Synthesis and *In Vitro* Anti-Helicobacter and Anti-Staphylococcal Activities of Novel Diaryldisulfides and Diarylthiosulfonates

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**Abstract**

Arylthiols were reacted with acrylonitrile under basic conditions to form the corresponding aryl sulfides which were oxidised with sodium metaperiodate in aqueous methanol to yield 3-arylsulphinylpropanenitriles that upon thermolysis in refluxing toluene produced a mixture of diarylthiosulfonates and diaryldisulfides. The mixture of the two products was easily separated by flash chromatography and characterized spectroscopically. The diarylthiosulfonates and diaryldisulfides, garlic-like organosulfur compounds, were tested for their antimicrobial properties against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Helicobacter pylori* and had been found to have good activity against *S. aureus* and *H. pylori* with no activity against the other two organisms.

**Keywords**

Diarylthiosulfonates, Diaryldisulfides, *Helicobacter pylori*, Arylsulfenic Acids, Michael Reaction

**1. Introduction**

The medicinal properties of crushed garlic and onions are well recognized [1][2]. The natural products in garlic responsible for the therapeutic actions are identified as the thiosulfinate esters alliin (S-allyl-L-cysteine sulfoxide) and allicin (di-allylthiosulfinate) (*Figure 1*) which have been reported to have a broad range of biological activities such as for example anti-inflammatory [1] and antibacterial [3][4] actions.

In the laboratory synthetic versions of thiosulfinate esters have usually been made by either the oxidation of diaryldisulfides [5][6][7][8] with peracids or by condensation of a sulfinyl chloride with a thiol in the presence of a tertiary
amine [8] [9] [10]. A third method which has been little used for making symmetrical diarylthiosulfonates involves the condensation of arylsulfenic acids generated in situ by thermolysis of aryloxides possessing β-hydrogens. The β-elimination of sulfides to yield sulfenic acids and the condensation of sulfenic acids to form thiosulfinate esters are well documented in the literature [11] (Figure 2).

In our previously reported synthesis of phenylsulfanyl alkene derivatives by the Markovnikov addition of benzenesulfenic acid to terminal alkynes, generated in situ by thermolysis of 3-phenylsulfanylpropanenitrile [12] the diphenylthiosulfinate produced as a by-product was always disposed of. In this article, we report this synthetic strategy of making diarylthiosulfonates by condensation of arylsulfenic acids in the absence of any terminal alkynes as trapping agents.

The antimicrobial activity of garlic is mainly due to the thiosulfinate ester called allicin and is reported to be three times more effective on Gram-positive bacteria than Gram-negative ones [13]. The antibacterial activity of aqueous garlic extract against 16 H. pylori strains has been assessed and MIC50 concentrations in the range 2 - 5 mg ml−1 were able to inhibit the strains. In most cases, the inhibitory concentrations [4] were also bactericidal.

*Helicobacter pylori* and its clinical association with the development of peptic ulcers has led to the development of various chemotherapeutic agents to eliminate infection caused by this pathogen [14] [15] [16] [17] [18]. *H. pylori* is also implicated in the development of acute and chronic gastritis, gastric adenocarcinoma and gastric lymphoma (MALT), and has been classified as a class 1 carcinogen in humans and is a major contributing factor in the development of gastric cancer [19]. Infection with *H. pylori* is typically treated with a combination of clarithromycin, ampicillin and a proton pump inhibitor, but this triple therapy approach is costly [20]. The infection is eradicated in up to 90% of patients but side effects, poor compliance and the development of antimicrobial resistance are common causes of treatment failure [21]. *H. pylori* infection has been implicated with increased COX-2 expression in gastric antral mucosa for both NSAID users and non-users [22] [23] [24]. We have been engaged with the search for compounds with anti-*H. pylori* activity and have recently reported anti-*H. pylori* activity of novel quinoline-derived propionic acid esters [25]. In this paper, we report the synthesis and antimicrobial activities of a set of diaryl disulfides 6 and diaryl thiosulfonates 7.

![Figure 1](image-url). Enzymatic conversion of alliin into diallylthiosulfinate (allicin).
Figure 2. β-Elimination arylsulphoxides to produce sulphenic acids.

_Staphylococcus aureus_ is a versatile pathogen that can cause a range of infectious diseases ranging from superficial skin infections to life-threatening septicaemia. The emergence of antibiotic-resistant strains of _S. aureus_ including MRSA, VISA, LRSA and multi-drug resistant isolates has led to a great deal of interest in developing novel anti-staphylococcal agents [26] [27] [28].

_Escherichia coli_ is a common commensal organism in the human gastrointestinal tract but it is also a significant pathogen that can cause a range of human infections including diarrheal diseases and urinary tract infections through wound infections and life-threatening ulcerative colitis and septicaemia [29] [30].

