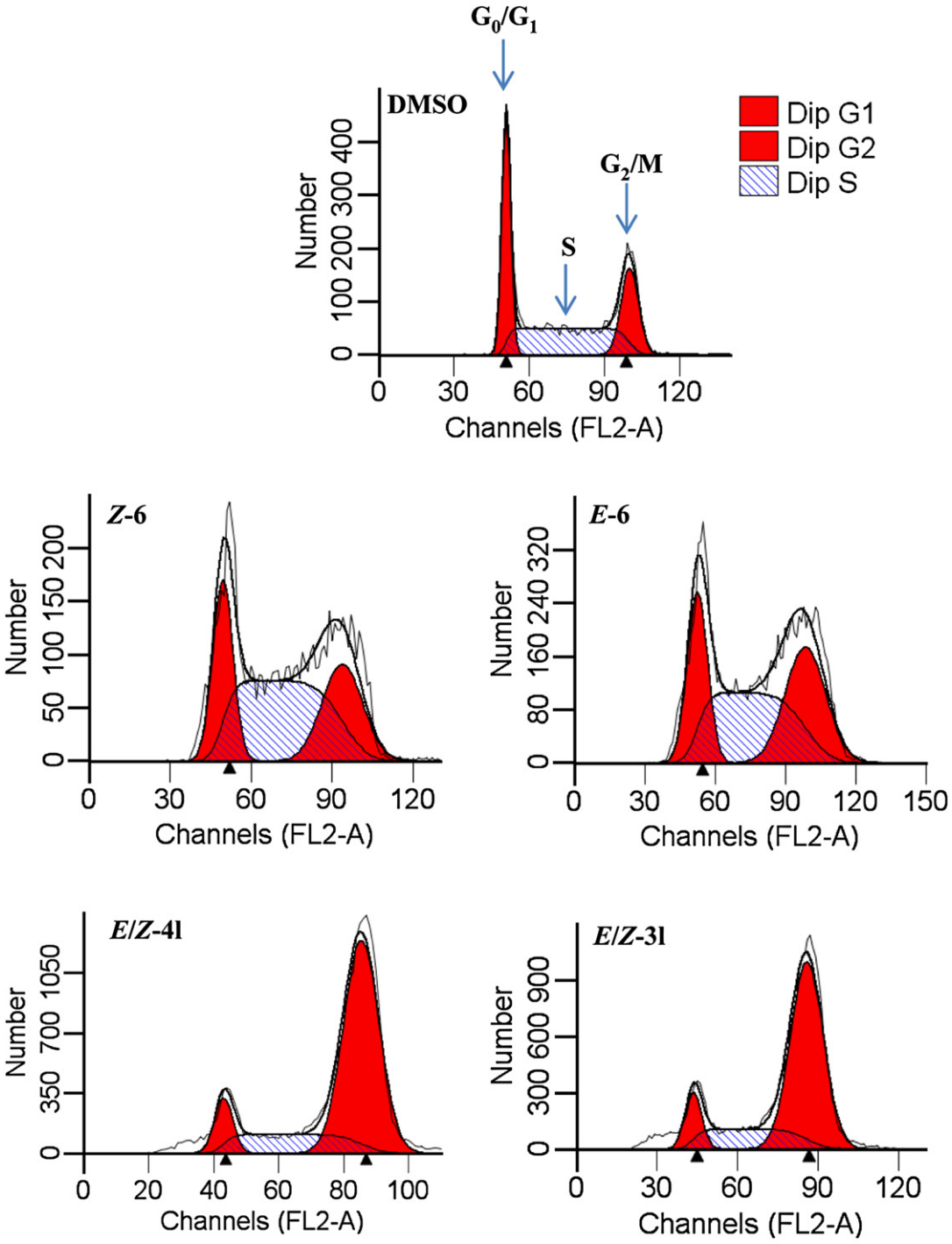


Fig. 3. WHCO1 cell growth following treatment with analogues of *E*/*Z*-**4l**, monitored by xCelligence. A: 24 h after plating, WHCO1 cells (5000 cells per well) were treated with 0.1% DMSO (control, dark-blue), *Z*-**6** (34 μM, light-green), *E*-**6** (34 μM, pink), **8** (34 μM, light-blue), *E*/*Z*-**3l** (34 μM, dark-green), and *E*/*Z*-**4l** (34 μM, red). Cell growth was then measured continuously for a further six days; B: WHCO1 cells (5000 cells per well) treated with 0.1% DMSO (control, dark-blue), **7** (113 μM, dark-green), **5** (113 μM light-blue), and *E*/*Z*-**4l** (100 μM, red). Each curve represents an average of three replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cys58 of GR and the vinyl sulfur of ajoene with the expulsion of the S-allyl group. No rationale of this unexpected regioselectivity was presented in the paper. We thus decided to investigate this reaction in solution, as it was considered more likely that the allyl sulfur of ajoene would be the more electrophilic sulfur of the two in view of the greater leaving ability of the vinylthio moiety in the disulfide exchange. To this end, *N*-Boc-L-cysteine ethyl ester as a limiting reagent was refluxed with the ajoene derivative *E*/*Z*-**4l** (2 equiv) for 24 h in THF. Only a single product spot was observed on TLC, and this was taken to be the primary product of the reaction. Column chromatographic purification gave a 52% yield (based on the cysteine derivative as limiting reagent and at 78% conversion) of *para*-methoxybenzyl *N*-Boc cysteine ethyl ester disulfide (**9**), indicating that the preferred site of attack in solution is indeed at the non-vinyl sulfur (Scheme 5). This finding is in agreement with those of Krauth-Siegel's laboratory results [34] on the same process, although Lawson [47] has reported that in addition to forming *S*-thioallylcysteine, reaction of excess cysteine (2 equiv) with ajoene also forms ajocysteine containing a disulfide bond with the vinyl sulfur of ajoene, possibly as a secondary product. Overall, such results lead to the conclusion that the more electrophilic sulfur of the vinyl disulfide is the allyl sulfur, and that the better leaving group in the thiol/disulfide exchange is the vinylthio moiety. This would imply that the product observed by Krauth-Siegel *in vitro* with the enzyme GR [34] with reverse regioselectivity was not the primary product of a direct thiolysis. Krauth-Siegel also demonstrated that GR reduces ajoene with the formation of single-electron reduced products and superoxide anion radical. Together with the thiolysis pathway, such single-electron processes of the disulfide grouping with production of ROS leading to apoptosis via the mitochondrial signalling cascade provide the likely mode of action of ajoene *in vitro* in which reactivity is enhanced by the presence of the vinyl disulfide double bond.

2.3.4. Structure–activity hypothesis for ajoene

Based on the findings in this paper, a structure–activity hypothesis for ajoene is proposed (see Fig. 7) in which the disulfide is the pharmacophore responsible for anti-proliferation activity, G_2/M cell-cycle arrest and induction of apoptosis, with the vinyl group playing a further activation role. The allyl sulfur is hypothesized to be the more electrophilic sulfur capable of reacting with a free cysteine residue in a protein to form a mixed disulfide. A *Z*-configuration about the vinyl disulfide, electron rich side groups and a lipophilic



Compound	Average population of cells (%) ± SD		
	G_0/G_1	S	G_2/M
DMSO	29.2 ± 5.2	45.3 ± 1.9	25.5 ± 3.7
***Z*-6**	19.5 ± 1.7	63.1 ± 6.8	17.4 ± 5.8
***E*-6**	21.5 ± 0.8	67.0 ± 1.1	11.4 ± 1.9
***E*/*Z*-4l**	8.03 ± 1.2	13.5 ± 2.7	78.6 ± 3.8
***E*/*Z*-3l**	9.73 ± 1.6	15.9 ± 2.4	74.4 ± 2.6

Fig. 4. Flow cytometry of WHCO1 cells treated with analogues of *E*/*Z*-**4l**. WHCO1 cells were treated either with 10 μM *E*/*Z*-**4l**, *E*/*Z*-**3l**, *E*-**6** or *Z*-**6** for 16 h. Cell cycle analysis was then performed using propidium iodide staining as described in the Experimental Section. Each sample was done in triplicate.

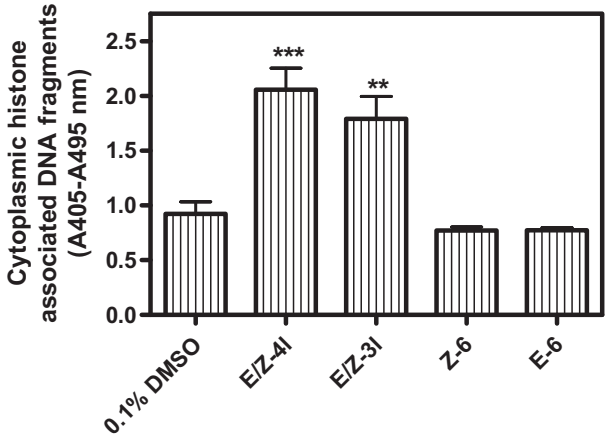


Fig. 5. Quantification of apoptosis in WHCO1 cells treated with analogues of *E*/*Z*-**4l**. WHCO1 cells were treated either with 10 μM *E*/*Z*-**4l**, *E*/*Z*-**3l**, *E*-**6** or *Z*-**6** for 16 h. Cell lysate was then collected and apoptosis was quantified by assaying for cytoplasmic histone-associated DNA fragments using the Roche Cell Death Elisa assay as described in Materials and Methods. Bars, SD for each sample analyzed in quadruplicate; $^{**}P < 0.01$, $^{***}P < 0.005$ relative to DMSO control.

surface is generally favourable for increased activity implying a level of stereospecificity between the target protein and ajoene.

3. Conclusions

A concise, non-stereoselective four-step synthesis has been developed for accessing derivatives of the natural product ajoene in which the terminal end-groups (R^1 and R^2) can be varied whilst retaining the vinyl disulfide and sulfoxide functional groups. A structure–activity study involving a small library of such modified derivatives against WHCO1 cancer-cell proliferation has revealed that end-group substitution retains and may enhance bioactivity. In general, *Z*-ajoene and the *Z*-analogues were found to be slightly more active than *E*-ajoene and the corresponding *E*-analogues. Benzylic end-groups increased activity in which an electron-rich PMB group at each end (derivative *E*/*Z*-**4l**) returned the highest activity of twelve-fold compared to the parent ajoene. Varying the vinyl disulfide and sulfoxide groupings indicates the disulfide to be the dominant pharmacophore at inhibiting WHCO1 cell proliferation, inducing G_2/M cell cycle arrest and apoptosis by caspase 3 activation, with the vinyl group appearing to play an activation role; by comparison, the sulfoxide group appears to play a minor role in the bioactivity in the ajoene series. Reaction of *E*/*Z*-**4l** with

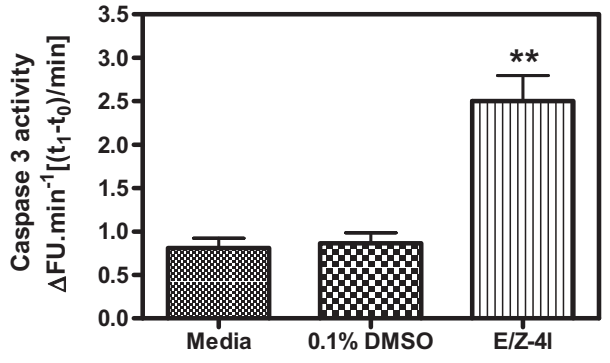
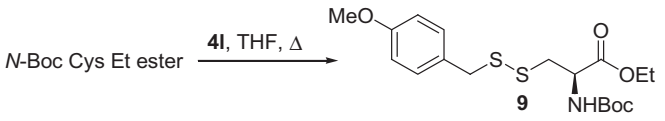


Fig. 6. Quantification of caspase 3 enzyme activity in WHCO1 cells treated with *E*/*Z*-**4l**. WHCO1 cells were treated with 25 μM *E*/*Z*-**4l** for 24 h. Cell lysate was then collected and assayed for caspase 3 enzyme activity using the Roche caspase 3 enzyme assay kit as described in Materials and Methods. Bars, SD for each sample analyzed in triplicate; $^{**}P < 0.01$, relative to DMSO control.



Scheme 5. Reaction of *N*-Boc cysteine Et ester with *E*/*Z*-**4l**.

cysteine in solution reveals a regioselectivity in the thiol/disulfide exchange favouring nucleophilic (thiol) attack on the allylic rather than the vinylic sulfur. However, the extent to which this might be relevant to events *in vitro* still remains to be elucidated as does the reason for the *in vitro* bioactivity dependency on the ajoene *E*/*Z*-geometry. Future studies will attempt to separate the *E*/*Z*-isomeric mixture of **4l** and evaluate the activity of individual isomers. The use of fluorescently labelled ajoene derivatives to identify molecular targets within the cancer cell will also be explored, as well as the activity of *E*/*Z*-**4l** in a mouse model.

4. Experimental section

4.1. Cell lines and cell proliferation assays

The oesophageal cancer cell-line WHCO1 was derived from a biopsy of primary oesophageal squamous cell carcinoma of South African origin [48]. Cells were all incubated at 37 °C under 5% CO_2 and cultured without antibiotics in DMEM (Dulbecco's Modified Eagle Medium, Sigma) containing 10% FBS (Fetal Bovine Serum, Gibco). IC_{50} determinations were carried out using the Roche MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, WHCO1 cells were seeded (2×10^3 cells/well) in 96-well plates in a total volume of 90 μL. The following day, DMSO solutions of each compound in media were added to the cells to give a final concentration of 0.5 200 μM compound and 0.1% DMSO. Control cells received 0.1% DMSO alone. After 48 h incubation, 10 μL of 5 mg/mL MTT was added to each well. After a further 4 h incubation, 100 μL of 10% SLS (sodium lauryl sulfate) in 0.01 M HCl was added to each well, and plates were incubated overnight. Plates were read at 595 nm on a Thermo Scientific Multiscan FC plate-reader and data was analysed by Graphpad prism 4 software using sigmoidal dose–response (variable slope) curve fitting. For the Roche xCelligence system, WHCO1 cells (2- or 5×10^3 cells per well) were plated in 96 well E-plates and growth was monitored as an impedance value continuously for seven days on the RTCA SP xCelligence system. 24 h after seeding, the compounds were added according to the method described for the MTT assay.

4.2. Flow cytometry

WHCO1 cells were seeded for triplicate experiments (1×10^6 cells, 8 mL) in 100 mm cell culture dishes. 6 h after plating, DMSO solutions of each compound in media were added to the cells to give a final concentration of 10 μM compound and 0.1% DMSO. Control cells received 0.1% DMSO alone. After 16 h treatment, the cells were lifted by trypsinisation and resuspended in 2 mL cold PBS, and fixed by adding 8 mL cold 70% ethanol. Cells were pelleted at 4000 rpm for 5 min, washed twice with 1 mL cold PBS, resuspended in 100 μL PBS containing 50 μg/mL RNase A (Roche), incubated for 15 min at 37 °C. 20 min prior to analysis, propidium iodide (10 μg/mL) was added and incubated with the sample at 4 °C. Individual samples were run on a Becton Dickinson FACSCalibur flowcytometer using a 488 nm coherent laser and the acquisition software Cellquest Pro version 5.2.1. The cell population was identified and gated (R1) on a forward scatter (FSC) vs. side scatter (SSC) dot plot in acquisition mode. Fluorescent Channel 2 (FL2) at 575 nm was used for Propidium Iodide detection. A dot plot of FL2A

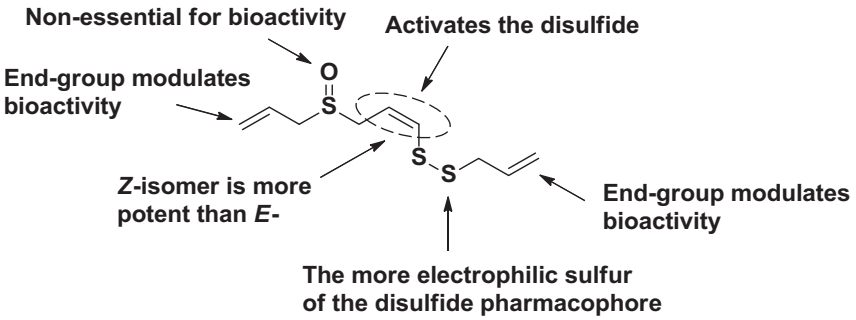


Fig. 7. Proposed structure–activity hypothesis for *Z*-ajoene in its inhibition of WHCO1 cancer-cell proliferation.

