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Substituted ajoenes as novel anti-cancer agents

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ABSTRACT

A new synthesis of the ajoene pharmacophore core is presented involving the regioselective radical addition of a thiyl radical to a terminal alkyne as the key step. The synthesis allows structural variation of the two end groups on sulfur, and a range of novel derivatives varying the R^1 group (sulfoxide end) has been prepared and tested against CT-1 transformed fibroblast cells for anti-cancer activity. The results indicate comparable or even improved activity compared to the parent natural product ajoene isomers. This opens up the way to systematically studying the biology of the ajoene core.

Ajoene is a stable sulfoxide rearrangement product of allicin, a natural product found in freshly crushed garlic (Fig. 1), whose structure was established in the mid 1980s following seminal work by Block and Apitz-Castro.[1] It possesses an intriguing allyl vinyl disulfide functional grouping, which is likely to account for its range of biological activities via acting as a sulfenylating agent towards protein sulfhydryl groups.[2] Originally, the focus of its biological activity centered around its anti-thrombotic activity,[3] but in subsequent years it has been demonstrated to possess a range of other biological activities to include antimicrobial,[4] anti-obesity,[5] antifungal,[6] and anti-cancer[7] activities.

Regarding the latter, while garlic dietary supplements and extracts have been reported to reduce the risk of cancer[8] as well as alter the activation of several carcinogens[9] and to cause growth inhibition and/or death of tumor cells,[10] the overall verdict on garlic is controversial. The fact that crude extracts of garlic contain numerous organosulfur compounds with varying stability and biological activity has made it impossible to reach any firm conclusions from clinical trials[11] about the chemopreventative effect of garlic.

By comparison, the fact that ajoene is a relatively stable compound has allowed more accurate data to be collected, and it has been demonstrated that it is able to induce apoptosis in a number of tumor cell-lines.[12] Ajoene has been shown to offer strong protection against TPA-promoted carcinogenesis on the mouse skin, and to strongly inhibit metastasis to lung in the B16/BL6 melanoma tumor model in C57BL/6 mice.[13] Topical application of ajoene to the tumors of 21 human patients with either nodular or superficial basal cell carcinoma caused a reduction in tumor size in 17/21 patients.[14] Ajoene has been shown to induce apoptosis and arrest HL60 leukemia cells in the G_2/M phase of the cell cycle in a dose-dependent manner,[12c,12d] and ajoene-treated leukemia cells have been shown to undergo a time-dependent reduction in the anti-apoptotic bcl-2 protein that results in release of cytochrome c and the activation of caspase-3.[1,2] These results support the hypothesis that ajoene-induced apoptosis in leukemia cells proceeds via the mitochondria-dependent caspase cascade pathway rather than the triggering of cell-surface death receptors. Ajoene has also been shown to decrease the expression of $\alpha_4\beta_1$ integrin in murine melanoma cells,[12e] and to induce complete disassembly of the microtubule network in HL60 cells.[12c]

The only synthesis published in the literature to date for ajoene is the biomimetic thermal rearrangement of allicin in aqueous acetone due to Block in his original work.[1] Although the synthesis is a one-pot conversion, the synthesis suffers from being low-yielding as well as not being realistically amenable to producing structural

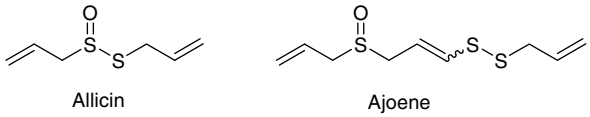

Figure 1. Structures of garlic-derived allicin and ajoene.

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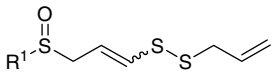
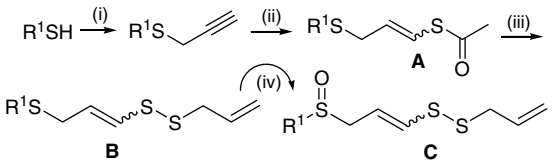


Figure 2. General structure of substituted ajoenes available from the present synthesis.



