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Studies on 3′-Quaternary Ammonium Cephalosporins—III. Synthesis and Antibacterial Activity of 3′-(3-Aminopyrazolium)cephalosporins

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Abstract—The synthesis and in vitro antibacterial activity of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins bearing *N*-mono or dialkyl and carbamoyl aminopyrazolium, and five- or six-membered rings fused to the 3-aminopyrazolium methyl groups at the 3-position, are described. Aminopyrazolium methyl cephalosporins (**23e**, **f**, **i**), with fused saturated and unsaturated rings were especially effective against *Staphylococcus* strains compared to 3-amino-2-methylpyrazolium methyl cephalosporin (**1**). Among the cephalosporins prepared in this work, 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(4,5,6,7-tetrahydro-1-pyrazolo[1,5-*a*]pyrimidinio)methyl-3-cephem-4-carboxylate (**23f**) showed a good balance of antibacterial activity against both Gram-positive bacteria including *Staphylococcus aureus* and Gram-negative bacteria including *P. aeruginosa*. An imidazopyrazolium group at the 3-position in, for example, cephalosporin (**23i**) was particularly effective for improving antibacterial activity against MRSA.

Introduction

In the last decade, a number of new parenteral cephalosporins with a broad spectrum of antibacterial activity and high stability against various β-lactamases have been marketed.[1] Most of them possess a 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-alkoxyiminoacetamido] side chain, for example ceftizoxime (CZX), and as a result they show excellent activity against Gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*, and moderate activity against Gram-positive bacteria, especially *Staphylococcus aureus*.

Recently, a number of 3′-quaternary ammonium cephalosporins, such as ceftazidime (CAZ), cefpirome (CPR)[2] and cefepime (CFPM),[3] which show an improved spectrum of activity against both Gram-positive bacteria including *S. aureus*, and Gram-negative bacteria including *P. aeruginosa* have been marketed or developed. As a result, our efforts in the last few years have been focused on synthesizing novel 3′-quaternary ammonium cephalosporins with enhanced activity against Gram-positive bacteria including *S. aureus* and Gram-negative bacteria including *P. aeruginosa*, since it is our contention that the optimum 3′-quaternary ammonium substituent remains to be discovered.

In our previous papers,[4,5] we reported the synthesis and antibacterial activity of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins having various heterocycles at the 3-position. As a result, we discovered that the introduction of an amino group to a pyrazolium ring imparts greatly increased potency against *S. aureus* and *P. aeruginosa*. For example, 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(3-amino-2-methylpyrazolio)methyl-3-cephem-4-carboxylate (**1**) showed extremely potent, broad-spectrum activity against both Gram-positive bacteria, including *S. aureus*, and Gram-negative bacteria, including *P. aeruginosa* (see Fig. 1).

In order to discover an aminopyrazolium cephalosporin with an ever better spectrum of activity, we next examined the synthesis of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins having various substituents attached to the aminopyrazolium ring and also with five- or six-membered rings fused to the aminopyrazolium ring at the 3-position. In this paper, we wish to disclose the synthesis and in vitro antibacterial activity of this novel series of aminopyrazolium cephalosporins.

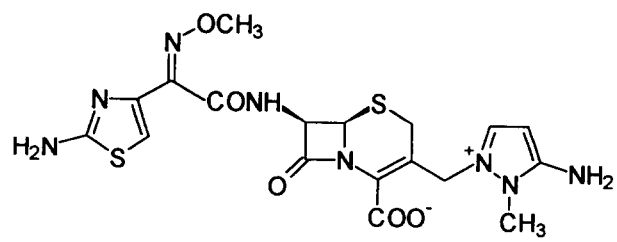

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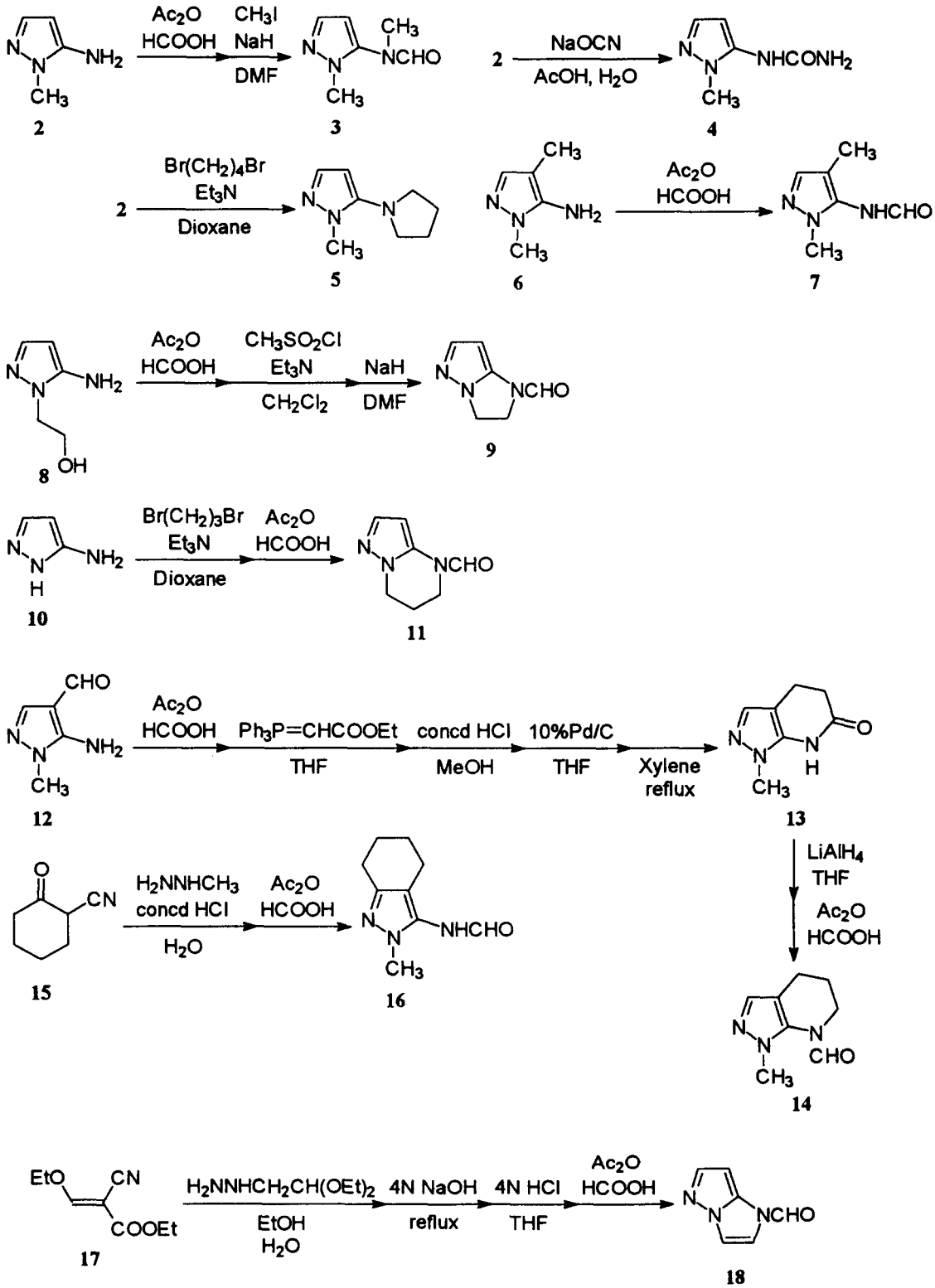
Figure 1. Structure of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(3-amino-2-methylpyrazolio)methyl-3-cephem-4-carboxylate (**1**).


