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Studies on Anti-*Helicobacter pylori* Agents. Part 2: New Cephem Derivatives

Yoshiki Yoshida,[a,*] Keiji Matsuda,[a] Hiroshi Sasaki,[a] Yoshimi Matsumoto,[b] Satoru Matsumoto,[b] Shuichi Tawara[b] and Hisashi Takasugi[a]

[a]*Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b]*Medicinal Biology Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

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Abstract—The synthesis and optimization of the anti-*Helicobacter pylori* activity of a novel series of cephem derivatives are described. Introduction of thio-heterocyclic groups containing N- and S-atoms to the 3-position and phenyl or thienyl acetamido groups to the 7-position of the cephem nucleus dramatically improved the activity. From this series of derivatives, compound **13i** was found to have extremely potent in vitro anti-*H. pylori* activity, superior therapeutic efficacy compared to AMPC and CAM, no cross-resistance between CAM or MNZ and low potential for causing diarrhea due to instability to β-lactamase.

Introduction

Since its discovery in the gastric mucosa of humans,[1] the clinical importance of eradication of *Helicobacter pylori* (*H. pylori*) has increased significantly[2–6] due to its relationship to diseases such as chronic gastritis, peptic ulcer, certain malignant peptic complications and the like. To date only a small number of multi-drug therapy regimens containing antibacterial agents, for example amoxicillin (AMPC) and clarithromycin (CAM), with a proton pump inhibitor such as omeprazole or lansoprazole, antiprotozoal agent or bismuth salt, are used for eradication of *H. pylori*.[7] Although these therapies have shown a reasonable response, they are not entirely successful, and furthermore, there remain problems such as drug resistance,[8,9] side effects[10,11] and non-compliance.[12] However, whilst new anti-*H. pylori* agents have been studied by many investigators,[13–16] there are currently no new anti-*H. pylori* compounds that show superior therapeutic eradication efficacy to AMPC and CAM. As a result, the need for alternative and novel structural types is evident, and has stimulated the search for novel agents that have potent therapeutic efficacy against *H. pylori* and resolve the problems with current treatment.

In a previous paper,[17] we reported a series of benzyloxyisoquinoline derivatives which show good in vitro anti-*H. pylori* activity, but which failed to show therapeutic efficacy, and a typical compound **1** is shown in Figure 1. Therefore, as part of a further screening program of various compounds, we were drawn to antibiotic compounds, especially cephem derivatives; even though generally it has been believed that the anti-*H. pylori* activity of cephem derivatives is rather poor and weaker than that of penicillin derivatives such as AMPC, we found in our preliminary study that cephem derivatives display high potential for anti-*H. pylori* activity. Moreover, they are fundamentally stable under acidic conditions and are less susceptible to degradation in the stomach compared to AMPC and, furthermore, much information is available on mechanism, bactericidal activity and toxicity. Furthermore, cephem compounds containing a non-oxime structure at the 7-position have low potential for causing diarrhea, a major side effect of AMPC, due to low stability towards β-lactamase,[18–20] and thereby reduced potential for disruption of intestinal microbial flora, due to rapid deactivation by β-lactamase in intestine. Therefore, using this design concept we searched for a compound having a non-oxime structure at the 7-position for low stability towards β-lactamase, along with potent anti-*H. pylori* activity, and investigated the preparation of novel cephem analogues substituted at the 3- and/or 7-position of the cephem nucleus. In this paper, we describe the synthesis and biological evaluation of

*Corresponding author. Present address. Intellectual Property Division, Fujisawa Pharmaceutical Co., Ltd., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan. Tel.: +81-6-6390-1226; fax: +81-6-6304-1264; e-mail: yoshiki_yoshida@po.fujisawa.co.jp

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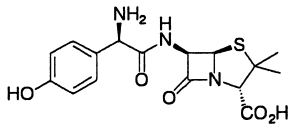
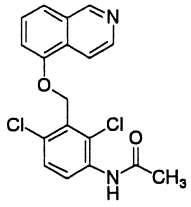
PII: S0968-0896(00)00163-2

cephem compounds with excellent in vitro activity and therapeutic efficacy.[21]



Amoxicillin(AMPC)

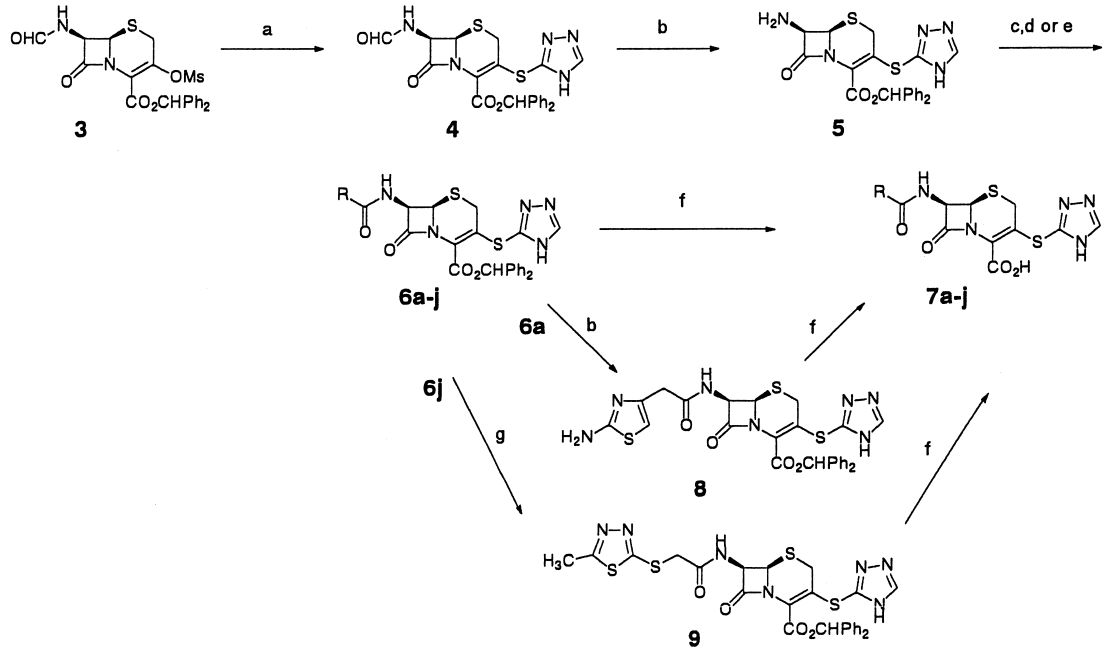


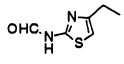
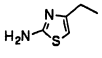
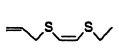
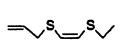
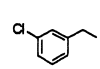
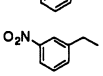
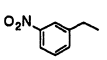
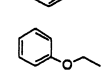
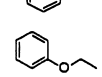
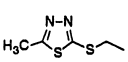
benzyloxyisoquinoline-type compound (1)

Figure 1. Anti-*H. pylori* agents.

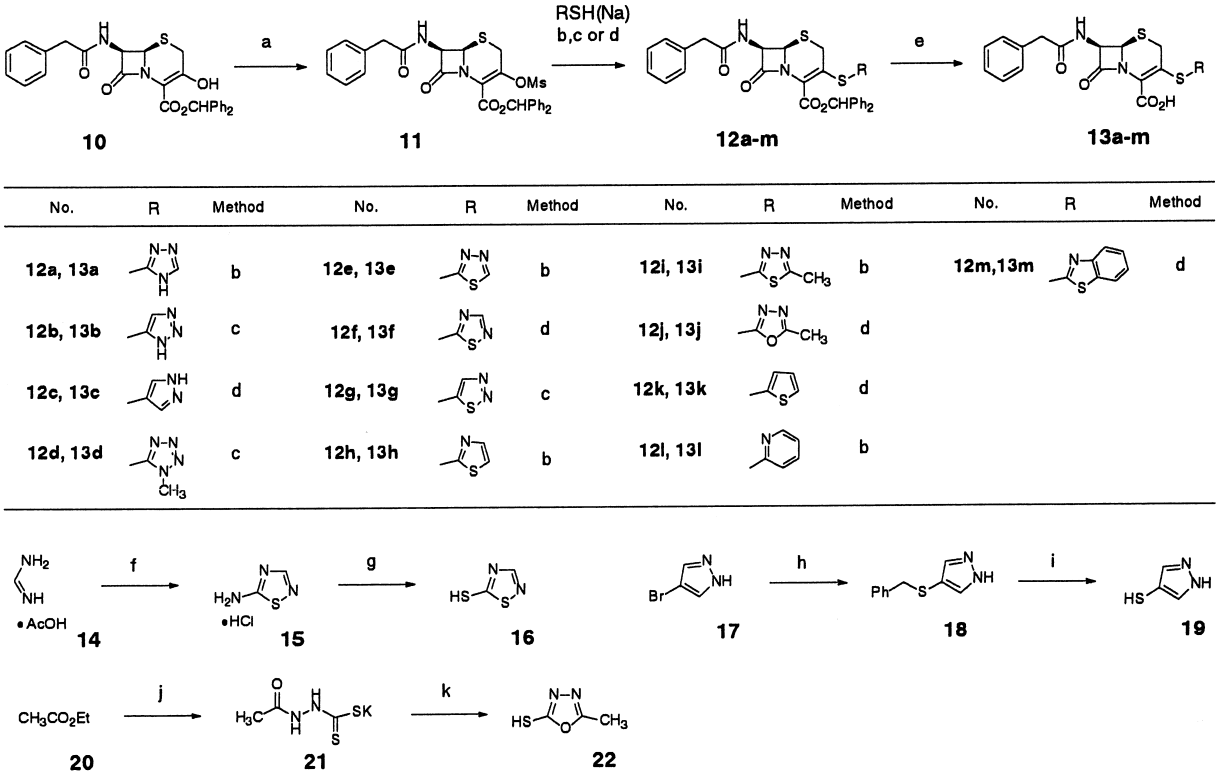
Chemistry

Cephem derivatives having various substituents at the 7-position and 1,2,4-triazol-3-ylthio moiety at the 3-position were synthesized by the typical route shown in Scheme 1. Treatment of 3-mesylated compound **3** having a benzhydryl ester at the 4-position and formamido at the 7-position with 3-mercapto-1,2,4-triazole using *i*-Pr_2NEt as a base gave triazolylthio derivative **4**, which was deformylated with c-HCl to yield 7-amino compound **5**. Acylation of **5** with various activated carboxy derivatives using PCl_5 or the Vilsmeier method gave 7-acylated compounds **6b–i**. Deprotection of the benzhydryl ester with TFA/anisole then afforded **7b–i** in good yield. In the case of a compound having a reactive amino moiety at the 7-position (**6a**), the acylation was performed using a formamido protected amino group. Subsequently, after deformylation of **6a**, deprotection of the benzhydryl ester of **8** yielded **7a**. Moreover, acylation of **5** with bromoacetyl bromide gave **6j** and subsequent reaction with 2-mercapto-5-methyl-1,3,4-thiadiazole gave **9**; deprotection of the benzhydryl ester then yielded **7j**.



No.	R	Method	No.	R	Method	No.	R	No.	R
6a		c	**6f**		c	**7a**		**7f**	
6b		c	**6g**		c	**7b**		**7g**	
6c		c	**6h**		e	**7c**		**7h**	
6d		d	**6i**		c	**7d**		**7i**	
6e		c	**6j**		d	**7e**		**7j**	

Scheme 1. Reagents: (a) 3-mercapto-1,2,4-triazole, *N,N*-diisopropylethylamine (DIPEA), DMF; (b) c-HCl, MeOH–THF; (c) RCO_2H, PCl_5, MSA, CH_2Cl_2–THF; (d) RCOX, MSA, THF; (e) RCO_2H, $POCl_3$, DMF, MSA, AcOEt–THF; (f) TFA, anisole, CH_2Cl_2; (g) *t*-BuOK, 2-mercapto-5-methyl-1,3,4-thiadiazole, THF–DME.



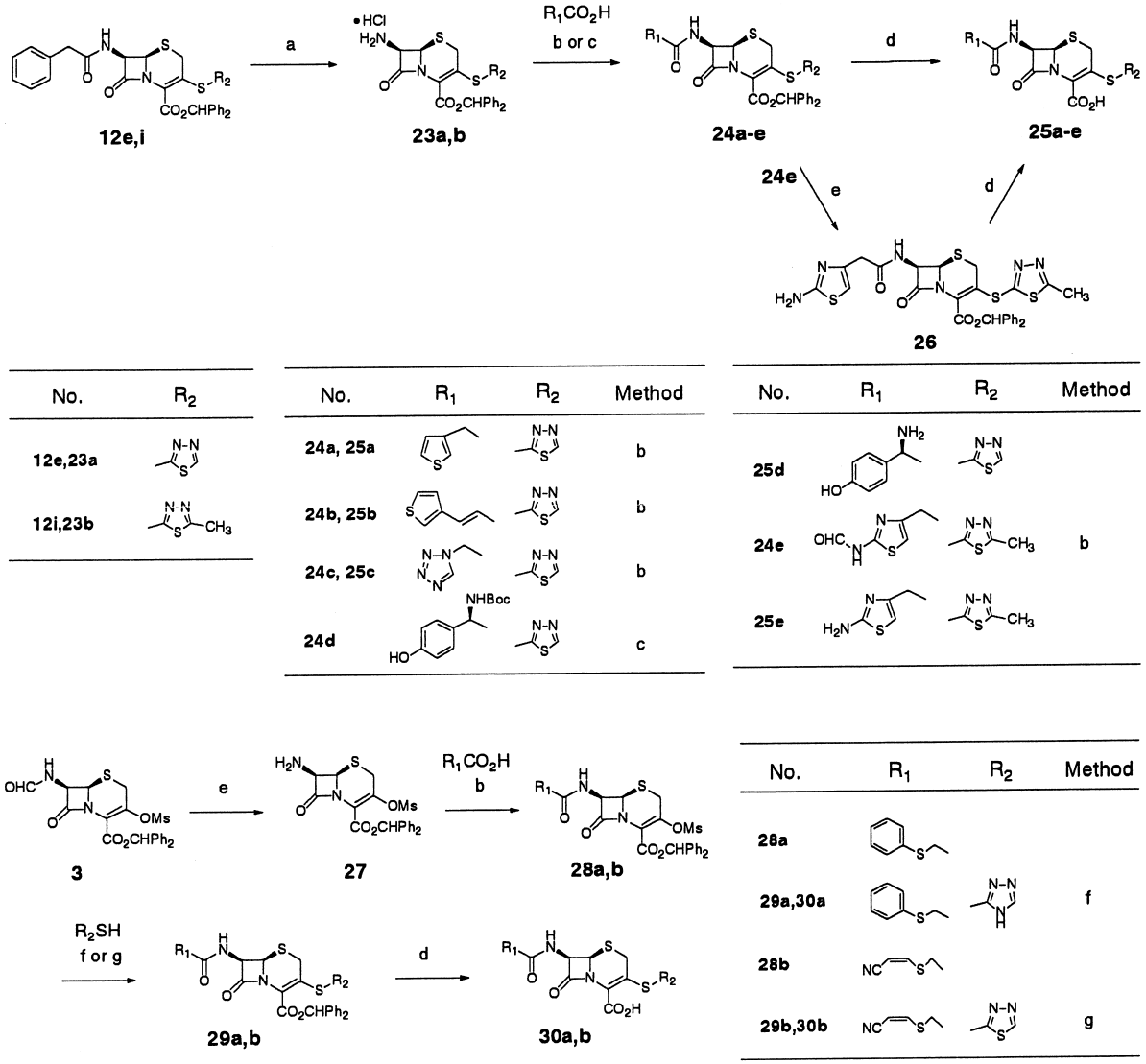
No.	R	Method	No.	R	Method	No.	R	Method	No.	R	Method
12a, 13a		b	12e, 13e		b	12i, 13i		b	12m,13m		d
12b, 13b		c	12f, 13f		d	12j, 13j		d			
12c, 13c		d	12g, 13g		c	12k, 13k		d			
12d, 13d		c	12h, 13h		b	12l, 13l		b			

Scheme 2. Reagents: (a) MsCl, K_2CO_3, DMF; (b) RSH, *t*-BuOK, THF–DME; (c) RSNa, THF–DME; (d) RSH, DIPEA, DMF; (e) TFA, anisole, CH_2Cl_2; (f) KSCN, $NaOCH_3$, Br_2, MeOH, HCl; (g) HBr, Cu, $NaNO_2$, thiourea; (h) benzyl disulfide, *n*-BuLi; (i) Na, EtOH; (j) H_2NNH_2, CS_2, MeOH, (k) pyridine.

