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Orally Active Cephalosporins. Part 2: Synthesis, Structure–Activity Relationships and Oral Absorption of Cephalosporins Having a C-3 Pyridyl Side Chain

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Abstract—A series of 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]cephalosporins having a pyridine ring connected through various spacer moieties at the C-3 position was designed and synthesized and evaluated for antibacterial activity and oral absorption in rats. All compounds showed potent antibacterial activity against *Staphylococcus aureus*, whereas antibacterial activity against Gram-negative bacteria was markedly influenced by the spacer moiety between the pyridine and cephem nucleus. Oral absorption was influenced by the position of the pyridine nitrogen as well as by the spacer moiety. Among these compounds, FR86830 (**14**), having a 4-pyridylmethylthio moiety at the C-3 position, showed the most well balanced activity and moderate oral absorption.

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 we discovered CFDN, continuous efforts have been made to find a more potent and well balanced compound, especially against *Haemophilus influenzae*, an important pathogen that is the cause of severe respiratory infections. As reported in a previous paper,[4] we modified the C-3 vinyl moiety of CFDN and discovered FR86524 (**2**), having a (*Z*)-2-(3-pyridyl)vinyl moiety at the C-3 position, which showed potent antibacterial activity against *H. influenzae* together with a well balanced profile. These SAR studies revealed that most cephems having a pyridine moiety in the C-3 position exhibit potent antibacterial activity against *H. influenzae*. Further, the C-3 double bond stereochemistry plays an important role in antibacterial activity and the oral absorption. These results prompted us to investigate the antibacterial activity and oral absorption of a series of cephems having a pyridine moiety connected through various spacer moieties at the C-3 position, as shown in Figure 1. In particular, we focused our attention on the investigation of 3- or 4-pyridyl cephems since 2-pyridyl cephems usually show less potent antibacterial activity than the corresponding 3- or 4-analogues.[5] We report herein the synthesis, structure–activity relationships and oral absorption of this series of novel 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]cephalosporins having a pyridine moiety attached through various spacers to the C-3 position.

Chemistry

Scheme 1 outlines the synthesis of a series of cephems with the carbon atom directly attached to the C-3 position. 3-Chloromethylcephem (**19**)[6] was activated with NaI in DMF and reacted with the corresponding mercaptopyridines or pyridylthiocarbinols to give **20a–d** in good yield (79–99%). Although mercaptopyridines reacted with **19** in the absence of base, *N*,*N*-diisopropylethylamine was necessary for pyridylthiocarbinols. Successive treatment of **20** with concentrated HCl in formic acid gave the deprotected compounds **21**, which were coupled with C-7 side chain HOBt ester (**22**) in the presence of trimethylsilyl chloride and Et_3N in THF or with (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetyl

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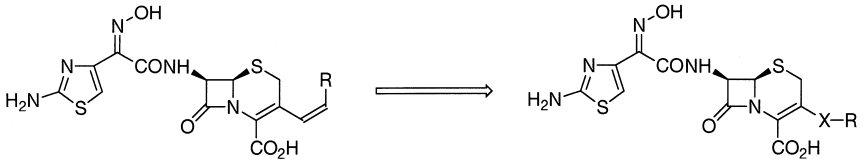
chloride hydrochloride (**23**) in the presence of *N*,*O*-bistrimethylsilyl acetamide (BSA) in CH_2Cl_2 to give partially protected cephems **24** and **25**. Trityl derivatives **24** were deprotected under acidic conditions whereas acetates **25** were deprotected under basic conditions without isolation to afford **3**–**6**. Scheme 2 shows the preparation of a series of cephems bearing an (*E*)-vinylthio moiety. Thus, the (*E*)-thiovinyl analogues were obtained by the reaction of **26**[7] with the corresponding mercaptopyridines or pyridylthiocarbinol in the presence of *N*,*N*-diisopropylethylamine. Subsequent reactions were accomplished using similar methods to those in Scheme 1 to afford **7**–**9**. Scheme 3 shows the synthesis of **10** with an oxygen atom directly attached to the C-3 position. **32d** was synthesized from diphenylmethyl 7β-formamido-3-hydroxy-3-cephem-4-carboxylate (**30**)[8] and 4-pyridylcarbinol (**31**) using a Mitsunobu reaction in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine.[9] After removal of the formyl group, 7β-aminocephem dihydrochloride (**33d**) was coupled with **23**, followed by deprotection of the acetyl moiety to give **34d**. Successive treatment of **34d** with TFA–anisole afforded **10**. Scheme



Cefdinir (**1**) R = H
FR86524 (**2**) R = 3-pyridyl

3-18

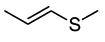
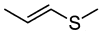
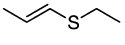
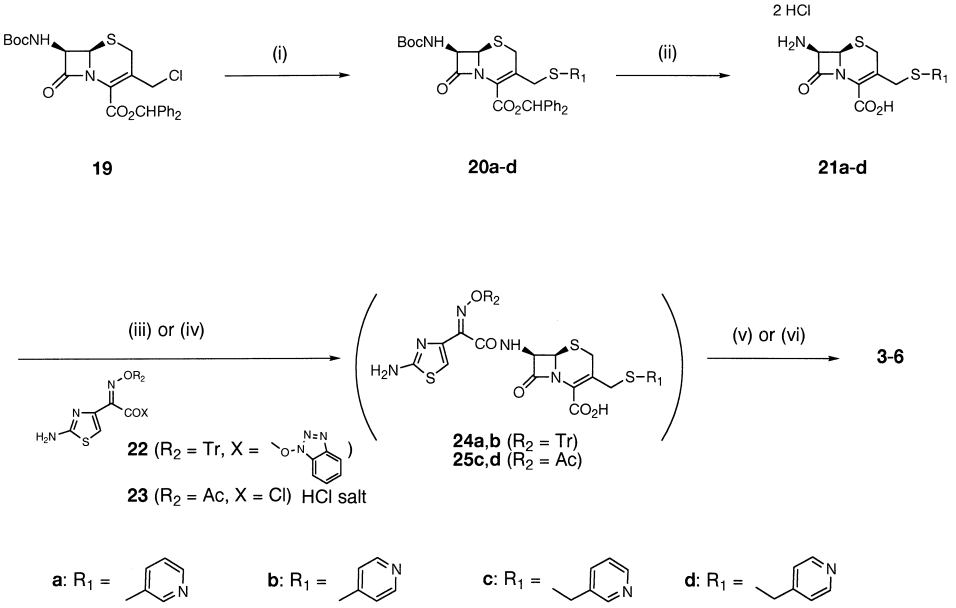
X	R	X	R
3: -CH_2S-	3-pyridyl	**11**: -S-	3-pyridyl
4: -CH_2S-	4-pyridyl	**12**: -S-	4-pyridyl
5: -CH_2SCH_2-	3-pyridyl	**13**: -SCH_2-	3-pyridyl
6: -CH_2SCH_2-	4-pyridyl	**14**: -SCH_2-	4-pyridyl
7: 	3-pyridyl	**15**: -SCH_2CH_2-	4-pyridyl
8: 	4-pyridyl	**16**: 	3-pyridyl
9: 	4-pyridyl	**17**: -SCH_2S-	3-pyridyl
10: -OCH_2-	4-pyridyl	**18**: -SCH_2S-	4-pyridyl

Figure 1. Structure of cephalosporins.



19 **20a-d** **21a-d**

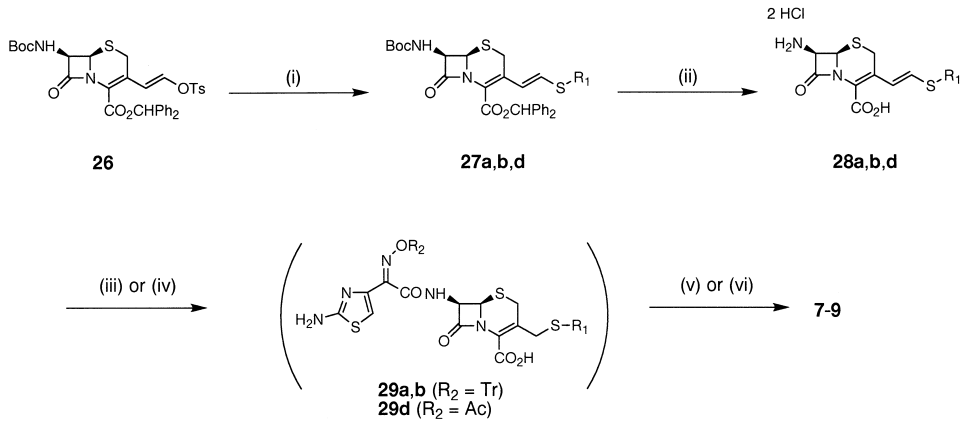
22 (R_2 = Tr, X =)
23 (R_2 = Ac, X = Cl) HCl salt

24a,b (R_2 = Tr)
25c,d (R_2 = Ac)

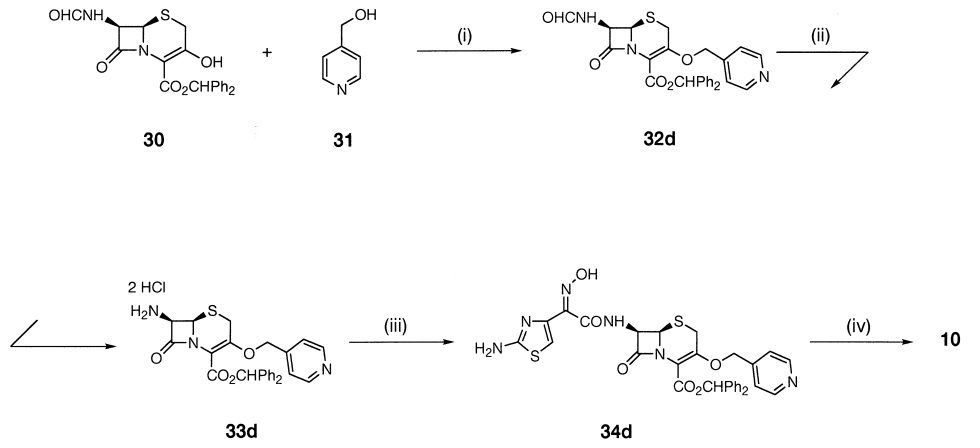
3-6

a: R_1 = **b**: R_1 = **c**: R_1 = **d**: R_1 =

Scheme 1. Reagents: (i) a. NaI, DMF, b. R_1SH (*N*,*N*-diisopropylethylamine). (ii) cHCl, HCO_2H. (iii) TMSCl, **22**, Et_3N, THF, DMF. (iv) *N*,*O*-Bistrimethylsilyl acetamide (BSA), **23**, CH_2Cl_2. (v) $NaHCO_3$, NH_4Cl, MeOH, H_2O. (vi) 90% HCO_2Haq.



