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Orally Active Cephalosporins. Part 4: Synthesis, Structure–Activity Relationships and Oral Absorption of Novel 3-(4-Pyrazolylmethylthio)cephalosporins with Various C-7 Side Chains

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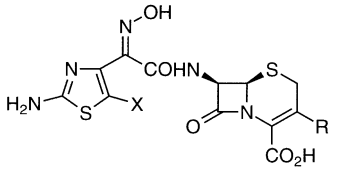
Abstract—A series of 3-(4-pyrazolylmethylthio)cephalosporins with various C-7 side chains was designed, synthesized and evaluated for antibacterial activity and oral absorption in rats. Antibacterial activity against *Haemophilus influenzae* was markedly increased by the C-7 oxime moiety. Deamination at the 2 position of, or introduction of a substituent such as halogen or methyl to, the 5 position of the (*Z*)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino) moiety improved oral absorption. Among these compounds, FR192752 (**40a**) having a (*Z*)-2-(2-amino-5-chlorothiazol-4-yl)-2-hydroxyiminoacetamido moiety, showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria including *H.influenzae* and penicillin G-resistant *Streptococcus pneumoniae* (PRSP). Further, it showed higher oral absorption than CFDN and FK041.

Introduction

Orally active cephalosporins such as cefixime (CFIX),[1] cefdinir (CFDN)(**1**)[2] and cefditoren pivoxil (CDTR-PI)[3] are of key clinical importance for the treatment of bacterial infections. Since the discovery of CFDN, further efforts have been made to find oral cephalosporins which have more potent and well balanced antibacterial activity, especially against *Haemophilus influenzae*, an important pathogen that is the cause of severe respiratory infections. In a previous paper,[4] we investigated a series of cephems having a heteroarylmethylthio moiety at the C-3 position and finally discovered FK041 (**2**) (Fig. 1), having a 4-pyrazolylmethylthio moiety which demonstrated potent and well-balanced antibacterial activity, including *H. influenzae*, and high oral absorption in rats.

Recently, PRSP has been encountered with increasing frequency. Since penicillin G-resistant isolates show increased resistance to other β-lactam antibiotics,[5,6] PRSP is a serious problem in the clinic. Therefore, our



	X	R
CFDN (**1**)	—H	—CH=CH$_2$
FK041(**2**)	—H	—SCH$_2$—
FR192752 (**40a**)	—Cl	—SCH$_2$—

Figure 1. Structures of CFDN, FK041 and FR192752.

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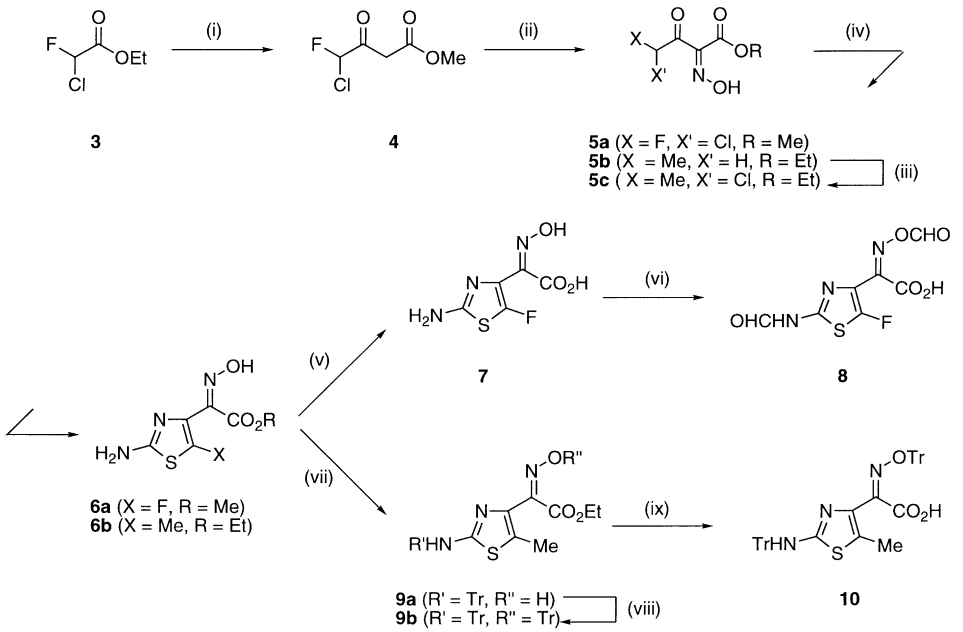
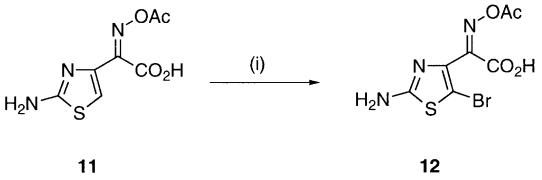


Scheme 1. Reagents and conditions: (i) (a) $CISO_3H$, phthaloyl chloride, 0 °C; (b) isopropylidene malonate, pyridine, 0 °C; (c) MeOH, reflux; (ii) isoamyl nitrate, CH_3COCl, CH_2Cl_2, 0 °C–rt; (iii) SO_2Cl_2, AcOH, 0 °C–rt; (iv) thiourea, DMAc, 0 °C–rt; (v) BCl_3, CH_2Cl_2, 0 °C; (vi) Ac_2O, HCO_2H, rt; (vii) TrCl, Et_3N, DMF, rt; (viii) NaH, TrCl, AcOEt, rt; (ix) NaOH, dioxane, H_2O, 100 °C.



Scheme 2. Reagents and conditions; (i) NBS, MeOH, rt.

next research objective was to discover a new cephalosporin having more potent and well-balanced antibacterial activity including PRSP and equal, or higher oral absorption than CFDN and FK041 without using a prodrug strategy. We directed our next efforts towards studies on the C-7 moiety of these derivatives and to maximize the effectiveness of this C-3 side chain. We report herein the synthesis, structure–activity relationships and oral absorption of novel 3-(4-pyrazolylmethylthio)cephalosporins with various C-7 side chains.

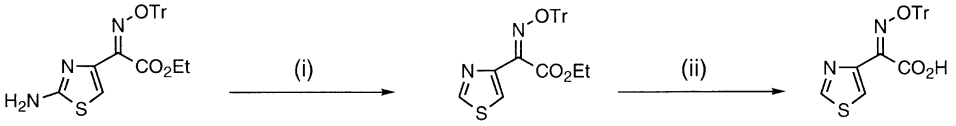
Chemistry

Scheme 1 outlines the synthesis of the C-7 acid fragments, (*Z*)-2-(2-amino-5-fluorothiazol-4-yl)-2-hydroxyiminoacetic acid (**8**) and (*Z*)-2-(2-amino-5-methylthiazol-4-yl)-2-hydroxyiminoacetic acid (**10**). Ethyl 2-chloro-2-fluoroacetate (**3**) was easily converted to the β-ketoester (**4**) in moderate yield.[7,8] The β-ketoester (**4**) was then treated with isoamylnitrate in the presence of acetyl chloride to afford hydroxyiminoester (**5a**). The hydroxyiminoester (**5a**) was reacted with thiourea to give 2-amino-5-fluorothiazole derivative (**6a**). Similarly, the known ethyl 2-hydroxyiminopropionyl-acetate (**5b**)[9] was treated with sulfuryl chloride and reacted with thiourea to give the 2-amino-5-methylthiazole derivative (**6b**). The ester **6a** was converted to the acid (**7**) using trichloroborane and treated with a mixture of acetic anhydride and formic acid to give fully protected C-7 acid fragment **8**. Similarly, **6b** was protected stepwise with trityl chloride followed by treatment with sodium hydroxide in aqueous dioxane to give fully protected C-7 acid fragment **10**.

Scheme 2 shows the synthesis of the (*Z*)-2-(2-amino-5-bromothiazol-4-yl)-2-hydroxyiminoacetic acid (**12**). The acid **11** [10] was brominated using *N*-bromosuccimide (NBS) in MeOH to give the bromo acid **12** in moderate yield.

Scheme 3 shows the synthesis of (*Z*)-2-(4-thiazolyl)-2-trityloxyiminoacetic acid (**15**). The ester (**13**)[11] was easily deaminated using *tert*-butylnitrate in THF and converted to the acid (**15**) using sodium hydroxide in aqueous dioxane.

