Synthesis and Antibacterial Activity of 1,3-Diallyltrisulfane Derivatives

Fang-Kui Ren, Xiao-Yan He, Li Deng, Bo-Heng Li,[†] Dong-Soo Shin,[‡,*] and Zhu-Bo Li[*]

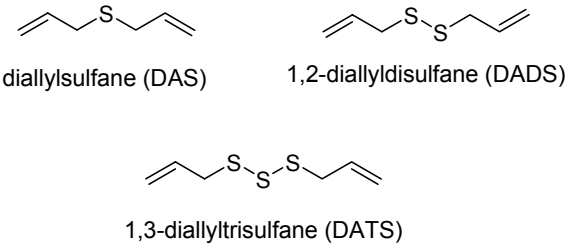
College of Pharmaceutical Sciences, Southwest University, Chongqing 400716, China
[†]High School Attached to Southwest Normal University, Chongqing 400715, China
[‡]Department of Chemistry, Changwon National University, Changwon, GN 641-733, Korea
[*]E-mail: dsshin@changwon.ac.kr
Received October 29, 2008, Accepted February 5, 2009

A series of novel 1,3-diallyltrisulfane analogues were synthesized and assayed *in vitro* for antimicrobial activity against Gram positive, Gram negative bacteria and fungi. The antimicrobial activity of the 1,3-diallyltrisulfane derivatives showed, on the whole, very potent towards all the tested Gram positive, Gram negative and fungi (MIC ranging from 4 to 256 μg/mL). 1,3-Di(pent-4-enyl)trisulfane **3b** and 1,3-bis(3-methylbut-2-enyl)trisulfane **3e** exhibited the strongest antibacterial activity among all the compounds, and both of them were more active than 1,3-diallyltrisulfane (DATS). Results indicated the relationship of either carbon number or lipophilicity with antimicrobial activity presented "V" shape. These observations provided some predictions in order to further design 1,3-diallyltrisulfane derivatives with antimicrobial activity.

Key Words: Diallylsulfane, Diallyldisulfane, Diallyltrisulfane, Antimicrobial activity, Biological activity

Introduction

Garlic (*Allium sativum* L.) has long been recognized to be nature plant medical.[1] Garlic contains abundance of organosulfur compounds, such as diallylsulfane (DAS), 1,2-diallyldisulfane (DADS) and 1,3-diallyltrisulfane (DATS) (Scheme 1**)**. On the other hand, thio ether and double bond has been indicated as an important pharmacophore and privileged structure in medicinal chemistry,[2-4] producing a diverse range of biological activities including antibacterical,[5] antiparasite,[6] antiviral,[7] anticancer,[8] antitumor,[9] antioxidation[10-11] and anticardiovascular activities.[12] Usually, 1,3-diallyltrisulfane was synthesized by unsaturated alkyl halide, sodium thiosulfate and sodium sulfide in DMSO, THF or H_2O-EtOH. Buffer solutions or expensive metal catalysts were used in some reactions.[13-16] Until now 1,3-diallyltrisulfane derivatives were not reported. Herein, we improved the method and synthesized novel 1,3-diallyltrisulfane derivatives **3a-3e**, containing longer chain and branched allyl moieties in the structures. All the compounds were tested for *in vitro* antimicrobial properties against Gram positive, Gram negative bacteria and fungi, and exhibited excellent activity. The structure-activity relationship of the 1,3-diallyltrisulfane derivatives was studied.



Scheme 1. Diallylsulfanes in garlic

Materials and Methods

All chemicals and solvents used were of AR grade and DATS was purchased from Institute of Biology, the Chinese Academy of Sciences. Extracted solvents were dried over anhydrous Na_2SO_4, followed by evaporation under vacuum. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, respectively) with TMS as the internal reference on Bruker Advance 400 FT spectrometer. Chemicals shifts were reported in parts per million. Mass spectra (MS) were measured by EPCI method. IR spectra were recorded, as KBr pellets, on a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Japan) and the reported wave numbers were given in cm^{-1}. Silica gel (200-300 mesh) was used for flash column chromatography. All the reactions were monitored by TLC using 0.25 mm silica gel plates (Merck 60F-254) with UV indicator. The ClogP values were calculated using ChemDraw Ultra 8.0.