Scheme 2. Reagents: (i) R_1SH, *N,N*-diisopropylethylamine, DMF. (ii) cHCl, HCO_2H. (iii) a. **39**, MsCl, K_2CO_3, DMF, b. BSA, DMAc. (iv) BSA, **23**, CH_2Cl_2. (v) $NaHCO_3$, NH_4Cl, MeOH, H_2O. (vi) 90% HCO_2Haq.



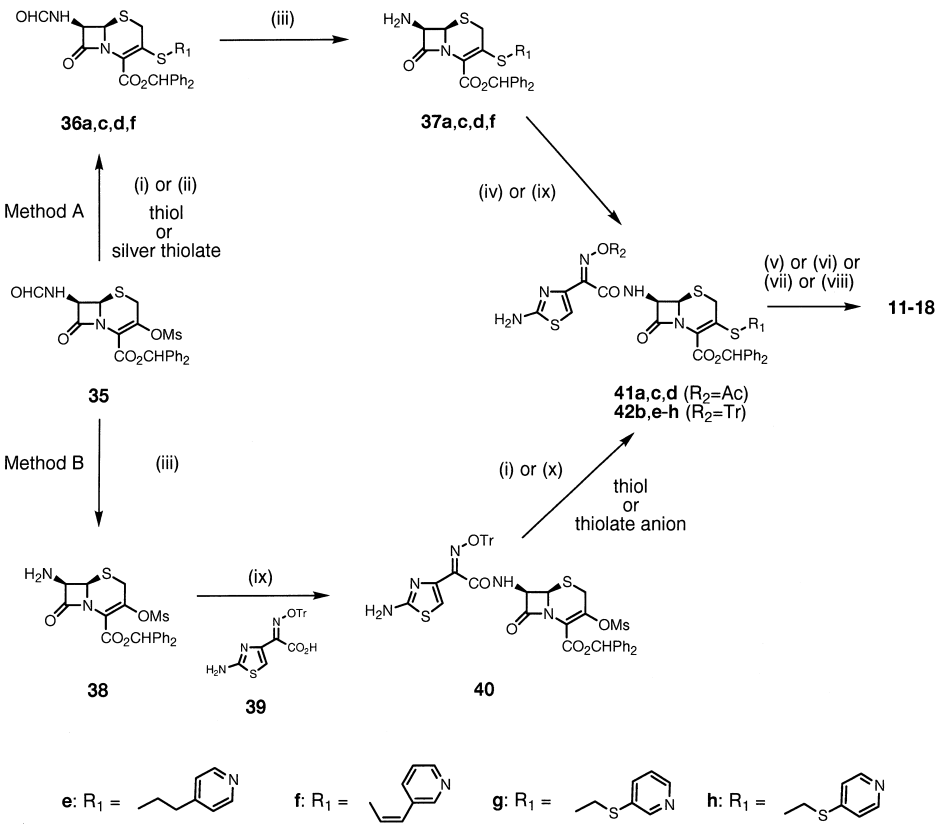
Scheme 3. Reagents: (i) PPh_3, diethyl azodicarboxylate (DEAD), THF. (ii) cHCl, MeOH. (iii) a. BSA, **23**, CH_2Cl_2, b. cHCl, MeOH. (iv) TFA, anisole, CH_2Cl_2.

4 shows the two methods used for the synthesis of cephems with the sulfur atom directly attached to the C-3 position. Method A involves introduction of the C-3 side chain in the first step and the C-7 side chain was introduced afterwards. Method B indicates the route via key intermediate **40**, to which was already introduced the C-7 side chain.[10] Thus, diphenylmethyl 7β-formamido-3-methanesulfonyloxy-3-cephem-4-carboxylate (**35**) was reacted with the corresponding mercaptopyridine or pyridylthiocarbinol in the presence of *N,N*-diisopropylethylamine in DMF to give **36a**,**c**,**d**. Although **36c**,**d** were obtained in good yield (88%), **36a** was obtained in low yield (29%) because of the generation of the undesired Δ^2 isomer. In the case of **36f**, silver thiolate was employed as the nucleophile as previously described.[11] Deprotection of **36** followed by coupling with **23** in the presence of BSA or coupling with (*Z*)-2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetic acid (**39**), which was activated with methanesulfonyl chloride in the presence of K_2CO_3, gave fully protected cephems **41a**,**c**,**d** and **42f**. Compound **35** was deprotected in a similar manner and coupled with **39** to give the key intermediate **40**, which was reacted with the corresponding pyridylthiocarbinols or thiolate anions generated in situ[12] to give **42**. In the case of thiolate anion, a low temperature (below −65 °C) was necessary. If a higher temperature was employed, the amount of undesired Δ^2 isomers was significantly increased. **41** and **42** were deprotected under various conditions to yield **11**–**18**.

Biological Results

The in vitro antibacterial activities of new cephalosporins against Gram-positive and Gram-negative bacteria are shown in Table 1. For comparison, CFDN (**1**) and FR86524 (**2**) were employed as reference drugs. As can be seen from these data, all of the synthesized compounds exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria. In particular, they all exhibited equal or improved antibacterial activity against *Staphylococcus aureus* compared with CFDN and FR86524. Thus, 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]cephalosporins having a pyridine at the C-3 side chain exhibited potent antibacterial activity against *S. aureus* irrespective of both the spacer moiety between the pyridine and cephem nucleus and the position of the pyridine nitrogen.

However, the effect of the spacer moiety on the antibacterial activity against Gram-negative bacteria was distinct from Gram-positive bacteria. Compounds with



Scheme 4. Reagents: (i) R_1SH (*N,N*-diisopropylethylamine), DMF. (ii) R_1SAg, NaI, CH_3CN. (iii) cHCl, MeOH. (iv) BSA, **23**, CH_2Cl_2. (v) TFA, anisole, CH_2Cl_2. (vi) a. TFA, anisole, CH_2Cl_2, b. $NaHCO_3$, NH_4Cl, MeOH, H_2O. (vii) cHCl, HCO_2H. (viii) $AlCl_3$, anisole, CH_3NO_2. (ix) BSA, **39**, MsCl, K_2CO_3, DMAc. (x) CH_3COSR_1, NaOMe, THF, DMF.

Table 1. Antibacterial activity of cephalosporins[a]

Drugs	MIC (μg/mL)					
	S.a.[b]	E.f.	M.c.	H.i.	E.c.	K.p.
3	0.21	25	0.53	0.21	0.78	0.57
4	0.14	13.5	0.62	0.11	0.18	0.17
5	0.20	40	0.57	0.49	3.9	2.7
6	0.20	31	0.72	0.59	3.4	2.5
7	0.18	1.3	0.31	0.071	0.16	0.14
8	0.20	0.9	0.29	0.062	0.083	0.11
9	0.25	1.8	0.36	0.23	0.46	0.39
10	0.20	46	0.25	0.93	0.21	N.T.
11	0.27	3.9	0.18	0.056	0.14	0.1
12	0.39	3.4	0.17	0.088	0.16	0.14
13	0.23	7.3	0.09	0.085	0.13	0.077
14	0.14	5.8	0.11	0.067	0.061	0.041
15	0.27	20	0.27	0.22	0.42	0.36
16	0.21	2.0	0.39	0.071	0.052	0.066
17	0.14	5.8	0.31	0.18	0.72	0.67
18	0.12	2.1	0.23	0.12	0.31	0.25
CFDN (1)	0.33	13.5	0.072	0.41	0.133	0.062
FR86524 (2)	0.29	7.3	0.195	0.047	0.097	0.041

[a]Müller-Hinton agar; 10^{-2}, stamp method; 37 °C, 20 h.
[b]S.a., *Staphylococcus aureus* (MSSA) (9); E.f., *Enterococcus faecalis* (9); M.c., *Morachisella catarrhalis* (9); H.i., *Haemophilus influenzae* (20); E.c., *Escherichia coli* (9); K.p., *Klebsiella pneumoniae* (9). CFDN: cefdinir.

a carbon atom directly attached to the C-3 position (**3**–**6**) showed decreased antibacterial activity against *Morachisella catarrhalis*, although **3** and **4** showed improved antibacterial activity against *H. influenzae* compared with CFDN. Comparing **3** with **4**, antibacterial activity against *H. influenzae*, *Escherichia coli* and *Klebsiella pneumoniae* was influenced by the position of the pyridine nitrogen. Thus, 4-pyridyl compound (**4**) was slightly superior to the corresponding 3-pyridyl compound (**3**) with regards to antibacterial activity. Further, insertion of methylene between pyridine and sulfur (**3** versus **5**, **4** versus **6**) resulted in decreased antibacterial activity against all strains tested, except *S. aureus*. Thus, we anticipate compounds having a bigger methylthioalkyl moiety than **5** or **6** would show further decreased antibacterial activity.

In the series of compounds bearing an (*E*)-vinylthio moiety at the C-3 position (**7**–**9**), all showed increased antibacterial activity against *H. influenzae* and *Enterococcus faecalis*, but decreased antibacterial activity against *M. catarrhalis*. Comparison of **7** and **8** indicated that they showed almost the same antibacterial activity. Thus, the position of the pyridine nitrogen had only a marginal effect on antibacterial activity. Insertion of methylene between 4-pyridine and sulfur (**8** versus **9**) resulted in decreased antibacterial activity against all tested strains. Thus, we abandoned synthesis of compounds having a bigger spacer moiety than **9**, since they were predicted to show further decreased antibacterial activity.

