SYNTHESIS AND ANTIBACTERIAL PROPERTIES OF 7-[2-(3-SUBSTITUTED-5-ISOXAZOLYL)-2-METHOXYIMINOACETAMIDO]CEPHALOSPORANIC ACID DERIVATIVES

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The synthesis of new 2-(3-substituted-5-isoxazolyl)-2-methoxyiminoacetic acids and their condensation derivatives with a suitable cephalosporanic nucleus, is reported. Their antibacterial properties were tested *in vivo* and *in vitro* also against β-lactamase producer microorganisms; particularly the oral bioavailability of some of these new derivatives was studied.

In the past few years many molecules in which a suitable cephalosporanic nucleus is condensed with alkoxyiminoacetic acid derivatives bearing a heterocycle such as furane or 2-aminothiazole[1], have been extensively studied in order to obtain new antibacterial drugs, especially those active against β-lactamase producer bacteria. However, these broad-spectrum antibiotics have the disadvantage of requiring parenteral administration; therefore the possibility of increasing their oral absorbtion remains one of the most important goals in chemotherapy.

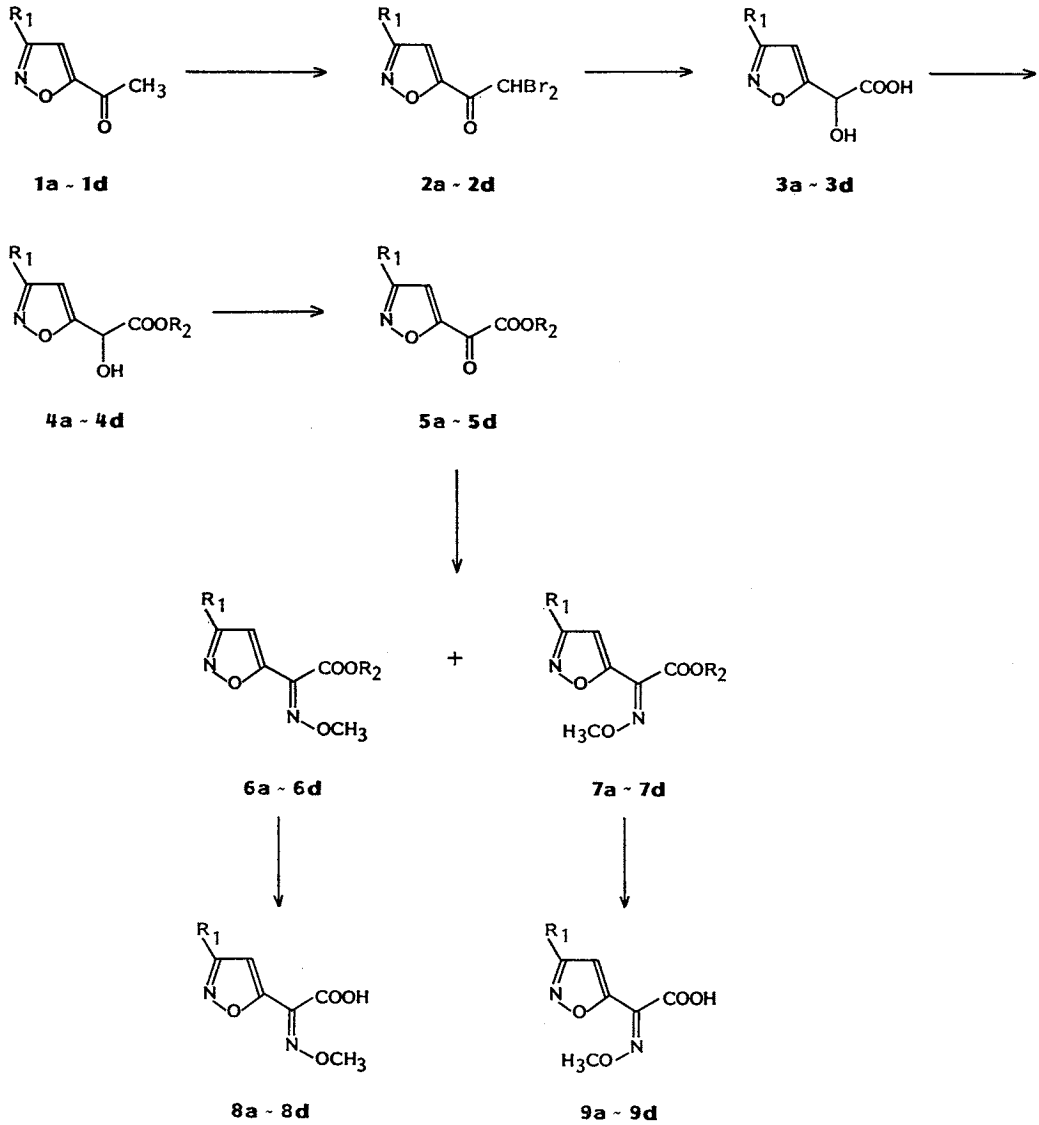
We were interested to determine if the choice of an isoxazole ring as the heteroaromatic moiety could improve the oral bioavailability as previous proved for the penicillin series[2]. As part of our investigations in the isoxazole field[3,4], we developed the synthesis of new 2-(3-substituted-5-isoxazolyl)-2-methoxyiminoacetic acids **8a**~**8d** and **9a**~**9d** for their use in synthesizing new cephalosporin derivatives potentially active in chemotherapy. Thus we prepared derivatives **10a**, **10b**, **11a**~**11d**, **12a** and **12c** and investigated the structure-activity relationships regarding their antibacterial properties; we were also interested in evaluating a possible improvement of their oral bioavailability compared with cefuroxime.