Due to the structural similarity of our synthetic compounds 6 and 7 to thiosulfinate esters found in garlic, we decided to investigate their preliminary antimicrobial activity against _Helicobacter pylori_ and a Gram-positive bacterium, _Staphylococcus aureus_ (including resistant strains of this species), and the Gram-negative bacterium, _Escherichia coli_.

2. Results and Discussion

2.1. Chemistry

Conjugate addition of thiolate anions to acrylonitrile furnished the arylsulfides 2a-i and 2k-m in excellent date yields (93% - 98%) with 2j obtained in 82% yield. Sodium metaperiodate is a specific oxidising agent for converting sulfides into sulfoxides. Thus oxidation of the sulfides 2a-m with one-molar equivalent of sodium metaperiodate in aqueous methanol yielded the corresponding sulfoxides 3a-m which were isolated in good yields (63% - 89%) and characterised spectroscopically. The FTIR spectra showed the >S=O absorbance at 1043 - 1093 cm⁻¹. Upon refluxing in toluene under nitrogen the sulfoxides 3a-m thermolized to furnish the disulfides 6a-m and diarylthiosulfonates 7a-m as the only reaction products according to TLC analysis. The mixtures diaryldisulfides 6a-m and diarylthiosulfonates 7a-m were separated and isolated by flash chromatography (Scheme 1).

Our disappointment in not obtaining the anticipated diarylthiosulfinates 5 as reaction products was shown by subsequent literature search that described diarylthiosulfinates 5 to be thermally unstable compounds due to the weak S-S bond (bond strength ~ 35 kcal) [31]. Thermolysis of the sulfoxides 3 initially generates arylsulfenic acids 4 which by virtue of their high reactivity condense to
form diarylthiosulfines 5 and water. Compounds of the formula RS(O)SR tend to be unstable and usually cannot be isolated. At the high temperature of the reaction the weak sulfinyl-sulfide S-S bond cleaves homolytically to generate radical pairs [32]. The recombination of arylsulfinyl radicals then produces the isolated symmetrical product 6 (Scheme 2).

The recombination of arylsulfinyl radicals did not produce any of the anticipated \( a,a' \)-diaryldisulfoxide 8 as reaction product [33] [34] but instead produced the thiosulfonate 7 as the isolated reaction product. The only evidence to date for the existence of stable \( a,a' \)-disulfoxides has been provided by bridged bicyclic compound 9 [35].

### 2.2. Microbiological Results and Discussion

An increase in resistance of bacteria and fungi towards currently available antibacterial and antifungal agents has resulted in a huge demand for the identification of novel antimicrobial agents. This is due to the rapid development of antimicrobial-resistant bacterial and fungal strains as well as a lack of new antimicrobial drugs that are effective against these resistant strains. Antimicrobial screening assays provide a robust method for the discovery of potential inhibitors of microbial growth. Due to the structural similarity of our synthetic compounds 6 and 7 to thiosulfinate esters found in garlic we decided to investigate their preliminary antimicrobial activity against \( Helicobacter pylori \) and a Gram-positive bacterium, \( Staphylococcus aureus \) (including resistant strains of this species, and the Gram-negative bacterium, \( Escherichia coli \)). The diarylthiosulfonates 7d, 7h, 7j, 7k, 7l and 7m and diaryldisulfides 6d, 6h, 6j, 6k, 6l and 6m were dissolved in 50% DMSO and tested for their antimicrobial activities using the broth microdilution method (Table 1).
Scheme 2. The disproportionation and recombination of thiol and arylsulfinyl radicals to form the products 7 and 8.

Table 1. Antimicrobial activity of compounds 6a-f, 6h, 6j, 6k-m and 7b-e, 7g-h, 7j-l.

