Synthesis of Ajoene Analogues by Novel Synthetic Strategies

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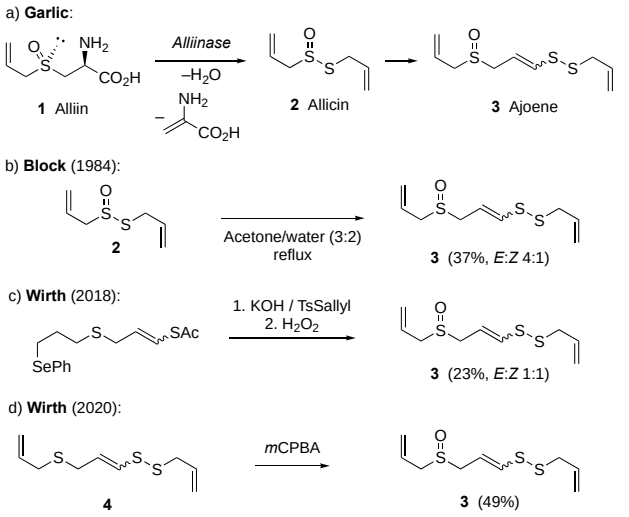
Abstract: Ajoene is a compound found in garlic extracts exhibiting a large range of biological activity. Novel ajoene analogues have been prepared in the search of compounds with superior bioactivity. Modifications include the alteration of the sulfoxide, the central alkene and the terminal allyl groups.

Garlic extracts are known for a very long time to exhibit a wide range of biological properties. Ajoene (**3**) is a molecule which is formed from alliin **1** and allicin **2** as naturally occurring compounds in garlic, and has potent antithrombotic inhibitory properties (Scheme 1a).[1] (*E*/*Z*)-Ajoene **3** was first observed in 1983 by Apitz-Castro[2] and was fully characterised by Block in 1984.[1] Block and co-workers have obtained (*E*/*Z*)-**3** through a thermal rearrangement of allicin (**2**) (Scheme 1b). Although Block isolated **3** in 37% yield as a mixture of its geometric isomers, many other rearrangement and decomposition products were also obtained, including mono and polysulfides as well as vinyl-dithiins.
In 2018, we reported the first total synthesis of **3** (Scheme 1c). The chemical total synthesis overcame obstacles such as the instability and volatility of **2** by replacing allicin with alternative starting materials to develop a reliable and robust five step synthesis.[3] More recently, the precursor **4** was synthesised in a flow process and also converted to **3** (Scheme 1d).[4]



Scheme 1. Synthetic routes to ajoene (**3**): a) Biosynthetic route in garlic. b) Synthesis described by Block *et al.* in 1984 by thermal rearrangement of **2** in aqueous acetone. c,d) Synthesis of **3** by Wirth and coauthors.

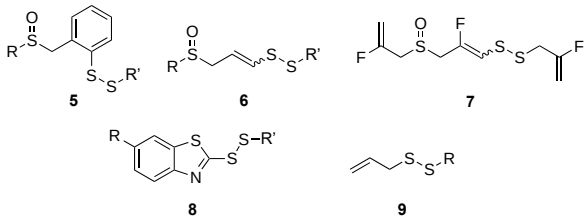


Figure 1. Reported analogues of Ajoene **3**.

In recent years there has been increased activities in the synthesis of non-natural compounds based on the core structure of **3** and attempts to generate compounds with superior biological activity (Figure 1). In 1986, Block *et al.* extended their work by developing ajoene analogues of type **5** containing a central aromatic moiety.[5] In 2008 and 2012, Hunter *et al.* reported the synthesis of analogues **6** with terminal end modifications.[6] More recently, Block *et al.* reported the rearrangement of fluorinated allicin to yield fluorinated ajoene **7**.[7] Additionally, Fong *et al.* reported ajoene analogues **8** and **9** which are based on disulfides.[8] An aryl-substituted analogue has also been investigated.[9] Gruhlke and co-authors have very recently reported sulfilimine analogues of allicin.[10] Compounds **5**–**9** have been biologically evaluated and compared to the activity of **3**. Different assays have been used and some derivatives have shown promising activity.