Ac2O
HCOOH
CH3I
NaH
DMF
NaOCN
AcOH, H2O
Br(CH2)4Br
Et3N
Dioxane
Ac2O
HCOOH
CH3SO2Cl
Et3N
CH2Cl2
NaH
DMF
Br(CH2)3Br
Et3N
Dioxane
Ac2O
HCOOH
Ph3P=CHCOOEt
THF
concd HCl
MeOH
10%Pd/C
THF
Xylene
reflux
LiAlH4
THF
Ac2O
HCOOH
H2NNHCH3
concd HCl
H2O
Ac2O
HCOOH
H2NNHCH2CH(OEt)2
EtOH
H2O
4N NaOH
reflux
4N HCl
THF
Ac2O
HCOOH
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Scheme 1.

Chemistry

The preparation of the various aminopyrazole compounds used as 3-side chains in the present study, possessing alkyl- and carbamoyl substituents or fused saturated and unsaturated rings, was performed according to the routes summarized in Scheme 1. 5-(*N*-Formyl-*N*-methylamino)-1-methylpyrazole (**3**) was obtained by formylation of 5-amino-1-methylpyrazole (**2**), followed by methylation. 5-Ureido-1-methylpyrazole (**4**) was obtained by treatment of **2** with sodium cyanate. 5-(1-Pyrrolidinyl)-1-methylpyrazole (**5**) was prepared by treatment of **2** with 1,4-dibromobutane and triethylamine. 5-Formylamino-1,4-dimethylpyrazole (**7**) was obtained by formylation of 1,4-dimethyl-5-aminopyrazole (**6**), prepared according to the literature procedure.[6] 5-Amino-1-(2-hydroxyethyl)pyrazole (**8**) was formylated, treated with methanesulfonyl chloride and triethylamine, and then followed by cyclization with sodium hydride, to give 1-formyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (**9**). 5-Aminopyrazole (**10**) was treated with 1,3-dibromopropane and triethylamine, followed by formylation, to give 4-formyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine (**11**). 5-Amino-4-formyl-1-methylpyrazole (**12**), prepared according to the literature procedure,[7] was formylated and treated with Wittig reagent, followed by deformylation, hydrogenation, and cyclization, to give 1-methyl-6-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine (**13**). **13** was reduced with lithium aluminum hydride and formylated to give 7-formyl-1-methyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]-pyridine (**14**). 2-Cyanocyclohexanone (**15**), prepared according to the literature procedure[8] was treated with methylhydrazine, followed by formylation, to give 3-formamido-2-methyl-4,5,6,7-tetrahydro-2*H*-indazole (**16**). Ethyl 2-ethoxymethylene-2-cyanoacetate (**17**) was treated with hydrazinoacetaldehyde diethylacetal, followed by hydrolysis, cyclization with decarboxylation and formylation, to give 1-formyl-1*H*-imidazo[1,2-*b*]pyrazole (**18**).

The synthesis of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(aminopyrazolio)methyl cephalosporins (**23b–i**) is outlined in Scheme 2.

7-ACA derivatives (**20b–d**, **21a**, **e–i**) were prepared according to similar procedures described in our previous paper.[5] The 7-ACA derivatives (**20b**, **d**, **21a**, **e–g**) were acylated by the Vilsmeier method with (*Z*)-2-(2-formylaminothiazol-4-yl)-2-methoxyiminoacetic acid (**22**), followed by deformylation, to give 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-pyrazoliomethylcephalosporin derivatives (**23a**, **b**, **d–g**). The other 7-ACA derivatives (**20c**, **21h**, **i**) were acylated with 1-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl)]-benzotriazol-3-oxide (**24**) to give 3-pyrazoliomethyl cephalosporin derivatives (**23c**, **h**, **i**).

Results and Discussion

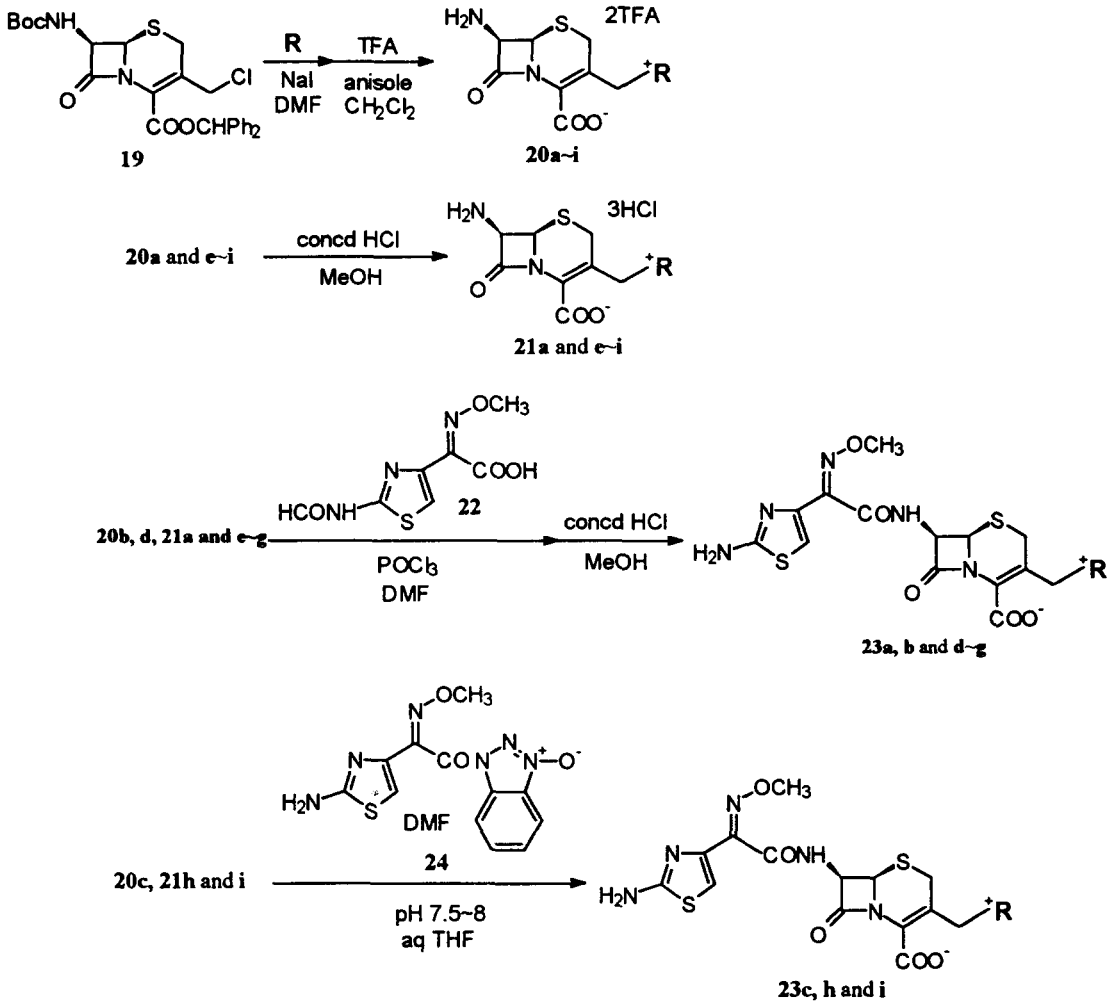
The antibacterial activity (MICs) of the prepared 3′-quaternary ammonium cephalosporins in comparison to cefpirome (CPR)[2] as reference compound, against various selected Gram-positive and Gram-negative bacteria are shown in Tables 1–3. MICs were determined by the standard serial twofold agar dilution method using Müeller–Hinton agar.

