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Synthesis and *In Vitro* Antibacterial Activity of Cephalosporins with a Pyridinium Substituent Carrying an Isoxazole Moiety at the C-3 Position

Haeil Park[1], Hyunjoo Choi[1], Jinhee Jang[1], Sunghak Choi[2], Jaekeol Rhee[2], and Minsun Chang[2]

[1]College of Pharmacy, Kangwon National University, Chunchon 200-701, Korea and [2]Department of Medicinal Chemistry, Dong-A Pharm. Co., Ltd., 47-5, Sanggal-ri, Kiheung-up, Yongin-si, Kyunggi-do 449-900, Korea

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Several quaternary pyridinium cephalosporin analogues were prepared and evaluated *in vitro* for antibacterial activity against selected Gram-positive and Gram-negative organisms. Most of the synthesized analogues were either as effective or less effective against the tested bacterial organisms than the reference compounds, Cefpirome and Ceftazidime.

Key words: Quaternary pyridinium cephalosporin, Isoxazole moiety, Anti-staphylococcal activity, Anti-pseudomonal activity

INTRODUCTION

Cephalosporins have been widely used for the treatment of infections in clinics because of their broad-spectrum antibacterial activity and low number of side effects. In addition the new group of cephalosporin analogues (Fig. 1) with a 2-(2-aminothiazol-4-yl)-2-oxyimino-acetamido substituent at the C-7 side chain such as cefotaxime, ceftriaxone, ceftizoxime, and cefmenoxime show both broad-spectrum and excellent antibacterial activity. However, they are less effective against *Staphylococci* than the 1st and 2nd generation cephalosporins. Furthermore, their activity against *Pseudomonas* still needs to improve (Schrinner, 1980; Muytjens, 1982).

Cephalosporins bearing a 1-pyridinium group at the C-3 position, Ceftazidime, Cefsulodine, Cefpirome and Cefepime (Fig. 2), were shown to be effective against pseudomonal infections. These quaternary cephalosporins exhibited excellent activity against Gram-negative bacteria including pseudomonal organisms but were only weakly active against staphylococci (Neu, 1981). In this regard, cephalosporin analogues with an α-oximino substituent at the C-7 side chain and a 1-pyridinium group at the C-3 position have been extensively studied over past two decades in order to launch cefpirome (HR-810, Seibert, 1983; Lattrell, 1988) and cefepime (BMY-28142, Kessler, 1985; Steele, 1985; Naito, 1986) on to the market (Fig. 2).

The primary research goal of this paper was to obtain C-3 pyridinium cephalosporins with improved antibacterial activity against *Staphylococci* while retaining their activity against *Pseudomonas*. This paper describes the synthesis and antibacterial profile of the new cephalosporins with a pyridinium substituent carrying an isoxazole moiety at the C-3 position (Fig. 3 and Fig. 4).

RESULTS AND DISCUSSIONS

Substituted pyridines bearing the isoxazole moiety at either the *meta* or *para* position (Fig. 3) were synthesized from methyl nicotinate or methyl isonicotinate with the corresponding oximes as described in Scheme 1 (He, 1994). Ring cyclization of the oxime dianions and esters followed by a dehydration process in acidic conditions resulted in the substituted pyridines **1-6** in moderate yields (30-58%). The substituted pyridines (**1-6**) were reacted with 3-iodomethyl cefotaxime generated *in situ* from cefotaxime and sodium iodide via a known procedure (Ejima, 1987) to produce quarternary C-3 pyridinium cephalosporins **7-12**. Purification of the crude products by silica gel column chromatography followed by freezing dry of the fractions yielded compounds **7-12** in 15-35% chemical yields.

