Synthesis and Pharmacological Evaluation of a New Class of 2-(2-Aminothiazol-4-yl)-2-hydrazonoacetamido Cephalosporins†

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A series of 2-(2-aminothiazol-4-yl)-2-hydrazonoacetamido cephalosporins **1a–h** was prepared. Whenever possible, *E* and *Z* isomers were isolated, and their relative stabilities and their interconversions were tested. The antibacterial activity was tested against Gram-positive and Gram-negative bacteria. For compound **1c**, whose *Z* and *E* forms do not interconvert rapidly, the *Z* form was the more active one. Among the other compounds, for which the *E* form is the only stable one for practical purposes, compound **1a** was the most active. When compared with cefuroxime and cefotaxime, compound **1a** showed slightly lower antibacterial activity but good serum level and half-life values.

Cephalosporins bearing a 2-(*Z*-substituted oximino)-2-(2-aminothiazol-4-yl)acetyl group at position 7 of the cephem nucleus have been widely studied in the last years, and some of them are now well-accepted in clinical medicine.[1] In contrast, to our knowledge, very little has been reported about the related cephalosporins with an unsubstituted or substituted 2-(2-aminothiazol-4-yl)-2-hydrazono side chain.[2,3] This paper describes the synthesis and the in vitro antibacterial activity of some cephalosporins of this kind (Table I, **1a–h**). For the more promising derivative (**1a**) we report also some preliminary in vivo data, as well as single-dose pharmacokinetics and urinary excretion data.

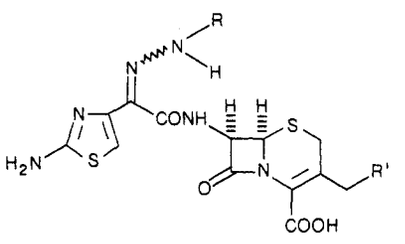
Chemistry

The synthesis of compounds **1a,c–g** has been carried out through a common intermediate, 7β-(2-aminothiazol-4-yl)glyoxylyl cephalosporin **2a**, whereas compounds **1b** and **1h** were prepared through **2b**. Compounds **2a** and **2b** were prepared, as outlined in Scheme I, by minor modifications of a reported synthesis[4] starting from the commercially available ethyl (2-aminothiazol-4-yl)acetate.

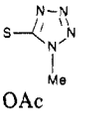
When compound **2a** as its sodium salt was allowed to react with a slight excess of *tert*-butyl carbazate in water solution, the desired *tert*-butoxycarbonylhydrazone **1g** was obtained as a mixture of *Z*/*E* isomers, approximately in a 7:3 ratio. The two isomers were separated by preparative TLC and their configurations were assigned through the ^{1}H NMR chemical shift of the 5-position of the thiazole ring, by analogy with the data for the corresponding oximino derivatives.[5] The *tert*-butyl group of the *E* form of **1g** was removed with TFA/anisole to yield **1a** (*E* form, NMR). However, when the *Z* form of **1g** was subjected to the same treatment, **1a** was obtained as a *Z*/*E* mixture in a nearly 4:6 ratio (NMR, HPLC) at the end of the deprotection reaction and recrystallization.

The *Z* form of **1a** was expected to be more biologically active than the *E* form, by analogy with what was known about the oximino derivatives.[5,6] Therefore, with the aim of obtaining a pure sample of the *Z* form of **1a**, the synthesis was carried out again with benzyl carbazate.[7] After separation of the two isomeric hydrazones, hydrogenolysis removed the protecting group and yielded **1a** as the pure *Z* form. ^{1}H NMR and HPLC analyses revealed that in water this isomer, as the sodium salt, was not stable enough for practical purposes because of its complete isomerization to the *E* form in nearly 3 h.

Table I



1a–h

no.	R	R′
1a	H	
1b	H	OAc
1c	$CONH_2$	
1d	CH_2COOEt	
1e	CH_2COOH	
1f	CH_2COO-*t*-Bu	
1g	COO-*t*-Bu	
1h	COO-*t*-Bu	OAc

The synthesis of **1b** from the reaction of **2b** with *tert*-butyl carbazate required the intermediacy of **1h** and was carried out as reported for the synthesis of **1a** from **2a**. As to the separation and the stability of the *E* and *Z* forms, we observed the same behavior as for **1a**.