Preparation of 7-phenylacetoamido derivatives having various heterocyclic thio moieties at the 3-position was performed according to the route shown in Scheme 2. 3-Mesylated compound **11** was obtained by mesylation ($MsCl–K_2CO_3$/DMF, −30 °C) of the corresponding 3-hydroxy cephem derivative **10**[22] in good yield, while mesylation using *i*-Pr_2NEt as a base did not afford good results. Subsequent treatment of **11** with various heterocyclic mercaptans or their salts using various methods (such as *t*-BuOK or *i*-Pr_2NEt as a base and DMF or THF–DME as a solvent) gave **12a**–**m**, followed by deprotection of the benzhydryl ester to afford **13a**–**m**. These mercapto derivatives, except for **16**, **19** and **22**, were commercially available. Thioles **16**, **19** and **22** were prepared as shown in Scheme 2. Aminothiadiazole **15** was obtained by cyclization of amidine with potassium thiocyanate and bromine. Treatment of **15** with HBr and $NaNO_2$ gave bromo derivative, followed by addition of thiourea to yield 5-mercapto-1,2,4-thiadiazole **16**. After coupling of 4-bromopyrazole **17** and benzyl disulfide using *n*-BuLi, reaction with Na afforded 4-mercaptopyrazole **19**. Treatment of AcOEt with hydrazine and carbon disulfide gave potassium acetylhydrazinodithioate **21**, which was cyclized using pyridine to yield oxadiazole derivative **22**.

Compounds **25a**–**e** and **30a**,**b** were prepared by the routes shown in Scheme 3, though these compounds could also be prepared by the same method as shown in Scheme 1. Regarding preparation of **25a**–**e**, 7-amino compounds having a 1,3,4-thiadiazol-2-ylthio moiety at the 3-position (**23a**,**b**) were obtained by de-phenylacetylation of **12e**,**i** using PCl_5/Py conditions. Treatment of **23a**,**b** with activated carboxylic acids gave the corresponding acylated compounds **24a**–**e**, which were deprotected to afford final compounds **25a**–**e**. In the case of compounds having a reactive amino moiety at the 7-position **24d**,**e**, the acylation was performed using a compound having a Boc or formyl protective group, respectively. After acylation of **23a** with D-2-(Boc-amino)-2-(4-hydroxyphenyl)acetic acid, simultaneous deprotection of the Boc and benzhydryl groups afforded **25d**. **25e** was prepared by a similar method to compound **7a** in Scheme 1. Next, concerning compounds **30a**,**b**, after de-formylation of 7-formamido derivative **3**, acylation with carboxylic acids using PCl_5 afforded 3-mesyl-7-acylated compounds **28a**,**b**. Treatment of **28a**,**b** with heterocyclic mercaptans using *i*-Pr_2NEt or *t*-BuOK as the base, respectively, followed by deprotection of the resulting benzhydryl ester **29a**,**b**, yielded **30a**,**b**.

Compounds having different spacer moieties between the cephem nucleus and the heterocyclic moiety **32**, **35**, **40** and **44** were prepared as shown in Scheme 4. Compound **32** which has a natural type partial structure at the 3-position was obtained by phenylacetylation of **31**.[23] Treatment of 3-mesylated compound **11** with 5-methylthiadiazol-2-ylmethyl thiobenzoate **33** using NaOMe to hydrolyze the thioester gave 5-methylthiadiazol-2-ylmethylthio derivative **34**, which was deprotected at the benzhydryl group to afford **35** having the opposite thiomethylene spacer part, compared to the natural type **32**. 2-Chloromethylthiothiazole **37**, a starting material for **40** having a thiomethylenethio spacer

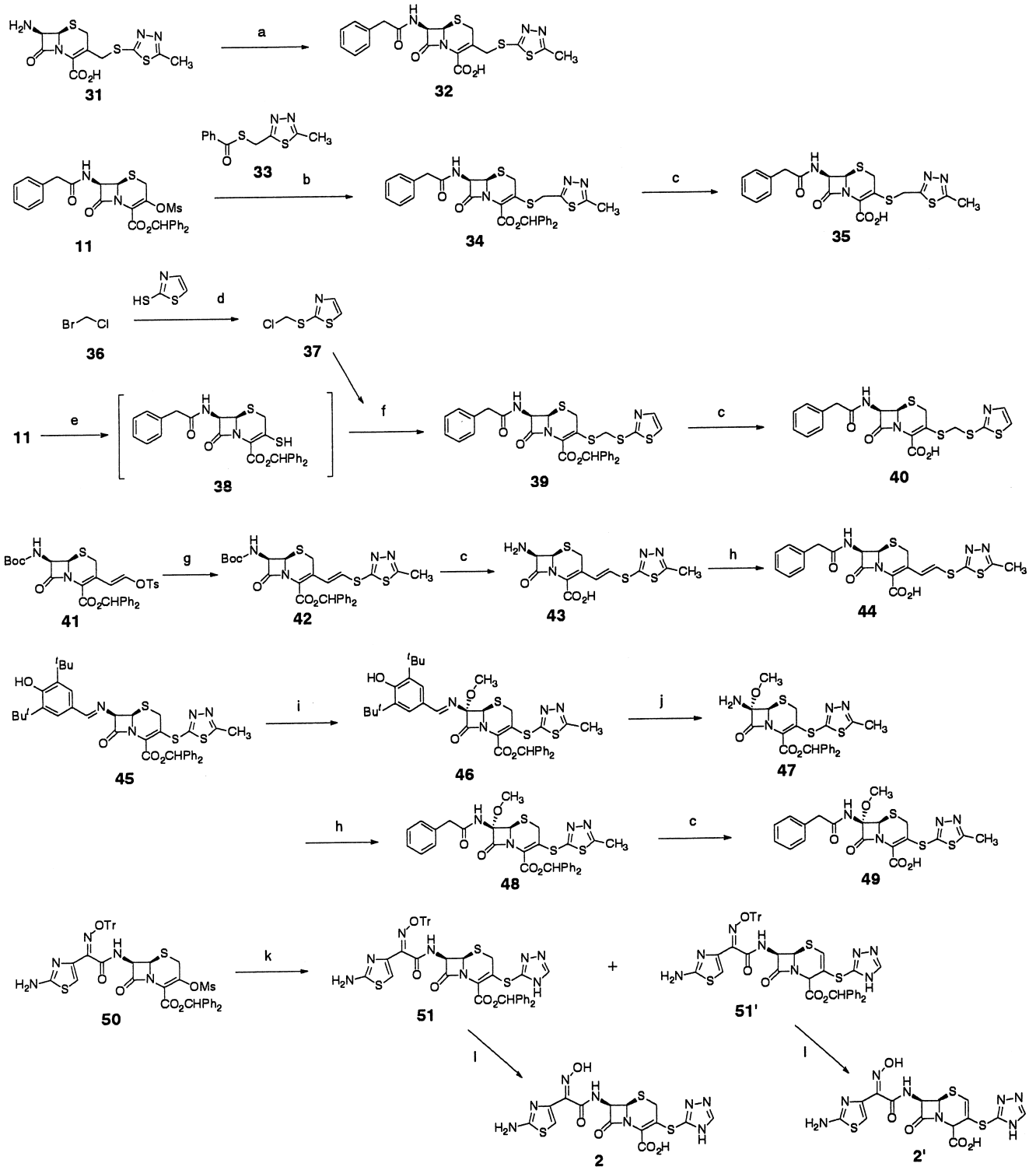


Scheme 3. Reagents: (a) PCl_5, Py, CH_2Cl_2; (b) PCl_5, MSA, CH_2Cl_2–THF; (c) DCC, CH_2Cl_2; (d) TFA, anisole, CH_2Cl_2; (e) c-HCl, MeOH–THF; (f) DIPEA, DMF; (g) *t*-BuOK, THF–DME.

moiety, was obtained by reaction of bromochloromethane **36** with 2-mercaptothiazole. Treatment of mesylate **11** with NaSH gave 3-mercapto derivative **38**; subsequent addition of **37** yielded **39**, which was deprotected at the benzhydryl group to yield **40**. Compound **42** was obtained by coupling reaction of 3-vinyltosylate **41** with 2-mercapto-5-methylthiadiazole. After deprotection of the Boc and benzhydryl protective groups at the 7- and 4-positions, respectively, using TFA/anisole conditions, acylation of **43** with phenylacetyl chloride afforded **44** having a vinylthio spacer moiety. Preparation of the cephamycin-type compound **49** is also shown in Scheme 4. Treatment of imino protected compound **45** with DDQ/MeOH gave **46** having a methoxy group at the 7α-position, which was deprotected with Girard's Reagent T to yield 7-amino compound **47**. After acylation with phenylacetyl chloride, deprotection afforded 7α-methoxy cephamycin-type compound **49**. Regarding compounds having a hydroxyimino structure at the 7-position (**2**, **2′**), treatment of 3-mesylated compound **50**[24] with triazolylmercaptan using *i*-Pr_2NEt as a base gave a mixture of **51** and its Δ2 isomer **51′**, after isolation, which were deprotected with HCO_2H/c-HCl to yield **2** and **2′**, respectively.

Results and Discussion

In the search for novel compounds with anti-*H. pylori* activity, we initiated a random screening effort and found in our preliminary study that cephem derivatives display some potential for anti-*H. pylori* activity, even though generally it has been believed that the anti-*H. pylori* activity of cephem derivatives is rather poor and inferior to penicillin derivatives. Subsequent intentional screening of the Fujisawa cephem library uncovered compound **2** having a 1,2,4-triazol-3-ylthio moiety at the 3-position of the cephem nucleus, which displayed good anti-*H. pylori* activity. Its activity was not only superior to that of CFDN[25] and FK041[24] having different partial structures at the 3-position and the same structure at the 7-position, but also was good compared

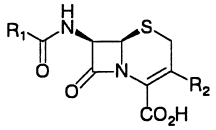


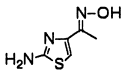
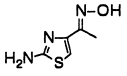
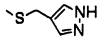
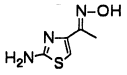
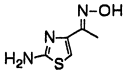
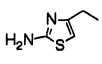
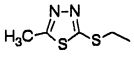
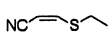
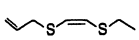
Scheme 4. Reagents: (a) $PhCH_2COCl$, Et_3N, acetone–H_2O; (b) NaOMe, MeOH–THF–DMF; (c) TFA, anisole, CH_2Cl_2; (d) *t*-BoOK, DMF; (e) NaSH, DIPEA, DMF; (f) NaI, DIPEA, DMF; (g) 2-mercapto-5-methyl-1,3,4-thiadiazole, DIPEA, DMF; (h) $PhCH_2COCl$, MSA, THF; (i) DDQ, MeOH; (j) Girard T, AcOEt–MeOH; (k) 3-mercapto-1,2,4-triazole, DIPEA, DMF; (l) HCO_2H, c-HCl.

with AMPC. The Δ2 isomer **2′** had much weaker activity, as is the case generally for antibacterial cephalosporins. Consequently, we adopted compound **2** with a characteristic heterocyclic thio moiety at the 3-position as a seed compound in order to investigate the potency of cephem compounds. We first prepared and evaluated the anti-*H. pylori* activity of a series of analogues.

First, we investigated various 7-acylated compounds having the 1,2,4-triazol-3-ylthio moiety at the 3-position of cephem nucleus. Table 1 shows the minimum inhibitory concentration values (MIC, μg/mL) against three strains of *H. pylori* including the somewhat insensitive strain *H. pylori* 9005. First of all, we evaluated compound **7a** having no hydroxyimino group at the 7-position, which thus

Table 1. Structure–activity relationships (1): 7-position optimization



Compound no.	R_1	R_2	MIC (μg/mL)[a] *Helicobacter pylori* 9005	13001	FP1757
CFDN			0.2	0.39	0.2
FK041			0.2	0.1	0.1
2			0.1	0.00625	0.00625
2′ (Δ2 isomer)			3.13	0.78	0.78
7a			0.05	0.00156	0.00313
7b			0.39	0.00156	0.00313
7c			0.05	0.00156	0.00156
13a			0.025	0.00313	0.00156
7d			3.13	0.1	0.78
7e			0.2	0.0125	0.025
30a			0.1	0.00625	0.00625
7j			0.2	0.00313	0.00313
7f			0.025	0.00313	0.00313
7g			3.13	0.39	0.39
7h			0.025	0.00313	0.00156
7i			0.1	0.00625	0.00625
AMPC			0.05	0.025	0.0125
CAM			0.2	0.05	0.1

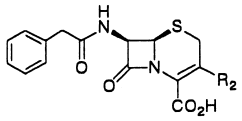
[a]MIC (μg/mL), brucella agar + 7% horse blood, 37 °C, 72 h, 10% CO_2, stamp method.

