Synthesis and Biological Activity of Quaternary Ammoniopropenylcephalosporins with Hydroxylated Alicyclic or Aliphatic Amines

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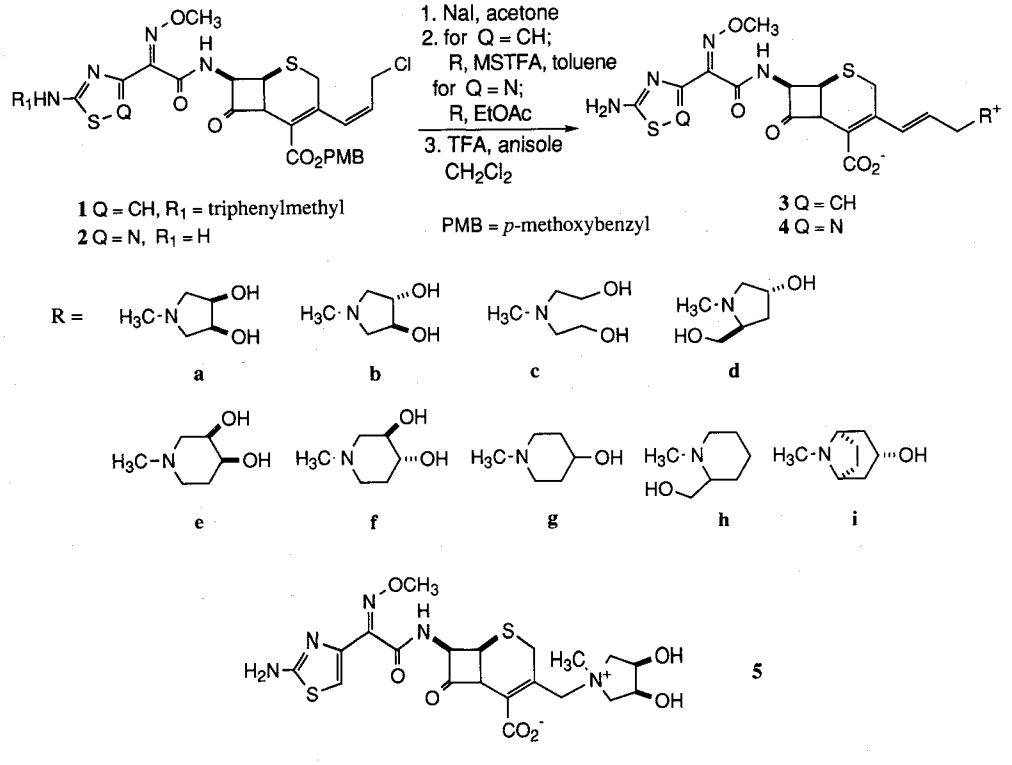
Recently, it has been demonstrated that aminothiazolylcephalosporins having the dihydroxy aromatic moiety as a catechol or its isosters at the C-3 position exhibit potent activity against Gram-negative bacteria including *Pseudomonas aeruginosa*[1~4]. In a previous paper[5], we reported that the introduction of hydroxy groups to aliphatic or alicyclic amine at the C-3 the position of the cephem nucleus yields a broad antibacterial spectrum and potent activity against Gram-negative bacteria including *Pseudomonas aeruginosa*. However, most of these cephalosporins (*e.g.* **5**) did not show satisfactory activity against *Staphylococcus aureus* in common with ceftazidime[6] and most of the catecholic cephalosporins[3,4]. It was reported[7] that introduction of a propenyl linkage at the C-3 position of the ammoniocephalosporins improves activity against Gram-positive bacteria such as *Staphylococcus aureus*. Thus, our efforts have been focused on synthesizing new cephalosporins possessing well-balanced antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* by inserting propenyl linkage to cephalosporins (*e.g.* **5**), which have a quaternary hydroxylated alicyclic ammonium groups in the 3-side chain. Herein we describe the synthesis and antibacterial activity of quaternary propenylammoniocephalosporins bearing hydroxylated aliphatic or alicyclic amine at the C-3 the position.

Preparation of new ammoniopropenylcephalosporins (**3a~4i**) having a quaternary hydroxylated alicyclic ammonium groups in the 3-side chain was performed by the slight modification of the known procedure[7] as shown in Scheme 1.