Scheme 4 outlines the two methods used for the synthesis of the cephems with various C-7 side chains. The preparation of compounds **38b** and **43** was carried out by Method B and the other derivatives were obtained by Method A. Method A involved the synthesis of the key intermediate **17** to which the C-3 side chain, the (4-pyrazolyl)methylthio moiety, was already introduced. Thus, diphenylmethyl 7β-amino-3-methanesulfonyloxy-3-cephem-4-carboxylate (**16**)[12] was treated with sodium hydrosulfide in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylacetamide (DMAc) at −20 °C followed by addition of 4-chloromethyl-1-tritylpyrazole to give the cephem (**17**). The coupling reaction of cephem (**17**) with various C-7 fragments[13–22] was accomplished by three different methods. Thus, the 7β-aminocephem (**17**) was treated with *N*,*O*-bistrimethylsilylacetamide (BSA) followed by addition of the C-7 acid chloride (**18c**) or the C-7 acid (**20**–**22**),



13 14 15

Scheme 3. Reagents: (i) *tert*-BuONO, THF, rt; (ii) NaOH, dioxane, H_2O.

which was activated with $POCl_3$ and DMF or methanesulfonyl chloride in the presence of K_2CO_3, to give **23**–**28**. The compounds **23c**, **24d**–**f** and **27b** were deprotected in one step using trifluoroacetic acid (TFA) in the presence of anisole in CH_2Cl_2 to give **38c**–**f** and **40b**, whereas the other compounds (**25g**,**i**,**26k**,**27l**,**28l**) were deprotected stepwise to give **38 h**,**j**, **39a**–**42a**. Method B involves the introduction of the C-7 side chain in the first step and the C-3 side chain is introduced afterwards. Thus, compounds **34b** and **35n** were obtained from the coupling reaction of 7β-aminocephem (**16**) with **18b** or **19n** in the presence of BSA according to the procedure that was used for **23c**. These compounds (**34b**,**35n**) were reacted with sodium thiolate, generated in situ by the methanolysis of 4-benzoylthiomethyl-1-(trityl)pyrazole,[4] to give **36b** and **37n**. In this reaction, a low temperature (below −65 °C) was necessary. If a higher temperature was employed, the amount of undesired Δ^2 isomer was significantly increased. Compounds **36b** and **37n** were deprotected in one step according to the manner described above to give **38b** and **43**.

Scheme 5 shows the synthesis of a hydroxyiminocephem (**45**) which has a deaminated C-7 side chain. The acid **15** described above was coupled with 7β-aminocephem (**17**) using the method described above and deprotected using $AlCl_3$ in the presence of anisole in CH_3NO_2 to afford **45**.

Biological Results

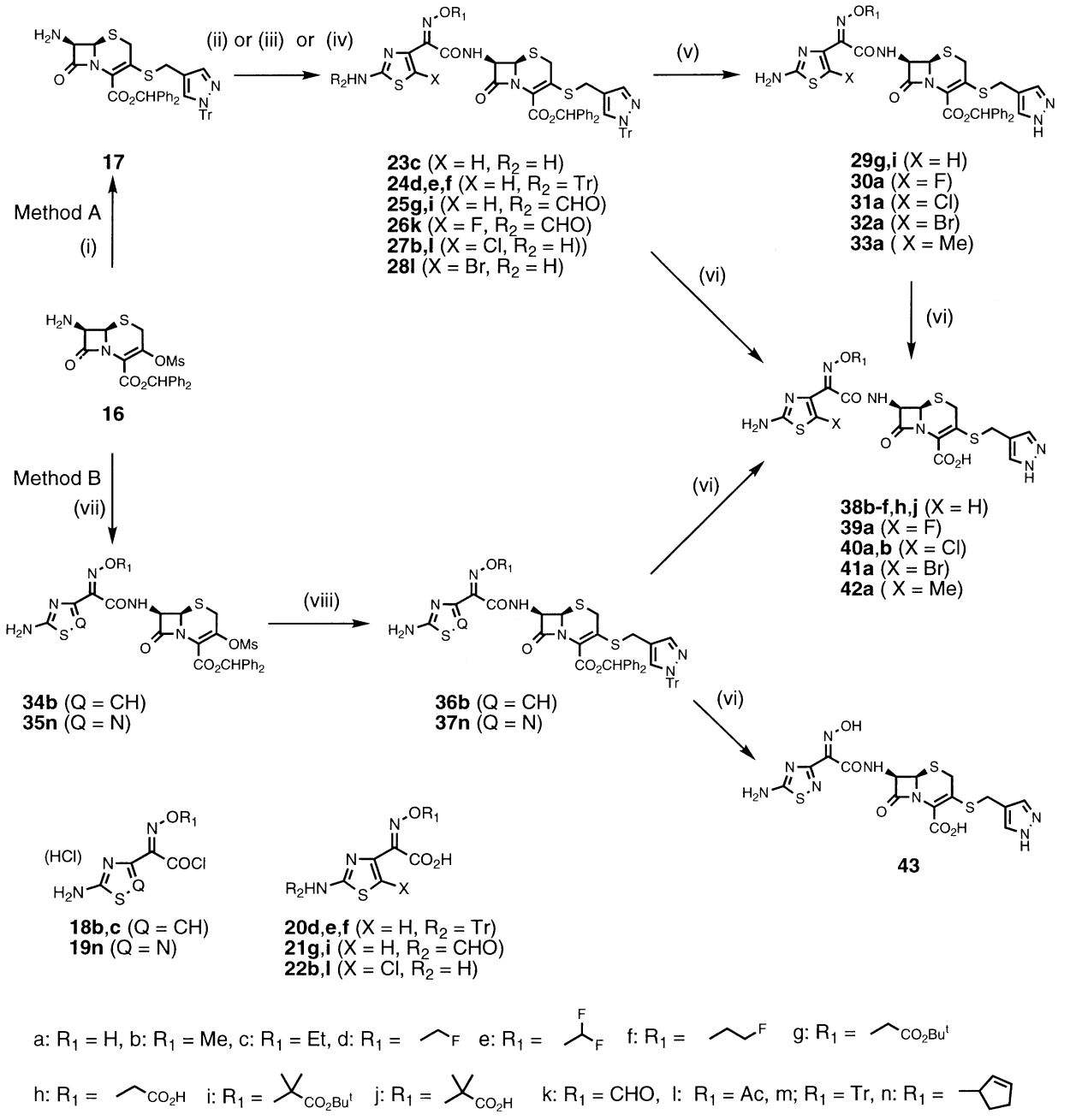
The in vitro antibacterial activity of the new cephalosporins against Gram-positive and Gram-negative bacteria is shown in Table 1. For comparison, CFDN (**1**) and FK041 (**2**) were employed as reference drugs. As can be deduced from this data, all of the synthesized compounds exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria.

Compounds **38b**–**f**,**j** and **40b** which have an alkoxyimino moiety have dramatically improved antibacterial activity against *H. influenzae* and similar potent antibacterial activity against the other Gram-negative bacteria compared with FK041. Thus, the effect of this alkoxyimino moiety on activity towards *H. influenzae* was significant in this series of cephalosporins. In contrast, these compounds had decreased antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis*. Especially, **38 h**,**j** which had a carboxymethoxyimino moiety showed dramatically decreased antibacterial activity against *S. aureus*. This result is in good agreement with our findings on the C-3 vinyl cephalosporins.[23] Comparison of **39a**, **40a**, **41a** with FK041 indicated the effect of introduction of an electron-withdrawing halogen substituent to the aminothiazole moiety. Although these compounds showed moderate antibacterial activity against *H. influenzae* and *Escherichia coli*, they showed similar potent antibacterial activity against *S. aureus* and improved antibacterial activity against *E. faecalis* and PRSP. In contrast, **42a** which had an electron-donating methyl substituent showed decreased antibacterial activity against all tested strains compared with FK041. Thus, introduction of an electron-donating substituent to the thiazole moiety appears to be an unfavorable modification in terms of activity. Comparison of **40a** with FK041 and of **40b** with **38b** showed the effect of introduction of the chloro substituent to the 5-position of the C-7 thiazole. These results revealed that this modification was independent of the oxime moiety. Compound **43** which had aminothiadiazole moiety, resulted in decreased antibacterial activity against all tested strains compared with FK041. Thus, conversion of the aminothiazole to aminothiadiazole had no beneficial effect on activity. Comparison of **45** with FK041 indicated the effect of an amino substituent at the 2-position of the C-7 thiazole. Deamination of FK041 resulted in much decreased antibacterial activity against *H. influenzae* although compound **45** showed moderate antibacterial activity against the other strains. Among all compounds prepared, **39a** and **40a** exhibited the most well balanced antibacterial activity against both Gram-positive and Gram-negative bacteria including PRSP, although they were slightly less active against *H. influenzae* and *E. coli* compared with FK041.

Table 1. Antibacterial activity of cephalosporins[a]

Drugs	MIC[b] (μg/mL)					
	S.a.	*E.f.*	*M.c.*	*H.i.*	*E.c.*	PRSP
38b	0.98	>100	0.036	0.025	0.057	1.21
38c	0.78	>100	0.028	0.03	0.181	0.66
38d	0.78	>100	0.033	≦0.025	0.053	0.72
38e	0.78	>100	0.031	≦0.025	0.144	0.72
38f	0.78	>100	0.033	≦0.025	0.144	0.93
38 h	7.9	>100	0.033	N.T.	0.078	N.T.
38j	12.5	>100	≦0.025	0.025	0.29	11.5
39a	0.33	6.3	0.039	0.182	0.039	0.78
40a (FR192752)	0.25	2.9	0.033	0.34	0.29	0.72
40b	0.78	19.8	≦0.025	0.051	0.57	0.36
41a	0.39	3.1	0.039	0.55	0.98	0.64
42a	0.67	15.7	0.181	5.4	5.0	3.5
43	0.42	7.87	0.078	0.68	0.098	4.96
45	0.25	5.0	0.144	2.2	0.91	3.9
FK041 **(2)**	0.23	7.9	0.072	0.129	0.033	1.56
CFDN **(1)**	0.33	13.5	0.072	0.41	0.133	6.25

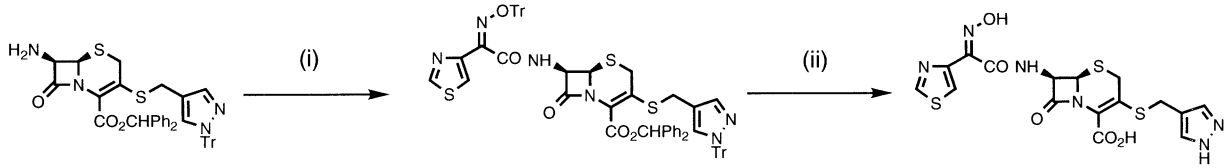
Müller–Hinton agar; 10^{-2}, stamp method; 37 °C, 20 h.
[a]*S.a.*, *Staphylococcus aureus* (MSSA); [9] *E.f.*, *Enterococcus faecalis*; [9] *M.c.*, *coli* [9] PRSP, *Streptococcus pneumoniae* (PC-resistant).[17]
[b]Mean MIC. N.T., not tested. CFDN, cefdinir.



