Cephalosporins with the Dichlorophenyl Group at C-7 Position and Pyrimidines at C-3 Position Exhibiting Potent Activity against Gram-positive Strains

GEUN TAE KIM,* EUN-JUNG RYU, YONG-JIN JANG,
CHANG-SEOK LEE,* HASIK YOUN,
YANG RAE CHO and HYUNG YEUL JOO

LG Life Science, Ltd.
R&D Park 104-1 Munji-dong, Yuseong-gu,
Taejon 305-380, Korea

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Nowadays, multidrug-resistant strains of Gram-positive bacteria caused nosocomial infection have great interest in the world.[1] In the hospitals, antibacterial drugs like vancomycin, quinupristin/dalfopristin (Synercid)[2] and linezolid (Zyvox)[3] have been utilized to treat the diseases such as complicated skin and skin structure infection and life-threatening bacteremia. The recently isolated pathogens from the hospitals are likely to reduce susceptibility to these drugs.[4] Especially, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resitant *Staphycoccous epidermidis* (MRSE), and vancomycin-resistant *Enterococcus* (VRE) among Gram-positive strains raised great concerns due to ineffectiveness of drugs already used. Therefore, requirement of a new agent with good safety and potent efficacy against MRSA and VRE has led us to search for a new cephalosporin.

Herein, we described the synthesis of cephalosporins bearing various linkers with pyrimidine rings at C-3 position and dichlorophenylthio groups at C-7 position.

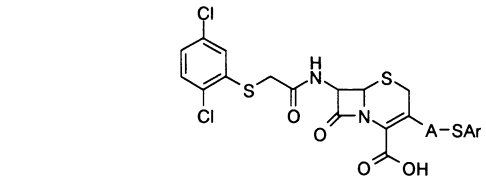
Chemistry

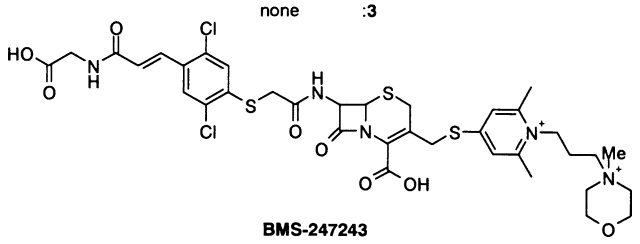
Installation of dichlorophenylthio group at C-7 position was known to improve antibacterial activities towards Gram-positive strains.[5] For instance, BMS-247243 was a good candidate which had good *in vitro* activity against MRSA (4 μg/ml) and *in vivo* efficacy in mice (PD_{50}: 5.4 mg/kg).[5c] Introduction of pyrimidine ring at C-3 position was planned to achieve not only good *in vitro* and *in vivo* activities, but also enhanced solubility.[6,7] Based on these results, our effort was dedicated to the investigation of the structure-activity relationship (SAR) of cephalosporins with dichlorophenylthio group at C-7 position and pyrimidines at C-3 position connected to various linkers such as the allyl and thiomethyl moieties.

At the beginning, we focused on the preparation of cephalosporin **1** having a thiomethyl linker (Scheme 1). In order to synthesize cephalosporon **1**, **4**[6] and the commercially available acid **5** was coupled with phosphorus oxychloride ($POCl_3$) and pyridine in dichloromethane at −20°C to afford thioacetate **6**. Deacetylation of thioacetate **6** with morpholine gave the corresponding thiol *in situ*, which was reacted with iodochloromethane to furnish chloromethyl derivative **7**. Displacement with pyrimidinethiols **9a**∼**9h**[7] in *N*,*N*-dimethylforamide (DMF) and the subsequent deprotection of diphenylmethyl (DPM) group with trifluoroacetic acid and triethylsilane afforded **1a**∼**1h**. Spectral data for the representatives **1f** and **1g** were given below.

Spectra for **1f**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.19 (1H, d, J=8.2 Hz), 7.49 (1H, d, J=2.3 Hz), 7.47 (1H, s), 7.46 (1H, s), 7.24 (1H, dd, J=8.2 Hz, 2.3 Hz), 6.15 (4H, br s), 5.46 (1H, dd, J=8.2 Hz, 4.6 Hz), 5.14 (1H, s), 4.92 (1H, d, J=4.6 Hz), 4.38 (2H, s), 3.91 (2H, s), 3.68 (1H, d, J=16.9 Hz), 3.49 (1H, d, J=16.5 Hz); MS m/z 604 $(M+1)^+$.