| Compound | MIC (μg/ml) H. pylori 3339 | MIC (μg/ml) H. pylori 26695 | MIC (μg/ml) E. coli | MIC (μg/ml) S. aureus |
|----------|---------------------------|----------------------------|---------------------|----------------------|
| 6a       | >256                      |                            |                     |                      |
| 6b       | 64                        | 32                         | >256                | 64                   |
| 6c       | 32                        | 64                         |                     |                      |
| 6d       | 64                        | 128                        |                     |                      |
| 6e       | 16                        | 32                         | >256                | >256                 |
| 6f       |                           |                            | >256                | >256                 |
| 6h       | 32                        | 32                         | >256                | 4                    |
| 6j       | 16                        | 32                         | >256                | 16                   |
| 6k       | 32                        | 32                         | >256                | 8                    |
| 6l       | 16                        | 64                         | >256                | 16                   |
| 6m       | 64                        | 64                         |                     |                      |
| 7b       | 16                        | 64                         | >256                | 32                   |
Although there was no antimicrobial activity of any of the compounds against the Gram-negative pathogen *E. coli* there was some very promising effect on the common Gram-positive pathogen *S. aureus*. Compounds 6 and 7 were significantly inactive in antimicrobial activity against *E. coli* showing activity only at > 256 μg/mL but displayed modest activity against the other two organisms *H. pylori* 3339 and *S. aureus*. All the derivatives 6e, 6j, 6l, 7b, 7c, 7g and 6g as a mixture, 7f and 7k have shown modest antimicrobial activity against *H. pylori* 3339 when compared with the standard anti-*Helicobacter* agents shown in Table 1. There is a noticeable common Structure-Activity Relationship (SAR) observed in inhibitory activity for the two different sets of compounds 6 and 7 on the *H. pylori* strain 3339. It was seen that when the aromatic rings in compounds 6 and 7 are ortho- and para-substituted with -OMe, -Cl and -Br groups the compounds tend to have the lowest concentration inhibitory effects overall. On the other hand some of the compounds 6 and 7 have shown modest to good antimicrobial activity against *S. aureus* In particular the diaryldisulfides 6h, 6k and diarylthiosulfonates 7h and 7k gave the lowest inhibitory responses that are either equal or better than ampicillin. Thus, it is interesting to note that diarylthiosulfonate 7k is the most active at 2 μg/mL whilst its counterpart diaryldisulfide 6k inhibits at a somewhat higher concentration of 8 μg/mL. Similarly the 2-chloro derivatives of 6 and 7 showed good inhibitory action against *S. aureus* at 4 μg/mL. Thus, a noticeable structure-activity feature of the most promising four active compounds 6h, 6k, 7h and 7k is the presence of chloro- or bromo-groups in the 2-position of the aromatic rings. We conclude that for good inhibitory activity against *S. aureus* compounds 6 and 7 require of an ortho-substituted chlorine or bromine atoms as best candidates for further antibacterial studies.

### 3. Experimental

#### 3.1. General Methods

Melting points are uncorrected and were determined on Stuart Scientific SMP3
apparatus. Infrared spectra were recorded with an ATI Mattson Genesis series FTIR spectrophotometer. $^1$H NMR and $^{13}$C NMR spectra were recorded in CDCl$_3$ using a Bruker AC 250 spectrometer operating at 250 and 62.9 MHz, respectively. Chemical shifts (δ) are in ppm downfield from Me$_4$Si as internal standard and J values are given in Hz. Mass spectra were recorded with EI-VG 7070E mass spectrometer. Accurate masses were determined on VG Autospec, EI mass spectrometer with magnetic sector instrument.