Scheme 4. Reagents: (i) NaSH, *N*,*N*-diisopropylethylamine, DMF, 4-chloromethyl-1-tritylpyrazole; (ii) **18c**, *N*,*O*-bistrimethylsilylacetamide (BSA), CH_2Cl_2; (iii) (a) **8** or **10** or **20** or **21**, $POCl_3$, DMF, AcOEt, THF; (b) BSA, DMAc; (iv) (a) **12** or **22**, MsCl, K_2CO_3, DMAc; (b) BSA, DMF; (v) cHCl, MeOH, THF; (vi) TFA, anisole, CH_2Cl_2; (vii) **18b** or **19n**, BSA, DMAc; (viii) 4-benzothiomethyl-1-tritylpyrazole, NaOMe, MeOH, THF, DMF.

Further, compared with CFDN, these compounds exhibited superior antibacterial activity against tested strains. Thus, these compounds are highly attractive in terms of antibacterial activity.

The urinary and biliary recoveries of these compounds after oral administration to rats are shown in Table 2. Among these compounds, all hydroxyiminocephems (**39a**,**40a**,**41a**,**42a**,**45**) except for **43** showed high oral absorption. In particular, **40a** (FR192752) exhibited the highest and improved oral absorption compared with FK041 and CFDN. Regarding the alkoxyiminocephems (**38b–f**,**h**,**j**,**40b**), all compounds except for **38h** showed moderate oral absorption. Among these compounds **38h** showed the highest oral absorption. Comparison of **45** with FK041 indicated that the amino substituent at the 2-position of the thiazole had only marginal effect on oral absorption. Comparing the results of **43** with FK041 indicates that conversion of the aminothiazole to an aminothiadiazole had no beneficial effect on either oral absorption or activity. Compounds **39a**, **40a**, **41a** to which an electron-withdrawing substituent was introduced at the 5-position of the thiazole, and **42a** to which an electron-donating methyl substituent was introduced, showed high oral absorption. Thus, oral absorption was not decreased by introduction of these



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Scheme 5. Reagents: (i) (a) $POCl_3$, DMF, AcOEt, THF; (b) **15**, BSA, CH_2Cl_2; (ii) $AlCl_3$, anisole, CH_3NO_2.

Table 2. 24-h urinary and biliary recovery of cephalosporins after oral administration (20 mg/kg) to rats

Drugs	Recovery (%)		Drugs	Recovery (%)	
	Urine	Bile		Urine	Bile
38b	3.66	6.48	**40a** (FR192752)	41.9	28.7
38c	4.41	6.67	**40b**	1.97	7.87
38d	5.22	4.75	**41a**	30.4	21.4
38e	3.35	6.38	**42a**	46.7	11.1
38f	3.83	5.54	**43**	4.98	0.37
38h	12.70	26.40	**45**	42.9	6.3
38j	0.28	14.1	FK041 **(2)**	42.9	6.81
39a	33.1	7.79	CFDN **(1)**	32.4	1.4

substituents to the 5-position of thiazole. Further, **40a** and **42a** resulted in increased oral absorption compared with FK041.

Consequently, these results indicate that for high oral absorption the (*Z*)-2-(2-aminothiazol-4-yl)-2-(hydroxyimino)acetamido moiety is the most important of this type of derivative and that introduction of a substituent of appropriate size to the 5-position of the thiazole is effective to increase oral absorption.

Although two compounds (**39a**,**40a**) showed attractive antibacterial activity, **40a** exhibited higher oral absorption than both CFDN and FK041. Thus, we selected **40a** (FR192752) as an optimal compound with regards to both antibacterial activity and oral absorption.

FR192752 (**40a**) was tested against a wide variety of clinical isolates of bacteria and exhibited broad spectrum activity. FR192752 also showed high in vivo efficacy, good oral absorption and pharmacokinetics in other animals.[24]

Conclusions

Based on the parent 3-[(4-pyrazolyl)methylthio]-cephalosporin (**2**; FK041), new analogues optimized and modified at the C-7 side chain were synthesized, and we thereby discovered FR192752 (**40a**) having a (*Z*)-2-(2-amino-5-chlorothiazol-4-yl)-2-(hydroxyimino)-acetamido moiety at the C-7 position, which exhibited potent and well balanced antibacterial activity against both Gram-positive and Gram-negative bacteria, including *H. influenzae* and PRSP. Further, oral absorption of FR192752 was distinctly higher than that of both CFDN and FK041 in rats. Our further studies to optimize the absorption and activity of compounds derived from FR192752 are now in progress in this laboratory.

Experimental

IR spectra were recorded on a Hitachi 260-10 spectrophotometer. NMR spectra were recorded at 90 MHz on a Varian EM-390 NMR spectrometer, a Hitachi R-90H NMR spectrometer or a Bruker AC200P at 200 MHz. Chemical shifts are reported in ppm downfield from TMS as internal standard. Mass spectra were obtained on a Hitachi Model M-80 mass spectrometer (EIMS), a Finnigan MAT TSQ-70 (FABMS) and elemental analyses were obtained on a Perkin–Elmer 2400 CHN Elemental Analyzer.

Methyl 4-chloro-4-fluoro-3-oxobutyrate (4). A mixture of ethyl chlorofluoroacetate (100 g, 712 mmol), phthaloyl chloride (102.5 mL, 712 mmol) and chlorosulfonic acid (47.3 mL, 712 mmol) was heated at reflux in a distillation still connected to an ice-cooled receiver backed up by a dry ice cooled trap. When the pot temperature reached 120 °C, distillation was started and the volatile material was distilled from the reaction mixture until the internal pot temperature reached 200 °C. The condensates in the receiver and the dry ice cooled trap were combined. Concurrently, isopropylidene malonate (51.3 g, 356 mmol) was dissolved in a solution of pyridine (69.7 mL) in CH_2Cl_2 (160 mL). To this solution was added dropwise the above obtained combined solution at 5–10 °C and the mixture was stirred for 3 h at the same temperature. The solution was poured into cooled 3 N aqueous HCl (200 mL). The separated organic phase was washed with brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was diluted with MeOH (300 mL) at room temperature with stirring and refluxed for 8 h. The mixture was cooled and concentrated in vacuo. The residue was purified by column chromatography on silica-gel eluting with a mixture of *n*-hexane and ethyl acetate to give **4**. Colorless oil. Yield: 43.2 g (36%). ^{1}H NMR (DMSO-d_6) δ 3.72 (3H, s), 3.88 (2H, d, J = 1.6 Hz), 6.99 (1H, d, J = 49 Hz).

Methyl 4-chloro-4-fluoro-2-(hydroxyimino)-3-oxobutyrate (5a). Under ice cooling, acetyl chloride (13 mL, 184 mmol) was added to a solution of methyl 4-chloro-4-fluoro-3-oxobutyrate (31 g, 184 mmol) and isopentylnitrate (25.8 mL, 193 mmol) in CH_2Cl_2 (120 mL) with stirring. The mixture was stirred for 3 h at the same

temperature and poured into water (300 mL). The solution was adjusted to pH 3 with saturated aqueous $NaHCO_3$, and the aqueous layer was separated. The organic layer was washed with water and dried over $MgSO_4$. Evaporation of the solvent afforded **5a** including residual solvent. Colorless oil. Yield: 44.5 g (quant). ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s), 7.39 (1 h, d, $J=49$ Hz), 14.14 (1H, s).

Ethyl (*Z*)-4-chloro-2-hydroxyimino-3-oxopentanoate (5c). A solution of ethyl 2-(*Z*)-hydroxyimino-3-oxopentanoate (**5b**) (10 g, 57.9 mmol) in AcOH (10 mL) was treated dropwise with SO_2Cl_2 (5.3 mL), and stirred for 3 h at room temperature. The reaction was quenched with ice-water, and the product was extracted with ethyl acetate. The extracts were washed with brine and dried over $MgSO_4$. Evaporation of the solvent afforded **5c**. Oil. Yield: 11.9 g (99%). IR (neat) cm^{-1} 3371, 1736, 1703; ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, $J=7.1$ Hz), 1.58 (3H, d, $J=6.7$ Hz), 4.29 (2H, q, $J=7.1$ Hz), 5.34 (1H, q, $J=6.7$ Hz), 13.71 (1H, s).

