Cephalosporins with the (E)-Thiovinyl Linker with Pyrimidine at C-3 Position Exhibiting Potent Activities against Gram-positive Strains

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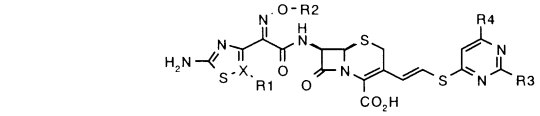
Cephalosporins have been used to treat many pathogens in the clinic. However, the emergence of multidrug-resistant strains of Gram-positive bacteria caused the nosocomial infection has become a major concern.[1,2] Antimicrobial agents like vancomycin, quinupristin/dalfopristin (Synercid)[3] and linezolid (Zyvox)[4] were developed to manage infections such as complicated skin and skin structure infection and life-threatening bacteremia. The recently isolated pathogens from hospitals are likely to reduce susceptibility to those drugs.[5] Therefore, urgent need 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 having the (E)-thiovinyl linker with pyrimidines ring at C-3 position and aminothiazole rings at C-7 position and their antibacterial activities as well.

Chemistry

Cephalosporin with the (E)-thiovinyl linker at C-3 position were known to possess good *in vitro* antibacterial activities against MRSA and vancomycin-resistant *Enterococcus faecalis*.[6] Therefore we applied the (E)-thiovinyl linker with pyrimidines at C-3 position to develop a new and effective cephalosporin with excellent activities against MRSA and VRE in our communication. This approach would show the scope of activities of cephalosporins with the (E)-thiovinyl linker having pyrimidines at C-3 position.

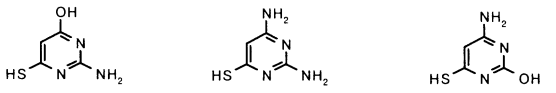
All the cephalosporins and pyrimidinethiols[7] we prepared were described in Fig. 1. Among those cephalosporins, representative **1c** was synthesized as described in Scheme 1. In the beginning, the commercially available cephalosporin **3** was coupled with acid **4** using phosphorus oxychloride ($POCl_3$) and pyridine in dichloromethane at −20°C to produce amide **5**. Treatment of **5** with Bredereck's reagent[8] *t*-BuOCH(NMe_2)$_2$ afforded enamine, which was hydrolyzed with 1 N HCl to give aldehyde **6**. The proper leaving group should be chosen for the formation of vinyl sulfide. Reaction of aldehyde **6** with *p*-toluenesulfonyl chloride, methanesulfonyl chloride, chlorine and trifluoromethanesulfonic anhydride gave the corresponding vinyl tosylate, vinyl mesylate, vinyl chloride and vinyl triflate, respectively. While displacement of vinyl tosylate, vinyl mesylate and vinyl chloride with pyrimidinethiols in Fig. 1 was too sluggish or turned out to give low chemical yield (5∼10%), transformation of vinyl triflate to vinyl sulfide was proven to be successful. It was worthwhile to note that vinyl triflate was an appropriate leaving group to handle with good stability. The generated vinyl triflate **7** was reacted with pyrimidinethiol **2a** to afford

Fig. 1.

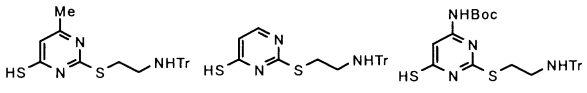


1

SAr=



2a **2b** **2c**



2d **2e** **2f**

No	X	R1	R2	R3	R4	SAr
1a	C	H	Me	NH_2	OH	**2a**
1b	C	H	H	NH_2	OH	**2a**
1c	C	Cl	H	NH_2	OH	**2a**
1d	C	Cl	H	NH_2	NH_2	**2b**
1e	C	Cl	Cyclo-pentyl	NH_2	NH_2	**2b**
1f	C	Cl	H	OH	NH_2	**2c**
1g	N	-	H	NH_2	NH_2	**2b**
1h	C	Cl	H	$SCH_2CH_2NH_2$	Me	**2d**
1i	C	Cl	H	$SCH_2CH_2NH_2$	H	**2e**
1j	C	Cl	H	$SCH_2CH_2NH_2$	NH_2	**2f**