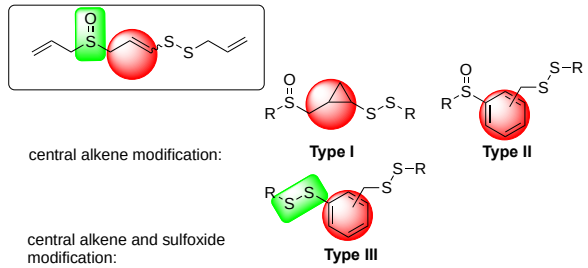


Figure 2. Library of ajoene analogues incorporating modifications at the central alkene and the sulfoxide (Types I-III).

We will expand upon current analogues to produce a wide library of novel ajoene analogues which are reported here. Biological activities of all analogues have been evaluated and compared to the parent compound ajoene **3**. The first series of analogues have incorporated a modification in the central alkene unit and at least one terminal group R. The central alkene has been replaced by a cyclopropyl moiety (Type I), or by an aromatic ring (Type II) in a total of 10 examples. In the third modification (Type III) the central alkene is replaced by an aromatic ring and a disulfide is installed in place of the original sulfoxide (Figure 2). At present, there are no methods to synthesise (*E*)-**3** and (*Z*)-**3** selectively and replacing the central double bond with another moiety could lead to a pharmaceutically relevant drug molecule. Although the selective synthesis alkenyl disulfides has been reported, it has not been used in the synthesis of ajoene derivatives.[11] Block observed that

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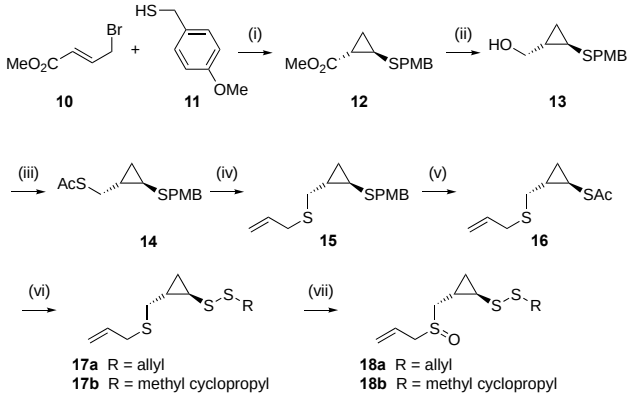
(*Z*)-**3** has a higher activity than (*E*)-**3** and thus, a selective synthesis for either isomer would be advantageous.[5] A central cyclopropyl moiety was chosen to replace the central olefin as this would significantly affect the electronic properties of the molecule, without greatly affecting the steric demand of the central part. Ajoene **3** is formed as a racemate in garlic extracts and investigations on the effect of the sulfoxide chirality on the biologic activity have yet to be reported.