Methyl (*Z*)-2-(2-amino-5-fluorothiazol-4-yl)-2-(hydroxyimino)acetate (6a). Thiourea (15 g, 197 mmol) was added to a solution of **5a** (7.8 g, 39.5 mmol) in DMF (80 mL) at 30 °C and stirred for 12 h at the same temperature. The mixture was poured into a mixture of water (400 mL) and ethyl acetate (300 mL), and adjusted to pH 3.0 with saturated aqueous $NaHCO_3$. The aqueous layer was separated and the organic layer was washed with water and brine, dried over $MgSO_4$. Evaporation of the solvent afforded **6a**. Amorphous solid. Yield: 2.2 g (25%). ^{1}H NMR (DMSO-d_6) δ 3.76 (3H, s), 7.11 (2H, br s), 11.92 (1H, s).

The following compound was obtained using a method similar to that used for **6a.**

Ethyl (*Z*)-2-(2-amino-5-methylthiazol-4-yl)-2-(hydroxyimino)acetate (6b). Amorphous solid. Yield: 2.8 g (62%). IR (KBr) cm^{-1} 3163, 1724, 1618; ^{1}H NMR (DMSO-d_6) δ 1.24 (3H, t, $J=7.1$ Hz), 2.35 (3H, s), 4.21 (2H, q, $J=7.1$ Hz), 6.89 (2H, s), 11.57 (1H, s).

(*Z*)-2-(2-Amino-5-fluorothiazol-4-yl)-2-(hydroxyimino)-acetic acid (7). Under ice-cooling, 1 M BCl_3 in CH_2Cl_2 (22.8 mL, 22.8 mmol) was added dropwise to a solution of **6a** (1 g, 4.56 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred for 3 h at the same temperature and poured into a mixture of THF (30 mL) and brine (15 mL), and the mixture was adjusted to pH 3 with saturated aqueous $NaHCO_3$ The aqueous layer was separated and the organic layer was dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with a mixture of ethyl acetate and MeOH to afford **7**. Amorphous solid. Yield: 330 mg (35%). ^{1}H NMR (DMSO-d_6) δ 6.94 (2H, br s). APCIMS m/z 206 [$(M+H)^+$].

(*Z*)-2-(5-Fluoro-2-formylaminothiazol-4-yl)-2-(formyloxyimino)acetic acid (8). A mixture of Ac_2O (2.7 mL, 29.2 mmol) and HCO_2H (2.2 mL, 58.5 mmol) was stirred for 30 min at 30 °C. The mixture was cooled with ice and **7** (1.2 g, 5.85 mmol) was added thereto with stirring. The whole mixture was stirred for 3 h at 25–35 °C and poured into isopropyl ether (50 mL). The resulting precipitate was collected by filtration, dried under reduced pressure to afford **8**. Amorphous solid. Yield: 0.65 g (43%). ^{1}H NMR (DMSO-d_6) δ 8.23 (1H, s), 8.50 (1H, s).

Ethyl (*Z*)-2-hydroxyimino-2-(5-methyl-2-tritylaminothiazol-4-yl)acetate (9a). Trityl chloride (11.7 g, 41.9 mmol) and triethylamine (6.4 mL, 45.7 mmol) was added successively to a solution of **6b** (8.73 g, 38.1 mmol) in DMF (60 mL), and stirred for 2 h at room temperature. The reaction was quenched with water and extracted with ethyl acetate. The extracts were washed with water and brine, and dried over $MgSO_4$. Evaporation of the solvent afforded **9a**. Amorphous solid. Yield: 16.15 g (90%). IR (KBr) cm^{-1} 1740, 1539, 1520; ^{1}H NMR (DMSO-d_6) δ 1.05 (3H, t, $J=7.1$ Hz), 2.25 (3H, s), 3.74 (2H, q, $J=7.1$ Hz), 7.16–7.40 (15H, m), 8.44 (1H, s), 11.43 (1H, s)

Ethyl (*Z*)-2-(5-methyl-2-tritylaminothiazol-4-yl)-2-(trityloxyimino)acetate (9b). Compound **9a** (16.15 g, 34.2 mmol) was added to a suspension of NaH (1.37 g, 34.2 mmol) in ethyl acetate (110 mL) at room temperature and stirred for 30 min A solution of trityl chloride (9.55 g, 34.2 mmol) in ethyl acetate (66 mL) was added to the mixture. The whole mixture was stirred for 20 h and the reaction was quenched with ice-water. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with *n*-hexane–ethyl acetate to afford **9b**. Amorphous solid. Yield: 9.8 g (40%). IR (KBr) cm^{-1} 1740, 1540, 1515; ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, $J=7.1$ Hz), 1.74 (3H, s), 3.93 (2H, q, $J=7.1$ Hz), 7.13–7.36 (30H, m), 8.51 (1H, s).

(*Z*)-2-(5-Methyl-2-tritylaminothiazol-4-yl)-2-(trityloxyimino)acetic acid (10). Sodium hydroxide (2.75 g, 68.6 mmol) was added to a solution of **9** (9.8 g, 13.7 mmol) in dioxane (40 mL) and water (40 mL). The mixture was allowed to warm to 100 °C and stirred for 3 h. After cooling to room temperature, the mixture was diluted with water (100 mL) and the resulting precipitate was collected by filtration. The precipitate was suspended in a mixture of ethyl acetate and water, and the mixture was adjusted to pH 2.5 with 1 N aqueous HCl. The aqueous layer was separated and the organic layer was washed with brine, dried over $MgSO_4$. Evaporation of the solvent afforded **10**. Amorphous solid. Yield: 9.15 g (90%). IR (KBr) cm^{-1} 1738, 702; ^{1}H NMR (DMSO-d_6) δ 1.68 (3H, s), 7.15–7.34 (30H, m), 8.47 (1H, s).

(*Z*)-2-(Acetoxyimino)-2-(2-amino-5-bromothiazol-4-yl)-acetic acid (12). *N*-Bromosuccimide (10.65 g, 60 mmol) was added portionwise to a solution of **11** (6.9 g, 30 mmol) in MeOH (75 mL) at room temperature. Stirring was continued for 30 min and the resulting precipitate was collected by filtration. The precipitate was dissolved in water (20 mL) and the pH was adjusted to pH 3.5

with saturated $NaHCO_3$. The resulting precipitate was collected by filtration, washed with water and dried to afford **12**. Amorphous solid. Yield: 3.8 g (42%). IR (KBr) cm^{-1} 3479, 3122, 1760, 1639, 1614; ^1H NMR (DMSO-d_6) δ 2.20 (3H, s), 7.58 (2H, s), 7.79 (1H, s).

Ethyl (*Z*)-2-(4-thiazolyl)-2-(trityloxyimino)acetate (14). To a solution of ethyl (2-aminothiazol-4-yl)-2-(*Z*)-(trityloxyimino)acetate (**13**) (9.15 g, 20 mmol) in THF (100 mL) *tert*-butylnitrate (3.7 mL, 30.8 mmol) in THF (20 mL) was added over 20 min at 50 °C. The mixture was stirred for 1 h at the same temperature until the gas evolution ceased, and poured into a mixture of ethyl acetate and water. The aqueous layer was separated, the organic layer was washed with water and brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **14**. Amorphous solid. Yield: 5.5 g (62%). IR (KBr) cm^{-1} 1733, 1587; ^1H NMR (DMSO-d_6) δ 1.38 (3H, t, $J=7.1$ Hz), 4.51 (2H, q, $J=7.1$ Hz), 6.98–7.40 (15H, m), 7.47 (1H, d, $J=2.1$ Hz), 8.73 (1H, d, $J=2.1$ Hz). FABMS m/z 443.1 [$(M+H)^+$].

The following compound was obtained using a method similar to that used for **10**.

(*Z*)-2-(4-Thiazolyl)-2-(trityloxyimino)acetic acid (15). Amorphous solid. Yield: 4.3 g (84%). IR (KBr) cm^{-1} 3446, 1735; ^1H NMR (DMSO-d_6) δ 7.25–7.40 (15H, m), 7.79 (1H, d, $J=1.9$ Hz), 9.12 (1H, d, $J=1.9$ Hz). FABMS m/z 415.2 [$(M+H)^+$].

Diphenylmethyl 7β-amino-3-[(1-tritylpyrazol-4-yl)-methylthio]-3-cephem-4-carboxylate (17). To a stirred solution of diphenylmethyl 7β-amino-3-methanesulfonyloxy-3-cephem-4-carboxylate (**16**) (5.0 g, 10.8 mmol) in DMF (60 mL) was added a mixture of sodium hydrosulfide (70%, 1.12 g, 14 mmol) and diisopropylethylamine (2.8 mL, 19.1 mmol) in DMF (42 mL) at −20 °C. The solution was stirred for 1 h and 4-chloromethyl-1-tritylpyrazole (5.45 g, 17.2 mmol) was added dropwise. The whole mixture was stirred for another hour below −5 °C. The mixture was poured into a mixture of water and ethyl acetate and the resulting precipitate was collected by filtration. The crude product was washed with a mixture of isopropyl ether and ethyl acetate (1:1) and dried under reduced pressure to afford **17**. Amorphous solid. Yield: 4.4 g (56%). IR (KBr) cm^{-1} 3419, 1772, 1731, 1689, 1600; ^1H NMR (DMSO-d_6) δ 3.75 (2H, s), 3.96 (2H, s), 4.70–4.80 (1H, m), 4.90 (1H, dd, $J=4.9$ Hz), 6.81 (1H, s), 7.00–7.60 (27H, m).