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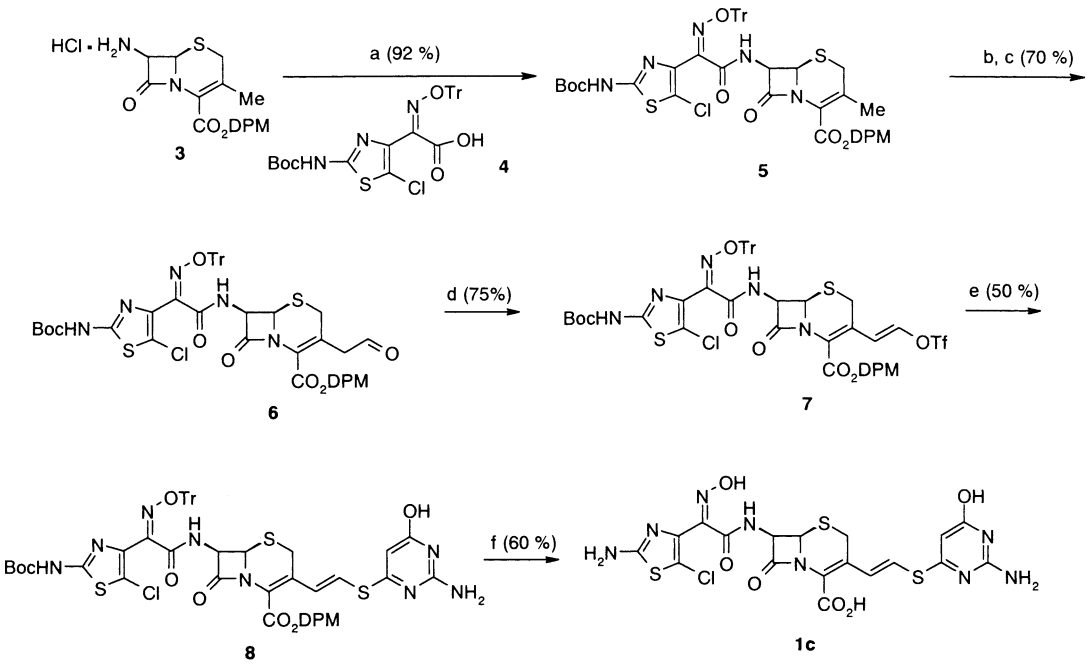
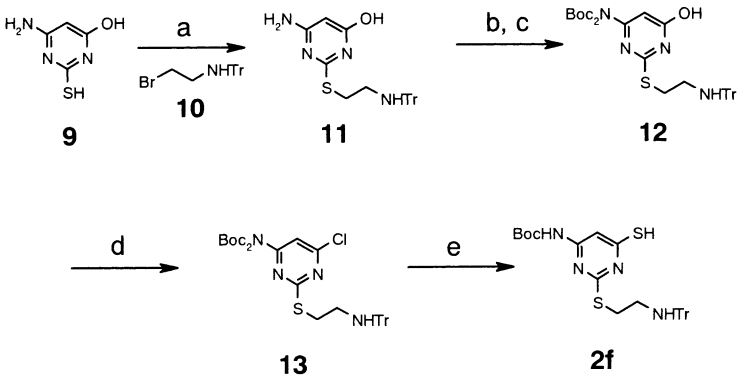
Scheme 1.



a) $POCl_3$, pyridine, CH_2Cl_2, -20 °C; b) t-$BuOCH(NMe_2)_2$, DMF, 50 °C; c) 1 N HCl, ethyl acetate, rt; d) Tf_2O, pyridine, CH_2Cl_2, -20 °C; e) DMF:DMSO=2:1, pyrimidinethiols, rt; f) CF_3COOH, Et_3SiH, anisole

Abbreviation: DPM= diphenylmethyl, Tr=trityl

Scheme 2.