(area) vs FL2W (width) was used to identify single cells (R2) and to thus eliminate doublets. A histogram plot of FL2A was used to enumerate G_1/G_0, S-phase and G_2 populations and a threshold of 52 on the FSC channel was set to remove sample debris. Nile Red Fluorescent particles were used for instrument standardization, stability and reproducibility. Data was analysed using MODFIT LT version 2.0, software.

4.3. Quantification of apoptosis

WHCO1 cells were seeded for quadruplicate experiments (5×10^3 cells, 90 μL) in a 96-well cell culture plate. The following day, DMSO solutions of each compound in media (10 μL) were added to the cells to give a final concentration of 10 μM compound and 0.1% DMSO. Control cells received 0.1% DMSO alone. After 16 h treatment, the cell lysate was collected and pelleted. The cytoplasmic fraction (supernatant, 20 μL) was transferred to a strepavadin coated plate for analysis by the Roche Cell Death Elisa Kit which was performed according to the manufacturer's instruction. Briefly, the freshly prepared immunoreagent (80 μL) containing anti-histone biotin and anti-DNA-POD was added to each well and the plate was incubated on a shaker at room temperature for 2 h. The solution was then removed by gentle tapping, washed (three times) and then incubated with ABTS (100 μL) for 15 min. The reaction was quenched by adding the ABTS stop solution (100 μL) after which the absorbance at 405 nm and 495 nm was read using a Thermoscientific multiskan FC plate reader.

4.4. Quantification of caspase-3 enzyme activity

WHCO1 cells were seeded (1.8×10^6 cells) for triplicate experiments in 145 mm cell culture dishes. The following day *E*/*Z*-**4l** in DMSO was added to give a final concentration of 25 μM and 0.1% DMSO. Control cells received media alone or 0.1% DMSO in media alone. Cells were incubated for 24 h and then lifted with trypsin, pelleted and washed with cold PBS. The cells were then resuspended in lysis buffer (300 μL) and stored at −20 °C ready for the Roche Caspase-3 Activity Assay which was followed according to the manufacturer's instruction. Briefly the provided 96 well plates were coated with the anti-caspase-3 coating solution (100 μL per well) at 4 °C overnight. The coating solution was removed by tapping and non-specific binding was blocked using the provided blocking buffer (200 μL, 30 min). The blocking buffer was removed by gentle tapping followed by washing (three times × 200 μL). The cell lysate was then added to the wells (200 μL) and incubated for 1 h at 37 °C. The lysate was then removed by tapping followed by washing (three times, 200 μL). The substrate solution was then added (freshly prepared, 100 μL) and the fluorescence reading for time zero immediately determined ($t_0 = 0$) on a Varian Cary Eclipse Fluorescence Spectrophotometer [$\lambda_{ex} = 390$ nm, $\lambda_{em} = 505$ nm]. The plate was then covered with an adhesive cover foil and incubated at 37 °C for 2 h after which the fluorescence was measured again ($t_1 = 120$ min). The caspase 3 enzyme activity is calculated as the rate of change in fluorescence (ΔFU) between time intervals t_0 and t_1.

4.5. Statistical analysis

Student's *t* test was used for statistical significance of differences in treated vs untreated samples. $P < 0.05$ was considered to be significant.

4.6. General synthesis methods

Thin layer chromatography (TLC) was used to monitor reactions using aluminium-backed plates coated with silica-gel F_{254}. Compounds on TLC plates were observed by a combination of ultraviolet light, iodine vapour, or by spraying with a 2.5% solution of anisaldehyde in a mixture of sulfuric acid and ethanol (1:10 v/v) and then heating at 150 °C. Column chromatography was performed using silica-gel 60 mesh. Infrared spectra were recorded in chloroform, dichloromethane or neat on caesium chloride or sodium chloride plates on an FT-IR spectrophotometer. Elemental analyses were performed using a CHN elemental analyser. High resolution mass spectrometry data was obtained using a micromass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C or at 400 MHz for ^{1}H and 100.6 MHz for ^{13}C in deuteriochloroform ($CDCl_3$) unless otherwise stated. Chemical shifts are quoted using residual chloroform (δ 7.26 in ^{1}H NMR and δ 77.00 in ^{13}C NMR) as an internal standard. All chemical shifts are reported in ppm and all coupling constants are quoted in Hz. All chemicals and reagents were commercially available. Dichloromethane (DCM) and THF were purified using standard methods and freshly distilled before use. Degassed methanol or toluene as stated in the text refers to purging the solvent of oxygen with nitrogen gas.

4.7. Experimental procedures for synthesis of the ajoene derivatives(i) Addition of the propargyl group to (**1**)

Thiol R^1SH or its corresponding isothiouronium salt ($R^1SC(=NH_2)NH_2^+\ Br^-$) was dissolved in degassed methanol (0.5 M) at 0 °C and solid KOH added (1.2 equiv for R^1SH or 2.5 equiv for the salt). After 5 min, propargyl bromide (1.5 equiv, 80% in toluene) was added and the mixture left to warm to room temperature. After a few hours, TLC had indicated complete propargylation to a less polar product, whereupon the methanol was removed under vacuum and the residue extracted from water with ethyl acetate or dichloromethane (3 times). Following drying and removal of solvent, the residue was chromatographed with toluene/hexane mixtures on silica-gel to afford the propargylic sulfide **1** product discernible by NMR and IR spectroscopy. In some cases the sulfide could be readily distilled *in vacuo*.

*4.7.1. 3-(Propylthio)-1-propyne (**1a**) [49]*

Obtained as an orange oil (80%): IR ν_{max} (neat)/cm^{-1} 3304 (C≡CH), 2967 (C≡C), 746 (C—S); δ_H (300 MHz, $CDCl_3$): 0.97 (3H, t, $J = 7.3$ Hz, H-3′), 1.61 (2H, sext, $J = 7.3$ Hz, H-2′), 2.20 (1H, t, $J = 2.7$ Hz, H-1), 2.63 (2H, t, $J = 7.3$ Hz, H-1′), 3.20 (2H, d, $J = 2.7$ Hz, H-3); δ_C (75 MHz, $CDCl_3$): 13.3 (C-3′), 19.0 (C-3), 22.2 (C-2′), 33.6 (C-1′), 70.7 (C-1), 80.1 (C-2).

*4.7.2. 3-[(1′,1′-Dimethylethyl)thio]-1-propyne (**1b**) [50]*

Obtained as a pale-yellow oil (57%): δ_H (400 MHz, $CDCl_3$): 1.36 (9H, s, H-2′), 2.16 (1H, t, $J = 3.0$ Hz, H-1), 3.24 (2H, d, $J = 3.0$ Hz, H-3); δ_C (100 MHz, $CDCl_3$): 16.4 (C-3), 30.7 (C-2′), 43.2 (C-1′), 70.4 (C-1), 81.5 (C-2).

*4.7.3. 3-[3′-((tert-Butyl)dimethylsilyloxy)-prop-1-ylthio]-1-propyne (**1c**)*

Obtained as a colourless oil (70%): IR ν_{max} (neat)/cm^{-1} 3314 (C≡CH), 2931 (C^C), 1256 (Si(CH_3)$_2$), 776 (C—S); δ_H (400 MHz, $CDCl_3$): 0.06 (6H, s, CH_3), 0.90 (9H, s, *t*-butyl), 1.82 (2H, m, H-2′), 2.20 (1H, t, $J = 2.6$ Hz, H-1), 2.76 (2H, t, $J = 7.3$ Hz, H-1′), 3.24 (2H, d, $J = 2.6$ Hz, H-3), 3.70 (2H, t, $J = 6.1$ Hz, H-3′); δ_C (100 MHz, $CDCl_3$): −5.3 (Si-($\underline{C}H_3$)$_2$), 18.3 ($\underline{C}$-(CH_3)$_3$), 19.2 (C-3), 25.9 (C-($\underline{C}H_3$)$_3$), 28.2 (C-2′), 32.1 (C-1′), 61.5 (C-3′), 70.8 (C-1), 80.1 (C-2).

*4.7.4. 3-((3′-p-Methoxybenzyloxy)prop-1-ylthio)-1-propyne (**1d**)*

Obtained as a colourless oil (57%): IR ν_{max} (neat)/cm^{-1} 3290 (C≡CH), 2858 (C^C), 1098 (C—O), 710 (C—S); δ_H (400 MHz, $CDCl_3$): 1.92 (2H, quint, $J = 6.6$ Hz, H-2′), 2.22 (1H, d, $J = 2.4$ Hz, H-1), 2.79 (2H, t, $J = 6.6$ Hz, H-1′), 3.24 (2H, d, $J = 2.4$ Hz, H-3), 3.55 (2H, t, $J = 6.6$ Hz, H-3′), 3.80 (3H, s, OCH_3), 4.44 (2H, s, Bn), 6.88 (2H, d, $J = 8.6$ Hz, H_m), 7.26 (2H, d, $J = 8.6$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 19.2 (C-3), 28.5 (C-2′), 29.2 (C-1′), 55.2 (OCH_3), 68.3 (C-3′), 70.9 (C-1), 72.6 (Bn), 80.0 (C-2), 113.8 (C_m), 129.2 (C_o), 130.5 (C_i), 159.2 (C_p); HRMS (EI): *m/z* 250.1010 [M]$^+$, $C_{14}H_{18}O_2S$ requires 250.1028.

4.7.5. N-(3-Bromopropyl)-phthalimide [51]

Obtained as a flakey-white solid (71%): δ_H (400 MHz, $CDCl_3$): 2.25 (2H, quint, $J = 6.8$ Hz, H-2), 3.41 (2H, t, $J = 6.8$ Hz, H-3), 3.83 (2H, t, $J = 6.8$ Hz, H-1), 7.71 (2H, m, Ar-H), 7.83 (2H, m, Ar-H).

*4.7.6. 3-(3′-Phthalimidoprop-1-ylthio)-1-propyne (**1e**)*

Obtained as a white solid (69%) which was recrystallized from petroleum ether/toluene, mp 61–62 °C; IR ν_{max} (neat)/cm^{-1} 3308 (C≡CH), 2944 (C≡C), 1709 (C]O), 665 (C—S); δ_H (400 MHz, $CDCl_3$): 2.01 (2H, quint, $J = 7.3$ Hz, H-2′), 2.17 (1H, t, $J = 2.6$ Hz, H-1), 2.73 (2H, t, $J = 7.4$ Hz, H-1′), 3.25 (2H, d, $J = 2.6$ Hz, H-3), 3.81 (2H, t, $J = 7.1$ Hz, H-3′), 7.69 (2H, m, Ar-H), 7.80 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 19.0 (C-3), 27.7 (C-2′), 28.7 (C-1′), 37.0 (C-3′), 71.1 (C-1), 79.6 (C-2), 123.2 (Ar-C), 132.1 (C_{qu}), 133.9 (Ar-C), 168.3 (C]O); HRMS (EI): *m/z* 260.0744 [M + H]$^+$, $C_{14}H_{14}NO_2S$ requires 260.0745; found: C, 64.57%; H, 4.99%; N, 5.17%; S, 12.12%; $C_{14}H_{13}NO_2S$ requires C, 64.84%; H, 5.05%; N, 5.40%; S, 12.37%.

*4.7.7. 3-Benzylthio-1-propyne (**1f**) [52]*

Obtained as a yellow solid (98%): δ_H (400 MHz, $CDCl_3$): 2.29 (1H, t, $J = 2.6$ Hz, H-1), 3.09 (2H, d, $J = 2.6$ Hz, H-3), 3.88 (2H, s, Bn), 7.25 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 18.4 (C-3), 35.3 (Bn), 71.2 (C-1), 79.8 (C-2), 127.2 (Ar-C), 128.5 (Ar-C), 129.0 (Ar-C),137.4 (Ar-C); HRMS (EI): *m/z* 163.0611 (M + H)$^+$, $C_{10}H_{11}S$ requires 163.0581.

*4.7.8. 1-Methoxy-4-[(2-propyn-1-ylthio)methyl]-benzene (**1g**) [53]*

Obtained as a light-yellow oil (66%): IR ν_{max} (neat)/cm^{-1} 3282 (C≡CH), 2860 (C≡C), 663 (C—S); δ_H (400 MHz, $CDCl_3$): 2.25 (1H, t, $J = 2.6$ Hz, H-1), 3.04 (2H, d, $J = 2.6$ Hz, H-3), 3.77 (2H, s, Bn), 3.80 (3H, s, OCH_3), 6.84 (2H, d, $J = 8.8$ Hz, H_m), 7.24 (2H, d, $J = 8.8$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 18.3 (C-3), 34.7 (Bn), 55.3 (OCH_3), 71.1 (C-1), 79.9 (C-2), 114.0 (C_m), 129.3 (C_i), 130.1 (C_o), 158.8 (C_p); HRMS (ES): *m/z* 193.0701 [M + H]$^+$, $C_{11}H_{13}OS$ requires 193.0687.

*4.8. (ii) Radical addition step to (**2**)*

The propargylic sulfide was dissolved in degassed toluene (0.5 M) and AIBN or an equivalent radical initiator (5 mol %) added. The mixture was heated to 85 °C whereupon thiolacetic acid (1.1 equiv) in toluene (1 M) was added slowly dropwise over 1 h. Thereafter, the mixture was left stirring until TLC indicated maximum conversion of starting material. In some cases, extra thiolacetic acid was added in order to maximize conversion but care had to be taken not to significantly promote secondary addition to afford the bis-addition product. On completion of reaction, the solvent was evaporated and the residue chromatographed directly on silica-gel using toluene or ethyl acetate/petroleum ether mixtures to afford vinyl thioacetate **2** as a ~2:1 mixture of *Z*:*E* stereoisomers by ^{1}H NMR spectroscopy.

*4.8.1. (E/Z)-S-[3-(Prop-1-ylthio)propen-1-yl] ethanethioate (**2a**)*

Obtained as a 2:1 *Z*:*E* mixture (73%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 1705 (C]O); (*Z*)-**2**$^{\mathbf{a}}$ δ_H (400 MHz, $CDCl_3$): 0.97 (3H, t, $J = 7.3$ Hz, H-6′), 1.60 (2H, sext, $J = 7.3$ Hz, H-5′), 2.38 (3H, s, H-1), 2.43 (2H, t, $J = 7.3$ Hz, H-4′), 3.17 (2H, dd, $J = 1.1$, 7.7 Hz, H-3′), 5.86 (1H, dt, $J = 7.7$, 9.6 Hz, H-2′), 6.65 (1H, dt, $J = 1.1$, 9.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): 13.4 (C-6′), 22.8 (C-5′), 30.8 (C-1), 30.9 (C-3′), 33.2 (C-4′), 119.1 (C-1′), 128.9 (C-2′), 191.3 (C-2); (*E*)-**2**$^{\mathbf{a}}$ δ_H (400 MHz, $CDCl_3$): 0.97 (3H, t, $J = 7.3$ Hz, H-6′), 1.58 (2H, sext, $J = 7.3$ Hz, H-5′), 2.34 (3H, s, H-1), 2.43 (2H, t, $J = 7.3$ Hz, H-4′), 3.20 (2H, dd, $J = 1.2$, 7.4 Hz, H-3′), 5.82 (1H, dt, $J = 7.4$, 15.6 Hz, H-2′), 6.53 (1H, dt, $J = 1.2$, 15.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): 13.4 (C-6′), 22.6 (C-5′), 30.3 (C-1), 33.0 (C-4′), 34.0 (C-3′), 118.8 (C-1′), 130.5 (C-2′), 192.8 (C-2); HRMS (EI): *m/z* 190.0485 [M]$^+$, $C_8H_{14}OS_2$ requires 190.0486.