Compounds with the sulfur atom directly attached to the C-3 position (**11**–**18**) also showed improved antibacterial

activity against *H. influenzae*. Comparison of **12** with **14** and **15** indicated that **14**, having a 4-pyridylmethylthio moiety showed the most potent and well balanced activity. **16**, having a (*Z*)-3-pyridylvinylthio moiety also exhibited potent antibacterial activity, but antibacterial activity against *M. catarrhalis* was 5-fold less than CFDN. Compounds having a thiomethylthio as a spacer moiety (**17**,**18**) showed decreased antibacterial activity against *M. catarrhalis*, *E. coli* and *K. pneumoniae*. Thus, we considered a methylthio moiety as the optimal spacer moiety with regards to antibacterial activity and balance. Comparison of **13** with **14** indicated that antibacterial activity was influenced by the position of pyridine nitrogen. Thus, the 4-pyridyl compound (**14**; FR86830) was superior to the corresponding 3-pyridyl compound (**13**). However, compound **10**, having a 4-pyridylmethyloxy moiety, showed decreased antibacterial activity against all tested strains, except *S. aureus*. From these results, sulfur is the most desirable linker atom.

Among all compounds prepared, FR86830 (**14**) exhibited the most well balanced activity against both Gram-positive and Gram-negative bacteria. In particular, FR86830 was 7-fold more active against *H. influenzae* than CFDN. Further, compared with FR86524, FR86830 was slightly less active against *H. influenzae*, but it exhibited equal or improved antibacterial activity against the other strains tested. Thus, this compound is highly attractive, at least as far as antibacterial activity is concerned.

The urinary and biliary recoveries of these compounds after oral administration to rats are shown in Table 2. Most of these compounds showed relatively low oral absorption in rats, except for **5**, **6** and **17**. In particular, **5**, having a 3-pyridylthiomethylthio moiety at the C-3 position, exhibited extraordinarily high and improved oral absorption. For oral absorption, 4-pyridyl cephems appear to be more favorable than the corresponding 3-pyridyl cephems, as shown from the data for **5** versus **6** and **17** versus **18**. However, among the compounds that showed relatively low oral absorption, the position of pyridine nitrogen had only a marginal effect on oral absorption, since there was little difference between 3-pyridyl compounds (**3**, **7**, **13**) and 4-pyridyl compounds (**4**, **8**, **14**). Further, oral absorption was also affected by the type of spacer rather than the size of spacer moiety. However, these results indicated that the spacer moiety as well as the position of the pyridine nitrogen affected oral absorption.

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

Drugs	Recovery (%)		Drugs	Recovery (%)	
	Urine	Bile		Urine	Bile
3	1.43	9.31	**13**	6.78	3.53
4	4.18	4.34	**14**	4.91	4.80
5	13.8	51.4	**15**	3.50	2.26
6	4.88	21.7	**16**	2.53	2.20
7	2.31	0.84	**17**	9.82	15.10
8	0.90	1.18	**18**	2.01	3.30
9	1.30	3.22	**CFDN (1)**	32.5	1.40
10	6.09	22.4	**FR86524 (2)**	3.76	0.62
11	7.61	0.30			

FR86830 (**14**) was selected as an optimal compound with regards antibacterial activity, and showed moderate oral absorption that was higher than FR86524 (**2**). To confirm higher oral absorbability and/or stability of FR86830, we further tested the urinary and biliary recovery of FR86830 (**14**) and FR86524 (**2**) in mice (Table 3). The results revealed that oral absorption of FR86830 was far superior to that of FR86524.

Conclusions

As a result of modification of the spacer moiety between the C-3 position of pyridine and cephem nucleus, we discovered FR86830 (**14**), having a 4-pyridylmethylthio moiety at the C-3 position, which exhibited potent and well balanced antibacterial activity against both Gram-positive and Gram-negative bacteria, including *H. influenzae*. Further, oral absorption of FR86830 was distinctly higher than that of FR86524 in both rats and mice. Thus, FR86830 was selected as a favorable parent compound for further optimization. Our further studies to optimize the absorption and activity of compounds derived from FR86830 will be presented in subsequent papers.

Experimental

Melting points were measured on a Büchi 535 apparatus in open capillaries and are uncorrected. 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 a Hewlett–Packard 1100LC-MSD instrument (ESIMS). Elemental analyses were carried out on a Perkin–Elmer 2400 CHN Elemental Analyzer.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(4-pyridyl)thiomethyl-3-cephem-4-carboxylate (20b). Under an N_2 atmosphere, NaI (1.46 g, 9.71 mmol) was added to a solution of **19** (5.0 g, 9.71 mmol) in DMF (25 ml) at room temperature. After 30 min the mixture was cooled with ice and 4-mercaptopyridine (1.19 g, 10.68 mmol) was added dropwise to the mixture. The whole mixture was stirred for 30 min at 5 °C and poured into a mixture

Table 3. 24 h urinary and biliary recovery after oral administration (20 mg/kg) to mice

Drugs	Recovery (%)	
	Urine	Bile
14	8.18	12.5
FR86524 (2)	4.85	2.51

of ethyl acetate and cold water. The aqueous layer was separated, and the organic layer was washed with water and brine and dried over $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by column chromatography on silica-gel eluting with ethyl acetate–CH_2Cl_2 to give **20b**. Amorphous solid. Yield: 4.53 g (79%). IR (KBr) cm^{-1} 1791, 1785, 1772, 1733; ^1H NMR (DMSO-d_6) δ 1.40 (9H, s), 3.58 and 3.68 (2H, ABq, $J=17.7$ Hz), 4.07 and 4.20 (2H, ABq, $J=12.8$ Hz), 5.12 (1H, d, $J=4.8$ Hz), 5.53 (1H, dd, $J=8.8$, 4.8 Hz), 6.95 (1H, s), 7.16 (2H, d, $J=6.0$ Hz), 7.2–7.5 (10H, m), 8.04 (1H, d, $J=8.8$ Hz), 8.30 (2H, d, $J=6.0$ Hz).

20a was obtained using a method similar to that used for **20b** and **20c**,**d** were also obtained in a similar manner as used for **20b** except for successive addition of an equal molar amount of *N*,*N*-diisopropylethylamine after addition of the corresponding pyridylthiocarbinol.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(3-pyridyl)thiomethyl-3-cephem-4-carboxylate (20a). Amorphous solid. Yield: 5.70 g (99%). IR (KBr) cm^{-1} 1785, 1720; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.60 and 3.70 (2H, ABq, $J=17.6$ Hz), 3.97 and 4.12 (2H, ABq, $J=13.0$ Hz), 5.11 (1H, d, $J=4.7$ Hz), 5.48 (1H, dd, $J=8.6$, 4.7 Hz), 6.74 (1H, s), 7.2–7.5 (11H, m), 7.66 (1H, dt, $J=8.0$, 2.2 Hz), 8.03 (1H, d, $J=8.6$ Hz), 8.35 (1H, dd, $J=4.7$, 1.5 Hz), 8.48 (1H, d, $J=1.8$ Hz).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(3-pyridyl)methylthiomethyl-3-cephem-4-carboxylate (20c). Amorphous solid. Yield: 23.8 g (85%). IR (Nujol) cm^{-1} 1766, 1700; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.46–3.68 (6H, m), 5.11 (1H, d, $J=4.8$ Hz), 5.50 (1H, dd, $J=8.8$, 4.8 Hz), 6.89 (1H, s), 7.2–7.5 (10H, m), 7.60 (1H, dt, $J=7.9$, 1.8 Hz), 8.02 (1H, d, $J=8.8$ Hz), 8.40–8.45 (2H, m). MS *m*/*e* 604 $[(M+H)^+]$.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-(4-pyridyl)methylthiomethyl-3-cephem-4-carboxylate (20d). Amorphous solid. Yield: 24.6 g (88%). IR (Nujol) cm^{-1} 1762, 1695; ^1H NMR (DMSO-d_6) δ 1.41 (9H, s), 3.5–3.7 (6H, m), 5.08 (1H, d, $J=4.8$ Hz), 5.51 (1H, dd, $J=8.8$, 4.8 Hz), 6.87 (1H, s), 7.18 (1H, dd, $J=4.5$, 1.5 Hz), 7.2–7.5 (10H, m), 8.03 (1H, d, $J=8.8$ Hz), 8.40–8.45 (2H, m). MS *m*/*e* 604 $[(M+H)^+]$.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(4-pyridyl)thiovinyl]-3-cephem-4-carboxylate (27b). To a solution of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(4-toluenesulfonyloxy)vinyl]-3-cephem-4-carboxylate (5.0 g) in DMF (100 mL) was added 4-mercaptopyridine (1.0 g) and *N*,*N*-diisopropylethylamine (1.3 mL) at 5 °C. The mixture was stirred at room temperature for 4.5 h and poured into ice-water (1 L). The resulting precipitate was collected by filtration and dissolved in a mixture of THF (200 mL) and ethyl acetate (200 mL). The solution 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 ethyl acetate–hexane to give **27b**. Amorphous solid. Yield: 2.66 g (59%). IR (KBr) cm^{-1} 1786, 1716; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.73 and 4.13 (2H, ABq, $J=18$ Hz), 5.18 (1H, d, $J=5$ Hz), 5.58 (1H, dd, $J=5$, 8 Hz), 6.95 (1H, s), 7.04 (1H, d, $J=16$ Hz), 7.2–7.5 (13H, m), 8.11 (1H, d, $J=8$ Hz), 8.46 (2H, d, $J=6$ Hz).

Compounds **27a**,**d** were obtained using a method similar to that used for **27b**.

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(3-pyridyl)thiovinyl]-3-cephem-4-carboxylate (27a). Amorphous solid. Yield: 3.8 g (83%). IR (KBr) cm^{-1} 1782, 1716; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.68 and 4.00 (2H, ABq, $J=17$ Hz), 5.15 (1H, d, $J=5$ Hz), 5.52 (1H, dd, $J=5$, 8 Hz), 6.91 (1H, s), 6.95 (1H, d, $J=15$ Hz), 7.24 (1H, d, $J=15$ Hz), 7.3–7.5 (11H, m), 7.8–7.9 (1H, m), 8.07 (1H, d, $J=8$ Hz), 8.4–8.5 (1H, m), 8.5–8.6 (1H, m).

Diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(4-pyridyl)methylthiovinyl]-3-cephem-4-carboxylate (27d). Amorphous solid. Yield: 12.6 g (68%). IR (KBr) cm^{-1} 3240, 1760, 1690, 1585; ^1H NMR (DMSO-d_6) δ 1.42 (9H, s), 3.60 and 3.80 (2H, ABq, $J=16.9$ Hz), 3.94 and 4.00 (2H, ABq, $J=13.1$ Hz), 5.14 (1H, d, $J=4.6$ Hz), 5.56 (1H, dd, $J=8.9$, 4.6 Hz), 6.84 (1H, d, $J=15.9$ Hz), 6.89 (1H, s), 7.18 (1H, d, $J=15.9$ Hz), 7.3–7.55 (12H, m), 8.05 (1H, d, $J=8.9$ Hz), 8.48 (2H, d, $J=4.4$ Hz). MS *m*/*e* 616 $[(M+H)^+]$.

7β-Amino-3-[(*E*)-2-(4-pyridyl)thiovinyl]-3-cephem-4-carboxylic acid dihydrochloride (28b). To a solution of diphenylmethyl 7β-*tert*-butoxycarbonylamino-3-[(*E*)-2-(pyridin-4-yl)thiovinyl]-3-cephem-4-carboxylate (2.64 g) in formic acid (10.6 mL) was added concd HCl (1.1 mL). The mixture was stirred at room temperature for 40 min. The reaction mixture was poured into a mixture of ethyl acetate (67 mL) and acetone (33 mL). The resulting precipitate was collected by filtration and dried in vacuo to give **28b**. Amorphous solid. Yield: 2.0 g (quant.). IR (Nujol) cm^{-1} 1782, 1711, 1624; ^{1}NMR (DMSO-d_6) δ 3.87 and 4.23 (2H, ABq, $J=17$ Hz), 5.23 (1H, d, $J=5$ Hz), 5.30 (1H, d, $J=5$ Hz), 7.37 (2H, s), 7.94 (2H, d, $J=7$ Hz), 8.67 (2H, d, $J=7$ Hz).

The following compounds were obtained using a method similar to that used for **28b**.

7β-Amino-3-(3-pyridyl)thiomethyl-3-cephem-4-carboxylic acid dihydrochloride (21a). Amorphous solid. Yield: 3.3 g (87%). IR (KBr) cm^{-1} 3600–3400, 1781, 1708, 1619; ^1H NMR (DMSO-d_6) δ 3.75 (2H, s), 4.20 and 4.30 (2H, ABq, $J=13.0$ Hz), 5.10 (1H, d, $J=4.9$ Hz), 5.21 (1H, d, $J=4.9$ Hz), 7.77 (1H, dd, $J=8.1$, 5.4 Hz), 8.34 (1H, d, $J=8.1$ Hz), 8.71 (1H, d, $J=4.6$ Hz), 8.86 (1H, d, $J=1.9$ Hz).

7β-Amino-3-(4-pyridyl)thiomethyl-3-cephem-4-carboxylic acid dihydrochloride (21b). Amorphous solid. Yield: 2.9 g (95%). IR (KBr) cm^{-1} 1781, 1716, 1623; ^1H NMR (DMSO-d_6) δ 3.71 and 3.77 (2H, ABq, $J=17.7$ Hz), 4.46 (2H, s), 5.17 (1H, d, $J=4.8$ Hz), 5.24 (1H, d, $J=4.8$ Hz), 7.94 (2H, d, $J=6.3$ Hz), 8.67 (2H, d, $J=6.3$ Hz).

7β-Amino-3-(3-pyridyl)methylthiomethyl-3-cephem-4-carboxylic acid dihydrochloride (21c). Amorphous solid.

Yield: 14.7 g (90%). IR (Nujol) cm^{-1} 3100–2700, 1750, 1670; ^1H NMR (DMSO-d_6) δ 3.57–3.83 (4H, m), 4.04 (2H, s), 5.10 (1H, d, $J=4.8$ Hz), 5.23 (1H, d, $J=4.8$ Hz), 8.00 (1H, m), 8.52 (1H, d, $J=8.1$ Hz), 8.82 (1H, d, $J=4.8$ Hz), 8.87 (1H, d, $J=1.6$ Hz).

7β-Amino-3-(4-pyridyl)methylthiomethyl-3-cephem-4-carboxylic acid dihydrochloride (21d). Amorphous solid. Yield: 14.4 g (86%). IR (Nujol) cm^{-1} 3200–2400, 1760, 1690; ^1H NMR (DMSO-d_6) δ 3.63 and 3.79 (2H, ABq, $J=17.7$ Hz), 3.70 (2H, d, $J=5.1$ Hz), 4.06 (2H, s), 5.09 (1H, d, $J=4.8$ Hz), 5.22 (1H, d, $J=4.8$ Hz), 7.99 (2H, d, $J=6.6$ Hz), 8.85 (2H, d, $J=6.6$ Hz).

7β-Amino-3-[(*E*)-2-(3-pyridyl)thiovinyl]-3-cephem-4-carboxylic acid dihydrochloride (28a). Amorphous solid. Yield: 2.2 g (95%). IR (Nujol) cm^{-1} 1774; ^1H NMR (DMSO-d_6) δ 3.80 and 4.05 (2H, ABq, $J=17$ Hz), 5.14 (1H, d, $J=5$ Hz), 5.24 (1H, d, $J=5$ Hz), 7.19 (1H, d, $J=16$ Hz), 7.35 (1H, d, $J=16$ Hz), 7.71 (1H, dd, $J=4.9$, 7.8 Hz), 8.2–8.3 (1H, m), 8.67 (1H, dd, $J=1.0$, 4.8 Hz), 8.83 (1H, d, $J=2$ Hz).

7β-Amino-3-[(*E*)-2-(4-pyridyl)methylthiovinyl]-3-cephem-4-carboxylic acid dihydrochloride (28d). Amorphous solid. Yield: 2.2 g (95%). IR (Nujol) cm^{-1} 3310, 1750, 1675, 1620, 1555; ^1H NMR (DMSO-d_6) δ 3.70 and 3.87 (2H, ABq, $J=16.5$ Hz), 4.47 (2H, br s), 5.05 (1H, d, $J=4.8$ Hz), 5.19 (1H, d, $J=4.8$ Hz), 6.99 (1H, d, $J=15.8$ Hz), 7.34 (1H, d, $J=15.8$ Hz), 8.01 (2H, d, $J=6.7$ Hz), 8.88 (2H, d, $J=6.7$ Hz).

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-(3-pyridyl)thiomethyl-3-cephem-4-carboxylic acid (24a). Et_3N (4.8 mL, 34.3 mmol) was added to a suspension of **21a** (3.3 g, 8.4 mmol) in THF (66 mL). Trimethylsilyl chloride (2.8 mL, 21.8 mmol) was added dropwise to the mixture under ice-cooling over 5 min. The mixture was stirred for 30 min at room temperature and cooled again with ice. To the mixture was added **22** (4.59 g, 8.4 mmol) in DMF (46 mL) over 10 min. The mixture was stirred overnight at room temperature and concentrated in vacuo to remove most of the THF. The concentrate was poured into ice-water (500 mL) with vigorous stirring. The resulting precipitate was collected by filtration and purified by column chromatography on silica-gel eluting with 5% aqueous THF to give **24a**. Amorphous solid. Yield: 3.5 g (57%). IR (KBr) cm^{-1} 3600–3300, 1762, 1675, 1618; ^1H NMR (DMSO-d_6) δ 3.4–3.6 (2H, m), 4.24 and 4.40 (2H, ABq, $J=12.8$ Hz), 5.06 (1H, d, $J=4.8$ Hz), 5.74 (1H, dd, $J=8.3$, 4.8 Hz), 6.60 (1H, s), 7.25–7.35 (28H, m), 7.89 (1H, m), 8.36 (1H, dd, $J=4.7$, 1.4 Hz), 8.56 (1H, d, $J=1.9$ Hz), 9.83 (1H, d, $J=8.3$ Hz).

Compounds **24b** and **29a**,**b** were obtained using a method similar to that used for **24a**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-(4-pyridyl)thiomethyl-3-cephem-4-carboxylic acid (24b). Amorphous solid. Yield: 2.9 g (55%). IR (KBr) cm^{-1} 3600–3300, 1758, 1677; ^1H NMR (DMSO-d_6) δ 3.59 (2H, s), 4.28 and 4.44 (2H, ABq, $J=13.2$ Hz), 5.09 (1H, d, $J=4.9$ Hz), 5.75 (1H, dd, $J=8.3$, 4.9 Hz), 6.59 (1H, s), 7.25–7.40 (17H, m), 7.39 (2H, d, $J=6.2$ Hz), 8.33 (2H, d, $J=6.2$ Hz), 9.84 (1H, d, $J=8.3$ Hz).

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-[(*E*)-2-(4-pyridyl)thiovinyl]-3-cephem-4-carboxylic acid (29b). To a mixture of (*Z*)-2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetic acid (2.03 g, 4.73 mmol) in DMAc (20 mL) were added K_2CO_3 (654 mg, 4.73 mmol) and methanesulfonyl chloride (0.73 mL, 9.46 mmol) at 5 °C. The mixture was stirred for 30 min at the same temperature. In another flask, to a solution of **28b** (1.93 g, 4.73 mmol) in DMF (19.3 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (8.2 mL, 33.1 mmol) at 5 °C and stirred for 10 min. To this solution was added the above mentioned solution of the activated acid. The mixture was stirred at 5 °C for 30 min and poured into 20% aqueous NaCl (400 mL). The resulting precipitate was collected by filtration, washed with water and dried in vacuo. The crude product was purified by column chromatography on silica-gel eluting with ethyl acetate–hexane to give **29b**. Amorphous solid. Yield: 1.7 g (49%). ^1H NMR (DMSO-d_6) δ 3.64 and 3.89 (2H, ABq, $J=17$ Hz), 5.21 (1H, d, $J=5.0$ Hz), 5.85 (1H, dd, $J=5.0$, 8.0 Hz), 6.62 (1H, s), 7.2–7.4 (18H, m), 7.47 (1H, d, $J=15.1$ Hz), 8.41 (2H, d, $J=5.9$ Hz), 9.92 (1H, d, $J=8.0$ Hz).