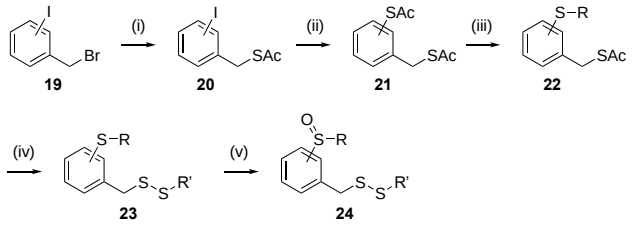
An eight-step route has been developed, with a Michael Induced Ring Closure (MIRC) for the cyclopropane synthesis being the key step. Ester **12** was obtained in 69% yield using an adapted method from the literature,[12] which is formed exclusively as *trans*-isomer. *J*-coupling values were in agreement in those reported by Bernard, who had confirmed the *trans*-configuration by 2D NMR and NOSEY experiments. After formation of the central moiety, the sulfide is installed over three steps (ii−iv), by reduction, Mitsunobu reaction[13] and deprotection/allylation to yield **15**. The challenging step of the synthesis was the subsequent deprotection of the *para*-methoxy benzyl (PMB) group. The deprotection (v) suffered from low yields due to the volatility and instability of the intermediate thiolate. A large excess of trifluoroacetic anhydride and acetic acid allowed a cleavage of the PMB group with direct trapping of the thiolate as thioacetate **16** in 20% yield. Despite the PMB deprotection resulting in low yields, there is an advantage in employing such a group. The advantage of having an acid sensitive protecting group is its stability in the previous step (iii) using basic conditions avoiding the formation of side products. Due to the low yield, other acid-sensitive protecting groups should be considered in the future. With thioacetate **16** synthesised, the disulfide bond was installed yielding **17a** and **17b** using a thiotosylate reagent as an electrophilic sulfur source. Finally, mono-oxidation of **17** with *m*CPBA furnished the desired sulfoxides **18a** and **18b** (Scheme 2).



Scheme 2. Synthesis of ajoene analogues **18**. Conditions and reagents: (i): *n*-BuLi (1.1 eq.) in THF at −40 °C, 69% yield; (ii) lithium aluminium hydride (1.1 equiv.) in THF at −40 °C, 85% yield; (iii): PPh_3 (2 eq.), diisopropylazodicarboxylate (2 eq.), thioacetic acid (3 eq.) in THF at −20 °C, 92% yield; (iv): KOH (2.5 e.), allylbromide (2 eq.) in MeOH, −78 to −40 °C, 54% yield; (v): trifluoroacetic acid anhydride (5 eq.), acetic acid (5 eq.) in CH_2Cl_2 at –78 °C, 20% yield; (vi): allyl/methyl cyclopropyl thiotosylate (2 eq.), KOH (2 eq.) in MeOH, −78 to −40 °C, 23–27% yield; (vii): *m*CPBA (1.1 eq.) in CH_2Cl_2 at −78 °C, 14-21% yield.

MIRC protocols using benzyl mercaptan or thiobenzoic acid were also investigated. While the MIRC product was obtained in 42% yield with benzyl mercaptan, the benzylic protecting group could not be cleaved under standard conditions. By employing thiobenzoic acid in the MIRC reaction, the thiobenzoic acid proved to be a harder nucleophile resulting in a direct bromide substitution (see supporting information).

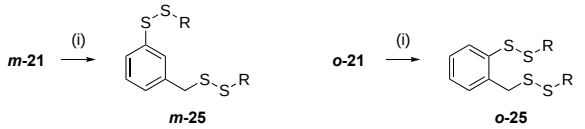
We have drawn inspiration from the work by Block who synthesised central aromatic analogues of type **5**.[5] We prepare aromatic compounds where the sulfoxide and disulfide are exchanged. This will alter their electronic properties and the reactivity of both moieties. Eight examples with *meta*- and *ortho*-substitution have been synthesised by a novel route as shown in Scheme 3.



Scheme 3. Synthesis of ajoene analogues **24**. Conditions and reagents: (i): AcSH (1 eq.), K_2CO_3 (1 eq.) THF, r.t., 4 h, >95% yield; (ii): CuI (0.2 eq.), phenanthroline (0.2 eq.), KSAc (1.5 eq.) in toluene 100 °C, >95% yield; (iii): K_2CO_3 (1 eq.), R-Br (2 eq.) in MeOH at 0 °C, 8–86% yield; (iv): KOH (1.5 eq.), R^1-STs (2 eq.) in MeOH at 0 °C, 51–88 % yield; (v): *m*CPBA (1 eq.) in CH_2Cl_2 at −78 °C, 40–78% yield. R = **a**: allyl, **b**: benzyl, **c**: *iso*butyl, **d**: methyl cyclopropyl.

