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Synthesis and *In Vitro* Antibacterial Activity of C-3' Pyridinium Cephalosporin Derivatives

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The quaternary ammonium cephalosporin derivatives were prepared with various pyridines substituted at the 3 or/and 4 position. Their *in vitro* antibacterial activities were determined and substituent effect on pyridine nucleus was studied. Preparation of substituted pyridines are also described.

Key words: Quaternary pyridinium cephalosporin derivatives, Antibacterial activity

INTRODUCTION

The quaternary ammonium cephalosporins have been widely used as one of the major parentral antibacterials in clinics for the treatment of infectious disease because of their broad-spectrum antibacterial activity and lower frequency of side effects. However, they are generally less potent against Gram-positive bacteria than older cephalosporins such as cefazolin and cefotiam.In particular, even though ceftazidime, which is a pyridinium cephalosporin derivative, has shown clinically acceptable efficacy to infectious diseases by Gram-negative and *Pseudomonas aureginosa*, it is still of interest to explore further derivatization of the ceftazidime analogs for enhancing activity against Gram-positive and β-lactamase resistant pathogens.

Over the past 10~15 years, many research groups have been interested in development of new pyridinium derivatives with improved activity against gram-positive bacteria and β-lactamase stability (Brown, *et al.*, 1990).

In general, antibacterial activity and pharmacokinetic profile of the quaternary ammonium cephalosporins might be influenced by amine group substituted at the 3-position of cephalosporin nucleus in addition to acyl moiety of the 7-amino group. In case of pyridine series as an amine substituent, Gram-negative and anti-pseudomonal activity could be enhanced by hydrophilic substituents on the pyridine ring, while lipophilic substituents are likely to increase activity against Gram-positive bacteria.

Therefore, our research objective was to discover a new pyridinium cephalosporin derivatives by varying the substituents of the pyridine ring to enhance activity against Gram-positive and β-lactamase resistant strains, and equal or better pharmacokinetic profile than commercially available antibiotic analogues.

In this paper, we describe the synthesis and structure-activity relationship of the new pyridinium cephalosporins bearing aminothiazolyl β-oxime acyl group at the 7 position.

MATERIALS AND METHODS

Materials

All starting materials were obtained from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from calcium hydride or PCl_5 under nitrogen. ^1H NMR spectra were recorded on a Gemini Varian-300 (300 MHz) spectrometer. Chemical shifts are reported in parts per million (δ) down field relative to TMS as an internal standard. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin Elmer 16F PC FT-IR spectrometer. Mass spectra were determined with a HP590 GC/MS 5972 MSD spectrometer. HPLC analyses were done at 254nm on a Waters 840 HPLC system equipped with a Waters WISP injection system and a Merck μ Bondpack C18 reverse-phase column (LiChroCART100RP-18,10 μm).Chromatographic purification was carried out by flash chromatography

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using Kieselgel 60 (230~400 mesh,Merck). Antibacterial activities were determined by agar dilution method.

Synthesis of substituted pyridines

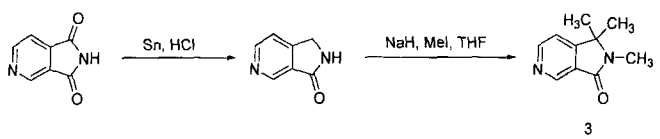
Most of pyridine derivatives as a quaternary ammonium salt were synthesized by general synthetic methods (Tayler, *et al.*, 1956) or purchased from a commercial supplier. 4-Morpholino-, 4-thiomorpholino- and 4-*N*-piperazinyl picoline(**23~25**) were prepared by the substitution reaction of 4-chloropicoline *N*-oxide as known in the literature (Erdtmon, *et al.*, 1963). 1,1,2-trimethyl-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-3-one (**3**) was prepared by methylation of 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-3-one (Kuthan *et al.*, 1977) suggested in Scheme 1.

4-Amino-3,5-dichloropyridine derivatives (**10, 11**) were synthesized from 4-amino-3,5-dichloropyridine (David *et al.*, 1998) as shown in Scheme 2. 4-Methyl-3-pyridine-carboxamide derivatives (**14, 15**, and **16**) were prepared with the corresponding amines from 4-methyl-3-pyridine-carboxylate (Bobbitt *et al.*, 1960).

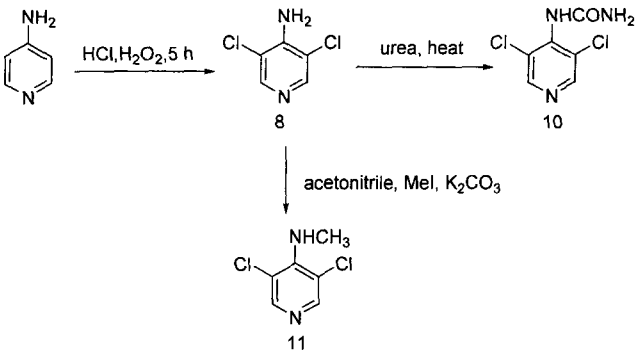
1,1,2-Trimethyl-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-3-one (3)