Chemistry

The synthesis of 2-(3-substituted-5-isoxazolyl)-2-methoxyiminoacetic acids **8a**~**8d** and **9a**~**9d**, key intermediates for the preparation of cephalosporanic derivatives, is reported in Scheme 1. The synthesis was carried out using known carbonyl compounds **1a**~**1d**[3,5,6] as starting materials.

The reaction between derivatives **1a**~**1d** and bromine in acetic acid gave dibrominated compounds **2a**~**2d** which were treated without any purification with aqueous sodium hydroxide in ethanol affording α-hydroxyacids **3a**~**3d**. These unstable compounds were directly transformed into corresponding esters **4a**~**4d** which were oxidized with chromic anhydride in acetic acid to give α-keto esters **5a**~**5d**. Analytical data of derivatives **4a**~**4d** and **5a**~**5d** are reported in Table 1. The reaction of intermediates **5a**~**5d** and methoxyamine hydrochloride in aqueous alcohol yielded the mixture of (*Z*)- and (*E*)-oximes which were easily separated by column chromatography. The study of the

Scheme 1.



^{1}H NMR spectra made possible the assignment of the (*Z*)-isomerism to compounds **6a**~**6d** and the (*E*)-isomerism to compounds **7a**~**7d** in agreement with the previously reported literature data[7]. Analytical data of derivatives **6a**~**6d** and **7a**~**7d** are described in Table 2. A mild basic hydrolysis in aqueous ethanol of compounds **6a**~**6d** and **7a**~**7d** gave respectively (*Z*)-methoxyiminoacetic acids **8a**~**8d** and (*E*)-methoxyiminoacetic acids **9a**~**9d**. Analytical data of key intermediates **8a**~**8d** and **9a**~**9d** are reported in Table 3.

The synthesis of 7-aminodesacetoxycephalosporanic acids **10a**, **10b** and 7-aminocephalosporanic acid **11a**~**11d** is described in Scheme 2. Acid chlorides of compounds **8a**~**8d**, generated *in situ* with oxalyl chloride in mild conditions, reacted with silylated cephalosporanic intermediates to afford desired 7-aminodesacetoxycephalosporanic acids **10a**, **10b** and 7-aminocephalosporanic acids **11a**~**11d**. Analytical data of compounds **10a**, **10b** and **11a**~**11d** are reported in Table 4. The synthesis

Table 1. 2-(3-Substituted-5-isoxazolyl)-2-hydroxyacetic esters **4a**~**4d** and 2-(3-substituted-5-isoxazolyl)-2-oxoacetic esters **5a**~**5d**.