Diphenylmethyl 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(ethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (23c). Compound **17** (2.88 g, 4.0 mmol) was dissolved in CH_2Cl_2 (40 mL) by addition of BSA (1.64 g, 8.06 mmol). To the resulting solution was added **18c** (1.1 g, 4.0 mmol) at 5 °C and the mixture was stirred at the same temperature for 2 h and at room temperature for 1 h. The mixture was diluted with water and adjusted to pH 7 by addition of 1 N aqueous sodium hydroxide. The aqueous layer was separated and the organic layer was washed with brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **23c**. Amorphous solid. Yield: 2.25 g (61%). IR (KBr) cm^{-1} 3332, 1785, 1681, 1612, 1533; ^1H NMR (DMSO-d_6) δ 1.22 (3H, t, $J=7.0$ Hz), 3.79 (2H, s), 4.04 (2H, s), 4.11 (2H, q, $J=7.0$ Hz), 5.16 (1H, d, $J=4.6$ Hz), 5.79 (1H, dd, $J=8.3$, 4.6 Hz), 6.78 (1H, s), 6.84 (1H, s), 6.8–7.6 (27H, m), 9.60 (1H, d, $J=8.3$ Hz).

The following compounds were obtained from **16** and the corresponding acid chloride (**18b** or **19n**) using a method similar to that used for **23c**.

Diphenylmethyl 7β-[(Z)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (34b). Amorphous solid. Yield: 365 mg (18%). IR (KBr) cm^{-1} 2925, 1780, 1741, 1665; ^1H NMR (DMSO-d_6) 3.17 (3H, s), 3.70 and 4.00 (2H, ABq, $J=18.0$ Hz), 3.83 (3H, s), 5.31 (1H, d, $J=4.9$ Hz), 5.92 (1H, dd, $J=8.3$, 4.9 Hz), 6.73 (1H, s), 6.92 (1H, s), 7.1–7.6 (10H, m), 9.69 (1H, d, $J=8.3$ Hz).

Diphenylmethyl 7β-[(Z)-2-(5-aminothiadiazol-3-yl)-2-(3-cyclopentenyloxyimino)acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (35n). Amorphous solid. Yield: 10.4 g (74%). IR (KBr) cm^{-1} 3342, 1793, 1733, 1683, 1621, 1525; ^1H NMR (DMSO-d_6) d 1.8–2.4 (4H, m), 3.18 (1H, s), 3.71 and 4.00 (2H, ABq, $J=18.2$ Hz), 5.30–5.35 (2H, m), 5.85–6.00 (2H, m), 6.11–6.14 (1H, m), 6.91 (1H, s), 7.2–7.60 (10H, m), 8.14 (2H, s), 9.63 (1H, d, $J=8.5$ Hz).

Diphenylmethyl 7β-[(Z)-2-(2-tritylaminothiazol-4-yl)-2-(fluoromethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)-methylthio]-3-cephem-4-carboxylate (24d). Under a N_2 atmosphere, $POCl_3$ (0.48 mL, 5.3 mmol) was added to a solution of DMF (0.4 mL, 5.3 mmol) in ethyl acetate (2.5 mL) with ice-cooling. After 10 min, the mixture was diluted with THF (25 mL) and then **20d** (2.2 g, 4.8 mmol) was added to the mixture. The mixture was stirred for 1 h at the same temperature (solution A). In another flask, BSA (5.9 mL, 24 mmol) was added to a solution of **17** (2.88 g) in DMF (30 mL) with ice-cooling (solution B). Solution A was added slowly to solution B under stirring for 30 min The whole mixture was stirred for 1 h at 0 °C, then it was poured into a mixture of water and ethyl acetate while the reaction pH was adjusted to between 6 and 7 with 10% aqueous K_2CO_3. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **24d**. Amorphous solid. Yield: 3.8 g (83%). IR (KBr) cm^{-1} 1785, 1693, 1594; ^1H NMR (DMSO-d_6) δ 3.74 (2H, s), 4.02 (2H, s), 5.13 (1H, d, $J=4.5$ Hz), 5.65 (1H, dd, $J=7.9$, 4.5 Hz), 5.70 (2H, d, $J=55.5$ Hz), 6.83 (1H, s), 6.9–7.6 (43H, m), 8.92 (1H, s), 9.75 (1H, d, $J=7.9$ Hz).

The following compounds were obtained using a method similar to that used for **24d**.

Diphenylmethyl 7β-[(*Z*)-2-(2-tritylaminothiazol-4-yl)-2-(difluoromethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (24e). Amorphous solid. Yield: 4.2 g (88%). IR (KBr) cm^{-1} 3388, 1785, 1695, 1527; ^1H NMR (DMSO-d_6) δ 3.74 (2H, s), 4.03 (2H, s), 5.15 (1H, d, $J=4.6$ Hz), 5.67 (1H, dd, $J=7.9$, 4.6 Hz), 6.83 (1H, s), 6.9–7.8 (45H, m), 9.89 (1H, d, $J=7.9$ Hz).

Diphenylmethyl 7β-[(*Z*)-2-(2-tritylaminothiazol-4-yl)-2-(2-fluoroethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)-methylthio]-3-cephem-4-carboxylate (24f). Amorphous solid. Yield: 3.0 g (64%). IR (KBr) cm^{-1} 1785, 1731, 1687, 1594; ^1H NMR (DMSO-d_6) δ 3.74 (2H, s), 4.02 (2H, s), 4.26 (2H, dt, $J=29.3$, 4.3 Hz), 4.62 (2H, dt, $J=47.7$, 3.8 Hz), 5.12 (1H, d, $J=4.5$ Hz), 5.66 (1H, dd, $J=8.0$, 4.5 Hz), 6.80 (1H, s), 6.83 (1H, s), 6.9–7.6 (42H, m), 8.83 (1H, s), 9.59 (1H, d, $J=8.0$ Hz).

Diphenylmethyl 7β-[(*Z*)-2-(2-formamidothiazol-4-yl)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (25g). Amorphous solid. Yield: 7.2 g (70%). IR (KBr) cm^{-1} 3266, 1783, 1727, 1691; ^1H NMR (DMSO-d_6) δ 1.43 (9H, s), 3.78 (2H, s), 4.04 (2H, s), 4.62 (2H, s), 5.19 (1H, d, $J=4.5$ Hz), 5.83 (1H, dd, $J=8.3$, 4.5 Hz), 6.84 (1H, s), 7.0–7.6 (28H, m), 8.53 (1H, s), 9.70 (1H, d, $J=8.3$ Hz), 12.66 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-formamidothiazol-4-yl)-2-(1-*tert*-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (25i). Amorphous solid. Yield: 1.8 g (41%). IR (KBr) cm^{-1} 1787, 1726, 1693, 1604; ^1H NMR (DMSO-d_6) δ 1.39 (9H, s), 1.44 (3H, s), 1.46 (3H, s), 3.81 (2H, s), 4.03 (2H, s), 5.18 (1H, d, $J=4.7$ Hz), 5.87 (1H, dd, $J=8.6$, 4.7 Hz), 6.84 (1H, s), 6.9-7.6 (28H, m), 8.49 (1H, s), 9.54 (1H, d, $J=8.6$ Hz), 12.66 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(5-fluoro-2-formylaminothiazol-4-yl)-2-(3-formyloxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (26k). Amorphous solid. Yield: 1.3 g (46%). ^1H NMR (DMSO-d_6) δ 3.80 (2H, br s), 4.05 (2H, br s), 5.15 (1H, d, $J=4.7$ Hz), 5.87 (1H, dd, $J=8.7$, 4.7 Hz), 6.84 (1H, s), 7.00–7.55 (27H, m), 7.95 (1H, s), 8.48 (1H, s), 9.6 (1H, $J=8.7$ Hz), 11.89 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-chlorothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (27b). To a solution of (*Z*)-2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid (**22b**) (1.70 g, 7.2 mmol) in DMAc (20 mL) was added K_2CO_3 (995 mg, 7.2 mmol) and methanesulfonyl chloride (1.1 mL, 14.4 mmol) at 5 °C. The mixture was stirred for 30 min at the same temperature. In another flask to a solution of **17** (4.32 g, 6 mmol) in DMAc (45 mL) was added BSA (8.9 mL, 36 mmol) at 5 °C and stirred for 20 min. To this solution was added the above mentioned solution of the activated acid. The mixture was stirred at 5 °C for 1 h and poured into a mixture of water (200 mL) and ethyl acetate (200 mL). The aqueous layer was separated and the organic layer was washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **27b**. Amorphous solid. Yield: 3.6 g (64%). IR (KBr) cm^{-1} 3324, 1781, 1689, 1612; ^1H NMR (DMSO-d_6) δ 3.80 (2H, s), 3.86 (3H, s), 4.02 (2H, s), 5.12 (1H, d, $J=4.6$ Hz), 5.81 (1H, dd, $J=8.7$, 4.6 Hz), 6.83 (1H, s), 6.9–7.6 (29H, m), 9.61 (1H, d, $J=8.7$ Hz).

The following compounds were obtained using a method similar to that used for **27b**.

