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Synthesis and evaluation of novel sulfenamides as novel anti Methicillin-resistant *Staphylococcus aureus* agents

Jian-Li Shang [a,†], Hui Guo [b,c,†], Zai-Shun Li [a], Biao Ren [b,c], Zheng-Ming Li [a], Huan-qin Dai [b], Li-Xin Zhang [b,*], Jian-Guo Wang [a,*]

[a] State-Key Laboratory and Research Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, PR China
[b] CAS Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100190, PR China
[c] Graduate University of Chinese Academy of Sciences, Beijing 100049, PR China

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ABSTRACT

A total of 29 novel sulfenamide compounds were synthesized, spectroscopically characterized and evaluated in vitro for antimicrobial activity against various infectious pathogens. Compounds **1b** and **2c** exhibited potent inhibition against clinical Methicillin-resistant *Staphylococcus aureus* (MRSA) strains with minimum inhibitory concentration (MIC) values of 1.56 μg/mL.

The majority of sulfur–nitrogen bonding compounds refer to the sulfonyl amino compounds.[1] For examples, sulfonylureas are a class of important herbicides,[2,3] while sulfanilamides are common drugs treating infections.[4,5] In some cases, the sulfinyl amides are also frequently mentioned.[6–8] However, compounds containing an N–S bond (referred as N-thiolated compound or sulfenamide) have seldom been explored. Previously, the N–S bond is mainly used in the protection of amino groups for peptide synthesis.[9] Very recently, some sulfenyl amino compounds were reported to display herbicidal activity or insecticidal activity for pesticide research.[10–13] Turos's research group has showed that some N-thiolated β-lactams possess considerable antibacterial activity.[14–21] It is interesting to further investigate the biological activities of the N–S bonding compounds.

Infections caused by resistant microbes such as Methicillin-resistant *Staphylococcus aureus* (MRSA) have become a major global health concern,[22] which makes it urgent to develop novel anti-MRSA agents.[23–25] Previously, we had identified some sulfenamide compounds of different skeletons with antibacterial activity via a high-throughput screening of a synthetic compound library (unpublished result). In the present study, we have designed and synthesized more derivatives of the sulfenamides and investigated their antimicrobial activity. With the expectation that compounds contaning an N–S bond may have diverse structural space, the N moieties of the sulfenamides are from L-tryptophan esters, L-glutamic acid esters or substituted isatins, while the S parts are from various arenesulfenyl chlorides. To our excitement, some of the target compounds display remarkable anti-MRSA activity.

The synthesis procedures for groups **1**, **2** and **3** of the sulfenamides are shown in Scheme 1. The chemical synthesis and characterizations of the compounds are detailed in the Supplementary data. For groups **1** and **2**, the solvent is anhydrous diethyl ether and the temperature is low using an ice–salt bath for the reaction. For group **3**, the solvent for the reactants is *N,N*-dimethylformamide and the reaction occurs at room temperature. Except **1i** and **1j**, most compounds in the previous two series have low melting points, while most compounds in the last series have relatively higher melting points. Generally, the yields of the reaction are >50%. The chemical structures of all compounds are listed in Table 1.

The preliminary antibacterial activity screening results of the target compounds are shown in Table 2. All compounds exhibit no measurable inhibition against the Gram-negative *Pseudomonas aeruginosa* (PA, PAO1) even at a high concentration of 100 μg/mL. Interestingly, some of the compounds display considerable inhibition against the Gram-positive *Bacillus subtilis* (BS, ATCC6633) and MRSA (a clinical isolate from Beijing Chaoyang Hospital). Most compounds in groups **1** and **2** have minimum inhibitory

* Corresponding authors. Tel./fax: +86 10 62566511 (L.-X. Z.); tel.: +86 22 23499414; fax: +86 22 23503627 (J.-G. W.).
E-mail addresses: zhanglixin@im.ac.cn (L.-X. Zhang), nkwjg@nankai.edu.cn (J.-G. Wang).
† These authors contributed equally to this work.