To a stirred suspension of 3,4-pyridinedicarboximide (1 g, 6.8 mmol) in 10 ml c-HCl was added tin powder (5 eq, 4 g) at 0°C. The reaction temperature was raised to reflux for 6 h after stirring 30 min at 0°C. After cooling the reaction mixture, precipitated solid was filtered off and the filter cake was basified with 50 ml aqueous $NaHCO_3$, and then extracted with chloroform (3 × 100 ml). Organic layer was dried with $MgSO_4$ and concentrated to obtain 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyri-din-3-one (0.175 g, 20%) as a white solid. mp 199-201°C(lit. 199-200); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.52(s, 2 H), 7.47(d, *J*=5.4 Hz, 1 H), 7.73(brs, 1 H), 8.77(d, *J*=4.8Hz, 1 H), 9.12(s, 1 H).

A solution of 2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-3-one (70.7 mg, 0.477 mmol) in dried THF was slowly added to a stirred suspension of NaH (1.2 eq, 0.02 g) in dried THF at 0°C. After stirring about 30min, MeI (1.2 eq, 0.035 ml) was slowly added to a solution, and then the mixture was stirred about 20 h at room temperature. After quenching with sat.-NH_4Cl solution, the solvent was removed under reduced pressure. The residue was extracted with $CHCl_3$ (350 ml), and dried with



Scheme 1. Synthesis of 1,1,2-trimethyl-2,3-dihydro-1*H*-pyrrolo [3,4-c] pyridin-3-one



Scheme 2. Synthesis of *N*-substituted 4-amino-3,5-dichloro-pyridine

$MgSO_4$. The organic layer was concentrated to obtain 1,1,2-trimethyl-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridine-3-one (0.18g, 47.2%) as a white solid. mp 134-135°C; IR (KBr) cm^{-1} 1391, 1686; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47(s, 6 H), 3.03(s, 3 H), 7.38(d, *J*=5.1 Hz, 1 H), 8.76(d, *J*=5.1 Hz, 1 H), 9.07(s, 1 H).

3,5-Dichloro-4-ureidopyridine (10)

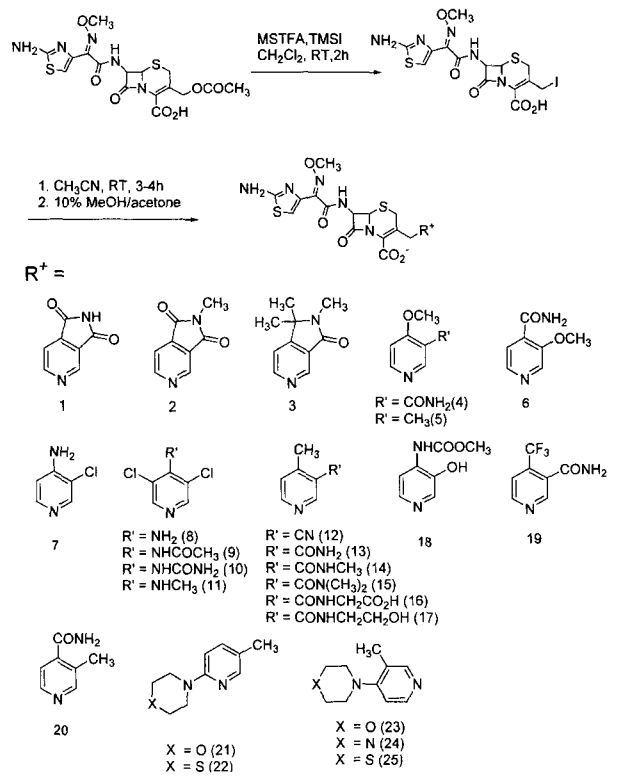
4-Amino-3,5-dichloropyridine (0.3 g, 1.8 mmol) and urea (2 eq, 0.22 g) was dissolved in 5 ml dried DMF, and reacted at 140°C about 8 h. After the reaction were proceeded completely, solvent was removed under reduced pressure to gain solid and it was recrystallized with MeOH to obtain pale yellow solid (0.3 g,78.9%). mp 162-164°C; IR (KBr) cm^{-1} 1312, 1648, 1726; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.21(s ,3 H), 4.35(br, 2 H), 8.21(s, 2 H), 11.2(br, 1 H).

3,5-Dichloro-*N*-methylaminopyridine(11)

4-Amino-3,5-dichloropyridine (0.5 g, 3.08 mmol) was dissolved in 10 ml acetonitrile, and K_2CO_3 (1.27 g, 9.24 mmol) was added to a solution. Then, CH_3I (0.66 g, 1.5eq) was slowly added to the reaction mixture, and stirred about 10 h at room temperature. The suspended solid was filtered off and filtrate was concentrated under reduced pressure to yield pale yellow solid (0.54 g, ≒ 100%). mp 148-151°C; IR (KBr) cm^{-1} 1121, 1435, 1521; ^{1}H NMR (300MHz, DMSO-d_6) δ 2.11(s, 3 H), 2.54(s, 3 H), 8.21(s, 2 H), 10.5(br, 1 H).