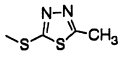
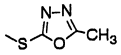
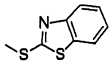
has low potential for causing diarrhea, a major side effect of AMPC, due to low stability towards β-lactamase. Encouragingly, **7a** showed increased anti-*H. pylori* activity compared to **2**, which has a hydroxyimino moiety at the 7-position, and we thus decided to prepare novel derivatives having a non-oxime structure at the 7-position. Regarding other heterocyclic groups, the 1,2,5-thiadiazolyl compound **7b** did not give improved activity especially against *H. pylori* 9005 and 2-thienyl compound **7c**, which is rather lipophilic, showed slightly improved activity. On the other hand, benzyl derivative **13a** showed good activity, in particular against *H. pylori* 9005. A benzamide derivative at the 7-position (**7d**) showed greatly decreased activity, and phenoxyacetamide derivative **7e** gave poor activity compared to the seed compound **2**. On the other hand, a compound having the phenylthioacetamide partial structure (**30a**) showed the same activity compared with seed compound **2**. Thus we examined compounds having other heterocyclic moieties in place of the phenylthio ring at the 7-position; however, compound **7j** did not show increased activity. Concerning non-cyclic derivatives at the 7-position, while **7g** showed decreased activity, cyanovinylthio derivative **7f** had good and nearly equipotent activity with **13a**. We also examined substituents on the phenyl ring, and as a result, 3-chlorophenyl compound **7h** showed the same activity as **13a**, but the 3-nitro derivative **7i** gave decreased activity.

Next, we examined various 3-heterocyclic thio derivatives having a phenylacetamido moiety as the best partial structure at the 7-position (Table 2). Regarding only N-atom-containing heterocyclic compounds, 1,2,3-triazol-4-yl derivative **13b** showed improved activity against all strains while pyrazole and tetrazole derivatives (**13c**,**d**) did not give improved activity. Subsequently, we introduced heterocyclic compounds containing N- and S-atoms (**13e-i**), and were surprised to find that all thiadiazole and thiazole derivatives had remarkably potent anti-*H. pylori* activity, especially against *H. pylori* 9005, which is a somewhat insensitive strain even with **13a** (MIC; 0.05 μg/mL). The in vitro activity of these compounds was superior to that of the reference compounds: 10-fold improved compared to AMPC and about 50-fold relative to CAM, against all strains tested. On the other hand, N- and O-atom-containing heterocyclic compounds, for example oxadiazole derivative **13j** and thiophene derivative **13k**, showed dramatically decreased activity. A pyridine derivative **13l** gave somewhat decreased activity compared with **13e**. We also examined a fused ring compound (**13m**); however, even though this fused cyclic compound had a thiazole partial ring structure, it showed poor activity. Therefore, these results showed that the anti-*H. pylori* activity of this type of compound is fairly specific for mono-heterocyclic rings containing N- and S-atoms.

Subsequently, we investigated the spacer group between the cephem nucleus and the heterocyclic moiety at the 3-position, a cephamycin type derivative, and compounds having other partial structures at the 7-position, together with a 1,3,4-thiadiazol-2-ylthio group at the 3-position (Table 3). Regarding spacer group, we evaluated several compounds having different spacer structures between the cephem nucleus and the 5-methyl-1,3,4-thiadiazol-2-yl or 2-thiazolyl moieties as typical heterocyclic groups. All prepared compounds (methylene-*S* **32**, *S*-methylene **35**, *S*-methylene-*S* **40**, vinyl-*S* **44**) gave decreased activity, though *S*-methylene derivative

Table 2. Structure–activity relationships (2): 3-position optimization



Compound no.	R_2	MIC (μg/mL)[a] *Helicobacter pylori*		
		9005	13001	FP1757
13a		0.05	0.00313	0.00156
13b		0.0125	0.00156	0.00078
13c		0.05	0.0125	0.0125
13d		0.05	0.05	0.0125
13e		0.00625	0.00313	0.00078
13f		0.00313	0.00313	0.00156
13g		0.00156	0.00078	0.00156
13h		0.00625	0.00156	0.00156
13i		0.00625	0.00078	0.00156
13j		0.78	0.1	0.1
13k		0.39	0.2	0.2
13l		0.025	0.00313	0.00313
13m		0.05	0.025	0.0125

[a]MIC (μg/mL), brucella agar + 7% horse blood, 37 °C, 72 h, 10% CO_2, stamp method.

35 was better than the other compounds. From these data it is clear that the anti-*H. pylori* activity of this class of compounds is specific in connection with the thioheterocyclic structure at the 3-position of the cephem nucleus. Moreover, cephamycin type derivative **49** which was expected to have good activity was inferior to compound **13i**. Next, we prepared compounds having other partial structures at the 7-position together with the 1,3,4-thiadiazol-2-ylthio group at the 3-position. Thienylvinyl derivative **25b** showed moderate activity against *H. pylori* 13001 and FP1757, although it was reasonable against *H. pylori* 9005, and tetrazolylmethyl compound **25c** had reasonable activity. Compound **25d**, having a D-2-amino-2-(4-hydroxyphenyl)acetamido moiety which is the 6-position structure of AMPC, showed dramatically decreased activity. We also estimated various combination compounds having 3- and 7-partial structures which had displayed good activity in the earlier compounds (Table 4, **25a**,**e**, **30b**). As expected, these compounds showed excellent in vitro anti-*H. pylori* activity against all strains tested.

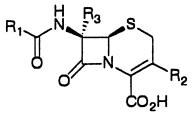
In the next phase, we examined the therapeutic effect of typical compounds in a mouse infection model (Table 4). A dosage regimen of two times per day for 4 days at 0.32 and 0.1 mg/kg, in *H. pylori* FP1757 infected mouse, followed by assessment of eradication was employed. It was noted that nearly all compounds tested showed therapeutic efficacy in parallel with the in vitro activity. Though compound **13b** showed an eradication effect at a dosage of 0.32 mg/kg in five out of seven mice, **25e** showed eradication in only one out of eight mice, and this eradication effect of **25e** was the same as AMPC. Compound **25a** having the most potent in vitro activity showed a good eradication effect at a dosage of 0.1 mg/kg in 6/8 mice but did not show complete eradication even at a dosage of 0.32 mg/kg, suggesting **25a** may have unfavorable properties such as poor solubility or stability. Though **30b** and **13g** showed decreased therapeutic effects compared with **25a**, **13h** showed excellent effect at a dosage of 0.32 mg/kg, but the effect did not extend to the dosage of 0.1 mg/kg. Compound **13i** showed superior therapeutic effect, reflecting the in vitro activity, yet **13i** did not necessarily give the best in vitro activity against the test strain. This compound showed eradication in all mice at a dosage of 0.32 mg/kg and in 5 out of 8 mice at a dosage of 0.1 mg/kg. This effect is superior to that of other test compounds and reference compounds. These results show that there are many unknown factors concerning therapeutic effect, namely permeation into mucosa, stability in mucosa, oral absorption and the like.

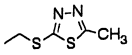
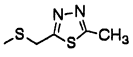
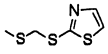
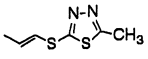
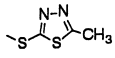
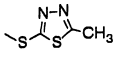
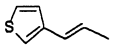
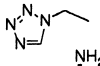
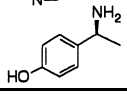
Additionally, we examined the time-dependence of the bactericidal activity (Fig. 2), because in a previous study we uncovered benzyloxyisoquinoline derivatives which show excellent in vitro anti-*H. pylori* activity, but no therapeutic efficacy.[17] With these compounds, we concluded that the poor bactericidal activity at early contact times may be the cause of poor in vivo effect. From the data shown in Figure 2, **13i** showed good bactericidal activity at 6 h even at 1MIC concentration and the bactericidal activity was slightly superior to AMPC, although benzyloxyisoquinoline derivative **1** expresses poor bactericidal activity at 6 h even at 16MIC. Consequently, the importance of bactericidal activity at early contact times was suggested more clearly.

Furthermore, we estimated the in vitro effect against CAM and metronidazole (MNZ) resistant strains (Table 5). **13i** showed excellent activity against *H. pylori* 16021 and 16043, which are highly resistant strains in the clinical environment, and there was no cross-resistance with CAM and MNZ.

In the next phase, we evaluated stability towards β-lactamase of **13i** in order to estimate the potential for causing diarrhea, a major side effect of AMPC (Table 5). The relative values of **13i** and AMPC against cephaloridine (CER), a typical unstable drug towards cephalosporinase, are shown. **13i** was 2-fold more unstable than CER towards *B. fragilis* FP784 (cephalosporinase) and 40-fold more unstable than AMPC. Towards *TEM* (penicillinase)

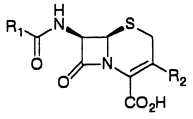
Table 3. Structure–activity relationships (3): spacer optimization and other derivatives

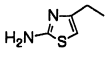
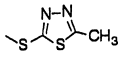
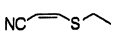
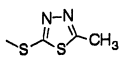


Compound no.	R_1	R_2	R_3	MIC (μg/mL)[a] *Helicobacter pylori* 9005	13001	FP1757
32			H	0.39	0.1	0.05
35			H	0.05	0.025	0.00625
40			H	0.1	0.2	0.05
44			H	0.025	0.78	0.39
13i			H	0.00625	0.00078	0.00156
49			OCH_3	0.05	0.0125	0.0125
25b			H	0.0125	0.05	0.05
25c			H	0.05	0.00156	0.00156
25d			H	0.39	0.39	0.39

[a]MIC (μg/mL), brucella agar + 7% horse blood, 37 °C, 72 h, 10% CO_2, stamp method.

Table 4. Structure–activity relationships: therapeutic efficacy



Compound no.	R_1	R_2	MIC (μg/mL)[a] *Helicobacter pylori* 9005	13001	FP1757	Therapeutic efficacy[b] (eradication ratio) Dose (mg/kg) 0.1	0.32
13b			0.0125	0.00156	0.00078	0/8	5/7
25e			0.00313	0.00039	0.00078	0/7	1/8
25a			0.00156	0.00078	0.00039	6/8	6/8
30b			0.00625	0.00313	0.00078	1/5	3/5
13g			0.00156	0.00078	0.00156	0/8	5/8
13h			0.00625	0.00156	0.00156	1/8	8/8
13i			0.00625	0.00078	0.00156	5/8	8/8
AMPC			0.05	0.025	0.0125	0/8	1/8
CAM			0.2	0.05	0.1	NT[c]	0/8

[a]MIC (μg/mL), brucella agar + 7% horse blood, 37 °C, 72 h, 10% CO_2, stamp method.
[b]Mouse, PO, infection; *H. pylori* FP1757, therapy; 2/day×4 days, termination; 2 weeks after final therapy.
[c]NT = not tested.

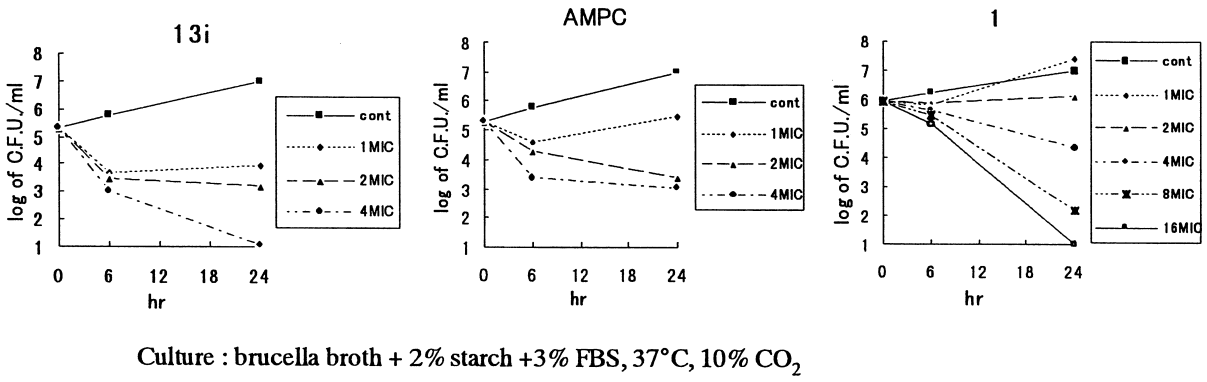


Figure 2. Bactericidal activity against *H. pylori* FP1757.

Table 5. MIC against clarithromycin- and metronidazole-resistant strains and stability towards β-lactamase

Compound	MIC (μg/mL)		Stability towards β-lactamase[b]					
	Helicobacter pylori		*B. fraglis* FP784			*TEM*		
	16021[a]	16043[a]	K_m (μg/ml)	V_{max}[c]	V_{max}/K_m[c]	K_m (μg/ml)	V_{max}[c]	V_{max}/K_m[c]
13i	0.0016	0.0016	14.5	0.351	1.98	47.4	3.61	6.33
AMPC	0.025	0.05	27.3	0.0175	0.0521	22.0	2.94	11.1
CAM	50	50	NT[d]	NT	NT	NT	NT	NT
MNZ	50	50	NT	NT	NT	NT	NT	NT
CER	NT	NT	31.7	1.0	1.0	276	1.0	1.0

[a]Clarithromycin- and metronidazole-resistant strain; underlined values indicate resistant strains.
[b]*B. fragilis* FP784, cephalosporinnase, *TEM*; penicillinase.
[c]Relative value (CER = 1.0), V_{max}/K_m; larger values mean more unstable.
[d]NT = not tested.

13i was 6-fold more unstable than CER though only 2-fold more stable compared to AMPC. From this result, **13i** showed considerable lability towards β-lactamase including cephalosporinase and penicillinase, so it was expected that this compound has low potential for causing diarrhea due to reduced potential for disruption of intestinal microbial flora.

Conclusion

In summary, we have prepared a novel series of cephem derivatives and evaluated them as anti-*H. pylori* compounds. The SAR study in this series of compounds revealed the following main features. (1) Concerning the 3-position, various thio-heterocyclic groups showed extremely potent activity, and especially mono-heterocyclic compounds containing N- and S-atoms gave excellent activity. (2) Regarding the 7-position groups, phenyl or thienyl acetamido groups showed good results and the presence of an oxime part gave decreased activity. (3) Compounds having good in vitro activity showed clear therapeutic efficacy, and **13i** containing a (5-methyl-1,3,4-thiadiazol-2-yl)thio moiety at the 3-position and a phenylacetamido group at the 7-position gave the best efficacy, superior to AMPC and CAM. (4) There was no cross-resistance between **13i** and CAM or MNZ. (5) **13i** has low potential for causing diarrhea due to instability to β-lactamase. Consequently, **13i** (FR182024) having excellent therapeutic efficacy was selected as a candidate compound for further development. Future publications will report optimized therapeutic efficacy as well as in vitro activity, and a study of factors that influence therapeutic efficacy.

Experimental

Melting points were determined using a Thomas-Hoover capillary melting apparatus and are uncorrected. IR spectra were recorded on a Horiba Spectradesk FT-210 spectrometer as KBr disks or Nujol as indicated. NMR spectra were measured on a Bruker AC200P (^{1}H, 200 MHz) and chemical shifts are expressed in δ ppm with TMS as internal standard. Mass spectra were measured on a Hitachi M-1000H mass spectrometer (APCI) or a Finnigan MAT TSQ-70 (FAB). Elemental analyses were carried out on a Perkin Elmer 2400 CHN Elemental Analyzer. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica-gel or non-ionic adsorption resin, Diaion HP-20, and reaction progress was determined by TLC analysis on silica-gel coated glass plates. Visualization was with UV light (254 nm) or iodine.

