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Synthesis and Structure–Activity Relationships of Quaternary Ammonium Cephalosporins with 3-Pyrazolylpyridinium Derivatives

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Abstract—Cephalosporins with 3-pyazolylpyridinium at C-3 position, which is supposed to exhibit synergic activity of ceftazidime and cefoselis, were synthesized and their antibacterial activity against Gram-positive and Gram-negative was inspected.

Since first launching ceftazidime to the market as a kind of pyridinium cephalosporin, many efforts have been dedicated to develop quaternary ammonium cephalosporin with broad spectrum against Gram-positive and Gram-negative microorganisms and to overcome the multidrug-resistance.[1–3] Representative ceftadizime have a tendency to be mainly used to treat opportunistic infection with *Pseudomonas aeruginosa* strains, meanwhile cefpirome exhibit more enhanced broad spectrum against both range of Gram-positive and Gram-negative.[2] Nevertheless its excellent broad spectrum, the limitation is not to overcome MRSA (methicillin-resistant *Staphylococcus aureus*). Recently, another type of quaternary ammonium cephalosporin, pyrazolium cephalosporin (cefoselis), was marketed.[4–6] It was found to have activity superior to that of cepirome, cefepime, and ceftazidime against clinical isolates of MRSA, but inferior to that of those compounds against some *Pseudomonas aeruginosa*.[6] The clinical results about activity against microorganism with ceftazidime and cefoselis led us to suppose that the combination of pyridine and pyrazole moiety will exhibit its individual excellent activity against Gram-positive and Gram-negative. To minimize the electronic effects of substituent, pyrazolyl system was introduced into meta position of pyridine.

In this paper, we describe the synthesis and antibacterial activities of novel series of cephalosporins having 3-(pyrazol-3-yl)pyridinium group at the C-3 side chain represented by Figure 1.

The preparation of the various 3-(pyrazol-3-yl)pyridine derivatives possessing hydrogen (**5a**), methyl (**5b**), carboxamide (**7a–c**), hydroxymethyl (**8a–c**), and amino group (**9a–c**) at the C-5 position and hydrogen, methyl, and hydroxyethyl group at the C-1 or C-2 position on pyrazole ring, was performed according to the modification of known procedure (Scheme 1).[7–10]

β-Enamino ketone **2** was obtained from the reaction of 3-acetylpyridine with dimethyl acetamide dimethylacetal (DMA) or DMF acetal. Subsequent reaction of **2** with hydrazine hydrate in ethanol under reflux gave **3**-(pyrazol-3-yl)pyridine (**5a**) in good to excellent yield. Sodium enolate **3** was obtained by the Claisen condensation of 3-acetyl pyridine with diethyloxalate. Reaction of 3 with hydrazine hydrate, methylhydrazine, or hydroxyethylhydrazine gave ethyl 3-(3-pyridyl)pyrazole-5-carboxylate (**6a**), 3-(3-pyridyl)-2-methylpyrazole-5-carboxylate (**6b**), or 3-(3-pyridyl)-2-hydroxyethyl-pyrazole-5-carboxylate (**6c**) in 83, 61, or 63% yield, respectively. Reduction of **6a–c** with LAH gave the corresponding 3-(3-pyridyl)-5-hydroxymethylpyrazole derivatives (**8a–c**) in 95, 90, or 90% yield. Converting to the corresponding amide derivatives (**7a–c**) was carried out by treatment of **6a–c** with ammonium hydroxide in excellent yield. The condensation of 3-cyanopyridine with acetonitrile using sodium hydride produced **4** in 64% yield, which subsequently underwent reaction with hydrazine, methylhydrazine, and 2-hydroxyhydrazine to