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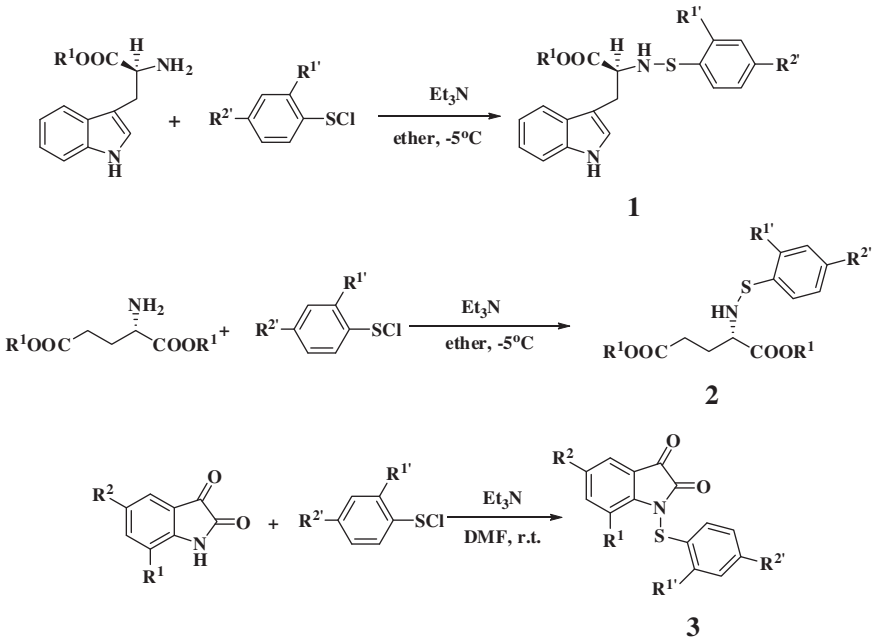

Scheme 1. Outline of the synthesis of the various sulfenamide compounds.

Table 1
Chemical structures of the sulfenamide compounds

Compd	R^1	R^2	$R^{1'}$	$R^{2'}$	Compd	R^1	R^2	$R^{1'}$	$R^{2'}$
1a	CH_3	—	NO_2	H	**2f**	$CH(CH_3)_2$	—	NO_2	NO_2
1b	CH_2CH_3	—	NO_2	H	**3a**	H	H	NO_2	H
1c	$CH(CH_3)_2$	—	NO_2	H	**3b**	H	H	H	H
1d	CH_3	—	H	H	**3c**	H	H	H	CH_3
1e	CH_2CH_3	—	H	H	**3d**	H	H	$COOCH_3$	H
1f	CH_2CH_3	—	H	CH_3	**3e**	H	H	$COOCH_2CH_3$	H
1g	$CH(CH_3)_2$	—	H	CH_3	**3f**	H	H	NO_2	NO_2
1h	CH_3	—	H	NO_2	**3g**	Cl	H	NO_2	H
1i	CH_3	—	NO_2	NO_2	**3h**	Cl	H	$COOCH_3$	H
1j	CH_2CH_3	—	NO_2	NO_2	**3i**	Cl	H	$COOCH_2CH_3$	H
2a	CH_3	—	NO_2	H	**3j**	Br	H	NO_2	H
2b	CH_2CH_3	—	NO_2	H	**3k**	Br	H	$COOCH_2CH_3$	H
2c	CH_3	—	H	NO_2	**3l**	Br	Br	NO_2	H
2d	CH_3	—	NO_2	NO_2	**3m**	Br	Br	$COOCH_2CH_3$	H
2e	CH_2CH_3	—	NO_2	NO_2					