Synthesized C-3 pyridinium cephalosporins were examined for their biological activity against some selected Gram-positive and negative bacterial strains by an agar plate dilution method (Table I). Compound **10** and **12** exhibited modest activity against *pseudomonas aeruginosa*

Correspondence to: Haeil Park, College of Pharmacy, Kangwon National University, Chunchon, Kangwon 200-701, Korea
E-mail: haeilp@cc.kangwon.ac.kr

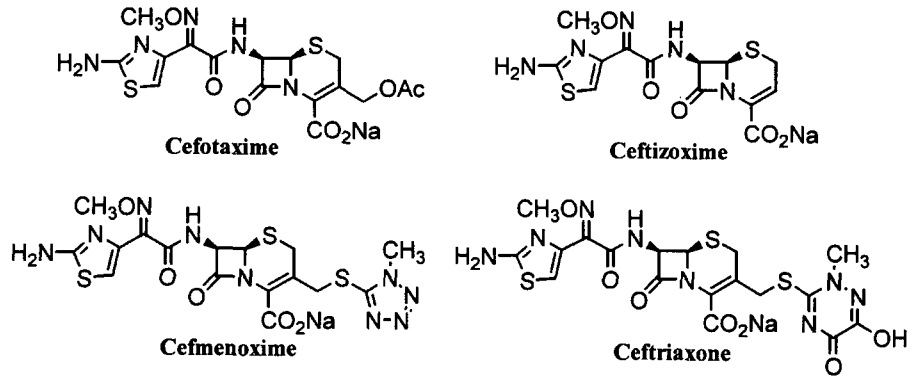


Fig. 1. Cephalosporins with a 2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetamido substituent at the C-7 side chain

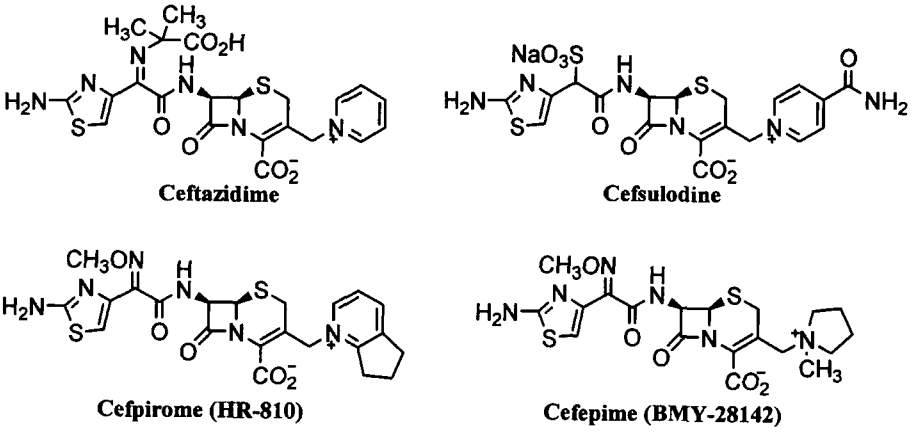


Fig. 2. Cephalosporins with a quarternary amine substituent at the C-3 position

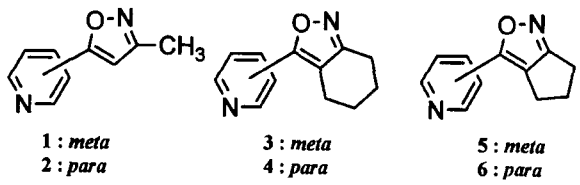


Fig. 3. Substituted pyridines bearing isoxazole moiety at *meta* or *para* position

while all of the six compounds showed good activities against the rest of the strains including *K. pneumoniae*, *P. mirabilis*, *S. marcescens* and *S. aureus*.

MATERIALS AND METHODS

Materials

All starting materials were obtained from commercial

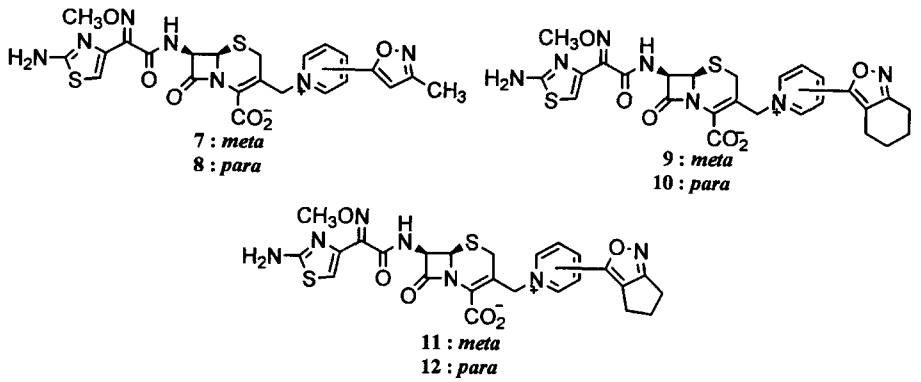


Fig. 4. Cephalosporins with a quarternary pyridinium substituent carrying a isoxazole moiety at the C-3 position