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give corresponding 5-amino pyrazole derivatives (**9a**–**c**) in good yield. The quaternary cephalosporin derivatives were prepared as follow: 7-[2-(2-Aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]cephalosporanic acid (456 mg, 1 mmol) was suspended in methylene chloride (10 mL) under nitrogen atmosphere. *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (0.7 mL, 3.8 mmol) was added, and the mixture was stirred for 1 h. Trimethylsilyl iodide (0.4 mL, 2.8 mmol) was added to the resulted pale yellow solution, and the solution was stirred for additional 30 min. The solvent was evaporated in vacuo to afford the 3-iodomethyl cephem as a viscous yellowish residue. The residue was dissolved in acetonitrile (4 mL) and tetrahydrofuran (0.37 mL), which make a role to destroy excess of trimethylsilyl iodide, was added. 3-(Pyridine-3-yl)-5-amino-1-methylpyrazole (**9b**) (175 mg, 1 mmol) dissolved in acetonitrile (4 mL) was added to the solution. The reaction mixture was stirred at room temperature for 16 h, followed by addition of 5% methanolic acetone (20 mL) to precipitate iodide salt of **1j**. The crude iodide salt was dissolved in water (1 mL) and neutralized with powder sodium bicarbonate (52 mg). The resulting solution was directly chromatographed on silica gel column with a mixture of acetonitrile and water (6:1, v/v). Lyophilization of collected solution gave 7-[(Z)-2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(3-(5-amino-1-methylpyrazol-3-yl)pyridinium)methyl] ceph-3-em-4-carboxylate (**1j**) as an amorphous colorless solid in 42% yield: mp 220–221 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.07, 3.52 (2H, ABq, J = 17.5 Hz, C_4–H), 3.62 (3H, s, OCH_3), 3.77 (3H, s, pyrazole-NCH_3), 5.05 (1H, d, J = 4.9 Hz, C_6–H), 5.09, 5.73 (2H, ABq, J = 17.5 Hz, C_3–CH_2-), 5.62 (1H, d, J = 4.9 Hz, C_7–H), 5.63 (2H, brs, pyrazole-NH_2), 5.95 (1H, s, pyrazole-H), 6.69 (1H, s, thiazole-H), 7.20 (2H, brs, thiazole-NH_2), 8.08 (1H, dd, J = 6.01 Hz, J = 8.13 Hz, pyridine-H), 8.72 (1H, d, J = 8.13 Hz, pyridine-H), 9.36 (1H, d, J = 6.01 Hz, pyridine-H), 9.53 (1H, d, J = 8.12 Hz, pyridine-H), 9.82 (1H, brs,-CONH-); anal. calcd for $C_{23}H_{23}N_9O_5S_2$: C, 48.50; H, 4.07; N, 22.13. Found C, 48.73; H, 3.95; N, 21.91. Cephalosporins prepared as described above are shown in Scheme 2.

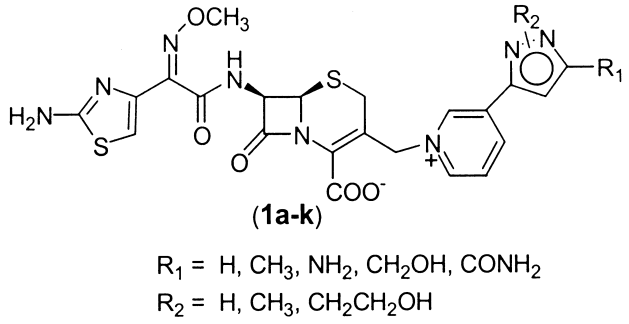
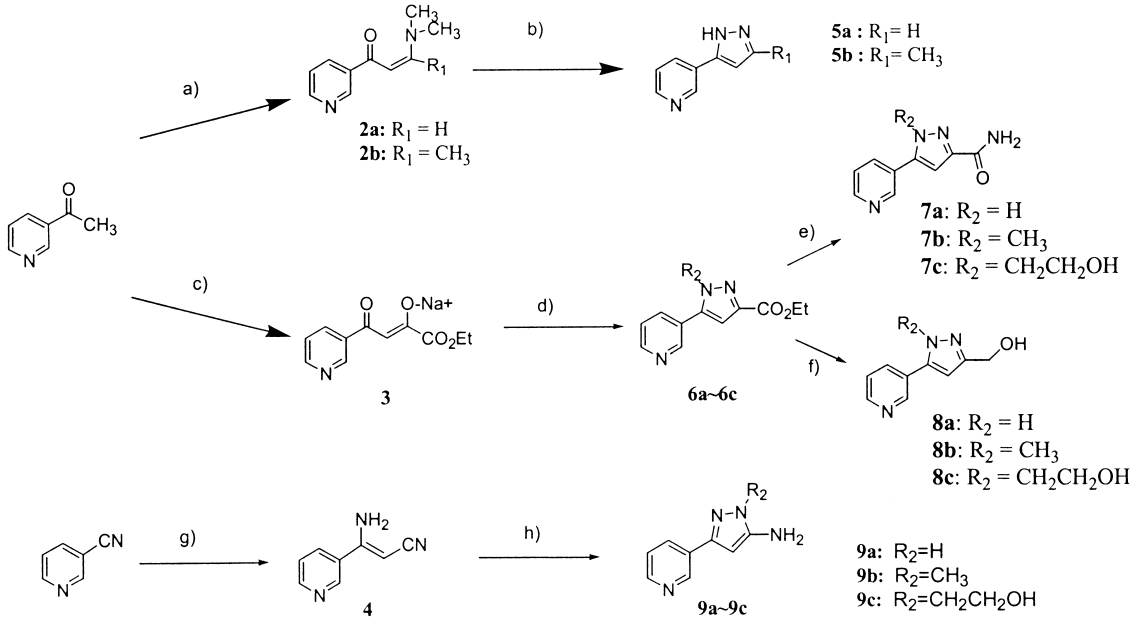
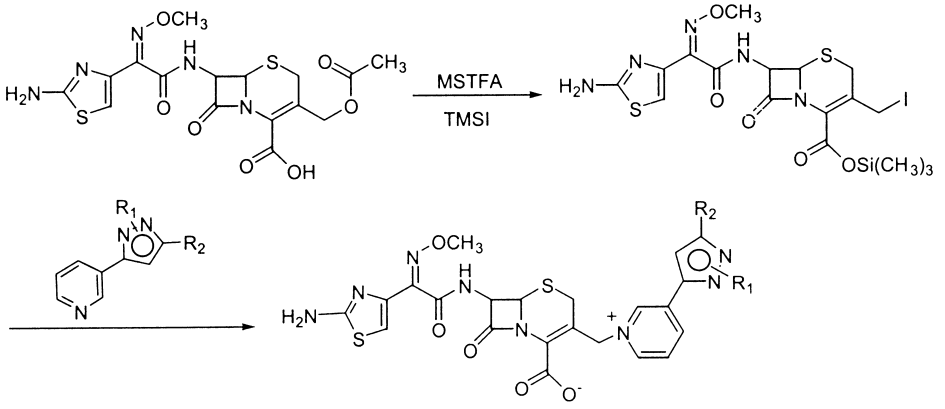