General procedure for the synthesis. To the saturated aqueous solution of sodium thiosulfate (0.13 mol), was added the unsaturated alkyl bromide (0.1 mol) dropwise at 50-60°C, kept stirring. When the solution turned clear, the reaction mixture was cooled to room temperature and standing for separation. To the under layer the sodium sulfide solution (30 mL, 0.001 mol/mL) was added, kept stirring at room temperature overnight. The upper layer of the reaction mixture was washed by water and dried over aqueous sodium sulfate. Crude products were purified by column chromatography, yielding 80-90% of the products.

1,3-Di(but-3-enyl)trisulfane(3a): light-yellow oil; IR (KBr) ν_{max} 3078, 2978, 2922, 2844, 1835, 1639, 1435, 1415, 1273, 1215, 992, 914, 749, 634, 478 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49-2.55 (m, 4H), 2.94 (t, J = 7.6 Hz, 4H), 5.05-5.14 (m, 4H), 5.79-5.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 33.0, 37.9, 116.5, 134.0; MS (APCI) m/z 206 [M^+].

1,3-Di(pent-4-enyl)trisulfane (3b): light-yellow oil; IR (KBr) ν_{max} 3074, 2974, 2929, 2848, 1828, 1640, 1434, 1414, 1272, 1208, 989, 913, 748, 638, 478 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.82-1.90 (m, 4H), 2.16-2.21 (m, 4H), 2.88 (t, J = 7.2 Hz, 4H), 4.99-5.08 (m, 4H), 5.75-5.85 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.0, 32.4, 38.1, 115.4, 137.5; MS (APCI) m/z 235 [M^+].

3-Di(hex-5-enyl)trisulfane (3c): light-yellow oil; IR (KBr) ν_{max} 3074, 2974, 2928, 2855, 1827, 1639, 1435, 1415, 1273, 1215, 992, 916, 741, 634, 478 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.48-1.56 (m, 4H), 1.73-1.80 (m, 4H), 2.06-2.12 (m, 4H), 4.65 (t, J = 7.2 Hz, 4H), 4.94-5.05 (m, 4H), 5.75-5.86 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 28.2, 38.7, 114.8, 138.4; MS (APCI) m/z 262 [M^+].

1,3-Bis(2-methylallyl)trisulfane (3d): light-yellow oil; IR (KBr) ν_{max} 3079, 2972, 2938, 2914, 2854, 1801, 1648, 1449, 1374, 1219, 1010, 897, 742, 641, 479 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ1.84 (s, 6H), 3.46 (s, 4H), 4.94 (d, J = 6.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 46.4, 115.6, 140.0; MS (APCI) m/z 206 [M^+].

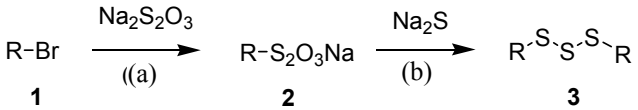
1,3-Bis(3-methylbut-2-enyl)trisulfane (3e): light-yellow oil; IR (KBr) ν_{max} 3025, 2969, 2935, 2857, 1665, 1634, 1448, 1376, 1207, 1104, 981, 888, 767, 693, 478 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.72 (s, 6H), 1.76 (s, 6H), 3.56 (d, J = 8.0 Hz, 4H), 5.30 (t, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0, 25.8, 36.8, 118.5, 138.2; MS (APCI) m/z 235 [M^+].

Microbiology. The *in vitro* antimicrobial activity was assayed by the twofold broth dilution technique against Gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 4698), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6896 and *Pseudomonas aeruginosa* ATCC 27853) and fungi (*Candida albicans* ATCC 76615, *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404). The minimal inhibitory concentration (MIC, μg/mL) was defined as the lowest concentration of compound that completely inhibited the growth of each strain.[17] DATS was used as reference antibacterial and antifungal substance. Test compounds were dissolved in H_2O containing 1% DMSO and diluted into different concentrations from 1 to 512 μg/mL with liquid medium. The concentration of mixture of 1,3-diallyltrisulfane derivatives and inocula was 5.00×10^5 bacteria/mL and 1.00×10^4 fungi/mL. It was incubated at 37 °C for 24 h for bacteria in broth medium, and at 25 °C for 48 h for the fungus in improved Sabouraud medium. Microbial growth was examined by measuring the absorbance at 655 nm with spectrophotometer. The H_2O/1% DMSO was used as a blank, inoculation bacterial not medicine as positive control. All experiments were run in triplicate.