Compound ***o-/m*-24** can be synthesised in a concise synthetic route starting from 2-iodobenzyl bromide ***o*-19** or 3-iodobenzyl bromide ***m*-19**, respectively. Employing a nucleophilic substitution of the labile bromide in the benzyl position the first thioacetate can be installed providing **20** in up to 99% yield. A copper(I) catalysed coupling between aryl iodides and potassium thioacetate[14] was employed to furnish **21** in very high yields. Since **21** has two acetate protecting groups, the first deprotection uses a mild base which cleaves the more labile acetate in the phenylic position with some selectivity in up to 86% yield. Cleaving the acetate will unmask the thiophenolate which will react with an alkyl bromide as electrophile to obtain thioether **22**. Treatment of **22** with potassium hydroxide as base cleaves the second acetate. The thiolate generates a disulfide with the thiotosylate reagent as electrophilic sulfur source to yield **23** in up to 85% yield. With **23** containing a disulfide and a sulfide moiety, the addition of recrystallised *m*CPBA at low temperatures enables the selective oxidation which proceeds quickly and cleanly to yield derivatives **24** in up to 79% yield. Different substituents R (allyl, benzyl, *iso*-butyl, methyl cyclopropyl) were investigated to cover a spectrum of electronic and steric factors (Scheme 3).

Disulfides are well known to possess biological activity. This is often the result of the instability of the disulfide bond and the subsequent interaction of the thiolate with cystine residues in proteins.[15]. We therefore synthesised a range of bis-disulfides with a central aromatic moiety. Analogues of **Type III** (Figure 2) are 1,3-disubstituted benzene derivatives which were developed to mimic ajoene with *E*-geometry, whereas 1,2-disubstitued benzene derivatives were prepared to mimic ajoene with *Z*-geometry. Their synthesis is summarised in Scheme 4.



Scheme 4. Synthesis of ajoene analogues **24**. Conditions and reagents: (i): KOH (3 equiv.), R-thiotosylate (4 eq.) in MeOH at 0 °C, 1–98% yield.

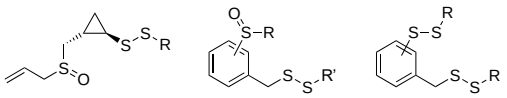
The bis-disulfides **25** could be obtained by cleaving both thioacetates in **24** with a strong base and reacting the thiolate with a thiotosylate as an electrophilic sulfur source. The efficiency and ease of this step (i) varied greatly depending on the thiotosylate employed in the reaction. A general trend shows that allyl and alkyl substituents gave higher yield than benzyl substituents. The yield suffered significantly when a strong electron withdrawing or donating group was attached to the *para*-position of the benzyl substituent. Generally, yields were higher for the ***m*-25** than ***o*-25**, presumably due to the lower steric hinderance. The low yields for ***m*-25f-h** was a result of difficulties during purification, as each compound required normal phase chromatography followed by reverse phase separation (Table 1).

Table 1. Yields for the synthesis of novel unsymmetrical bis-disulfides **25**.

Entry	Compound	Substituent R	Yield [%]
1	***m*-25a**	allyl	69
2	***m*-25b**	benzyl	21
3	***m*-25c**	*iso*-butyl	81
4	***m*-25d**	methyl cyclopropyl	98
5	***m*-25e**	4-methyl benzyl	70
6	***m*-25f**	4-CF_3-C_6H_4-CH_2	30
7	***m*-25g**	4-OCH_3-C_6H_4-CH_2	15
8	***m*-25h**	4-(CO_2CH_3)-C_6H_4-CH_2	1
9	***o*-25a**	allyl	48
10	***o*-25b**	benzyl	41
11	***o*-25c**	*iso*-butyl	65
12	***o*-25d**	methyl cyclopropyl	55