*4.8.2. (E/Z)-S-[3-[(1,1-Dimethylethyl)thio)propen-1-yl] ethanethioate (**2b**)*

Obtained as a 2:1 *Z*:*E* mixture (73%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 1705 (C]O); (*Z*)-**2b** δ_H (400 MHz, $CDCl_3$): 1.33 (9H, s, *t*-butyl), 2.39 (3H, s, H-1), 3.22 (2H, dd, $J = 1.2$, 7.5 Hz, H-3′), 5.90 (1H, dt, $J = 7.5$, 9.6 Hz, H-2′), 6.61 (1H, dt, $J = 1.2$, 9.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): 28.5 (C-3′), 30.8 (C-1), 30.9 (*t*-butyl), 42.8 ($\underline{C}$(CH_3)$_3$), 118.8 (C-1′), 129.3 (C-2′), 191.2 (C-2); (*E*)-**2b** δ_H (400 MHz, $CDCl_3$): 1.32 (9H, s, *t*-butyl), 2.33 (3H, s, H-1), 3.30 (2H, dd, $J = 1.2$, 7.5 Hz, H-3′), 5.88 (1H, dt, $J = 7.5$, 15.6 Hz, H-2′), 6.62 (1H, dt, $J = 1.2$, 15.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): 30.3 (C-1), 31.0 (C($\underline{C}H_3$)$_3$), 31.4 (C-3′), 42.9 ($\underline{C}$(CH_3)$_3$), 118.8 (C-1′), 131.2 (C-2′), 192.8 (C-2); HRMS (ES): *m/z* 221.0696 [M + O + H]$^+$, $C_9H_{17}O_2S_2$ requires 221.0670.

*4.8.3. (E/Z)-S-[3-(3-tert-Butyldimethylsilyloxy-prop-1-ylthio) propen-1-yl] ethanethioate (**2c**)*

Obtained as a 1:1 *Z*:*E* mixture (31%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1704 (C]O); (*Z*)-**2c** δ_H (400 MHz, $CDCl_3$): 0.05 (6H, s, CH_3), 0.89 (9H, s, *t*-butyl), 1.77 (2H, m, H-5′), 2.39 (3H, s, H-1), 2.54 (2H, t, $J = 7.3$ Hz, H-4′), 3.18 (2H, dd, $J = 1.0$, 7.7 Hz, H-3′), 3.67 (2H, t, $J = 6.1$ Hz, H-6′), 5.87 (1H, dt, $J = 7.7$, 9.6 Hz, H-2′), 6.66 (1H, dt, $J = 1.0$, 9.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): −5.3 (Si(CH_3)$_2$), 18.3 ($\underline{C}$(CH_3)$_3$), 25.9 (C($\underline{C}H_3$)$_3$), 27.8 (C-5′), 30.8 (C-1), 31.1 (C-3′), 32.5 (C-4′), 61.6 (C-6′), 119.3 (C-1′), 128.7 (C-2′), 191.2 (C-2); (*E*)-**2c** δ_H (400 MHz, $CDCl_3$): 0.05 (6H, s, CH_3), 0.89 (9H, s, *t*-butyl), 1.77 (2H, m, H-5′), 2.34 (3H, s, H-1), 2.54 (2H, t, $J = 7.3$ Hz, H-4′), 3.22 (2H, dd, $J = 1.2$, 7.4 Hz, H-3′), 3.68 (2H, t, $J = 6.1$ Hz, H-6′), 5.83 (1H, dt, $J = 7.4$, 15.6 Hz, H-2′), 6.54 (1H, dt, $J = 1.2$, 15.6 Hz, H-1′); δ_C (100 MHz, $CDCl_3$): −5.3 (Si(CH_3)$_2$), 18.3 ($\underline{C}$(CH_3)$_3$), 25.9 (C($\underline{C}H_3$)$_3$), 27.5 (C-5′), 30.4 (C-1), 32.4 (C-4′), 34.2 (C-3′), 61.5 (C-6′), 118.9 (C-1′), 130.4 (C-

2′), 193.9 (C-2); HRMS (EI): *m/z* 320.1297 $[M]^+$, $C_{14}H_{28}O_2S_2Si$ requires 320.1300.

4.8.4. (E/Z)-S-[3-(3-p-Methoxybenzyl prop-1-ylthio)-propen-1-yl] ethanethioate (**2d**)

Compound **2e** was obtained as a 3:2 *Z*:*E* mixture (64%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1702 (C]O); (*Z*)-**2d** δ_H (300 MHz, $CDCl_3$): 1.86 (2H, m, H-5′), 2.34 (3H, s, H-1), 2.56 (2H, t, $J = 7.2$ Hz, H-4′), 3.18 (2H, dd, $J = 1.1$, 7.7 Hz, H-3′), 3.52 (2H, t, $J = 6.2$ Hz, H-6′), 3.80 (3H, s, OCH_3), 4.43 (2H, s, Bn), 5.86 (1H, dt, $J = 7.7$, 9.6 Hz, H-2′), 6.66 (1H, dt, $J = 1.1$, 9.6 Hz, H-1′), 6.87 (2H, d, $J = 8.6$ Hz, H_m), 7.25 (2H, d, $J = 8.6$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 28.0 (C-5′), 29.7 (C-4′), 30.8 (C-1), 31.0 (C-3′), 55.2 (OCH_3), 68.5 (C-6′), 72.6 (Bn), 113.8 (C_m), 119.3 (C-1′), 128.7 (C-2′), 129.2 (C_o), 130.3 (C_i), 159.2 (C_p), 192.8 (C-2); (*E*)-**2d** δ_H (300 MHz, $CDCl_3$): 1.86 (2H, m, H-5′), 2.37 (3H, s, H-1), 2.56 (2H, t, $J = 7.2$ Hz, H-4′), 3.21 (2H, dd, $J = 1.2$, 7.4 Hz, H-3′), 3.52 (2H, t, $J = 6.2$ Hz, H-6′), 3.80 (3H, s, OCH_3), 4.43 (2H, s, Bn), 5.82 (1H, dt, $J = 7.4$, 15.6 Hz, H-2′), 6.54 (1H, dt, $J = 1.2$, 15.6 Hz, H-1′), 6.87 (2H, d, $J = 8.6$ Hz, H_m), 7.25 (2H, d, $J = 8.6$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 27.7 (C-5′), 29.5 (C-4′), 30.3 (C-1), 34.1 (C-3′), 55.2 (OCH_3), 68.4 (C-6′), 72.6 (Bn), 113.8 (C_m), 119.0 (C-1′), 129.2 (C_o), 130.3 (C_i), 130.5 (C-2′), 159.2 (C_p), 191.2 (C-2); HRMS (EI): *m/z* 326.1035 $[M]^+$, $C_{16}H_{22}O_3S_2$ requires 326.1010.

4.8.5. (E/Z)-S-[3-(3-N-Phthalimidoprop-1-ylthio]propen-1-yl] ethanethioate (**2e**)

Obtained as a 3:2 *Z*:*E* mixture (66%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1713 (C]O); (*Z*)-**2e** δ_H (300 MHz, $CDCl_3$): 1.93 (2H, q, $J = 7.3$ Hz, H-5′), 2.36 (3H, s, H-1), 2.49 (2H, t, $J = 7.3$ Hz, H-4′), 3.16 (2H, dd, $J = 0.8$, 7.6 Hz, H-3′), 3.74 (2H, t, $J = 7.3$ Hz, H-6′), 5.82 (1H, dt, $J = 7.6$, 9.6 Hz, H-2′), 6.62 (1H, dt, $J = 0.8$, 9.6 Hz, H-1′), 7.68 (2H, m, Ar-H), 7.81 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 28.2 (C-5′), 28.5 (C-4′), 29.7 (C-1), 30.9 (C-3′), 37.1 (C-6′), 119.6 (C-1′), 123.2 (Ar-C), 128.4 (C-2′), 132.1 (C_{qu}), 133.9 (Ar-C), 168.3 (C]O), 191.2 (C]O); (*E*)-**2e** δ_H (300 MHz, $CDCl_3$): 1.93 (2H, q, $J = 7.3$ Hz, H-5′), 2.29 (3H, s, H-1), 2.47 (2H, t, $J = 7.3$ Hz, H-4′), 3.21 (2H, dd, $J = 1.0$, 7.6 Hz, H-3′), 3.76 (2H, t, $J = 7.3$ Hz, H-6′), 5.79 (1H, dt, $J = 7.6$, 15.6 Hz, H-2′), 6.48 (1H, dt, $J = 1.0$, 15.6 Hz, H-1′), 7.68 (2H, m, Ar-H), 7.81 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 28.1 (C-5′), 28.3 (C-4′), 30.3 (C-1), 34.0 (C-3′), 37.0 (C-6′), 119.2 (C-1′), 123.2 (Ar-C), 130.3 (C-2′), 132.1 (C_{qu}), 133.9 (Ar-C), 168.3 (C]O), 192.7 (C]O); HRMS (EI): *m/z* 335.0659 $[M]^+$, $C_{16}H_{17}NO_3S_2$ requires 335.0650.

4.8.6. (E/Z)-S-[3-(Benzylthio)propen-1-yl] ethanethioate (**2f**)

Obtained as a 2:1 *Z*:*E* mixture (53%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1702 (C]O); (*Z*)-**2f** δ_H (300 MHz, $CDCl_3$): 2.38 (3H, s, CH_3), 3.11 (2H, dt, $J = 1.3$, 7.5, H-3′), 3.69 (2H, s, Bn), 5.87 (1H, dt, $J = 7.5$, 9.6 Hz, H-2′), 6.68 (1H, dt, $J = 1.3$, 9.6 Hz, H-1′), 7.32 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 30.7 (C-3′), 30.8 (C-1), 35.8 (C-Bn), 119.7 (C-1′), 128.5 (Ar-C), 128.6 (Ar-C) 129.0 (Ar-C), 129.1 (C-2′), 137.9 (Ar-C), 191.2 (C-2); (*E*)-**2f** δ_H (300 MHz, $CDCl_3$): 2.37 (3H, s, CH_3), 3.11 (2H, dt, $J = 1.3$, 7.5 Hz, H-3′), 3.68 (2H, s, Bn), 5.83 (1 H, dt, $J = 7.5$, 15.6 Hz, H-2′), 6.52 (1H, dt, $J = 1.3$, 15.6 Hz, H-1′), 7.26 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 30.4 (C-1), 33.3 (C-3′), 35.0 (Bn), 119.5 (C-1′), 127.0 (Ar-C), 128.5 (Ar-C), 128.6 (Ar-C) 130.3 (C-2′), 137.9 (Ar-C), 191.2 (C-2); HRMS (EI): *m/z* 255.0545 $[M + O + H]^+$, $C_{12}H_{15}O_2S_2$ requires 255.0513.

4.8.7. (E/Z)-S-[3-(p-Methoxybenzylthio)propen-1-yl] ethanethioate (**2g**)

Obtained as a 3:2 *Z*:*E* mixture (55%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 1700 (C]O); (*Z*)-**2g** δ_H (400 MHz, $CDCl_3$): 2.36 (3H, s, H-1), 3.09 (2H, dd, $J = 1.0$, 7.4 Hz, H-3′), 3.63 (2H, s, Bn), 3.78 (3H, s, OCH_3), 5.84 (1H, dt, $J = 7.4$, 9.5 Hz, H-2′), 6.66 (1H, dt, $J = 1.0$, 9.5 Hz, H-1′), 6.83 (2H, d, $J = 8.3$ Hz, H_m), 7.20 (2H, d, $J = 8.3$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 30.7 (C-1), 30.8 (C-3′), 35.2 (Bn), 55.2 (OCH_3), 113.9 (C_m), 119.5 (C-1′), 128.6 (C-2′), 129.8 (C_i), 130.0 (C_o), 158.7 (C_p), 191.2 (C-2); (*E*)-**2g** δ_H (400 MHz, $CDCl_3$): 2.35 (3H, s, H-1), 3.08 (2H, dd, $J = 1.1$, 7.4 Hz, H-3′), 3.61 (2H, s, Bn), 3.78 (3H, s, OCH_3), 5.80 (1H, td, $J = 7.4$, 15.2 Hz, H-2′), 6.49 (1H, dt, $J = 1.1$, 15.2 Hz, H-1′), 6.83 (2H, d, $J = 8.7$ Hz, H_m), 7.22 (2H, d, $J = 8.7$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 30.4 (C-1), 33.1 (C-3′), 34.4 (Bn), 55.2 (OCH_3), 114.0 (C_m), 119.3 (C-1′), 129.8 (C_i), 130.1 (C_o), 130.3 (C-2′), 158.7 (C_p), 192.9 (C-2); HRMS (ES): *m/z* 307.0404 $[M + Na + O]^+$, $C_{13}H_{16}NaO_3S_2$ requires 307.0439.

4.9. (iii) Sulfenylation step to vinyl disulfide (**3**)

Vinyl thioacetate **2** dissolved in methanol (1 M) was cooled to −40 °C using a cooling bath with acetonitrile/liquid nitrogen. KOH in methanol (1.05 equiv, 1 M) was added slowly via syringe and the mixture stirred for 20 min before cooling the mixture to −78 °C (acetone/liquid nitrogen bath), whereupon *S*-allyl *p*-toluenesulfonylthioate in methanol (1.1 equiv, 1 M) was syringed into the solution. The solution was allowed to warm to 0 °C and stirred at this temperature for 2 h before adding aqueous NH_4Cl after which the organic product was extracted with ethyl acetate or dichloromethane (3 times). Following drying and evaporation of solvent, the residue was subjected to column chromatography to afford vinyl disulfide **3**.

4.9.1. (E,Z)-4,5,9-Trithiadodeca-1,6-diene (**3a**)

Obtained as a 5:4 *Z*:*E* mixture (97%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 498 (S–S); (*Z*)-**3**[a] δ_H (400 MHz, $CDCl_3$): 0.99 (3H, t, $J = 7.3$ Hz, H-12), 1.61 (2H, sext, $J = 7.3$ Hz, H-11), 2.47 (2H, t, $J = 7.3$ Hz, H-10), 3.27 (2H, dd, $J = 1.1$, 7.6 Hz, H-8), 3.36 (2H, dt, $J = 0.9$, 7.0 Hz, H-3), 5.18 (2H, m, H-1), 5.68 (1H, dt, $J = 7.6$, 9.2 Hz, H-7), 5.85 (1H, m, H-2), 6.22 (1H, dt, $J = 1.1$, 9.2 Hz, H-6); δ_C (100 MHz, $CDCl_3$): 13.5 (C-12), 22.9 (C-11), 29.4 (C-8), 33.4 (C-10), 42.0 (C-3), 118.8 (C-1), 128.7 (C-7), 131.7 (C-6), 132.8 (C-2); (*E*)-**3**[a] δ_H (400 MHz, $CDCl_3$): 0.99 (3H, t, $J = 7.3$ Hz, H-12), 1.61 (2H, sext, $J = 7.3$ Hz, H-11), 2.44 (2H, t, $J = 7.3$ Hz, H-10), 3.18 (2H, dd, $J = 1.1$, 7.6 Hz, H-8), 3.34 (2H, dt, $J = 0.9$, 7.0 Hz, H-3), 5.18 (2H, m, H-1), 5.85 (1H, m, H-2), 5.88 (1H, dt, $J = 7.6$, 14.6 Hz, H-7), 6.11 (1H, dt, $J = 1.1$, 14.6 Hz, H-6); δ_C (100 MHz, $CDCl_3$): 13.5 (C-12), 22.7 (C-11), 33.1 (C-10), 33.5 (C-8), 41.3 (C-3), 118.9 (C-1), 127.5 (C-6), 128.6 (C-7), 132.8 (C-2); HRMS (ES): *m/z* 237.0460 $[M + O + H]^+$, $C_9H_{17}OS_3$ requires 237.0442.