The new cephalosporins (**3** or **4**) were prepared by quaternization of 3-(*E*)-iodopropenylcephalosporin derivatives, which were derived from 3-(*Z*)-chloropropenylcephalosporin derivatives (**1** or **2**), with hydroxylated tertiary amine followed by the removal of protecting groups. In a previous paper[7], quaternizations of *N*-tritylaminothiazolyl iodopropenylcephalosporin derivatives were carried out in toluene or ether to prevent the formation of its undesired Δ-2 isomer. In some cases, quaternizations of *N*-tritylaminothiazolyl iodopropenylcephalosporin derivatives did not proceeded effectively due to the low solubility of most of the hydroxylated tertiary amines except *N*-methyl-diethanolamine, 2-hydroxymethyl-1-methylpiperidine, and tropine in the reaction solvent. Longer reaction time or using DMF as solvent resulted in the formation of Δ-2 isomer. However,

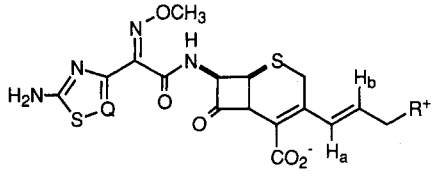
Scheme 1. Synthesis of quaternary hydroxylated ammoniopropenylcephalosporins.



the reaction proceeded cleanly when the silylated hydroxylated tertiary amine was used in quaternization. The general procedure is as follows; To a stirred solution of *p*-methoxybenzyl 7β-[2(*Z*)-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*Z*)-3-chloro-1-propen-1-yl]-3-cephem-4-carboxylate (**1**, 300 mg, 0.36 mmol) in acetone (15 ml) was added sodium iodide (165 mg, 1.10 mmol) in one portion at room temperature followed by stirring for 2 hours. The reaction mixture was evaporated and dissolved in ethyl acetate. The ethyl acetate solution was washed successively with 10% aqueous $Na_2S_2O_3$ solution and brine and then dried ($MgSO_4$). After evaporation of solvent, the residue was dissolved in toluene (5 ml) and treated with a solution of *N*-methyltrimethylsilylacetamide (MSTFA, 436 mg, 2.19 mmol) and *meso*-3,4-dihydroxy-1-methylpyrrolidine (86 mg, 0.73 mmol) in toluene at −10°C. The reaction mixture was kept in a refrigerator (*ca.* −10°C) for 12 hours. The resulting precipitate was filtered and washed several times with ether to afford a quaternary ammonium salt as a white solid (*ca.* 290 mg). The quaternary salt was dissolved in dichloromethane (0.5 ml) and treated with trifluoroacetic acid (1 ml) and anisole (0.5 ml) and stirred for 2 hours at room temperature. The mixture was evaporated and treated with isopropyl ether (20 ml). The resulting solid was washed several times with isopropyl ether and neutralized with saturated aqueous $NaHCO_3$ solution and purified by flash column chromatography (acetonitrile - water = 4 : 1 to 2 : 1) to provide 7β-[2(*Z*)-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[(*E*)-3-(*meso*-3,4-dihydroxy-1-methyl-1-pyrrolidinio)-1-propen-1-yl]-3-cephem-4-carboxylate (**3a**) as a white solid (74 mg, 38%). IR (KBr) 3406, 1766, 1602, 1534 cm^{-1}; ^1H NMR (D_2O) δ 7.02 (1H, s), 6.93 (1H, d, J = 15.4 Hz), 5.97 (1H, dt, J = 15.4, 7.4 Hz), 5.83 (1H, d, J = 4.6 Hz), 5.26 (1H, d, J = 4.6 Hz), 4.21 (1H, d, J = 7.4 Hz), 3.50 ~ 4.08 (4H, m), 4.08 (3H, s), 3.87 (2H, s), 3.12 and 3.31 (3H, two s).

The quaternizations of aminothiadiazolyl iodopropenylcephalosporin derivatives were carried out in a similiar way in ethyl acetate solvent except the silylation step since hydroxylated tertiary amines and aminothiadiazolyl

Table 1. Yield, IR and ^1H NMR data of the aminothiazolylcephalosporins (**3a** ~ **4i**).



Compound	Q	Yield from **1** (%)	IR (KBr) β-lactam (cm^{-1})	^1H NMR (300 MHz, δ in D_2O, ppm)						
				Thiazole-H (s)	6-H, 7-H (d, J=4.0 ~ 4.7 Hz)		H_a (d, J= 15 ~ 16 Hz)	H_b (dt, J=15 ~ 16, 7 Hz)	OCH_3 (s)	N^+CH_3 (s)
3a	CH	38	1766	7.02	5.26	5.83	6.93	5.97	4.08	3.12
3b	CH	25	1766	7.03	5.27	5.83	6.93	5.97	4.00	3.24
3c	CH	26	1766	7.05	5.30	5.86	6.96	5.97	4.02	3.17
3d	CH	28	1766	7.03	5.28	5.84	6.94	6.02	4.00	3.06
3e	CH	26	1764	7.02	5.28	5.83	6.94	5.97	3.99	3.08, 3.12
3f	CH	49	1766	7.01	5.26	5.82	6.93	5.95	3.98	3.03, 3.15
3g	CH	40	1766	7.09	5.34	5.90	6.95	6.05	4.07	3.08, 3.11
3h	CH	28	1766	7.03	5.27	5.83	6.93	5.93	4.00	3.03, 3.13
3i	CH	6	1764	7.03	5.28	5.84	6.95	5.98	4.01	3.03
4a	N	18	1764	-	5.31	5.89	6.97	6.03	5.31	3.15
4b	N	24	1764	-	5.32	5.90	6.97	6.10	5.32	3.30
4c	N	16	1762	-	5.30	5.89	6.95	5.95	5.30	3.17
4d	N	54	1764	-	5.27	5.81	6.95	6.03	5.27	3.07
4e	N	16	1765	-	5.29	5.87	6.95	6.00	5.29	3.05, 3.17
4f	N	10	1766	-	5.31	5.89	6.97	6.03	5.31	3.12, 3.17
4i	N	24	1764	-	5.29	5.88	6.95	6.03	5.29	3.05

Table 2. *In vitro* antimicrobial activity (MIC: μg/ml) of the cephalosporins (**3a**～**4i**).