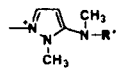
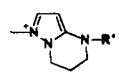
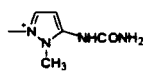
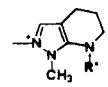
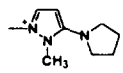
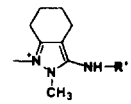
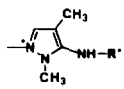
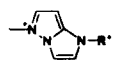
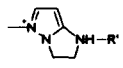
Table 1 shows the in vitro antibacterial activity of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]cephalosporins bearing aminopyrazolium groups with alkyl- or carbamoyl substituents at the 3-position. 3-Methylaminopyrazolium derivative (**23a**) exhibited potency similar to the parent compound (**1**). The antibacterial activity of 3-pyrrolidinylpyrazolium derivative (**23c**), bearing a more hydrophobic substituent compared to **1**, was more potent against *S. aureus*, but activity against *P. aeruginosa* was decreased a little. As already reported by Hoechst in structure–activity studies related to CPR,[2,9] increasing the size and/or hydrophobicity of the 3′-substituent is associated with a decrease in the antibacterial activity against *P. aeruginosa*. 3-Ureidopyrazolium derivative (**23b**), bearing a more hydrophilic substituent compared to **1** showed less activity against *S. aureus* than **1**.

Table 2 shows the in vitro antibacterial activity of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido] cephalosporins bearing aminopyrazolium groups with a methyl substituent at the 4-position or a five- or six-membered ring fused to the aminopyrazolium ring. 4-Methyl-3-aminopyrazolium derivative (**23d**) exhibited potency similar to the parent compound (**1**). MICs of **23e**, **23f** and **23i** bearing a five- or six-membered ring fused between the 2-nitrogen and 3-amine on the aminopyrazolium ring were more potent than **1** against *S. aureus* and similar to **1** against *P. aeruginosa*. In particular, the imidazopyrazolium cephalosporin (**23i**) showed good activity against MRSA. Compounds **23g** and **23h**, bearing a six-membered ring fused at the 3–4 and 4–5 position, respectively on the aminopyrazolium ring were less active against *P. aeruginosa* than **1**. The above results are clearly different from the structure–activity relationships for CPR,[2] i.e., that with increasing ring size, the activity against *P. aeruginosa* is diminished. However, in the case of the pyrazolium series, the fusion position to the pyrazole moiety is shown to be more important.

Table 3 shows the antibacterial activity (MIC_{50} and MIC_{80}) of cephalosporins (**23f** and **23i**) against some clinically isolated strains. **23f** displayed more potent activity than CPR against *S. aureus* (MSSA) and the same activity as CPR against the other strains. Antibacterial activity against MRSA for **23i** was apparently superior to that of CPR, whilst antibacterial activity against the other strains was similar to that of CPR. Thus, **23f** and **23i** displayed more potent activity than CPR against MSSA and MRSA, respectively.

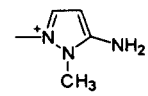
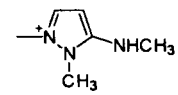
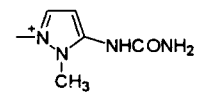
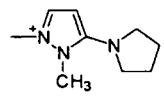
In conclusion, 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(4,5,6,7-tetrahydro-1-pyrazolo[1,5-*a*]pyrimidinio)methyl-3-cephem-4-carboxylate (**23f**) showed good antibacterial activity against both Gram-



	+R	R'(20)	R' (21 and 23)		+R	R'(20)	R' (21 and 23)
a		CHO	H	f		CHO	H
b				g		CHO	H
c				h		CHO	H
d		CHO	H	i		CHO	H
e		CHO	H				

Scheme 2.

Table 1. Antibacterial activity (MIC μg/mL) of 3′-aminopyrazolium cephalosporins (**1** and **23a–c**). 10^6 cfu/mL

Compound No.	$^+$R	*S.a.* 1	*S.a.* 2	*E.c.*	*K.p.*	*P.a.**
1		0.78	>100	≤ 0.025	0.05	12.5
23a		0.78	>100	≤ 0.025	≤ 0.025	12.5
23b		1.56	>100	0.05	0.05	12.5
23c		0.39	>100	≤ 0.025	≤ 0.025	25
CPR		0.78	>100	0.05	0.1	6.25

**S.a.* 1; *Staphylococcus aureus* 209P JC-1, *S.a.* 2; *Staphylococcus aureus* 3004 (MRSA), *E.c.*; *Escherichia coli* NIHJ JC-2, *K.p.*; *Klebsiella pneumoniae* 12, *P.a.*; *Pseudonmonas aeruginosa* IAM 1095.

positive bacteria, including *S. aureus*, and Gram-negative bacteria, including *P. aeruginosa*. The imidazopyrazole (**23i**) is an effective moiety for improving antibacterial activity against MRSA. Our further studies to optimize this activity will be the subject of subsequent papers.

Experimental

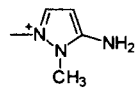
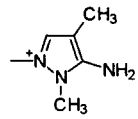
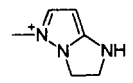
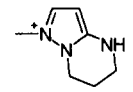
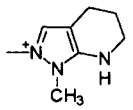
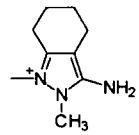
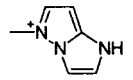
MPs were determined using a Thomas–Hoover capillary melting apparatus and are uncorrected. IR spectra were taken on a Hitachi 260-10 spectrophotometer. NMR spectra were recorded at 90 MHz on Varian EM-390 NMR spectrometer, a Hitachi R-90H NMR spectrometer or a Bruker AC200P at 200 MHz. Chemical shifts are reported in ppm from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS, in D_2O) or TMS (in $CDCl_3$ and DMSO-d_6) as internal standard.

Preparation of aminopyrazole compounds

5-(*N*-Formyl-*N*-methylamino)-1-methylpyrazole (3). A mixture of formic acid (15.5 mL, 0.41 mol) and acetic anhydride (38.9 mL, 0.41 mol) was stirred at 40 °C for 45 min. To the reaction mixture was added 5-amino-1-methylpyrazole (**2**; 10 g, 0.103 mol) in several portions at room temperature, and the mixture was stirred for 30 min. The mixture was added to a mixture of ethyl acetate and water and adjusted to pH 8 with potassium carbonate. The organic layer was separated, and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to give 5-formamido-1-methylpyrazole (12.9 g, 100%). MP 71–73 °C, IR (Nujol) cm^{-1} 3300, 3200, 1705, 1590, ^{1}H NMR ($CDCl_3$) δ 3.69 and 3.74 (3H, each s), 6.04 and 6.23 (1H, each d, J = 3 Hz), 7.34 (1H, s), 8.21 (1H, s).

