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Design, synthesis and antibacterial activities of a series of new 2-oxaisocephems

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ABSTRACT

A series of 2-oxaisocephems with a thio-substituted methyl group at the 3-position and a [2-(5-amino-1,2,4-thiadizol-3-yl)-2-(*Z*)-alkoxyimino]acetamido moiety at 7-position were synthesized and tested for their antibacterial activities. The analogs **17c** and **17f** have well-balanced potency and significantly enhanced activity as compared with the reference compound ceftazidime.

Over the past decades, a large variety of antibiotics have become available for clinical use. Cephalosporin antimicrobial agents continue to be an important role in the treatment of bacterial infections for their favorable safety profile and generally bactericidal mode of action. These antibiotics share a common structure and mechanism of action by inhibiting peptidoglycan synthesis in the cell wall. Their clinical efficacy and favorable safety profiles have spurred the development of new products that practitioners can now add to their armamentarium to fight infections. Unfortunately, the wide-spread and indiscriminant use of cephalosporins has accelerated the selection of resistant strains and led to a rapid increase in the number of bacterial strains which are resistant to these compounds.[1] Furthermore, the introduction of new antibiotics has been decreasing recently compared with the period from 1945 to 1970, when a variety of new antibiotics with new structures were discovered.[2] Thus it is urgent to develop new agents which can solve the problem of resistant bacteria.

2-Oxaisocephem is a special group among β-lactam antibacterial agents and a class of new compounds possessing an oxygen atom at the 2-position of the cephalosporin frame. Doyle et al. reported the synthesis and activity of 2-oxaisocephems, but the results showed that 2-oxaisocephems lacked comprehensive antibacterial activity.[3–11] Later, Mastalerz et al. proposed that new orally absorbable 2-oxaisocephems were effective against Gram-positive organisms.[12] Until the application of the 2-(2-aminothiazol-4-y1)-2-(*Z*)-(methoxyimino)acetamido side chain at the 7-position, a great progress has been made in the development of 2-oxaisocephems.[13] Tsubouchi et al. further developed the side chains at 7-position from this standpoint and presented the synthesis of parenteral 2-oxaisocephems with the side chains of third-generation cephalosporins at 7-position, which apparently enhanced the activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus faecalis* and broadened anti-Gram-positive spectrum while maintaining Gram-negative activity.[14–20] Whereas, there was no extensive study about 2-oxaisocephems reported in recent decades. As the development of the antibiotics, more new side-chains with good activity and broad antibacterial spectrum appeared, therefore we speculated that introduction of the side-chains of new-generation cephalosporins into the 3-position and 7-position of 2-oxaisocehems might further enhance the activity and broaden the antibacterial spectrum. We herein reported the synthesis and antibacterial activity of new 2-oxaisocephems possessing thio-substituted methyl groups at the 3-position and the 5-amino-1,2,4-thiadizol-3-yl moiety at 7-position.

First, compound **10**, a key intermediate in the synthesis of new 2-oxaisocephems, was prepared. The starting material **2** was prepared in 12 steps from L-ascorbic acid **1** according to a modified literature procedure.[21] Typical procedures to obtain **10** from **2** are as follows. Protection of the alcohol group of **2** as a silyl ether **3** was achieved by treatment with *tert*-butyldimethylsilyl chloride in DMF. Removal of the sulfuryl group in the presence of trifluoroacetic acid gave **4** followed by hydrogenolysis and reaction with trityl chloride to accomplish the replacement of the benzyloxycarbonyl protecting group on **6** with a trityl group. Attachment of the allyl acetate group to the nitrogen atom of the azetidinone **6** was easy to achieve in the presence of strong base sodium