3.2. Typical Experimental Procedure for the Formation of Sulfides 2 a-m

To a magnetically stirred solution of thiol 1 (0.10 mol) and acrylonitrile (20 ml, an excess) in THF (40 ml) at 0°C was added a solution of tetrabutylammonium fluoride in THF (2 ml) and the mixture was allowed to stir and come to room temperature overnight. The solvent was rotary evaporated and the residue extracted with DCM (160 ml). The organic solution was washed with 2M NaOH solution (2 × 40 ml) and water (50 ml) before being dried over MgSO$_4$ and evaporated to yield the crude sulfide 2 which according to TLC [1: 5 EtOAc-petrol] did not require any further purification. $2a$ [12], $2b$: oil, 98% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.43 (3H, s, CH$_3$), 2.56 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.10 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.10 - 7.40 (4H, m, Ar); MS m/z 177 (M', 73%), 137 (M-C$_2$H$_4$CN, 100%); $2c$: oil, 93% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.35 (3H, s, CH$_3$), 2.58 (2H, t, J = 7.8 Hz, S$CH_2$-), 3.08 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.03 - 7.30 (4H, m, Ar); EI-MS: m/z 177 (M', 64%), 137 (M-C$_2$H$_4$CN, 100%); $2d$: oil, 93% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.37 (3H, s, CH$_3$), 2.55 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.10 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.15 (2H, d, J = 7.5 Hz, AB system Ar), 7.34 (2H, d, J = 7.5 Hz, AB system Ar); EI-MS: m/z 177 (M', 64%), 137 (M-C$_2$H$_4$CN, 100%); $2e$: oil, 98% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.58 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.11 (2H, t, J = 7.8 Hz, -CH$_2$CN), 3.90 (3H, s, OCH$_3$), 6.83 - 6.94 (2H, m, Ar); 7.23 - 7.40 (2H, m, Ar); EI-MS: m/z 193 (M', 100%), 153 (M-C$_2$H$_4$ CN, 69%); $2f$: oil, 98% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.62 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.13 (2H, t, J = 7.8 Hz, -CH$_2$CN), 3.80 (3H, s, OCH$_3$), 6.80 (1H, d, J = 7.5 Hz, Ar H-4); 6.88 - 7.00 (2H, m, Ar); (1H, t, J = 7.5 Hz, Ar H-5); EIMS: m/z 193 (M', 100%), 153 (M-C$_2$H$_4$CN, 68%), 140 (M-C$_2$H$_4$CH$_2$CN, 63%); $2g$: oil, 93% yield, IR ν 2248 (CN) cm$^{-1}$; $^1$HNMR δ 2.52 (2H, t, J = 7.8 Hz, SCH$_2$-), 2.98 (2H, t, J = 7.8 Hz, -CH$_2$CN), 3.80 (3H, s, OCH$_3$), 6.87 (2H, d, J = 7.5 Hz, AB system Ar H-3 and H-5), 7.40 (2H, d, J = 7.5 Hz, AB system Ar H-2 and H-6); EIMS: m/z 193 (M', 100%), 153 (M-C$_2$H$_4$CN, 77%), 140 (M-C$_2$H$_4$CH$_2$CN, 70%); $2h$: oil, 98% yield, IR ν 2252 (CN) cm$^{-1}$; $^1$HNMR δ 2.64 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.17 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.15 - 7.30 (2H, m, Ar); 7.35 - 7.45 (2H, m, Ar); EI-MS: m/z 197.5 (M', 52%), 157.5 (M-C$_2$H$_4$CN, 100%); $2i$: oil, 98% yield, IR ν 2252 (CN) cm$^{-1}$; $^1$HNMR δ 2.63 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.12 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.17 - 7.30 (3H, m, Ar), 7.37 (1H, s, Ar); EI-MS: m/z 197.5 (M', 60%), 157.5 (M-C$_2$H$_4$CN, 100%); $2j$: oil, 82% yield, IR ν 2243 (CN) cm$^{-1}$; $^1$HNMR δ 2.58 (2H, t, J = 7.8 Hz, SCH$_2$-), 3.12 (2H, t, J = 7.8 Hz, -CH$_2$CN), 7.20
Typical experimental procedure for the formation of 3-arylsulfinylpropanenitriles 3a-m:

To a vigorously stirred solution of sodium metaperiodate (0.0763 mol) in water (135 ml) at 0°C was quickly added a solution of the sulfide 2 (0.0763 mol) in methanol (135 ml) and the mixture was stirred for 22 h and allowed to come to RT. The precipitated inorganic solid was filtered at the pump and the mother liquor extracted with DCM (3 × 200 ml). After washing with water (100 ml) the organic layer was dried (MgSO₄) and evaporated to yield the crude sulfoxide 3 which was purified by flash chromatography [2:3 ethyl acetate-petrol followed by ethyl acetate]. 3a [12], 3b: 82% yield, IR ν 2246 (CN), 1035, 1068 (>S=O) cm⁻¹; 1H NMR δ 2.30 (3H, s, CH₃), 2.40 - 2.60 (1H, m, -CHCN), 2.75 - 2.93 (2H, m, -SO-CH₂-), 3.05 - 3.22 (1H, m, -CHCN), 7.10 - 7.25 (1H, m, Ar), 7.30 - 7.45 (2H, m, Ar), 7.67 - 7.80 (1H, m, Ar); EIMS: m/z 193 (M⁺, 29%), 140 (M-CH₂=CH-CN, 46%), 139 (M-CH₂CH₂CN, 46%), 77 (100%); HRMS: Found 193.0565. Calcd. for C₁₀H₁₁NOS 193.0561; 3c: 85% yield, IR ν 2246 (CN), 1049, 1085 (>S=O) cm⁻¹; 1H NMR δ 2.36 (3H, s, CH₃), 2.32 - 2.50 (1H, m, -CHCN), 2.72 - 2.95 (2H, m, -SO-CH₂-), 3.10 - 3.25 (1H, m, -CHCN), 7.20 - 7.40 (4H, m, Ar); EIMS: m/z 193 (M⁺, 14%), 140 (M-CH₂=CH-CN, 16%), 139 (M-CH₂CH₂CN, 67%), 66 (100%); HRMS: Found 193.0568. Calcd. for C₁₀H₁₁NOS 193.0561; 3d: 76% yield, IR ν 2246 (CN), 1045, 1085 (>S=O) cm⁻¹; 1H NMR δ 2.33 (3H, s, CH₃), 2.27 - 2.50 (1H, m, -CHCN), 2.70 - 2.95 (2H, m, -SO-CH₂-), 3.05 - 3.20 (1H, m, -CHCN), 7.30 (2H, d, J = 7.5 Hz, AB system Ar), 7.43 (2H, d, J = 7.5 Hz, AB system Ar); EIMS: m/z 193 (M⁺, 13%), 140 (M-CH₂=CH-CN, 23%), 139 (M-CH₂CH₂CN, 100%), 91 (43%); HRMS: Found 193.0557. Calcd. for C₁₀H₁₁NOS 193.0561; 3e: 65% yield, IR ν 2246 (CN), 1039, 1068 (>S=O) cm⁻¹; 1H NMR δ 2.25 - 2.45 (1H, m, -CHCN), 2.64 - 2.85 (1H, m, -SO-CH₂-), 2.97 - 3.13 (1H, m, -SOCH₂-), 3.15 - 3.34 (1H, m, -CHCN), 2.83 (3H, s, OCH₃), 6.86 (1H, d, J = 7.5 Hz, Ar), 7.10 (1H, t, J = 7.5 Hz, Ar), 7.43 (1H, t, J = 7.5 Hz, Ar), 7.60 (1H, d, J = 7.5Hz, Ar); EIMS: m/z 209 (M⁺, 18%), 156 (M-CH₂=CH-CN, 44%), 155 (M-CH₂CH₂CN, 100%); HRMS: Found 209.0517. Calcd. for C₁₀H₁₁NO₂S 209.0510; 3f: 89% yield, IR ν 2246 (CN), 1043 (>S=O) cm⁻¹; 1H NMR δ 2.32 - 2.52 (1H, m, -CHCN), 2.67 - 2.94 (2H, m, -SO-CH₂-), 3.05 - 3.22 (1H, m, -CHCN), 2.75 (3H, s, OCH₃), 6.88 - 7.10 (3H, m, Ar), 7.28 - 7.43 (1H, t, J = 7.5 Hz, Ar); EIMS: m/z 209
Typical experimental procedure for the formation of diaryldisulfides 6a-m and diarylthiosulfonates 7a-m:

The sulfoxide 3 (0.03 mol) in dry toluene (80 ml) was heated at reflux under nitrogen for 2h after which the solvent was evaporated and the residue showing two spots by TLC [1:5 ethyl acetate-petrol] was separated by flash chromatography to yield firstly 6 followed by 7. 6a [12] and 7a [12]. 6b: 32% yield, oil, IR ν 1641, 1041, 1149 cm⁻¹; ¹H NMR δ 2.35 - 2.53 (1H, m, -CHCN), 2.78 - 3.08 (2H, m, -SO-CH₂-), 3.10 - 3.35 (1H, m, -CHCN), 7.37 - 7.70 (3H, m, Ar), 7.70 - 8.05 (3H, m, Ar), 8.15 (1H, s, Ar); EIMS: m/z 229 (M⁺, 13%), 176 (M-CH₂=CH-CN, 35%), 175 (M-CH₂=CH-CN, 100%); HRMS: Found 229.0571 Calcd for C₁₀H₉NO₂S 229.0561.
7.55 - 7.65 (2H, d, J = 7.50 Hz, Ar); \textsuperscript{13}C NMR δ 20.85, 125.38, 126.05, 130.96, 133.32, 142.08; EIMS: m/z 246 (M\textsuperscript{+}, 83%), 123 (M – SC\textsubscript{6}H\textsubscript{4}-Me, 100%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}S\textsubscript{2} 246.0537; Found 246.0530; \textbf{7b}: 30% yield, oil, IR ν 1315, 1145 cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 2.15 (3H, s, CH\textsubscript{3}), 2.69 (3H, s, CH\textsubscript{3}), 7.02 - 7.50 (8H, m, Ar); \textsuperscript{13}C NMR δ 21.26, 21.64, 125.45, 126.10, 126.80, 128.25, 129.29, 131.24, 132.30, 133.58, 137.65, 139.18, 142.10; EIMS: m/z 278 (M\textsuperscript{+}, 50%), 123 (M – SC\textsubscript{6}H\textsubscript{4}-Me, 85%); HRMS: Calcd  for C\textsubscript{14}H\textsubscript{14}O\textsubscript{2}S\textsubscript{2} 278.04352; Found 278.04356; \textbf{6c}: 30% yield, oil, IR ν 1079, 1141 cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 2.34 (6H, s, 2x CH\textsubscript{3}), 7.05 (2H, d, J = 7.80 Hz, Ar), 7.22 (2H, t, J = 7.80 Hz, Ar), 7.34 - 7.47 (4H, m, Ar); \textsuperscript{13}C NMR δ 21.02, 124.22, 125.78, 126.75, 136.05, 140.59; EIMS: m/z 246 (M\textsuperscript{+}, 100%), 123 (M – SC\textsubscript{6}H\textsubscript{4}-Me, 77%); HRMS: Found 246.0526. Calcd for C\textsubscript{14}H\textsubscript{14}S\textsubscript{2} 246.0537; \textbf{7c}: 24% yield, oil, IR ν 1318(-SO\textsubscript{2}), 1146 (S -O) cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 2.30 (3H, s, CH\textsubscript{3}), 2.35 (3H, s, CH\textsubscript{3}), 7.15 (2H, s, Ar), 7.20 - 7.45 (6H, m, Ar); 13C NMR δ 21.42, 124.99, 128.28, 128.95, 132.54, 133.89, 134.72, 137.41, 137.42, 143.42, 143.05; EIMS: m/z 278 (M\textsuperscript{+}, 47%), 139 (SO\textsubscript{2}C\textsubscript{6}H\textsubscript{4}-Me, 100%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}O\textsubscript{2}S\textsubscript{2} 278.04352; Found 278.0447; \textbf{6d}: 35% yield, mp 45˚C - 47˚C, IR ν 1340, 1116 cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 2.34 (6H, s, 2x CH\textsubscript{3}), 7.13 (4H, d, J = 7.80 Hz, AB system Ar), 7.41 (4H, d, J = 7.80 Hz, AB system Ar); \textsuperscript{13}C NMR δ 21.48, 128.47, 129.70, 133.85, 137.33; EIMS: m/z 246 (M\textsuperscript{+}, 100%), 123 (M – SC\textsubscript{6}H\textsubscript{4}-Me, 86%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}S\textsubscript{2} 246.0537; \textbf{7d}: 36% yield, mp 82˚C - 84˚C, IR