Table 2
Antimicrobial activity of the sulfenamide compounds

Compd	BS[a]	MRSA[a]	PA[a]	Compd	BS[a]	MRSA[a]	PA[a]
1a	12.5	3.13	>100	**2f**	25	>100	>100
1b	6.25	1.56	>100	**3a**	>100	25	>100
1c	12.5	3.13	>100	**3b**	25	50	>100
1d	25	50	>100	**3c**	>100	>100	>100
1e	25	25	>100	**3d**	>100	50	>100
1f	25	>100	>100	**3e**	>100	12.5	>100
1g	25	>100	>100	**3f**	>100	>100	>100
1h	25	>100	>100	**3g**	>100	6.25	>100
1i	25	>100	>100	**3h**	>100	12.5	>100
1j	25	>100	>100	**3i**	>100	>100	>100
2a	25	12.5	>100	**3j**	>100	12.5	>100
2b	25	25	>100	**3k**	>100	>100	>100
2c	3.13	1.56	>100	**3l**	>100	25	>100
2d	50	>100	>100	**3m**	>100	25	>100
2e	50	>100	>100	Van[b]	0.5	1.0	>100

The MIC data are expressed in μg/mL.
[a] BS = *Bacillus subtilis* (ATCC6633), PA = *Pseudomonas aeruginosa* (PAO1), MRSA is a clinical isolate from Chaoyong hospital, Beijing.
[b] Van = vancomycin.

concentrations (MICs) <50 μg/mL against BS, while most compounds in group **3** do not have a measureable MIC value even at 100 μg/mL concentration. It is noteworthy that both compounds **1b** and **2c** exhibit remarkable MICs of 1.56 μg/mL against MRSA, comparable to the reference drug vancomycin (MIC = 1.0 μg/mL). The MICs of compounds **1a** and **1c** against MRSA are 3.13 μg/mL, and the MIC of compound **3g** is 6.25 μg/mL. The remaining compounds exhibit very weak activity against MRSA. The MIC of compound **2c** against BS is 3.13 μg/mL, and compound **1b** has an MIC 6.25 μg/mL. For compounds **1a** and **1c**, their MIC data against BS are both 12.5 μg/mL. There is a general correlation between the anti-MRSA and anti-BS activity.

Since the sulfenamides display desirable anti-MRSA activity, we reevaluated the biological activity of compounds **1a**, **1b**, **1c**, **2c** and **3g** with ATCC standard strain and various clinical isolated Methicillin-susceptible *Staphylococcus aureus* (MSSA) and MRSA strains. On the other hand, it is interesting to judge whether these compounds belong to bactereostatic or bactericidal agents, the minimum bactericidal concentrations (MBCs) were also determined in this study. Table 3 lists the MICs and the MBCs of the selected compounds. For SA ATCC6538 and MSSA 309-1, the MICs are in the

Table 3
MIC data of selected sulfenamides and related compounds against vaious clinical isolated MRSA and MSSA strains

Compd.	SA ATCC6538		MSSA 309-1[a]		MRSA Chaoyang[a]		MRSA 6-42[a]		MRSA 309-4[a]		MRSA 309-7[a]		MRSA 309-8[a]	
	MIC[b]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1a	6.25	>100	25	>100	3.13	>100	3.13	>100	25	>100	12.5	>100	25	>100
1b	6.25	>100	12.5	>100	1.56	>100	1.56	>100	12.5	>100	6.25	>100	6.25	>100
1c	6.25	>100	25	>100	3.13	>100	3.13	>100	25	>100	6.25	>100	6.25	>100
2c	6.25	>100	6.25	>100	1.56	>100	1.56	>100	6.25	>100	3.13	>100	3.13	>100
3g	12.5	>100	12.5	>100	6.25	>100	6.25	>100	12.5	>100	6.25	>100	12.5	>100
Van[c]	1	2	1	1	1	1	1	1	0.5	1	1	1	0.5	1
L-Trp[c]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
L-Glu[c]	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4-Nitrobenzenethiol	3.13	>100	1.56	>100	1.56	>100	1.56	>100	1.56	>100	1.56	>100	1.56	>100