Preparation of quaternary ammonium cephalosporins

Formation of quaternary pyridinium salt was carried out by reaction of the intermediate iodide, generated *in situ* from cefotaxime, and the corresponding substituted pyridine in the similiar manner reported in the literature (Ejima *et al.*, 1986) as shown in Scheme 3. All the products were isolated as hydrogen iodide salt, and these compounds were converted to pure inner salt by freeze drying after purification with chromatograph. All



Scheme 3. Preparations of quaternary ammonium cephalosporin derivatives

the purified compounds were subjected to examine their antibacterial activity.

General procedure to form the pyridinium cephalosporin derivatives

7-[2-(2-Aminothiazol-4-yl)-2(*Z*)-methoxyiminoacetamido]cephalosporanic acid (cefotaxim; 0.16 g, 0.36 mmol) was suspended in methylene chloride (5 ml) under nitrogen atmosphere. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (2 ml, 3 eq) was added. After the mixture was stirred about 1 h, trimethylsilyl iodide (0.15 ml, 3 eq) was added to the resulting pale yellow solution, and the solution was stirred for 1 h. Then, the solvent was evaporated *in vacuo* to afford the 3-iodomethyl cephem as a viscous yellowish residue. The residue was dissolved in acetonitrile (1 ml), and tetrahydrofuran (0.1 ml) was added to destroy excess of trimethylsilyl iodide. The substituted pyridines (1.5 eq) dissolved in acetonitrile (5ml) were added to the solution. The reaction mixture was stirred at room temperature for 3-4 h, and then 5% methanol/acetone (0.5:1, 5 ml) was added to hydrolyze the trimethylsilyl group. Hydrogen iodide salt of the precipitated product was obtained by filteration, and washed with ethyl ether (5 ml× 2) followed by methylene chloride (5 ml× 2) and acetone (5 ml × 2). The crude hydrogen iodide salt was dissolved in 10% aqueous sodium bicarbonate, and chromatographed by eluting with acetonitrile/water mixture (4:1). Eluted solution containing the product was freeze-dried to obtain an amorphous colorless or pale yellow inner salt. The purity was examined on a HPLC (eluent solvent : 10% acetonitrile/water; flow rate:1 ml/min).

7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(1,3-dioxo-2,3-dihydro-1*H*-pyrrolo[3,4-c]-pyridin-5-ium-5-yl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (1)

Purity 95%; mp 188°C(dec.); IR (KBr) cm^{-1} 3842, 1715; ^1H NMR (300 MHz, DMSO-d_6) δ 3.80(s, 3 H), 5.03(m, 2 H), 5.58(m, 2 H), 6.73(s, 1 H), 7.17(brs, 2 H), 7.85(d, J=4.2 Hz, 1 H), 7.90(s, 1 H), 9.35(d, J=5.1 Hz, 1 H), 9.44(brs, 1 H), 9.55(d, J=3.8 Hz, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(2-methyl-1,3-dioxo-2,3-dihydro-1H-pyrrolo-[3,4-c]pyridin-5-ium-5-yl)methyl]-6-oxo-7,7a-dihydro-2H,6H-azeto[2,1-b][1,3]thiazine-4-carboxylate (2)

Purity 95%; mp 189°C(dec.); IR (KBr) cm^{-1} 1740; ^1H NMR (300 MHz, DMSO-d_6) δ 2.78(s, 3 H), 3.63(q, 2 H), 3.81(s, 3 H), 5.06(s, 2 H), 5.60(d, *J*=1.2 Hz, 1 H), 5.62(d, *J*=6.2 Hz, 1 H), 6.69(s, 1 H), 7.18(brs, 2 H), 7.95(d, *J*=3.5 Hz, 1 H), 9.41(d, *J*=3.5Hz, 1 H), 9.43(s, 1 H), 9.47(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-3-[(1,1,2-trimethyl-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-c]pyridin-5-ium-5-yl)methyl]-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (3)

Purity 91%; mp 180°C(dec.); IR (KBr) cm^{-1} 1735; ^1H NMR (300 MHz, DMSO-d_6) δ 1.58(s, 6 H), 2.98(s, 3 H), 3.75(q, 2 H), 3.81(s, 3 H), 5.06(m, 2 H), 5.60(d, *J*=4.2 Hz, 1 H), 5.62(d, *J*=4.2 Hz, 1 H), 6.72(s, 1 H), 7.20(brs, 2 H), 8.61(d, *J*=5.1 Hz, 1 H), 9.41(d, *J*=3.2 Hz, 1 H), 9.43(s, 1 H), 9.47(brs, 2 H).