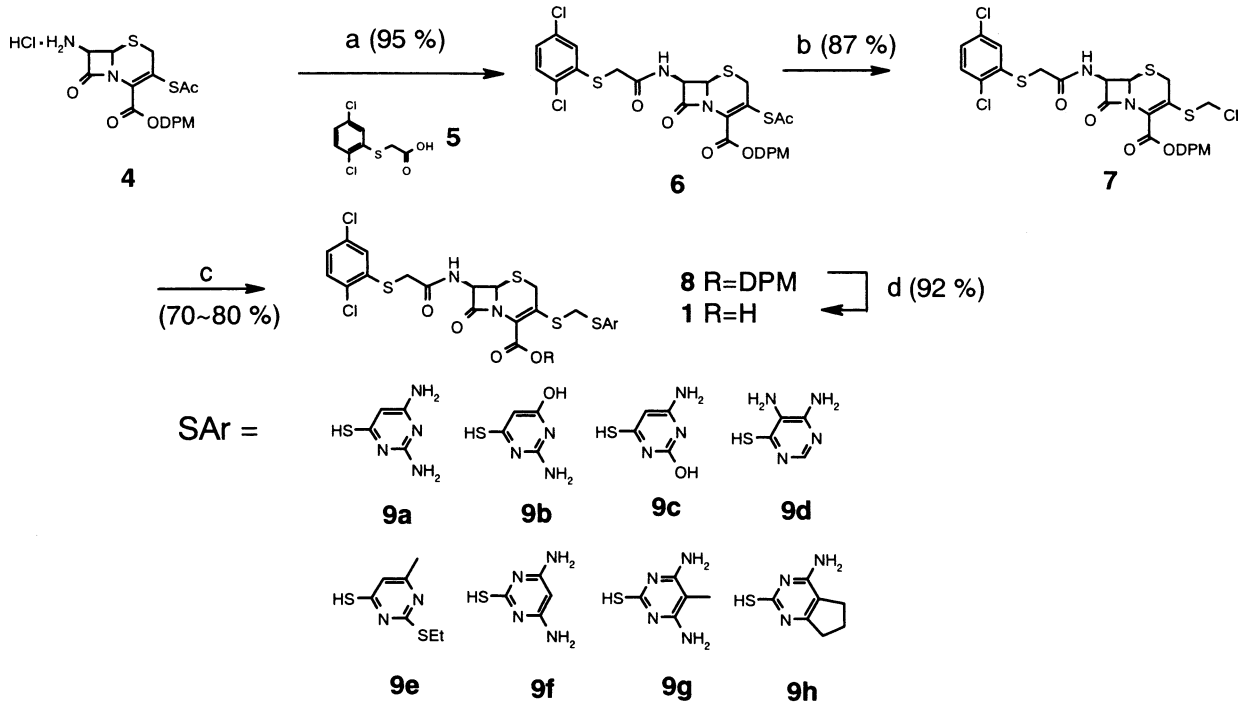
Spectra for **1g**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.19 (1H, d, J=8.2 Hz), 7.50 (1H, d, J=2.3 Hz), 7.47 (1H, s), 7.46 (1H, s), 7.24 (1H, dd, J=8.2 Hz, 2.3 Hz), 5.98 (4H,





* Corresponding author: gtakim@lgls.co.kr

Scheme 1.



a) $POCl_3$, pyridine, CH_2Cl_2, -20 °C; b) morpholine, -20 °C, iodochloromethane, DMF; c) DMF, NaI, pyrimidinethiols **9a~9h**; d) CF_3COOH, anisole, triethylsilane; Abbreviation: DPM= diphenylmethyl, Ac=acetyl

br s), 5.47~5.45 (1H, m), 4.91 (1H, d, J=5.0 Hz), 4.39 (2H, dd, J=12.8 Hz, 15.1 Hz), 3.91 (2H, s), 3.68 (1H, d, J=13.2 Hz), 3.50 (1H, d, J=16.5 Hz), 1.73 (3H, s); MS *m/z* 619 $(M+1)^+$.