To a solution of 5-formamido-1-methylpyrazole (5 g, 40 mmol) in DMF (50 mL) was added 62% sodium hydride (1.6 g, 41 mmol) in several portions at 4 °C and stirred for 30 min at the same temperature, and then treated with methyl iodide (2.5 mL, 40 mmol). The reaction mixture was stirred for 1 h at rt and then added to a mixture of ethyl acetate and water. The organic layer was separated, dried over anhydrous $MgSO_4$, and the filtrate concentrated under reduced pressure, the resulting residue was chromatographed on a column of silica gel. The column was eluted with ethyl acetate–diisopropyl ether (3:1). The fractions containing the desired product were collected and evaporated to give 5-(*N*-formyl-*N*-methylamino)-1-methylpyrazole (2.5 g, 50%). IR (Nujol) cm^{-1} 1660–1680, 1550, 1320, ^{1}H

Table 2. Antibacterial activity (MIC, μg/mL) of 3′-aminopyrazolium cephalosporins (**1** and **23a–c**). 10^6 cfu/mL

Compound No.	$^+$R	*S.a.* 1	*S.a.* 2	*E.c.*	*K.p.*	*P.a.**
1		0.78	>100	≤ 0.025	0.05	12.5
23d		0.78	100	≤ 0.025	0.05	6.25
23e		0.39	100	≤ 0.025	≤ 0.025	12.5
23f		0.2	100	≤ 0.025	≤ 0.025	12.5
23g		0.78	>100	≤ 0.025	0.05	25
23h		0.78	>100	0.05	0.39	50
23i		0.39	50	≤ 0.025	0.05	12.5
CPR		0.78	>100	0.05	0.1	6.25

*Abbreviations: see Table 1.

Table 3. Antibacterial activity (MIC, μg/mL) of **23f** and **23i** against clinical isolates

Organism (No. of strains)		(MIC, μg/mL) 50%	80%
S. aureus (10) (MSSA)	**23f**	0.39	0.39
	23i	0.39	0.78
	CPR	0.39	0.78
S. aureus (10) (MRSA)	**23f**	50	100
	23i	12.5	50
	CPR	50	100
P. aeruginosa (10)	**23f**	12.5	12.5
	23i	12.5	12.5
	CPR	6.25	12.5

NMR (DMSO-d_6) δ 3.07 (3H, s), 3.67 (3H, s), 6.28 (1H, d, J = 2 Hz), 7.44 (1H, d, J = 2 Hz), 8.20 (1H, s).

5-Ureido-1-methylpyrazole (4). To a solution of **2** (19.4 g, 0.2 mol) in a mixture of acetic acid (96 mL) and water (192 mL) was added dropwise a solution of sodium cyanate (52 g, 0.8 mol) in water (400 mL) at 4 °C and stirred for 7 h at 4 °C. To the reaction mixture was added a mixture of ethyl acetate and water, and the organic layer separated, and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to give 5-ureido-1-methylpyrazole (14.6 g, 52.3%). MP 146–149 °C, IR (Nujol) cm^{-1} 3300, 1735, 1600, ^{1}H NMR (DMSO-d_6) δ 3.63 and 3.67 (3H, each s), 6.00 (1H, s), 6.10 and 6.22 (1H, each d, J = 2 Hz), 6.86 (1H, s), 7.28 and 7.35 (1H, each d, J = 2Hz), 8.35 (1H, s).

5-(1-Pyrrolidinyl)-1-methylpyrazole (5). To a solution of **2** (3 g, 31 mmol) and triethylamine (9.5 mL, 68 mmol) in 1,4-dioxane (50 mL) was added 1,4-dibromobutane (4.1 mL, 34 mmol) and stirred for 24 h at 100 °C. After insoluble material was filtered off, the filtrate was evaporated under reduced pressure, and the residue chromatographed on a column of silica gel. The column was eluted with ethyl acetate. The fractions containing the desired product were collected and evaporated to give 5-(1-pyrrolidinyl)-1-methylpyrazole (1.2 g, 26%). IR (Nujol) cm^{-1} 3400, 2970, 1550, 1490, 1460, ^1H NMR ($CDCl_3$) δ 1.86–2.03 (4H, m), 3.05–3.12 (4H, m), 3.76 (3H, s), 5.60 (1H, d, J = 3 Hz), 7.26 (1H, d, J = 3 Hz).

5-Formamido-1,4-dimethylpyrazole (7). Preparation of **7** was carried out by a method similar to formylation described for **3**, using **6** instead of **2**. IR (Nujol) cm^{-1} 3200, 1665, 1585, ^1H NMR ($CDCl_3$) δ 1.90 and 1.98 (3H, each s), 3.64 and 3.72 (3H, each s), 7.29 and 7.31 (1H, each s), 8.10 (1H, br s), 8.33 and 9.03 (1H, each s).

1-Formyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (9). 1-(2-Hydroxyethyl)-5-aminopyrazole (**8**) was formylated by a method similar to that described for **3** to give 1-(2-hydroxyethyl)-5-formamidopyrazole. IR (Nujol) cm^{-1} 3230, 1695, 1570, 1540, ^1H NMR (DMSO-d_6) δ 3.62–3.95 (2H, m), 3.98–4.32 (2H, m), 6.22 and 6.36 (1H, each d, J = 3 Hz), 7.42 (1H, d, J = 3 Hz), 8.32 and 8.36 (1H, each s).

To a solution of 1-(2-hydroxyethyl)-5-formamidopyrazole (10 g, 64 mmol) and triethylamine (18 mL, 130 mol) in CH_2Cl_2 (100 mL) was added dropwise methanesulfonyl chloride (8.5 mL, 110 mol) at 4 °C and the mixture stirred for 1.5 h at 0 °C. The reaction mixture was then added to water (20 mL) and the organic layer was separated, and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a column of silica gel. The column was eluted with ethyl acetate. The fractions containing the desired product were collected and evaporated to give 1-(2-methylsulfonyloxyethyl)-5-formamidopyrazole (3.2 g, 21.4%). MP 101–104 °C, IR (Nujol) cm^{-1} 3300, 1780, 1730, 1660–1680, 1540 1560, ^1H NMR (DMSO-d_6) δ 2.93 (3H, s), 4.18–4.60 (4H, m), 6.08–6.40 (1H, m), 7.33 (1H, d, J = 2 Hz), 8.17–8.43 (1H, m), 9.97–10.63 (1H, m).

To a solution of 1-(2-methylsulfonyloxyethyl)-5-formamidopyrazole (6.2 g, 27 mmol) in DMF (60 ml) was added 62% sodium hydride (1.03 g, 27 mmol) in several portions at 4 °C and the mixture stirred for 3 h at the same temperature. The reaction mixture was added to ethyl acetate, and after insoluble material was filtered off, the filtrate was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel. The column was eluted with ethyl acetate–diisopropyl ether. The fractions containing the desired product were collected and evaporated to give 1-formyl-2,3-dihydro-1*H*-imidazo[1,2-*b*]pyrazole (3.91 g, 100%). MP 78–80 °C, IR (Nujol) cm^{-1} 1650, 1570, 1520, ^1H NMR ($CDCl_3$) δ 4.10–4.70 (4H, m), 5.77 (1H, d, J = 2 Hz), 7.37 (1H, d, J = 2 Hz), 8.61 (1H, s).