Benzhydryl 7β-formamido-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (4). To a solution of 3-mercapto-1,2,4-triazole (6.76 g, 66.6 mmol) in DMF (200 mL) was added *N,N*-diisopropylethylamine (DIPEA, 6.62 g, 51.2 mmol) and the mixture was stirred at room temperature for one hour. To a solution of benzhydryl 7β-formamido-3-methanesulfonyloxy-3-cephem-4-carboxylate (25.0 g, 51.2 mmol) in DMF (100 mL) was added the thiol

solution obtained above at −30 °C. After stirring under ice-cooling for 4 h, the reaction mixture was poured into a mixture of AcOEt and water, and the mixture was adjusted to pH 5.0 with 1N HCl. The separated organic layer was washed with brine (3×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane, 2:1) to give 13.2 g of **4** (52%): ^{1}H NMR ($CDCl_3$) δ 2.92 (d, 2H, J = 15.3 Hz), 4.94 (d, 1H, J = 4.9 Hz), 5.88 (dd, 1H, J = 4.9, 9.0 Hz), 6.93 (s, 1H), 7.1–7.5 (m, 11H), 8.19 (s, 1H); APCI-MS m/z 494 (MH^+).

Preparation of **12c**, **12f**, **12j–k**, **12m**, **29a**, **42**, **51** and **51′** was carried out by a similar method to that described for **4**.

Benzhydryl 7β-(2-phenylacetamido)-3-(pyrazol-4-yl)thio-3-cephem-4-carboxylate (12c). Yield 45%; ^{1}H NMR (DMSO-d_6) δ 3.13 (d, 2H, J = 4.0 Hz), 3.53 (d, 2H, J = 2.6 Hz), 5.12 (d, 1H, J = 4.6 Hz), 5.65 (dd, 1H, J = 4.6, 8.3 Hz), 6.92 (s, 1H), 7.1–7.7 (m, 16H), 8.06 (s, 1H), 9.09 (d, 1H, J = 8.3 Hz), 13.39 (s, 1H).

Benzhydryl 7β-(2-phenylacetamido)-3-(1,2,4-thiadiazol-5-yl)thio-3-cephem-4-carboxylate (12f). Yield 56%; ^{1}H NMR ($CDCl_3$) δ 3.65 (d, 2H, J = 2.7 Hz), 3.47 and 3.85 (ABq, 2H, J = 18.1 Hz), 5.08 (d, 1H, J = 5.1 Hz), 5.93 (dd, 1H, J = 5.1, 9.0 Hz), 6.15 (d, 1H, J = 9.0 Hz), 6.97 (s, 1H), 7.1–7.5 (m, 15H), 8.48 (s, 1H); FAB–MS m/z 601.1 (MH^+).

Benzhydryl 7β-(2-phenylacetamido)-3-(5-methyl-1,3,4-oxadiazol-2-yl)thio-3-cephem-4-carboxylate (12j). Yield 49%; ^{1}H NMR (DMSO-d_6) δ 3.32 (s, 3H), 3.54 (s, 2H), 3.76 and 3.99 (ABq, 2H, J = 18.1 Hz), 5.26 (d, 1H, J = 4.9 Hz), 5.82 (dd, 1H, J = 4.9, 8.4 Hz), 6.92 (s, 1H), 7.2–7.6 (m, 15H), 9.21 (d, 1H, J = 8.4 Hz).

Benzhydryl 7β-(2-phenylacetamido)-3-(thiophen-2-yl)thio-3-cephem-4-carboxylate (12k). Yield 61%; ^{1}H NMR (DMSO-d_6) δ 3.32 (s, 2H), 3.53 (d, 2H, J = 2.7 Hz), 5.18 (d, 1H, J = 4.7 Hz), 5.70 (dd, 1H, J = 4.7, 8.3 Hz), 6.95 (s, 1H), 7.1–7.6 (m, 17H), 7.88 (d, 1H, J = 4.1 Hz), 9.12 (d, 1H, J = 8.3 Hz).

Benzhydryl 7β-(2-phenylacetamido)-3-(benzothiazol-2-yl)-thio-3-cephem-4-carboxylate (12m). Yield 70%; ^{1}H NMR (DMSO-d_6) δ 3.55 (d, 2H, J = 4.1 Hz), 3.75 and 4.02 (ABq, 2H, J = 17.9 Hz), 5.33 (d, 1H, J = 5.1 Hz), 5.89 (dd, 1H, J = 5.1, 8.3 Hz), 6.96 (s, 1H), 7.1–8.1 (m, 19H), 9.31 (d, 1H, J = 8.3 Hz).

Benzhydryl 7β-[2-(phenythio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (29a). Yield 39%; ^{1}H NMR ($CDCl_3$) δ 3.39 and 3.76 (ABq, 2H, J = 18.0 Hz), 3.59 (d, 2H, J = 4.4 Hz), 4.94 (d, 1H, J = 4.9 Hz), 5.81 (dd, 1H, J = 4.9, 9.0 Hz), 6.92 (s, 1H), 7.1–7.4 (m, 15H), 7.68 (d, 1H, J = 9.0 Hz), 8.12 (s, 1H).

(*E*)-Benzhydryl 7β-*t*-butoxycarbonylamino-3-[2-(5-methyl-1,3,4-thiadiazol-2-yl)thio]vinyl-3-cephem-4-carboxylate (42). Yield 42%; ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 9H), 2.71 (s, 3H), 3.67 and 3.98 (ABq, 2H, J = 17.6 Hz), 5.17 (d, 1H, J = 4.9 Hz), 5.57 (dd, 1H, J = 4.9, 9.0 Hz), 6.96 (s, 1H), 7.01 (d, 1H, J = 15.6 Hz), 7.25 (d, 1H, J = 15.6 Hz), 7.3–7.6 (m, 10H), 8.08 (d, 1H, J = 9.0 Hz).

(*Z*)-Benzhydryl 7β-[2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetoamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (51). Yield 13%; IR (KBr) 1788, 1685, 1531 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.47 (s, 2H), 5.38 (d, 1H, J = 5.0 Hz), 5.99 (dd, 1H, J = 5.0, 8.4 Hz), 6.63 (s, 1H), 6.96 (s, 1H), 7.2–7.6 (m, 27H), 8.75 (s, 1H), 9.90 (d, 1H, J = 8.4 Hz), 14.6 (brs, 1H).

(*Z*)-Benzhydryl 7β-[2-(2-aminothiazol-4-yl)-2-trityloxyiminoacetoamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate Δ2-isomer (51′). Yield 14%; IR (KBr) 1784, 1680, 1533 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.29 (d, 1H, J = 5.0 Hz), 5.55 (s, 1H), 5.83 (dd, 1H, J = 5.0, 8.4 Hz), 6.68 (s, 1H), 6.84 (s, 1H), 7.2–7.5 (m, 27H), 8.64 (s, 1H), 10.01 (d, 1H, J = 8.4 Hz), 14.3 (brs, 1H).

Benzhydryl 7β-amino-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (5). To a solution of **4** (13.1 g, 0.30 mol) in a mixture of MeOH (65 mL) and THF (10 mL) was added concd HCl (11 mL). After stirring at room temperature for 1.5 h, the reaction mixture was poured into a mixture of AcOEt and water, and the mixture was adjusted to pH 6.5 with an aqueous sodium hydrogen carbonate solution. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt) to give 1.79 g of **5** (15%): ^{1}H NMR ($CDCl_3$) δ 3.44 and 3.78 (ABq, 2H, J = 17.9 Hz), 4.73 (d, 1H, J = 5.0 Hz), 4.91 (d, 1H, J = 5.0 Hz), 6.96 (s, 1H), 7.1–7.5 (m, 10H), 8.14 (s, 1H).

Preparation of **26** and **27** was carried out by a similar method to that described for **5**.

Benzhydryl 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (26). Yield 69%; ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3H), 3.38 (s, 2H), 3.56 and 3.79 (ABq, 2H, J = 17.8 Hz), 5.27 (d, 1H, J = 5.0 Hz), 5.87 (dd, 1H, J = 5.0, 8.5 Hz), 6.25 (s, 1H), 6.88 (s, 2H), 6.97 (s, 1H), 7.2–7.5 (m, 10H), 9.05 (d, 1H, J = 8.5 Hz).

Benzhydryl 7β-amino-3-methanesulfonyloxy-3-cephem-4-carboxylate (27). Yield 27%; ^{1}H NMR ($CDCl_3$) δ 2.73 (s, 3H), 3.70 and 3.89 (ABq, 2H, J = 18.5 Hz), 4.79 (d, 1H, J = 5.1 Hz), 5.01 (d, 1H, J = 5.1 Hz), 6.97 (s, 1H), 7.2–7.5 (m, 10H).

Benzhydryl 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (8). To a solution of phosphorus pentachloride (738 mg, 3.54 mmol) in CH_2Cl_2 (8 mL) was added (2-formylaminothiazol-4-yl)acetic acid (601 mg, 3.23 mmol) at −15 °C and the mixture was stirred under ice-cooling for 30 minutes. To a solution of **5** (1.50 g, 3.23 mmol) in THF (23 mL) was added *N*-trimethylsilylacetamide (1.27 g, 9.67 mmol) under ice-cooling, and the mixture was stirred at the same temperature for 30 min. Thereto was dropwise

added the acid chloride solution obtained above under ice-cooling and the mixture was stirred at the same temperature for one hour. The reaction mixture was poured into a mixture of aqueous sodium hydrogen carbonate solution and AcOEt with stirring. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue (crude **6a**) was dissolved in a mixture of MeOH (10 mL) and THF (2 mL) and concd HCl (1.3 mL) was added to the solution. After stirring at room temperature for 1.5 h, the reaction mixture was poured into a mixture of AcOEt and water, and the mixture was adjusted to pH 6.5 with an aqueous sodium hydrogen carbonate solution. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt) to give 639 mg of **8** (33%): ^{1}H NMR (DMSO-d_6) δ 3.34 (s, 2H), 3.47 and 3.77 (ABq, 2H, J = 17.3 Hz), 5.24 (d, 1H, J = 4.8 Hz), 5.77 (dd, 1H, J = 4.8, 8.6 Hz), 6.25 (s, 1H), 6.8–7.6 (m, 12H), 8.73 (brs, 1H), 8.98 (d, 1H, J = 8.6 Hz).

7β-[2-(1,2,5-Thiadiazol-3-yl)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7b). To a solution of phosphorus pentachloride (738 mg, 3.54 mmol) in a mixture of CH_2Cl_2 (8 mL) and THF (4 mL) was added (1,2,5-thiadiazol-3-yl)acetic acid (465 mg, 3.23 mmol) at −15 °C and the mixture was stirred under ice-cooling for 30 min. To a solution of **5** (1.5 g, 3.23 mmol) in THF (23 mL) was added *N*-trimethylsilylacetamide (1.27 g, 9.67 mmol) under ice-cooling, and the mixture was stirred at the same temperature for 30 min. Thereto was dropwise added the acid chloride solution obtained above under ice-cooling and the mixture was stirred at the same temperature for 1 h. The reaction mixture was poured into a mixture of aqueous sodium hydrogen carbonate solution and AcOEt with stirring. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. To a solution of the residue (crude **6b**) in a mixture of CH_2Cl_2 (6 mL) and anisole (2 mL) was added trifluoroacetic acid (4 mL) with stirring under ice-cooling. The mixture was stirred at the same temperature for one hour. The reaction mixture was poured into diisopropyl ether (IPE, 120 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of a sodium hydrogen carbonate solution and THF, adjusted to pH 5.0 with 1N HCl with stirring and evaporated under reduced pressure to remove the organic solvent. The resulting solution was subjected to column chromatography on Diaion HP-20 (eluent 20% aqueous isopropyl alcohol). The desired fractions were evaporated under reduced pressure to remove the organic solvent and the residue was adjusted to pH 2.0 with 1N HCl and extracted twice with a mixture of THF and AcOEt. The organic layer was dried over magnesium sulfate and evaporated, and the resulting precipitate was collected by filtration and washed with AcOEt to give 577 mg of **7b** (42%): IR (Nujol) 3263, 1757, 1660, 1554, 1298, 1232 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.45 and 3.81 (ABq, 2H, J = 17.2 Hz), 4.02 (s, 2H), 5.19 (d, 1H, J = 4.8 Hz), 5.69 (dd, 1H, J = 4.8, 8.2 Hz), 8.72 (brs, 1H), 8.75 (s, 1H), 9.31 (d, 1H, J = 8.2 Hz), 14.5 (brs, 1H); FAB–MS m/z 425.9 (MH^+).

Preparation of **7c** and **7i** was carried out by a similar method to that described for **7b**.

7β-(2-Thienyl)acetamido-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7c). Yield 53%; ^{1}H NMR (DMSO-d_6) δ 3.45 and 3.79 (ABq, 2H, J = 17.3 Hz), 3.75 (s, 1H), 5.18 (d, 1H, J = 4.8 Hz), 5.67 (dd, 1H, J = 4.8, 8.3 Hz), 6.90–6.96 (m, 2H), 7.35 (dd, 1H, J = 1.6, 4.8 Hz), 8.67 (brs, 1H), 9.15 (d, 1H, J = 8.3 Hz), 14.4 (brs, 1H).

7β-[2-(3-Nitrophenyl)acetamido]-3-(1,2,4-triazol-3-yl)-thio-3-cephem-4-carboxylic acid (7i). Yield 40%; ^{1}H NMR (DMSO-d_6) δ 3.44 and 3.79 (ABq, 2H, J = 17.2 Hz), 3.72 (s, 2H), 5.17 (d, 1H, J = 4.8 Hz), 5.66 (dd, 1H, J = 4.8, 8.2 Hz), 7.55–7.75 (m, 2H), 8.05–8.20 (m, 2H), 8.70 (brs, 1H), 9.26 (d, 1H, J = 8.2 Hz), 14.5 (brs, 1H); FAB–MS m/z 463.0 (MH^+).

Benzhydryl 7β-benzoylamino-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (6d). To a solution of **5** (1.0 g, 2.15 mmol) in THF (15 mL) was added *N*-trimethylsilylacetamide (846 mg, 6.46 mmol) at ambient temperature, and the mixture was stirred for 30 min. Thereto was dropwise added a solution of benzoyl chloride (0.26 ml, 2.23 mmol) in CH_2Cl_2 (1 mL) under ice-cooling and the mixture was stirred for 1 h. The reaction mixture was poured into a mixture of aqueous sodium hydrogen carbonate solution, THF and AcOEt with stirring, and adjusted to pH 2.0 with 1N HCl. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give 1.30 g of **6d** (100%): ^{1}H NMR ($CDCl_3$) δ 3.17 and 3.67 (ABq, 2H, J = 18.2 Hz), 5.01 (d, 1H, J = 4.9 Hz), 6.16 (dd, 1H, J = 4.9, 9.2 Hz), 6.76 (s, 1H), 7.1–7.5 (m, 15H), 7.98 (d, 1H, J = 9.2 Hz), 8.17 (s, 1H).