Compound	R_1	R_2	Yield (%)	MP[a] (°C) or bp (°C/Torr)	Molecular formula[b]	^{1}H NMR ($CDCl_3$) δ
4a	Br	C_2H_5	65	50~51	$C_7H_8BrNO_4$	1.31 (3H, t), 4.37 (2H, q), 5.40 (1H, d), 6.50 (1H, s)
4b	C_6H_5	C_2H_5	63	82~83	$C_{13}H_{13}NO_4$	1.22 (3H, t), 4.26 (2H, q), 5.56 (1H, d), 7.15 (1H, s), 7.40~8.20 (5H, m)
4c	CH_3	CH_3	55	130/0.15	$C_7H_9NO_4$	2.24 (3H, s), 3.78 (3H, s), 5.42 (1H, d), 6.40 (1H, s)
4d	OCH_3	CH_3	66	130/0.2	$C_7H_9NO_5$	3.88 (3H, s), 4.00 (3H, s), 5.30 (1H, d), 6.04 (1H, s)
5a	Br	C_2H_5	80	115/0.3	$C_7H_6BrNO_4$	1.47 (3H, t), 4.50 (2H, q), 7.54 (1H, s)
5b	C_6H_5	C_2H_5	91	41~42	$C_{13}H_{11}NO_4$	1.46 (3H, t), 4.50 (2H, q), 7.50~8.20 (6H, m)
5c	CH_3	CH_3	70	82~83	$C_7H_7NO_4$	2.46 (3H, s), 4.04 (3H, s), 7.37 (1H, s)
5d	OCH_3	CH_3	71	97~98	$C_7H_7NO_5$	4.01 (3H, s), 4.08 (3H, s), 7.03 (1H, s)

[a] Crystallization solvent: Isopropyl ether.
[b] Microanalyses were in satisfactory agreement with calculated values: C ±0.32, H ±0.18, N ±0.20.

Table 2. 2-(3-Substituted-5-isoxazolyl)-(*Z*)-2-methoxyiminoacetic esters **6a**~**6d** and 2-(3-substituted-5-isoxazolyl)-(*E*)-2-methoxyiminoacetic esters **7a**~**7d**.

Compound	R_1	R_2	Yield (%)	MP[a] (°C) or bp (°C/Torr)	Molecular formula[b]	^{1}H NMR ($CDCl_3$) δ
6a	Br	C_2H_5	35	135/0.2	$C_8H_9BrN_2O_4$	1.41 (3H, t), 4.15 (3H, s), 4.21 (2H, q), 6.67 (1H, s)
6b	C_6H_5	C_2H_5	36	44~45	$C_{14}H_{14}N_2O_4$	1.40 (3H, t), 4.13 (3H, s), 4.47 (2H, q), 6.93 (1H, s), 7.30~8.10 (5H, m)
6c	CH_3	CH_3	39	22~23	$C_8H_{10}N_2O_4$	2.37 (3H, s), 3.98 (3H, s), 4.15 (3H, s), 6.47 (1H, s)
6d	OCH_3	CH_3	33	165/0.1	$C_8H_{10}N_2O_5$	3.98 (3H, s), 4.05 (3H, s), 4.13 (3H, s), 6.24 (1H, s)
7a	Br	C_2H_5	56	140/0.15	$C_8H_9BrN_2O_4$	1.41 (3H, t), 4.24 (3H, s), 4.43 (2H, q), 7.07 (1H, s)
7b	C_6H_5	C_2H_5	57	46~47	$C_{14}H_{14}N_2O_4$	1.40 (3H, t), 4.28 (3H, s), 4.50 (2H, q), 7.42 (1H, s), 7.40~8.10 (5H, m)
7c	CH_3	CH_3	50	59~60	$C_8H_{10}N_2O_4$	2.38 (3H, s), 3.98 (3H, s), 4.24 (3H, s), 6.91 (1H, s)
7d	OCH_3	CH_3	58	69~70	$C_8H_{10}N_2O_5$	3.96 (3H, s), 4.05 (3H, s), 4.23 (3H, s), 6.65 (1H, s)

[a] Crystallization solvent: Isopropyl ether.
[b] Microanalyses were in satisfactory agreement with calculated values: C ±0.28, H ±0.15, N ±0.18.

of 7-aminocephalosporanic acids **12a**, **12c** is described in Scheme 3. The reaction was carried out in the same mild conditions indicated for (*Z*)-isomer. Analytical data of compounds **12a**, **12c** are reported in Table 4.