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-chlorothiazol-4-yl)-2-(acetoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)-methylthio]-3-cephem-4-carboxylate (27l). Amorphous solid. Yield: 3.9 g (81%). IR (KBr) cm^{-1} 3331, 1780, 1693, 1616; ^1H NMR (DMSO-d_6) δ 2.18 (3H, s), 3.83 (2H, s), 4.04 (2H, s), 5.19 (1H, d, $J=4.6$ Hz), 5.81 (1H, dd, $J=8.5$, 4.6 Hz), 6.84 (1H, s), 6.9–7.6 (29H, m), 9.91 (1H, d, $J=8.5$ Hz). FABMS m/z 968 [$(M+H)^+$].

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-bromothiazol-4-yl)-2-(acetoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)-methylthio]-3-cephem-4-carboxylate (28l). Amorphous solid. Yield: 4.6 g (90%). IR (KBr) cm^{-1} 1780, 1695, 1614; ^1H NMR (DMSO-d_6) δ 2.19 (3H, s), 3.83 (2H, s), 4.04 (2H, s), 5.19 (1H, d, $J=4.7$ Hz), 5.83 (1H, dd, $J=8.4$, 4.7 Hz), 6.84 (1H, s), 6.95–7.6 (29H, m), 9.90 (1H, d, $J=8.4$ Hz). FABMS m/z 1012[$(M+H)^+$].

Diphenylmethyl 7β-[(*Z*)-(4-thiazolyl)-2-(trityloxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (44). Amorphous solid. Yield: 4.8 g (quant). IR (KBr) cm^{-1} 1791, 1689; ^1H NMR (DMSO-d_6) δ 3.80 (2H, s), 4.05 (2H, s), 5.27 (1H, d, $J=4.7$ Hz), 5.99 (1H, dd, $J=8.6$, 4.7 Hz), 6.86 (1H, s), 6.98–7.60 (42H, m), 7.76 (1H, d, $J=1.9$ Hz), 9.12 (1H, d, $J=1.9$ Hz), 9.95 (1H, d, $J=8.6$ Hz). FABMS m/z 1117 [M^+].

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(*tert*-butoxycarbonylmethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylate (29g). To a solution of **25g** (7.2 g, 6.95 mmol) in a mixture of MeOH (70 mL) and THF (30 mL) was added dropwise concentrated aqueous HCl (2.9 mL, 34.7 mmol) at room temperature. After the mixture was stirred for 3 h, the solvent was evaporated. The residue was diluted with a mixture of ethyl acetate and water, while the pH was adjusted to between 8 and 8.5 with saturated aqueous $NaHCO_3$. The aqueous layer was separated and the organic layer was washed with water and brine, and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **29g**. Amorphous solid. Yield: 4.4 g (83%). IR (KBr) cm^{-1} 3293, 3201, 1783, 1726, 1685, 1618; ^1H NMR (DMSO-d_6) δ 1.43 (9H, s), 3.85 (2H, s), 4.07 (2H, s), 4.56 (2H, s), 5.23 (1H, d, $J=4.6$ Hz), 5.78 (1H, dd, $J=8.3$, 4.6 Hz), 6.83 (1H, s), 6.85 (1H, s), 7.2–7.7 (14H, m), 9.58 (1H, d, $J=8.3$ Hz), 12.76 (1H, s).

The following compounds were obtained using a method similar to that used for **26g**.

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(1-*tert*-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio] - 3 - cephem - 4 - carboxylate (29i). Amorphous solid. Yield: 0.83 g (63%). IR (KBr) cm^{-1} 3357, 1783, 1722, 1685, 1618; ^1H NMR (DMSO-d_6) δ 1.40 (9H, s), 1.43 (3H, s), 1.44 (3H, s), 3.89 (2H, s), 4.06 (2H, s), 5.24 (1H, d, $J=4.6$ Hz), 5.83 (1H, dd, $J=8.6$, 4.6 Hz), 6.76 (1H, s), 6.84 (1H, s), 7.2–7.8 (14H, m), 9.46 (1H, d, $J=8.6$ Hz), 12.76 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-fluorothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylate (30a). Amorphous solid. Yield: 1.1 g (quant). ^1H NMR (DMSO-d_6) δ 3.85 (2H, br s), 4.07 (2H, br s), 5.19 (1H, d, $J=4.7$ Hz), 5.80 (1H, dd, $J=8.7$, 4.5 Hz), 6.83 (1H, s), 7.10–7.23 (14H, m), 9.48 (1H, d, $J=8.7$ Hz), 11.55 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-chlorothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylate (31a). Amorphous solid. Yield: 0.55 g (81%). IR (KBr) cm^{-1} 3365, 1776, 1679; ^1H NMR (DMSO-d_6) δ 3.85 (2H, br s), 4.01 (2H, br s), 5.19 (1H, d, $J=4.7$ Hz), 5.82 (1H, dd, $J=8.7$, 4.5 Hz), 6.83 (1H, s), 7.10–7.80 (15H, m), 9.46 (1H, d, $J=8.7$ Hz), 11.73 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-bromothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylate (32a). Amorphous solid Yield: 2.8 g (86%). IR (KBr) cm^{-1} 3330, 1776, 1677, 1612; ^1H NMR (DMSO-d_6) δ 3.85 (2H, s), 4.04 (2H, s), 5.19 (1H, d, $J=4.7$ Hz), 5.81 (1H, dd, $J=8.7$, 4.7 Hz), 6.83 (1H, s), 7.2–7.6 (14H, m), 9.43 (1H, d, $J=8.7$ Hz), 11.73 (1H, s).

Diphenylmethyl 7β-[(*Z*)-2-(2-amino-5-methylthiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylate (33a). Under a N_2 atmosphere, $POCl_3$ (0.25 mL, 2.7 mmol) was added to a solution of DMF (0.21 mL, 2.7 mmol) in ethyl acetate (2 mL) with ice-cooling. After 10 min, the mixture was diluted with ethyl acetate (5 mL) and then **10** (1.7 g, 2.5 mmol) was added to the mixture. The mixture was stirred for 1 h at the same temperature (solution A). BSA (5.4 mL) was added to a solution of **17** (1.6 g, 2.2 mmol) in DMAc (16 mL) with ice-cooling (solution B).

Solution A was added slowly to solution B under stirring for 30 min The whole mixture was stirred for 2 h at 0 °C, then it was poured into a mixture of water and ethyl acetate while the reaction pH was adjusted to 7 with 10% aqueous K_2CO_3. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was dissolved in MeOH (20 mL) and concentrated aqueous HCl (0.5 mL) was added at room temperature. After the mixture was stirred for 4 h, the solvent was evaporated. The residue was diluted with a mixture of ethyl acetate and water, while the pH was adjusted to 6.5 with saturated aqueous $NaHCO_3$. The aqueous layer was separated and the organic layer was washed with water and brine, dried over $MgSO_4$. After evaporation of the solvent, the residue was triturated with isopropyl ether to afford **33a**. Amorphous solid. Yield: 0.6 g (41%). IR (KBr) cm^{-1} 1761, 1686, 1674, 1605. ^1H NMR (DMSO-d_6) δ 1.84 (3H, s), 3.86 (2H, s), 3.98 and 4.10 (2H, ABq, $J=16.1$ Hz), 5.30 (1H, d, $J=4.7$ Hz), 6.02 (1H, dd, $J=9.0$, 4.7 Hz), 6.86 (1H, s), 7.20–7.60 (14H, m), 9.70 (1H, d, $J=9.0$ Hz). FABMS; 662 [$(M+H)^+$].

Diphenylmethyl 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio]-3-cephem-4-carboxylate (36b). Under a N_2 atmosphere, 1.2 N sodium methoxide in MeOH (2.0 mL, 2.4 mmol) was added slowly to a solution of 1-methyl-4-benzoylthiomethylpyrazole (1.1 g, 2.4 mmol) in a mixture of THF (4 mL) and DMF (12 mL) at 0 °C. The mixture was stirred for 1 h and cooled to −78 °C with a dry ice–acetone bath. In another flask, **34b** (1.54 g, 2.4 mmol) was dissolved in a mixture of THF (1.5 mL) and DMF (4.5 mL) and cooled with a dry ice-acetone bath. To this solution, the above sodium thiolate generated in situ was added dropwise while the temperature was maintained below −65 °C. After stirring for 1 h, the reaction was quenched with 10% aqueous HCl. The mixture was poured into a mixture of water and ethyl acetate and the aqueous layer was separated. The organic layer was washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **36b**. Amorphous solid. Yield: 690 mg (32%). ^1H NMR (DMSO-d_6) δ 3.79 (2H, s), 3.85 (2H, s), 4.05 (2H, s), 5.16 (1H, d, $J=4.6$ Hz), 5.77 (1H, dd, $J=8.3$, 4.6 Hz), 6.75 (1H, s), 6.80 (1H, s), 6.9–7.6 (27H, m), 9.66 (1H, d, $J=8.3$ Hz).

The following compound was obtained using a method similar to that used for **36b**.

Diphenylmethyl 7β-[(*Z*)-2-(5-aminothiadiazol-3-yl)-2-(3-cyclopentenyloxyimino)acetamido]-3-[(1-tritylpyrazol-4-yl)methylthio] - 3 - cephem - 4 - carboxylate (37n). Amorphous solid. Yield: 1.50 g (39%). IR (KBr) cm^{-1} 1781, 1683, 1618, 1519; ^1H NMR (DMSO-d_6) δ 3.39 (2H, s), 3.79 (2H, s), 4.03 (2H, s), 5.12 (1H, d, $J=4.7$ Hz), 5.3–5.4 (1H, m), 5.8–5.95 (2H, m), 7.55 (2H, s), 6.05–6.15 (1H, m), 6.83 (1H, s), 7.0–7.60 (12H, m), 8.15 (2H, s), 9.57 (1H, d, $J=8.8$ Hz).