Compounds	*S.p.* 308 A	*S.f.* MD	*S.a.* SG 511	*S.a.* 285	*S.a.* 503	*E.c.* 055	*P.a.* 9027	*S.t.*	*En.c.* P 99
3a	0.013	50	0.39	0.78	0.2	0.013	3.13	0.013	6.25
3b	0.013	50	0.39	0.78	0.2	0.025	3.13	0.025	3.13
3c	0.013	50	0.39	0.78	0.2	0.013	3.13	0.025	6.25
3d	0.013	50	0.39	0.78	0.2	0.013	6.25	0.025	3.13
3e	0.013	50	0.39	0.78	0.2	0.025	6.25	0.025	6.25
3f	0.013	50	0.39	0.78	0.2	0.025	3.13	0.025	12.5
3g	0.013	50	0.39	0.78	0.2	0.025	6.25	0.049	3.13
3h	0.013	25	0.39	0.39	0.2	0.013	6.25	0.025	3.13
3i	0.013	50	0.39	0.78	0.2	0.025	6.25	0.049	6.25
4a	0.049	100	0.78	1.56	0.39	0.098	3.13	0.098	12.5
4b	0.049	>100	0.78	1.56	0.39	0.049	3.13	0.098	12.5
4c	0.049	>100	0.78	1.56	0.78	0.098	6.25	0.098	12.5
4d	0.049	100	1.56	1.56	0.78	0.098	6.25	0.098	12.5
4e	0.049	100	0.78	1.56	0.39	0.049	3.13	0.098	6.25
4f	0.049	100	0.78	1.56	0.39	0.098	3.13	0.098	12.5
4i	0.049	100	0.39	1.56	0.2	0.049	3.13	0.098	6.25
5	0.025	100	3.13	25	3.13	0.049	3.13	0.098	6.25
CFZ	0.098	100	12.5	12.5	3.13	0.098	3.13	0.2	100

Abbreviations: *S.p.* 308 A, *Streptococcus pyogenes* 308 A; *S.f.* MD, *Streptococcus faecium* MD; *S.a.* SG 511, *Staphylococcus aureus* SG 511; *S.a.* 285, *Staphylococcus aureus* 285; *S.a.* 503, *Staphylococcus aureus* 503; *E.c.* 055, *Escherichia coli* 055; *P.a.* 9027, *Pseudomonas aeruginosa* 9027; *S.t.*, *Salmonella typhimurium*; *En.c.* P 99, *Enterobacter cloacae* P 99; CFZ, Ceftazidime

iodopropenylcephalosporin derivatives are soluble in ethyl acetate. The spectral data and overall yield from **1** or **2** of new cephalosporins (**3a**～**4i**) are summarized in Table 1.

The activity of the new cephalosporins against 9 test organisms selected from Gram-positive and Gram-negative bacteria was determined by the standard 2-fold serial *in vitro* agar dilution method (Table 2). For comparison, the MIC values of ceftazidime and 3,4-dihydroxypyrrolidiniomethylcephalosporin derivative (**5**) are also listed in Table 2.

Most of the propenylammoniocephalosporins having a hydroxylated aliphatic or alicyclic amine (**3a**～**4i**) at C-3 side chain exhibit highly enhanced activity against Gram-positive bacteria (apart from *Streptococcus faecium* MD) in comparison with ammoniomethyl cephalosporin derivative **5** (Table 2). These propenylammoniocephalosporins derivatives were 2- to 32-fold more active than **5** and ceftazidime against three strains of *Staphylococcus aureus*. They were 8- to 32-fold more active than ceftazidime against *Enterobacter cloacae* P99. The anti-pseudomonal activities of **3a**～**4i** were nearly equal to **5** and ceftazidime. The nature of individual C-3 substituents did not markedly influence antimicrobial activity. However, aminothiazolyl derivatives (**3a**～**3i**) were 2-fold more active than aminothiadiazolyl derivatives (**4a**～**4i**) against Gram-positive bacteria.

In conclusion, propenylammoniocephalosporins having a hydroxylated aliphatic or alicyclic amine (**3a**～**4i**) at the C-3 the side chain possessed a well-balanced antibacterial spectrum and potent activity against Gram-positive and Gram-negative bacteria.

Acknowledgments

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