ν 1321 (-SO\textsubscript{2}), 1146 (S -O) cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 3.91 (6H, s, 2x CH\textsubscript{3}), 6.80 - 7.00 (4H, m, Ar), 7.13 - 7.30 (2H, m, Ar), 7.54 (2H, d, J = 7.60 Hz, Ar); \textsuperscript{13}C NMR δ 56.25, 110.92, 121.70, 128.14, 138.68, 156.99; EIMS: m/z 278 (M\textsuperscript{+}, 82%), 139 (SO\textsubscript{2}C\textsubscript{6}H\textsubscript{4}-OMe, 100%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}O\textsubscript{4}S\textsubscript{2} 278.0333; Found 278.0340; \textbf{6f}: 31% yield, oil, IR ν 1249, 1085,1152 cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 3.79 (6H, s, 2x CH\textsubscript{3}), 6.78 (2H, d, J = 7.80 Hz, Ar), 7.05 - 7.15 (4H, m, Ar), 7.23 (2H, d, J = 7.8 Hz, Ar); \textsuperscript{13}C NMR δ 55.84, 111.09, 112.19, 114.09, 119.36, 120.72, 121.63, 130.14, 130.62, 133.50, 139.64, 160.06, 161.00; EIMS: m/z 278 (M\textsuperscript{+}, 41%), 139 (M-SC\textsubscript{6}H\textsubscript{4}-OMe, 100%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}O\textsubscript{4}S\textsubscript{2} 278.0435; Found 278.0439; \textbf{7f}: 45% yield, oil, IR ν 1286(-SO\textsubscript{2}), 1139,1183 (S-O) cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 3.70 (6H, s, 2x OCH\textsubscript{3}), 6.80 - 7.38 (8H, m, Ar); \textsuperscript{13}C NMR δ 55.85, 111.20, 112.19, 114.09, 119.36, 120.72, 121.63, 130.10, 130.62, 133.50, 139.64, 160.06, 161.00; EIMS: m/z 310 (M\textsuperscript{+}, 44%), 177 (SO\textsubscript{2}C\textsubscript{6}H\textsubscript{4}-OMe, 22%), 155 (SO\textsubscript{2}C\textsubscript{6}H\textsubscript{4}-OMe, 62%), 139 (SC\textsubscript{6}H\textsubscript{4}-OMe, 100%); HRMS: Calcd for C\textsubscript{14}H\textsubscript{14}O\textsubscript{4}S\textsubscript{2} 310.0333; Found 310.0340; \textbf{6g}: 34% yield, oil, IR ν 1030, 1171 cm\textsuperscript{-1}; \textsuperscript{1}H NMR δ 3.81 (6H, s, 2x OCH\textsubscript{3}), 6.84 (4H, m, Ar), 7.54 (2H, d, J = 7.75 Hz, AB system Ar), 7.60 (2H, d, J = 7.50 Hz, Ar).
1H NMR δ 5.59, 11.40, 128.54, 130.21, 157.10; EIMS: m/z 278 (M+, 95%), 139 (SC₆H₄-OMe, 100%); HRMS: Calcd. for C₁₄H₁₂O₄S₂ 278.0435; Found 278.0435; 31% yield, oil, IR ν 1288 (ω-SO₂), 1170, 1032 (ν-SO₂) cm⁻¹; 1H NMR δ 7.38 (4H, d, J = 7.80 Hz, Ar), 7.50 (2H, d, J = 7.80 Hz, Ar); 13C NMR δ 55.88, 114.64, 115.28, 124.90, 129.61, 130.37, 130.77, 130.81, 131.80, 134.31; EIMS: m/z 286 (Cl₃⁵) (M⁺, 90%), 143 (SC₆H₄-Cl₃⁵, 58%); HRMS: Calcd. for C₁₂H₈S₂Cl₂ 285.9445; Found 285.9451; 32% yield, mp 70 - 72°C, IR ν 1151 cm⁻¹; 1H NMR δ 7.28 (2H, t, J = 7.80 Hz, Ar), 7.54 (4H, d, J = 7.80 Hz, Ar); 13C NMR δ 121.00, 126.88, 127.83, 128.08, 132.80, 136.06; EIMS: m/z 378 (Br₈¹) (M⁺, 28%), 374 (Br₇₉) (M⁺, 25%), 189 (SC₆H₄-Br₈¹, 18%), 187 (SC₆H₄-Br₇₉, 17%); HRMS: Calcd. for C₁₂H₈S₂Br₂ 373.8443 (Br₈¹); Found 373.8445; 26% yield, mp 131°C - 135°C, IR ν 1326 (ω-SO₂), 1151 (ν-SO₂) cm⁻¹; 1H NMR δ 7.25 - 7.47 (4H, m, Ar), 7.57 (2H, s, Ar); 13C NMR δ 121.07, 121.25, 127.18, 128.20, 128.95, 131.07, 131.22, 132.93, 133.52, 134.56, 135.95, 141.82; 13C NMR δ 130.71, 131.20, 