[a] MSSA 309-1 is a clinical isolated strain of MSSA from the 309th hospital of Chinese People's Liberation Army in Beijing, MRSA Chaoyang is a MRSA clinical isolated strain from Chaoyang hospital in Beijing (the same with the MRSA isolate in Table 2), MRSA 6-42 is a MRSA clinical isolated strain from the 306th hospital of Chinese People's Liberation Army in Beijing, MRSA 309-4, MRSA 309-7 and MRSA 309-8 are three MRSA clinical isolated strains from the 309th hospital of Chinese People's Liberation Army in Beijing.
[b] MIC and MBC values are expressed in μg/mL.
[c] Van = vancomycin, L-Trp = L-tryptophan ethyl ester, L-Glu = L-glutamic acid dimethyl ester.

range of 6.25–25 μg/mL, while for the different MRSA clinical strains, the MIC data range from 1.56 μg/mL to 25 μg/mL. It should be noted that for compounds **1b** and **2c**, their MIC data against MRSA chaoyang strain and MRSA 6-42 strain are all 1.56 μg/mL. When tested against the MRSA strains of 309-4, 309-7 and 309-8, **2c** shows better MIC data than **1b**, however, the best MIC data of **2c** increase to 3.13 μg/mL. It can be seen that the MBC data of all compounds are >100 μg/mL, suggesting that these sulfenamides are bactereostatic compounds. It is clear that vancomycin is bactericidal, and the MICs of which are 0.5–1 μg/mL and the MBC/MIC ratios for vancomycin are 1:2.

Actually, this is not the first report indicating that compounds containing an N–S bond possess inspiring antimicrobial activity, Turos et al. have systematically investigated the antibacterial activity of the N–S linking compounds.[14–21] However, they only focus on the N-thiolated β-lactams and N-thiolated 2-oxazolidinones, and the most potent compound they have found exhibits similar ani-MRSA MIC value with vancomycin.[16] Turos et al. found that the anti-MRSA activity of N-thiolated β-lactams has relationship with fatty acid synthesis (FAS),[17] and their latest research indicated that these agents are unique since their effects do not unimpeded by exogenous FAs.[16]

As the N-thiolated β-lactams were proposed as prodrugs,[17] it is necessary to investigate whether the N part or S part plays a major role in MRSA inhibition, which has not been experimentally studied by any previous literature. Thus we further evaluated the biological activity of L-tryptophan ethyl ester (L-Trp), L-glutamic acid dimethyl ester (L-Glu) and 4-nitrobenzenethiol against the MSSA and MRSA strains in our laboratory in that they have close structural relationship with compounds **1b** and **2c** in this study. The bioassay data are also shown in Table 3, from which L-Trp and L-Glu do not show anti-MRSA or anti-MSSA activity even at 100 μg/mL concentration, while 4-nitrobenzenethiol shows strong inhibitory activity. For the standard strain of SA (ATCC6538), the MIC data of 4-nitrobenzenethiol is 3.13 μg/mL, and for the clinical isolated MSSA and MRSA strains, the corresponding MIC data are all 1.56 μg/mL. This result strongly indicates that, the anti-MRSA activity of the N-thiolated compounds is most likely due to the N–S bond cleavage within the cell.

From a view of the structure–activity relationship, although it is supposed that the true active component of the sulfonamide is the S moiety of the molecule after the intracellular cleavage of the N–S bond, the attached N part of the compound also influence the antibacterial activity significantly. For example, compound **1h** and **2c** have the same S parts while their N parts differ a lot, and **2c** is 60 times more potent than **1h** for the anti-MRSA activity. For **1a**, **1b** and **1c**, their whole molecular structures are very similar, thus their anti-MRSA activities are at similar level. It is quite possible that the disassociation of the N–S bond is highly influenced by the different N moiety of the compounds. For the S part of the molecule, from the preliminary result, it seems that a single $-NO_2$ group in either the $R^{1'}$ or $R^{2'}$ position leads to desirable activity. However, when both the $R^{1'}$ and $R^{2'}$ positions are $-NO_2$ groups, the sulfonamides do not display good inhibition of MRSA. The $-CH_3$ in $R^{2'}$ position or –COOR in $R^{1'}$ position of the compound offers poor activity. It is expected that some groups such as $-CF_3$ will lead to promising activity. More derivatives of the sulfonamides will be synthesized in the future and detailed study on their biological activity and cytotoxic data will be carried out for the discovery of new anti-MRSA agents.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.103.