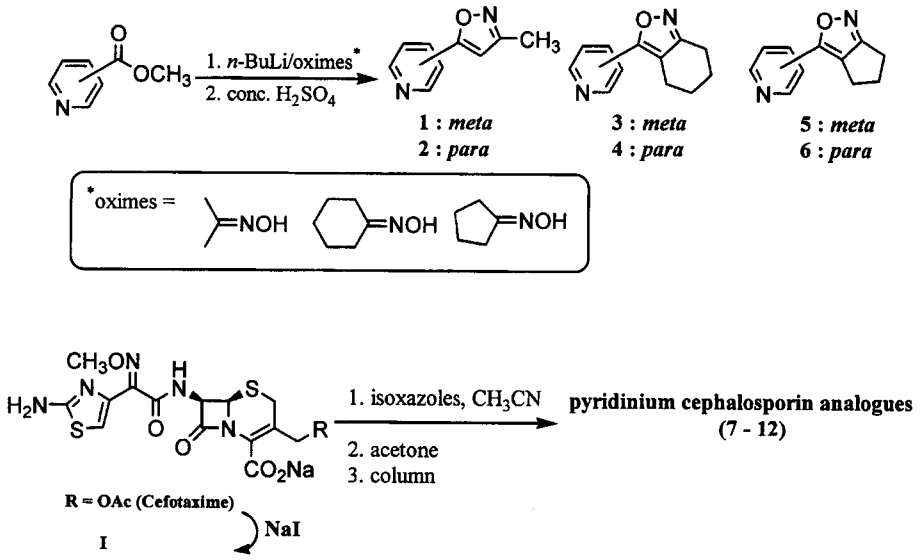
Table I. Antibacterial activities (MIC: μg/mL) of synthesized compounds against standard strains