a) DMF, H_2O, Et_3N; b) Et_3N, Boc_2O, CH_2Cl_2; c) EtOH, NaOH; d) oxalyl chloride, DMF(cat.), CH_2Cl_2; e) EtOH, NaSH

8 in good yield (50~80%), and then deprotection with trifluoroacetic acid afforded **1c**. For the preparation of **1j**, pyrimidine **2f** with a basic chain could be synthesized as shown in Scheme 2. The commercially available pyrimidinethiol **9** was subjected to bromide **10**, followed by protection with Boc_2O and selective hydrolysis with sodium

hydroxide. Vilsmeyer reaction of bis-Boc protected amine **12** with oxalyl chloride in the presence of DMF afforded **13**. Displacement of **13** with sodium sulfide and the concomitant deprotection of Boc group furnished **2f**. Cephalosporins **1a**, **1b** and **1d**~**g** were prepared by using the same procedure of the formation of **1c**. Spectra data of **1c** were given as below.

Spectra for **1c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (1H, s), 9.30 (1H, d, J=8 Hz), 7.20~7.00 (2H, br s), 7.10 (1H, d, J=16 Hz), 6.95 (1H, d, J=16 Hz), 6.76~6.64 (2H, br s), 5.64 (1H, dd, J=8.8 Hz, 4.6 Hz), 5.48 (1H, s), 5.01 (1H, d, J=4.8 Hz), 3.84 (1H, d, J=17.6 Hz), 3.49 (1H, d, J=17.6 Hz); MS m/z 571 $(M+1)^+$.

Spectra for **1f**: ^{1}H NMR (500 MHz, D_2O) δ 11.58 (1H, s), 9.30 (1H, d, J=8 Hz), 7.20~7.00 (2H, br s), 7.10 (1H, d, J=16 Hz), 6.95 (1H, d, J=16 Hz), 6.76~6.64 (2H, br s), 5.64 (1H, dd, J=8.8 Hz, 4.6 Hz), 5.48 (1H, s), 5.01 (1H, d, J=4.8 Hz), 3.84 (1H, d, J=17.6 Hz), 3.49 (1H, d, J=17.6 Hz); MS m/z 571 $(M+1)^+$.

Spectra for **1j**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.05 (1H, d, J=16 Hz), 6.65 (1H, d, J=16 Hz), 6.07 (1H, s), 5.65~5.64 (1H, m), 5.04 (1H, d, J=5 Hz), 3.25~3.07 (6H, m); MS m/z 629 $(M+1)^+$.

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 are shown for comparison. In general all the compounds showed good activities against Gram-positive strains except **1a** and **1b**, in which antibacterial activities against MRSA were poorly active (32 μg/ml, 8 μg/ml). Installation of chloride at an aminothiazole ring resulted in improved antibacterial activities in **1c**, **1d**, **1e** and **1f** significantly. Among those cephalosporins, **1c**, **1d** and **1f** having the same aminothiazole ring and the different pyrimidine rings exhibited excellent and well-balanced antibacterial activities against MRSA, MRSE and vancomycin-resistant *Enterococcus faecalis*. Especially, antibacterial activities of **1c** having 2-amino-4-hydroxy-substituted pyrimidine against MRSA were comparable to those of vancomycin, and antibacterial activities of **1c** against all the MRSE strains were twice more active than those of vancomycin. In addition, cephalosporin **1c** displayed excellent activity against vancomycin-resistant *E. faecalis* 2009 (MIC: 0.25 μg/ml). Cephalosporin **1f** with 2-hydroxy-4-amino-substituted pyrimidine also possessed good activities against Gram-positive strains. When R_2 was introduced with bulky group like cyclopentyl group in **1e**, **1e** was twice to four times less active against MRSA than cephalosporin **1c**. Switching an aminothiazole ring in **1c** with an aminothiadiazole ring in **1g** resulted in the reduction of *in-vitro* antibacterial activities against all the Gram-positive strains in Table 1.