4-Formyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine (11). Preparation of 4,5,6,7-tetrahydropyrazolo[1,5-*a*]-pyrimidine was carried out by a method similar to that described for **5**, using 5-aminopyrazole (**10**) and 1,3-dibromopropane instead of **2** and 1,4-dibromobutane respectively. IR (Nujol) cm^{-1} 3220, 1570, 1460, ^1H NMR (DMSO-d_6) δ 1.78–2.01 (2H, m), 3.0–3.21 (2H, m), 3.90 (2H, t, J = 6 Hz), 5.10 (1H, d, J = 2 Hz), 5.86 (1H, br s), 6.97 (1H, d, J = 2 Hz).

Preparation of **11** was carried out by a method similar to formylation described for **3**, using 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidine instead of **2**. IR (Nujol) cm^{-1} 1670, 1535, 1500, 1450, 1430, 1400, ^1H NMR (DMSO-d_6) δ 1.97–2.27 (2H, m), 3.62–3.91 (2H, m), 3.97–4.24 (2H, m), 6.22 and 6.48 (1H, each d, J = 3 Hz), 7.29 (1H, d, J = 3 Hz), 8.19 and 8.77 (1H, each s).

7-Formyl-1-methyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine (14). 4-Formyl-1-methyl-5-aminopyrazole (**12**) was formylated by a method similar to that described for **3** to give 4-formyl-5-formamido-1-methylpyrazole. IR (Nujol) cm^{-1} 3340, 1700, 1660, 1130, 810, ^1H NMR (DMSO-d_6) δ 3.70 (3H, s), 7.91 (1H, br s), 8.40 (1H, br s), 9.69 (1H, s), 10.7 (1H, s).

To a solution of 4-formyl-5-formamido-1-methylpyrazole (12 g, 78 mmol) in THF (120 ml) was added (carboethoxymethylene)triphenylphosphorane (30 g, 86 mmol) and the mixture stirred for 5 h at rt. The reaction mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel, eluting with ethyl acetate. The fractions containing the desired product were collected and evaporated. The residue was dissolved in MeOH (100 mL) and treated with concd HCl (13 ml, 157 mmol) at 4 °C. The mixture was stirred for 2 h at rt. The resulting precipitate was collected by filtration. The solid was then added to a mixture of ethyl acetate and water and neutralized with saturated sodium bicarbonate aqueous solution. The organic layer was separated, dried over anhydrous $MgSO_4$, and the filtrate concentrated under reduced pressure to give ethyl *trans*-3-(5-amino-1-methylpyrazol-4-yl)-2-propenate (10.8 g, 71%). IR (Nujol) cm^{-1} 3380, 3220, 1680, 1170, ^1H NMR (DMSO-d_6) δ 1.23 (3H, t, J = 6 Hz), 3.51 (3H, s), 4.11 (2H, q, J = 6 Hz), 5.94 (1H, d, J = 15 Hz), 6.10 (2H, s), 7.53 (1H, s), 7.58 (1H, d, J = 15 Hz).

To a solution of ethyl *trans*-3-(5-amino-1-methylpyrazol-4-yl)-2-propenate (15.7 g, 80 mmol) in THF (350 mL) was added 10% Pd-C (3.2 g) and stirred for 3 h at rt under hydrogen atmosphere. The catalyst was filtered off, and the filtrate evaporated under reduced pressure to give ethyl 3-(5-amino-1-methylpyrazol-4-yl)-2-propionate (15.3 g, 96.5%). IR (Nujol) cm^{-1} 1730, 1660, 1470, 1380, 1170, ^1H NMR (DMSO-d_6) δ 1.17 (3H, t, J = 7.1 Hz), 3.47 (1H, s), 4.04 (2H, q, J = 7.1 Hz), 4.89 (2H, s), 6.87 (1H, s).

A suspension of ethyl 3-(5-amino-1-methylpyrazol-4-yl)-2-propionate (0.7 g, 3.5 mmol) in xylene (3.5 ml) was refluxed for 4 h. The solution was cooled and the resulting precipitate collected by filtration to give 1-methyl-6-oxo-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine (**13**; 0.29 g, 51.9%). IR (Nujol) cm^{-1} 1680, 1580, 1460, 1180, 1000, ^{1}H NMR (DMSO-d_6) δ 2.3–2.5 (2H, m), 2.55–2.7 (2H, m), 3.62 (1H, s), 7.09 (1H, s), 10.69 (1H, s).

To a suspension of lithium aluminum hydride (0.15 g, 3.97 mmol) in THF (6 mL) was added dropwise a solution of **13** (0.5 g, 3.3 mmol) in THF (3 mL) and the mixture refluxed for 1.5 h. The reaction mixture was cooled and treated with sodium fluoride (0.67 g, 16.0 mmol) and water (0.21 mL, 12 mmol) and stirred for 1 h at 4 °C. After insoluble material was filtered off, the filtrate was evaporated under reduced pressure to give 1-methyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine (0.32 g, 70.3%). IR (Nujol) cm^{-1} 3180, 2900, 1590, 1560, 1340, ^{1}H NMR (DMSO-d_6) δ 1.55–1.75 (2H, m), 2.35–2.45 (2H, m), 3.05–3.15 (2H, m), 3.45 (3H, s), 5.32 (1H, br s), 6.91 (1H, s).

1-Methyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine was formylated by a method similar to that described for **3** to give 7-formyl-1-methyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine (**14**). IR (Nujol) cm^{-1} 1680, 1600, 1475, 1380, ^{1}H NMR (DMSO-d_6) δ 1.70–1.90 (2H, m), 2.50–2.60 (2H, m), 3.60–3.75 (2H, m), 3.67 and 3.81 (3H, each s), 7.23 and 7.25 (1H, each s), 8.25 and 8.69 (1H, each s).

3-Formamido-2-methyl-4,5,6,7-tetrahydro-2*H*-indazole (16). To a mixture of 2-cyanocyclohexanone (**15**; 8 g, 65 mmol) and methylhydrazine (4.5 mL, 84.6 mmol) in water (80 mL was added concd HCl (8 mL) and the mixture stirred for 1 h at 60–70 °C. The mixture was added to ethyl acetate and water and adjusted to pH 7 with saturated sodium bicarbonate aqueous solution. The organic layer was separated, dried over anhydrous $MgSO_4$, and the filtrate concentrated under reduced pressure to give 3-amino-2-methyl-4,5,6,7-tetrahydro-2*H*-indazole (6.1 g, 62.2%). MP 55–58 °C, IR (Nujol) cm^{-1} 3300, 1640, 1580, 1530, 1400, ^{1}H NMR (DMSO-d_6) δ 1.4–1.8 (4H, m), 2.1–2.6 (4H, m), 3.43 (3H, s), 4.73 (2H, s).