Biological Results and Discussion

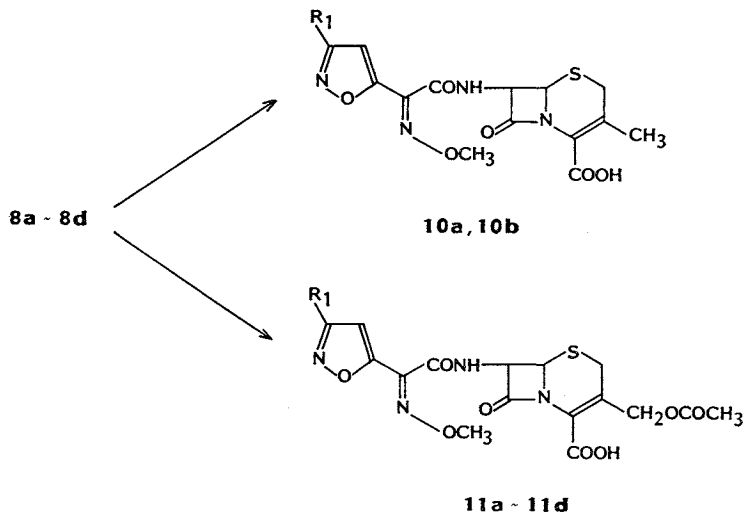
The *in vitro* antibacterial activities of 7-aminodesacetoxycephalosporanic acid derivatives **10a**, **10b** and 7-aminocephalosporanic acid derivatives **11a**~**11d**, **12a** and **12c** are shown in Table 5. Cefuroxime was used as a reference compound. According to the data obtained by the *in vitro* studies, the substitution of the heterocyclic moiety such as furane, with an isoxazole ring gave derivatives

Table 3. 2-(3-Substituted-5-isoxazolyl)-(*Z*)-2-methoxyiminoacetic acids **8a**~**8d** and 2-(3-substituted-5-isoxazolyl)-(*E*)-2-methoxyiminoacetic acids **9a**~**9d**.

Compound	R_1	Yield (%)	MP (°C) (crystallization solvent)[a]	Molecular formula[b]	^{1}H NMR (solvent) δ
8a	Br	94	136~137 (A)	$C_6H_5BrN_2O_4$	(DMSO-d_6), 4.05 (3H, s), 7.32 (1H, s)
8b	C_6H_5	92	156~157 (B)	$C_{12}H_{10}N_2O_4$	(DMSO-d_6), 4.10 (3H, s), 7.50~6.30 (6H, m)
8c	CH_3	94	147~148 (C)	$C_7H_8N_2O_4$	(DMSO-d_6), 2.31 (3H, s), 4.05 (3H, s), 6.79 (1H, s)
8d	OCH_3	96	147~148 (A)	$C_7H_8N_2O_5$	(CD_3OD), 4.02 (3H, s), 4.12 (3H, s), 6.27 (1H, s)
9a	Br	95	141~142 (A)	$C_6H_5BrN_2O_4$	(DMSO-d_6), 4.20 (3H, s), 7.48 (1H, s)
9b	C_6H_5	94	108~109 (A)	$C_{12}H_{10}N_2O_4$	(DMSO-d_6), 4.23 (3H, s), 7.50~8.30 (6H, m)
9c	CH_3	97	134~135 (C)	$C_7H_8N_2O_4$	(DMSO-d_6), 2.34 (3H, s), 4.14 (3H, s), 7.03 (1H, s)
9d	OCH_3	98	121~122 (A)	$C_7H_8N_2O_5$	(CD_3OD), 4.00 (3H, s), 4.21 (3H, s), 6.62 (1H, s)

[a] Crystallization solvent: (A) Benzene, (B) 1,2-dichloroethane, (C) isopropyl ether.
[b] Microanalyses were in satisfactory agreement with calculated values: C ±0.31, H ±0.18, N ±0.17.

Scheme 2.



Scheme 3.

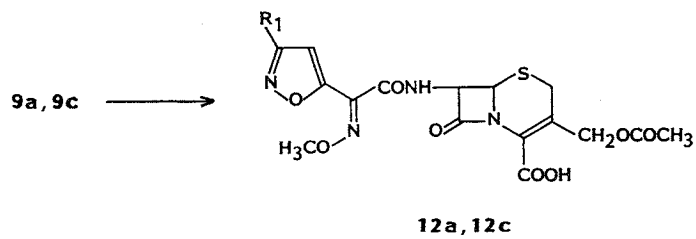


Table 4. 7-Aminodesacetoxycephalosporanic acid derivatives **10a**, **10b** and 7-aminocephalosporanic acid derivatives **11a**~**11d**, **12a** and **12c**.