3-[3-(aminocarbonyl)-4-methoxy-1-pyridiniumyl]methyl-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (4)

Purity 90%; mp 190°C(dec.); IR (KBr) cm^{-1} 1775, 3246; ^1H NMR (300 MHz, DMSO-d_6) δ 3.62(q, 2 H), 3.82(s, 3 H), 3.87(s, 3 H), 5.38(s, 2 H), 5.68(d, *J*=4.2 Hz, 1 H), 5.95(s, 1H), 6.87(s, 1 H), 7.17(brs, 2 H), 8.39(s, 1 H), 8.69(d, *J*=4.1 Hz, 1 H), 9.35(d, *J*=4.1 Hz, 1 H), 9.56(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(4-methoxy-3-methyl-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (5)

Purity 96%; mp 187°C(dec.); IR (KBr) cm^{-1} 1780; ^1H

NMR (300 MHz, DMSO-d_6) δ 2.21(s, 3 H), 3.82(s, 3 H), 4.19(s, 3 H), 5.12(s, 2 H), 5.30(d, *J*=4.1 Hz, 1 H), 5.52(s, 1 H), 6.77(s, 1 H), 7.19(brs, 2 H), 7.67(d, *J*=4.2 Hz, 1 H), 8.76(s, 1 H), 9.09(d, *J*=2.8 Hz, 1 H), 9.49(brs, 2 H).

3-[4-(aminocarbonyl)-3-methoxy-1-pyridiniumyl]methyl-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-7,7a-dihydro-2H,6H-azeto[2,1-b][1,3]thiazine-4-carboxylate (6)

Purity 90%; mp 193°C(dec.); IR (KBr) cm^{-1} 1740; ^1H NMR (300 MHz, DMSO-d_6) δ 3.62(2 H, ABq), 3.89(s, 3 H), 3.87(s, 3 H), 5.38(m, 2 H), 5.68(m, 1 H), 5.95(d, *J*=4.2 Hz, 1 H), 6.87(s, 1 H), 7.15(brs, 2 H), 8.39(s, 1 H), 8.69(d, *J*=6.7 Hz, 1 H), 9.35(d, *J*=4.1 Hz, 1 H), 9.56(brs, 2 H).

3-[(4-amino-3-chloro-1-pyridiniumyl)methyl]-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-6-oxo-7,7a-dihydro-2H,6H-azeto[2,1-b][1,3]thiazine-4-carboxylate (7)

Purity 93%; mp 218-220°C(dec.); IR (KBr) cm^{-1} 1122, 1391, 1654, 1760, 3400; ^1H NMR (300 MHz, D_2O-d_2) δ 3.18(m, 2 H), 3.95(s, 3 H), 5.24(d, *J*=1.2 Hz, 1H), 5.84(t, *J*=1.5 Hz, 1 H), 6.86(s, 1 H), 7.01(d, *J*=3 Hz, 1 H),7.97(d, *J*=3 Hz, 1 H), 8.49(s, 1 H).

3-[(4-amino-3,5-dichloro-1-pyridiniumyl)methyl]-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate (8)

Purity 96%; mp 205-208°C (dec.); IR (KBr) cm^{-1} 1180, 1530, 1618, 1768, 3404; ^1H NMR(300 MHz, D_2O) δ 3.18(dd, J=18.0 Hz, J'=4.2 Hz, 2 H), 3.97(s, 3H), 5.14(d, J=2.7Hz, 1H), 5.27(d, J=2.1 Hz, 1 H), 5.86(t, J=2.4 Hz, 1 H), 7.01(s, 1 H), 8.56(s, 2 H).

3-[(4-acetoamino-3,5-dichloro-1-pyridiniumyl)methyl]-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-*b*][1,3]thiazine-4-carboxylate (9)

Purity 95.7%; mp 203-205°C(dec.), IR (KBr,cm^{-1} 1384, 1638, 1772, 3442, ^1H NMR(DMSO-d_6) δ 2.24(s, 3 H), 3.25(m, 1 H), 3.61(m, 1 H), 3.97(s, 3 H), 4.95(m, 2 H), 5.16(m, 1 H,), 5.89(m, 1 H), 7.01(s, 1 H), 8.56(s, 2 H), 9.19(s, 1 H).

3-({4-[(aminocarbonyl)amino]-3,5-dichloro-1-pyridiniumyl}methyl)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]amino}-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto-[2,1-b][1,3]thiazine-4-carboxylate (10)

Purity 96%; mp 198-201°C(dec.); IR (KBr) cm^{-1} 1192, 1656, 1762, 3404, ^1H NMR (300 MHz, DMSO-d_6) δ 3.62(d, *J*=9.2 Hz, 2 H), 3.91(s, 3 H), 5.01(d, *J*=2.5 Hz, 1 H), 5.28(d, *J*=2.5 Hz, 1 H), 5.87(t, *J*=2.4 Hz, 1 H), 6.91(s, 1 H), 8.52(s, 2 H), 9.51(brs, 2 H).

3-{[4-(methylamino)-3,5-dichloro-1-pyridiniumyl]methyl}-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)-acetyl]amino}-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (11)

Purity 96%; mp 212-216°C(dec.); IR (KBr) cm^{-1} 1382, 1612, 1758, 3308; ^1H NMR (300 MHz, DMSO-d_6) δ 1.81(s, 3 H), 3.21(m, 1 H), 3.63(m, 1 H), 3.98(s, 3 H), 4.95(m, 2 H), 5.21(m, 1 H), 5.89(m, 1 H), 6.91(s, 1 H), 8.56(s, 2 H), 9.19(s, 1 H).