4.9.2. (E,Z)-10,10-Dimethyl-4,5,9-trithiaundeca-1,6-diene (**3b**)

Obtained as a 3:1 *Z*:*E* mixture (89%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 500 (S–S); (*Z*)-**3b** δ_H (300 MHz, $CDCl_3$): 1.34 (9H, s, *t*-butyl), 3.32 (2H, dd, $J = 1.2$, 7.4 Hz, H-8), 3.35 (2H, dt, $J = 1.2$, 7.4 Hz, H-3), 5.15 (2H, m, H-1), 5.72 (1H, dt, $J = 7.4$, 9.5 Hz, H-7), 5.83 (1H, m, H-2), 6.15 (1H, dt, $J = 1.2$, 9.5 Hz, H-6); δ_C (75 MHz, $CDCl_3$): 26.9 (C-8), 31.0 (C($\underline{C}H_3)_3$), 42.0 (C-3), 42.8 ($\underline{C}(CH_3)_3$), 118.9 (C-1), 129.2 (C-7), 131.1 (C-6), 132.8 (C-2); (*E*)-**3b** δ_H (300 MHz, $CDCl_3$): 1.33 (9H, s, *t*-butyl), 3.26 (2H, dd, $J = 1.2$, 7.4 Hz, H-8), 3.33 (2H, dt, $J = 1.2$, 7.4 Hz, H-3), 5.21 (2H, m, H-1), 5.82 (1H, m, H-2), 5.93 (1H, dt, $J = 7.4$, 14.7 Hz, H-7), 6.17 (1H, dt, $J = 1.2$, 14.7 Hz, H-6); δ_C (75 MHz, $CDCl_3$): 30.8 (C-8), 31.0 (C($\underline{C}H_3)_3$), 41.2 (C-3), 42.8 ($\underline{C}(CH_3)_3$), 118.9 (C-1), 127.3 (C-6), 129.2 (C-7), 132.8 (C-2); HRMS (ES): *m/z* 251.0615 $[M + O + H]^+$, $C_{10}H_{19}OS_3$ requires 251.0598.

4.9.3. (E,Z)-12-(t-Butyldimethylsilyloxy)-4,5,9-trithiadodeca-1, 6-diene (**3c**)

Compound **3c** was obtained as a 2:1 *Z*:*E* mixture (89%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 498 (S–S); (*Z*)-**3c** δ_H (400 MHz, $CDCl_3$): 0.05 (6H, s, CH_3), 0.89 (9H, s, *t*-butyl), 1.80 (2H, m, H-11), 2.57 (2H, t, $J = 7.0$ Hz, H-10), 3.27 (2H, dd, $J = 1.1$, 7.7 Hz, H-8), 3.36 (2H, dt, $J = 1.0$, 7.4 Hz, H-3), 3.69 (2H, t, $J = 6.2$ Hz, H-12), 5.18 (2H,

m, H-1), 5.69 (1H, dt, $J = 7.7$, 9.3 Hz, H-7), 5.83 (1H, m, H-2), 6.21 (1H, dt, $J = 1.1$, 9.3 Hz, H-6); δ_C (100 MHz, $CDCl_3$): −5.3 ($Si(CH_3)_2$), 18.3 ($\underline{C}(CH_3)_3$), 26.0 ($C(\underline{C}H_3)_3$), 27.9 (C-11), 29.5 (C-8), 32.6 (C-10), 42.1 (C-3), 61.6 (C-12), 118.9 (C-1), 128.5 (C-7), 131.9 (C-6), 132.8 (C-2); (*E*)-**3c** δ_H (400 MHz, $CDCl_3$): 0.06 (6H, s, CH_3), 0.90 (9H, s, *t*-butyl), 1.80 (2H, m, H-11), 2.54 (2H, t, $J = 7.4$ Hz, H-10), 3.18 (2H, dd, $J = 1.1$, 7.4 Hz, H-8), 3.34 (2H, dt, $J = 1.0$, 7.4 Hz, H-3), 3.69 (2H, t, $J = 6.2$ Hz, H-12), 5.18 (2H, m, H-1), 5.83 (1H, m, H-2), 5.88 (1H, dt, $J = 7.4$, 14.7 Hz, H-7), 6.11 (1H, dt, $J = 1.1$, 14.7 Hz, H-6); δ_C (100 MHz, $CDCl_3$): −5.3 ($Si(CH_3)_2$), 18.3 ($\underline{C}(CH_3)_3$), 26.0 ($C(\underline{C}H_3)_3$), 27.5 (C-11), 32.5 (C-10), 33.6 (C-8), 41.3 (C-3), 61.6 (C-12), 118.9 (C-1), 127.6 (C-6), 128.4 (C-7), 132.8 (C-2); HRMS (ES): *m*/*z* 277.1131 $[M - SC_3H_5]^+$, $C_{12}H_{25}OS_2Si$ requires 277.1116.

4.9.4. (E,Z)-12-(p-Methoxybenzyloxy)-4,5,9-trithiadodeca-1,6-diene (**3d**)

Obtained as a 3:2 *Z*:*E* mixture (89%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 498 (S–S); (*Z*)-**3d** δ_H (400 MHz, $CDCl_3$): 1.87 (2H, m, H-11), 2.60 (2H, t, $J = 7.2$ Hz, H-10), 3.27 (2H, dd, $J = 1.1$, 7.8 Hz, H-8), 3.35 (2H, m, H-3), 3.53 (2H, t, $J = 6.2$ Hz, H-12), 3.80 (3H, s, OCH_3), 4.43 (2H, s, Bn), 5.17 (2H, m, H-1), 5.67 (1H, dt, $J = 7.8$, 9.2 Hz, H-7), 5.83 (1H, m, H-2), 6.22 (1H, dt, $J = 1.1$, 9.2 Hz, H-6), 6.88 (2H, d, $J = 8.8$ Hz, H_m), 7.25 (2H, d, $J = 8.8$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 28.2 (C-11), 29.5 (C-8), 29.8 (C-10), 42.0 (C-3), 55.3 (OCH_3), 68.5 (C-12), 72.6 (Bn), 113.8 (C_m), 118.9 (C-1), 128.4 (C-7), 129.2 (C_o), 130.4 (C_i), 131.9 (C-6), 132.8 (C-2), 159.2 (C_p); (*E*)-**3d** δ_H (400 MHz, $CDCl_3$): 1.87 (2H, m, H-11), 2.56 (2H, t, $J = 7.4$ Hz, H-10), 3.18 (2H, dd, $J = 1.1$, 7.2 Hz, H-8), 3.33 (2H, m, H-3), 3.53 (2H, t, $J = 6.2$ Hz, H-12), 3.80 (3H, s, OCH_3), 4.43 (2H, s, Bn), 5.17 (2H, m, H-1), 5.83 (1H, m, H-2), 5.87 (1H, dt, $J = 7.4$, 14.4 Hz, H-7), 6.10 (1H, dt, $J = 1.1$, 14.4 Hz, H-6), 6.88 (2H, d, $J = 8.8$ Hz, H_m), 7.25 (2H, d, $J = 8.8$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 27.8 (C-11), 29.6 (C-10), 33.5 (C-8), 41.2 (C-3), 55.3 (OCH_3), 68.4 (C-12), 72.6 (Bn), 113.8 (C_m), 118.9 (C-1), 127.7 (C-6), 128.3 (C-7), 129.2 (C_o), 130.4 (C_i), 132.8 (C-2), 159.2 (C_p); HRMS (ES): *m*/*z* 356.0949 $[M]^+$, $C_{17}H_{24}O_2S_3$ requires 356.0938.

4.9.5. (E,Z)-12-Phthalimido-4,5,9-trithiadodeca-1,6-diene (**3e**)

Obtained as a 1:1 *Z*:*E* mixture (67%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1713 (C]O), 499 (S–S); (*Z*)-**3e** δ_H (400 MHz, $CDCl_3$): 1.85 (2H, q, $J = 7.1$ Hz, H-11), 2.42 (2H, t, $J = 7.1$ Hz, H-10), 3.15 (2H, dd, $J = 1.0$, 7.7 Hz, H-8), 3.22 (2H, dt, $J = 1.0$, 7.2 Hz, H-3), 3.67 (2H, t, $J = 7.1$ Hz, H-12), 5.05 (2H, m, H-1), 5.55 (1H, dt, $J = 7.7$, 9.4 Hz, H-7), 5.70 (1H, m, H-2), 6.09 (1H, dt, $J = 1.0$, 9.4 Hz, H-6), 7.60 (2H, m, Ar-H), 7.73 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 28.0 (C-11), 28.5 (C-10), 29.3 (C-8), 37.0 (C-12), 42.0 (C-3), 118.9 (C-1), 123.1 (Ar-C), 128.1 (C-7), 132.0 (C-2), 132.1 (C-6), 132.6 (C_{qu}), 133.8 (Ar-C), 168.1 (C]O); (*E*)-**3e** δ_H (400 MHz, $CDCl_3$): 1.85 (2H, t, $J = 7.1$ Hz, H-11), 2.38 (2H, q, $J = 7.1$ Hz, H-10), 3.07 (2H, dd, $J = 1.0$, 7.7 Hz, H-8), 3.19 (2H, dt, $J = 1.0$, 7.2 Hz, H-3), 3.67 (2H, t, $J = 7.1$ Hz, H-12), 5.05 (2H, m, H-1), 5.70 (2H, m, H-2, H-7), 5.97 (1H, dt, $J = 1.0$, 14.8 Hz, H-6), 7.60 (2H, m, Ar-H), 7.72 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 28.0 (C-11), 28.4 (C-10), 33.2 (C-8), 37.0 (C-12), 41.2 (C-3), 118.8 (C-1), 123.1 (Ar-C), 127.7 (C-6), 127.8 (C-7), 132.1 (C-2), 132.7 (C_{qu}), 133.9 (Ar-C), 168.1 (C]O); HRMS (EI): *m*/*z* 221.0511 $[M - C_4H_7S_2 + H]^+$, $C_{11}H_{11}NO_2S$ requires 221.0510.

4.9.6. (E,Z)-10-Phenyl-4,5,9-trithiadeca-1,6-diene (**3f**)

Obtained as a 4:3 *Z*:*E* mixture (86%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 490 (S–S); (*Z*)-**3f** δ_H (400 MHz, $CDCl_3$): 3.19 (2H, dd, $J = 1.0$, 7.6 Hz, H-8), 3.33 (2H, dt, $J = 1.0$, 7.2 Hz, H-3), 3.69 (2H, s, Bn), 5.15 (2H, m, H-1), 5.66 (1H, dt, $J = 7.6$, 9.8 Hz, H-7), 5.80 (1H, m, H-2), 6.20 (1H, dt, $J = 1.0$, 9.8 Hz, H-6), 7.27 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 29.4 (C-8), 36.0 (Bn), 42.1 (C-3), 118.9 (C-1), 127.0 (Ar-C), 128.2 (C-7), 128.5 (Ar-C), 128.9 (Ar-C), 132.3 (C-6), 132.8 (C-2), 138.0 (Ar-C); (*E*)-**3f** δ_H (400 MHz, $CDCl_3$): 3.06 (2H, dd, $J = 1.0$, 7.2 Hz, H-8), 3.33 (2H, dt, $J = 1.0$, 7.2 Hz,, H-3), 3.65 (2H, s, Bn), 5.15 (2H, m, H-1), 5.80 (1H, m, H-2), 5.85 (1H, dt, $J = 7.6$, 14.8 Hz, H-7), 6.05 (1H, dt, $J = 1.0$, 14.8, Hz, H-6), 7.27 (5H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 32.8 (C-8), 35.2 (Bn), 41.3 (C-3), 118.9 (C-1), 127.0 (Ar-C), 127.9 (C-6), 128.1 (C-7), 128.5 (Ar-C), 128.9 (Ar-C), 132.8 (C-2), 138.0 (Ar-C); HRMS (EI): *m*/*z* 285.0439 $[M + O + H]^+$, $C_{13}H_{17}OS_3$ requires 285.0442.

4.9.7. (E,Z)-10-(p-Methoxyphenyl)-4,5,9-trithiadeca-1,6-diene (**3g**)

Obtained as a 3:2 *Z*:*E* mixture (84%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 495 (S–S); (*Z*)-**3g** δ_H (300 MHz, $CDCl_3$): 3.20 (2H, dd, $J = 1.1$, 7.7 Hz, H-8), 3.35 (2H, dd, $J = 0.9$, 7.5 Hz, H-3), 3.67 (2H, s, Bn), 3.80 (3H, s, OCH_3), 5.15 (2H, m, H-1), 5.68 (1H, dt, $J = 7.7$, 9.5 Hz, H-7), 5.81 (1H, m, H-2), 6.21 (1H, dt, $J = 1.1$, 9.5 Hz, H-6), 6.84 (2H, d, $J = 9.0$ Hz, H_m), 7.20 (2H, d, $J = 9.0$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 29.4 (C-8), 35.4 (C-Bn), 42.1 (C-3), 55.3 (OCH_3), 114.0 (C_m), 118.9 (C-1), 128.2 (C-7), 128.7 (C_i), 130.0 (C_o), 132.1 (C-6), 132.8 (C-2), 158.7 (C_p); (*E*)-**3g** δ_H (300 MHz, $CDCl_3$): 3.07 (2H, dd, $J = 1.1$, 7.4 Hz, H-8), 3.35 (2H, dd, $J = 0.9$, 7.5 Hz,, H-3), 3.63 (2H, s, Bn), 3.80 (3H, s, OCH_3), 5.15 (2H, m, H-1), 5.81 (2H, m, H-2, H-7), 6.06 (1H, dt, $J = 1.1$, 14.7, Hz, H-6), 6.84 (2H, d, $J = 9.0$ Hz, H_m), 7.20 (2H, d, $J = 9.0$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 32.7 (C-8), 34.6 (C-Bn), 41.3 (C-3), 55.3 (OCH_3), 114.0 (C_m), 118.9 (C-1), 128.0 (C-6), 128.2 (C-7), 128.7 (C_i), 130.0 (C_o), 132.8 (C-2), 158.7 (C_p); HRMS (ES): *m*/*z* 225.0436 $[M - C_3H_5S]^+$, $C_{11}H_{13}OS_2$ requires 225.0408.

4.9.8. (E,Z)-4,5,9-Trithiadodeca-6-ene (**3h**)

Obtained as a 1:1 *Z*:*E* mixture (76%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 499 (S–S); (*Z*)-**3h** δ_H (300 MHz, $CDCl_3$): 0.97 (3H, t, $J = 7.3$ Hz, H-1/ H-12), 0.99 (3H, t, $J = 7.3$ Hz, H-1/H-12), 1.61 (2H, m, H-2/H-11), 1.69 (2H, m, H-2/H-11), 2.47 (2H, t, $J = 7.3$ Hz, H-10), 2.70 (2H, t, $J = 7.3$ Hz, H-3), 3.26 (2H, dd, $J = 1.1$, 7.4 Hz, H-8), 5.66 (1H, dt, $J = 7.4$, 9.3 Hz, H-7), 6.23 (1H, dt, $J = 1.1$, 9.3 Hz, H-6); δ_C (75 MHz, $CDCl_3$): 12.9 (C-1/C-12), 13.4 (C-1/C-12), 22.2 (C-2/C-11), 22.9 (C-2/C-11), 29.4 (C-8), 33.2 (C-10), 41.1 (C-3), 128.2 (C-7), 132.2 (C-6); (*E*)-**3h** δ_H (300 MHz, $CDCl_3$): 0.97 (3H, t, $J = 7.3$ Hz, H-1/H-12), 0.99 (3H, t, $J = 7.3$ Hz, H-1/H-12), 1.61 (2H, m, H-2/H-11), 1.69 (2H, m, H-2/H-11), 2.43 (2H, t, $J = 7.3$ Hz, H-10), 2.68 (2H, t, $J = 7.3$ Hz, H-3), 3.17 (2H, dd, $J = 1.1$, 7.8 Hz, H-8), 5.87 (1H, dt, $J = 7.8$, 14.4 Hz, H-7), 6.11 (1H, dt, $J = 1.1$, 14.4 Hz, H-6); δ_C (75 MHz, $CDCl_3$): 13.0 (C-1/C-12), 13.4 (C-1/C-12), 22.3 (C-2/C-11), 22.6 (C-2/C-11), 33.0 (C-10), 33.5 (C-8), 40.4 (C-3), 127.8 (C-6), 127.9 (C-7); HRMS (EI): *m*/*z* 222.0566 $[M]^+$, $C_9H_{18}S_3$ requires 222.0571.