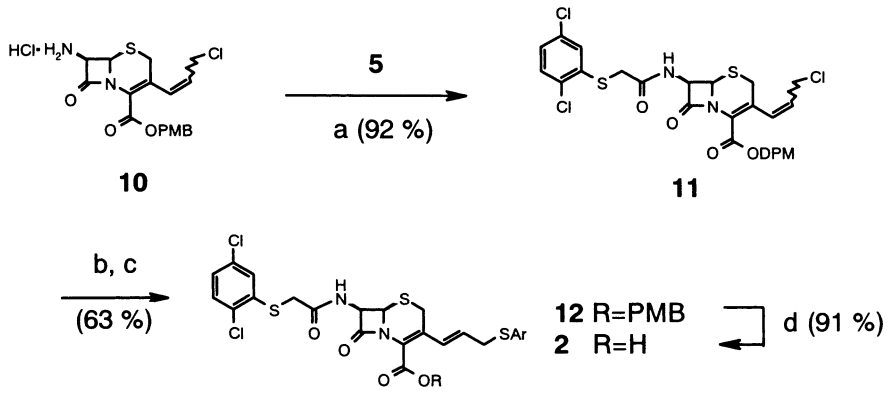
For the preparation of **2** having the allyl linker, cephalosporin **10**[7] was coupled with the commercially available acid **5** to afford **11** by using the same procedure of formation of **6** in Scheme 1. Isomerization of *cis*-isomer **11** into *trans*-isomer **11** was carried out with sodium iodide in acetone, and then treatment with pyrimidinethiol **9a**, **9b** and **9f** in DMF produced **12a**, **12b** and **12f** respectively. Deprotection of **12a**, **12b** and **12h** with trifluoroacetic acid gave **2a**, **2b** and **2f**. Spectral data for the representative **2a** were given below.

Specta for **2a**: ^{1}H NMR (500 MH?z, CD_3OD) δ 8.64 (1H, s), 8.15~8.13 (1H, d, J=7.8 Hz), 7.46 (1H, s), 6.69~6.66 (1H, d, J=15.6 Hz), 5.94~5.91 (1H, m), 5.69 (1H, s), 5.50~5.49 (1H, d, J=4.6 Hz), 5.02~5.01 (1H, d, J=4.55 Hz), 3.72~3.71 (2H, q), 3.59~3.52 (2H, m); MS *m/z* 599 $(M^+ + 1)$.

To investigate the influence of the linker size, cephalosporin **3a** devoid of linker was obtained as shown in Scheme 3. Cephalosporin **13** was treated with **9a** and then deprotected with trifluoroacetic acid to give **14a**. Coupling of **14a** with the corresponding acyl chloride of **5** was carried out using *N*,*O*-bis(trimethylsilyl)-acetamide and pyridine in dichloromethane. Acyl chloride of **5** was prepared *in situ* with thionyl chloride. **3b** and **3c** were also obtained by the same procedure of formation of **3a**. Spectral data for the representative **3b** were given below. All the cephalosporins with different linkers to be tested have been prepared in hand.

Spectra for **3a**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.32 (1H, s), 7.24 (1H, d, J=8.2 Hz), 7.07 (1H, d, J=8.2 Hz), 5.75 (1H, s), 5.57 (1H, d, J=5.0 Hz), 5.13 (1H, d, J=5.0 Hz), 3.89~3.81 (2H, m), 3.68 (1H, d, J=17.4 Hz), 3.25 (1H, d, J=17.4 Hz); MS *m/z* 559 $(M^+ + 1)$.

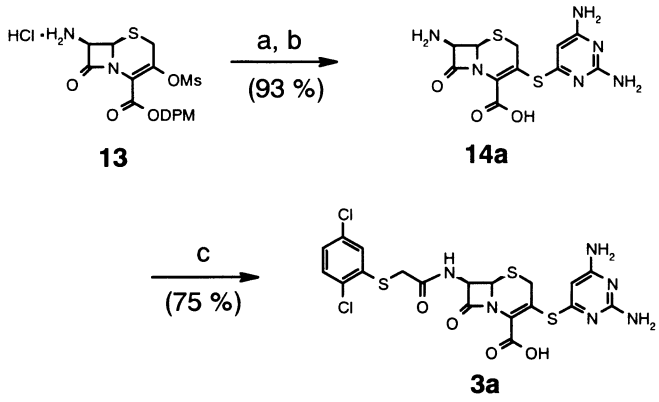
Scheme 2.