7-[3-(2-amino-1,3-thiazol-4-yl)-3-(methoxyimino)-2-oxo-propyl]-3-[(3-cyano-4-methyl-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6H-azeto[2,1-b][1,3]thiazine-4-carboxylate (12)

Purity 95%; mp 185°C(dec.); IR (KBr) cm^{-1} 1735; ^1H NMR (300 MHz, DMSO-d_6) δ 2.13(s, 3 H), 3.97(s, 3 H), 5.12(s, 2 H), 5.28(d, *J*=4.2 Hz, 1 H), 5.95(s, 1 H), 6.87(s, 1 H), 7.37(brs, 2 H), 8.65(s, 1H),8.69(d, *J*=4.1 Hz, 1 H), 8.91(d, *J*=4.2 Hz, 1 H), 9.51(brs, 2 H).

3-[3-(aminocarbonyl)-4-methyl-1-pyridiniumyl]methyl-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (13)

Purity 96%; mp 175°C(dec.); IR (KBr) cm^{-1} 1721, 3705; ^1H NMR(300 MHz, DMSO-d_6) δ 3.36(s, 3 H), 3.62(q, 2), 3.91(s, 3 H), 5.08(s, 2 H), 526(d, *J*=4.2 Hz, 1 H), 5.69(s, 1 H), 6.87(s, 1 H), 7.25(brs, 2 H), 8.21(s, 1 H), 8.36(d, *J*=4.0Hz, 1 H), 9.35(d, *J*=5.1 Hz, 1 H), 9.56(brs, 4 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-(4-methyl-3-[(methylamino)carbonyl]-1-pyridiniumylmethyl)-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thia-zine-4-carboxylate (14)

Purity 97%; mp 190°C(dec.); IR (KBr) cm^{-1} 1740; ^1H NMR (300MHz, DMSO-d_6) δ 2.82(s, 3 H), 3.35(s, 3 H), 3.65(q, 2 H), 3.95(s, 3 H), 5.02(s, 2 H), 5.22(d, *J*=4.2Hz, 1 H), 5.69(d, *J*=3.6Hz, 1 H), 6.87(s, 1 H), 7.25(brs, 2 H), 8.21(s, 1 H), 8.36(d, *J*=4.6Hz, 1 H), 9.35(d, *J*=4.1 Hz, 1 H), 9.52(brs, 4 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-(3-[(dimethylamino)carbonyl]-4-methyl-1-pyridiniumylmethyl)-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (15)

Purity 89%; mp 195°C(dec.); IR (KBr) cm^{-1} 1740; ^1H NMR (300 MHz, DMSO-d_6) δ 2.62(s, 3 H), 3.12(s, 6 H), 3.62(q, 2 H), 3.85(s, 3 H), 5.02(s, 2 H), 5.22(d, *J*=4.5 Hz, 1 H), 5.69(d, *J*=4.5 Hz, 1 H), 6.87(s, 1 H), 7.25(brs, 2 H), 8.21(s, 1 H), 8.36(d, *J*=4.1 Hz, 1 H), 9.35(d, *J*=4.2 Hz, 1 H),9.41(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(3-[(2-hydroxyethyl)amino]carbonyl-4-methyl-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto-[2,1-b][1,3]thiazine-4-carboxylate (16)

Purity 93% mp <250°C(dec.); IR (KBr) cm^{-1} 1740; ^1H NMR (300 MHz, DMSO-d_6) δ 2.41(s, 3 H), 2.65(t, *J*=4.2 Hz, 4.0Hz, 2 H), 3.21(m, 2 H), 3.58(m, 2 H), 3.81(s, 3 H), 5.01(m, 2 H), 5.18(d, *J*=4.2 Hz, 1 H), 5.68(d, *J*=4.8 Hz, 1 H), 6.81(s, 1 H), 7.17(brs, 2 H), 8.09(d, *J*=4.6 Hz, 1 H), 9.05(brs, 1 H), 9.31(d, *J*=4.5 Hz, 1 H), 9.35(s, 1 H), 9.58(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(3-[(carboxymethyl)amino]carbonyl-4-methyl-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate(17)

Purity 95%; mp 219°C(dec.); IR (KBr) cm^{-1} 1790; ^1H NMR (300 MHz, DMSO-d_6) δ 2.41(s, 3 H), 3.21(d, *J*=4.5 Hz, 2 H), 3.58(m, 2 H), 3.89(s, 3 H), 5.01(m, 2 H), 5.15(d, *J*=4.1 Hz, 1 H), 5.71(d, *J*=4.3 Hz, 1 H), 6.76(s, 1 H), 7.15(brs, 2 H), 8.09(d, *J*=4.5 Hz, 1 H), 9.05(brs, 1 H), 9.31(d, *J*=4.1 Hz, 1 H), 9.42(s, 1 H), 9.62(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-(3-hydroxy-4-[(methoxycarbonyl)amino]-1-pyridiniumylmethyl)-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (18)

Purity 92%; mp 196°C(dec.); IR (KBr) cm^{-1} 1740, 1780; ^1H NMR (300 MHz, DMSO-d_6) δ 2.31(s, 3H), 3.62(m, 2 H), 3.87(s, 3 H), 5.08(m, 2 H), 5.68(d, *J*=4.2Hz, 1 H), 5.95(s, 1 H), 6.87(s, 1 H), 7.17(brs, 2 H), 8.39(s, 1 H), 8.69(d, 1 H), 8.92(d, 1 H), 9.35(d, 2H), 9.56(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[3-(aminocarbonyl)-4-(trifluromethyl)-1-1-pyridiniumyl]methyl-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate(19)