4.9.9. (E,Z)-2,3,7-Trithiadeca-4-ene (**3i**)

Obtained as a 3:2 *Z*:*E* mixture (76%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 498 (S–S); (*Z*)-**3i** δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, $J = 7.2$ Hz, H-10), 1.60 (2H, m, H-9), 2.44 (3H, s, H-1), 2.47 (2H, t, $J = 7.4$ Hz, H-8), 3.26 (2H, dd, $J = 1.0$, 7.4 Hz, H-6), 5.74 (1H, dt, $J = 7.4$, 9.3 Hz, H-5), 6.24 (1H, dt, $J = 1.0$, 9.3 Hz, H-4); δ_C (100 MHz, $CDCl_3$): 13.4 (C-10), 22.9 (C-1/C-9), 22.9 (C-1/ C-9), 29.3 (C-6), 33.2 (C-8), 129.2 (C-5), 130.9 (C-4); (*E*)-**3i** δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, $J = 7.2$ Hz, H-10), 1.60 (2H, m, H-9), 2.40 (3H, s, H-1), 2.46 (2H, t, $J = 7.4$ Hz, H-8), 3.20 (2H, dd, $J = 1.0$, 7.4 Hz, H-6), 5.90 (1H, dt, $J = 7.4$, 14.6 Hz, H-5), 6.12 (1H, dt, $J = 1.0$, 14.6, Hz, H-4); δ_C (100 MHz, $CDCl_3$): 13.4 (C-10), 22.1 (C-1), 22.6 (C-9), 33.0 (C-8), 33.4 (C-6), 126.4 (C-4), 128.5 (C-5); HRMS (EI): *m*/*z* 194.0248 $[M]^+$, $C_7H_{14}S_3$ requires 194.0258.

4.9.10. (E,Z)-8-(p-Methoxyphenyl)-1-phenyl-2,3,7-trithiaocta-4-ene (**3j**)

Obtained as a 3:2 *Z*:*E* mixture (88%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 499 (S–S); (*Z*)-**3j** δ_H (300 MHz, $CDCl_3$): 3.18 (2H, d, $J = 7.6$ Hz, H-6), 3.67 (2H, s, H-8), 3.81 (3H, s, OCH_3), 3.94 (2H, s, H-1), 5.61 (1H, dt, $J = 7.6$, 9.3 Hz, H-5), 5.99 (1H, d, $J = 9.3$ Hz, H-4), 6.87 (2H, m, Ar_{OMe}-H_m), 7.25 (2H, m, Ar_{OMe}-H_o), 7.32 (5H, m, Ar-H);

δ_C (75 MHz, $CDCl_3$): 29.2 (C-6), 35.3 (C-8), 43.5 (C-1), 55.2 (OCH_3), 113.9 (Ar_{OMe}-C_m), 127.5 (Ar-C), 128.1 (C-5), 128.5 (Ar-C), 129.3 (Ar-C), 129.8 (Ar_{OMe}-C_i), 129.9 (Ar_{OMe}-C_o), 131.6 (C-4), 136.8 (Ar-C), 158.6 (Ar_{OMe}-C_i); (*E*)-**3j** δ_H (300 MHz, $CDCl_3$): 3.02 (2H, d, $J = 7.0$ Hz, H-6), 3.61 (2H, s, H-8), 3.82 (3H, s, OCH_3), 3.94 (2H, s, H-1), 5.82 (1H, dt, $J = 7.0$, 14.6 Hz, H-5), 5.92 (1H, d, $J = 14.6$ Hz, H-4), 6.87 (2H, m, Ar_{OMe}-H_m), 7.25 (2H, m, Ar_{OMe}-H_o), 7.32 (5H, m, Ar-H); δ_C (75 MHz, $CDCl_3$): 32.6 (C-6), 34.5 (C-8), 42.7 (C-1), 55.2 (OCH_3), 113.9 (Ar_{OMe}-C_m), 127.5 (C-4), 127.5 (Ar-C), 128.1 (C-5), 128.5 (Ar-C), 129.3 (Ar-C), 129.8 (Ar_{OMe}-C_i), 130.0 (Ar_{OMe}-C_o), 136.8 (Ar-C), 158.6 (Ar_{OMe}-C_p); HRMS (ES): *m*/*z* 365.0695 $[M + O + H]^+$, $C_{18}H_{21}O_2S_3$ requires 365.0704.

4.9.11. (E,Z)-1-(p-Fluorophenyl)-8-(p-methoxyphenyl)-2,3,7-trithiaocta-4-ene (**3k**)

Obtained as a 4:3 *Z*:*E* mixture (70%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 498 (S—S); (*Z*)-**3k** δ_H (300 MHz, $CDCl_3$): 3.17 (2H, d, $J = 7.7$ Hz, H-6), 3.66 (2H, s, H-8), 3.80 (3H, s, OCH_3), 3.89 (2H, s, H-1), 5.61 (1H, dt, $J = 7.7$, 9.2 Hz, H-5), 5.96 (1H, dt, $J = 1.1$, 9.2 Hz, H-4), 6.86 (2H, m, Ar_{OMe}-H_m), 7.00 (2H, m, Ar_F-H_m), 7.25 (4H, m, Ar_{OMe}-H_o and Ar_F-H_o); δ_C (75 MHz, $CDCl_3$): 29.2 (C-6), 35.3 (C-8), 42.5 (C-1), 55.2 (OCH_3), 113.9 (Ar_{OMe}-C_m), 115.4 (d, $J_{CF} = 21.1$ Hz, Ar_F-C_m), 128.2 (C-5), 129.8 (Ar_{OMe}-C_i), 129.9 (Ar_{OMe}-C_o), 130.9 (d, $J_{CF} = 8.0$ Hz, Ar_F-C_o), 131.4 (C-4), 132.6 (d, $J_{CF} = 3.0$ Hz, Ar_F-C_i), 158.6 (Ar_{OMe}-C_p), 162.2 (d, $J_{CF} = 245.3$ Hz, Ar_F-C_p); (*E*)-**3k** δ_H (300 MHz, $CDCl_3$): 3.02 (2H, dd, $J = 1.1$, 7.1 Hz, H-6), 3.60 (2H, s, H-8), 3.81 (3H, s, OCH_3), 3.89 (2H, s, H-1), 5.80 (1H, dt, $J = 7.1$, 14.7 Hz, H-5), 5.90 (1H, d, $J = 14.7$ Hz, H-4), 6.86 (2H, m, Ar_{OMe}-H_m), 7.00 (2H, m, Ar_F-H_m), 7.25 (4H, m, Ar_{OMe}-H_o and Ar_F-H_o); δ_C (75 MHz, $CDCl_3$): 32.6 (C-6), 34.6 (C-8), 41.8 (C-1), 55.2 (OCH_3), 113.9 (Ar_{OMe}-C_m), 115.4 (d, $J_{CF} = 21.1$ Hz, Ar_F-C_m), 127.3 (C-4), 128.3 (C-5), 129.8 (Ar_{OMe}-C_i), 129.9 (Ar_{OMe}-C_o), 130.9 (d, $J_{CF} = 9.5$ Hz, Ar_F-C_o), 132.6 (d, $J_{CF} = 3.0$ Hz, Ar_F-C_i), 158.6 (Ar_{OMe}-C_p), 162.2 (d, $J_{CF} = 245.3$ Hz, Ar_F-C_p); HRMS (ES): *m*/*z* 405.0450 $[M + O + Na]^+$, $C_{18}H_{19}FNaO_2S_3$ requires 405.0429.

4.9.12. (E,Z)-1,8-(Bis-p-methoxyphenyl)-2,3,7-trithia-octa-4-ene (E/Z-**3l**)

Obtained as a 1:1 *Z*:*E* mixture (83%) as a pale-yellow oil: IR ν_{max} (neat)/cm^{-1} 500 (S—S); (*Z*)-*E*/*Z*-**3l** δ_H (400 MHz, $CDCl_3$): 3.20 (2H, d, $J = 0.8$, 7.6 Hz, H-6), 3.68 (2H, s, H-8), 3.80 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 3.91 (2H, s, H-1), 5.63 (1H, dt, $J = 7.6$, 9.4 Hz, H-5), 6.03 (1H, d, $J = 9.4$ Hz, H-4), 6.87 (4H, m, H_m), 7.23 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 29.4 (C-6), 35.4 (C-8), 43.1 (C-1), 55.3 (OCH_3), 114.0 (C_m), 114.1 (C_m), 128.0 (C-5), 128.8 (C_i), 130.0 (C_i), 130.1 (C_o), 130.6 (C_o), 131.9 (C-4), 158.7 (C_p), 159.2 (C_p); (*E*)-*E*/*Z*-**3l** δ_H (400 MHz, $CDCl_3$): 3.03 (2H, d, $J = 7.3$ Hz, H-6), 3.61 (2H, s, H-8), 3.78 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.91 (2H, s, H-1), 5.83 (1H, dt, $J = 7.3$, 14.6 Hz, H-5), 5.94 (1H, d, $J = 14.6$ Hz, H-4), 6.87 (4H, m, H_m), 7.23 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 32.7 (C-6), 34.7 (C-8), 42.3 (C-1), 55.3 ($OCH_3 \times 2$), 114.0 (C_m), 114.1 (C_m), 127.8 (C-4), 128.1 (C-5), 128.8 (C_i), 130.0 (C_i), 130.1 (C_o), 130.6 (C_o), 158.7 (C_p), 159.2 (C_p); HRMS (ES): *m*/*z* 395.0807 $[M + O + H]^+$, $C_{19}H_{23}O_3S_3$ requires 395.0809.

4.10. (iv) Oxidation step to (**4**)

The vinyl disulfide **3** dissolved in dichloromethane (0.2 M) was cooled to −78 °C under nitrogen, and *m*-CPBA (1.1 equiv) added in one portion. The reaction was allowed to warm to room temperature over a few hours until TLC (40% ethyl acetate/petroleum ether) indicated the consumption of starting material. Saturated aqueous $NaHCO_3$ was then added and the product extracted into ethyl acetate or dichloromethane (3 times). The combined organic layers were dried and concentrated *in vacuo* to afford a residue, which was purified on a silica-gel column using petroleum ether/ethyl acetate mixtures to afford the ajoene derivative as an *E*/*Z* mixture. In some cases (see Tables 3 and 4), isomers could be separated by gravity chromatography using a low flow rate. Yields varied in the 60—90% range and reaction temperatures for optimal conversion were substrate-specific.

4.10.1. (E,Z)-4,5,9-Trithiadodeca-1,6-diene 9-oxide (**4a**) [54]

Obtained as a 3:2 *Z*:*E* mixture (82% and separable) as colourless oils: IR ν_{max} (neat)/cm^{-1} 1021 (S]O), 498 (S—S); (*Z*)-**4**[a] δ_H (400 MHz, $CDCl_3$): 1.07 (3H, t, $J = 7.4$ Hz, H-12), 1.79 (2H, m, H-11), 2.60 (2H, dt, $J = 8.2$, 13.7 Hz, H-10[a]), 2.70 (2H, dt, $J = 8.2$, 13.7 Hz, H-10b), 3.36 (2H, d, $J = 7.3$ Hz, H-3), 3.55 (1H, ddd, $J = 0.8$, 8.0, 13.2 Hz, H-8a), 3.62 (1H, ddd, $J = 0.8$, 8.0, 13.2 Hz, H-8b), 5.16 (2H, m, H-1), 5.75 (1H, dt, $J = 8.0$, 9.2 Hz, H-7), 5.82 (1H, m, H-2), 6.53 (1H, d, $J = 9.2$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): 13.3 (C-12), 16.2 (C-11), 42.0 (C-3), 50.9 (C-8), 53.4 (C-10), 118.4 (C-7), 119.2 (C-1), 132.5 (C-2), 138.2 (C-6); HRMS (ES): *m*/*z* 259.0263 $[M + Na]^+$, $C_9H_{16}ONaS_3$ requires 259.0261; (*E*)-**4**[a] δ_H (400 MHz, $CDCl_3$): 1.06 (3H, t, $J = 7.3$ Hz, H-12), 1.80 (2H, m, H-11), 2.58 (1H, m, H-10-a), 2.67 (1H, m, H-10-b), 3.33 (2H, d, $J = 7.4$ Hz, H-3), 3.42 (1H, ddd, $J = 0.9$, 7.9, 13.2 Hz, H-8a), 3.51 (1H, ddd, $J = 0.9$, 7.9, 13.2 Hz, H-8b), 5.15 (2H, m, H-1), 5.80 (1H, m, H-2), 5.90 (1H, dt, $J = 7.9$, 14.6 Hz, H-7), 6.34 (1H, d, $J = 14.6$ Hz, H-4); δ_C (100 MHz, $CDCl_3$): 13.3 (C-12), 16.1 (C-11), 41.2 (C-3), 53.0 (C-10), 54.5 (C-8), 117.1 (C-7), 119.1 (C-1), 132.4 (C-2), 134.2 (C-6); HRMS (ES): *m*/*z* 259.0263 $[M + Na]^+$, $C_9H_{16}ONaS_3$ requires 259.0261.

4.10.2. (E,Z)-10,10-Dimethyl-4,5,9-trithiaundeca-1,6-diene 9-oxide (**4b**)

Obtained as a 2:1 *Z*:*E* mixture (78%) and as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1043 (S]O), 498 (S—S); (*Z*)-**4b** δ_H (400 MHz, $CDCl_3$): 1.30 (9H, s, CH_3), 3.31 (1H, ddd, $J = 1.0$, 8.1, 13.2 Hz, H-8[a]), 3.37 (2H, m, H-3), 3.51 (1H, ddd, $J = 1.0$, 8.1, 13.2 Hz, H-8b), 5.19 (2H, m, H-1), 5.85 (2H, m, H-2 and H-7), 6.50 (1H, d, $J = 9.2$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): 22.9 (CH_3), 42.0 (C-3), 45.8 (C-8), 54.0 ($\underline{C}(CH_3)_3$), 119.1 (C-1), 121.1 (C-7), 132.7 (C-2), 136.7 (C-6); HRMS (ES): *m*/*z* 273.0417 $[M + Na]^+$, $C_{10}H_{18}ONaS_3$ requires 273.0418; (*E*)-**4b** δ_H (400 MHz, $CDCl_3$): 1.28 (9H, s, CH_3), 3.24 (1H, ddd, $J = 1.0$, 7.5, 13.0 Hz, H-8[a]), 3.37 (2H, m, H-3), 3.37 (1H, ddd, $J = 1.0$, 7.5, 13.0 Hz, H-8b), 5.19 (2H, m, H-1), 5.85 (1H, m, H-2), 6.02 (1H, dt, $J = 7.5$, 14.8 Hz, H-7), 6.37 (1H, d, $J = 14.8$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): 23.0 (CH_3), 41.1 (C-3), 49.5 (C-8), 53.7 ($\underline{C}(CH_3)_3$), 119.2 (C-1), 119.8 (C-7), 132.5 (C-2), 133.0 (C-6); HRMS (ES): *m*/*z* 273.0417 $[M + Na]^+$, $C_{10}H_{18}ONaS_3$ requires 273.0418.