Preparation of **6j**, **32**, **44** and **48** was carried out by a similar method to that described for **6d**.

Benzhydryl 7β-(2-bromoacetamido)-3-(1,2,4-triazol-3-yl)-thio-3-cephem-4-carboxylate (6j). Yield 66%; ^{1}H NMR ($CDCl_3$) δ 3.42 and 3.75 (ABq, 2H, J = 17.9 Hz), 3.71 (s, 2H), 4.96 (d, 1H, J = 4.7 Hz), 5.82 (dd, 1H, J = 4.7, 9.0 Hz), 6.91 (s, 1H), 7.10–7.55 (m, 10H), 7.74 (d, 1H, J = 9.0 Hz), 8.27 (s, 1H).

7β-(2-Phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl) thiomethyl-3-cephem-4-carboxylic acid (32). Yield 42%; IR (Nujol) 1768, 1712, 1659, 1533 cm^{-1}.

(*E*)-7β-(2-Phenylacetamido)-3-[2-(5-methyl-1,3,4-thiadiazol-2-yl)thio]vinyl-3-cephem-4-carboxylic acid (44). Yield 62%; IR (KBr) 1776, 1664, 1537 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.72 (s, 3H), 3.54 (d, 2H, J = 5.0 Hz), 3.67 and 3.96 (ABq, 2H, J = 17.4 Hz), 5.14 (d, 1H, J = 4.9 Hz), 5.70 (dd, 1H, J = 4.9, 8.2 Hz), 7.13 (d, 1H, J = 15.6 Hz), 7.17 (d, 1H, J = 15.6 Hz), 7.2–7.6 (m, 5H), 9.16 (d, 1H, J = 8.2 Hz); FAB–MS m/z 475.0 (MH^+).

7α-Methoxy-7β-(2-phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (48). Yield 30%; IR (KBr) 1782, 1738, 1695, 1497 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.69 (s, 3H), 3.23 (s, 3H), 3.48 (s, 2H), 3.55 and 3.83 (ABq, 2H, $J=17.8$ Hz), 5.61 (s, 1H), 6.98 (s, 1H), 7.16 (d, 1H, $J=3.0$ Hz), 7.2–7.7 (m, 15H), 8.54 (s, 1H).

Benzhydryl 7β-(2-phenoxyacetamido)-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (6e). To a solution of phosphorus pentachloride (492 mg, 2.36 mmol) in CH_2Cl_2 (5 mL) was added 2-phenoxyacetic acid (343 mg, 2.25 mmol) at −15 °C and the mixture was stirred under ice-cooling for 30 min. To a solution of **5** (1.0 g, 2.15 mmol) in THF (15 mL) was added *N*-trimethylsilylacetamide (846 mg, 6.46 mmol) under ice-cooling, and the mixture was stirred at the same temperature for 30 min. Thereto was dropwise added the acid chloride solution obtained above under ice-cooling and the mixture was stirred for 1 h. The reaction mixture was poured into a mixture of aqueous sodium hydrogen carbonate solution and AcOEt with stirring. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane) to give 1.26 g of **6e** (98%): ^1H NMR ($CDCl_3$) δ 3.29 and 3.52 (ABq, 2H, $J=18.0$ Hz), 4.52 (s, 2H), 4.99 (d, 1H, $J=4.8$ Hz), 5.87 (dd, 1H, $J=4.8$, 9.2 Hz), 6.8–7.5 (m, 17H), 8.13 (s, 1H).

Preparation of **6f–g**, **24a–c**, **24e** and **28a,b** was carried out by a similar method to that described for **6e**.

(*Z*)-Benzhydryl 7β-[2-(2-cyanoethenylthio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (6f). Yield 43%; ^1H NMR ($CDCl_3$) δ 3.25 (s, 2H), 3.43 and 3.82 (ABq, 2H, $J=17.9$ Hz), 4.99 (d, 1H, $J=4.8$ Hz), 5.30 (d, 1H, $J=10.4$ Hz), 5.82 (dd, 1H, $J=4.8$, 8.8 Hz), 6.95 (s, 1H), 7.1–7.5 (m, 11H), 7.72 (d, 1H, $J=8.8$ Hz), 8.20 (s, 1H).

(*Z*)-Benzhydryl 7β-[2-(2-allylthioethenylthio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (6g). Yield 46%; ^1H NMR ($CDCl_3$) δ 3.29 and 3.61 (ABq, 2H, $J=18.0$ Hz), 3.30 (d, 2H, $J=6.9$ Hz), 3.34 (s, 2H), 5.00 (d, 1H, $J=4.8$ Hz), 5.08 (d, 1H, $J=2.8$ Hz), 5.14 (d, 1H, $J=9.8$ Hz), 5.75 (dd, 1H, $J=4.8$, 8.9 Hz), 5.7–5.9 (m, 2H), 6.12 (dd, 1H, $J=8.2$, 40.2 Hz), 6.94 (s, 1H), 7.2–7.5 (m, 10H), 7.63 (d, 1H, $J=8.9$ Hz), 8.17 (s, 1H).

Benzhydryl 7β-[2-(3-thienyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (24a). Yield 22%; ^1H NMR ($CDCl_3$) δ 3.62 and 3.88 (ABq, 2H, $J=17.9$ Hz), 3.67 (s, 2H), 5.04 (d, 1H, $J=5.1$ Hz), 5.91 (dd, 1H, $J=5.1$, 9.2 Hz), 6.37 (d, 1H, $J=9.2$ Hz), 6.9–7.4 (m, 13H), 9.11 (s, 1H).

(*E*)-Benzhydryl 7β-[3-(3-thienyl)acryloylamino]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (24b). Yield 59%; ^1H NMR ($CDCl_3$) δ 3.63 and 3.88 (ABq, 2H, $J=18.0$ Hz), 5.09 (d, 1H, $J=5.0$ Hz), 6.07 (dd, 1H, $J=5.0$, 9.0 Hz), 6.34 (d, 1H, $J=15.5$ Hz), 6.75 (d, 1H, $J=9.0$ Hz), 6.97 (s, 1H), 7.2–7.5 (m, 13H), 7.78 (d, 1H, $J=15.5$ Hz), 9.12 (s, 1H).

Benzhydryl 7β-[2-(1-tetrazolyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (24c). Yield 67%; ^1H NMR (DMSO-d_6) δ 3.67 and 3.90 (ABq, 2H, $J=17.8$ Hz), 5.32 (d, 1H, $J=5.1$ Hz), 5.37 (s, 2H), 5.93 (dd, 1H, $J=5.1$, 8.4 Hz), 6.97 (s, 1H), 7.2–7.5 (m, 10H), 9.36 (s, 1H), 9.64 (s, 1H), 9.66 (d, 1H, $J=8.4$ Hz).

Benzhydryl 7β-[2-(2-formamidothiazol-4-yl)acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (24e). Yield 63%; ^1H NMR (DMSO-d_6) δ 2.67 (s, 3H), 3.60 (s, 2H), 3.72 and 3.90 (ABq, 2H, $J=17.7$ Hz), 5.28 (d, 1H, $J=5.1$ Hz), 5.88 (dd, 1H, $J=5.1$, 8.4 Hz), 6.96 (s, 1H), 6.97 (s, 1H), 8.45 (s, 1H), 9.17 (d, 1H, $J=8.4$ Hz), 12.2 (s, 1H).

Benzhydryl 7β-[2-(phenylthio)acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (28a). Yield 80%; ^1H NMR ($CDCl_3$) δ 2.78 (s, 3H), 3.65 and 3.88 (ABq, 2H, $J=18.5$ Hz), 3.70 (d, 2H, $J=3.9$ Hz), 5.02 (d, 1H, $J=5.0$ Hz), 5.85 (dd, 1H, $J=5.0$, 9.3 Hz), 6.95 (s, 1H), 7.2–7.5 (m, 15H), 7.51 (d, 1H, $J=9.3$ Hz).

(*Z*)-Benzhydryl 7β-[2-(2-cyanoethenylthio)acetamido]-3-methanesulfonyloxy-3-cephem-4-carboxylate (28b). Yield 46%; ^1H NMR (DMSO-d_6) δ 3.19 (s, 3H), 3.74 (s, 2H), 3.77 and 3.90 (ABq, 2H, $J=18.0$ Hz), 5.30 (d, 1H, $J=4.9$ Hz), 5.74 (d, 1H, $J=10.5$ Hz), 5.84 (dd, 1H, $J=4.9$, 8.2 Hz), 6.93 (s, 1H), 7.2–7.5 (m, 10H), 7.66 (d, 1H, $J=10.5$ Hz), 9.32 (d, 1H, $J=8.2$ Hz).

Benzhydryl 7β-[2-(3-chlorophenyl)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylate (6h). Phosphorus oxychloride (440 mg, 2.87 mmol) was added dropwise to a mixture of DMF (220 mg, 2.87 mmol) and AcOEt (700 μL) under ice-cooling. After stirring for 20 min at the same temperature, the mixture was cooled until a precipitate appeared. To the suspension were added THF (10 mL) and 3-chlorophenylacetic acid (403 mg, 2.36 mmol). The mixture was stirred at the same temperature for 30 min to give an activated acid solution. To a suspension of **5** (1.0 g, 2.15 mmol) in THF (10 mL) was added *N*-trimethylsilylacetamide (845 mg, 6.46 mmol). The suspension was stirred at room temperature for 30 min to give a clear solution. To this solution was added the activated acid solution prepared above at −20 °C and the mixture was stirred at −25 to −10 °C for an hour. The mixture was poured into aqueous sodium hydrogen carbonate solution. The extract was washed with water and brine, dried over magnesium sulfate and evaporated in vacuo. The residue was triturated with diethyl ether to give 955 mg of **6h** (72%): IR (KBr) 1790, 1730, 1670, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.3–3.7 (m, 4H), 5.24 (d, 1H, $J=5.0$ Hz), 5.74 (dd, 1H, $J=5.0$, 8.0 Hz), 6.93 (s, 1H), 7.2–7.6 (m, 14H), 8.70 (brs, 1H), 9.23 (d, 1H, $J=8.0$ Hz), 14.5 (brs, 1H); APCI-MS *m/z* 618 (MH^+).

7β-[2-{(5-Methyl-1,3,4-thiadiazol-2-yl)thio}acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7j). To a solution of 2-mercapto-5-methyl-1,3,4-thiadiazole (2.0 g, 15.2 mmol) in a mixture of THF (10 mL) and 1,2-dimethoxyethane (DME, 10 mL) was added *t*-BuOK (1.70 g, 15.2 mmol) at −10 °C, and the mixture was

stirred under ice-cooling for 30 min. The resulting precipitate was collected by filtration, washed with DME and dried in vacuo to give a solid. This solid was added to a solution of **6j** (500 mg, 0.854 mmol) in DME (5 mL) at a temperature from 0 °C to −10 °C, and the mixture was stirred for 1 h. The reaction mixture was poured into a mixture of AcOEt and 1N HCl with stirring. The organic layer was separated, washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. To a solution of the residue (crude **9**) in a mixture of CH_2Cl_2 (1.2 mL) and anisole (0.4 mL) was added trifluoroacetic acid (0.8 mL) with stirring under ice-cooling. The mixture was stirred at said temperature for 1 h. The reaction mixture was poured into IPE (24 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of a sodium hydrogen carbonate solution and THF, adjusted to pH 5.0 with 1N HCl with stirring and evaporated under reduced pressure to remove the organic solvent. The resulting solution was subjected to column chromatography on Diaion HP-20 (eluent 20% aqueous isopropyl alcohol). The desired fractions were evaporated under reduced pressure to remove the organic solvent and the residue was adjusted to pH 2.0 with 1N HCl and extracted twice with a mixture of THF and AcOEt. The organic layer was dried over magnesium sulfate and evaporated, and the resulting precipitate was collected by filtration and washed with AcOEt to give 249 mg of **7j** (62%): ^{1}H NMR (DMSO-d_6) δ 3.44 and 3.78 (ABq, 2H, J = 17.3 Hz), 4.12 (d, 2H, J = 13.5 Hz), 5.19 (d, 1H, J = 4.8 Hz), 5.68 (dd, 1H, J = 4.8, 8.2 Hz), 8.72 (brs, 1H), 9.28 (d, 1H, J = 8.2 Hz), 14.5 (brs, 1H); FAB–MS m/z 471.8 (MH^+).

7β-[2-(2-Aminothiazol-4-yl)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7a). To a solution of **8** (620 mg, 1.02 mmol) in a mixture of CH_2Cl_2 (1.8 mL) and anisole (0.6 mL) was added trifluoroacetic acid (1.2 mL) with stirring under ice-cooling and the mixture stirred for 1 h. The reaction mixture was poured into IPE (36 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of a sodium hydrogen carbonate solution and THF, adjusted to pH 5.0 with 1N HCl with stirring and evaporated under reduced pressure to remove the organic solvent. The resulting solution was subjected to column chromatography on Diaion HP-20 (eluent 20% aqueous isopropyl alcohol). The desired fractions were evaporated under reduced pressure to remove the organic solvent and the residue was adjusted to pH 2.0 with 1N HCl and extracted twice with a mixture of THF and AcOEt. The organic layer was dried over magnesium sulfate and evaporated, and the resulting precipitate was collected by filtration and washed with AcOEt to give 228 mg of **7a** (51%): ^{1}H NMR (DMSO-d_6) δ 3.37 (s, 2H), 3.44 and 3.72 (ABq, 2H, J = 17.3 Hz), 5.17 (d, 1H, J = 4.7 Hz), 5.67 (dd, 1H, J = 4.7, 8.3 Hz), 6.24 (s, 1H), 6.91 (s, 2H), 8.67 (brs, 1H), 8.93 (d, 1H, J = 8.3 Hz); FAB–MS m/z 439.9 (MH^+).

Preparation of **7d,e** was carried out by a similar method to that described for **7a**.

7β-Benzamido-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7d). Yield 45%; ^{1}H NMR (DMSO-d_6) δ 3.48 (s, 2H), 5.28 (d, 1H, J = 4.7 Hz), 5.83 (dd, 1H, J = 4.7, 8.0 Hz), 7.4–7.6 (m, 3H), 7.8–8.0 (m, 2H), 8.71 (brs, 1H), 9.42 (d, 1H, J = 8.0 Hz); FAB–MS m/z 403.9 (MH^+).

7β-(2-Phenoxyacetamido)-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7e). Yield 41%; IR (KBr) 1756, 1674, 1462 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.45 (d, 2H, J = 3.6 Hz), 4.61 (d, 2H, J = 2.0 Hz), 5.21 (d, 1H, J = 4.6 Hz), 5.68 (dd, 1H, J = 4.6, 8.4 Hz), 6.8–7.0 (m, 3H), 7.2–7.4 (m, 2H), 8.72 (brs, 1H), 9.15 (d, 1H, J = 8.4 Hz); FAB–MS m/z 433.9 (MH^+).