133.31, 133.93, 135.36, 140.10; EIMS: m/z 318 (Cl₃⁵) (M⁺, 41%), 159 (SO₂C₆H₄-Cl₃⁵, 100%), 143 (SC₆H₄-Cl₃⁵, 64%); HRMS: Calcd. for C₁₂H₈O₂S₂Cl₂ 317.9343 (Cl₃⁵); Found 317.9343; 43% yield, mp 70 - 72°C, IR ν 1127, 1456 cm⁻¹; 1H NMR δ 7.17 - 7.30 (4H, m, Ar), 7.30 - 7.42 (2H, m, Ar), 7.49 (2H, s, Ar); 13C NMR δ 125.58, 126.39, 127.63, 127.61, 129.10, 130.71, 131.20, 131.80, 134.31, 137.38; EIMS: m/z 286 (Cl₃⁵) (M⁺, 100%), 143 (SC₆H₄-Cl₃⁵, 78%); HRMS: Calcd. for C₁₂H₈S₂Cl₂ 285.9445 (Cl₃⁵); Found 285.9455; 7j: 31% yield, oil, IR ν 1294 (ω-SO₂), 1117 (ν-SO₂) cm⁻¹; 1H NMR δ 5.59, 11.40, 128.54, 130.21, 157.10; EIMS: m/z 278 (M⁺, 95%), 139 (SC₆H₄-OMe, 100%); HRMS: Calcd. for C₁₄H₁₂O₄S₂ 278.0435; Found 278.0435; 31% yield, oil, IR ν 1288 (ω-SO₂), 1170, 1032 (ν-SO₂) cm⁻¹; 1H NMR δ 7.38 (4H, d, J = 7.80 Hz, Ar), 7.50 (2H, d, J = 7.80 Hz, Ar); 13C NMR δ 55.90, 114.50, 128.54, 130.21, 157.10; EIMS: m/z 278 (M⁺, 95%), 139 (SC₆H₄-OMe, 100%); HRMS: Calcd. for C₁₄H₁₂O₄S₂ 278.0435; Found 278.0435; 7i: 32% yield, oil, IR ν 1294 (ω-SO₂), 1117 (ν-SO₂) cm⁻¹; 1H NMR δ 5.59, 11.40, 128.54, 130.21, 157.10; EIMS: m/z 278 (M⁺, 95%), 139 (SC₆H₄-OMe, 100%); HRMS: Calcd. for C₁₄H₁₂O₄S₂ 278.0435; Found 278.0435; 7h: 41% yield, oil, IR ν 1113, 1446 cm⁻¹; 1H NMR δ 7.38 (4H, m, Ar), 7.50 (2H, d, J = 7.80 Hz, Ar), 7.56 (2H, d, J = 7.80 Hz, Ar); 13C NMR δ 55.88, 114.64, 115.28, 124.90, 129.61, 130.37, 130.77, 130.81, 131.80, 134.31; EIMS: m/z 286 (Cl₃⁵) (M⁺, 90%), 143 (SC₆H₄-Cl₃⁵, 85%); HRMS: Calcd. for C₁₂H₈S₂Cl₂ 285.9444 (Cl₃⁵); Found 285.9455; 7g: 31% yield, oil, IR ν 1127, 1456 cm⁻¹; 1H NMR δ 5.59, 11.40, 128.54, 130.21, 157.10; EIMS: m/z 278 (M⁺, 95%), 139 (SC₆H₄-OMe, 100%); HRMS: Calcd. for C₁₄H₁₂O₄S₂ 278.0435; Found 278.0435;
EIMS: m/z 410 (Br⁸¹) (M⁺, 17%), 406 (Br⁷⁹) (M⁺, 15%), 221 (SO₂C₆H₄-Br⁸¹, 18%), 219 (SO₂C₆H₄-Br⁷⁹, 19%), 205 (SO₂C₆H₄Br⁸¹, 64%), 203 (SO₂C₆H₄Br⁷⁹, 61%), 108 (100%); HRMS: Calcd. for C₁₂H₈O₂S₂Br₂ 405.8332 (Br⁷⁹); Found 405.8344; 6l: 57% yield, 87˚C - 88˚C, IR ν 1149 cm⁻¹; ¹H NMR δ 7.34 (4H, d, J = 7.80 Hz, AB system Ar), 7.43 (4H, d, J = 7.80 Hz, AB system Ar); ¹³C NMR δ 121.43, 129.28, 132.09, 135.62; HRMS: Calcd. for C₁₂H₈S₂Br₂ 373.8434 (Br⁷⁹); Found 373.8439; 7l: 28% yield, mp 105˚C - 107˚C, IR ν 1323 (-SO₂), 1142 (S-O) cm⁻¹; ¹H NMR δ 7.24 (2H, d, J = 7.80 Hz, AB system, Ar), 7.44 (2H, d, J = 7.80 Hz, AB system, Ar), 7.52 (2H, d, J = 7.80 Hz, AB system Ar), 7.60 (2H, d, J = 7.80 Hz, AB system Ar); ¹³C NMR δ 126.49, 126.88, 128.82, 129.06, 132.10, 132.78, 137.70, 141.75; EIMS: m/z 318 (M⁺, 48%), 159 (SC₁₀H₇, 59%), 115 (100%); HRMS: Calcd. for C₁₀H₁₄O₂S₂ 318.0537; Found 318.0536; 7m: 40% yield, IR ν 1132 (SO₂) cm⁻¹; ¹H NMR δ 7.24 (1H, s, H -1'); 7.60 (5H, m, H-1', H-6, H-6', H-7, H-7'); 7.85 (5H, m, H-4', H-5, H-5', H-8, H-8'); 8.15 (1H, d, J = 7.80 Hz, H-3); 8.38 (1H, d, J = 7.80 Hz, H-4); 8.85 (1H, s, H-1); ¹³C NMR δ 126.05, 126.60, 126.88, 127.06, 128.10, 128.71, 129.30, 129.35, 129.41, 129.69, 129.80, 129.90, 130.2; EIMS: m/z 350 (M⁺, 10%), 159 (SC₁₀H₇, 51%), 115 (100%); HRMS: Calcd. for C₁₀H₁₄O₂S₂ 350.0435; Found 350.0436.