Purity 95%; mp 168°C(dec.); IR (KBr) cm^{-1} 1775, 3366; ^1H NMR (300 MHz, DMSO-d_6) δ 3.80(s, 3 H), 5.03(s, 2 H), 5.38(d, 1 H), 5.58(d, 1 H), 6.73(s, 1 H), 7.17(brs, 2H), 7.85(d, *J*=4.2Hz, 1 H), 7.90(s, 1 H), 9.35(d, *J*=4.2 Hz, 1 H), 9.44(brs, 2 H), 9.55(d, *J*=4.1 Hz, 2 H).

3-[4-(aminocarbonyl)-3-methyl-1-pyridiniumyl]methyl-7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (20)

Purity 90%; mp186°C(dec.); IR (KBr) cm^{-1} 1785, 3166; ^1H NMR (300 MHz, DMSO-d_6) δ 2.24(s, 3 H), 3.92(s, 3H), 5.22(s, 2 H), 5.38(d, *J*=4.2Hz, 1 H), 5.68(d, 1H), 6.86(s, 1 H), 7.15(brs, 2 H), 8.29(s, 1 H), 8.45(d, *J*=4.1 Hz, 1 H), 8.86(d, 1 H), 9.35(brs, 2 H), 9.56(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[(2-morpholino-5-nitro-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (21)

Purity 91%; mp 229°C(dec.); IR (KBr) cm^{-1} 1645, 1775; ^1H NMR (300 MHz, DMSO-d_6) δ 3.29(m, 4 H), 3.62(dd, *J*=4.2Hz, 4.0 Hz, 2 H), 3.79(m, 4 H), 3.91(s, 3 H), 5.08(m, 2 H), 5.15(d, *J*=4.8 Hz, 1 H), 5.65(d, *J*=4.3 Hz, 1 H), 6.54(d, *J*=9.0 Hz, 1 H), 6.68(s, 1 H), 6.98(d, *J*=9Hz, 1 H), 7.17(brs, 2 H), 7.75(s, 1 H), 9.35(d, *J*=4.2 Hz, 1 H), 9.56(brs, 2 H).

7-[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino-3-[5-nitro-2-(1,4-thiazinan-4-yl)-1-pyridiniumyl]-methyl-6-oxo-7,7a-di-hydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (22)

Purity 93%; mp215°C(dec.); IR (KBr) cm^{-1} 1660, 1771, 3443; ^1H NMR (300 MHz, DMSO-d_6) δ 3.62(dd, *J*=8.2 Hz, 3.5 Hz, 2 H), 3.72(m, 4 H), 3.91(s, 3 H), 4.05(m, 4 H), 5.08(m, 2 H), 5.15(d, *J*=4.2 Hz, 1 H), 5.65(d, *J*=4.0 Hz, 1 H),6.51(d, *J*=9 Hz, 1 H), 6.67(s, 1 H), 6.91(d, *J*=9 Hz, 1 H), 7.17(brs, 2 H), 7.73(s, 1 H), 9.35(d, 1 H), 9.56(brs, 2 H).

7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino}-3-[(3-methyl-4-morpholino-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-carboxylate (23)

Purity 95.5%; mp 201-205°C(dec.); IR (KBr) cm^{-1} 1378, 1622, 1762, 3406; ^1H NMR(300 MHz. DMSO-d_6) δ 2.32(s, 3 H), 3.17(t, *J*=4.2 Hz, 4 H), 3.23(d, *J*=4.5 Hz, 2 H), 3.92(s, 3 H), 3.93(t, *J*=4.2 Hz, 2 H), 5.03(m, 1 H), 5.21(m, 2 H), 5.61(m, 1 H), 6.97(s, 1 H), 7.10(d, *J*=7.8 Hz, 1 H), 7.21(brs, 2 H), 8.05(m, 3 H).

7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino}-3-[(3-methyl-4-piperazino-1-pyridiniumyl)methyl]-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]thiazine-4-car-boxylate (24)

Purity 94.2%; mp 186-190°C(dec.); IR (KBr) cm^{-1} 1534, 1632, 1758, 3416; ^1H NMR (300 MHz, DMSO-d_6) δ 2.31(s, 3H), 3.17(t, *J*=4.5 Hz, 4 H), 3.23(d, *J*=4.5 Hz, 2 H), 3.91(s, 3 H), 3.98(t, *J*=4.5 Hz, 4 H), 5.01(m, 1 H), 5.12(m, 2 H), 5.21(brs, 1 H), 5.76(m, 1 H), 6.97(s, 1 H), 7.10(d, *J*=7.8 Hz, 1 H), 7.22(brs, 2 H), 8.11(m, 3 H).