Figure 1.

The in vitro activity of the compounds (**1a**–**k**) against Gram-positive and Gram-negative bacteria were determined by an agar dilution method and was summarized in Table 1. The MIC values for cefpirome against the same strains are shown for comparison. In Table 1, almost all the cephalosporins showed well-balanced antibacterial activity against Gram-positive and Gram-negative bacteria except for *Enterobacter cloacae P99*. As indicated by comparison of MICs of **1c**–**e** and **1f**–**h**, the overall activity of **1c**–**e**, **1f-h** and **1i**–**k** has a tendency to diminish in 2- to 4-fold with increasing chain length at the C-2 position of pyrazole ring. Replacement of hydrogen (**1a**) with hydroxymethyl (**1c**), carbamoyl (**1f**), and amino (**1i**) at the C-5 position of pyrazole ring beared fruitful in improving the activity in 2- to 6-fold. Among new series, **1i** has the best activity against Gram-positive and Gram-negative bacteria including *P.*



Scheme 1. Reagents and conditions: (a) DMA acetal or DMF acetal, 110 °C, 5 h, 98%; (b) NH_2NH_2 H_2O, EtOH, 80 °C, 4 h, 98%; (c) $(CO_2Et)_2$, EtONa, 78 °C, 13 h, 84%; (d) NH_2NHR_2, AcOH, 105 °C, 7 h, (**6a**, 83%; **6b**, 61%; **6c**, 63%); (e) NH_4OH, rt, 35 h, 95%; (f) LAH, THF, rt, 5 h, (**8a**, 95%; **8b**, **8c**, 90%); (g) CH_3CN, 55%, NaH, *t*-BuOH, abs-ether, 40 °C, 24 h, 65%; (h) NH_2NHR_2, 2N-HCl, IPA, 80 °C, 4 h, (**9a**, 74%; **9b**, 57%; **9c**, 30%).



	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k
R_1	H	CH_3	CH_2OH	CH_2OH	CH_2OH	$CONH_2$	$CONH_2$	$CONH_2$	NH_2	NH_2	NH_2
R_2	H	H	H	CH_3	CH_2CH_2OH	H	CH_3	CH_2CH_2OH	H	CH_3	CH_2CH_2OH

Scheme 2.