(*Z*)-7β-[2-(2-Cyanoethenylthio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7f). To a solution of **6f** (720 mg, 1.19 mmol) in a mixture of CH_2Cl_2 (2.2 mL) and anisole (0.72 mL) was added trifluoroacetic acid (1.44 mL) with stirring under ice-cooling and the mixture stirred for 1 h. The reaction mixture was poured into IPE (40 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of a sodium hydrogen carbonate solution and THF, adjusted to pH 5.0 with 1N HCl with stirring and evaporated under reduced pressure to remove the organic solvent. The resulting solution was subjected to column chromatography on Diaion HP-20 (eluent 20% aqueous isopropyl alcohol). The desired fractions were concentrated under reduced pressure and freeze-dried to give 365 mg of **7f** (70%): IR (KBr) 2214, 1772, 1670, 1616 cm^{-1}; ^{1}H NMR (D_2O) δ 3.47 and 3.82 (ABq, 2H, J = 17.4 Hz), 3.77 (s, 2H), 5.20 (d, 1H, J = 4.7 Hz), 5.57 (d, 1H, J = 10.5 Hz), 5.65 (d, 1H, J = 4.7 Hz), 7.48 (d, 1H, J = 10.5 Hz), 8.46 (s, 1H); FAB–MS m/z 425.0 (MH^+).

Preparation of **7g,h**, **13c,d**, **13f**, **13h**, **13j–m**, **25a–e** and **30a,b** was carried out by a similar method to that described for **7f**.

(*Z*)-7β-[2-(2-Allylthioethenylthio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (7g). Yield 55%; IR (KBr) 1770, 1670, 1608, 1508 cm^{-1}; ^{1}H NMR (D_2O) δ 3.1–4.1 (m, 6H), 5.02 (m, 1H), 5.05 (d, 1H, J = 4.6 Hz), 5.65 (d, 1H, J = 4.6 Hz), 5.80 (m, 1H), 6.6–7.3 (m, 3H), 8.45 (s, 1H).

7β-[2-(3-Chlorophenyl)acetamido]-3-(1,2,4-triazol-3-yl)-thio-3-cephem-4-carboxylic acid (7h). Yield 68%; IR (KBr) 3270, 1780, 1660, 1550 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.37 and 3.58 (ABq, 2H, J = 17.0 Hz), 3.56 and 3.58 (total 1H, each s), 5.17 (d, 1H, J = 5.0 Hz), 5.66 (dd, 1H, J = 5.0, 8.0 Hz), 7.2–7.4 (m, 4H), 8.68 (brs, 1H), 9.19 (d, 1H, J = 8.0 Hz), 14.4 (brs, 1H); FAB–MS m/z 451.9 (MH^+).

7β-(2-Phenylacetamido)-3-(pyrazol-4-yl)thio-3-cephem-4-carboxylic acid (13c). Yield 41%; IR (KBr) 1759, 1659, 1537 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.27 (d, 2H, J = 2.7 Hz), 3.48 and 3.54 (ABq, 2H, J = 13.9 Hz), 5.04 (d, 1H, J = 4.6 Hz), 5.55 (dd, 1H, J = 4.6, 8.2 Hz), 7.1–7.4 (m, 5H), 7.85 (s, 2H), 9.05 (d, 1H, J = 8.2 Hz), 13.32 (brs, 1H); FAB–MS m/z 417.0 (MH^+).

7β-(2-Phenylacetamido)-3-(1-methyltetrazol-5-yl)thio-3-cephem-4-carboxylic acid (13d). Yield 58%; IR (KBr) 1784, 1666, 1531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.42 and 3.62 (ABq, 2H, $J=17.4$ Hz), 3.52 (d, 2H, $J=5.2$ Hz), 4.05 (s, 3H), 5.18 (d, 1H, $J=4.9$ Hz), 5.74 (dd, 1H, $J=4.9$, 8.3 Hz), 7.1–7.4 (m, 5H), 9.17 (d, 1H, $J=8.3$ Hz); FAB–MS m/z 433.0 (MH^+).

7β-(2-Phenylacetamido)-3-(1,2,4-thiadiazol-5-yl)thio-3-cephem-4-carboxylic acid (13f). Yield 61%; IR (KBr) 1790, 1724, 1666, 1531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.54 (d, 2H, $J=4.5$ Hz), 3.69 and 4.01 (ABq, 2H, $J=17.8$ Hz), 5.27 (d, 1H, $J=5.1$ Hz), 5.84 (dd, 1H, $J=5.1$, 8.4 Hz), 7.1–7.4 (m, 5H), 8.80 (s, 1H), 9.30 (d, 1H, $J=8.4$ Hz); FAB–MS m/z 435.0 (MH^+).

7β-(2-Phenylacetamido)-3-(thiazol-2-yl)thio-3-cephem-4-carboxylic acid (13h). Yield 52%; IR (KBr) 1782, 1660, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 (d, 2H, $J=4.3$ Hz), 3.50 and 3.74 (ABq, 2H, $J=17.5$ Hz), 5.21 (d, 1H, $J=4.9$ Hz), 5.75 (dd, 1H, $J=4.9$, 8.3 Hz), 7.1–7.4 (m, 5H), 7.88 (s, 2H), 9.22 (d, 1H, $J=8.3$ Hz); FAB–MS m/z 434.0 (MH^+). Anal. calcd for $C_{18}H_{15}N_3O_4S_3$: C, 49.87; H, 3.49; N, 9.69. Found: C, 49.55; H, 3.43; N, 9.56.

7β-(2-Phenylacetamido)-3-(5-methyl-1,3,4-oxadiazol-2-yl)-thio-3-cephem-4-carboxylic acid (13j). Yield 36%; IR (KBr) 1782, 1724, 1659, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.41 (s, 3H), 3.53 (d, 2H, $J=5.6$ Hz), 3.65 and 3.95 (ABq, 2H, $J=18.0$ Hz), 5.18 (d, 1H, $J=4.9$ Hz), 5.72 (dd, 1H, $J=4.9$, 8.3 Hz), 7.2–7.4 (m, 5H), 9.17 (d, 1H, $J=8.3$ Hz); FAB–MS m/z 433.0 (MH^+).

7β-(2-Phenylacetamido)-3-(2-thienyl)thio-3-cephem-4-carboxylic acid (13k). Yield 79%; IR (KBr) 1767, 1632, 1531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.37 (s, 2H), 3.51 (d, 2H, $J=4.2$ Hz), 5.11 (d, 1H, $J=4.7$ Hz), 5.61 (dd, 1H, $J=4.7$, 8.3 Hz), 7.1–7.5 (m, 7H), 7.85 (d, 1H, $J=4.1$ Hz), 9.08 (d, 1H, $J=8.3$ Hz); FAB–MS m/z 432.9 (MH^+).

7β-(2-Phenylacetamido)-3-(2-pyridyl)thio-3-cephem-4-carboxylic acid (13l). Yield 55%; IR (KBr) 1782, 1660, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.53 (s, 2H), 3.51 and 3.88 (ABq, 2H, $J=17.5$ Hz), 5.26 (d, 1H, $J=4.9$ Hz), 5.77 (dd, 1H, $J=4.9$, 8.4 Hz), 7.1–7.4 (m, 7H), 7.75 (dt, 1H, $J=1.8$, 7.8 Hz), 8.45 (d, 1H, $J=4.0$ Hz), 9.26 (d, 1H, $J=8.4$ Hz); FAB–MS m/z 427.9 (MH^+).

7β-(2-Phenylacetamido)-3-(benzothiazol-2-yl)thio-3-cephem-4-carboxylic acid (13m). Yield 32%; IR (KBr) 1782, 1659, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.55 (d, 2H, $J=4.2$ Hz), 3.68 and 4.00 (ABq, 2H, $J=17.8$ Hz), 5.28 (d, 1H, $J=5.0$ Hz), 5.82 (dd, 1H, $J=5.0$, 8.4 Hz), 7.1–7.6 (m, 7H), 7.91 (d, 1H, $J=7.4$ Hz), 8.07 (d, 1H, $J=7.2$ Hz), 9.27 (d, 1H, $J=8.4$ Hz); FAB–MS m/z 483.8 (MH^+).

7β-[2-(3-Thienyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (25a). Yield 81%; ^1H NMR (DMSO-d_6) δ 3.54 (s, 2H), 3.73 and 3.96 (ABq, 2H, $J=17.6$ Hz), 5.24 (d, 1H, $J=5.0$ Hz), 5.79 (dd, 1H, $J=5.0$, 8.3 Hz), 7.01–7.04 (m, 1H), 7.26 (m, 1H), 7.43–7.48 (m, 1H), 9.19 (d, 1H, $J=8.3$ Hz), 9.65 (s, 1H); FAB–MS m/z 441.0 (MH^+). Anal. calcd for $C_{15}H_{12}N_4O_4S_4$: C, 40.90; H, 2.75; N, 12.72. Found: C, 41.16; H, 2.74; N, 12.46.

(*E*)-7β-[3-(3-Thienyl)acryloylamino]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (25b). Yield 49%; ^1H NMR (DMSO-d_6) δ 3.75 and 3.97 (ABq, 2H, $J=17.6$ Hz), 5.30 (d, 1H, $J=5.0$ Hz), 5.93 (dd, 1H, $J=5.0$, 8.4 Hz), 6.55 (d, 1H, $J=15.7$ Hz), 7.33 (d, 1H, $J=5.0$ Hz), 7.54 (d, 1H, $J=15.7$ Hz), 7.62 (dd, 1H, $J=2.9$, 5.0 Hz), 7.86 (d, 1H, $J=2.9$ Hz), 9.13 (d, 1H, $J=8.4$ Hz), 9.65 (s, 1H); MS m/z 453.0 (MH^+).

7β-[2-(1-Tetrazolyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)-thio-3-cephem-4-carboxylic acid (25c). Yield 32%; IR (KBr) 1778, 1693, 1666, 1542 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.61 and 3.89 (ABq, 2H, $J=17.6$ Hz), 5.28 (d, 1H, $J=5.1$ Hz), 5.38 (s, 2H), 5.86 (dd, 1H, $J=5.1$, 8.3 Hz), 9.38 (s, 1H), 9.64 (d, 1H, $J=8.3$ Hz), 9.66 (s, 1H); FAB–MS m/z 427.0 (MH^+).

(D)-7β-[2-Amino-2-(4-hydroxyphenyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (25d). Yield 36%; IR (KBr) 1770, 1614, 1516 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.48 and 3.79 (ABq, 2H, $J=17.8$ Hz), 5.00 (s, 1H), 5.22 (d, 1H, $J=4.8$ Hz), 5.53 (dd, 1H, $J=4.8$, 8.5 Hz), 6.74 (d, 2H, $J=8.5$ Hz), 7.09 (d, 2H, $J=8.5$ Hz), 8.66 (d, 1H, $J=8.5$ Hz), 9.43 (s, 1H); FAB–MS m/z 466.0 (MH^+).

7β-[2-(2-Aminothiazol-4-yl)acetamido]-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (25e). Yield 55%; ^1H NMR (DMSO-d_6) δ 2.72 (s, 3H), 3.39 (s, 2H), 3.69 and 3.92 (ABq, 2H, $J=17.6$ Hz), 5.23 (d, 1H, $J=5.0$ Hz), 5.78 (dd, 1H, $J=5.0$, 8.4 Hz), 6.28 (s, 1H), 7.06 (brs, 2H), 9.02 (d, 1H, $J=8.4$ Hz); FAB–MS m/z 471.0 (MH^+).

7β-[2-(Phenylthio)acetamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (30a). Yield 47%; IR (KBr) 1763, 1608, 1540 cm^{-1}; ^1H NMR (D_2O) δ 3.35 and 3.83 (ABq, 2H, $J=17.3$ Hz), 3.77 (d, 2H, $J=3.5$ Hz), 5.09 (d, 1H, $J=4.7$ Hz), 5.57 (d, 1H, $J=4.7$ Hz), 7.2–7.5 (m, 5H), 8.46 (s, 1H); FAB–MS m/z 449.8 (MH^+).

(*Z*)-7β-[2-(2-Cyanoethenylthio)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (30b). Yield 45%; IR (KBr) 2212, 1770, 1616 cm^{-1}; ^1H NMR (D_2O) δ 3.70 and 3.97 (ABq, 2H, $J=17.5$ Hz), 3.80 (s, 2H), 5.29 (d, 1H, $J=4.9$ Hz), 5.60 (d, 1H, $J=10.5$ Hz), 5.72 (d, 1H, $J=4.9$ Hz), 7.51 (d, 1H, $J=10.5$ Hz), 9.43 (s, 1H); FAB–MS m/z 442.0 (MH^+).

7β-(2-Phenylacetamido)-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (13a). To a solution of **12a** (348 mg, 0.597 mmol) in a mixture of CH_2Cl_2 (1.5 mL) and anisole (0.5 mL) was added trifluoroacetic acid (1.0 mL) with stirring under ice-cooling and the mixture stirred for 1 h. The reaction mixture was poured into IPE (30 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of a sodium hydrogen carbonate solution, AcOEt and THF at pH 7.5, and the aqueous layer was washed with a mixture of AcOEt and THF. Then AcOEt and THF were added to the aqueous layer and the mixture

was adjusted to pH 2.0 with 1N HCl with stirring. The organic layer was washed twice with brine, dried over magnesium sulfate and evaporated under reduced pressure to give a precipitate. The precipitate was collected by filtration, washed with AcOEt and dried in vacuo to give 154 mg of **13a** (62%): IR (Nujol) 3250, 1755, 1660, 1530 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.3–3.6 (m, 4H), 5.16 (d, 1H, J= 4.7 Hz), 5.60 (dd, 1H, J=4.7, 8.3 Hz), 7.1–7.3 (m, 5H), 8.69 (brs, 1H), 9.14 (d, 1H, J=8.3 Hz), 14.4 (brs, 1H).

Preparation of **13b**, **13e**, **13g**, **13i**, **35**, **40** and **49** was carried out by a similar method to that described for **13a**.

7β-(2-Phenylacetamido)-3-(1,2,3-triazol-4-yl)thio-3-cephem-4-carboxylic acid (13b). Yield 51%; IR (Nujol) 1745, 1695, 1650, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.27 (s, 2H), 3.51 and 3.80 (ABq, 2H, J=18.3 Hz), 5.09 (d, 1H, J=4.7 Hz), 5.62 (dd, 1H, J=4.7, 8.2 Hz), 7.1–7.3 (m, 5H), 8.32 (brs, 1H), 9.11 (d, 1H, J=8.2 Hz); FAB–MS m/z 417.9 (MH^+).

7β-(2-Phenylacetamido)-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (13e). Yield 65%; IR (Nujol) 1782, 1693, 1659, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 (d, 2H, J=4.4 Hz), 3.57 and 3.89 (ABq, 2H, J=17.0 Hz), 5.23 (d, 1H, J=5.0 Hz), 5.79 (dd, 1H, J=5.0, 8.3 Hz), 7.2–7.4 (m, 5H), 9.25 (d, 1H, J=8.3 Hz), 9.65 (s, 1H); FAB–MS m/z 434.9 (MH^+).