A. CHO *et al.* reported that the incorporation of a basic aminopyridine at C-7 position afforded good potency against MRSA and acceptable solubility for intravenous administration.[9] When **1h**, **1i** and **1j** attached with a basic chain in pyrimidines were tested, they showed excellent antibacterial activities against all the Gram-positive strains in Table 1 respectively. Especially **1j** was highly active against MRSA and twice more active against MRSE and VREF than **1c**. Overall, most of the cephalosporins **1** with the (E)-thiovinyl linker attached pyrimidines were found to show excellent and well-balanced activities against Gram-

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

compound	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	Vancomycin
S.a.1	32	8	1	2	4	1	4	2	2	1	2
S.a.2	32	8	0.5	1	2	0.5	2	2	2	1	2
S.a.3	32	8	1	1	2	1	4	1	4	1	0.5
S.a.4	32	8	1	1	2	1	4	2	4	1	0.5
S.a.5	32	8	0.5	1	2	1	2	2	2	1	0.5
S.e.1	32	2	0.5	1	2	0.5	1	0.5	1	0.25	1
S.e.2	16	2	0.5	1	1	0.5	2	0.5	1	0.25	1
S.e.3	64	16	0.5	1	1	1	4	1	2	0.5	1
E.f.1	32	0.5	0.25	0.5	2	0.25	1	0.25	0.5	0.13	>64
E.f.2	8	0.25	0.25	0.5	2	0.25	1	0.25	0.5	0.13	1

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.a.4:methicillin-resistant *Staphylococcus aureus* K367, S.a.5:methicillin-resistant *Staphylococcus aureus* K372, S.e.1: methicillin-resistant *Staphylococcus epidermidis* 887E1, S.e.2: methicillin-resistant *Staphylococcus epidermidis* R005, S.e.3: methicillin-resistant *Staphylococcus epidermidis* R025, E.f.1:vancomycin-resistant *Enterococcus faecalis* 2009, E.f.2:*Enterococcus faecalis* EFS004

Table 2. Protective effect against MRSA K283 systemic infection.

compound	PD_{50}(mg/kg)	MIC(μg/ml)
1c	7.5	1
1d	9.8	2
1e	>30	2
1f	4.7	1
1h	9	2
1i	>30	2
1j	2.5	1
Vancomycin	2.5	1

Systemic infection was caused by methicillin-resistant *S. aureus* K283 in 4-week-old male mice (18~21 g) by injection a single 0.5 ml inoculum containing various lethal doses of bacteria, intraperitoneally. Control and treatment group at each dose were composed of 6 mice each. The 50% protective dose (PD_{50}, mg/kg) was calculated by Probit analysis from the survival rates on day 5 after the infection. Compounds were administrated subcutaneously with 5 min interval and 2 h interval after infection.

positive strains.

Based on the MIC values, cephalosporins **1c**, **1d**, **1e**, **1f**, **1h**, **1i** and **1j** were selected to adjust the systemic infection model against MRSA K283 for the further evaluation as shown in Table 2. Among them, **1e** and **1i** displayed disappointing *in vivo* activities due to low pharmacokinetic profile in mice. **1c**, **1d** and **1f** showed moderate *in vivo* activity but inferior *in vivo* activity to vancomycin although they have good *in vitro* activity against MRSA K283. Cephalosporin **1j** having a basic chain in pyrimidine was proven to exhibit almost the same *in vivo* activity (PD_{50}: 2.5 mg/kg) with vancomycin in this model. Among them, intravenous administration of **1c**, **1f** and **1j** in rats with 20 mg/kg resulted in AUC (mg min/ml) of 4972, 5573 and 924, and half time (minute) of 47, 55 and 16, respectively.

1c, **1f** and **1j** were selected for the further investigation as our new candidates against nosocomial infection based on *in vivo* experiment and pharmacokinetic data.

In conclusion, cephalosporin having the (E)-2-thiovinyl linker attached with pyrimidines at C-3 position was found to possess excellent *in vitro* activities and good *in vivo* activity against MRSA, MRSE and VREF.

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