4.10.3. (E,Z)-4,8,9-Trithiadodeca-6,11-dien-1-ol 4-oxide (**4c**)

(*E*,*Z*)-12-(*t*-Butyldimethylsilyloxy)-4,5,9-trithiadodeca-1,6-diene 4-oxide was obtained from the oxidation as a 3:2 *Z*:*E* mixture (86% and separable) as colourless oils: IR ν_{max} (neat)/cm^{-1} 1027 (S]O), 497 (S—S); (*Z*)-isomer δ_H (400 MHz, $CDCl_3$): 0.05 (6H, s, $Si(CH_3)_2$), 0.89 (9H, s, CH_3), 1.97 (2H, m, H-11), 2.79 (2H, m, H-10), 3.37 (2H, dt, $J = 1.0$, 7.2 Hz, H-3), 3.56 (1H, ddd, $J = 1.0$, 8.0, 13.3 Hz, H-8[a]), 3.64 (1H, ddd, $J = 1.0$, 8.0, 13.3 Hz, H-8b), 3.75 (2H, m, H-12), 5.18 (2H, m, H-1), 5.79 (2H, m, H-2 and H-7), 6.55 (1H, d, $J = 9.6$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): −5.4 ($Si(\underline{C}H_3)_2$), 18.2 ($\underline{C}(CH_3)_3$), 25.7 (C-11), 25.9 ($C(\underline{C}H_3)_3$), 42.1 (C-3), 48.4 (C-10), 51.0 (C-8), 61.4 (C-12), 118.4 (C-7), 119.2 (C-1), 132.3 (C-2), 138.3 (C-6); HRMS (ES): *m*/*z* 366.1169 $[M]^+$, $C_{15}H_{30}O_2S_3Si$ requires 366.1177; (*E*)-isomer δ_H (300 MHz, $CDCl_3$): 0.05 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, CH_3), 1.96 (2H, m, H-11), 2.76 (2H, m, H-10), 3.34 (2H, dt, $J = 1.0$, 7.5 Hz, H-3), 3.46 (1H, ddd, $J = 1.1$, 8.0, 12.7 Hz, H-8a), 3.54 (1H, ddd, $J = 1.1$, 8.0, 12.7 Hz, H-8b), 3.74 (2H, m, H-12), 5.17 (2H, m, H-1), 5.82 (1H, m, H-2), 5.93 (1H, dt, $J = 7.5$, 15.0 Hz, H-7), 6.36 (1H, dt, $J = 1.1$, 14.7 Hz, H-6); δ_C (75 MHz, $CDCl_3$): −5.4 ($Si(\underline{C}H_3)_2$), 18.2 ($\underline{C}(CH_3)_3$), 25.7 (C-11), 25.9 ($C(\underline{C}H_3)_3$), 41.3 (C-3), 48.1 (C-10), 54.6 (C-8), 61.4 (C-12), 117.1 (C-7), 119.2 (C-1), 132.5 (C-2), 134.3 (C-6); HRMS (ES): *m*/*z* 366.1171 $[M]^+$, $C_{15}H_{30}O_2S_3Si$ requires 366.1177.

The TBS ethers were individually deprotected with HF in CH_3CN to afford individual isomers of **4c**:

(*Z*)-**4c** was obtained as a colourless oil (84%): IR ν_{max} (neat)/cm^{-1} 3378 (OH), 1615 (C]C), 1015 (S]O), 497 (S–S); δ_H (400 MHz, $CDCl_3$): 2.07 (2H, m, H-11), 2.85 (2H, m, H-10), 2.87 (1H, br s, OH), 3.37 (2H, dt, $J = 1.0$, 7.3 Hz, H-3), 3.61 (1H, ddd, $J = 1.0$, 8.0, 13.3 Hz, H-8**a**), 3.67 (1H, ddd, $J = 1.0$, 8.0, 13.3 Hz, H-8b), 3.75 (2H, m, H-12), 5.18 (2H, m, H-1), 5.73 (1H, dt, $J = 8.0$, 9.6 Hz, H-7), 5.81 (1H, ddt, $J = 7.3$, 10.0, 16.5 Hz, H-2), 6.56 (1H, dt, $J = 1.0$, 9.6 Hz, H-6); δ_C (100 MHz, $CDCl_3$): 26.5 (C-11), 42.1 (C-3), 48.7 (C-10), 50.8 (C-8), 61.0 (C-12), 118.0 (C-7), 119.3 (C-1), 132.6 (C-2), 138.7 (C-6); HRMS (ES): *m/z* 275.0229 $[M + Na]^+$, $C_9H_{16}NaO_2S_3$ requires 275.0210; (*E*)-**4c** was obtained as a colourless oil (89%): IR ν_{max} (neat)/cm^{-1} 3481 (OH), 1630 (C]C), 1010 (S]O), 495 (S–S); δ_H (400 MHz, $CDCl_3$): 2.03 (2H, m, H-11), 2.82 (2H, m, H-10), 3.25 (1H, br s, OH), 3.33 (2H, d, $J = 7.2$ Hz, H-3), 3.53 (2H, m, H-8), 3.72 (2H, t, $J = 5.6$ Hz, H-12), 5.15 (2H, m, H-1), 5.81 (1H, ddt, $J = 7.2$, 9.8, 16.9 Hz, H-2), 5.89 (1H, dt, $J = 7.6$, 14.8 Hz, H-7), 6.37 (1H, d, $J = 14.8$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): 26.2 (C-11), 41.2 (C-3), 48.2 (C-10), 54.3 (C-8), 60.8 (C-12), 116.6 (C-7), 119.2 (C-1), 132.4 (C-2), 134.7 (C-6); HRMS (ES): *m/z* 275.0201 $[M + Na]^+$, $C_9H_{16}NaO_2S_3$ requires 275.0210.

4.10.4. (E,Z)-12-(p-Methoxybenxyloxy)-4,5,9-trithiadodeca-1,6-diene 9-oxide (**4d**)

Obtained as a 2:1 *Z*:*E* mixture (92% and separable) as colourless oils: IR ν_{max} (neat)/cm^{-1} 1030 (S]O), 498 (S–S); (*Z*)-**4d** δ_H (300 MHz, $CDCl_3$): 2.06 (2H, m, H-11), 2.79 (2H, m, H-10), 3.36 (2H, dt, $J = 1.1$, 7.2 Hz, H-3), 3.58 (4H, m, H-8, H-12), 3.80 (3H, s, OCH_3), 4.44 (2H, s, Bn), 5.17 (2H, m, H-1), 5.74 (1H, dt, $J = 8.0$, 9.3 Hz, H-7), 5.82 (1H, m, H-2), 6.54 (1H, dt, $J = 1.1$, 9.3 Hz, H-6), 6.87 (2H, d, $J = 8.7$ Hz, H_m), 7.24 (2H, d, $J = 8.7$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 23.2 (C-11), 42.1 (C-3), 48.7 (C-10), 51.0 (C-8), 55.3 (OCH_3), 68.1 (C-12), 72.7 (Bn), 113.8 (C_m), 118.4 (C-7), 119.2 (C-1), 129.3 (C_o), 130.2 (C_i), 132.6 (C-2), 138.3 (C-6), 159.3 (C_p); HRMS (ES): *m/z* 395.0791 $[M + Na]^+$, $C_{17}H_{24}NaO_3S_3$ requires 395.0785; (*E*)-**4d** δ_H (300 MHz, $CDCl_3$): 2.05 (2H, m, H-11), 2.77 (2H, m, H-10), 3.33 (2H, dt, $J = 1.0$, 7.5 Hz, H-3), 3.44 (1H, ddd, $J = 1.0$, 7.8, 13.1 Hz, H-8**a**), 3.53 (1H, ddd, $J = 1.0$, 7.8, 13.1 Hz, H-8b), 3.59 (2H, m, H-12), 3.80 (3H, s, OCH_3), 4.44 (2H, s, Bn), 5.17 (2H, m, H-1), 5.82 (1H, m, H-2), 5.92 (1H, dt, $J = 7.8$, 14.7 Hz, H-7), 6.35 (1H, dt, $J = 1.0$, 14.7 Hz, H-6), 6.88 (2H, d, $J = 8.7$ Hz, H_m), 7.24 (2H, d, $J = 8.7$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 23.1 (C-11), 41.2 (C-3), 48.3 (C-10), 54.6 (C-8), 55.3 (OCH_3), 68.0 (C-12), 72.7 (Bn), 113.9 (C_m), 117.1 (C-7), 119.2 (C-1), 129.3 (C_o), 130.1 (C_i), 132.5 (C-2), 134.3 (C-6), 159.3 (C_p); HRMS (ES): *m/z* 395.0790 $[M + Na]^+$, $C_{17}H_{24}NaO_3S_3$ requires 395.0785.

4.10.5. (E,Z)-12-Phthalimido-4,5,9-trithiadodeca-1,6-diene 9-oxide (**4e**)

Obtained as a 2:1 *Z*:*E* mixture (64% and separable) as colourless oils: IR ν_{max} (CH_2Cl_2)/cm^{-1} 1047 (S]O), 499 (S–S); (*Z*)-**4e** δ_H (300 MHz, $CDCl_3$): 2.19 (2H, m, H-11), 2.75 (2H, m, H-10), 3.36 (2H, dt, $J = 1.0$, 7.5 Hz, H-3), 3.55 (1H, ddd, $J = 0.9$, 8.0, 13.2 Hz, H-8**a**), 3.64 (1H, ddd, $J = 0.9$, 8.0, 13.2 Hz, H-8b), 3.85 (2H, m, H-12), 5.17 (2H, m, H-1), 5.73 (1H, dt, $J = 8.0$, 9.5 Hz, H-7), 5.82 (1H, m, H-2), 6.54 (1H, dt, $J = 0.9$, 9.5 Hz, H-6), 7.73 (2H, m, Ar-H), 7.86 (2H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 22.3 (C-11), 36.9 (C-12), 42.1 (C-3), 48.9 (C-10), 51.1 (C-8), 118.0 (C-7), 119.2 (C-1), 123.4 (Ar-C), 132.0 (C-2), 132.6 (Ar_{qu}), 134.1 (Ar-C), 138.7 (C-6), 168.2 (C]O); HRMS (ES): *m/z* 382.0583 $[M + H]^+$, $C_{17}H_{20}NO_3S_3$ requires 382.0605; (*E*)-**4e** δ_H (300 MHz, $CDCl_3$): 2.18 (2H, m, H-11), 2.71 (2H, m, H-10), 3.33 (2H, d, $J = 7.3$ Hz, H-3), 3.46 (1H, ddd, $J = 1.1$, 7.9, 13.1 Hz, H-8**a**), 3.54 (1H, ddd, $J = 1.1$, 7.9, 13.1 Hz, H-8b), 3.86 (2H, m, H-12), 5.17 (2H, m, H-1), 5.78 (1H, dt, $J = 7.3$, 9.9 Hz,, H-2), 5.90 (1H, dt, $J = 7.9$, 14.9 Hz, H-7), 6.35 (1H, dt, $J = 0.9$, 14.9 Hz, H-6), 7.73 (2H, m, Ar-H), 7.85 (2H, m, Ar-H), δ_C (100 MHz, $CDCl_3$): 22.2 (C-11), 36.9 (C-12), 41.3 (C-3), 48.5 (C-10), 54.7 (C-8), 116.7 (C-7), 119.2 (C-1), 123.4 (Ar-C), 132.0 (C-2), 132.5 (Ar_{qu}), 134.2 (Ar-C), 134.7 (C-6), 168.3 (C]O); HRMS (ES): *m/z* 382.0583 $[M + H]^+$, $C_{17}H_{20}NO_3S_3$ requires 382.0605.

4.10.6. (E,Z)-10-Phenyl-4,5,9-trithiadeca-1,6-diene 9-oxide (**4f**)

Obtained as a 6:5 *Z*:*E* mixture (65%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1047 (S]O), 493 (S–S); (*Z*)-**4f** δ_H (300 MHz, $CDCl_3$): 3.36 (2H, m, H-3), 3.44 (1H, m, H-8**a**), 3.56 (1H, m, H-8b), 3.98 (2H, s, Bn), 5.18 (2H, m, H-1), 5.76 (1H, m, H-7), 5.84 (1H, m, H-2), 6.57 (1H, d, $J = 9.6$ Hz, H-6), 7.34 (5H, m, Ar-H); δ_C (75 MHz, $CDCl_3$): 42.1 (C-3), 49.8 (C-8), 57.6 (Bn), 118.3 (C-7), 119.2 (C-1), 128.4 (Ar-C), 129.0 (Ar-C), 129.9 (Ar-C), 130.1 (Ar-C), 132.6 (C-2), 138.5 (C-6); HRMS (ES): *m/z* 285.0452 $[M + H]^+$, $C_{13}H_{17}OS_3$ requires 285.0442; (*E*)-**4f** IR ν_{max} (neat)/cm^{-1} 1634 (C]C), 1047 (S]O), 493 (S–S); δ_H (300 MHz, $CDCl_3$): 3.30 (1H, m, H-8**a**), 3.36 (2H, m, H-3), 3.49 (1H, m, H-8b), 3.98 (2H, s, Bn), 5.18 (2H, m, H-1), 5.84 (1H, m, H-2), 5.96 (1H, dt, $J = 7.5$, 14.7 Hz, H-7), 6.36 (1H, d, $J = 14.7$ Hz, H-6), 7.34 (5H, m, Ar-H); δ_C (75 MHz, $CDCl_3$): 41.3 (C-3), 52.9 (C-8), 57.0 (Bn), 117.0 (C-7), 119.2 (C-1), 128.5 (Ar-C), 129.1 (Ar-C), 129.7 (Ar-C), 130.0 (Ar-C), 132.5 (C-2), 134.7 (C-6); HRMS (ES): *m/z* 285.0452 $[M + H]^+$, $C_{13}H_{17}OS_3$ requires 285.0442.

4.10.7. (E,Z)-10-(p-Methoxyphenyl)-4,5,9-trithiadeca-1,6-diene 9-oxide (**4g**)

Obtained as a 2:1 *Z*:*E* mixture (85%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1015 (S]O), 495 (S–S); (*Z*)-**4g** δ_H (300 MHz, $CDCl_3$): 3.37 (2H, m, H-3), 3.43 (1H, ddd, $J = 1.1$, 7.6, 13.5 Hz, H-8**a**), 3.54 (1H, ddd, $J = 1.1$, 7.6, 13.5 Hz, H-8b), 3.80 (3H, s, OCH_3), 3.92 (2H, s, Bn), 5.17 (2H, m, H-1), 5.77 (1H, dt, $J = 7.6$, 9.5 Hz, H-7), 5.83 (1H, m, H-2), 6.56 (1H, dt, $J = 1.1$, 9.5 Hz, H-6), 6.90 (2H, m, H_m); 7.22 (2H, m, H_o); δ_C (100 MHz, $CDCl_3$): 42.1 (C-3), 49.5 (C-8), 55.3 (OCH_3), 56.9 (Bn), 114.5 (C_m), 118.4 (C-7), 119.2 (C-1), 121.6 (C_i), 131.2 (C_o), 132.6 (C-2), 138.2 (C-6), 159.8 (C_p). HRMS (ES): *m/z* 315.0551 $[M + H]^+$, $C_{14}H_{19}O_2S_3$ requires 315.0547; (*E*)-**4g** δ_H (300 MHz, $CDCl_3$): 3.30 (1H, ddd, *J* 1.1, 8.0, 13.5 Hz, H-8**a**), 3.36 (2H, m, H-3), 3.45 (1H, ddd, $J = 1.1$, 8.0, 13.5 Hz, H-8b), 3.80 (3H, s, OCH_3), 3.92 (2H, s, Bn), 5.17 (2H, m, H-1), 5.83 (1H, m, H-2), 5.92 (1H, dt, $J = 8.0$, 14.7 Hz, H-7), 6.35 (1H, dt, $J = 1.1$, 14.7 Hz, H-6), 6.90 (2H, m, H_m), 7.22 (2H, m, H_o); δ_C (100 MHz, $CDCl_3$): 41.3 (C-3), 52.7 (C-8), 55.3 (OCH_3), 56.3 (Bn), 114.5 (C_m), 117.2 (C-7), 119.2 (C-1), 121.5 (C_i), 131.2 (C_o), 132.5 (C-2), 134.4 (C-6), 159.8 (C_p); HRMS (ES): *m/z* 315.0551 $[M + H]^+$, $C_{14}H_{19}O_2S_3$ requires 315.0547.