Results & Discussion

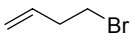
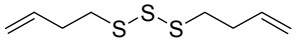
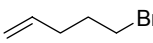
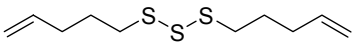
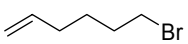
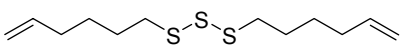
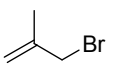
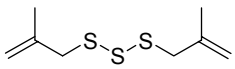
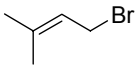
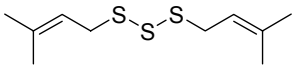
Chemistry. Unsaturated alkyl bromide **1** (1.0 equiv) was slowly added to saturated solution of sodium thiosulfate at 50-60 °C. The second step involved addition of sodium sulfide (1.0 equiv) to the product of salt **2** in solution obtained in the first step, to afford 1,3-diallyltrisulfane derivatives **3**, yielding 80-90%. In all the reactions, only water was used as solvent instead of any other organic solvents, which led to "green chemistry" compared with other methods (Scheme 2). As shown in Table 1, the reaction time and carbon number of compound **1** had significant effect on the yield of the products. As the carbon number of unsaturated alkyl bromide increased, the reaction time increased accordingly (**3a** to **3c**). Longer reaction time resulted in lower yield of product **3** (89.2% to 82.0%). The experimental data of **3d** and **3e** were also in accordance with such conclusion. The structures of 1,3-diallyltrisulfane derivatives, **3a-3e** were confirmed by IR, ^1H NMR, ^{13}C NMR and MS spectral data.

Antimicrobial activity. The 1,3-diallyltrisulfane deriva tives, **3a-3e** were assayed *in vitro* for their antimicrobial activity against the Gram positive, Gram negative bacteria and fungi (Table 2), in comparison with 1,3-diallyltrisulfane and the minimal inhibitory concentrations that inhibited the growth of the tested microorganisms (MIC) were detected.



Scheme 2. Design of the target compounds **3a-3e**. Reagents and condition: (a): H_2O, 50-60 °C; (b): H_2O, r.t., 80-90%

Table 1. Synthesis 1,3-diallyltrisulfane derivatives

Entry	Bromide	Product[a]	Reaction Time (h)[b]	Yield (%)[c]
3a			4	89.2
3b			6	87.5
3c			8	82.0
3d			4	85.0
3e			5	80.0

[a]All products were characterized by IR, MS, ^1H and ^{13}C NMR spectra. [b]The reaction time of the first step. [c]Isolated yields after column purification.

All the compounds displayed good inhibition of the growth of Gram positive and Gram positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* and Fungi, *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*. Most compounds exhibited MIC values in the range of 4-256 μg/mL, and the MIC values were represented (Table 2). Some of novel 1,3-diallyltrisulfane derivatives exhibited activity stronger than DATS. The data obtained indicated that the antibacterial activity against Gram positive bacteria was weaker than Gram negative bacteria, and fungi activity was the lowest (Figure 1). It is noteworthy that, among the 1,3-diallyltrisulfane derivatives the inhibitory effect appeared to be dependent on chain length and carbon number.

As shown in Table 2, all the compounds except **3c** were more active than DATS. Compounds **3b** and **3c** containing ten carbon atoms showed stronger antibacterial activity than **3a** and **3e** with eight carbons, and **3a** and **3e** exhibited stronger activity than DATS which contains only six carbon atoms. Whereas, the antimicrobial activity of **3c,** which contains twelve carbons, became lower than DATS and other derivatives (**3a**, **3b, 3d** and **3e**). In summary, the relationship of carbon number of product **3** with the antibacterial activity presented "V" shape (Figure 2). In addition, branched 1,3-diallyltrisulfane analogues (**3d** and **3e**) showed higher antimicrobial activity than straight chained 1,3-diallyltrisulfane analogues (**3a** and **3b**). MIC value of straight chained compounds from DATS to **3a** and **3b** decreased, with increase of corresponding ClogP value (Table 2), which indicated the increase of antibacterial activity. Whereas, the MIC value of compound **3c** was higher than DATS, **3a** and **3b**, also with higher ClogP than DATS, **3a** and **3b**. The lipophilicity of branched compounds from **3d** (ClogP = 4.52) to **3e** (ClogP = 5.58) showed an increase tendency, in accordance with the increase of antibacterial activity.[18] This meant the relationship of lipophilicity and antibacterial activity also presented "V" shape.