The semicarbazone **1c** was obtained from the sodium salt of **2a** and semicarbazide hydrochloride in ethanol/water. It was easily separated in the *Z* and *E* forms by preparative HPLC (as the sodium salt on reverse-phase RP8 eluted with 99:1 H_2O/*t*-BuOH). The pure isomers, in contrast with other related compounds (**1a,b,d–f**) did not interconvert in H_2O solution and also in rat and rabbit serum for at least 24 h, as monitored by HPLC.

† In grateful memory of Prof. Luigi Canonica, who did a fine job of teaching us organic chemistry.

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(1) For a review, see: Newall, C. E. In *Recent Advances in the Chemistry of β-Lactam Antibodies*: Brown, A. G., Roberts, S. M., Eds.; The Royal Society of Chemistry: London, 1984.
(2) Bellani, P.; Corsi, G. B.; Innocenti, S.; Broccali, G.; Valcavi, U. (Craf-Sud) Brit. U.K. Pat. Appl. 2 102 423, 1983; *Chem. Abstr.* **1983**, *99*, 139641*x*.
(3) Konig, H. B.; Metzger, K. G. (Bayer A.G.) Ger. Offen. De. 3 021 373, 1981; *Chem. Abstr.* **1982**, *96*, 85330*n*.
(4) Kamiya, T.; Takaya, T.; Teraji, T.; Hashimoto, M.; Nakaguti, O.; Oku, T. (Fujisawa Pharm. Co. Ltd.) Ger. Offen. 2 728 766, 1978; *Chem. Abstr.* **1978**, *88* 105388*c*.
(5) Bucourt, R.; Heymes, R.; Lutz, A.; Penasse, L.; Perronet J. *Tetrahedron* **1978**, *34*, 2233.
(6) Ochiai, M.; Morimoto, A.; Toshio, M.; Yoshihiro, M.; Taiiti, O.; Hideaki, N.; Makodo, K. *J. Antibiot.* **1981**, *34*, 171.
(7) Carpino, L. A. *J. Am. Chem. Soc.* **1957**, *79*, 98.

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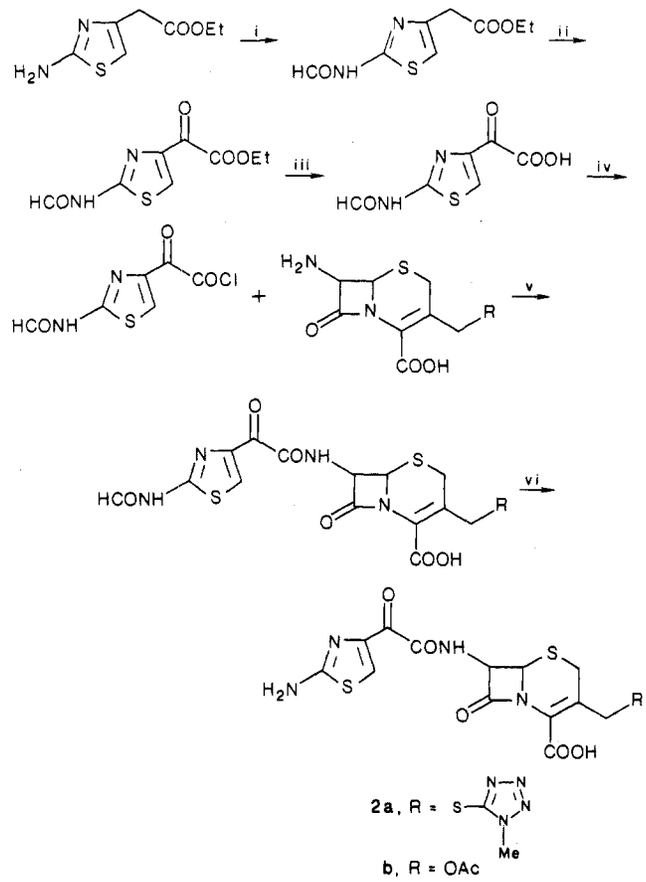
Scheme I[a]



[a] Reagents: (i) $HCOOCOCH_3$; (ii) $KMnO_4$, $Mn(OAc)_2 \cdot 4H_2O$ in CH_3COOH; (iii) 1, NaOH; 2, HCl; (iv) $SOCl_2$ in CH_2Cl_2; (v) Me_2NPh, Me_3SiCl; (vi) $POCl_3$, MeOH.