3-Amino-2-methyl-4,5,6,7-tetrahydro-2*H*-indazole was formylated by a method similar to that described for **3** to give 3-formamido-2-methyl-4,5,6,7-tetrahydro-2*H*-indazole (**16**). MP 115–118 °C, IR (Nujol) cm^{-1} 3200, 1675, 1640, 1580, 1540, 1380, ^{1}H NMR (DMSO-d_6) δ 1.4–1.8 (4H, m), 2.1–2.6 (4H, m), 3.53 (3H, s), 8.15 (1H, s), 9.75 (1H, s).

1-Formyl-1*H*-imidazo[1,2-*b*]pyrazole (18). To a suspension of ethyl 2-ethoxymethylene-2-cyanoacetate (**17**; 21.7 g, 0.13 mol) in ethanol (65 mL) was added dropwise a solution of hydrazinoacetaldehyde diethylacetal (19 g, 0.13 mol) in water (19 mL) under ice-cooling and the mixture then stirred for 1.5 h at 80 °C. The reaction mixture was evaporated under reduced pressure. To the residue was added 4 N sodium hydroxide aqueous solution (64 mL) and refluxed for 1 h. The reaction mixture was cooled and adjusted to pH 3.5 with concd HCl, and the precipitate collected by filtration to give 1-(2,2-diethoxyethyl)-4-carboxy-5-aminopyrazole (8.60 g, 27.2%). IR (Nujol) cm^{-1} 3460, 3360, 1645, 1620, 1545, ^{1}H NMR (DMSO-d_6) δ 1.05 (6H, t, J = 7 Hz), 3.41 (2H, q, J = 7 Hz), 3.63 (2H, q, J = 7 Hz), 3.98 (2H, d, J = 5.5 Hz), 4.80 (1H, t, J = 5.5 Hz), 6.14 (2H, br s), 7.44 (1H, s).

To a solution of 1-(2,2-diethoxyethyl)-4-carboxy-5-aminopyrazole (1 g, 4.1 mmol) in THF (20 mL) was added 4 N HCl (10 mL) and the mixture refluxed for 1 h. The reaction mixture was cooled and adjusted to pH 8 with 5 N sodium hydroxide aqueous solution, and thereto added to a mixture of THF and ethyl acetate. The organic layer was separated, and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to give 1*H*-imidazo[1,2-*b*]pyrazole (235 mg, 53.5%). ^{1}H NMR (DMSO-d_6) δ 5.62 (1H, d, J = 2 Hz), 7.14 (1H, br s), 7.45 (1H, d, J = 2 Hz), 7.49 (1H, br s), 10.97 (1H, br s).

1*H*-Imidazo[1,2-*b*]pyrazole was formylated by a method similar to that described for **3** to give 1-formyl-1*H*-imidazo[1,2-*b*]pyrazole (**18**). ^{1}H NMR (DMSO-d_6) δ 6.20 and 6.28 (1H, each d, J = 2 Hz), 7.65–7.72 (1H, m), 7.71 (1H, d, J = 2 Hz), 7.87–7.92 (1H, m), 9.02 (1H, br s).

Preparation of 3-pyrazoliomethyl cephalosporin derivatives

7β-Amino-3-(3-ureido-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate bistrifluoroacetate (20b). To a suspension of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-chloromethyl-3-cephem-4-carboxylate (10 g, 19.4 mmol) and sodium iodide (2.91 g, 19.4 mmol) in DMF (10 mL) was added 5-ureido-1-methylpyrazole (6.8 g, 48.5 mmol) and stirred for 24 h at rt. The reaction mixture was added to a mixture of THF, ethyl acetate and water. The organic layer was separated, and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to give diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(3-ureido-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate iodide (12.1 g, 83.5%). IR (Nujol) cm^{-1} 3250, 1780, 1705, ^{1}H NMR (DMSO-d_6) δ 1.43 (9H, s), 3.39 (2H, br s), 3.67 (3H, s), 5.18 (1H, d, J = 5 Hz), 5.38 (2H, br s), 5.68 (1H, dd, J = 5 Hz, 8 Hz), 6.88 (1H, d, J = 3 Hz), 7.00 (1H, s), 7.18–7.68 (10H, m), 8.01 (1H, d, J = 8 Hz), 8.22 (1H, d, J = 3 Hz).

To a mixture of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(3-ureido-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate iodide (12.0 g, 16.1 mmol) and anisole (12 mL) in dichloromethane (36 mL) was added dropwise trifluoroacetic acid (24 mL) under ice-cooling and then stirred for 1 h at the same temperature. The reaction mixture was added to diisopropyl ether

(700 ml) and the produced precipitate collected by filtration to give 7β-amino-3-(3-ureido-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate bistrifluoroacetate (9.2 g, 98.5%). ^{1}H NMR (DMSO-d_6) δ 3.53 (2H, br s), 3.90 (3H, s), 5.33 (2H, s), 5.68 (2H, br s), 6.89 (1H, d, J = 2 Hz), 8.32 (1H, d, J = 2 Hz), 8.36 (1H, s).

Preparation of **20a** and **c–i** was carried out by a method similar to that described for **20b**.

7β-Amino-3-[3-(*N*-formyl-*N*-methyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate bistrifluoroacetate (20a). 1H NMR (D_2O) δ 3.46 (3H, s), 3.87 and 4.00 (3H, each s), 3.13–3.77 (2H, m), 5.05–5.47 (4H, m), 6.89 (1H, d, J = 2 Hz), 8.32 (1H, d, J = 2 Hz), 8.36 (1H, s).

7β-Amino-3-[3-(1-pyrrolidinyl)-2-methyl-1-pyrazolio]-methyl-3-cephem-4-carboxylate bistrifluoroacetate (20c). IR (Nujol) cm^{-1} 1780, 1665, 1595, ^{1}H NMR (DMSO-d_6) δ 1.82–2.05 (4H, m), 3.42 (2H, br s), 3.43–3.61 (4H, m), 3.79 (3H, s), 5.23 (2H, br s), 5.23 and 5.45 (2H, ABq, J = 15 Hz), 6.15 (1H, d, J = 3 Hz), 8.20 (1H, d, J = 3 Hz).

7β-Amino-3-(3-formamido-2,4-dimethyl-1-pyrazolio)-methyl-3-cephem-4-carboxylate bistrifluoroacetate (20d). ^{1}H NMR (DMSO-d_6) δ 2.01 (3H, s), 3.48 (2H, br s), 3.83 (3H, s), 5.24 (2H, s), 5.50 (2H, br s), 8.26 (1H, s), 8.41 (1H, s).

7β-Amino-3-(4-formyl-4,5,6,7-tetrahydro-1-pyrazolo[1,5-*a*]pyrimidinio)methyl-3-cephem-4-carboxylate bistrifluoroacetate (20f). IR (Nujol) cm^{-1} 3350, 1780, 1650, ^{1}H NMR (DMSO-d_6) δ 2.04–2.45 (2H, m), 3.49 (2H, br s), 3.72–4.38 (4H, m), 5.22 (2H, br s), 5.49 (2H, br s), 7.07 (1H, br s), 8.35 (1H, d, J = 3 Hz), 8.47 and 9.07 (1H, each s).