7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(methoxyimino)acetyl]-amino}-3-{[3-methyl-4-(1,4-thiazinan-4-yl)-1-pyridinium-yl]-methyl}-6-oxo-7,7a-dihydro-2*H*,6*H*-azeto[2,1-b][1,3]-thiazine-4-carboxylate (25)

Purity 95%; mp 210-213°C(dec.); IR (KBr) cm^{-1} 1384, 1654, 1778, 3258; ^1H NMR (300 MHz, DMSO-d_6) δ 2.31(s,3 H), 3.17(t, *J*=4.5 Hz, 4 H), 3.23(d, *J*=4.5 Hz, 2 H), 3.91(s, 3H), 3.98(t, *J*=4.5 Hz, 4 H), 5.01(m, 1 H),

Table I. *In vitro* antimicrobial activity of cephalasporins (MIC, μg/ml)

Compds	*S.p.1*	*S.p.2*	*S.f.*	*S.a.1*	*S.a.2*	*S.a.3*	*E.c.1*	*E.c.2*	*E.c.3*	*E.c.4*	*E.c.5*	*P.a.1*	*P.a.2*	*P.a.3*	*P.a.4*	*S.t.*	*K.o.*	*K.a.*	*En.c.1*	*En.c.2*
CPR	0.013	0.007	1.563	0.781	0.781	0.098	0.025	0.013	0.049	0.049	0.025	3.125	1.563	0.781	0.391	0.049	1.563	0.025	3.125	0.013
1	0.098	0.098	100	25	25	6.25	0.098	0.098	0.195	1.563	0.195	25	12.5	6.25	0.781	0.391	50	0.098	12.5	0.098
2	0.049	0.049	25	12.5	12.5	6.25	0.049	0.098	0.098	3.125	0.098	25	12.5	3.125	0.195	0.098	100	0.049	>100	0.049
3	0.013	0.025	100	3.125	12.5	3.125	0.025	0.098	0.098	0.098	0.098	12.5	25	6.25	1.563	0.049	6.25	0.049	50	0.025
4	0.391	0.195	100	12.5	25	3.125	0.391	0.195	0.781	1.563	0.781	50	25	12.5	3.125	0.391	25	0.391	>100	0.195
5	0.013	0.013	12.5	1.563	3.125	0.781	0.025	0.049	0.049	0.049	0.049	6.25	3.125	1.563	0.781	0.049	6.25	0.049	25	0.025
6	0.013	0.013	100	1.563	3.125	0.781	0.049	0.098	0.195	0.391	0.098	6.25	6.25	3.125	1.563	0.098	6.25	0.098	100	0.049
7	0.025	0.049	25	6.25	6.25	3.125	0.195	0.391	0.098	0.391	0.391	25	25	3.125	3.125	0.195	12.5	0.195	25	0.098
8	0.013	0.013	6.25	1.563	1.563	1.563	0.049	0.098	0.025	0.098	0.098	6.25	3.125	0.781	1.563	0.049	12.5	0.049	50	0.025
9	0.025	0.025	25	3.125	6.25	1.563	0.098	0.195	0.049	0.391	0.195	25	12.5	3.125	3.125	0.195	25	0.195	100	0.098
10	0.025	0.049	50	6.25	12.5	3.125	0.195	0.391	0.098	0.781	0.391	25	12.5	6.25	6.25	0.391	25	0.195	100	0.195
11	0.049	0.098	>100	50	100	50	0.781	1.563	0.195	1.563	1.563	>100	>100	>100	6.25	0.391	50	0.391	>100	0.391
12	0.049	0.049	25	3.125	6.25	1.563	0.098	0.195	0.195	0.195	0.195	12.5	6.25	6.25	0.781	0.195	25	0.098	25	0.049
13	0.025	0.025	12.5	1.563	3.125	0.781	0.025	0.049	0.098	0.098	0.049	6.25	3.125	1.563	0.391	0.098	3.125	0.049	50	0.025
14	0.098	0.098	100	25	50	12.5	0.098	0.195	0.195	0.391	0.195	100	>100	12.5	0.781	0.195	25	0.049	>100	0.049

Abbreviations:*S.p.1,Streptococcus pyogenes* 308A; *S.p.2,Streptococcus pyogenes* 77A;*S.f.,Streptococcus faecium* MD8b; *S.a.1,Streptococcus aureus* SG511; *S.a.2,Streptococcus aureus* 285; *S.a.3,Streptococcus aureus* 503; *E.c.1,Escherichia coli* 055; *E.c.2,Escherichia coli* DC 0; *E.c.3,Escherichia coli* DC2; *E.c.4,Escherichia coli* TEM; *E.c.5,Escherichia coli* 1507E; *P.a.1,Pseudomonas aeruginosa* 9027; *P.a.2,Pseudomonas aeruginosa* 1592E; *P.a.3,Pseudomonas aeruginosa* 1771; *P.a.4,Pseudomonas aeruginosa* 1771M; *S.t.,Salmonella typhimurium*; *K.o.,Klebsiella oxytoca* 1082E; *K.a.,Klebsiella aerogenes* 1522E; *En.c.1,Enterobacter cloacae* P99; *En.c.2,Enterobacter cloacae* 1321E

Table II. *In vitro* antimicrobial activity of cephalasporins (MIC, μg/ml)