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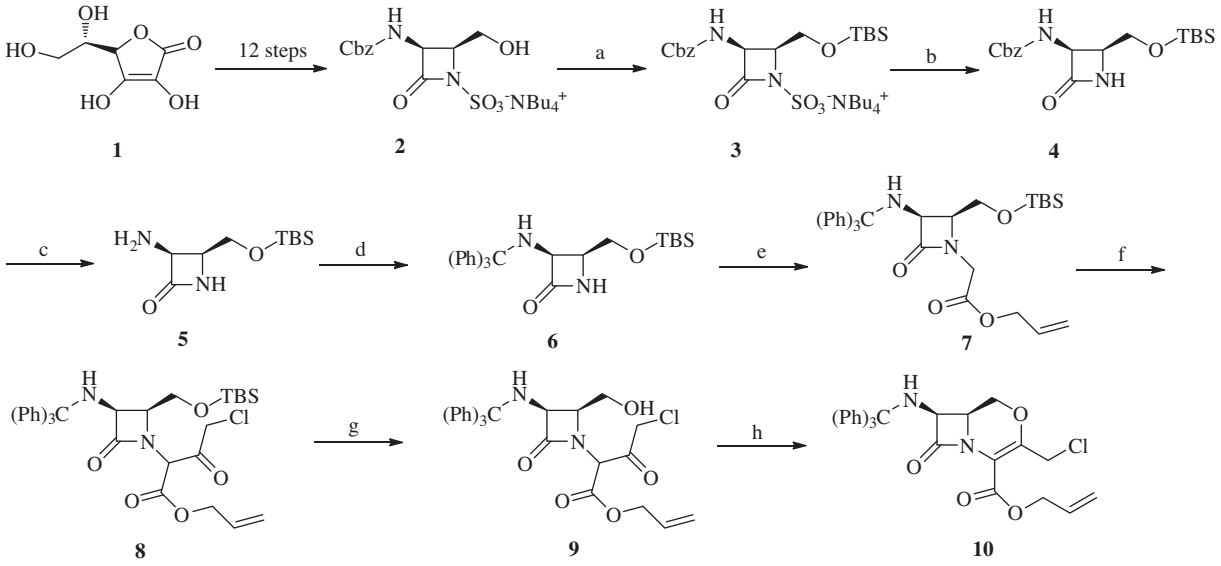
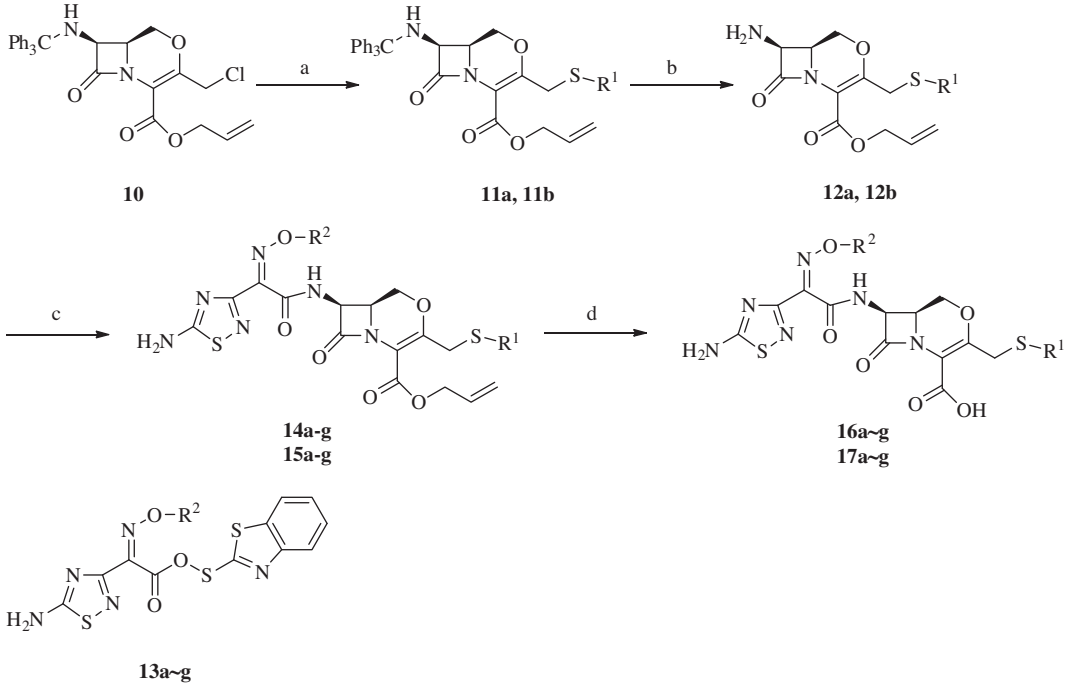