7β-Amino-3-[7-formyl-1-methyl-4,5,6,7-tetrahydro-2-(1*H*-pyrazolo[3,4-*b*]pyridinio)]methyl-3-cephem-4-carboxylate bistrifluoroacetate (20g). ^{1}H NMR (D_2O + $NaHCO_3$) δ 1.6–2.0 (2H, m), 2.6–2.8 (2H, m), 3.12 and 3.25 (2H, ABq, J = 17 Hz), 3.3–3.5 (2H, m), 3.83 (3H, s), 5.13 (1H, d, J = 4.6 Hz), 5.22 and 5.47 (2H, ABq, J = 16 Hz), 5.50 (1H, d, J = 4.6 Hz), 8.12 (1H, s), 8.36 and 8.73 (1H, each s).

7β-Amino-3-[3-formamido-2-methyl-4,5,6,7-tetrahydro-1-(2*H*-indazolio)]methyl-3-cephem-4-carboxylate bistrifluoroacetate (20h). IR (Nujol) cm^{-1} 1780, 1660, 1200, ^{1}H NMR (D_2O + $NaHCO_3$) δ 1.6–2.0 (4H, m), 2.2–2.6 (2H, m), 2.6–2.9 (2H, m), 3.03 and 3.36 (2H, ABq, J = 16 Hz), 3.80 (3H, s), 5.00 (2H, s), 5.03 (1H, d, J = 5 Hz), 5.31 (1H, d, J = 5 Hz), 8.36 (1H, s).

7β-Amino-3-[1-formyl-5-(1*H*-imidazo[1,2-*b*]pyrazolio)]-methyl-3-cephem-4-carboxylate bistrifluoroacetate (20i). IR (Nujol) cm^{-1} 1780, 1660, 1590, ^{1}H NMR (DMSO-d_6) δ 3.40 and 3.56 (2H, ABq, J = 18 Hz), 5.05–5.5 (4H, m), 6.64 (1H, d, J = 3 Hz), 7.78 (1H, br s), 8.07 (1H, br s), 8.27 (1H, d, J = 3 Hz), 8.50 (1H, br s).

7β-Amino-3-[3-(*N*-methyl)amino-2-methyl-1-pyrazolio]-methyl-3-cephem-4-carboxylate trishydrochloride (21a). To a solution of **20a** (5.3 g, 9.1 mmol) in methanol (26 mL) was added concd HCl (2.6 mL) and stirred for 2 h at rt. The reaction mixture was added dropwise to ethyl acetate (260 mL) and collected the produced precipitate by filtration to give 7β-amino-3-[3-(*N*-methyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate trishydrochloride (2.45 g, 62.2%). ^{1}H NMR (D_2O + $NaHCO_3$) δ 2.93 (3H, s), 3.25–3.38 (2H, m), 3.63 (3H, s), 5.07–5.33 (4H, m), 5.97 (1H, d, J = 2 Hz), 7.89 (1H, d, J = 2 Hz).

Preparation of **21e–i** was carried out by a method similar to that described for **21a**.

7β-Amino-3-[2,3-dihydro-5-(1*H*-imidazo[1,2-*b*]pyrazolio)]-methyl-3-cephem-4-carboxylate trishydrochloride (21e). ^{1}H NMR (D_2O) δ 3.37 and 3.63 (1H, ABq, J = 18 Hz), 3.93–4.47 (4H, m), 5.12 (2H, s), 5.14 (1H, d, J = 5 Hz), 5.30 (1H, d, J = 5 Hz), 5.86 (1H, d, J = 2 Hz), 7.92 (1H, d, J = 2 Hz).

7β-Amino-3-(4,5,6,7-tetrahydro-1-pyrazolo[1,5-*a*]pyrimidinio)methyl-3-cephem-4-carboxylate trishydrochloride (21f). IR (Nujol) cm^{-1} 3350, 1780, 1700, 1620, ^{1}H NMR (DMSO-d_6) δ 1.87–2.17 (2H, m), 3.27 (2H, br s), 3.36–3.55 (2H, m), 3.85–4.17 (2H, m), 5.23 (2H, br s), 5.29 (2H, br s), 5.76 (1H, d, J = 3 Hz), 8.08 (1H, d, J = 3 Hz).

7β-Amino-3-[1-methyl-4,5,6,7-tetrahydro-2-(1*H*-pyrazolo[3,4-*b*]pyridinio)]methyl-3-cephem-4-carboxylate trishydrochloride (21g). ^{1}H NMR (D_2O) δ 1.6–2.0 (2H, m), 2.4–2.6 (2H, m), 3.12 and 3.23 (2H, ABq, J = 18 Hz), 3.35–3.5 (2H, m), 3.57 (3H, s), 4.86 and 5.19 (2H, ABq, J = 16 Hz), 5.04 (1H, d, J = 4.6 Hz), 5.46 (1H, d, J = 4.6 Hz), 7.65 (1H, s).

7β-Amino-3-[3-amino-2-methyl-4,5,6,7-tetrahydro-1-(2*H*-indazolio)]methyl-3-cephem-4-carboxylate trishydrochloride (21h). IR (Nujol) cm^{-1} 3150, 1780, 1700, 1640, ^{1}H NMR (D_2O) δ 1.6–2.0 (4H, m), 2.2–2.5 (2H, m), 2.5–2.8 (2H, m), 2.96 and 3.23 (2H, ABq, J = 17 Hz), 3.65 (3H, s), 4.80 and 5.16 (2H, ABq, J = 17 Hz), 5.03 (1H, d, J = 5 Hz), 5.45 (1H, d, J = 5 Hz).

7β-Amino-3-[5-(1*H*-imidazo[1,2-*b*]pyrazolio)]methyl-3-cephem-4-carboxylate trishydrochloride (21i). ^{1}H NMR (D_2O+$NaHCO_3$) δ 3.09 and 3.40 (2H, ABq, J = 18 Hz), 4.40–5.5 (4H, m), 6.42 (1H, d, J = 3 Hz), 7.45 (1H, d, J = 2 Hz), 7.86 (1H, d, J = 2 Hz), 7.99 (1H, d, J = 3 Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2,3-dihydro-5-(1*H*-imidazo[1,2-*b*]pyrazolio)]-methyl-3-cephem-4-carboxylate (23e). A mixture of DMF (0.26 mL, 3.36 mmol) and phosphoryl chloride (0.3 mL, 3.22 mmol) was stirred for 30 min at rt. To the mixture were added THF (6 mL) and (*Z*)-2-(2-formylaminothiazol-4-yl)-2-methoxyiminoacetic acid (**22**; 0.64 g, 2.79 mmol) at 4 °C and the reaction mixture stirred

for 1 h at the same temperature. Meanwhile, a mixture of **21e** (1.2 g, 2.79 mmol) and *N*-trimethylsilylacetamide (3.66 g, 27.9 mmol) in CH_2Cl_2 (25 mL) was warmed to make a clear solution. The solution was then cooled to -20 °C and added to the activated acid solution obtained above. The reaction mixture was stirred for 2 h at -10–0 °C and then poured into ethyl acetate (25 mL) and the produced precipitate collected by filtration, and dried under reduced pressure. This powder and concd HCl (0.74 mL) in methanol (17 mL) were stirred for 2 h at rt and then poured into ethyl acetate (170 mL) and the produced precipitate collected by filtration. The powder was dissolved in water and adjusted to pH 2.5 with saturated sodium bicarbonate aqueous solution and then subjected to column chromatography on Diaion HP-20. The column was washed with water and eluted with 5% aqueous isopropanol. The fractions containing the desired compound were evaporated to remove isopropanol and then lyophilized to give 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[2,3-dihydro-5-(1*H*-imidazo[1,2-*b*]pyrazolio)]methyl-3-cephem-4-carboxylate (0.35 g, 24.9%). IR (Nujol) cm^{-1} 1770, 1660, 1600, 1530, ^1H NMR (D_2O) δ 3.21 and 3.51 (2H, ABq, J = 18 Hz), 3.98 (3H, s), 3.97–4.47 (4H, m), 4.89 and 5.12 (2H, ABq, J = 13 Hz), 5.21 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 5.82 (1H, d, J = 3 Hz), 6.96 (1H, s), 7.89 (1H, d, J = 3 Hz).