3.3. Microbiological Methods

Microorganisms: E. coli (JM 109) and S. aureus (SH1000) control strains were used to test antimicrobial activity. The series of compounds 6 and 7 were dissolved in DMSO to produce various concentrations which were refrigerated.

Inoculum: E. coli and S. aureus microorganisms were grown on Muller-Hinton Broth (MHB) for 24 hours at 37˚C. Starting inocula were prepared by diluting overnight cultures in fresh MHB to a culture density of 1 × 10⁶ cfu/ml. Minimum Inhibitory Concentrations (MIC) were determined according to the BSAC broth microdilution MIC methodology described by Andrews (2001).

4. Conclusion

We have shown how diarylthiosulfonates and diaryldisulfides, garlic-like organosulphur compounds can be conveniently synthesised in two steps in good yields. These compounds were tested for their antimicrobial properties against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Helicobacter pylori and had been found to have good activity against S. aureus and H. pylori with no activity against the other two organisms. SAR analysis of the results has shown the presence of chloro- or bromo-groups in the 2-position of the
aromatic rings to be crucial for antimicrobial activities. By SAR analysis the most promising four active compounds were \textit{6h, 6k, 7h} and \textit{7k} against Gram-positive organisms \textit{H. pylori} and \textit{S. aureus}. These compounds offer the promising prospects for development into clinical candidates by further studies.

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**Conflicts of Interest**

These studies were supported by the BMRC and there is no conflict of interest to disclose.

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