Compound **29a** was obtained using a method similar to that used for **29b**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-[(*E*)-2-(3-pyridyl)thiovinyl]-3-cephem-4-carboxylic acid (29a). Amorphous solid. Yield: 2.2 g (55%). IR (KBr) cm^{-1} 1763, 1678, 1605; ^1H NMR (DMSO-d_6) δ 3.60 and 3.84 (2H, ABq, $J=17.1$ Hz), 5.19 (1H, d, $J=4.9$ Hz), 5.84 (1H, dd, $J=4.9$, 7.8 Hz), 6.62 (1H, s), 6.69 (1H, d, $J=16$ Hz), 7.2–7.4 (19H, m), 7.8–7.9 (19H, m), 8.45 (1H, dd, $J=1.0$, 4.9 Hz), 8.56 (1H, d, $J=2.1$ Hz), 9.91 (1H, d, $J=7.8$ Hz).

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(3-pyridyl)methylthiomethyl-3-cephem-4-carboxylic acid (5). To a solution of **21c** (2.05 g, 5.0 mmol) and BSA (3.05 g, 15.0 mmol) in CH_2Cl_2 (50 mL) was added (*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetylchloride hydrochloride (**23**) (1.70 g, 6 mmol) under ice-cooling. After stirring for 4 h at the same temperature, the mixture was added dropwise to IPE (400 mL). The precipitate containing 7β-[(*Z*)-2-(2-aminothiazol-4-yl)-2-acetoxyiminoacetamido]-3-[(*Z*)-2-(3-pyridyl)vinyl]-3-cephem-4-carboxylic acid was collected by filtration and dried in vacuo. The precipitate was dissolved in 10% MeOH aqueous solution (160 mL), and thereto NH_4Cl (802 mg, 15.0 mmol) was added and the mixture adjusted to pH 8 with saturated aq $NaHCO_3$ solution. The mixture was stirred for 30 min maintaining pH 8 with saturated aq $NaHCO_3$ solution. The solution was adjusted to pH 6 with 1 N HCl, and evaporated in vacuo to remove MeOH. The solution was subjected to column chromatography on HP-20 and eluted with 15% aqueous IPA. 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 **5.**

Mp 174 °C. Yield: 730 mg (28%). IR (Nujol) cm^{-1} 3000–2800, 1745, 1650; ^1H NMR (DMSO-d_6) δ 3.48 and 3.49 (2H, ABq, $J=17.6$ Hz), 3.64 (2H, s), 3.69 and 3.78 (2H, ABq, $J=13.4$ Hz), 5.13 (1H, d, $J=4.8$ Hz), 5.74 (1H, dd, $J=8.2$, 4.8 Hz), 6.67 (1H, s), 7.13 (2H, br s), 7.34 (1H, dd, $J=7.8$, 4.8 Hz), 7.72 (1H, dt, $J=7.8$, 1.9 Hz), 8.4–8.5 (2H, m), 9.44 (1H, d, $J=8.2$ Hz), 11.31 (1H, s). ESIMS (neg.) *m/e* 505[$(M-H)^+$]. Anal. calcd for $C_{19}H_{18}N_6O_5S_3 \cdot 1.7H_2O$: C, 42.48; H, 4.02; N, 15.64; found: C, 42.52; H, 3.76; N, 15.52.

The following compounds were obtained using a method similar to that used for **5**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(4-pyridyl)methylthiomethyl-3-cephem-4-carboxylic acid (6). Amorphous solid. Yield: 816 mg (16%). IR (Nujol) cm^{-1} 3300–2800, 1740; ^1H NMR (DMSO-d_6) δ 3.48 and 3.59 (2H, ABq, $J=17.6$ Hz), 3.62 (2H, s), 3.67 and 3.76 (2H, ABq, $J=17.6$ Hz), 5.11 (1H, d, $J=4.8$ Hz), 5.75 (1H, dd, $J=8.2$, 4.8 Hz), 6.66 (1H, s), 7.14 (2H, br s), 7.31 (2H, dd, $J=4.5$, 1.5 Hz), 8.49 (2H, dd, $J=4.5$, 1.5 Hz), 9.45 (1H, d, $J=8.2$ Hz), 11.31 (1H, s). ESIMS (neg.) *m/e* 505 [$(M-H)^+$]. Anal. calcd for $C_{19}H_{18}N_6O_5S_3 \cdot 1.8H_2O$: C, 42.34; H, 4.04; N, 15.59; found: C, 42.19; H, 3.79; N, 15.53.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(*E*)-2-(4-pyridyl)methylthiovinyl]-3-cephem-4-carboxylic acid (9). Amorphous solid. Yield: 288 mg (11%). IR (Nujol) cm^{-1} 1750, 1620, 1590, 1530; ^1H NMR (DMSO-d_6) δ 3.56 and 3.77 (2H, ABq, $J=17.3$ Hz), 4.10 (2H, s), 5.15 (1H, d, $J=4.7$ Hz), 5.72 (1H, dd, $J=4.7$, 8.2 Hz), 6.68 (1H, s), 6.86 (1H, d, $J=15.7$ Hz), 6.99 (1H, d, $J=15.7$ Hz), 7.14 (2H, br s), 7.40 (1H, dd, $J=4.4$, 1.6 Hz), 8.51 (2H, dd, $J=4.4$, 1.6 Hz), 8.62 (1H, s), 9.47 (1H, d, $J=8.2$ Hz), 11.3 (1H, s). ESIMS (neg.) *m/e* 517 [$(M-H)^+$]. Anal. calcd for $C_{20}H_{18}N_6O_5S_3 \cdot 3.1H_2O$: C, 41.82; H, 4.25; N, 14.63; found: C, 41.73; H, 3.99; N, 14.52.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(*E*)-2-(4-pyridyl)thiovinyl]-3-cephem-4-carboxylic acid (8). A solution of **29b** (1.72 g) in 90% aqueous formic acid (6.5 ml) was stirred at room temperature for 1 h. The insoluble material was filtered off and the filtrate was adjusted to pH 7 with $NaHCO_3$. The aqueous solution was washed with ethyl acetate and the aqueous layer was separated. The aqueous layer was adjusted to pH 5 and chromatographed with HP-20 eluting with aqueous IPA. The eluent was concentrated in vacuo and the resulting precipitate was collected by filtration, washed with water and dried in vacuo to give **8**. Amorphous solid. Yield: 332 mg (29%). IR (KBr) cm^{-1} 1767, 1624; ^1H NMR (DMSO-d_6) δ 3.69 and 4.09 (2H, ABq, $J=18$ Hz), 5.21 (1H, d, $J=5$ Hz), 5.82 (1H, dd, $J=5$, 8 Hz), 6.68 (1H, s), 7.11 (1H, d, $J=16$ Hz), 7.43 (2H, d, $J=6$ Hz), 8.46 (2H, d, $J=6$ Hz), 9.52 (1H, d, $J=8$ Hz), 11.3 (1H, s). ESIMS (neg.) *m/e* 503 [$(M-H)^+$]. Anal. calcd for $C_{19}H_{16}N_6O_5S_3 \cdot 3.8H_2O$: C, 39.95; H, 4.13; N, 14.71; found: C, 39.65; H, 3.86; N, 14.48.

The following compounds were obtained using a method similar to that used for **8**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(3-pyridyl)thiomethyl-3-cephem-4-carboxylic acid (3). Mp 188 °C. Yield: 209 mg (17%). IR (KBr) cm^{-1} 3600–3300, 1768, 1668; ^1H NMR (DMSO-d_6) δ 3.53 and 3.69 (2H, ABq, $J=17.6$ Hz), 4.01 and 4.19 (2H, ABq, $J=13.1$ Hz), 5.12 (1H, d, $J=4.8$ Hz), 5.72 (1H, dd, $J=8.2$, 4.8 Hz), 6.66 (1H, s), 7.13 (2H, s), 7.82 (1H, m), 8.45 (1H, m), 8.56 (1H, d, $J=1.9$ Hz), 9.45 (1H, d, $J=8.2$ Hz), 11.30 (1H, s). ESIMS (neg.) *m/e* 491 [$(M-H)^+$]. Anal. calcd for $C_{18}H_{16}N_6O_5S_3 \cdot 2.4H_2O$: C, 40.35; H, 3.91; N, 15.69; found: C, 40.26; H, 3.69; N, 15.79.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(4-pyridyl)thiomethyl-3-cephem-4-carboxylic acid (4). Amorphous solid. Yield: 253 mg (19%). IR (KBr) cm^{-1} 3600–3300, 1766, 1664, 1625; ^1H NMR (DMSO-d_6) δ 3.52 and 3.67 (2H, ABq, $J=17.8$ Hz), 4.15 and 4.27 (2H, ABq, $J=12.9$ Hz), 5.15 (1H, d, $J=4.8$ Hz), 5.76 (1H, dd, $J=8.2$, 4.8 Hz), 6.65 (1H, s), 7.12 (2H, s), 7.31 (2H, d, $J=6.1$ Hz), 8.38 (2H, d, $J=6.1$ Hz), 9.45 (1H, d, $J=8.2$ Hz), 11.29 (1H, s). ESIMS (neg.) *m/e* 491 [$(M-H)^+$]. Anal. calcd for $C_{18}H_{16}N_6O_5S_3 \cdot 3.2H_2O$: C, 39.30; H, 4.10; N, 15.27; found: C, 39.27; H, 3.95; N, 15.45.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(*E*)-2-(3-pyridiyl)thiovinyl]-3-cephem-4-carboxylic acid (7). Mp 172 °C. Yield: 970 mg (30%). IR (KBr) cm^{-1} 1767, 1666, 1601; ^1H NMR (DMSO-d_6) δ 3.63 and 3.96 (2H, ABq, $J=17.1$ Hz), 5.17 (1H, d, $J=4.9$ Hz), 5.77 (1H, dd, $J=4.9$, 8.0 Hz), 6.67 (1H, s), 7.03 (2H, s), 7.14 (2H, br s), 7.44 (1H, dd, $J=5.0$, 7.9 Hz), 7.90 (1H, d, $J=7.8$ Hz), 8.52 (1H, d, $J=4.0$ Hz), 8.62 (1H, s), 9.49 (1H, d, $J=8.0$ Hz), 11.3 (1H, s). ESIMS (neg.) *m/e* 503 [$(M-H)^+$]. Anal. calcd for $C_{19}H_{16}N_6O_5S_3 \cdot 2.9H_2O$: C, 40.99; H, 3.95; N, 15.09; found: C, 40.74; H, 3.65; N, 15.24.