Preparation of **23a**, **b**, **d**, **f**, and **g** was carried out by a method similar to that described for **23e**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(*N*-methyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (23a). IR (Nujol) cm^{-1} 3200–3300, 1770, 1670, 1620, 1530, ^1H NMR (D_2O) δ 2.90 (3H, s), 3.08 and 3.34 (2H, ABq, J = 18 Hz), 3.60 (3H, s), 3.97 (3H, s), 5.03 and 5.27 (2H, ABq, J = 12 Hz), 5.18 (1H, d, J = 5 Hz), 5.79 (1H, d, J = 5 Hz), 5.91 (1H, d, J = 3 Hz), 6.96 (1H, s), 7.86 (1H, d, J = 3 Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(3-ureido-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate (23b). IR (Nujol) cm^{-1} 1765, 1700, 1660, ^1H NMR (DMSO-d_6) δ 3.29 (2H, br s), 3.83 (3H, s), 3.85 (3H, s), 5.08 (1H, d, J = 5 Hz), 5.33 (2H, br s), 5.67 (1H, dd, J = 5 Hz, 8 Hz), 6.78 (1H, d, J = 3 Hz), 7.16 (2H, br s), 8.18 (1H, d, J = 3 Hz), 9.54 (1H, d, J = 8 Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(3-amino-2,4-dimethyl-1-pyrazolio)methyl-3-cephem-4-carboxylate (23d). IR (Nujol) cm^{-1} 3300, 1770, 1640, 1600, ^1H NMR (D_2O) δ 1.93 (3H, s), 3.08 and 3.33 (2H, ABq, J = 18 Hz), 3.65 (3H, s), 3.98 (3H, s), 4.88 and 5.21 (2H, ABq, J = 15 Hz), 5.18 (1H, d, J = 5 Hz), 5.81 (1H, d, J = 5 Hz), 6.97 (1H, s), 7.66 (1H, s).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-(4,5,6,7-tetrahydro-1-pyrazolo[1,5-*a*]pyrimidinio)methyl-3-cephem-4-carboxylate (23f). IR (Nujol) cm^{-1} 3250, 1760, 1660, 1610, ^1H NMR (D_2O) δ 1.92–2.28 (2H, m), 3.13–3.49 (4H, m), 3.88–4.24 (2H, m), 3.97 (3H, s), 4.85 and 5.20 (2H, ABq, J = 15 Hz), 5.17 (1H, d, J = 5 Hz), 5.77 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 3 Hz), 6.95 (1H, s), 7.72 (1H, d, J = 3 Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[1-methyl-4,5,6,7-tetrahydro-2-(1*H*-pyrazolo[3,4-*b*]pyridinio)]methyl-3-cephem-4-carboxylate (23g). IR (Nujol) cm^{-1} 3300, 1760, 1600, ^1H NMR (DMSO-d_6) δ 1.5–2.0 (2H, m), 2.3–2.7 (2H, m), 3.03 and 3.23 (2H, ABq, J = 17 Hz), 3.35–3.5 (2H, m), 3.70 (3H, s), 3.83 (3H, s), 4.93 and 5.25 (2H, ABq, J = 16 Hz), 5.02 (1H, d, J = 5 Hz), 5.58 (1H, dd, J = 5 Hz, 8 Hz), 6.72 (1H, s), 7.20 (2H, s), 7.91 (1H, s), 8.13 (1H, s), 9.47 (1H, d, J = 8 Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(1-pyrrolidinyl)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (23c). **20c** (1.5 g, 2.54 mmol) was dissolved in a mixture of water (15 mL) and THF (30 mL). The solution was adjusted to pH 6.5 with aqueous sodium bicarbonate and treated with 1-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetyl)]-benzotriazol-3-oxide monodimethylformamide (**24**; 1.49 g, 3.81 mmol) and then maintained at pH 7 with aqueous sodium bicarbonate for 5 h at rt. The reaction mixture was washed with ethyl acetate and the aqueous solution was adjusted to pH 3 with 1 N HCl and washed with ethyl acetate. The aqueous solution was subjected to column chromatography on Diaion HP-20. The column was washed with water and eluted with 10% aqueous isopropanol. The fractions containing the desired compound were evaporated to remove isopropanol and then lyophilized to give 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-(1-pyrrolidinyl)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (0.683g, 49.2%). IR (Nujol) cm^{-1} 3300, 1770, 1660, ^1H NMR (D_2O) δ 1.83–2.07 (4H, m), 3.07 and 3.33 (2H, ABq, J = 18 Hz), 3.37–3.58 (4H, m), 3.73 (3H, s), 3.96 (3H, s), 4.87 and 5.23 (2H, ABq, J = 15 Hz), 5.18 (1H, d, J = 5 Hz), 5.78 (1H, d, J = 5 Hz), 5.88 (1H, d, J = 3 Hz), 6.93 (1H, s), 7.85 (1H, d, J = 3 Hz).

Preparation of **23h** and **i** was carried out by a method similar to that described for **23c**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[3-amino-2-methyl-4,5,6,7-tetrahydro-1-(2*H*-indazolio)]methyl-3-cephem-4-carboxylate (23h). IR (Nujol) cm^{-1} 3340, 1770, 1660, 1600, ^1H NMR ($D_2O + NaHCO_3$) δ 1.5–1.9 (4H, m), 2.0–2.35 (2H, m), 2.35–2.7 (2H, m), 2.90 and 3.20 (2H, ABq, J = 18 Hz), 3.60 (3H, s), 4.00 (3H, s), 5.05 (2H, s), 5.16 (1H, d, J = 5 Hz), 5.80 (1H, d, J = 5 Hz), 6.90 (1H, s).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-[5-(1*H*-imidazo[1,2-*b*]pyrazolio)]methyl-3-cephem-4-carboxylate (23i). IR (Nujol) cm^{-1} 1770, 1670, 1600, ^1H NMR ($D_2O + NaHCO_3$) δ 2.93 and 3.23 (2H, ABq, J = 18 Hz), 3.83 (3H, s), 5.02 (1H, d, J = 5 Hz), 5.11 (2H, br s), 5.65 (1H, d, J = 5 Hz), 6.21 (1H, d, J = 3 Hz), 6.74 (1H, s), 7.20 (1H, d, J = 2 Hz), 7.70 (1H, d, J = 2 Hz), 7.80 (1H, d, J = 3 Hz).

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