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38b). To a solution of **36b** (0.5 g, 0.55 mmol) in CH_2Cl_2 (1.5 mL) and anisole (0.5 mL) was added trifluoroacetic acid (1.0 mL) at 5 °C. The mixture was stirred for 1.5 h at the same temperature and poured into IPE (30 mL). The resulting precipitate was collected by filtration, washed with IPE and dried in vacuo. The crude product was dissolved in pH 6.86 buffer (150 mL) and the solution was stirred for 2 h maintaining pH 6.8 with aqueous $NaHCO_3$ solution. The solution was concentrated in vacuo and adjusted to pH 5 with 1 N

HCl and chromatographed on HP-20 (100 mL) eluting with 80% aqueous MeOH. The fractions containing the object compound were collected and lyophilized to give crude product, which was purified by preparative HPLC utilizing a C18 μ Bondapak resin to afford **38b**. Amorphous solid. Yield: 171 mg (63%). IR (KBr) cm^{-1} 1780, 1745, 1673, 1635; ^1H NMR (DMSO-d_6) δ 3.21 and 3.43 (2H, ABq, $J=17.4$ Hz), 3.70 and 3.78 (2H, ABq, $J=13.9$ Hz), 3.79 (3H, s), 4.95 (1H, d, $J=4.7$ Hz), 5.56 (1H, dd, $J=8.3$, 4.6 Hz), 6.74 (1H, s), 7.23 (2H, s), 7.52 (2H, s), 9.54 (1H, d, $J=8.3$ Hz). Anal. calcd for $C_{17}H_{17}N_7O_5S_3$ $3.0H_2O$: C, 37.15; H, 4.22; N, 17.84; found: C, 37.21; H, 4.11; N, 17.78.

The following compounds were obtained using a method similar to that used for **38b**.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(ethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38c). Amorphous solid. Yield: 544 mg (44%). IR (KBr) cm^{-1} 3318, 1764, 1660, 1608, 1533; ^1H NMR (DMSO-d_6) δ 1.22 (3H, t, $J=7.0$ Hz), 3.74 (2H, s), 4.00 (2H, s), 4.10 (2H, q, $J=7.0$ Hz), 5.13 (1H, d, $J=4.6$ Hz), 5.68 (1H, dd, $J=8.3$, 4.6 Hz), 6.74 (1H, s), 7.22 (2H, s), 7.55 (2H, s), 9.55 (1H, d, $J=8.3$ Hz). Anal. calcd for $C_{18}H_{19}N_7O_5S_3$ $1.6H_2O$: C, 40.16; H, 4.16; N, 18.21; found: C, 40.08; H, 3.97; N, 17.97.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(fluoromethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38d). Yield: 555 mg (34%). IR (KBr) cm^{-1} 3311, 1768, 1675, 1621; ^1H NMR (DMSO-d_6) δ 3.70 and 3.80 (2H, ABq, $J=17.0$ Hz), 4.00 and 4.05 (2H, ABq, $J=13.3$ Hz), 5.17 (1H, d, $J=4.6$ Hz), 5.69 (1H, dd, $J=8.0$, 4.6 Hz), 5.73 (1H, d, $J=55.6$ Hz), 6.95 (1H, s), 7.31 (2H, s), 7.55 (2H, s), 9.80 (1H, d, $J=8.0$ Hz), 13.0 (1H, br s). Anal. calcd for $C_{17}H_{16}N_7O_5S_3$ $1.3H_2O$: C, 38.03; H, 3.49; N, 18.26; found: C, 38.03; H, 3.43; N, 17.99.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(difluoromethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38e). Yield: 485 mg (26%). IR (KBr) cm^{-1} 3332, 1770, 1677, 1618; ^1H NMR (DMSO-d_6) δ 3.74 and 3.80 (2H, ABq, $J=17.1$ Hz), 4.00 and 4.06 (2H, ABq, $J=13.1$ Hz), 5.18 (1H, d, $J=4.6$ Hz), 5.71 (1H, dd, $J=8.0$, 4.6 Hz), 7.04 (1H, s), 7.13 (1H, t, $J=71.1$ Hz), 7.37 (2H, s), 7.55 (2H, s), 9.93 (1H, d, $J=8.0$ Hz). Anal. calcd for $C_{17}H_{15}F_2N_7O_5S_3$ $1.3H_2O$: C, 36.79; H, 3.20; N, 17.67; found: C, 36.74; H, 3.04; N, 17.41.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(2-fluoroethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38f). Yield: 430 mg (31%) IR (KBr) cm^{-1} 3320, 1766, 1670, 1533; ^1H NMR (DMSO-d_6) δ 3.76 and 3.78 (2H, ABq, $J=17.1$ Hz), 4.02 and 4.03 (2H, ABq, $J=13.3$ Hz), 4.29 (2H, dt, $J=29.7$, 4.20 Hz), 4.65 (2H, dt, $J=47.8$, 3.8 Hz), 5.15 (1H, d, $J=4.6$ Hz), 5.70 (1H, dd, $J=8.10$, 4.6 Hz), 6.80 (1H, s), 7.25 (2H, s), 7.55 (2H, s), 9.64 (1H, d, $J=8.1$ Hz), 12.97 (1H, br s). Anal. calcd for $C_{18}H_{18}FN_7O_5S_3$ $1.7H_2O$: C, 38.73; H, 3.86; N, 17.57; found: C, 38.82; H, 3.73; N, 17.31.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(carboxymethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38 h). Amorphous solid. Yield: 314 mg (30%). IR (KBr) cm^{-1} 3351, 1766, 1672, 1635; ^1H NMR (DMSO-d_6) δ 3.73 and 3.80 (2H, ABq, $J=17.0$ Hz), 4.03 (2H, s), 4.59 (2H, s), 5.16 (1H, d, $J=4.6$ Hz), 5.70 (1H, dd, $J=8.2$, 4.6 Hz), 6.83 (1H, s), 7.26 (2H, s), 7.55 (1H, s), 9.55 (1H, d, $J=8.2$ Hz). Anal. calcd for $C_{18}H_{17}N_7O_7S_3$ $1.7H_2O$: C, 37.92; H, 3.61; N, 17.20; found: C, 38.00; H, 3.59; N, 16.97.

7β-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (38j). Amorphous solid. Yield: 207 mg (35%). IR (KBr) cm^{-1} 1770, 1675, 1637; ^1H NMR (DMSO-d_6) δ 1.45 (3H, s), 1.47 (3H, s), 3.80 (2H, s), 4.03 (2H, s), 5.18 (1H, d, $J=4.7$ Hz), 5.74 (1H, dd, $J=8.6$, 4.7 Hz), 6.79 (1H, s), 7.56 (2H, s), 9.47 (1H, d, $J=8.7$ Hz). Anal. calcd for $C_{20}H_{21}N_7O_7S_3$ $3.1H_2O$: C, 38.53; H, 4.40; N, 15.73; found: C, 38.83; H, 4.27; N, 15.32.

7β-[(*Z*)-2-(2-Amino-5-fluorothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (39a). Amorphous solid. Yield: 142 mg (19%). IR (KBr) cm^{-1} 1766, 1673, 1621, 1535, 1390, 1218; ^1H NMR (DMSO-d_6) δ 3.74 (2H, br s), 4.05 and 3.97 (2H, ABq, $J=13.4$ Hz), 5.11 (1H, d, $J=4.7$ Hz), 5.16 (1H, d, $J=8.7$, 4.7 Hz), 7.00 (2H, br s), 7.55 (2H, s), 9.41 (1H, d, $J=8.7$ Hz), 11.50 (1H, s). FABMS *m*/*z* 499.9 [$(M)^+$].

7β-[(*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (40a). Amorphous solid. Yield: 418 mg (35%). IR (KBr) cm^{-1} 3236, 1764, 1648, 1616; ^1H NMR (DMSO-d_6) δ 3.75 (2H, s), 4.00 (2H, s), 5.11 (1H, d, $J=4.7$ Hz), 5.72 (1H, dd, $J=8.6$, 4.7 Hz), 7.29 (2H, s), 7.55 (2H, s), 9.39 (1H, d, $J=8.6$ Hz), 11.70 (1H, s). FABMS *m*/*z* 515.9 [$(M+H)^+$]. Anal. calcd for $C_{16}H_{14}ClN_7O_5S_3$ $2.3H_2O$: C, 34.48; H, 3.36; N, 17.59; found: C, 34.61; H, 3.14; N, 17.36.

7β-[(*Z*)-2-(2-Amino-5-chlorothiazol-4-yl)-2-(methoxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (40b). Amorphous solid. Yield: 510 mg (37%). IR (KBr) cm^{-1} 3324, 1770, 1673, 1619; ^1H NMR (DMSO-d_6) δ 3.77 (2H, s), 3.86 (3H, s), 4.01 (2H, s), 5.12 (1H, d, $J=4.6$ Hz), 5.71 (1H, dd, $J=8.6$, 4.6 Hz), 7.39 (2H, s), 7.55 (2H, s), 9.57 (1H, d, $J=8.6$ Hz). Anal. calcd for $C_{17}H_{16}ClN_7O_5S_3$ $1.2H_2O$: C, 37.02; H, 3.36; N, 17.77; found: C, 36.97; H, 3.32; N, 17.58.