References and notes

1. Davis, F. A. *J. Org. Chem.* **2006**, *71*, 8993.
2. Chaleff, R. S.; Mauvais, C. J. *Science* **1984**, *224*, 1443.
3. Wang, J. G.; Li, Z. M.; Ma, N.; Wang, B. L.; Jiang, L.; Pang, S. S.; Lee, Y. T.; Guddat, L. W.; Duggleby, R. G. *J. Comput. Aided Mol. Des.* **2005**, *19*, 801.
4. Kneeland, Y.; Mulliken, B. *J. Clin. Invest.* **1940**, *19*, 735.
5. Cooper, L. Z.; Madoff, M. A.; Weinstein, L. *Antibiot. Chemother.* **1962**, *12*, 618.
6. Jin, S. S.; Wang, H.; Xu, M. H. *Chem. Commun. (Camb)* **2011**, *47*, 7230.
7. Schenkel, L. B.; Ellman, J. A. *Org. Lett.* **2004**, *6*, 3621.
8. Mukade, T.; Dragoli, D. R.; Ellman, J. A. *J. Comb. Chem.* **2003**, *5*, 590.
9. Zervas, L.; Hamalidis, C. *J. Am. Chem. Soc.* **1965**, *87*, 99.
10. Wang, J. G.; Xiao, Y. J.; Li, Y. H.; Ma, Y.; Li, Z. M. *Bioorg. Med. Chem.* **2007**, *15*, 374.
11. Sun, R.; Zhang, Y.; Chen, L.; Li, Y.; Li, Q.; Song, H.; Huang, R.; Bi, F.; Wang, Q. *J. Agric. Food Chem.* **2009**, *57*, 3661.
12. Zhao, Q.; Ou, X.; Huang, Z.; Bi, F.; Huang, R.; Wang, Q. *J. Agric. Food Chem.* **2008**, *56*, 10799.

13. Zhao, Q.; Shang, J.; Liu, Y.; Wang, K.; Bi, F.; Huang, R.; Wang, Q. *J. Agric. Food Chem.* **2007**, *55*, 9614.
14. Turos, E.; Konaklieva, M. I.; Ren, R.; Shi, H.; Gonzalez, J.; Dickey, S.; Lim, D. V. *Tetrahedron* **2000**, *56*, 5571.
15. O'Driscoll, M.; Greenhalgh, K.; Young, A.; Turos, E.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem.* **2008**, *16*, 7832.
16. Prosen, K. R.; Carroll, R. K.; Burda, W. N.; Krute, C. N.; Bhattacharya, B.; Dao, M. L.; Turos, E.; Shaw, L. N. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5293.
17. Revell, K. D.; Heldreth, B.; Long, T. E.; Jang, S.; Turos, E. *Bioorg. Med. Chem.* **2007**, *15*, 2453.
18. Heldreth, B.; Long, T. E.; Jang, S.; Reddy, G. S.; Turos, E.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem.* **2006**, *14*, 3775.
19. Turos, E.; Long, T. E.; Heldreth, B.; Leslie, J. M.; Reddy, G. S.; Wang, Y.; Coates, C.; Konaklieva, M.; Dickey, S.; Lim, D. V.; Alonso, E.; Gonzalez, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2084.
20. Turos, E.; Long, T. E.; Konaklieva, M. I.; Coates, C.; Shim, J. Y.; Dickey, S.; Lim, D. V.; Cannons, A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2229.
21. Mishra, R. K.; Revell, K. D.; Coates, C. M.; Turos, E.; Dickey, S.; Lim, D. V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2081.
22. Gootz, T. D. *Crit. Rev. Immunol.* **2010**, *30*, 79.
23. Appelbaum, P. C. *Clin. Microbiol. Infect.* **2006**, 3.
24. Rice, L. B. *J. Infect. Dis.* **2008**, *197*, 1079.
25. Deurenberg, R. H.; Stobberingh, E. E. *Infect. Genet. Evol.* **2008**, *8*, 747.