Table 1. In vitro antimicrobial activity (MIC: μg/mL) of the cephalosporins (**1a**–**1k**)[a]

Strains	CPR	CAZ	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k
S.p. 308	0.007	0.098	0.049	0.007	0.025	0.049	0.049	0.013	0.025	0.049	0.007	0.013	0.025
S.p. 77A	0.007	0.049	0.025	0.007	0.013	0.025	0.049	0.013	0.013	0.025	0.007	0.007	0.013
S.f. MD 8b	1.563	>100.0	6.250	3.125	3.125	6.250	12.50	3.125	3.125	6.250	1.563	1.563	1.563
S.p. SG 511	0.391	12.5	3.125	0.781	1.563	3.125	6.250	0.781	1.563	1.563	0.391	0.781	0.781
*S.*a. 285	0.781	12.5	3.125	0.781	1.563	3.125	6.250	1.563	3.125	3.125	0.781	0.781	1.563
S.a. 503	0.098	3.125	0.781	0.195	0.391	0.781	1.563	0.391	0.391	0.781	0.195	0.391	0.391
MRSA[b]	25.00	>100.0	N/D[c]	25.00	N/D	N/D	25.00	50.00	100.0	25.00	25.00	50.00	12.50
E.c. 055	0.025	0.098	0.049	0.025	0.049	0.049	0.098	0.025	0.049	0.098	0.013	0.025	0.025
E.c. DC 0	0.013	0.098	0.098	0.025	0.049	0.049	0.195	0.025	0.049	0.098	0.025	0.025	0.025
E.c. DC 2	0.013	0.098	0.049	0.013	0.025	0.049	0.098	0.025	0.025	0.098	0.013	0.013	0.013
E.c. TEM	0.049	0.195	0.195	0.098	0.195	0.195	0.781	0.195	0.195	0.391	0.049	0.098	0.098
E.c. 1507 E	0.025	0.195	0.195	0.098	0.098	0.098	0.391	0.098	0.098	0.195	0.025	0.049	0.098
P.a. 9027	3.125	3.125	25.00	6.250	6.250	25.00	25.00	6.250	25.00	25.00	3.125	6.25	12.50
P.a. 1592 E	1.563	1.563	12.50	3.125	3.125	12.50	12.50	3.125	12.50	12.50	1.563	3.125	6.250
P.a. 1771	0.781	0.781	6.250	1.563	1.563	3.125	6.250	1.563	3.125	3.125	0.781	1.563	3.125
P.a. 1771M	0.195	0.195	3.125	0.781	0.391	0.391	0.781	0.781	0.391	0.781	0.195	0.391	0.391
S.t.	0.025	0.195	0.195	0.098	0.098	0.195	0.391	0.049	0.098	0.098	0.049	0.049	0.098
K.o. 1082 E	1.563	0.391	6.250	1.563	3.125	6.25	12.50	1.563	3.125	6.250	1.563	1.563	3.125
K.a. 1522 E	0.025	0.098	0.098	0.049	0.049	0.098	0.195	0.049	0.049	0.098	0.025	0.025	0.049
En.c. P99	3.125	>100.0	>25.00	50.00	>12.50	>25.00	>50.00	50.00	25.00	50.00	50.00	25.00	50.00
En.c. 1321 E	0.013	0.025	0.098	0.013	0.049	0.049	0.098	0.025	0.025	0.049	0.013	0.025	0.025

[a]Abbeviations: CPR, cefpirome; CAZ, ceftazidime; *S.p., Streptococcus pyogenes; S.f., Streptococcus faecium; S.a., Staphylococcus aureus; E.c., Escherichia coli; P.a., Pseudomonas aeruginosa; S.t., Salmonella typhimurium; K.o., Klebsiella oxytoca; K.a., Klebsiella aerogenes; En.c., Enterobacter cloacae.*

[b]Collected from Hospital affilated with Seoul National University.

[c]N/D, not determined.

aeruginosa. The antibacterial activity against MRSA was checked. Most compounds did not show potent value as expected. Compound **1k** exhibited the best activity of compounds including cefpirome tested against MRSA. The inspection to influence the activity against MRSA was undergone through the comparison of **1f**–**h** and **1i**–**k**. NH_2 group at the C-5 position affected to improve the antibacterial activity against MRSA in 2-fold as compared with $CONH_2$ group at the same position. In summary, the order of substituent at 1 or 2 positon of pyrazole ring to influence the overall activity against Gram-positive and Gram-negative microorganism is H >CH_3 >CH_2CH_2OH,

while the order against MRSA is $CH_2CH_2OH > H > CH_3$. The tendency of activity on 5-substituent of pyrazole ring is also increased in order of $NH_2 > CH_3 > CONH_2 > -CH_2OH > H$.

In conclusion, the new cephalosporins with 3-pyrazolylpyridinium moiety exhibited well-balanced broad spectrum against Gram-positive and Gram-negative microorganism. However, the activity against MRSA was not largely improved as compared with cefpirome.

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