7β-[(*Z*)-2-(2-Amino-5-bromothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (41a). Amorphous solid. Yield: 1.31 g (61%). IR (KBr) cm^{-1} 3236, 1766, 1648; ^1H NMR (DMSO-d_6) δ 3.75 (2H, s), 4.00 (2H, s), 5.11 (1H, d, $J=4.7$ Hz), 5.71 (1H, dd, $J=8.6$, 4.7 Hz), 7.31 (2H, s), 7.54 (2H, s), 9.36 (1H, d, $J=8.6$ Hz), 11.70 (1H, s). FABMS *m*/*z* 558.1[M^+].

7β-[(*Z*)-2-(2-amino-5-methylthiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (42a). Amorphous solid. Yield: 140 mg (31%). IR (KBr) cm^{-1} 1765, 1668, 1610, 1535, 1392, 1354; ^1H NMR (DMSO-d_6) δ 2.33 (3H, s), 3.75 (2H, s), 3.90–4.10 (2H, m), 5.10 (1H, d, $J=4.7$ Hz), 5.72 (1H, dd, $J=8.7$, 4.7 Hz), 6.83 (2H, s), 7.55 (2H, s), 9.27 (1H, d, $J=8.7$ Hz), 11.29 (1H, s). FABMS m/z 495.9 [M^+].

7β-[(*Z*)-2-(5-Aminothiadiazol-3-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (43). Amorphous solid. Yield: 323 mg (43%). IR (KBr) cm^{-1} 3307, 1764, 1670, 1619; ^1H NMR (DMSO-d_6) δ 3.76 (2H, s), 4.02 (2H, s), 5.12 (1H, d, $J=4.7$ Hz), 5.75 (1H, dd, $J=8.6$, 4.7 Hz), 7.55 (2H, s), 8.06 (2H, s), 9.45 (1H, d, $J=8.6$ Hz). Anal. calcd for $C_{15}H_{14}N_8O_5S_3$ $1.8H_2O$: C, 34.99; H, 3.44; N, 21.76; found: C, 35.25; H, 3.28; N, 21.39.

7β-[(*Z*)-2-(4-thiazolyl)-2-(hydroxyimino)acetamido]-3-[(4-pyrazolyl)methylthio]-3-cephem-4-carboxylic acid (45). Under a N_2 atmosphere, a solution of $AlCl_3$ (2.86 g, 21.3 mmol) in anisole (8.5 mL) was added slowly to a solution of **44** (4.8 g, 4.29 mmol) in a mixture of anisole (8.5 mL) and CH_3NO_2 (35 mL) at −20 to −30 °C. The mixture was stirred for 1 h at the same temperature and the reaction was quenched with 1 N HCl (35 mL). The mixture was poured into a mixture of ethyl acetate and water, and the aqueous layer was separated. The organic layer was reextracted with water several times and the combined aqueous layer was concentrated in vacuo, chromatographed on a HP-20 (200 mL) column eluting with aqueous MeOH. The fractions containing the object compound were collected and lyophilized to give crude product, which was purified by preparative HPLC described above afforded **45**. Amorphous solid. Yield: 770 mg (39%). IR (KBr) cm^{-1} 3381, 1768, 1668; ^1H NMR (DMSO-d_6) δ 3.75 (2H, s), 4.01 (2H, s), 5.16 (1H, d, $J=4.7$ Hz), 5.76 (1H, dd, $J=8.2$, 4.7 Hz), 7.55 (2H, s), 7.85 (1H, d, $J=1.9$ Hz), 9.14 (1H, d, $J=1.9$ Hz), 9.54 (1H, d, $J=8.6$ Hz), 11.67 (1H, s), 12.98 (1H, br s). FABMS m/z 467.0 [M^+]. Anal. calcd for $C_{16}H_{14}N_6O_5S_3$ $1.0H_2O$: C, 39.66; H, 3.33; N, 17.12; found: C, 39.59; H, 3.30; N, 17.12.

Measurement of in vitro antibacterial activity

According to the method of the Japan Society of Chemotherapy, the MICs of compounds were determined by the 2-fold agar dilution method using heart infusion agar (Eiken). The inoculum size was adjusted to 10^6 cfu/mL, and incubation was carried out at 37 °C for 20 h.

Urinary and biliary recovery

ICR mice and Sprague–Dawley rats were fasted overnight and orally dosed with 20 mg/kg of the test drugs. Urine samples were collected for 24 h after dosing. For bile collection another group of mice and rats was cannulated with a polystyrene tube into the bile duct and test drugs were given orally at doses of 20 mg/kg. The samples were assayed by a disc-agar diffusion method using *E. coli* NIHJ JC-2 or *E. coli* ATCC 33546 as test organism and nutrient agar (Difco) as the test medium.

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References and Notes

1. Yamanaka, H.; Chiba, T.; Kawabata, K.; Takasugi, H.; Masugi, T.; Takaya, T. *J. Antibiot*. **1985**, *38*, 1738.
2. Inamoto, Y.; Chiba, T.; Kamimura, T.; Takaya, T. *J. Antibiot*. **1988**, *41*, 828.
3. Sakagami, K.; Atsumi, K.; Tamura, A.; Yoshida, T.; Nishihata, K.; Fukayasu, S. *J. Antibiot*. **1990**, *43*, 1047.
4. Yamamoto, H.; Terasawa, T.; Nakamura, A.; Kawabata, K.; Takasugi, H.; Tanaka, H.; Matsumoto, S.; Matsumoto, Y.; Tawara, S. *Bioorg. Med. Chem*. **2001**, *9*, 465.
5. Appelbaum, P. C. *Clin. Infect. Dis*. **1992**, *15*, 77.
6. Klugman, K. P. *Clin. Microbiol*. **1990**, *3*, 171.
7. Middleton, W. J. *J. Org. Chem*. **1979**, *44*, 2281.
8. Oikawa, Y.; Yoshioka, T.; Sugano, K.; Yonemitsu, O. *Org. Synth*. **1985**, 198.
9. Blumbach, J.; Duerckheimer, W.; Schrinner, E. Ger. Often., DE2758001-A1 **1979**; *Chem. Abstr*., **1980**, *92*, 58439.
10. Kachi, H.; Okita, T.; Okuyama, S.; Hoshi, H.; Nakatsu, T. *J. Antibiot*. **1990**, *43*, 1564.
11. For the synthesis of **13**. Kamachi, H.; Okita, T.; Okuyama, S.; Hoshi, H.; Naito, T. *J. Antibiot*. **1990**, *43*, 1564.
12. Yokoo, C.; Goi, M.; Onodera, A.; Murata, M.; Nagate, T.; Watanabe, Y. *J. Antibiot*. **1991**, *44*, 498.
13. For the synthesis of **18b**. Marc, M.; Roland, R. Eur. Pat. Appl., EP29966-A2. *Chem. Abstr*. **1981**, *95*, 150689.
14. For the synthesis of **18c**. Mandel, M.; Novak, L.; Rajsner, M.; Holubek, J.; Hola, V. *Collect. Czech. Chem. Commun*. **1989**, *54*, 1734.
15. For the synthesis of **19n**. Goto, J.; Sakane, K.; Teraji, T. *J. Antibiot*. **1984**, *37*, 557.
16. For the synthesis of **20d**: Bell, R., Foxton, Michael W., Looker, B., Eur. Pat. Appl., EP181172-A2 1986; *Chem Abstr*. **1986**, *105*, 152823.
17. For the synthesis of **20e**. Vignau, M.; Heymes, R. Ger. Offen., DE3027281-A1 1981. *Chem. Abstr*. **1981**, *95*, 7310.
18. For the synthesis of **20f**. Yoshida, C.; Tanaka, K.; Todo, Y.; Hattori, R.; Fukuoka, Y.; Komatsu, M.; Saikawa, I. *J. Antibiot*. **1986**, *39*, 90.
19. For the synthesis of **21g**. Takasugi, H.; Kochi, H.; Masugi, T.; Nakano, H.; Takaya, T. *J. Antibiot*. **1983**, *36*, 846.
20. For the synthesis of **21i**. Kenten, J. H.; Von Borstel, R.; Casadei, J. M.; Kamireddy, B.; Martin, M. T.; Massey, R. J.; Napper, A. D.; Simpson, D. M.; Smith, R. G. PCT Int. Appl., WO9302703-A1 1993. *Chem. Abstr*. **1993**, *119*, 210716.
21. For the synthesis of **22b**. Blumbach, J.; Duerckheimer, W.; Schrinner, E. Ger. Offen., DE2758001-A1 1979. *Chem. Abstr*. **1980**, *92*, 58439.
22. For the synthesis of **22l**. Jung, F. H. Eur. Pat. Appl., EP127992-A2 1984. *Chem. Abstr*. **1985**, *102*, 220651.
23. Sakane, K.; Kawabata, K.; Inamoto, Y.; Yamanaka, H.; Takaya, T. *Yakugaku Zassi* **1993**, *113*, 605.
24. Yamamoto, H.; Kawabata, K.; Tawara, S.; Takasugi, H.; Tanaka, H. *J. Antibiot*. **2000**, *53*, 1223.