Compound	R_1	Yield (%)	Dec point[a] (°C)	Molecular formula[b]	^{1}H NMR (DMSO-d_6) δ, typical signals
10a	Br	81	71~73	$C_{14}H_{13}BrN_4O_6S$	2.00 (3H, s, CH_3), 4.05 (3H, s, CH_3ON), 7.15 (1H, s, isoxazol), 10.00 (1H, d, CONH)
10b	C_6H_5	58	92~94	$C_{20}H_{18}N_4O_6S$	2.07 (3H, s, CH_3), 4.07 (3H, s, CH_3ON), 7.40 (1H, s, isoxazol), 10.10 (1H, d, CONH)
11a	Br	68	70~72	$C_{16}H_{15}BrN_4O_8S$	2.04 (3H, s, CH_3CO), 4.04 (3H, s, CH_3ON), 7.13 (1H, s, isoxazol), 10.00 (1H, d, CONH)
11b	C_6H_5	46	75~77	$C_{22}H_{20}N_4O_8S$	2.08 (3H, s, CH_3CO), 4.07 (3H, s, CH_3ON), 7.42 (1H, s, isoxazol), 10.10 (1H, d, CONH)
11c	CH_3	73	80~82	$C_{17}H_{18}N_4O_8S$	2.05 (3H, s, CH_3CO), 2.28 (3H, s, CH_3), 4.02 (3H, s, CH_3ON), 6.65 (1H, s, isoxazol), 10.00 (1H, d, CONH)
11d	OCH_3	79	81~83	$C_{17}H_{18}N_4O_9S$	2.07 (3H, s, CH_3CO), 3.98 (3H, s, CH_3O), 4.04 (3H, s, CH_3ON), 6.55 (1H, s, isoxazol), 10.00 (1H, d, CONH)
12a	Br	76	88~90	$C_{16}H_{15}BrN_4O_8S$	2.05 (3H, s, CH_3CO), 4.16 (3H, s, CH_3ON), 7.51 (1H, s, isoxazol), 9.80 (1H, d, CONH)
12c	CH_3	68	73~75	$C_{17}H_{18}N_4O_8S$	2.08 (3H, s, CH_3CO), 2.33 (3H, s, CH_3), 4.13 (3H, s, CH_3ON), 7.10 (1H, s, isoxazol), 9.70 (1H, d, CONH)

[a] Trituration solvent: *n*-Pentane.
[b] HPLC study showed all compounds had a purity >95%.

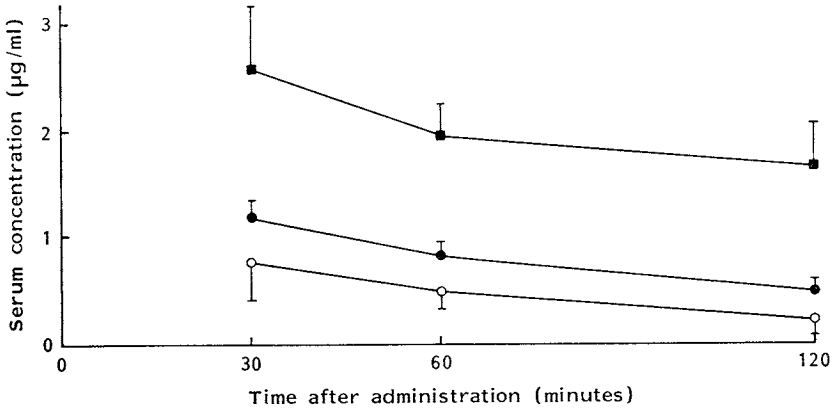
Table 5. *In vitro* antibacterial activity of compounds **10a**, **10b**, **11a**~**11d**, **12a** and **12c** (MIC, μg/ml).