7β-(2-Phenylacetamido)-3-(1,2,3-thiadiazol-5-yl)thio-3-cephem-4-carboxylic acid (13g). Yield 52%; ^1H NMR (DMSO-d_6) δ 3.52 and 3.83 (ABq, 2H, J=18.0 Hz), 3.55 (d, 2H, J=14.0 Hz), 5.20 (d, 1H, J=5.0 Hz), 5.79 (dd, 1H, J=5.0, 8.0 Hz), 7.2–7.3 (m, 5H), 8.92 (s, 1H), 9.24 (d, 1H, J=8.0 Hz).

7β-(2-Phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (13i). Yield 64%; IR (KBr) 1784, 1663, 1535 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.73 (s, 3H), 3.52 and 3.62 (ABq, 2H, J=14.0 Hz), 3.55 and 3.85 (ABq, 2H, J=17.6 Hz), 5.22 (d, 1H, J=5.0 Hz), 5.78 (dd, 1H, J=5.0, 8.3 Hz), 7.15–7.35 (m, 5H), 9.24 (d, 1H, J=8.3 Hz); FAB–MS m/z 448.9 (MH^+). Anal. calcd for $C_{18}H_{16}N_4O_4S_3$: C, 48.20; H, 3.60; N, 12.49. Found: C, 48.65; H, 3.68; N, 12.45.

7β-(2-Phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)methylthio-3-cephem-4-carboxylic acid (35). Yield 55%; IR (KBr) 1776, 1678, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.69 (s, 3H), 3.49 and 3.56 (ABq, 2H, J=15.0 Hz), 3.78 and 3.82 (ABq, 2H, J=17.4 Hz), 4.54 and 4.61 (ABq, 2H, J=15.0 Hz), 5.06 (d, 1H, J=4.9 Hz), 5.61 (dd, 1H, J=4.9, 8.3 Hz), 7.20–7.35 (m, 5H), 9.13 (d, 1H, J=8.3 Hz); FAB–MS m/z 463.1 (MH^+).

7β-(2-Phenylacetamido)-3-(thiazol-2-yl)thiomethylthio-3-cephem-4-carboxylic acid (40). Yield 66%; IR (KBr) 1776, 1660, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.50 and 3.56 (ABq, 2H, J=13.8 Hz), 3.87 (s, 2H), 4.74 (s, 2H), 5.11 (d, 1H, J=4.8 Hz), 5.64 (dd, 1H, J=4.8, 8.2 Hz), 7.2–7.4 (m, 5H), 7.75 (d, 1H, J=3.4 Hz), 7.81 (d, 1H, J=3.4 Hz), 9.13 (d, 1H, J=8.2Hz); FAB–MS m/z 480.0 (MH^+).

7α-Methoxy-7β-(2-phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylic acid (49). Yield 23%; IR (KBr) 1782, 1664, 1564 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.71 (s, 3H), 2.35 (s, 3H), 3.59 (d, 2H, J=3.4 Hz), 3.57 and 3.75 (ABq, 2H, J=17.6 Hz), 5.31 (s, 1H), 7.2–7.4 (m, 5H), 9.53 (s, 1H).

Benzhydryl 7β-(2-phenylacetamido)-3-methanesulfonyloxy-3-cephem-4-carboxylate (11). To a solution of **10** (800 mg, 1.6 mmol) in DMF (5 mL) were added methanesulfonyl chloride (421 mg, 3.68 mmol) and K_2CO_3 (310 mg, 2.24 mmol) at −35 °C with stirring and the mixture was stirred at −20 °C for 1 h. The resulting mixture was poured into a mixture of H_2O and AcOEt and the organic layer was sequentially washed with brine (3×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was triturated with a mixture of AcOEt and IPE, and the precipitate was collected by filtration to give 850 mg of **11** (92%): ^1H NMR ($CDCl_3$) δ 2.79 (s, 3H), 3.52 and 3.79 (ABq, 2H, J=18.4 Hz), 3.62 (d, 2H, J=2.4 Hz), 5.02 (d, 1H, J=5.0 Hz), 5.87 (dd, 1H, J=5.0, 9.2 Hz), 6.19 (d, 1H, J=9.2 Hz), 6.92 (s, 1H), 7.2–7.5 (m, 15H).

Benzhydryl 7β-(2-phenylacetamido)-3-(1,2,4-triazol-3-yl) thio-3-cephem-4-carboxylate (12a). To a solution of 3-mercapto-1,2,4-triazole (490 mg, 4.85 mmol) in a mixture of THF (5 mL) and DME (5 mL) was added *t*-BuOK (427 mg, 3.81 mmol) at −10 °C with stirring and the mixture was stirred at the same temperature for 20 min. A solution of **11** (2.0 g, 3.46 mmol) in a mixture of THF (10 mL) and DME (10 mL) was added dropwise to the solution obtained above at −20 °C, and the mixture was stirred at a temperature from −10 °C to 5 °C for 2 hours. The reaction mixture was poured into a mixture of 0.1N HCl (20 mL) and AcOEt (30 mL). The organic layer was sequentially washed with water, aqueous sodium hydrogen carbonate solution and brine, and dried over magnesium sulfate and evaporated under reduced pressure. The residue was subjected to silica-gel chromatography (eluent *n*-hexane:AcOEt) to give 351 mg of **12a** (17%): ^1H NMR ($CDCl_3$) δ 3.21 and 3.59 (ABq, 2H, J=17.8 Hz), 3.47 (s, 2H), 4.90 (d, 1H, J=4.5 Hz), 5.84 (dd, 1H, J=4.5, 9.3 Hz), 6.87 (s, 1H), 7.1–7.4 (m, 16H), 8.34 (s, 1H).

Preparation of **12e**, **12h,i**, **12l** and **29b** was carried out by a similar method to that described for **12a**.

Benzhydryl 7β-(2-phenylacetamido)-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (12e). Yield 40%; ^1H NMR ($CDCl_3$) δ 3.43 and 3.78 (ABq, 2H, J=18.0 Hz), 3.64 (d, 2H, J=1.9 Hz), 5.03 (d, 1H, J=5.0 Hz), 5.90 (dd, 1H, J=5.0, 9.1 Hz), 6.27 (d, 1H, J=9.1 Hz), 6.97 (s, 1H), 7.2–7.4 (m, 15H), 9.11 (s, 1H).

Benzhydryl 7β-(2-phenylacetamido)-3-(thiazol-2-yl)thio-3-cephem-4-carboxylate (12h). Yield 47%; ^1H NMR (DMSO-d_6) δ 3.53 (d, 2H, J=3.8 Hz), 3.54 and 3.77 (ABq, 2H, J=17.7 Hz), 5.26 (d, 1H, J=5.0 Hz), 5.83 (dd, 1H, J=5.0, 8.4 Hz), 6.97 (s, 1H), 7.2–7.5 (m, 15H), 7.90 (s, 2H), 9.26 (d, 1H, J=8.4 Hz).

Benzhydryl 7β-(2-phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (12i). Yield 58%; ^{1}H NMR (DMSO-d_6) δ 2.71 (s, 3H), 3.53 (d, 2H, J = 3.7 Hz), 3.57 and 3.87 (ABq, 2H, J = 17.7 Hz), 5.26 (d, 1H, J = 5.0 Hz), 5.85 (dd, 1H, J = 5.0, 8.3 Hz), 6.97 (s, 1H), 7.2–7.5 (m, 15H), 9.28 (d, 1H, J = 8.3 Hz).

Benzhydryl 7β-(2-phenylacetamido)-3-(2-pyridylthio)-3-cephem-4-carboxylate (12l). Yield 29%; ^{1}H NMR (DMSO-d_6) δ 3.53 (d, 2H, J = 4.0 Hz), 3.56 and 3.89 (ABq, 2H, J = 17.6 Hz), 5.29 (d, 1H, J = 5.0 Hz), 5.83 (dd, 1H, J = 5.0, 8.4 Hz), 6.92 (s, 1H), 7.1–7.4 (m, 7H), 7.77 (dt, 1H, J = 1.9, 7.7 Hz), 8.48 (d, 1H, J = 3.9 Hz), 9.29 (d, 1H, J = 8.4 Hz).

(*Z*)-Benzhydryl 7β-[2-(2-cyanoethenylthio)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (29b). Yield 25%; ^{1}H NMR (DMSO-d_6) δ 3.57 (s, 2H), 3.64 and 3.92 (ABq, 2H, J = 17.9 Hz), 5.09 (d, 1H, J = 5.0 Hz), 5.38 (d, 1H, J = 10.3 Hz), 5.87 (dd, 1H, J = 5.0, 9.0 Hz), 6.99 (s, 1H), 7.2–7.5 (m, 11H), 7.54 (d, 1H, J = 9.0 Hz), 9.15 (s, 1H).

Benzhydryl 7β-(2-phenylacetamido)-3-(1,2,3-triazol-4-yl)-thio-3-cephem-4-carboxylate (12b). To a solution of **11** (1.5 g, 2.60 mmol) in a mixture of THF (12 mL) and DME (12 mL) was added dropwise 4-mercapto-1,2,3-triazole sodium salt (443 mg, 3.60 mmol) with stirring under ice-cooling. After stirring at the same temperature for 3 h, to the reaction mixture were added AcOEt (100 mL) and 1N HCl (20 mL) with stirring. The organic layer was separated, sequentially washed with water, aqueous sodium hydrogen carbonate solution and brine, dried over magnesium sulfate and evaporated under reduced pressure to give 1.47 g of **12b** (97%): ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 2H), 3.59 and 3.73 (ABq, 2H, J = 17.9 Hz), 4.88 (d, 1H, J = 4.6 Hz), 5.75 (dd, 1H, J = 4.6, 8.4 Hz), 6.91 (s, 1H), 7.05–7.35 (m, 15H), 7.45 (d, 1H, J = 8.4 Hz), 7.64 (s, 1H).

Preparation of **12d** and **12g** was carried out by a similar method to that described for **12b**.

Benzhydryl 7β-(2-phenylacetamido)-3-(1-methyl-tetrazol-5-yl)thio-3-cephem-4-carboxylate (12d). Yield 77%; ^{1}H NMR ($CDCl_3$) δ 3.39 and 3.76 (ABq, 2H, J = 18.0 Hz), 3.55 (s, 2H), 3.79 (s, 3H), 5.04 (d, 1H, J = 5.0 Hz), 5.91 (dd, 1H, J = 5.0, 9.1 Hz), 6.22 (d, 1H, J = 9.1 Hz), 6.95 (s, 1H), 7.1–7.4 (m, 15H).

Benzhydryl 7β-(2-phenylacetamido)-3-(1,2,3-thiadiazol-5-yl)thio-3-cephem-4-carboxylate (12g). Yield 63%; ^{1}H NMR (DMSO-d_6) δ 3.4–3.6 (m, 2H), 3.50 and 3.85 (ABq, 2H, J = 18.0 Hz), 5.24 (d, 1H, J = 5.0 Hz), 5.87 (dd, 1H, J = 5.0, 8.0 Hz), 6.98 (s, 1H), 7.2–7.4 (m, 15H), 8.84 (s, 1H), 9.28 (d, 1H, J = 8.0 Hz).

5-Amino-1,2,4-thiadiazole hydrochloride (15). To a solution of amidine acetate (25.0 g, 0.24 mol) in MeOH (250 mL) was added potassium thiocyanate (23.3 g, 0.24 mol). After stirring at the room temperature for 10 min, 28% sodium methoxide in MeOH solution (92.6 g, 0.48 mol) was added dropwise to the above solution at 0 °C and then bromine (38.4 g, 0.24 mol) was added dropwise to the solution at −15 °C. After stirring at −10 °C for 30 min, 0 °C for 30 min and at the room temperature for 3 h, MeOH was evaporated in vacuo and then AcOEt (200 mL) was added to the residue and the resulting insoluble material was filtered off. The filtrate was poured into brine and the aqueous layer was extracted with AcOEt (3×), dried over magnesium sulfate and evaporated under reduced pressure. The residual gum was extracted with Et_2O (4×) and hydrogen chloride gas was passed through the Et_2O solution, and the resulting precipitate was collected by filtration, washed with Et_2O and dried in vacuo to give 5.34 g of **15** (16%): ^{1}H NMR (DMSO-d_6) δ 7.97 (brs, 2H), 8.00 (s, 1H).

5-Mercapto-1,2,4-thiadiazole (16). To a mixture of **15** (5.33 g, 38.8 mmol) in water (53 mL) were added 47% hydrobromic acid (64.5 mL) and Cu powder (38 mg), and the mixture was cool to −15 °C, and then sodium nitrite (5.35 g, 77.6 mmol) in water (7.75 mL) was added dropwise at −15 °C. After stirring at −10 °C for 2.5 h and at 40 °C for another hour, the mixture was adjusted to pH 6.2 with 3N NaOH with stirring at room temperature and poured into Et_2O. The extracted Et_2O layer was washed with aqueous $NaHSO_3$ solution and brine, dried over magnesium sulfate and evaporated under reduced pressure to give crude bromothiadiazole derivative. To a mixture of this crude bromide in THF (13.3 mL) and water (4.4 mL) was added thiourea (2.03 g, 26.7 mmol). After stirring at 65 °C for an hour, THF was evaporated and Et_2O was added to the mixture. The mixture was adjusted to pH 7.0 with $NaHCO_3$ solution and the separated aqueous layer was adjusted to pH 3.0 with 1N HCl. The resulting aqueous layer was extracted with Et_2O (3×), washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. To the residue in EtOH (8.3 mL) was added 1N KOH/EtOH solution (17 mL), and then Et_2O (100 mL) was added to the solution. The resulting potassium salt was collected by filtration, washed with Et_2O and dried in vacuo. To a mixture of potassium salt in water (12 mL) was added Et_2O (16 mL) and the solution was adjusted to pH 3.3 with 6N HCl. The aqueous layer was extracted with Et_2O (3×), washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give 0.85 g of **16** (19%): ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1H), 11.93 (brs, 1H).

4-Benzylthiopyrazole (18). To a solution of 4-bromopyrazole (15 g, 102 mmol) in THF (225 mL) was added dropwise 1.64 M *n*-BuLi hexane solution (205 mL, 337 mmol) at 0 °C. After stirring at room temperature for 1 hour, the solution was cooled to 0 °C, benzyl disulfide (25.1 g, 102 mmol) was added and the resulting solution was stirred for 2 h at ice cooling. The solution was poured into a mixture of AcOEt and ice–water, and adjusted to pH 5.6, and the separated organic layer was washed with water and brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was triturated with hexane and the resulting precipitate was collected by filtration, washed with hexane and dried in vacuo to give 13.0 g of **18** (67%): IR (KBr) 3153, 1493,

1452, 1369, 1138 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.86 (s, 1H), 7.1–7.4 (m, 6H), 7.65 (s, 1H), 12.93 (brs, 1H).