Scheme 1. Synthesis of key intermediate **10**. Reagents and conditions: (a) TBSCl, DMAP, Et_3N, DMF, rt, 1 h,; (b) TFA, THF, rt, 5 h; (c) Pd/C, H_2, EA, rt, 5 h; (d) Ph_3CCl, Et_3N, CH_2Cl_2, rt, 5 h; (e) allyl bromoacetate, NaHMDS, −50 °C, 30 min; (f) chloracetyl chloride, LiHMDS, THF, −78 °C; (g) TBAF, THF, rt; (h) PPh_3, DIAD, THF, rt, 15 min.

hexamethyldisiloxane and allyl bromoacetate to afford **7**. Conversion of **7** to its lithium salt in the presence of a strong base made it readily react with chloroacetyl chloride to give the α-keto ester **8**. Removal of the silyl protecting group furnished the 3-hydroxymethyl compound **9**, which was subsequently subjected to a Mitsunobu reaction to obtain the key intermediate **10** (Scheme 1).

With the key intermediate **10** in hand, next we carried out the synthesis of the targeting 2-oxaisocephems derivatives (series 1: **16a–g**, series 2: **17a–g**) according to the modified literature method.[22] C-3′ substituents were introduced by treatment of **10** with sodium salts of the thiol derivatives in the presence of the catalyst tetra-*n*-butyl ammonium bromide (TBAB) in methylene dichloride and H_2O to afford **11a** and **11b**. After deprotection of the trityl group by *p*-toluene sulfonic acid (PTSA), the generated amines were allowed to react with 2-mercaptobenzothiazole active ester **13a–g** to provide the ally esters **14a–g** and **15a–g**, which were subjected to deprotection with $Pd(PPh_3)_4$ to give the desired 2-oxaisocephems derivatives **16a–g** and **17a–g** (Scheme 2 and Table 1).

All of the compounds **16a**–**g** and **17a**–**g** prepared in this investigation were tested for their in vitro antibacterial activity against Gram-positive (*S. aureus*, and *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) bacterial strains. The minimum inhibitory concentrations (MICs) of the tested compounds compared with ceftazidime as reference compounds are displayed in Table 2.



Scheme 2. Synthesis of 2-oxaisocephems. Reagents and conditions: (a) $NaSR^1$, TBAB, CH_2Cl_2/H_2O, 30 °C, overnight; (b) PTSA, acetone, 0 °C, 30 h; (c) 2-mercaptobenzothiazole active esters **13a–g**, THF, 30 °C, 6 h; (d) PPh_3, $Pd(PPh_3)_4$, THF, 30 °C, 4 h.

Table 1
2-Oxaisocephems **16a–g** and **17a–g**

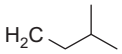
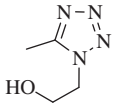
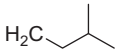
Compd	**16a**	**16b**	**16c**	**16d**	**16e**	**16f**	**16g**
R^1							
R^2	—CH_3						
Compd	**17a**	**17b**	**17c**	**17d**	**17e**	**17f**	**17g**
R^1							
R^2	—CH_3						

Table 2
In vitro antibacterial activity (MIC[a], μg mL^{-1}) of the compounds **16a–g** and **17a–g**

Compd	Gram-positive bacteria[b]		Gram-negative bacteria[b]		
	S. au.	*S. ep.*	*E. c.*	*P. ae.*	*K. pn.*
16a	64	16	64	32	8
16b	64	128	128	128	128
16c	32	1	128	4	1
16d	64	64	128	16	32
16e	128	128	128	128	128
16f	128	8	128	16	4
16g	128	16	128	16	16
17a	0.5	16	2	16	32
17b	0.5	1	2	64	64
17c	0.5	0.125	0.5	8	32
17d	16	0.125	8	4	32
17e	4	0.125	16	8	64
17f	1	0.125	4	2	8
17g	2	64	32	64	128
Ceftazidime	2	16	4	16	32