Compound	*S.a.* 209P	*S.a.* Z2[a]	*E.f.*	*S.p.* A-32	*K.p.* 381[b]	*E.c.* 128[b]	*E.c.* 078	*E.cl.* 122[b]	*P.m.* Vi	*S.f.*[b]
10a	64	32	>128	4	>128	>128	>128	>128	8	>128
10b	32	32	>128	1	>128	>128	>128	>128	>128	>128
11a	2	1	>128	0.25	32	16	8	16	1	8
11b	2	2	>128	0.125	>128	128	64	128	16	128
11c	2	2	>128	0.5	16	16	8	16	0.5	8
11d	4	4	>128	0.5	32	16	16	32	2	16
12a	16	16	>128	4	>128	>128	>128	>128	64	>128
12c	16	32	>128	2	>128	>128	>128	>128	16	>128
Cefuroxime	2	2	>128	0.0312	4	4	4	4	0.25	4

Test organisms and abbreviations: *S.a.* 209P, *Staphylococcus aureus* 209P; *S.a.* Z2, *Staphylococcus aureus* Z2; *E.f.*, *Enterococcus faecalis* ATCC 10541; *S.p.* A-32, *Streptococcus pyogenes* A-32; *K.p.* 381, *Klebsiella pneumoniae* 381; *E.c.* 128, *Escherichia coli* 128; *E.c.* 078, *Escherichia coli* 078; *E. cl.* 122, *Enterobacter cloacae* 122; *P.m.* Vi, *Proteus mirabilis* Vi; *S.f.*, *Shigella flexneri* 31172.
[a] Penicillinase producer.
[b] Cephalosporinase producer.

active against β-lactamase producer microorganisms. However, the nature of the substituent in position 3 at the isoxazole ring influenced significantly the activity. In fact, the presence of Br (**11a**) and CH_3 (**11c**) led to derivatives with significant antibacterial activity, whereas the presence of OCH_3 (**11d**) displayed a reduction in the activity against both Gram-positive and Gram-negative microorganisms. The introduction of Ph (**11b**) resulted in the decrease of the activity, especially against Gram-negative bacteria.

Fig. 1. Mean serum concentrations (±SE), $AUC_{0\sim\infty}$ and $T_{1/2}$ of compounds **11a**, **11c** and cefuroxime after oral administration (100 mg/kg) in rats.
● **11a**, ■ **11c**, ○ cefuroxime.



	$AUC_{0\sim\infty}$ (μg·minutes/ml)	$T_{1/2}$ (minutes)
11a	133.3	75.3
11c	386.9	97.5
Cefuroxime	64.2	55.8

Our studies confirmed the importance of (*Z*)-isomerism of the oximino group of derivatives **11a**~**11d**; in fact, when (*E*)-isomers **12a**, **12c** were tested *in vitro*, a remarkable reduction of the activity was found. The replacement of the 7-aminocephalosporanic nucleus of compounds **11a**~**11d** with the 7-aminodesacetoxycephalosporanic nucleus of derivatives **10a**, **10b** resulted again in a decrease of the *in vitro* antibacterial properties. While the *in vitro* antibacterial profile of our compounds generally denoted less potency than cefuroxime, the oral bioavailability of derivatives **11a**, **11c** was found higher than that shown by the reference drug. The mean serum concentration time curves of compounds **11a**, **11c** and cefuroxime, after oral administration in rats, are reported in Fig. 1. In the same figure the values of area under the curves ($AUC_{0\sim\infty}$) and elimination half-life ($T_{1/2}$) of these three compounds are also described. Particularly, derivative **11c** produced higher levels in serum, higher $AUC_{0\sim\infty}$ and longer $T_{1/2}$ than those found in compound **11a** and cefuroxime.

Experimental

Melting points were determined on a Buchi SMP-20 and are uncorrected; boiling points are also uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-360L spectrometer using TMS as internal standard. Solutions were dried over sodium sulfate and solvents were removed by evaporation under reduced pressure using a rotatory evaporator.

Biological Methods

Minimun inhibitory concentrations (MICs) were determined by the broth dilution method in microtiter trays, as already described[8], with Mueller-Hinton broth as the test medium, after incubation for 20 hours at 37°C with an inoculum size of about 10^6 cfu/ml. The serum concentrations of compounds **11a**, **11c** and cefuroxime were measured after oral administration, in rats (n=3), of 100 mg/kg of drugs. The serum samples were assayed by a disc-plate diffusion method, using *Bacillus subtilis* NCIB 8993 as test organism. The test medium was Lab-Lemco (Oxoid) supplemented with NaCl 0.5% and sodium citrate 0.25%. Standards were prepared with pooled normal rat antibiotic-

free serum. The plates were incubated overnight at 37°C. Standards, controls and samples were run at least four times. The sensitivity of the assay was 0.25 μg/ml for compounds **11a** and **11c**, while in the case of cefuroxime the lowest assayable concentration was 0.1 μg/ml.