4-Mercaptopyrazole (19). To a solution of **18** (1.19 g, 6.25 mmol) in EtOH (24 mL) was added sodium (2.88 g, 125 mmol) under reflux. After stirring under reflux for 1.5 h, the mixture was poured into a mixture of AcOEt and water, and the separated aqueous layer was adjusted to pH 5.5. and poured into AcOEt. The separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give 0.56 g of **19** (89%): IR (KBr) 3263, 1709, 1373 cm^{-1}.

Potassium acetylhydrazinodithioate (21). A mixture of AcOEt (25.8 g, 293 mmol) and hydrazine monohydrate (13.3 g, 266 mmol) was stirred under reflux for 5 h and then cooled to room temperature. To the solution were added a solution of KOH (17.6 g, 314 mmol) in MeOH (100 mL) and carbon disulfide (22.1 g, 290 mmol). After stirring at room temperature for 5 h, the resulting precipitate was collected by filtration, washed with MeOH and dried in vacuo to give 13.0 g of **21** (59%): IR (KBr) 2910, 1670, 1527, 1259 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.33 (s, 3H), 9.31 (brs, 1H), 9.74 (brs, 1H).

2-Mercapto-5-methyl-1,3,4-oxadiazole (22). A solution of **21** (29.6 g, 157 mmol) in pyridine (89 mL) was refluxed for 18 h. After cooling to room temperature, pyridine was evaporated, water was added to the residue, and the solution was adjusted to pH 1.0 and extracted with THF/AcOEt (3×). The combined organic layer was washed with brine (2×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was triturated with hexane and the resulting precipitate was collected by filtration, washed with hexane and dried in vacuo to give 12.0 g of **22** (66%): ^1H NMR ($CDCl_3$) δ 2.44 (s, 3H), 11.50 (brs, 1H).

Benzhydryl 7β-amino-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate hydrochloride (23a). A suspension of phosphorus pentachloride (14.2 g, 68.2 mmol) in CH_2Cl_2 (210 mL) was stirred at ambient temperature for 30 min. To the mixture was added pyridine (553 mL) at −15 °C with stirring. After stirring at −20 °C to −15 °C for 30 minutes, to the resulting mixture was added **12e** (27.9 g, 46.5 mmol) at the same temperature. After stirring at −10 to −5 °C for 1.5 h, MeOH (28 mL) was added dropwise to the mixture at −20 to −10 °C for 10 min. After being stirred at ambient temperature for 50 min, to the reaction mixture was added water (56 mL) under ice-cooling, and the mixture was stirred at the same temperature for 30 min. The separated organic layer was washed with water, dried over magnesium sulfate and evaporated in vacuo. The residue was triturated with AcOEt to give 15.3 g of **23a** (64%): ^1H NMR (DMSO-d_6) δ 3.82 (m, 2H), 5.29 (d, 1H, J = 5.0 Hz), 5.40 (d, 1H, J = 5.0 Hz), 6.99 (s, 1H), 7.2–7.5 (m, 10H), 9.71 (s, 1H).

Preparation of **23b** was carried out by a similar method to that described for **23a**.

Benzhydryl 7β-amino-3-(5-methyl-1,3,4-thiadiazol-2-yl)-thio-3-cephem-4-carboxylate hydrochloride (23b). Yield 62%; ^1H NMR (DMSO-d_6) δ 2.73 (s, 3H), 3.78 (brs, 2H), 5.27 (d, 1H, J = 5.0 Hz), 5.39 (d, 1H, J = 5.0 Hz), 6.98 (s, 1H), 7.2–7.5 (m, 10H).

(D)-Benzhydryl 7β-[2-(*t*-butoxycarbonylamino)-2-(4-hydroxyphenyl)acetamido]-3-(1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (24d). To a mixture of **23a** (1.93 g, 3.73 mmol), D-2-(*t*-butoxycarbonylamino)-2-(4-hydroxyphenyl)acetic acid (1.28 g, 4.79 mmol) and CH_2Cl_2 (40 mL) was added 1,3-dicyclohexylcarbodiimide (825 mg, 4.00 mmol) at −10 °C. After stirring at room temperature for 2 hours, AcOEt was added to the mixture and insoluble material was filtered off. The filtrate was adjusted to pH 6.8 and the separated organic layer was washed with brine, dried over magnesium sulfate and evaporated in vacuo. The residue was subjected to column chromatography on silica gel (eluent AcOEt:hexane, 2:1) to give 1.43 g of **24d** (52%): ^1H NMR (DMSO-d_6) δ 1.38 (s, 9H), 3.53 and 3.81 (ABq, 2H, J = 17.8 Hz), 5.16 (d, 1H, J = 7.7 Hz), 5.21 (d, 1H, J = 5.1 Hz), 5.56 (d, 1H, J = 7.7 Hz), 5.90 (dd, 1H, J = 5.1, 8.5 Hz), 6.66 (d, 2H, J = 8.5 Hz), 6.97 (s, 1H), 7.1–7.6 (m, 12H), 9.23 (d, 1H, J = 8.5 Hz), 9.36 (s, 1H), 9.62 (s, 1H).

Benzhydryl 7β-(2-phenylacetamido)-3-(5-methyl-1,3,4-thiadiazol-2-yl)methylthio-3-cephem-4-carboxylate (34). To a solution of (5-methyl-1,3,4-thiadiazol-2-yl)methyl thiobenzoate **33** (300 mg, 1.2 mmol) in a mixture of THF (1 mL) and DMF (3 mL) was added 0.5 M sodium methoxide methanol solution (2.4 mL, 1.2 mmol) at ice-cooling and the mixture was stirred at the same temperature for 40 min. The resulting mixture was cooled to −70 °C and a solution of **11** (579 mg, 1.0 mmol) in a mixture of THF (1.2 mL) and DMF (3.6 mL) was added dropwise. After stirring at the same temperature for 1.5 h, the solution was poured into a mixture of AcOEt and water, and adjusted to pH 7.0 with 1N HCl. The separated organic layer was washed with brine (2×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane, 2:1) to give 420 mg of **34** (67%): ^1H NMR (DMSO-d_6) δ 2.66 (s, 3H), 3.50 and 3.58 (ABq, 2H, J = 14.0 Hz), 3.84 and 3.92 (ABq, 2H, J = 17.2 Hz), 4.61 and 4.66 (ABq, 2H, J = 15.4 Hz), 5.14 (d, 1H, J = 4.9 Hz), 5.69 (dd, 1H, J = 4.9, 8.3 Hz), 6.87 (s, 1H), 7.2–7.6 (m, 15H), 9.17 (d, 1H, J = 8.3 Hz).

2-(Chloromethylthio)thiazole (37). To a solution of *t*-BuOK (8.98 g, 80 mmol) in DMF (160 mL) was added 2-mercaptothiazole (9.38 g, 80 mmol) under ice-cooling. After stirring at ambient temperature for 20 min, to the mixture was added a solution of bromochloromethane (11.4 g, 88 mmol) in DMF (5 mL) at −5 °C. After 1 h at the same temperature, the reaction mixture was poured into a mixture of AcOEt and water, and the separated organic layer was washed with brine (3×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane, 1:9) to give 9.83 g of **37** (68%): ^1H NMR ($CDCl_3$) δ 5.22 (s, 1H), 7.37 (d, 1H, J = 3.4 Hz), 7.82 (d, 1H, J = 3.4 Hz).

Benzhydryl 7β-(2-phenylacetamido)-3-(thiazol-2-yl)thiomethylthio-3-cephem-4-carboxylate (39). To a mixture of NaSH (62 mg, 1.1 mmol) in DMF (2.3 mL) was added DIPEA (194 mg, 1.5 mmol) at ambient temperature. After stirring at the same temperature for 30 min, this solution was added to a solution of **11** (579 mg, 1.0 mmol) in DMF (5.2 mL) at −5 °C and stirred at 0 °C for 1 h. A solution of **37** (215 mg, 1.3 mmol) in acetone (4.3 mL) was treated with sodium iodide (390 mg, 2.6 mmol) and heated. After stirring at 50 °C for 2 h, the mixture was poured into a mixture of AcOEt and water, and the separated organic layer was washed with brine (3×), dried over magnesium sulfate and evaporated under reduced pressure. The residue in DMF (2 mL) was added to the mercapto solution prepared above at −5 °C. After stirring at 0 °C for 40 min, the reaction mixture was poured into a mixture of AcOEt and water, and the separated organic layer was washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane, 1:1) to give 427 mg of **39** (66%): IR (KBr) 1782, 1680, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 and 3.56 (ABq, 2H, J = 14.0 Hz), 3.94 (s, 2H), 4.77 (s, 2H), 5.18 (d, 1H, J = 4.6 Hz), 5.71 (dd, 1H, J = 4.6, 8.4 Hz), 6.85 (s, 1H), 7.1–7.5 (m, 15H), 7.72 (d, 1H, J = 3.4 Hz), 7.80 (d, 1H, J = 3.4 Hz), 9.17 (d, 1H, J = 8.4 Hz); APCI-MS m/z 646 (MH^+).

(*E*)-7β-Amino-3-[2-(5-methyl-1,3,4-thiadiazol-2-yl)thio]-vinyl-3-cephem-4-carboxylic acid (43). To a solution of **42** (2.0 g, 3.21 mmol) in a mixture of CH_2Cl_2 (6.0 mL) and anisole (2.0 mL) was added trifluoroacetic acid (4.0 mL) with stirring under ice-cooling. The mixture was stirred at the same temperature for 1 h. The reaction mixture was poured into IPE (100 mL) and the resulting precipitate was collected by filtration and dried in vacuo. The precipitate was dissolved in a mixture of sodium hydrogen carbonate solution and AcOEt at pH 7.5, and the separated aqueous layer was adjusted to pH 2.5 with 1N HCl with stirring. The resulting precipitate was collected by filtration, washed with water and dried in vacuo to give 800 mg of **43** (70%): ^1H NMR (DMSO-d_6) δ 2.72 (s, 3H), 3.62 and 3.92 (ABq, 2H, J = 17.4 Hz), 4.85 (d, 1H, J = 9.2 Hz), 5.06 (d, 1H, J = 9.2 Hz), 7.12 (d, 1H, J = 15.4 Hz), 7.17 (d, 1H, J = 15.4 Hz).

Benzhydryl 7β-(3,5-di-*t*-butyl-4-hydroxybenzylidene amino)-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (46). To a mixture of benzhydryl 7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate **45** (8.1 g, 11.3 mmol) in MeOH (325 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2.57 g, 11.3 mmol) portionwise at −20 °C. After stirring at the same temperature for 30 min, AcOEt (325 mL) was added to the mixture and stirred another 30 min at −20 °C. The resulting mixture was evaporated and the residue was chromatographed over silica-gel (eluent AcOEt:hexane, 1:2) to give 4.58 g of **46** (54%): IR (KBr) 1776, 1737, 1627, 1429, 1373 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.40 (s, 18H), 2.69 (s, 3H), 3.60 (s, 3H), 3.55 and 3.83 (ABq, 2H, J = 17.4 Hz), 5.60 (s, 1H), 6.98 (s, 1H), 7.2–7.5 (m, 10H), 7.69 (s, 2H), 8.54 (s, 1H).

Benzhydryl 7β-amino-7α-methoxy-3-(5-methyl-1,3,4-thiadiazol-2-yl)thio-3-cephem-4-carboxylate (47). To a solution of **46** (4.5 g, 6.04 mmol) in AcOEt (45 mL) was added Girard T (2.03 g, 12.08 mmol) at ambient temperature. After stirring at the same temperature for 8 h, the mixture was poured into a mixture of AcOEt and water, and the separated organic layer was washed with brine (2×), dried over magnesium sulfate and evaporated under reduced pressure to give 3.18 g of **47** (100%): IR (KBr) 1781, 1737, 1697, 1606, 1377 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.68 (s, 3H), 3.35 (s, 3H), 3.51 and 3.78 (ABq, 2H, J = 17.4 Hz), 5.13 (s, 1H), 6.96 (s, 1H), 7.2–7.5 (m, 10H); FAB–MS m/z 527.0 (MH^+).

(*Z*)-7β-[2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetoamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid (2). A mixture of **51** (720 mg, 0.821 mmol), formic acid (2.88 mL) and concd HCl (0.21 mL) was stirred at room temperature for 2 h, then poured into a mixture of AcOEt:acetone (2:1) and the resulting precipitate was collected by filtration. The precipitate was dissolved in sodium hydrogen carbonate solution (30 mL) and adjusted to pH 3.0 with 1N HCl. The resulting solution was subjected to column chromatography on Diaion HP-20 (eluent 5% aqueous isopropyl alcohol), and the desired fractions were evaporated under reduced pressure. The resulting precipitate was collected by filtration, washed with water and dried in vacuo to give 185 mg of **2** (48%): IR (KBr) 1772, 1664, 1625, 1587 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.34 and 3.49 (ABq, 2H, J = 17.0 Hz), 5.21 (d, 1H, J = 5.0 Hz), 5.76 (dd, 1H, J = 5.0, 8.3 Hz), 6.64 (s, 1H), 7.10 (s, 2H), 8.68 (brs, 1H), 9.45 (d, 1H, J = 8.3 Hz).

Preparation of **2′** was carried out by a similar method to that described for **2**.

(*Z*)-7β-[2-(2-Aminothiazol-4-yl)-2-hydroxyiminoacetoamido]-3-(1,2,4-triazol-3-yl)thio-3-cephem-4-carboxylic acid Δ2-isomer (2′). Yield 37%; IR (KBr) 1765, 1658, 1633, 1531 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.19 (s, 1H), 5.24 (d, 1H, J = 5.0 Hz), 5.61 (dd, 1H, J = 5.0, 8.4 Hz), 6.71 (s, 1H), 7.19 (brs, 2H), 7.26 (s, 1H), 8.58 (brs, 1H), 9.59 (d, 1H, J = 8.4 Hz), 11.33 (brs, 1H).

Biological methods

MIC. MICs were determined by the agar dilution method. *H. pylori* cell suspensions (0.5 McFarland) were prepared from cells grown on Brucella agar containing 2% starch and 3% fetal bovine serum (FBS) under 10% CO_2, 37 °C for 72 h. Ten-fold dilutions of these suspensions were inoculated on Brucella agar plates containing 7% defibrinated horse blood and serial dilutions of test compounds. MICs were read after 72 h incubation under 10% CO_2, 37 °C.

Therapeutic efficacy in a mouse model. ICR mouse infected with 10^8 CFU of *H. pylori* FP1757 were orally treated with drugs two times per day for 4 days. The number of viable organisms in the gastric mucosa 2 weeks after final treatment were grown on a Brucella agar plate containing 3% horse serum, 2% starch,

Skirrow's antibiotics, 10 μg/mL of nalidixic acid and 30 μg/mL of bacitracin.

Stability to β-lactamase. Stability to β-lactamase was assayed photometrically.

Bactericidal effect. Bactericidal activity against *H. pylori* was evaluated by monitoring viable cell counts in Brucella broth containing 2% starch and 3% FBS.

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