[a] Minimum inhibitory concentrations (10^6 cells/mL).
[b] Definitions of organism abbreviations: *S. au.* = *S. aureus* ATCC25923, *S. ep.* = *S. epidermidis*, *E. c.* = *E. coli* ATCC25922, *P. ae.* = *P. aeruginosa* ATCC27853, *K. pn.* = *K. pneumoniae*.

Generally speaking, compounds in the series 1 with the C-3′ (1,3,4-thiadiazol-2-yl)thiomethyl group, 2-oxaisocephem derivatives (**16a–g**), most of which was less active against the microorganisms including the Gram-positive and Gram-negative bacterial strains, especially against the Gram-positive bacteria, than the ones in series 2 (**17a–g**) possessing a (1-(2-hydroxyethyl)-1*H*-tetrazol-5-yl)thiomethyl group at C-3. Among those compounds in series 1, there was generally no compound more potent against *S. aureus* and *E. coli* than the reference. The activity of **16a** against *S. epidermidis* was equal to that of the reference, but against *K. pneumoniae*, it was fourfold more potent than that of ceftazidime. Compound **16d** displayed about the same activity against *P. aeruginosa* and *K. pneumoniae* as reference compound. Compounds **16b** and **16e** showed poor activity against all the tested strains, whereas **16c** showed significantly enhanced activity against *S. epidermidis*, *P. aeruginosa* and *K. pneumoniae* as compared with the reference. In particular, against *K. pneumoniae*, it was the most potent compound in all the synthetic 2-oxaisocephems with the MIC value 1 μg mL^{-1}, which was 32-fold lower than that of ceftazidime. While against the three bacterial strains, the activity of compounds **16f** and **16g** was not apparently enhanced as **16c**, only **16f** showed an MIC value eightfold lower than the reference against *K. pneumoniae*.

Compounds in series 2 except **17g** showed much more activity against Gram-positive bacteria, on the other hand, the (1-(2-hydroxyethyl)-1*H*-tetrazol-5-yl)thiomethyl group in combination with the [2-(5-amino-1,2,4-thiadizol-3-yl)-2-(*Z*)-alkoxyimino]-acetamido moiety, which is often used as the side-chain at the 7-position of fourth-generation cephalosporins, contributes more to the enhancement of the activity against Gram-positive bacteria. Compound **17a** displayed the potency fourfold more than the reference against *S. aureus* and **17b** showed encouraging activity against *S. epidermidis*, with the MIC value 0.125 μg mL^{-1}, which was 128-fold lower than that of ceftazidime. Against the Gram-negative bacteria strains, the antibacterial activity of **17c** was equal or superior to that of the reference, especially against *E. coli*, **17c** was the most potent agent in all the new 2-oxaisocephems. Compounds **17c–f** were 2- to 16-fold more potent than the reference compound against *P. aeruginosa*. And compound **17f**, the most potent compound in series 2, was found to be fourfold more potent than ceftazidime in its ability to inhibit *K. pneumoniae*.

In conclusion, introductions of the side-chains used of fourth-generation cephalosporins into the 7-position of the 2-oxaisocephem nucleus and the alteration of the 3-subtituents into the side chains which were often used at 3-position of oxacephems gave new 2-oxaisocephems with good activity and broad antibacterial spectrum. Among them, particularly **17c** and **17f** had well-balanced potency and were found to have excellent antibacterial activities against Gram-positive organism while maintaining good Gram-negative activities. Encouraged by these findings, we are continuing to conduct the evaluation of extensive antibacterial activity and search more effective 2-oxaisocephem antibiotics, while the results will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.040. These data include MOL files and InChiKeys of the most important compounds described in this article.

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