Diphenylmethyl 7β-formamido-3-[(4-pyridyl)methyloxy]-3-cephem-4-carboxylate (32d). Under an N_2 atmosphere, a solution of 4-pyridylcarbinol (1.36 g, 12.5 mmol) in THF (25 mL) was added to a mixture of diphenylmethyl 7β-formamido-3-hydroxy-3-cephem-4-carboxylate (4.1 g, 10 mmol) and triphenylphosphine (3.28 g, 12.5 mmol) in THF (50 mL) at 0 °C. After 10 min, a solution of diethyl azodicarboxylate (1.9 mL, 12.1 mmol) in THF (25 mL) was added dropwise to the mixture. The whole mixture was stirred for 2 h at the same temperature and the solvent was evaporated. The residue was diluted with a mixture of ethyl acetate and water and the aqueous layer was separated. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with ethyl acetate–hexane to give **32d**. Amorphous solid. Yield: 4.6 g (93%). IR (KBr) cm^{-1} 1784, 1762; ^1H NMR (DMSO-d_6) δ 3.73 and 3.85 (2H, ABq, $J=16.6$ Hz), 5.19 (1H, d, $J=4.3$ Hz), 5.28 (2H, s), 5.61 (1H, dd, $J=9.0$, 4.3 Hz), 6.90 (1H, s), 7.15–7.45 (12H, m), 8.19 (1H, s), 8.53 (2H, d, $J=5.8$ Hz), 9.07 (1H, d, $J=9.0$ Hz).

Diphenylmethyl 7β-amino-3-[(4-pyridyl)methyloxy]-3-cephem-4-carboxylate dihydrochloride (33d). To a solution of **32d** (4.64 g, 9.3 mmol) in MeOH (50 mL) was added dropwise concd HCl (3.9 mL, 46.5 mmol) at room temperature. The mixture was stirred for 1.5 h and the solvent was evaporated. The residue was triturated with ether and the resulting precipitate was collected by filtration, washed with ether and dried in vacuo to give **33d**. Amorphous solid. Yield: 2.1 g (40%). IR (KBr) cm^{-1} 3303, 1783, 1768, 1695; ^1H NMR (DMSO-d_6) δ 3.86 and 4.02 (2H, ABq, $J=15.9$ Hz), 5.06 (1H, d, $J=4.4$ Hz), 5.30 (1H, d, $J=4.3$ Hz), 5.63 (1H, s), 6.91 (1H, s), 7.2–7.45 (10H, m), 7.96 (1H, d, $J=6.4$ Hz), 8.87 (1H, d, $J=6.6$ Hz).

Diphenylmethyl 7β-[2-(2-aminothiazol-4-yl)-2-(*Z*)-(hydroxyimino)acetamido]-3-[(4-pyridyl)methyloxy]-3-cephem-4-carboxylate (34d). BSA (9.5 mL, 38 mmol) was added to a solution of **33d** (2.6 g, 4.81 mmol) in DMAc (30 mL). After 30 min, **23** (2.2 g, 7.7 mmol) was added to the solution with ice cooling. The mixture was stirred for 1.5 h at the same temperature and poured into a mixture of ethyl acetate and water. 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 resulting solid (1.0 g) was suspended in MeOH (20 mL). To this suspension was added dropwise concd HCl (0.6 mL, 7.2 mmol) at room temperature. The mixture was stirred for 2 h at the same temperature and poured into a mixture of ethyl acetate and water. The aqueous layer was separated and the organic layer was washed with water and brine and dried over $MgSO_4$. The solvent was evaporated in vacuo to give **34d**. Amorphous solid. Yield: 752 mg (24%). ^1H NMR (DMSO-d_6) δ 3.63 (2H, s), 5.23 (1H, d, $J=$ 4.3 Hz), 5.28 (1H, s), 5.66 (1H, dd, $J=8.3$, 4.3 Hz), 6.75 (1H, s), 6.84 (1H, s), 7.1–7.6 (14H, m), 8.53 (2H, d, $J=6.0$ Hz), 9.43 (1H, d, $J=8.3$ Hz), 11.31 (1H, s).

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyridyl)methyloxy]-3-cephem-4-carboxylic acid (10). To a suspension of **34d** (750 mg, 1.2 mmol) in CH_2Cl_2 (3 mL) and anisole (1 mL) was added trifluoroacetic acid (2 mL) with ice-cooling. After stirring at the same temperature for 2 h, the mixture was poured into cold IPE. The resulting precipitate was collected by filtration to give crude **10.** The crude product was purified using a method similar to that used for **5** to afford **10**. Amorphous solid. Yield: 361 mg (65%). IR (KBr) cm^{-1} 3409, 1764, 1641, 1616; ^1H NMR (DMSO-d_6) δ 3.63 and 3.67 (2H, ABq, $J=16.7$ Hz), 5.16 (1H, d, $J=4.4$ Hz), 5.18 (2H, s), 5.59 (1H, dd, $J=8.2$, 4.4 Hz), 6.71 (1H, s), 7.10 (2H, s), 7.42 (2H, d, $J=6.0$ Hz), 8.57 (2H, d, $J=6.0$ Hz), 9.39 (1H, d, $J=8.3$ Hz), 11.28 (1H, s). ESIMS (neg.) *m/e* 475 [$(M-H)^+$]. Anal. calcd for $C_{18}H_{16}N_6O_6S_2 \cdot 3.2H_2O$: C, 40.48; H, 4.23; N, 15.73; found: C, 40.69; H, 4.01; N, 15.46.

Diphenylmethyl 7β-formamido-3-[(4-pyridyl)methylthio]-3-cephem-4-carboxylate (36d). To a solution of diphenylmethyl 7β-formamido-3-methanesulfonyloxy-3-cephem-4-carboxylate (14.66 g, 30 mmol) in DMF (103 mL) was added 4-(mercaptomethyl)pyridine (4.13 g, 33 mmol) at −20 °C, followed by dropwise addition of *N*,*N*-diisopropylethylamine (3.88 g, 30 mmol). The mixture was stirred for 1.9 h and poured into ice water (500 mL). The resulting precipitate was collected by filtration and washed with water. The powder was dissolved in THF, and ethyl acetate and water were then added. The separated organic layer was washed with brine, dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography on silica-gel eluting with ethyl acetate–hexane to give **36d**. Amorphous solid. Yield: 13.7 g (88%). IR (KBr) cm^{-1} 1750, 1660, 1590; ^1H NMR (DMSO-d_6) δ 3.82 (2H, br s), 4.19 (2H, br s), 5.18 (1H, d, $J=4.7$ Hz), 5.77 (1H, dd, $J=8.9$, 4.7 Hz), 6.87 (1H, s), 7.3–7.6 (12H, m), 8.17 (1H, s), 8.45–8.55 (2H, m), 9.12 (1H, d, $J=8.9$ Hz). FABMS *m/e* 518 [$(M+H)^+$].

The following compounds were obtained using a method similar to that used for **36d**.

Diphenylmethyl 7β-formamido-3-(3-pyridyl)thio-3-cephem-4-carboxylate (36a). Amorphous solid. Yield: 4.3 g (29%). IR (KBr) cm^{-1} 1778, 1660; ^1H NMR (DMSO-d_6) δ 3.60 and 3.73 (2H, ABq, $J=17.3$ Hz), 5.27 (1H, d, $J=4.9$ Hz), 5.89 (1H, dd, $J=8.9$, 4.9 Hz), 6.95 (1H, s), 7.2–7.5 (11H, m), 7.79 (1H, dt, $J=8.6$, 1.6 Hz), 8.15 (1H, s), 8.52–8.57 (1H, m), 9.19 (1H, d, $J=8.9$ Hz). MS *m/e* 504 [(M + H)].

Diphenylmethyl 7β-formamido-3-[(3-pyridyl)methylthio]-3-cephem-4-carboxylate (36c). Amorphous solid. Yield: 2.3 g (63%). IR (KBr) cm^{-1} 1765, 1680; ^1H NMR (DMSO-d_6) δ 3.89 (2H, s), 4.17 and 4.24 (2H, ABq, $J=13$ Hz), 5.19 (1H, d, $J=4.8$ Hz), 5.77 (1H, dd, $J=$ 8.9, 4.8 Hz), 6.85 (1H, s), 7.2–7.5 (11H, m), 7.6–7.7 (1H, m), 8.17 (1H, s), 8.4–8.5 (2H, m), 9.14 (1H, d, $J=8.9$ Hz).

Diphenylmethyl 7β-formamido-3-[(*Z*)-2-(3-pyridyl)vinylthio]-3-cephem-4-carboxylate (36f). To a suspension of crude silver (*Z*)-2-(3-pyridyl)vinylthiolate (18.7 g, 76.8 mmol) in CH_3CN (374 mL) was added NaI (57.5 g, 384 mmol) at room temperature in the dark. The mixture was stirred for 30 min at room temperature in the dark and cooled to −20 °C. To the mixture was added **35** (30 g, 61.4 mmol) in CH_3CN (300 mL) below 0 °C. The mixture was stirred for 30 min and the resulting precipitate was removed by filtration. The filtrate was concentrated in vacuo and the residue was diluted with a mixture of ethyl acetate and water. 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 ethyl acetate to give **36f**. Amorphous solid. Yield: 20.1 g (62%). ^1H NMR (DMSO-d_6) δ 3.84 and 4.09 (2H, ABq, $J=17.5$ Hz), 5.24 (1H, d, $J=4.9$ Hz), 5.87 (1H, dd, $J=8.9$, 4.9 Hz), 6.79 (1H, d, $J=3.8$ Hz), 6.93 (1H, s), 7.2–7.6 (12H, m), 8.16 (1H, s), 8.48 (1H, dd, $J=4.8$, 1.6 Hz), 8.60 (1H, d, $J=$ 1.6 Hz), 9.15 (1H, d, $J=8.9$ Hz).