Compounds **1d** and **1f** were obtained upon reaction of **2a** with the corresponding substituted hydrazine (ethyl α-hydrazinoacetate and *tert*-butyl α-hydrazinoacetate) in H_2O/EtOH solution. Removal of the protecting group from **1f** by TFA/anisol yielded **1e**.

Before testing (see Biological Results), compounds **1a,b,d,e** were checked for the presence of the *E* form only (HPLC) and purified on a short reverse-phase column.

Owing to the very interesting antibacterial activity of **1a** (see Biological Results), a more suitable synthesis was developed, as reported in the Experimental Section.

Biological Results and Discussion

The minimum inhibitory concentration (MIC) of the previously unreported cephalosporins **1a–e** against representative strains of Gram-negative and Gram-positive bacteria are reported in Table II, together with those of cefotaxime and cefuroxime as reference standards. Among the oximino derivatives, it is known that isomerization from the *Z* form to the *E* form causes a substantial loss of activity, depending on the bacterial strains.[5,6] In the hydrazone derivatives series, we were able to confirm this observation only for compound **1c**, whose *E* and *Z* isomers are obtained and are stable enough to allow a MIC evaluation. In any case, the *E* form of the hydrazone derivatives, prepared by us, showed an appreciable antibacterial activity.

In contrast with what could be expected, since in the oxime series[5,8] O-substituted oximes are often more active than the unsubstituted ones, the unsubstituted hydrazone

(8) Takasugi, H.; Kochi, H.; Masugi, T.; Nakano, H.; Takaia, T. *J. Antibiot.* **1983**, *36*, 846.

Table II. Geometrical Averages of the MIC Values (μg/mL) in Isosensitest Broth

compd	Gram-negative: $\bar{M}$ (29 strains)[a]	Gram-positive; $\bar{M}$ (11 strains)[b]
1a	1.74	4.95
1b	1.96	7.35
1c (*Z*)	3.54	18.65
1c (*E*)	16.82	76.46
1d	5.45	13.22
1e	8.59	18.01
cefotaxime	0.17	2.09
cefuroxime	10.64	1.11

[a] 15 *E. coli*, four *Enterobacter cloacae*, five *Klebsiella pneumoniae*, two *Proteus mirabilis*, three *Salmonella thyphimurium*. [b] Six *Staphylococcus*, one *Bacillus subtilis*, one *Bacillus pumilus*, one *Sarcina lutea*, two *Enterococcus*.

1a showed the most interesting activity when compared to that of the reference standards. Therefore, compound **1a** was further investigated against different strains, as reported in Table III. This study showed that **1a** has a good activity especially toward Gram-negative bacteria including strains of *Serratia* and *Enterobacter*, which commonly produce high levels of chromosomally mediated β-lactamase, but that it was inactive against *Pseudomonas*. Moreover, **1a** was evaluated in vivo, compared with the same standards in mice infected with selected strains. The results are reported as 50% protective dose (ED_{50}, mg/kg) in Table IV. Compound **1a** shows high affinity toward serum proteins, as shown by the fact that in the dog nearly 56% of **1a** was bound and in the rat up to 91% was found to be bound. In the rat, high serum levels are obtained after intravenous (iv) or intramuscular (im) administration. They were measurable for 4 h (Table V), with an elimination half-life of 45 and 69 min, by iv and im, respectively. In our hands the half-life of **1a** was much longer than that of standard molecules (cefotaxime, 32 min; cefuroxime, 30 min). In Table VI the tissue distributions at various time intervals are reported. They were taken following the iv and im administration of 50 mg/kg of **1a** in rats.

Only a part of the administrated dose (24% of the im and 28% of the iv dose) was found in urine. This fact suggests that **1a** is eliminated also by the biliary tract, like several recently described high molecular weight cephalosporins, or metabolized. It is indeed found that, when a 50 mg/kg dose is administered im to rats with cannulated bile duct, 30% of the administered dose was found in the bile secreted within 8 h.