a) pyridine, $POCl_3$, CH_2Cl_2, -20 °C; b) NaI, acetone; c) DMF, pyrimidinethiols; d) CF_3COOH, anisole

Abbreviation: PMB=*p*-Methoxybenzyl

Scheme 3.



a) DMF, **9a**; b) CF_3COOH, anisole; c) pyridine, CH_2Cl_2, N, O-bis(trimethylsilyl)acetamide, 0 °C

Abbreviation: Ms=methanesulfonyl

Antibacterial Activities and Discussion

Agar dilution method was used to determine the minimal inhibitory concentration (MIC) of compounds against the selected organisms. The MIC values for vancomycin and BMS-247243 were shown for comparison. BMS-247243 was prepared by the published method in the references[5~7]

In general, all the cephalosporins in Table 1 exhibited better activities against Gram-positive strains than BMS-247243 surprisingly. Among cephalosporins **1** bearing the thiomethyl linker, the antibacterial activities of compounds **1a**, **1b**, **1d** and **1f** against MRSA showed comparable activities to those of vancomycin and better activities than those of BMS-247243 and they exhibited much better activities against *S. epidermidis* strains than vancomycin and BMS-247243 as well. It is worthwhile to mention that all of the series (**1a**~**1f**) showed excellent activities against vancomycin-resistant *E. faecalis* caused great concerns recently.

Table 1. Antibacterial activities of cephalosporins (MIC, μg/ml).

Compound	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**2a**	**2b**	**2f**	**3a**	**3b**	**3c**	**Vancomycin**	**BMS-247243**
S.a.1	2	1	4	2	4	2	4	4	4	4	4	2	2	4	2	4
S.a.2	0.13	0.25	0.5	0.25	0.5	0.25	0.5	0.5	1	0.5	0.5	0.25	0.25	1	2	2
S.a.3	2	2	4	2	2	2	4	2	4	2	4	1	1	2	0.5	4
S.e.1	0.5	0.5	2	0.5	1	0.5	1	2	2	2	1	0.5	1	2	1	2
S.e.2	0.063	0.13	0.25	0.25	0.25	0.063	0.25	0.5	0.13	0.13	0.13	0.25	0.25	0.5	1	1
E.f.1	0.25	0.25	1	0.5	0.5	0.5	0.5	0.25	0.25	0.25	0.5	2	2	1.	>64	4
E.f.2	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.25	2	1	2	1	4

Abbreviation: Sa.1:methicillin-resistant *Staphylococcus aureus* 241, S.a.2:methicillin-resistant *Staphylococcus aureus* K311, S.a.3:methicillin-resistant *Staphylococcus aureus* K364, S.e.1: methicillin-resistant *Staphylococcus epidermidis* 887E1, S.e.2: methicillin-resistant *Staphylococcus epidermidis* R005, E.f.1:vancomycin-resistant *Enterococcus faecalis* 2009, E.f.2:*Enterococcus faecalis* EFS004.

As seen in Table 1, cephalosporins with the thiomethyl linker had good acitivties against Gram-positive strains irrespective of the pyrimidines at C-3 position in Scheme 1. Among cephalosporins **2** having the allyl linker, cephalosporin **2b** displayed comparable activities against MRSA and MRSE to vancomycin and BMS-247243, and better activities against vancomycin-resistant *E. faecalis*. However, the series **2** having the allyl linker exhibited in general less potent activities than **1** having the thiomethyl linker. It seemed that the flexibility at the C-3 position in the series of cephalosporins **1** might enhance the antibacterial activities against Gram-positive strains.

Compounds **3** devoid of linkers such as the allyl and thiomethyl groups showed good activity against Gram-positive strains. Especially **3a** and **3b** showed the highly antibacterial activities against MRSA, and exhibited the reduced antibacterial activities against VRE. *faecalis* as compared with **1a**.

Concerning *in-vitro* activities against Gram-positive strains, cephalosporins **1a**, **1b**, **1f**, **3a** and **3b** turned out to show the balanced antibacterial activities. Especially **3a** displayed excellent pharmacokinetic profile (AUC: 605 μg·min/ml and half time: 159 minutes) when administrated in rats with 20 mg/kg.

Based on the MIC value and the pharmacokinetic data, **3a** will be evaluated further for the new antibiotics against nosocomial infection.

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