Compds	*S.p.1*	*S.p.2*	*S.f.*	*S.a.1*	*S.a.2*	*S.a.3*	*E.c.1*	*E.c.2*	*E.c.3*	*E.c.4*	*E.c.5*	*P.a.1*	*P.a.2*	*P.a.3*	*P.a.4*	*S.t.*	*K.o.*	*K.a.*	*En.c.1*	*En.c.2*
CPR	0.013	0.007	1.563	0.781	0.781	0.098	0.025	0.013	0.049	0.049	0.025	3.125	1.563	0.781	0.391	0.049	1.563	0.025	3.125	0.013
15	0.781	0.781	>100	>100	>100	100	6.25	12.5	6.25	25	12.5	>100	>100	>100	50	12.5	>100	6.25	>100	3.125
16	0.781	0.781	>100	>100	>100	50	1.563	6.25	6.25	6.25	3.125	>100	>100	100	25	6.25	>100	3.125	>100	1.563
17	0.025	0.025	50	6.25	6.25	1.563	0.098	0.195	0.195	0.195	0.195	12.5	12.5	3.125	1.563	0.195	3.125	0.098	>100	>100
18	0.025	0.025	100	6.25	6.25	3.125	0.049	0.098	0.049	0.195	0.098	>100	>100	>100	6.25	0.098	25	0.049	>100	0.025
19	0.195	0.049	100	25	50	12.5	0.781	0.391	0.781	1.563	0.781	100	100	12.5	3.125	1.563	50	0.391	>100	0.391
20	0.049	0.049	25	3.125	6.25	1.563	0.098	0.195	0.195	0.781	0.195	6.25	3.125	0.781	25	0.195	25	0.195	>100	0.049
21	0.098	0.098	>100	12.5	25	6.25	0.098	0.391	0.049	0.391	0.391	>100	>100	50	0.781	0.195	6.25	0.195	>100	0.049
22	0.781	0.781	>100	100	>100	100	6.25	12.5	3.125	25	12.5	>100	>100	>100	50	6.25	25	6.25	>100	3.125
23	0.781	0.781	>100	100	>100	100	3.125	6.25	1.563	12.5	6.25	>100	>100	>100	50	6.25	50	3.125	>100	1.563
24	1.563	3.125	100	100	100	100	12.5	25	6.25	50	50	50	50	50	50	25	50	12.5	100	12.5
25	0.007	0.013	25	1.563	3.125	0.781	0.025	0.049	0.013	0.049	0.049	25	12.5	3.125	0.098	0.025	0.781	0.025	>100	0.013

Abbreviations:*S.p.1,Streptococcus pyogenes* 308A; *S.p.2,Streptococcus pyogenes* 77A;*S.f.,Streptococcus faecium* MD8b; *S.a.1,Streptococcus aureus* SG511; *S.a.2,Streptococcus aureus* 285; *S.a.3,*Streptococcus aureus 503; *E.c.1,Escherichia coli* 055; *E.c.2,Escherichia coli* DC 0; *E.c.3,Escherichia coli* DC2; *E.c.4,Escherichia coli* TEM; *E.c.5,Escherichia coli* 1507E; *P.a.1,Pseudomonas aeruginosa* 9027; *P.a.2,Pseudomonas aeruginosa* 1592E; *P.a.3,Pseudomonas aeruginosa* 1771; *P.a.4,Pseudomonas aeruginosa* 1771M; *S.t.,Salmonella typhimurium*; *K.o.,Klebsiella oxytoca* 1082E; *K.a.,Klebsiella aerogenes* 1522E; *En.c.1,Enterobacter cloacae* P99; *En.c.2,Enterobacter cloacae* 1321E

5.12(m, 2 H), 5.76(m, 1 H), 6.97(s, 1 H), 7.10(d, J=7.8 Hz, 1 H), 7.22(brs, 2 H), 8.11(m, 3 H).

Antibacterial activity

Minimal inhibitory concentrations (MIC) of the compounds were determined by an agar dilution method and were summarized in Table I, II.

RESULTS AND DISCUSSION

Pyridinium cephalosporin compounds (**1~25**) were easily prepared by quarternization reaction of intermediate cephalosporin iodide, generated *in situ* from cefotaxime, with substituted or bicyclic pyridines to examine structure-activity relationship for their antibacterial activity. In general, all compounds showed broad antibacterial spectra against Gram-positive and negative strains except *Streptococcus faecium, Pseudomonas aeruginosa* and *Enterobacter cloacae*. Especially, the compound **3**, **5**, **8** and **25** showed excellent activity against *Escherichia coli,* and the compound **5**, **6**, **8**, **13** and **20** reveal better activity than others against *Pseudomonas*. In general, substitution of *para* position with an electron donating group increases activity while an electron withdrawing group at *meta* position of pyridine ring decreases activity. However, activity profile does not show any advantages over cefpirome which is being clinically used as the 3rd or 4th generation parenteral cephalosporin antibiotic.

In conclusion, bicyclic structure(**1~3**) and position of substituent on pyridine ring did not meaningfully influence the activity compared with the character of substituent on the pyridine ring.

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