Among all the tested compounds, **3b** and **3e** exhibited the best antibacterial activity against Gram positive, Gram negative bacteria and Fungi. The MIC value of **3b** against Gram positive, *Staphylococcus aureus* was 8 μg/mL, and compound **3e** showed the highest activity against *Micrococcus luteus* (8 μg/mL). As regarding for the activity against the Gram negative bacteria, **3b** exhibited 8 μg/mL of MIC value against *Pseudomonas aeruginosa*, and the MIC of **3e** was 4 μg/mL against *Pseudomonas aeruginosa*. The lowest MIC value against fungi was obtained by compound **3e**, which gave MIC value of 8 μg/mL against *Candida albicans* and *Aspergillus niger* (Figure 2). Branched **3e** showed stronger antibacterial activity than straight chained **3b** (Figure 2). Among all the tested compounds, **3c** showed the lowest antibacterial activity. All the synthesized novel compounds showed lower activity than DATS against *Saccharomyces cerevisiae*. These observations provided some predictions in order to design further antimicrobial active compounds prior to their synthesis following with molecular modeling studies.

Table 2. Antimicrobial activity of **3a-3e** expressed as MIC (μg/mL)

Entry	CLogP[a]	Microorganisms								
		Gram-positive[b]			Gram-negative[c]			Fungi[d]		
		SA	BS	MI	EC	PV	PA	CA	SC	AN
DATS	3.72	64	32	64	32	64	64	64	64	64
3a	4.38	16	32	32	32	32	32	32	256	64
3b	5.44	8	32	16	32	64	8	16	128	16
3c	6.50	32	32	64	64	256	32	256	256	128
3d	4.52	32	32	64	32	32	32	64	128	64
3e	5.58	16	32	8	32	32	4	8	128	8

[a]Lipophilicity reported as calculated log of partition coefficient (ClogP values). [b]Gram positive bacteria: SA, *Staphylococcus aureus* (ATCC 25923); BS, *Bacillus subtilis* (ATCC 6633); MI, *Micrococcus luteus* (ATCC 4698). [c]Gram negative bacteria: EC, *Escherichia coli* (ATCC 25922); PV, *Proteus vulgaris* (ATCC 6896); PA, *Pseudomonas aeruginosa* (ATCC 27853). [d]Yeasts (Fungi): CA, *Candida albicans* (ATCC 76615); SC, *Saccharomyces cerevisiae* (ATCC 9763); AN, *Aspergillus niger* (ATCC 16404).

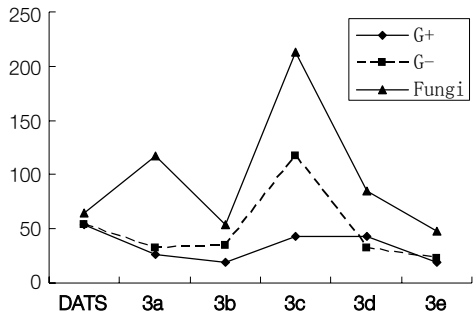


Fig 1. The average activity (μg/mL) against G+, G- and fungi of 1,3-diallyltrisulfane derivatives (**3a-3e**).

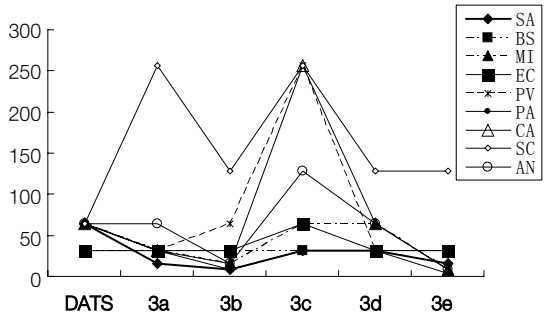


Fig 2. Antibacterial activity (μg/mL) of 1,3-diallyltrisulfane derivatives (**3a-3e**).