In total, 22 novel analogues were biologically evaluated by Minimum Biofilm Inhibitory Concentration (MBIC) studies using *S. Aureus* and *P. Aeruginosa* bacteria (Table 2). For compounds **18a** and **18b**, the MBIC for *S. Aureus* was at least 85 times higher than for ajoene (**3**). This suggests that the central olefin in **3** plays an important role in its mode of action, one which the central cyclopropyl cannot replicate. A similar pattern is seen for *P. Aeruginosa* where analogue **18a** and **18b** showed MBIC values at least over 2.6 times higher. Therefore, the potency of **18a**–**b** analogues is significantly reduced with the central cyclopropyl modification (Table 2, entries 1–3). Type II analogues show a more complex pattern where the MBIC results suggest that there may be several factors at play. In general, ***o*-24a**–**d** showed higher MBIC results than ***m*-24a**–**d**. In both the *ortho*- and the *meta*-analogues, a benzyl terminal group shows significantly superior potency against *S. Aureus* in comparison to allyl, *iso*-butyl and methyl cyclopropyl substituents. This observation is not replicated in type III analogues, suggesting there is an interaction between the benzyl and sulfoxide group (Table 2, entries 4–11). Interestingly, for bis-disulfide compounds of type III **25a**–**d**, the MBIC is significantly lower in the cases of *ortho*-compounds over the corresponding *meta*-compounds against *S. Aureus* and where ***o*-25a**, ***o*-25c**, ***o*-25d** all gave superior MBIC values in comparison to ajoene. This shows the importance of the disulfide moiety and suggests that the terminal group has a great influence on its mode of action, where a more sterically hindered disulfide shows higher biological potency (Table 2, entries 12–23). A standout result can be seen by both ***m*-25a** and ***o*-25a**, which also shows impressive MBIC values in comparison to ajoene (**3**). Although ***m*-25a** inhibits biofilm production in *S. Aureus*, the result of particular interest is seen against *P. Aeruginosa.* Here, the MBIC value is substantially lower, suggesting that analogue ***m*-25a** is up to 30 times more potent than **3**. Out of the 22 substrates tested, it was only ***o*-24b** and ***m*-25a** that gave an MBIC value of single digit magnitude against *P. Aeruginosa* (Table 2, entry 12).

Table 2. Biological evaluation of type I analogues (entries 2−3), type II analogues (entries 4−11) and type IIII analogues (entries 12−23) in comparison to ajoene (**3**, entry 1) against *S. Aureus* and *P. Aeruginosa* bacteria.



Entry	Compound	*S. Aureus* MBIC IC_{50} (μM)	*P. Aeruginosa* MBIC IC_{50} (μM)
1	**3**	0.565	49.2
2	**18a**	>48	>128
3	**18b**	>48	131
4	***m*-24a**	9.08	69
5	***m*-24b**	0.92	>128
6	***m*-24c**	8.28	36.3
7	***m*-24d**	7.9	113
8	***o*-24a**	12.5	>128
9	***o*-24b**	4	8.3
10	***o*-24c**	14.5	54.8
11	***o*-24d**	16.2	>128
12	***m*-25a**	0.223	1.64
13	***m*-25b**	36	>144
14	***m*-25c**	42	>144
15	***m*-25d**	44	>144
16	***m*-25e**	5.6	>128
17	***m*-25f**	17	>128
18	***m*-25g**	7.2	>128
19	***m*-25h**	1.3	>128
20	***o*-25a**	0.172	15.8
21	***o*-25b**	1.4	63.4
22	***o*-25c**	0.48	>128
23	***o*-25d**	0.425	65.5

In conclusion, 22 substrates of novel ajoene analogues have been synthesised over three novel routes. These analogues have been biologically evaluated in their potency to inhibit the biofilm production of two bacteria. Promising results were found in the MBIC value of ***o*-25a** against *S. Aureus* and ***m*-25a** against *P. Aeruginosa* which showed superior potency of 3 and 30 times greater than ajoene, respectively.