In conclusion, we have found that the replacement of the methoxyimino group of cefotaxime or cefmenoxime with a hydrazone group leads to compounds **1a,b**, which in the *E* form, have a good spectrum of antibacterial activity. Since, in our opinion, the slightly lower antibacterial activity of **1a** with respect to the standards is balanced by the superior values of serum levels and half-life, further research on this compound is in progress.

Experimental Section

Melting points were determined on a Büchi capillary apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with the assigned structure. ^1H NMR spectra were recorded on a Perkin-Elmer R 24B spectrometer. IR spectra were recorded on a Perkin-Elmer Model 681 spectrophotometer. TLC analyses were carried out on Merck glass plates precoated with silica gel (0.25-mm layer and 2-mm layer for preparative purposes) eluted with EtOAc/H_2O/AcOH (60:20:20), unless otherwise indicated. HPLC analyses were performed on a Perkin-Elmer Series 3 liquid chromatograph (5-μm column RP8 HS, eluted with 0.01 M $NH_4H_2PO_4$/CH_3OH (75:25) at 30 °C, flow rate 1 mL/min; λ 260 nm).

Protein Binding in Vitro. The compound was dissolved in serum at a concentration of 100 μg/mL, incubated for 1 h at 37

Table III. Geometrical Average of MIC (μg/mL) and MBC (μg/mL) in Isosensitest Broth

strains (no.)	1a		cefotaxime		cefuroxime	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli (4)	0.60	1.01	0.17	0.25	2.20	4.42
Proteus (10)	1.08	2.45	0.08	0.30	1.97	3.90
Salmonella (10)	0.86	1.79	0.24	0.38	3.12	6.70
Klebsiella (6)	1.17	2.78	0.12	0.14	3.71	4.68
Pseudomonas (6)	>100	>100	21.82	22.27	>100	>100
Serratia (3)	1.75	3.12	0.09	0.49	12.4	28.02
Enterobacter (3)	1.96	3.12	0.31	1.97	7.01	25
Alcaligenes (1)	8.83	12.5	1.56	3.12	50	100
Neisseria perfl. (1)	<0.09	<0.09	<0.09	<0.09	0.39	0.78
Staphilococcus (10)	6.69	11.26	1.61	3.23	1.06	1.67
Staphilococcus (P) (2)[a]	35.3	50	5.25	25	10.51	17.68
Bacillus (2)	42.01	49.96	25	29.73	35.36	35.36
Streptococcus (5)	25	70.71	12.33	35.36	15.14	59.46
Sarcina (1)	1.56	2.21	0.04	0.04	0.39	0.55

[a] (P) = β-lactamase producers.

Table IV. In Vivo Activity of 1a: ED_{50} in Infected Mice[a]

strain	inoculum size	compd	ED_{50}, mg/kg	MIC, μg/mL
E. coli ISM	*b*	1a	1.45 (0.90–2.34)	1.10
		cefotaxime	0.27 (0.118–0.615)	0.55
		cefuroxime	4.8 (3.47–6.63)	2.20
K. pneunomoniae 102	*c*	1a	15.5 (10.47–22.94)	1.56
		cefotaxime	3.8 (1.82–7.92)	0.13
		cefuroxime	9.0 (5.27–15.35)	12.5
S. typhimurium T.O.	*b*	1a	9.3 (5.47–15.81)	1.10
		cefuroxime	>90	1.56

[a] Mice Swiss (120/strain compound) were infected ip with mucin suspension of microorganisms in groups of 20. Therapy was given sc 1 h after the challenge. Each experiment was carried out for 7 days. [b] 0.5 mL of 10^6 CFU/mL of suspension. [c] 0.5 mL of 10^5 CFU/mL of suspension.

Table V. Serum Levels of 1a at Various Intervals following an Intravenous or Intramuscular Injection of 50 mg/kg in Rats

time, min	serum levels,[a] μg/mL	
	iv	im
5	231.30 ± 11.14	34.64 ± 0.97
15	136.57 ± 6.13	86.18 ± 8.37
30	87.15 ± 3.19	96.20 ± 4.47
60	52.47 ± 2.37	64.38 ± 4.57
120	20.50 ± 2.69	32.88 ± 2.97
240	3.65 ± 0.47	10.18 ± 0.50
360		3.43 ± 0.35
av C,[b] μg mL^{-1} h^{-1}	154.22	178.7
$t_{1/2}$, min	45	69

[a] The values represent the mean and standard error of the mean of five rats. [b] Calculated by the trapezoidal rule.