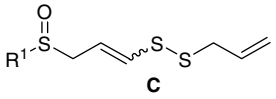
Scheme 1. Reagents and conditions: (i) KOH, MeOH, propargyl bromide; (ii) CH_3COSH (1.1 equiv), AIBN (2 mol%), Tol, 85 °C; (iii) (a) KOH (1.05 equiv), MeOH, −78 °C; (b) *p*-$TolSO_2Sallyl$ (1.1 equiv); (iv) *m*-CPBA (1.1 equiv), CH_2Cl_2, −78 °C to rt.

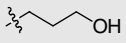
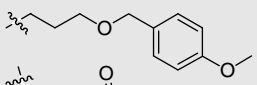
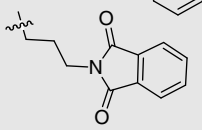
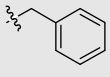
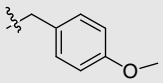
variants. In this communication, we report on the first synthetic sequence[15] that can access a range of ajoene derivatives containing the central vinyl disulfide/sulfoxide core while varying the end group R^1 (Fig. 2).

For the purpose of preliminary biological investigation, it was elected to change only the R^1 group and maintain the other end-group as allyl as in the parent ajoene. The key step in the synthesis utilizes a regioselective radical addition of thiolacetic acid to a terminal alkyne to generate a vinyl thioacetate based on the known addition[16] of thiolacetic acid to 1-hexyne as a model reaction. Scheme 1 summarizes the overall reaction sequence.

Thus, propargylation of thiol R^1SH available commercially or synthesized via an isothiouronium salt formed from the bromide or tosylate (R^1X), followed by regioselective radical addition of thiolacetic acid to the terminus of the triple bond generated vinylthioacetate **A**. Optimal conditions for the key step were identified in order to maximize both alkyne conversion as well as the yield of the mono-addition product. These involved dropping thiolacetic acid (1.1 equiv) into the alkyne and the initiator in deoxygenated toluene at 85 °C over 1 h and continuing heating (1–2 h) until TLC indicated maximum conversion. Yields for the radical addition step ranged routinely in around the 60–70% region after chromatography, and the vinylthioacetates **A** were obtained as a mixture of *E*/*Z* stereoisomers, with the *Z*-isomer predominating (~2:1) as reported for the free-radical addition of thiolacetic acid to 1-hexyne.[16] *E*/*Z*-Stereochemistry was assigned on the basis of the vinyl coupling constants as ~15 Hz for the *E*-isomer and ~10 Hz for the *Z*-isomer. The stereoselectivity is consistent with the quenching of the intermediate vinyl radicals (*E* and *Z*) by thiolacetic acid to be kinetically favoured for the *Z*-radical intermediate in view of minimized steric interaction between the attached thioacetate

Table 1
IC_{50} of ajoene derivatives of **C** against CT-1 fibroblast cells



No.	Name	$-R^1$	N[b]	CT-1	
				IC_{50} (M)	95% CI[c] (M)
1	*E*-ajoene		6	17.6	17.4–17.8
2	*Z*-ajoene		6	15.5	15.3–15.6
3	*E*-propyl		5	26.7	26.5–26.9
4	*Z*-propyl		5	17.0	16.8–17.2
5	*E*/*Z*-*tert*-butyl		5	33.1	32.5–33.6
6	*E*-OH[a]		5	23.1	23.0–23.3
7	*Z*-OH[a]		6	22.8	22.5–23.1
8	*E*-OPMB		5	23.5	23.2–23.8
9	*Z*-OPMB		6	21.7	21.6–21.9
10	*E*-phthal		4	95.9	95.0–96.8
11	*Z*-phthal		4	34.6	34.3–34.9
12	*E*/*Z*-benzyl		5	16.6	16.5–16.7
13	*E*/*Z*-PMB		12	11.2	11.1–11.3

[a] Prepared via deprotection of its TBDMS ether with HF/CH_3CN.
[b] Number of independent experimental observations.
[c] 95% Confidence interval in micromolar.