Standard strains	CAZ	CPR	7	8	9	10	11	12
S. aureus SG 511	6.25	0.39	3.13	0.78	0.78	0.78	0.78	0.78
S. aureus 209 P	25	1.56	12.5	1.56	1.56	3.13	1.56	6.25
S. aureus 285	6.25	0.39	3.13	0.78	0.78	0.78	0.78	0.78
S. aureus 5031	3.13	0.2	3.13	0.39	0.39	0.78	0.78	0.39
S. aureus Smith	12.5	0.78	12.5	1.56	1.56	1.56	1.56	1.56
E. coli TEM	0.1	≤ 0.025	0.39	0.39	0.1	0.05	0.1	0.05
E. coli DC 0	0.1	≤ 0.025	0.39	0.05	0.05	0.05	≤ 0.025	0.1
E. coli DC 2	0.05	≤ 0.025	0.1	0.05	≤ 0.025	≤ 0.025	≤ 0.025	≤ 0.025
E. coli 29	0.05	≤ 0.025	0.2	0.39	0.05	≤ 0.025	0.2	0.05
E. coli 011	3.13	1.56	12.5	12.5	0.39	3.13	12.5	3.13
P. aeruginosa A9027	1.56	3.13	>100	50	25	12.5	50	6.25
P. aeruginosa 1592 E	0.78	1.56	>100	50	12.5	6.25	50	6.25
P. aeruginosa 1771	0.78	1.56	>100	50	12.5	6.25	50	6.25
P. aeruginosa 1771 M	0.1	0.39	6.25	6.25	3.13	0.39	3.13	0.39
P. aeruginosa 93	0.78	3.13	100	25	12.5	12.5	50	25
P. aeruginosa PAO 1	0.78	1.56	>100	50	12.5	6.25	50	6.25
K. pneumoniae A0031	0.1	≤ 0.025	0.2	0.05	0.05	≤ 0.025	≤ 0.025	0.05
K. pneumoniae A9977	0.1	≤ 0.025	0.78	0.78	0.1	0.05	0.2	0.05
K. pneumoniae 477	0.05	≤ 0.025	0.1	0.05	0.05	≤ 0.025	≤ 0.025	0.05
K. pneumoniae 01	0.1	≤ 0.025	0.78	0.78	0.1	0.1	0.39	0.1
K. pneumoniae 020	1.56	0.2	12.5	6.25	3.13	3.13	3.13	3.13
P. mirabilis A 14273	0.05	0.1	0.78	1.56	0.2	0.1	1.56	0.1
P. mirabilis 112/3	0.1	0.2	0.78	6.25	0.78	0.78	6.25	0.2
P. mirabilis 174/3	0.05	0.05	0.39	0.39	0.1	0.1	0.78	0.1
P. mirabilis 4	0.05	0.05	0.78	1.56	0.39	0.2	1.56	0.2
P. vulgaris 867	0.05	0.05	0.78	1.56	0.39	0.2	1.56	0.2
P. vulgaris 868	≤ 0.025	≤ 0.025	0.2	0.2	0.05	≤ 0.025	0.1	0.05
P. vulgaris 5	0.05	0.2	1.56	1.56	1.56	0.1	0.39	0.1
E. cloacae	≤ 0.025	≤ 0.025	0.2	0.1	≤ 0.025	≤ 0.025	≤ 0.025	≤ 0.025
E. cloacae 417	≤ 0.025	≤ 0.025	0.2	0.2	≤ 0.025	≤ 0.025	0.2	0.05
E. cloacae P 99	100	3.13	100	12.5	12.5	50	25	25
E. cloacae 1321 E	≤ 0.025	≤ 0.025	0.2	0.1	≤ 0.025	0.05	0.05	0.05
C. freundii A 9090	0.1	≤ 0.025	0.2	0.05	≤ 0.025	0.2	0.1	0.2
S. marcescens 370	0.2	0.05	1.56	1.56	0.2	0.39	1.56	0.05
S. marcescens A 217	0.2	0.05	1.56	1.56	0.2	0.39	1.56	0.05
S. marcescens A 216	0.2	0.05	0.78	1.56	0.1	0.39	0.78	0.05
S. marcescens 6093	≤ 0.025	≤ 0.025	0.78	1.56	0.1	0.2	0.78	0.05

suppliers, and used without further purification. All the solvents used for reaction were freshly distilled using a proper dehydrating agent under nitrogen gas. All the solvents used for chromatography were used as supplied without further purification. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini 2000 (200 MHz) spectrometer. The chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) as an internal standard. The peak splitting patterns are abbreviated as m (multiplet), s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), and dd (doublet of doublets). ^{13}C-NMR spectra were recorded on a Varian Gemini 2000 (50 MHz) spectrometer, fully decoupled and the chemical shifts are reported in parts per million (ppm) downfield relative to TMS. Analytical thin-layer chromatography (TLC) was performed using a commercial glass plate with silica gel 60F 254 purchased from Merck. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230-400 mesh, Merck).

General synthetic procedure for the isoxazole analogues

LDA (2.1 equivalent, 1.0 M solution) in dry tetrahydrofuran, generated *in situ* from distilled diisopropylamine and *n*-BuLi at -78°C under nitrogen, was added to a stirring solution of an oxime (2.0 equivalent) in dry tetrahydrofuran (0.3 M solution). The reaction mixture was stirred at 0-5°C for 2 h. A solution (0.5 M) of methyl nicotinate or methyl isonicotinate (1.0 equivalent) in dry tetrahydrofuran was then added by cannula to the reaction mixture at -78°C under nitrogen gas. The reaction mixture was reacted at -78°C for 2 h then



Scheme 1. Synthesis of isoxazole analogues and pyridinium cephalosporin analogues

allowed to warm up to room temperature. After 12 h, the reaction mixture was subsequently cooled to 0-5°C and sulfuric acid was then added. The reaction mixture was stirred for 2 h at room temperature and neutralized by adding a saturated aqueous sodium bicarbonate solution. The reaction mixture was extracted with chloroform, washed with brine and dried over anhydrous sodium sulfate. After filtration, the removal of solvents yielded a crude product. Purification of the crude product by silica gel column chromatography resulted in the isoxazole analogues **1-6** in pure form.