Diphenylmethyl 7β-amino-3-[(4-pyridyl)methylthio]-3-cephem-4-carboxylate (37d). To a solution of **36d**

(13.7 g, 26.5 mmol) in MeOH (65 mL) was added dropwise concd HCl (11.0 mL) at room temperature and the mixture was stirred at the same temperature for 2.8 h. The mixture was poured into a mixture of ethyl acetate and ice water and adjusted to pH 7 by addition of 5 N aqueous NaOH. The aqueous layer was separated and the organic layer washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent, the residue was triturated with ethyl acetate to give **37d**. Amorphous solid. Yield: 5.3 g (41%). IR (Nujol) cm^{-1} 1755, 1720, 1595; ^1H NMR (DMSO-d_6) δ 2.37 (2H, br s), 3.73 and 3.83 (2H, ABq, $J=17.6$ Hz), 4.11 (2H, s), 4.79 (1H, d, $J=5.0$ Hz), 5.00 (1H, d, $J=5.0$ Hz), 6.85 (1H, s), 7.2–7.5 (12H, m), 8.48 (2H, dd, $J=4.4$, 1.6 Hz).

The following compounds were obtained using a method similar to that used for **37d**.

Diphenylmethyl 7β-amino-3-(3-pyridyl)thio-3-cephem-4-carboxylate (37a). Amorphous solid. Yield: 3.3 g (82%). IR (Nujol) cm^{-1} 1753, 1723, 1605; ^1H NMR (DMSO-d_6) δ 3.27 and 3.65 (2H, ABq, $J=17.7$ Hz), 4.88 (1H, d, $J=5.1$ Hz), 5.12 (1H, d, $J=5.1$ Hz), 6.84 (1H, s), 7.2–7.5 (11H, m), 7.74 (1H, dt, $J=8.6$, 1.6 Hz), 8.49–8.54 (1H, m).

Diphenylmethyl 7β-amino-3-[(3-pyridyl)methylthio]-3-cephem-4-carboxylate (37c). Amorphous solid. Yield: 2.7 g (94%). IR (Nujol) cm^{-1} 1730, 1690; ^1H NMR (DMSO-d_6) δ 2.37 (2H, br s), 3.79 and 3.89 (2H, ABq, $J=18$ Hz), 4.13 (2H, s), 4.80 (1H, d, $J=4.8$ Hz), 5.02 (1H, d, $J=4.8$ Hz), 6.83 (1H, s), 7.2–7.4 (10H, m), 7.4–7.5 (1H, m), 7.6–7.7 (1H, m), 8.4–8.5 (2H, m).

Diphenylmethyl 7β-amino-3-[(*Z*)-2-(3-pyridyl)vinylthio]-3-cephem-4-carboxylate (37f). Amorphous solid. Yield: 5.5 g (83%). ^1H NMR (DMSO-d_6) δ 3.76 and 4.04 (2H, ABq, $J=17.7$ Hz), 4.87 (1H, d, $J=5.1$ Hz), 5.08 (1H, d, $J=5.1$ Hz), 6.71 (1H, d, $J=10.9$ Hz), 6.80 (1H, d, $J=10.9$ Hz), 6.90 (1H, s), 7.2–7.5 (11H, m), 7.74 (1H, dt, $J=8.0$, 2.0 Hz), 8.47 (1H, dd, $J=4.8$, 1.6 Hz), 8.58 (1H, d, $J=2.0$ Hz).

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(acetoxyimino)acetamido]-3-[(4-pyridyl)methylthio]-3-cephem-4-carboxylate (41d). 37d (2.15 g, 4.39 mmol) was dissolved in CH_2Cl_2 (50 mL) by addition of BSA (1.79 g, 8.78 mmol). To the resulting solution was added **23** (1.50 g, 5.27 mmol) at 5 °C and the mixture was stirred at the same temperature for 1.5 h and at room temperature for 16 h. The mixture was poured into a mixture of water and MeOH and adjusted to pH 7 by addition of 1 N aqueous NaOH. The aqueous layer was separated and the organic layer was washed with brine and dried over $MgSO_4$ and the solvent was evaporated in vacuo to give **41d**. Amorphous solid. Yield: 2.4 g (80%). ^1H NMR (DMSO-d_6) δ 2.14 (3H, s), 3.75–3.85 (2H, m), 4.22 (2H, s), 5.27 (1H, d, $J=4.8$ Hz), 5.76 (1H, dd, $J=4.8$, 8.0 Hz), 6.87 (1H, s), 7.2–7.6 (15H, m), 8.53 (2H, d, $J=6.0$ Hz), 9.90 (1H, d, $J=8.0$ Hz).

The following compounds were obtained using a method similar to that used for **41d**.

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(acetoxyimino)acetamido]-3-(3-pyridylthio)-3-cephem-4-carboxylate (41a). Amorphous solid. Yield: 3.8 g (79%). ^1H NMR (DMSO-d_6) δ 2.10 (3H, s), 3.3–3.7 (2H, m), 5.29 (1H, d, $J=4.8$ Hz), 5.91 (1H, dd, $J=8.2$, 4.8 Hz), 6.90 (1H, s), 7.07 (1H, s), 7.2–7.5 (13H, m), 7.7–7.85 (1H, m), 8.5–8.6 (2H, m), 9.93 (1H, d, $J=8.2$ Hz).

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(acetoxyimino)acetamido]-3-[(3-pyridyl)methylthio]-3-cephem-4-carboxylate (41c). Amorphous solid. Yield: 3.4 g (91%). IR (Nujol) cm^{-1} 1759, 1672, 1598, 1520; ^1H NMR (DMSO-d_6) δ 2.19 (3H, s), 3.90 (2H, s), 4.21 (2H, s), 5.28 (1H, d, $J=4.9$ Hz), 5.85 (1H, dd, $J=4.9$, 7.8 Hz), 6.85 (1H, s), 7.14 (1H, s), 7.2–7.4 (10H, m), 7.4–7.5 (1H, m), 7.6–7.8 (1H, m), 8.4–8.5 (2H, m), 9.93 (1H, d, $J=7.8$ Hz).

Compound **42f** was obtained using a method similar to that used for **29b**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-[(*Z*)-2-(3-pyridyl)vinylthio]-3-cephem-4-carboxylate (42f). Amorphous solid. Yield: 2.1 g (58%). IR (Nujol) cm^{-1} 1785, 1675; ^1H NMR (DMSO-d_6) δ 3.88 and 4.09 (2H, ABq, $J=17.6$ Hz), 5.36 (1H, d, $J=4.9$ Hz), 6.08 (1H, dd, $J=8.5$, 4.9 Hz), 6.64 (1H, s), 6.79 (1H, d, $J=10.9$ Hz), 6.87 (1H, d, $J=10.9$ Hz), 6.95 (1H, s), 7.25–7.55 (28H, m), 7.81 (1H, dt, $J=8.0$, 2.0 Hz), 8.59 (1H, d, $J=3.4$ Hz), 8.61 (1H, d, $J=1.6$ Hz), 9.97 (1H, d, $J=8.5$ Hz).

The following compounds were obtained using a method similar to that used for **36d**.

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-(4-pyridylthio)-3-cephem-4-carboxylate (42b). Amorphous solid. Yield: 211 mg (21%). IR (KBr) cm^{-1} 1781, 1741, 1677; ^1H NMR (DMSO-d_6) δ 3.46 and 3.93 (2H, Abq, $J=17.8$ Hz), 5.31 (1H, d, $J=4.0$ Hz), 5.88 (1H, dd, $J=8.2$, 4.0 Hz), 6.86 (1H, s), 7.2–7.5 (27H, m), 8.46 (2H, d, $J=6.1$ Hz), 9.96 (1H, d, $J=8.2$ Hz). ESIMS (neg.) *m/e* 886 [$(M-H)^+$].

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-[2-(4-pyridyl)ethylthio]-3-cephem-4-carboxylate (42e). Amorphous solid. Yield: 293 mg (27%). IR (Nujol) cm^{-1} 1784, 1682; ^1H NMR (DMSO-d_6) δ 2.85 (2H, t, $J=7.1$ Hz), 3.20 (2H, t, $J=7.1$ Hz), 3.88 (2H, s), 5.33 (1H, d, $J=5.0$ Hz), 5.95 (1H, dd, $J=8.4$, 8.0 Hz), 6.71 (1H, s), 6.89 (1H, s), 7.2–7.6 (19H, m), 8.45 (2H, d, $J=6.2$ Hz), 9.90 (1H, d, $J=8.0$ Hz).

Diphenylmethyl 7β-[2-(2-aminothiazol-4-yl)-2-(*Z*)-(trityloxyimino)acetamido]-3-[(3-pyridyl)thiomethylthio]-3-cephem-4-carboxylate (42g). Under an N_2 atmosphere, 1.2 N sodium methoxide in MeOH (5 mL, 6 mmol) was added slowly to a solution of (3-pyridyl)thiomethylthioacetate (1.06 g, 6 mmol) in a mixture of THF (4 mL) and DMF (12 mL) at 0 °C, and stirred for 1 h. The mixture was cooled to −78 °C with a dry ice–acetone bath, and a solution of **40** (4.4 g, 5 mmol) in a mixture of THF (4.5 mL) and DMF (13.5 mL) was added dropwise

to the mixture while the temperature was maintained under −70 °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 $MgSO_4$. Afer evaporation of the solvent, the residue was purified by column chromatography on silica-gel eluting with CH_2Cl_2–acetone to afford **42g**. Amorphous solid. Yield: 2.5 g (53%). IR (KBr) cm^{-1} 1780; ^1H NMR (DMSO-d_6) δ 3.95 (2H, br s), 4.66 (2H, s), 5.33 (1H, d, J = 4.7 Hz), 5.97 (1H, dd, J = 8.5, 4.7 Hz), 6.69 (1H, s), 6.85 (1H, s), 7.1–7.6 (27H, m), 7.84 (1H, dt, J = 8.8, 1.6 Hz), 8.46 (1H, m), 8.59 (2H, d, J = 1.9 Hz), 9.90 (1H, d, J = 8.5 Hz).

Compound **42h** was obtained using a method similar to that used for **42g**.

Diphenylmethyl 7β-[2-(*Z*)-(2-aminothiazol-4-yl)-2-(trityloxyimino)acetamido]-3-[(4-pyridyl)thiomethylthio]-3-cephem-4-carboxylate (42h). Amorphous solid. Yield: 1.5 g (80%). IR (KBr) cm^{-1} 1778; ^1H NMR (DMSO-d_6) δ 3.94 (2H, br s), 4.76 (2H, s), 5.35 (1H, d, J = 4.7 Hz), 5.98 (1H, dd, J = 8.5, 4.7 Hz), 6.69 (1H, s), 6.85 (1H, s), 7.25–7.6 (29H, m), 8.41 (2H, d, J = 1.9 Hz), 9.92 (1H, d, J = 8.5 Hz).