Table VI. Tissue Distribution of 1a (at Various Intervals) following an Intravenous or Intramuscular Administration of 50 mg/kg in Rats

time, min	μg/g ± SEM		
	liver	lung	kidney
Intravenous Administration			
5	43.20 ± 7.56	26.42 ± 1.76	106.70 ± 12.86
15	25.85 ± 8.71	17.53 ± 2.04	50.60 ± 1.69
30	18.80 ± 1.64	8.97 ± 0.75	19.65 ± 0.92
60	9.22 ± 0.41	≤5	8.90 ± 0.88
120	≤5	*a*	*a*
240	*a*	*a*	*a*
Intramuscular Administration			
5	*a*	≤5	6.42 ± 0.66
15	15.36 ± 1.61	10.73 ± 0.89	25.13 ± 4.15
30	22.12 ± 2.13	12.17 ± 1.74	36.15 ± 3.01
60	15.12 ± 1.85	7.66 ± 0.91	22.92 ± 2.67
120	5.27 ± 0.18	≤5	10.19 ± 2.25
240	*a*	*a*	*a*

[a] Undetectable (detection limit 0.5 μg/mL).

°C, and ultrafiltered on membrane (Sartorius) with a molecular weight cut-off of 5000. The amount of the nonbound substance was assayed microbiologically in the ultrafiltrate and the percentage of binding was calculated.

Animal Studies. Pharmacokinetics. The product dissolved in sterile water was administered intravenously or intramuscularly with a dose of 50 mg/kg to groups of five Wistar rats weighing 200–250 g. The animals were sacrified at intervals of 5, 15, 30, 60, 120, 240, and 360 min after injection, and the blood was collected and centrifugated in order to separate serum. Samples of lungs, liver, and kidney were also taken, homogenized in 0.1 M, pH 7.3 phosphate buffer (tissue/buffer ratio 1:5), and assayed for their antibiotic concentration. Urine samples up to 24 h from injection were also collected. Bile levels were determined in anesthetized Wistar rats with cannulated bile duct. The animals were injected intramuscularly with a dose of 50 mg/kg, and the bile was collected for 8 h after injection and assayed for antibiotic levels.

Bioassay. Concentrations in serum, urine, bile, and tissue samples were determined microbiologically with an agar diffusion test, using Nutrient Agar (Difco) as a culture medium; *Escherichia coli* ATCC 10536 incubated at 37 °C for 18 h was the test organism. The lowest drug level detectable in serum was 1 μg/mL while in the other cases it was 0.5 μg/mL. The standard and diluted solutions were prepared in serum for serum levels or in phosphate buffer (pH 7.3, 0.1 M) for the assay of urine, tissue homogenates, and bile samples. The assays were performed as described by Bennet et al.[9]

7β-[2-Hydrazono-2-(2-aminothiazol-4-yl)acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1a) (and Sodium Salt). To a suspension of **2a** (20.0 g, 41.4 mmol) in anhydrous acetonitrile (800 mL) at −5 °C were added hexamethyldisilazane (HMDS) (20.7 mL, 129.5 mmol) and trimethylchlorosilane (16 mL, 129.5 mmol), and the solution was stirred for 1 h. Then at −5 °C 100 mL of a 0.5 M solution of silylhydrazine in CH_3CN [prepared by refluxing hydrazine hydrate (12.7 g) in CH_3CN with HMDS (81 mL) and then diluting to 500 mL with CH_3CN] was added dropwise and the mixture stirred for 2 h at this temperature. A second portion of the above-mentioned 0.5 M silylhydrazine solution (20 mL) was added and the solution stirred for 90 min. The mixture was then

(9) Bennet, I. V.; Brodie, I. L.; Benner, E. J.; Kirby, W. M. *Appl. Microbiol.* **1966**, *14*, 170.