3-(3-Methyl-isoxazol-5-yl)-pyridine (1)

34%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH3), 6.49 (s, 1H. isox-H), 7.39-7.45 (dd, *J*=6 Hz, 12 Hz, 1H, Py-H), 8.08 (d, *J*=12 Hz, 1H, Py-H), 8.67 (d, *J*=6 Hz, 1H. Py-H) 9.00 (s, 1H, Py-H).

4-(3-Methyl-isoxazol-5-yl)-pyridine (2)

30%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3), 6.57 (s, 1H, isox-H), 7.63 (d, *J*=6 Hz, 2H, 2x Py-H), 8.77 (d, *J*=6 Hz, 2H, 2x Py-H)

3-Pyridin-3-yl-4,5,6,7-tetrahydro-benzo[c]isoxazole (3)

40%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.75-2.02 (m, 4H, 2xCH_2), 2.71-2.92 (m. 4H, 2xCH_2), 7.27-7.46 (m, 1H, Py-H), 8.09 (d, *J*=10 Hz, 1H, Py-H), 8.64 (d, *J*=6 Hz, 1H, Py-H), 8.99 (s, 1H, Py-H).

3-Pyridin-4-yl-4,5,6,7-tetrahydro-benzo[c]isoxazole (4)

36%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.77-1.87 (m, 4H, 2xCH_2), 2.83-2.92 (m, 4H, 2xCH_2), 7.62 (d, *J*=6 Hz, 2 H, 2x Py-H), 8.73 (d, *J*=6 Hz, 2H, 2xPy-H).

3-Pyridin-3-yl-5,6-dihydro-4*H*-cyclopenta[c]isoxazole (5)

58%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 2.52-2.58 (m, 2H, CH_2), 3.32-3.34 (m, 4H, 2xCH_2), 7.61 (dd, *J*=5 Hz, 8 Hz, 1H, Py-H), 8.47 (d, *J*=8 Hz, 1H, Py-H), 8.76 (d, *J*=5 Hz, 1H, Py-H), 9.19 (s, 1H, Py-H).

3-Pyridin-4-yl-5,6-dihydro-4*H*-cyclopenta[c]isoxazole (6)

45%, ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.81-1.96 (m, 2H, CH_2), 2.64-2.81 (m, 4H, 2xCH_2), 7.63 (d, *J*=6 Hz, 2H, 2x Py-H), 8.56 (d, *J*=6 Hz, 2H, 2x Py-H).

General synthetic procedure for the quaternary pyridinium cephalosporins

A reaction mixture of cefotaxime sodium salt (1 equivalent), an isoxazole (2.2 equivalent), and sodium iodide (10 equivalent) in 4N-aquoues HCl and acetonitrile (1:3) was refluxed at 80°C for 2 h. After cooling to 0-5°C in an ice-bath, acetone was then added to the reaction mixture and the resulting solid was filtered and washed with acetone. Purification of the solid by silica gel column chromatography in an acetonitrile-water mixture as the mobile phase and freezing dry of the fractions containing the product yielded pyridinium cephalosporin as a white solid.

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(4-methylisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (7)

12%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 3.42 (ABq, 2H, *J*=17 Hz, cephem ring 2-H), 3.73 (s, 3H, methoxy group), 5.04 (d, 1H, *J*=5 Hz, cephem

ring 6-H), 5.51 (ABq, 2H, J=16 Hz, cephem ring 3-CH_2), 5.62 (q, 1H, J=13 Hz, cephem ring 7-H), 6.64 (s, 1H, thiazole ring 5-H), 7.20 (s, 2H, -NH_2) 8.27 (t, 1H, J=14 Hz, Py-H), 8.94 (d, 1H, J=9 Hz, Py-H), 9.49 (d, 1H, J=8 Hz, -CONH) 9.65 (d, 1H, J=6 Hz, Py-H), 10.08 (s, 1H, Py-H).