4.10.8. (E,Z)-4,5,9-Trithiadodeca-6-ene 9-oxide (**4h**)

Obtained as a 2:3 *Z*:*E* mixture (68% and separable) as colourless oils: IR ν_{max} (neat)/cm^{-1} 1045 (S]O), 498 (S–S); (*Z*)-**4h** δ_H (300 MHz, $CDCl_3$): 0.98 (3H, t, $J = 7.2$ Hz, H-1), 1.07 (3H, t, $J = 7.5$ Hz, H-12), 1.69 (2H, sext, $J = 7.2$ Hz, H-2), 1.80 (2H, m, H-11), 2.66 (2H, m, H-10), 2.70 (2H, t, $J = 7.2$ Hz, H-3), 3.54 (1H, ddd, $J = 0.8$, 8.0, 13.2 Hz, H-8**a**), 3.64 (1H, ddd, $J = 0.8$, 8.0, 13.2 Hz, H-8b), 5.74 (1H, dt, $J = 8.0$, 9.5 Hz, H-7), 6.56 (1H, d, $J = 9.5$ Hz, H-6); δ_C (75 MHz, $CDCl_3$): 12.9 (C-1), 13.3 (C-12), 16.2 (C-11), 22.2 (C-2), 41.2 (C-3), 50.8 (C-8), 53.3 (C-10), 117.9 (C-7), 138.7 (C-6); HRMS (ES): *m/z* 146.0211 $[M - PrSOH]^+$, $C_6H_{10}S_2$ requires 146.0224; (*E*)-**4h** δ_H (400 MHz, $CDCl_3$): 0.98 (3H, t, $J = 7.3$ Hz, H-1), 1.08 (3H, t, $J = 7.4$ Hz, H-12), 1.69 (2H, sext, $J = 7.3$ Hz,, H-2), 1.81 (2H, m, H-11), 2.62 (4H, m, H-10), 2.69 (2H, t, $J = 7.3$ Hz, H-3), 3.47 (1H, ddd, $J = 1.0$, 7.8, 13.0 Hz, H-8**a**), 3.53 (1H, ddd, $J = 1.0$, 7.8, 13.0 Hz, H-8b), 5.92 (1H, dt, $J = 7.8$, 14.8 Hz, H-7), 6.38 (1H, d, $J = 14.8$ Hz, H-6); δ_C (100 MHz, $CDCl_3$): 13.0 (C-1), 13.4 (C-12), 16.2 (C-11), 22.4 (C-2), 40.4 (C-3), 53.0 (C-10), 54.6 (C-8), 116.6 (C-7), 134.7 (C-6); HRMS (ES): *m/z* 146.0211 $[M - PrSOH]^+$, $C_6H_{10}S_2$ requires 146.0224.

*4.10.9. (E,Z)-2,3,7-Trithiadeca-4-ene 7-oxide (**4i**) [55]*

Obtained as a 3:2 *Z*:*E* mixture (68%) as a colourless oil: IR ν_{max} (neat)/cm^{-1} 1035 (S]O), 499 (S—S); (*Z*)-**4j** δ_H (400 MHz, $CDCl_3$): 1.03 (3H, t, *J* = 7.4 Hz, H-10), 1.76 (2H, m, H-9), 2.41 (3H, s, H-1), 2.58 (2H, m, H-8), 3.50 (1H, dd, *J* = 7.9, 13.2 Hz, H-6**a**), 3.59 (1H, dd, *J* = 7.9, 13.2 Hz, H-6b), 5.77 (1H, dt, *J* = 7.9, 9.2 Hz, H-5), 6.55 (1H, d, *J* = 9.2 Hz, H-4); δ_C (100 MHz, $CDCl_3$): 13.2 (C-10), 16.1 (C-9), 23.0 (C-1), 50.6 (C-6), 53.2 (C-8), 118.9 (C-5), 137.3 (C-4); HRMS (ES): *m*/*z* 117.9892 $[M - PrSOH]^+$, $C_4H_6S_2$ requires 117.9911; (*E*)-**4j** δ_H (400 MHz, $CDCl_3$): 1.04 (3H, t, *J* = 7.4 Hz, H-10), 1.76 (2H, m, H-9), 2.37 (3H, s, H-1), 2.65 (2H, m, H-8), 3.43 (1H, dd, *J* = 7.7, 13.1 Hz, H-6**a**), 3.52 (1H, dd, *J* = 7.7, 13.1 Hz, H-6b), 5.91 (1H, dt, *J* = 7.7, 15.0 Hz, H-5), 6.34 (1H, d, *J* = 15.0 Hz, H-4); δ_C (100 MHz, $CDCl_3$): 13.2 (C-10), 16.0 (C-9), 21.9 (C-1), 53.0 (C-8), 54.4 (C-6), 116.9 (C-5), 132.9 (C-4); HRMS (ES): *m*/*z* 117.9892 $[M - PrSOH]^+$, $C_4H_6S_2$ requires 117.9911.

*4.10.10. (E,Z)-8-(p-Methoxyphenyl)-1-phenyl-2,3,7-trithiaocta-4-ene 7-oxide (**4j**)*

Obtained as a 3:2 *Z*:*E* mixture (71%) as a white solid which was recrystallized from ethanol, mp 76—78 °C: IR ν_{max} (neat)/cm^{-1} 1034 (S]O), 499 (S—S); (*Z*)-**4j** δ_H (400 MHz, $CDCl_3$): 3.37 (1H, ddd, *J* = 1.1, 7.8, 13.6 Hz, H-6**a**;), 3.47 (1H, ddd, *J* = 1.1, 7.8, 13.6 Hz, H-6b), 3.80 (3H, s, OCH_3), 3.88 (1H, s, H-8**a**), 3.88 (1H, s, H-8b), 3.94 (2H, s, H-1), 5.64 (1H, dt, *J* = 7.8, 9.6 Hz, H-5), 6.25 (1H, dt, *J* = 1.1, 9.6 Hz, H-4), 6.90 (2H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.29 (4H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 43.5 (C-1), 49.5 (C-6), 55.3 (OCH_3), 56.8 (C-8), 114.4 (Ar-C_{OMe}), 118.2 (C-5), 121.6 (Ar-C_{OMe}), 127.6 (Ar-C_H), 128.6 (Ar-C_H), 129.4 (Ar-C_H), 131.2 (Ar-C_{OMe}), 136.6 (Ar-C_H), 137.8 (C-4), 159.8 (Ar-C_{OMe}); HRMS (ES): *m*/*z* 365.0714 $[M + H]^+$, $C_{18}H_{21}O_2S_3$ requires 365.0704; found: C, 59.01%; H, 5.48%; S, 26.49%. $C_{18}H_{20}O_2S_3$ requires C, 59.31%; H, 5.53%; S, 26.38%; (*E*)-**4j** δ_H (400 MHz, $CDCl_3$): 3.20 (1H, ddd, *J* = 1.1, 7.7, 13.4 Hz, H-6**a**), 3.34 (1H, ddd, *J* = 1.1, 7.7, 13.4 Hz, H-6b), 3.81 (3H, s, OCH_3), 3.86 (1H, s, H-8**a**), 3.87 (1H, s, H-8b), 3.93 (2H, s, H-1), 5.82 (1H, dt, *J* = 7.7, 14.8 Hz, H-5), 6.14 (1H, dt, *J* = 1.1, 14.8 Hz, H-4), 6.90 (2H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.29 (4H, m, Ar-H); δ_C (100 MHz, $CDCl_3$): 42.8 (C-1), 52.7 (C-6), 55.3 (OCH_3), 56.2 (C-8), 114.4 (Ar-C_{OMe}), 117.2 (C-5), 121.5 (Ar-C_{OMe}), 127.6 (Ar-C_H), 128.6 (Ar-C_H), 129.4 (Ar-C_H), 131.2 (Ar-C_{OMe}), 134.1 (C-4), 136.6 (Ar-C_H), 159.8 (Ar-C_{OMe}); HRMS (ES): *m*/*z* 365.0714 $[M + H]^+$, $C_{18}H_{21}O_2S_3$ requires 365.0704; found: C, 59.01%; H, 5.48%; S, 26.49%; $C_{18}H_{20}O_2S_3$ requires C, 59.31%; H, 5.53%; S, 26.38%.

*4.10.11. (E,Z)-1-(p-Fluorophenyl)-8-(p-methoxyphenyl)-2,3,7-trithiaocta-4-ene 7-oxide (**4k**)*

Obtained as a 4:3 *Z*:*E* mixture (74%) as a white solid which was recrystallized from ethanol, mp 89—91 °C: IR ν_{max} (neat)/cm^{-1} 1035 (S]O), 497 (S—S); (*Z*)-**4k** δ_H (300 MHz, $CDCl_3$): 3.37 (1H, ddd, *J* = 1.0, 7.8, 13.4 Hz, H-6**a**), 3.46 (1H, ddd, *J* = 1.0, 7.8, 13.4 Hz, H-6b), 3.79 (3H, s, OCH_3), 3.88 (2H, s, H-8), 3.88 (2H, s, H-1), 5.65 (1H, dt, *J* = 7.8, 9.3 Hz, H-5), 6.25 (1H, dt, *J* = 1.0, 9.3 Hz, H-4), 6.89 (2H, m, Ar-H), 6.98 (2H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.26 (2H, m, Ar-H); δ_C (75 MHz, $CDCl_3$): 42.5 (C-1), 49.5 (C-6), 55.2 (OCH_3), 56.8 (C-8), 114.4 (Ar-C_{OMe}), 115.4 (d, J_{CF} = 22.6 Hz, Ar-C_F), 118.5 (C-5), 121.5 (Ar-C_{OMe}), 131.0 (d, J_{CF} = 9.5 Hz, Ar-C_F), 131.2 (Ar-C_{OMe}), 132.4 (d, J_{CF} = 3.3 Hz, Ar-C_F), 137.6 (C-4), 159.7 (Ar-C_{OMe}), 162.3 (d, J_{CF} = 245.1 Hz, Ar-C_F); HRMS (ES): *m*/*z* 383.0610 $[M + H]^+$, $C_{18}H_{20}FO_2S_3$ requires 383.0609; found: C, 56.40%; H, 5.11%; S, 25.25%. $C_{18}H_{19}FO_2S_3$ requires C, 56.68%; H, 5.01%; S, 25.14%; (*E*)-**4k** δ_H (300 MHz, $CDCl_3$): 3.19 (1H, ddd, *J* = 1.1, 7.7, 13.2 Hz, H-6**a**), 3.34 (1H, ddd, *J* = 1.1, 7.7, 13.2 Hz, H-6b), 3.80 (3H, s, OCH_3), 3.88 (2H, s, H-1), 3.89 (2H, s, H-8), 5.83 (1H, dt, *J* = 7.7, 14.9 Hz, H-5), 6.14 (1H, dt, *J* = 1.1, 14.9 Hz, H-4), 6.89 (2H, m, Ar-H), 6.98 (2H, m, Ar-H), 7.20 (2H, m, Ar-H), 7.26 (2H, m, Ar-H); δ_C (75 MHz, $CDCl_3$): 41.7 (C-1), 52.6 (C-6), 55.2 (OCH_3), 56.3 (C-8), 114.4 (Ar-C_{OMe}), 115.4 (d, J_{CF} = 22.6 Hz, Ar-C_F), 117.5 (C-5), 121.4 (Ar-C_{OMe}), 131.0 (d, J_{CF} = 9.5 Hz, Ar-C_F), 131.1 (Ar-C_{OMe}), 132.4 (d, J_{CF} = 3.3 Hz, Ar-C_F), 133.7 (C-4), 159.7 (Ar-C_{OMe}), 162.3 (d, J_{CF} = 245.1 Hz, Ar-C_F); HRMS (ES): *m*/*z* 383.0610 $[M + H]^+$, $C_{18}H_{20}FO_2S_3$ requires 383.0609; found: C, 56.40%; H, 5.11%; S, 25.25%. $C_{18}H_{19}FO_2S_3$ requires C, 56.68%; H, 5.01%; S, 25.14%.

*4.10.12. (E,Z)-1,8-(Bis-p-methoxyphenyl)-2,3,7-trithiaocta-4-ene 7-oxide (E/Z-**4l**)*

Obtained as a 2:3 *Z*:*E* mixture (78%) as a white solid which was recrystallized from ethanol, mp 111—113 °C: IR ν_{max} (neat)/cm^{-1} 1035 (S]O), 499 (S—S); (*Z*)-*E*/*Z*-**4l** δ_H (400 MHz, $CDCl_3$): 3.41 (1H, ddd, *J* = 1.0, 7.8, 13.4 Hz, H-6**a**), 3.51 (1H, ddd, *J* = 1.0, 7.8, 13.4 Hz, H-6b), 3.82 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 3.89 (1H, s, H-8**a**), 3.90 (1H, s, H-8b), 3.93 (2H, s, H-1), 5.69 (1H, dt, *J* = 7.8, 9.6 Hz, H-5), 6.30 (1H, dt, *J* = 1.0, 9.6 Hz, H-4), 6.86 (2H, d, *J* = 8.8 Hz, H_m), 6.92 (2H, d, *J* = 8.8 Hz, H_m), 7.23 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 43.1 (C-1), 49.5 (C-6), 55.2 (OCH_3), 55.3 (OCH_3), 56.8 (C-8), 114.0 (C_m), 114.5 (C_m), 118.1 (C-5), 121.7 (C_i), 128.6 (C_i), 130.6 (C_o), 131.3 (C_o), 138.0 (C-4), 159.2 (C_p), 159.8 (C_p); HRMS (ES): *m*/*z* 395.0808 $[M + H]^+$, $C_{19}H_{23}O_3S_3$ requires 395.0809; found: C, 57.76%; H, 5.60%; S, 24.21%; $C_{19}H_{22}O_3S_3$ requires C, 57.84%; H, 5.62%; S, 24.38%; (*E*)-*E*/*Z*-**4l** IR ν_{max} (neat)/cm^{-1} 1611 (C]C), 1035 (S]O), 499 (S—S); δ_H (400 MHz, $CDCl_3$): 3.24 (1H, ddd, *J* = 1.0, 7.8, 13.2 Hz, H-6**a**), 3.38 (1H, ddd, *J* = 1.0, 7.8, 13.2 Hz, H-6b), 3.79 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.91 (1H, s, H-8**a**), 3.92 (1H, s, H-8b), 3.92 (2H, s, H-1), 5.86 (1H, dt, *J* = 7.8, 14.8 Hz, H-5), 6.19 (1H, dt, *J* = 1.0, 14.8 Hz, H-4), 6.86 (2H, d, *J* = 8.8 Hz, H_m), 6.93 (2H, d, *J* = 8.8 Hz, H_m), 7.23 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 42.3 (C-1), 52.7 (C-6), 55.2 (OCH_3), 55.3 (OCH_3), 56.1 (C-8), 114.0 (C_m), 114.5 (C_m), 117.0 (C-5), 121.5 (C_i), 128.6 (C_i), 130.6 (C_o), 131.2 (C_o), 134.2 (C-4), 159.2 (C_p), 159.8 (C_p); HRMS (ES): *m*/*z* 395.0808 $[M + H]^+$, $C_{19}H_{23}O_3S_3$ requires 395.0809; found: C, 57.76%; H, 5.60%; S, 24.21%. $C_{19}H_{22}O_3S_3$ requires C, 57.84%; H, 5.62%; S, 24.38%.

4.11. Synthesis of sulfenylating agents p-TsSR^2

To a solution of potassium *p*-toluenethiosulfonate (1.3 equiv) dissolved in DMF (1 M) was added R^2X (X = Cl or Br; 1.0 equiv) neat or in solution (DMF) slowly via syringe. The reaction was stirred at room temperature for 3 h or heated according to conversion of R^2X by TLC before being quenched with saturated aqueous $NaHCO_3$. The resulting mixture was extracted with dichloromethane (3 times) and the combined organic extracts were dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue purified on a silica-gel column using hexane/ethyl acetate mixtures to afford the desired compound.

4.11.1. Benzenesulfonothioic acid, S-2-propen-1-yl ester [56]

Obtained as a clear oil (96%): δ_H (300 MHz, $CDCl_3$): 2.44 (3H, s, CH_3), 3.66 (2H, dd, *J* = 1.0, 7.2 Hz, H-1′), 5.08 (1H, d, *J* = 10.5 Hz, H-3′), 5.19 (1H, d, *J* = 17.1 Hz, H-3′) 5.70 (1H, m, H-2′), 7.33 (2H, d, *J* = 7.8 Hz, H_m), 7.79 (2H, d, *J* = 7.8 Hz, H_o).