evaporated in vacuo and the residue dissolved in water with 5% $NaHCO_3$ (pH 8). At 5 °C 1 N HCl was added and the crude precipitate collected by filtration. The wet product was dissolved in THF and stirred for 2 h to cause complete isomerization (monitored by TLC, elution with 92:8 THF/H_2O). The organic solution was dried on Na_2SO_4 and poured in diisopropyl ether. The dried product was crystallized from THF/diisopropyl ether, giving 11.3 g (54.8% yield) of the title compound: mp 176 °C dec. This product was suspended in water and dissolved by bringing the pH value to 7.2 with 2 N NaOH. The solution was filtered through a short column of RP8 and washed with water, and the clear solution was lyophilized: IR 3400, 1770, 1650–1600, 1550, 1500 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.5 (br s, 2 H), 3.9 (s, 3 H), 4.35 (br, dd, 2 H), 5.0 (d, 1 H), 5.7 (dd, 1 H), 7.1 (s, 2 H), 7.3 (s, 1 H), 7.7 (d, 1 H), 9.4 (s, 2 H).

General Procedure for the Preparation of Substituted Hydrazono Cephalosporins 1c,d,f,g,h. To a solution of **2a** (or **2b** for **1h**) as the sodium salt (10 mmol) in water (100 mL) was added at 0 °C the solution of the appropriate hydrazine hydrochloride (15.0 mmol) in EtOH or H_2O (100 mL), and the pH of the resulting solution was adjusted to 6. The reaction mixture was stirred at room temperature for 8–16 h and then the precipitate was filtered and purified as the sodium salt by chromatography on a short reverse-phase column by elution with 99:1 H_2O/*t*-BuOH, followed by reprecipitation at pH 2 and recrystallization (THF/Et_2O).

7β-[2-(2-Aminothiazol-4-yl)-2-semicarbazonoacetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1c). From semicarbazide hydrochloride; yield 82% (*Z* + *E*). *Z* isomer: mp > 200 °C dec; IR (Nujol) 3450 (sh), 3310, 3200, 1770, 1680–1650, 1550 cm^{-1}; ^1H NMR (Me_2SO-d_6 + CF_3COOH) 3.65 (br s, 2 H), 3.92 (s, 3 H), 4.32 (br dd, 2 H), 5.15 (d, 1 H), 5.70 (dd, 1 H), 6.92 (s, 1 H). *E* isomer: mp > 200 °C dec; ^1H NMR (Me_2SO-d_6 + CF_3COOH) δ 3.65 (br s, 2 H), 3.82 (s, 3 H), 4.32 (br dd, 2 H), 5.15 (d, 1 H), 5.70 (dd, 1 H), 7.30 (s, 1 H).

7β-[2-(2-Aminothiazol-4-yl)-2-[[(ethoxycarbonyl)methyl]hydrazono]acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1d). From ethyl hydrazinoacetate hydrochloride: yield 51%; mp 207 °C dec; IR (Nujol) 3300, 1770, 1735, 1650–1620 cm^{-1}; ^1H NMR (Me_2SO-d_6 + CF_3COOD) δ 1.23 (t, 3 H), 3.60 (br s, 2 H), 3.90 (s, 3 H), 3.95–4.40 (m, 6 H), 5.10 (d, 1 H), 5.75 (dd, 1 H), 7.3 (s, 1 H).

7-[2-(2-Aminothiazol-4-yl)-2-[[(*tert*-butoxycarbonyl)methyl]hydrazono]acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1f). From *tert*-butyl hydrazinoacetate hydrochloride; yield 57%; mp 182–184 °C dec; IR (Nujol) 3300, 1770, 1730, 1650–1620 cm^{-1}; ^1H NMR (Me_2SO-d_6 + CF_3COOD) δ 1.45 (s, 9 H), 3.75 (br s, 2 H), 3.95 (s, 3 H), 4.15 (s, 2 H), 4.35 (s, 2 H), 5.1 (d, 1 H), 5.75 (dd, 1 H), 7.1 (s, 1 H).