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-(4-methylisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (8)

28%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 3.32 (ABq, 2H, J=17 Hz, cephem ring 2-H), 3.78 (s, 3H, methoxy group), 5.06 (d, 1H, J=5 Hz, cephem ring 6-H), 5.38 (ABq, 2H, J=18 Hz, cephem ring 3-CH_2), 5.66 (q, 1H, J=17 Hz, cephem ring 7-H), 6.70 (s, 1H, thiazole ring 5-H), 7.23 (s, 2H, -NH_2), 8.59 (d, 1H, J=7 Hz, 2xPy-H), 9.62 (d, 1H, J=8 Hz -CONH), 9.66 (d, 1H, J=7 Hz, 2xPy-H).

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(3,4-cyclohexenoisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (9)

10%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.77 (s, 4H, cyclohexeno-ring) 3.35 (ABq, 2H, J=17 Hz, cephem ring 2-H), 3.82 (s, 3H, methoxy group), 5.08 (d, 1H, J=5 Hz, cephem ring 6-H), 5.48 (ABq, 2H, J=13 Hz cephem ring 7-H), 5.66 (d, 1H, J=13 Hz, Cephem ring 7-H), 6.69 (s, 1H, thiazole ring 5-H), 7.25 (s, 2H, -NH_2), 8.30 (t, 1H, J=14 Hz, Py-H), 8.86 (d, 2H, J=8 Hz, Py-H), 9.57 (d, 1H, J=8 Hz, -CONH), 9.60 (d, 1H, J=6 Hz, Py-H), 9.99 (s, 1H, Py-H).

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-(3,4-cyclohexenoisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (10)

21%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.78 (bs, 4H, cyclohexeno-ring), 3.34 (ABq, 2H, J=18 Hz, cephem ring 2-H), 3.82 (s, 3H, methoxy group), 5.05 (d, 1H, J=5 Hz, cephem ring 6-H), 5.36 (ABq, 2H, J=11 Hz cephem ring 3-CH_2), 5.67 (q, 1H, J=22 Hz, Cephem ring 7-H), 6.69 (s, 1H, thiazole ring 5-H), 7.22 (s, 2H, -NH_2) 8.42 (d, 2H, J=7 Hz, 2xPy-H), 9.58 (1H, d, J=8 Hz, -CONH), 9.61 (d, 2H, J=7 Hz, 2xPy-H).

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(3,4-cyclopentenoisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (11)

7.5%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.77-2.05 (m, 4H, cyclopenteno-ring), 2.57 (t, 2H, J=7.0 Hz, cyclopentenoring), 3.30 (ABq, 2H, J=17.4 Hz, cephem ring 2-H), 3.88 (s, 3H, methoxy group), 5.02 (d, 1H, J=4.9 Hz, cephem ring 6-H), 5.19-5.69 (ABq, 2H, J=13.2 Hz, cephem ring 3-CH_2), 5.61 (dd, 1H, J=4.9 Hz, 5.5 Hz, Cephem ring 7-H), 6.65 (s, 1H, thiazole ring 5-H), 7.20 (s, 2H, -NH_2), 8.22 (t, 1H, J=14.4 Hz, Py-H), 8.75 (d, 1H, J=8.4 Hz, Py-H), 9.33 (d, 1H, J=5.4 Hz, Py-H), 9.59 (d, 1H, J=5.5 Hz, -CONH), 9.82 (s, 1H, Py-H).

7β-[2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[4-(3,4-cyclopentenoisoxazol-2-yl)-1-pyridino]methyl-3-cephem-4-carboxylate (12)

8.5%, ^{1}H-NMR (200 MHz, DMSO-d_6): δ 1.81-1.96 (m, 2H, cyclopenteno-ring), 2.64-2.81 (m, 4H, cyclopenteno-ring), 3.38 (ABq, 2H, J=18.0 Hz, cephem ring 2-H), 3.79 (s, 3H, methoxy group), 4.98(d, 1H, J=4.4 Hz, cephem ring 6-H), 5.05-5.53 (ABq, 2H, J=10.2 Hz, cephem ring 3-CH_2), 5.61 (dd, 1H, J=4.4 Hz, 7.2 Hz, Cephem ring 7-H), 6.84 (s, 1H, thiazole ring 5-H), 7.32 (s, 2H, -NH_2), 8.38 (d, 2H, J=6.6 Hz, 2xPy-H), 9.55 (d, 2H, J=6.8 Hz, 2xPy-H), 9.64 (d, 1H, J=7.2 Hz, -CONH).

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