4.11.2. Benzenesulfonothioic acid, S-propyl ester [56]

Obtained as a pale-yellow oil (96%): δ_H (400 MHz, $CDCl_3$): 0.93 (3H, t, *J* = 7.3 Hz, H-3′), 1.64 (2H, s, *J* = 7.3 Hz, H-2′), 2.45 (3H, s, CH_3), 2.97 (2H, t, *J* = 7.3 Hz, H-1′), 7.34 (2H, d, *J* = 8.0 Hz, H_m), 7.81 (2H, d, *J* = 8.0 Hz, H_o); δ_C (100 MHz, $CDCl_3$): 13.1 (C-3′), 21.5 (C-2′), 22.1 (CH_3), 37.9 (C-1′), 126.9 (C_o), 129.7 (C_m), 142.1 (C_i), 144.6 (C_p).

4.11.3. Benzenesulfonothioic acid, S-methyl ester [57]

Obtained as a colourless oil (96%): δ_H (300 MHz, $CDCl_3$): 2.43 (3H, s, CH_3), 2.48 (3H, s, CH_3), 7.34 (2H, d, *J* = 8.1 Hz, H_m), 7.78 (2H, d, *J* = 8.1 Hz, H_o); δ_C (100 MHz, $CDCl_3$): 17.9 (CH_3), 21.5 (CH_3), 127.0 (C_o), 129.8 (C_m), 140.8 (C_i), 144.8 (C_p).

4.11.4. Benzenesulfonothioic acid, S-[(4-fluorophenyl)methyl] ester [58]

Obtained as a white solid (78%): δ_H (400 MHz, $CDCl_3$): 2.43 (3H, s, CH_3), 4.23 (2H, s, CH_2), 6.91 (2H, m, H-3′), 7.15 (2H, m, H-2′), 7.27 (2H, d, $J = 8.6$ Hz, H_m), 7.71 (2H, d, $J = 8.6$ Hz, H_o).

4.11.5. Benzenesulfonothioic acid, S-[(4-methoxyphenyl)methyl] ester [59]

Obtained as a white solid (74%): δ_H (400 MHz, $CDCl_3$): 2.44 (3H, s, CH_3), 3.77 (3H, s, OCH_3), 4.21 (2H, s, CH_2), 6.76 (2H, d, $J = 8.6$ Hz, H-3′), 7.10 (2H, d, $J = 8.6$ Hz, H-2′), 7.28 (2H, d, $J = 8.6$ Hz, H_m), 7.74 (2H, d, $J = 8.6$ Hz, H_o).

4.11.6. Benzenesulfonothioic acid, S-benzyl ester [60]

Obtained as a white solid (95%): δ_H (400 MHz, $CDCl_3$): 2.43 (3H, s, CH_3), 4.25 (2H, s, CH_2), 7.20 (5H, m, Ph), 7.27 (2H, d, $J = 8.6$ Hz, H_m), 7.73 (2H, d, $J = 8.6$ Hz, H_o).

4.12. Synthesis of derivatives of E/Z-4l

4.12.1. (E,Z)-1,8-(Bis-p-methoxyphenyl)-2,6-dithiahepta-3-ene (5) [61]

A solution of *S*-3-(*p*-methoxyphenylthio)-prop-1enyl ethanethioate **2g** (295 mg, 1.1 mmol) in methanol (2 mL) was slowly added at 0 °C to a degassed solution of KOH (123 mg, 2.2 mmol) in methanol (3 mL). The resulting mixture was stirred at 0 °C under a nitrogen atmosphere for 10 min. *p*-Methoxybenzyl chloride (157 mg, 1.0 mmol) in methanol (1 mL) was then added and the mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the resultant residue was added to water (20 mL) and the solution acidified with hydrochloric acid. The compound was extracted with dichloromethane (3×20 mL), dried with $MgSO_4$ and the solvent removed by rotary evaporation. The crude product was purified by silica-gel column chromatography using petroleum ether/ethyl acetate mixtures to afford a 3:2 *Z*:*E* mixture of compound **5** (0.277 g, 80%) as a clear oil: IR ν_{max} (CH_2Cl_2)/cm^{-1} 690 (C—S); (*Z*)-**5** δ_H (400 MHz, $CDCl_3$): 3.08 (2H, d, $J = 7.2$ Hz, H-5), 3.48 (2H, s, H-7), 3.66 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 3.73 (2H, s, H-1), 5.50 (1H, m, H-4), 5.99 (1H, d, $J = 9.2$ Hz, H-3), 6.74 (4H, m, H_m), 7.11 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 30.0 (C-5), 35.1 (C-7), 37.6 (C-1), 55.2 ($OCH_3 \times 2$), 114.0 ($C_m \times 2$), 126.1 (C-4), 127.0 (C-3), 129.2 (C_i),130.0 ($C_o \times 2$), 130.4 (C_i), 158.6 (C_p), 158.8 (C_p); (*E*)-**5** δ_H (400 MHz, $CDCl_3$): 2.92 (2H, d, $J = 7.2$ Hz, H-5), 3.43 (2H, s, H-7), 3.67 (3H, s, OCH_3), 3.69 (3H, s, OCH_3),3.75 (2H, s, H-1), 5.50 (1H, m, H-4), 5.89 (1H, d, $J = 15.6$ Hz, H-3), 6.74 (4H, m, H_m), 7.11 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 33.5 (C-5), 34.3 (C-7), 36.5 (C-1), 55.2 ($OCH_3 \times 2$), 113.8 ($C_m \times 2$), 125.2 (C-4), 126.1 (C-3), 129.2 (C_i), 129.9 ($C_o \times 2$), 130.4 (C_i), 158.6 (C_p), 158.8 (C_p); HRMS (ES): *m*/*z* 347.1174 $(M + H)^+$, $C_{19}H_{23}O_2S_2$ requires 347.1139.

4.12.2. (E,Z)-1,7-(Bis-p-methoxyphenyl)-2,6-dithiahepta-3-ene 6-oxide (6)

To a 0 °C solution of (*E*,*Z*)-1,8-(bis-*p*-methoxyphenyl)-2,6-dithiahepta-3-ene (115 mg, 0.33 mmol) in dichloromethane (3 mL) was added solid *m*-chloroperoxybenzoic acid (67 mg, 0.30 mmol). The solution was stirred for 1 h, after which aqueous $NaHCO_3$ (8 mL) was added and the compound extracted with dichloromethane (3×15 mL). The combined organic extracts were dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was purified by silica-gel chromatography using petroleum ether/ethyl acetate mixtures to afford compound (*Z*/*E*)-**6** as separate isomers. (*Z*)-**6** (0.054 g, 50%) was obtained as a white solid which was recrystallized from ethanol, mp 78—80 °C; IR ν_{max} (CH_2Cl_2)/cm^{-1} 1033 (S]O), 690 (C—S); δ_H (300 MHz, $CDCl_3$): 3.41 (1H, dd, $J = 7.8$, 13.2 Hz, H-5a), 3.52 (1H, dd, $J = 7.8$, 13.2 Hz, H-5b), 3.75 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.81 (2H, m, H-7), 3.87 (2H, s, H-1), 5.70 (1H, dt, $J = 7.8$, 9.6 Hz, H-4), 6.42 (1H, d, $J = 9.6$ Hz, H-3), 6.83 (2H, d, $J = 8.7$ Hz, H_m), 6.87 (2H, d, $J = 8.7$ Hz, H_m), 7.18 (2H, d, $J = 8.7$ Hz, H_o), 7.21 (2H, d, $J = 8.7$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 37.6 (C-1), 50.4 (C-5), 55.2 (OCH_3), 56.6 (C-7), 114.1 (C_m), 114.3 (C_m), 116.2 (C-4), 122.0 (C-3), 129.3 (C_i), 129.9 (C_o), 131.3 (C_o), 132.9 (C_i), 158.9 (C_p), 159.6 (C_p); HRMS (ES): *m*/*z* 363.1082 $(M + H)^+$, $C_{19}H_{23}O_3S_2$ requires 363.1089; found: C, 62.79%; H, 6.09%; S, 17.50%. $C_{19}H_{22}O_3S_2$ requires C, 62.95%; H, 6.12%, S; 17.69%; (*E*)-**6** (0.035 g, 32%) was obtained as a white solid which was recrystallized from ethanol, mp 90—92 °C; IR ν_{max} (CH_2Cl_2)/cm^{-1} 1033 (S]O), 690 (C—S); δ_H (300 MHz, $CDCl_3$): 3.24 (1H, dd, $J = 7.6$, 13.2 Hz, H-5a), 3.38 (1H, dd, $J = 7.6$, 13.2 Hz, H-5b), 3.76 (3H, s, OCH_3), 3.80 (5H, m, OCH_3 and H-7), 3.90 (2H, s, H-1), 5.56 (1H, dt, $J = 7.6$, 15.3 Hz, H-4), 6.29 (1H, d, $J = 15.3$ Hz, H-3), 6.85 (2H, d, $J = 8.6$ Hz, H_m), 6.88 (2H, d, $J = 8.6$ Hz, H_m), 7.13 (2H, t, $J = 8.6$ Hz, H_o), 7.26 (2H, t, $J = 8.6$ Hz, H_o); δ_C (75 MHz, $CDCl_3$): 36.2 (C-1), 53.6 (C-5), 55.2 (OCH_3), 55.7 (C-7), 114.1 (C_m), 114.2 (C-4), 114.4 (C_m), 121.7 (C-3), 128.6 (C_i), 129.9 (C_o), 131.2 (C_o), 132.9 (C_i), 158.9 (C_p), 159.7 (C_p); HRMS (ES): *m*/*z* 363.1082 $(M + H)^+$, $C_{19}H_{23}O_3S_2$ requires 363.1089; found: C, 62.61%; H, 5.97%; S, 17.32%. $C_{19}H_{22}O_3S_2$ requires C, 62.95%; H, 6.12%; S, 17.69%.

4.12.3. 1,8-(Bis-p-methoxyphenyl)-2,3,7-trithiaoctane (7) [62]

A solution of *p*-methoxybenzyl chloride (157 mg, 1.00 mmol) in ethanol (2 mL) was added dropwise at room temperature to a solution of 1,3-propanedithiol (119 mg, 1.10 mmol) and KOH (112 mg, 2.00 mmol) in ethanol (3 mL) under a N_2 atmosphere. The mixture was allowed to stir for 3 h after which a solution of *S*-*p*-methoxybenzyl *p*-toluenesulfonylthioate (339 mg, 1.1 mmol) in ethanol (2 mL) was added. The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was added to water (20 mL) and extracted with dichloromethane (3×20 mL). The combined organic fractions were then washed with water (30 mL), brine (20 mL) and aqueous KOH (20 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude residue was purified by silica-gel column chromatography using petroleum ether/ethyl acetate mixtures to afford compound **7** (0.323 g, 85%) as a white solid which was recrystallized from ethanol, mp 41—43 °C; IR ν_{max} (CH_2Cl_2)/cm^{-1} 516 (S—S); δ_H (400 MHz, $CDCl_3$): 1.86 (2H, quint, $J = 7.1$ Hz, H-5), 2.46 (2H, t, $J = 7.1$ Hz, H-6), 2.51 (2H, t, $J = 7.1$ Hz, H-4), 3.68 (2H, s, H-8), 3.83 (6H, s, OCH_3), 3.88 (2H, s, H-1), 6.88 (4H, d, $J = 8.4$ Hz, H_m), 7.26 (4H, m, H_o); δ_C (100 MHz, $CDCl_3$): 28.2 (C-5), 29.6 (C-6), 35.5 (C-8), 37.0 (C-4), 43.0 (C-1), 55.2 ($OCH_3 \times 2$), 113.8 (C_m), 113.9 (C_m), 129.3 (C_i), 129.8 (C_o), 130.2 (C_i), 130.3 (C_o), 158.5 (C_p), 159.0 (C_p); HRMS (ES): *m*/*z* 419.0794 $(M + Na + O)^+$, $C_{19}H_{24}NaO_3S_3$ requires 419.0785; found: C, 59.95%; H, 6.33%; S, 25.30%. $C_{19}H_{24}O_2S_3$ requires C, 59.96%; H, 6.36%; S, 25.27%.

4.12.4. 1,8-(Bis-p-methoxyphenyl)-2,3,7-trithiaoctane 7-oxide (8)

To a 0 °C solution of 1,8-(bis-*p*-methoxyphenyl)-2,3,7-trithiaoctane (75 mg, 0.20 mmol) in dichloromethane (2 mL) was added solid *m*-chloroperoxybenzoic acid (32 mg, 0.14 mmol). The solution was stirred for 1 h, after which aqueous $NaHCO_3$ (8 mL) was added and the compound extracted with dichloromethane (3×15 mL). The combined organic extracts were dried with $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was purified by silica-gel chromatography using petroleum ether/ethyl acetate mixtures to afford compound **8** (0.050 g, 89%) as a white solid: recrystallized from ethanol/CH_2Cl_2, mp 108—110 °C; IR ν_{max} (CH_2Cl_2)/cm^{-1} 1033 (S]O), 517 (S—S); δ_H (400 MHz, $CDCl_3$): 2.06 (2H, m, H-5), 2.50 (2H, t, $J = 6.8$ Hz, H-4), 2.57 (1H, m, H-6a), 2.65 (1H, m, H-6b), 3.81 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.86 (2H, s, H-1), 3.92 (1H, d, $J = 13.2$ Hz, H-8a), 3.96 (1H, d, $J = 13.2$ Hz, H-8b), 6.87 (2H, d, $J = 8.0$ Hz, H_m), 6.92 (2H, d, $J = 8.0$ Hz, H_m), 7.22 (2H, d, $J = 8.0$ Hz, H_o), 7.23 (2H, d, $J = 8.0$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 21.8

(C-5), 36.9 (C-4), 42.9 (C-1), 48.9 (C-6), 55.2 ($OCH_3 \times 2$), 57.6 (C-8), 114.0 (C_m), 114.4 (C_m), 121.5 (C_i), 129.2 (C_i), 130.4 (C_o), 131.1 (C_o), 159.1 (C_p), 159.7 (C_p); HRMS (ES): m/z 419.0737 $(M+Na)^+$, $C_{19}H_{24}NaO_3S_3$ requires 419.0785; found: C, 57.71%; H, 6.17%; S, 24.30%. $C_{19}H_{24}O_3S_3$ requires C, 57.54%; H, 6.10%; S, 24.25%.

*4.12.5. N-Boc-L-cysteine methoxybenzyl disulfide (**9**)*

To a solution of *E*/*Z*-**4l** (174 mg, 0.44 mmol) in THF (5 mL) was added *N*-Boc-L-cysteine (55 mg, 0.22 mmol) dissolved in THF (0.5 mL) at room temperature under a nitrogen atmosphere. The solution was heated to reflux (70 °C) for 25 h. The mixture was evaporated under reduced pressure and the residue purified by silica-gel column chromatography to afford recovered starting materials *N*-Boc-L-cysteine (12 mg, 22% recovered) and *E*/*Z*-**4l** (96 mg, 55% recovered) as well as product **9** (36 mg, 52% based on a 78% conversion of the cysteine) as a clear oil: IR ν_{max} $(CHCl_3)/cm^{-1}$ 3364 (NH), 1716 (C]O), 1634 (C]O), 497 (S–S); δ_H (400 MHz, $CDCl_3$): 1.25 (3H, t, $J = 7.1$ Hz, H-3^b), 1.42 (9H, s, -C($\underline{C}H_3)_3$), 2.85 (2H, m, H-1), 3.77 (3H, s, OCH_3), 3.84 (2H, s, Bn), 4.16 (2H, qu, $J = 7.1$ Hz, H-3^a), 4.46 (1H, m, H-2), 5.24 (1H, br. s., NH), 6.82 (2H, d, $J = 8.8$ Hz, H_m), 7.21 (2H, d, $J = 8.8$ Hz, H_o); δ_C (100 MHz, $CDCl_3$): 14.1 (C-3^b), 28.3 (–C($\underline{C}H_3)_3$), 40.9 (C-1), 43.0 (Bn), 53.0 (C-2), 55.2 (OCH_3), 61.7 (C-3^a), 80.1 (–$\underline{C}(CH_3)_3$), 114.0 (C_m), 128.9 (C_i), 130.5 (C_o), 155.0 (C]O), 159.2 (C_p), 170.7 (C-3); HRMS (ES): m/z 424.1244 $(M+Na)^+$, $C_{18}H_{27}NNaO_5S_2$ requires 424.1228.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.01.058.

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