Acknowledgements

We thank Neem Biotech Ltd., especially Danielle Williams, and Lucy Andrews for the biological assays and the KESS 2 program (MYR) and the School of Chemistry, Cardiff University, for financial support. We thank James Silk and Alexander Lees, School of Chemistry, Cardiff University, for the synthesis of intermediates. Knowledge Economy Skills Scholarships (KESS) is a pan-Wales higher level skills initiative led by Bangor University on behalf of the HE sector in Wales. It is part funded by the Welsh Government's European Social Fund (ESF) convergence programme for West Wales and the Valleys.

Keywords: ajoene • biologic activity • disulfides • garlic • organosulfur compounds

[1] E. Block, S. Ahmad, M. K. Jain, R. W. Crecely, R. Apitz-Castro, M. R. J. Cruz, *J. Am. Chem. Soc.* **1984**, *106*, 8295–8296.
[2] R. Apitz-Castro, S. Cabrera, M. R. Cruz, E. Ledezma, M. K. Jain, *Thromb. Res.* **1983**, *32*, 155–169.
[3] F. Silva, S. S. Khokhar, D. M. Williams, R. Saunders, G. J. S. Evans, M Graz, T. Wirth, *Angew. Chem.* **2018**, *130*, 12470–12473; *Angew. Chem. Int. Ed.* **2018**, *57*, 12290–12293.
[4] M. Yamamoto Raynbird, F. Silva, H. Smallman, S. S. Khokhar, D. Neef, G. J. S. Evans, T. Wirth, *Chem. Eur. J.* **2020**, *26*, 8363–8367.
[5] E. Block, S. Ahmad, J. L. Catalfamo, M. K. Jain, R. Apitz-Castro, *J. Am. Chem. Soc.* **1986**, *108*, 7045–7055.
[6] a) R. Hunter, C. H. Kaschula, I. M. Parker, M. R. Caira, P. Richards, S. Travis, F. Taute, T. Qwebani, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5277–5279. b) C. H. Kaschula, R. Hunter, N. Stellenboom, M. R. Caira, S. Winks, T. Ogunleye, P. Richards, J. Cotton, K. Zilbeyaz, Y. Wang, V. Siyo, E. Ngarande, M. I. Parker, *Eur. J. Med. Chem.* **2012**, *50*, 236–254.
[7] E. Block, B. Bechand, S. Gundala, A. Vattekkatte, K. Wang, S. S. Mousa, K. Godugu, M. Yalcin, S. A. Mousa, *Molecules* **2017**, *22*, 2081−3001.
[8] J. Fong, M. Yuan, T. H. Jakobsen, K. T. Mortensen, M. M. S. D. Santos, S. L. Chua, L. Yang, C. H. Tan, T. E. Nielsen, M. Givskov, *J. Med. Chem.* **2017**, *60*, 215–227.
[9] J. W. Hwang, H. Cho, J. Y. Lee, Y. Jeon, S.-N. Kim, S. J. Lee, G.-U. Bae, S. Yoon, R. Jeon, Y. K. Kim, *Food Chem. Toxicol.* **2016**, *96*, 35–42.
[10] T. Horn, W. Bettray, U. Noll, F. Krauskopf, M. R. Huang, C. Bolm, A. J. Slusarenko, M. C. H. Gruhlke, *Antioxidants* **2020**, *9*, 1086.
[11] M. Musiejuk, J. Doroszuk, D. Witt, *RSC Adv.* **2018**, *8*, 9718–9722.
[12] A. M. Bernard, A. Frongia, P. P. Piras, *Synth. Commun.* **2003**, *33*, 801−817.
[13] A. Dion, P. Dubé, C. Spino, *Org. Lett.* **2005**, *7*, 5601–5604.
[14] S. M. Soria-Castro, A. B. Peñéñory, *Beilstein J. Org. Chem.* **2013**, *9* 467–475.
[15] J. V. Cook, A. McGregor, T. Lee, G. Milligan, K. A. Eidne, *Endocrinology* **1996**, *137*, 2851–2858.