Conclusion

Novel 1,3-diallyltrisulfane analogues **3a-3e** were synthesized by improved method and assayed *in vitro* for the evaluation of their antimicrobial activity against Gram positive, Gram negative bacteria and fungi. The antimicrobial activity of the 1,3-diallyltrisulfane derivatives showed, on the whole, very potent towards a wide spectrum of Gram positive, Gram negative and fungi (MIC ranging from 4 to 256 μg/mL). Compounds **3b** and **3e** exhibited the best antibacterial activity among all the new compounds. Data

obtained suggested that chain length and carbon number played an important role on the antimicrobial properties of this class of compounds. The good properties of the novel class of antibacterial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for deeper insight into structure-activity relationship and to optimize the effectiveness of the 1,3-diallyltrisulfane derivatives.

Acknowledgments. This study was supported by the Key Project of the Municipal Natural Science Foundation of Chongqing, China (grant No. CSTC, 2008 A A1001).

References

1. Corzo-Martinez, M.; Corzo, N.; Villamiel, M. *Trends Food Sci. Tech.* **2007**, *18*, 609-625.
2. Moriguchi, T.; Matsuura, H.; Kodera, Y.; Itakura, Y.; Katsuki, H.; Saito, H.; Nishiyama, N. *Neurochemical Research* **1997**, *22*, 1449-1452.
3. Augusti, K. T.; Sheela, C. G. *Cell Mol. Life Sci*. **1966**, *52*, 115-119.
4. Oi, Y.; Kawada, T.; Shishido, C.; Wada, K.; Kominato, Y.; Nishimura, S.; Ariga, T.; Iwa, K. *J. Nutrition* **1999**, *129*, 336-342.
5. Curtis, H.; Noll, U.; Störmann, J.; Slusarenko, A. J. *Physiol. Mol. Plant Pathol*. **2004**, *65*, 79-89.
6. Saleheen, D.; Ali, S. A.; Yasinzai, M. M. *Fitoterapia* **2004**, *75*, 9-13.
7. Weber, N. D.; Anderson, D. O.; North, J. A.; Murray, B. K.; Lawson, L. D.; Hughes, B. G. *Planta Med*. **1999**, *58*, 417-423.
8. Oommen, S.; Anto, R. J.; Srinivas, G.; Karunagaran, D. *Eur. J. Pharmacol*. **2004**, *485*, 97-103.
9. Wu, C. C.; Chung, J. G.; Tsai, S. J.; Yang, J. H.; Sheen, L. Y. *Food Chem. Toxicol*. **2004**, *42*, 1937-1947.
10. Wu, C. C.; Sheen, L. Y.; Chen, H. W.; Tsai, S. J.; Lii, C. K. *Food Chem. Toxicol*. **2001**, *39*, 563-569.
11. Yin, M. C.; Cheng, W. S. *Meat Sci*. **2003**, *63*, 23-28.
12. Gargner, C. D.; Chatterjee, L. M.; Carlson, J. J. *Atherosclerosis* **2001**, *154*, 213–220.
13. Zhu, Y.; Zhao, M. Z.; Xu, M. X. *Chinese J. Syn. Chem*. **2001**, *9*, 541-542.
14. Sinha, P.; Kundu, A.; Roy, S. *Organometallics* **2001**, *20*, 157-162.
15. Block, E.; Lyer, R.; Grisoni, S.; Saha, C.; Belman, S.; Lossing, F. P. *J. Am. Chem. Soc.* **1988**, *110*, 7813-7827.
16. Block, E.; Ahmad, S.; Catalfamo, J. L.; Jain, M. K.; Apitz-Castro, R. *J. Am. Chem. Soc*. **1986**, *108*, 7045-7055.
17. Ma, X. R. *Beijing: People Health Publishing House* **2001**, 50-53.
18. Phillips, O. A.; Udo, E. E.; Samuel, S. M. *Eur. J. Med. Chem*. **2008**, *43*, 1095-1104.