7β-[2-(2-Aminothiazol-4-yl)-2-[(*tert*-butoxycarbonyl)hydrazono]acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1g). From *tert*-butyl carbazate; yield 61%. The pure *Z* form was isolated by preparative TLC (silica gel eluted with 92:8 THF/H_2O): IR (Nujol) 3300, 3190, 1772, 1720, 1690 (sh), 1650–1600, 1520 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.5 (s, 9 H), 3.65 (br s, 2 H), 3.95 (s, 3 H), 4.35 (dd, 2 H), 5.2 (d, 1 H), 5.7 (dd, 1 H), 6.5 (s, 1 H), 7.4 (d, 1 H). The *E* isomer showed an identical NMR spectrum except for the thiazole H-5 proton at 6.8 ppm (s, 1 H) instead of 6.5.

7β-[2-(2-Aminothiazol-4-yl)-2-[(*tert*-butoxycarbonyl)hydrazono]acetamido]-3-(acetoxymethyl)-3-cephem-4-carboxylic Acid (1h). From *tert*-butyl carbazate; yield 56%; IR (Nujol) 3300, 1770, 1730, 1680, 1650–1615, 1510 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.5 (s, 9 H), 2.02 (s, 3 H), 3.6 (dd, 2 H), 5.0 (dd, 2 H), 5.15 (d, 1 H), 5.85 (dd, 1 H), 7.18 (s, 0.6 H), 7.85 (s, 0.4 H), 8.1 (d, 1 H).

7β-[2-(2-Aminothiazol-4-yl)-2-hydrazonoacetamido]-3-(acetoxymethyl)-3-cephem-4-carboxylic Acid (1b). A solution of **1h** (3.8 g, 7.2 mmol) in 1:1 TFA/anisole (24 mL) was stirred at room temperture for 15 min. Then, the solution was poured in Et_2O (250 mL), and the yellow product was filtered, suspended in water, dissolved by bringing the pH to 7.5 with 1 N NaOH, washed with EtOAc, and precipitated at pH 2 with 1 N HCl. After drying in vacuo, 2.7 g (yield 85%) of pure **1b** was obtained: mp 130–132 °C dec; IR (KBr) 3250, 1770, 1730 (br), 1660, 1630 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.02 (s, 3 H), 3.58 (dd, 2 H), 4.80 (dd, 2 H), 5.15 (d, 1 H), 5.85 (dd, 1 H), 7.15 (s, 1 H), 8.10 (d, 1 H).

7β-[2-(2-Aminothiazol-4-yl)-2-[(carboxymethyl)hydrazono]acetamido]-3-[[(1-methyl-1*H*-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (1e). A solution of **1f** (13.5 g, 22 mmol) in TFA (80 mL) was stirred for 10 min at 0 °C. Then Et_2O (1 L) was added and the yellow crude product collected by filtration. The product was suspended in water and dissolved by bringing the pH to 8 with 2 N NaOH. The solution was washed with EtOAc and acidified to pH 2. The product was filtered and dried in vacuo (7.1 g, yield 58.4%): mp 148 °C dec; IR (Nujol) 3300, 1765, 1770, 1630, 1510 cm^{-1}; ^1H NMR (Me_2SO-d_6 + CF_3COOD) δ 3.72 (br s, 2 H), 3.95 (s, 3 H), 4.20 (m, 4 H), 5.1 (d, 1 H), 5.75 (d, 1 H), 7.4 (s, 1 H).

Registry No. (*E*)-**1a**, 87328-96-9; (*Z*)-**1a**, 87328-87-8; (*E*)-**1a**·Na, 106626-04-4; (*E*)-**1a** (R = $C(O)OCH_2Ph$), 106626-14-6; (*Z*)-**1a** (R = $C(O)OCH_2Ph$), 106026-15-7; (*E*)-**1b**, 106709-17-5; (*E*)-**1c**, 106626-06-6; (*Z*)-**1c**, 106626-05-5; (*E*)-**1d**, 106626-07-7; (*E*)-**1e**, 106626-13-5; (*E*)-**1f**, 106626-08-8; (*Z*)-**1g**, 106626-10-2; (*E*)-**1g**, 106626-11-3; (*E*)-**1h**, 106626-12-4; **2a**, 64987-51-5; **2b**, 68363-46-2; semicarbazide hydrochloride, 563-41-7; ethyl hydrazinoacetate hydrochloride, 6945-92-2; *tert*-butyl hydrazinoacetate hydrochloride, 106626-09-9; *tert*-butyl carbazate, 870-46-2; benzyl carbazate, 5331-43-1.