group and incoming thiolacetic acid in the hydrogen-quenching propagation step. Generally, the stereoisomers could not be separated by column chromatography and were characterized as a mixture. Subsequent thioacetate deprotection with hydroxide in methanol at low temperature (−78 °C) followed by sulfenylation of the enethiolate with S-allyl p-toluenesulfonylthioate[17] afforded a high yield (>90%) of the vinyl disulfide **B** (R^2 = allyl) as the same E/Z-mixture, the latter indicating sulfenylation to be faster than isomerization of the intermediate enethiolate. Finally, chemoselective oxidation of **B** with *m*-CPBA (1.1 equiv) afforded the target ajoene derivative **C** in the same *E*/*Z*-ratio as that in **B**. Yield for the latter step varied (60–90%) and the optimal temperature range for the reaction was highly substrate specific. Thus, the overall yield for the synthesis was usually no less than about 35%. In most cases (exceptions = entries 5, 12, and 13 in Table 1), the *E*- and *Z*-isomers of **C** could be separated by slow column chromatography or preparative TLC. This was considered to be important because the two stereoisomers of ajoene have been shown[1] to have significantly different biological activities. Unfortunately, the parent *E*- and *Z*-ajoenes could not be prepared via this synthetic sequence because the intermediate vinyl radical precursor to **A** presumably cyclizes onto the allyl group double bond of R^1 via either a 5-*exo*- *or* 6-*endo*-*trig* process but this aspect was not investigated further. A preliminary investigation into the use of a protecting group on R^1 to circumvent this problem failed to resolve the issue. Thus, the parent ajoenes (**1** and **2** in Table 1) were prepared by the method of Block.[1] The final products **C** were characterized (see Annexure Supplementary data) by the normal range of spectroscopic (^{1}H and ^{13}C NMR) and analytical techniques including high resolution mass spectrometry in view of their nature as oils. A particularly diagnostic set of resonances for **C** pertained to the methylene and two vinyl protons of the vinyl disulfide grouping in the ^{1}H NMR spectrum in which a consistent coupling set could be identified for each derivative. Finally, the synthesis should also be amenable to changing the other allyl end-group that was kept constant in this particular study.

The R^1 group was varied in order to investigate the influence of lipophilicity on the anti-cancer activity against CT-1 transformed fibroblast cells with the individual ajoene isomers as the reference standard. Table 1 summarizes the various derivatives synthesized and their IC50 values against the CT-1 cell-line.

A number of interesting points emerge from analysis of the IC_{50} values depicted for the small library listed in Table 1. Firstly, in general, retention of anti-cancer activity for the derivatives at a level similar to that of the parent ajoenes was noted, supporting the idea that the central pharmacophore resides in the vinyl disulfide grouping. Moreover, there are various literature reports on the anti-cancer activity of *Z*-ajoene[7,12] but no general data on the activity of the *E*-isomer. The literature indicates that *Z*-ajoene is generally more potent than its *E*-isomer for a range of diseases, but interestingly in the current study, the anti-cancer activity of *E*-ajoene was found to be only marginally less than that of *Z*-ajoene (17.6 vs 15.5 μM). This trend was also observed (Table 1) for most of the synthesized ajoene analogues, namely entries **3** and **4**, (26.7 vs 17.0 μM), **6** and **7** (23.1 vs 22.8 μM), and **8** and **9** (23.5 vs 21.7 μM). A notable exception were the *E*- and *Z*-phthalimidopropyl derivatives in entries **10** and **11** (95.9 vs 34.6 μM). The data suggests that a shape-selectivity at a binding site may be an important parameter for anti-cancer activity. Only one of the derivatives as the E/Z-mixture of entry **13** (R^1 = *p*-methoxybenzyl) returned a higher activity (11.2 μM) than that of *Z*-ajoene (15.5 μM), suggesting this compound to be a lead for further study.

Ajoene itself degrades over time when stored at −20°C neat, as observed by the formation of a less polar TLC product. Based on TLC studies, in general, R^1 substitution of allyl in ajoene offers an increased chemical stability of the analogues over the parent ajoene. In summary, the synthesis described herein[18] opens up the way for a more comprehensive study to be carried out involving a broader range of derivatives and cell-lines in the hope of identifying synthetic ajoenes with significantly improved anti-cancer activity. Furthermore, it will allow an appraisal of substituted ajoenes as chemosensitizing agents for established drugs like cytarabine towards drug-resistant tumors to be carried out.[7d]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.056.

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18. A patent on the synthesis and the derivatives prepared has been filed under the name of the University of Cape Town entitled 'Compounds useful for the inhibition of the growth of tumor cells'. South African Patent Application No 2008/06780. Inventors: Roger Hunter, Mohamed Iqbal Parker, Catherine Hart Kaschula.