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(4-pyridylthio)-3-cephem-4-carboxylic acid (12). To a suspension of **42b** (1.37 g, 1.21 mmol) in anisole (2.7 mL) and CH_2Cl_2 (8.1 mL) was added trifluoroacetic acid (5.4 mL) at 5 °C. The resulting solution was stirred for 4 h at room temperature and poured into IPE (150 mL). The resulting precipitate was collected by filtration, washed with IPE and dried in vacuo. The powder was dissolved in water (100 mL) by addition of saturated aqueous $NaHCO_3$. The solution was adjusted to ca. pH 6 by addition of 1 N HCl and chromatographed on HP-20 (50 mL) eluting with 10% aqueous IPA. The eluent was lyophilized and the crude product was purified by preparative HPLC to give **12**. Amorphous solid. Yield: 52 mg (9%). IR (Nujol) cm^{-1} 1750, 1620, 1590, 1530; ^1H NMR (DMSO-d_6) δ 3.32 and 3.86 (2H, ABq, J = 17.6 Hz), 5.33 (1H, d, J = 5.0 Hz), 5.89 (1H, dd, J = 8.2, 5.0 Hz), 6.65 (1H, s), 7.13 (2H, br s), 7.21 (2H, dd, J = 4.6, 1.6 Hz), 8.45 (2H, dd, J = 4.6, 1.6 Hz), 9.59 (1H, d, J = 8.2 Hz), 11.33 (1H, s). FABMS m/e 479[$(M+H)^+$].

Compounds **17** and **18** were obtained using a method similar to that used for **12**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(3-pyridyl)thiomethylthio]-3-cephem-4-carboxylic acid (17). Yield: 291 mg (48%). IR (KBr) cm^{-1} 3303, 1768, 1664, 1612, 1533; ^1H NMR (DMSO-d_6) δ 3.84 (2H, s), 4.58 (2H, s), 5.17 (1H, d, J = 4.7 Hz), 5.75 (1H, dd, J = 8.2, 4.7 Hz), 6.68 (1H, s), 7.14 (2H, br s), 7.35–7.42 (1H, m), 8.44–8.48 (1H, m), 8.59–8.61 (1H, m), 9.49 (1H, d, J = 8.3 Hz), 11.32 (1H, s). ESIMS m/e 525 [$(M+H)^+$]. Anal. calcd for $C_{18}H_{16}N_6O_5S_4{\cdot}2.4H_2O$: C, 38.07; H, 3.69; N, 14.80; found: C, 37.93; H, 3.44; N, 14.73.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyridyl)thiomethylthio]-3-cephem-4-carboxylic acid (18). Yield: 325 mg (39%). IR (KBr) cm^{-1} 3313, 1766, 1664, 1623, 1533; ^1H NMR (DMSO-d_6) δ 3.86 (2H, s), 4.67 (2H, s), 5.18 (1H, d, J = 4.7 Hz), 5.75 (1H, dd, J = 8.2, 4.7 Hz), 6.68 (1H, s), 7.14 (2H, br s), 7.36 (2H, dd, J = 4.7, 1.6 Hz), 8.41 (2H, dd, J = 4.7, 1.6 Hz), 9.49 (1H, d, J = 8.2 Hz), 11.32 (1H, s). ESIMS (neg.) m/e 523 [$(M-H)^+$]. Anal. calcd for $C_{18}H_{16}N_6O_5S_4{\cdot}2.6H_2O$: C, 37.83; H, 3.74; N, 14.71; found: C, 37.91; H, 3.53; N, 14.51.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(3-pyridyl)methylthio]-3-cephem-4-carboxylic acid (13). To a solution of **41c** (3.32 g, 4.83 mmol) in CH_2Cl_2 (9.9 mL) and anisole (3.3 mL) was added trifluoroacetic acid (6.6 mL) at 5 °C. The mixture was stirred for 1.5 h at the same tempetature and poured into IPE (100 mL). The resulting precipitate was collected by filtration, washed with IPE and dried in vacuo. The crude product (3.85 g, 5.05 mmol) was dissolved in a mixture of water (116 mL) and MeOH (11.6 mL), and thereto NH_4Cl (810 mg, 15.2 mmol) was added and the mixture was adjusted to pH 8 with aq $NaHCO_3$ solution. The solution was stirred for 2 h maintaining pH 8 with aq $NaHCO_3$ solution. The reaction mixture was adjusted to pH 6 by addition of 1 N HCl. The mixture was concentrated in vacuo and adjusted to pH 5 with 1 N HCl and chromatographed on HP-20 (100 mL) eluting with 5–10% aqueous IPA. 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 **13.** Amorphous solid. Yield: 445 mg (18%). IR (Nujol) cm^{-1} 1770, 1650, 1579, 1521; ^1H NMR (DMSO-d_6) δ 3.76 (2H, s), 4.13 and 4.19 (2H, ABq, J = 13.0 Hz), 5.14 (1H, d, J = 4.9 Hz), 5.17 (1H, dd, J = 4.9, 7.8 Hz), 6.68 (1H, s), 7.14 (2H, br s), 7.3–7.4 (1H, m), 7.7–7.8 (1H, m), 8.4–8.6 (2H, m), 9.48 (1H, d, J = 7.8 Hz), 11.3 (1H, s). ESIMS (neg.) m/e 491 [$(M-H)^+$].

Compounds **11** and **14** were obtained using a method similar to that used for **13**.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-(3-pyridylthio)-3-cephem-4-carboxylic acid (11). Amorphous solid. Yield: 322 mg (12%). IR (Nujol) cm^{-1} 1750, 1600; ^1H NMR (DMSO-d_6) δ 3.22 and 3.62 (2H, ABq, J = 17.3 Hz), 5.22 (1H, d, J = 4.9 Hz), 5.79 (1H, dd, J = 8.2, 4.9 Hz), 6.64 (1H, s), 7.12 (2H, br s), 7.35–7.45 (1H, m), 7.8–7.9 (1H, m), 8.5–8.6 (2H, m), 9.52 (1H, d, J = 8.2 Hz), 11.32 (1H, s). ESIMS (neg.) m/e 476 [$(M-H)^+$].

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(4-pyridyl)methylthio]-3-cephem-4-carboxylic acid (14). Amorphous solid. Yield: 295 mg (17%). IR (Nujol) cm^{-1} 1750, 1600, 1505; ^1H NMR (DMSO-d_6) δ 3.70 (2H, s), 4.14 (2H, s), 5.13 (1H, d, J = 4.7 Hz), 5.71 (1H, dd, J = 8.2, 4.7 Hz), 6.67 (1H, s), 7.13 (2H, br s), 7.35 (2H, dd, J = 4.5, 1.6 Hz), 8.51 (2H, dd, J = 4.5, 1.6 Hz), 9.46 (1H, d, J = 8.2 Hz), 11.31 (1H, s). FABMS m/e 493 [$(M+H)^+$].

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[2-(4-pyridyl)ethylthio)-3-cephem-4-carboxylic acid (15). To a solution of **42e** (760 mg, 0.83 mmol) in formic acid (3 mL) was added concd HCl (0.21 mL, 2.5 mmol) at 5 °C. The mixture was stirred for 1 h at room temperature and then poured into a mixture of ethyl acetate (20 mL) and acetone (10 mL). The resulting precipitate was collected by filtration and dried in vacuo. The crude product was purified using a method similar to that used for **13** to give **15**. Mp 161 °C. Yield: 215 mg (51%). IR (KBr) cm^{-1} 1767, 1668, 1639, 1618; ^1H NMR (DMSO-d_6) δ 2.7–2.9 (2H, m), 3.13 (2H, t, $J=7$ Hz), 3.73 and 3.83 (2H, ABq, $J=17.1$ Hz), 5.18 (1H, d, $J=5.0$ Hz), 5.73 (1H, dd, $J=8.2$, 5.0 Hz), 6.69 (1H, s), 7.15 (2H, br s), 7.33 (2H, d, $J=5.0$ Hz), 8.51 (2H, d, $J=5.0$ Hz), 9.48 (1H, d, $J=8.0$ Hz), 11.3 (1H, s). ESIMS (neg.) *m/e* 505 [$(M-H)^+$]. Anal. calcd for $C_{19}H_{18}N_6O_5S_3 \cdot 3.5H_2O$: C, 40.60; H, 4.42; N, 14.75; found: C, 40.60; H, 4.16; N, 14.75.

7β-[2-(*Z*)-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetamido]-3-[(*Z*)-2-(3-pyridyl)vinylthio]-3-cephem-4-carboxylic acid (16). Under an N_2 atmosphere, a solution of $AlCl_3$ (1.53 g, 11.5 mmol) in anisole (3.6 mL) was added to a solution of **42f** (2.1 g, 2.30 mmol) in a mixture of CH_3NO_2 (14.3 mL) and anisole (3.6 mL) at −24 °C. The mixture was stirred for 1 h at the same temperature and the reaction was quenched with 1 N aq HCl (14.3 mL). The mixture was poured into a mixture of water and ethyl acetate and the aqueous layer was separated. The organic layer was reextracted with water and the combined aqueous layer was concentrated in vacuo and chromatographed on HP-20 eluting with 30% aqueous IPA. The fractions containing the object compound were collected and concentrated in vacuo. The resulting precipitate was collected by filtration and dried in vacuo to give **16**. Amorphous solid. Yield: 244 mg (21%). IR (KBr) cm^{-1} 1770, 1664, 1610; ^1H NMR (DMSO-d_6) δ 3.71 and 4.05 (2H, ABq, $J=17.4$ Hz), 5.21 (1H, d, $J=4.9$ Hz), 5.82 (1H, dd, $J=8.1$, 4.9 Hz), 6.67 (1H, s), 6.76 (1H, d, $J=10.9$ Hz), 6.81 (1H, d, $J=10.9$ Hz), 7.13 (2H, s), 7.45 (1H, m), 7.87 (1H, m), 8.47 (1H, dd, $J=4.8$, 1.5 Hz), 8.65 (1H, d, $J=1.5$ Hz), 9.51 (1H, d, $J=8.1$ Hz), 11.31 (1H, s).

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 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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