2-(3-Bromo-5-isoxazolyl)-2-hydroxyacetic Acid Ethyl Ester (**4a**)

A solution of bromine (33.6 g, 0.21 mol) in AcOH (70 ml) was added dropwise at 60°C to a stirred solution of 3-bromo-5-acetylisoxazole (**1a**, 19.0 g, 0.1 mol) in AcOH (190 ml). After 10 minutes at 60°C, the solvent was evaporated; the residue was taken up with water (100 ml) and treated at 0°C with a solution of NaOH (20.0 g, 0.5 mol) in water (100 ml). The solution was stirred at room temp for 2 hours, neutralized with AcOH and extracted with ethyl ether (3×100 ml). The aqueous layer was then acidified with concd HCl to pH 2 and extracted with ethyl ether (3×100 ml). The organic layer was dried and evaporated to give an oily residue (19.0 g) which was stirred at room temp for 20 hours in EtOH (200 ml) with a catalytic amount of concd H_2SO_4. The solvent was removed and the residue was poured into water (150 ml) and extracted with ethyl ether (300 ml). The organic layer was washed with 10% aq $NaHCO_3$, dried and evaporated. The solid residue was crystallized from isopropyl ether to give pure **4a** as a white solid (16.2 g, 65%, mp 50~51°C).

Compounds **4b**~**4d** were similarly prepared.

2-(3-Bromo-5-isoxazolyl)-2-oxoacetic Acid Ethyl Ester (**5a**)

To a stirred solution of **4a** (21.5 g, 0.086 mol) in AcOH (300 ml) was added portionwise CrO_3 (10.3 g, 0.103 mol) at room temp. The mixture was kept at 100°C for 2 hours; the solvent was removed and the residue was treated with water (300 ml) and neutralized with $NaHCO_3$. The aqueous mixture was extracted with ethyl ether (3×200 ml); the organic layer was washed with water (2×50 ml), dried and evaporated. The residue was purified by distillation to afford pure **5a** as a colorless oil (17.1 g, 80%, bp 115°C/0.3 Torr).

Compounds **5b**~**5d** were similarly prepared.

2-(3-Bromo-5-isoxazolyl)-(*Z*)-2-methoxyiminoacetic Acid Ethyl Ester (**6a**) and 2-(3-Bromo-5-isoxazolyl)-(*E*)-2-methoxyiminoacetic Acid Ethyl Ester (**7a**)

A mixture of **5a** (14.9 g, 0.06 mol) and methoxyamine hydrochloride (5.3 g, 0.063 mol) in EtOH (200 ml) and water (200 ml) was refluxed for 2 hours. After evaporation of the solvent, the residue was treated with water (200 ml) and extracted with ethyl ether (3×150 ml). The organic layer was washed twice with water, dried and evaporated. The two isomers were separated by column chromatography (silica gel 1,000 g, eluent hexane - isopropyl ether, 9 : 1) to give pure **6a** (5.8 g, 35%, bp 135°C/0.2 Torr) and pure **7a** (9.3 g, 56%, bp 140°C/0.15 Torr).

Compounds **6b**~**6d** and **7b**~**7d** were similarly prepared.

2-(3-Bromo-5-isoxazolyl)-(*Z*)-2-methoxyiminoacetic Acid (**8a**)

To a stirred solution of **6a** (5.54 g, 0.02 mol) in MeOH (30 ml) was added dropwise at room temp a solution of NaOH (0.88 g, 0.022 mol) in water (30 ml). The mixture was stirred at room temp for 2 hours; the solvent was removed and the residue was treated with water (80 ml) and extracted with ethyl ether (2×30 ml). The aqueous layer was acidified with concd HCl to pH 2 and extracted with ethyl ether (3×50 ml) which was dried and evaporated. The solid residue was crystallized from benzene (30 ml) to give pure **8a** as a white solid (4.68 g, 94%, mp 136~137°C).

Compounds **8b**~**8d** and **9a**~**9d** were similarly prepared.

3-Acetoxymethyl-7-[2-(3-bromo-5-isoxazolyl)-(*Z*)-2-(methoxyimino)acetamido]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic Acid (**11a**)

80% NaH dispersion in oil (0.15 g, 0.005 mol) was added portionwise at 5°C to a stirred solution of **8a** (1.24 g, 0.005 mol) in benzene (25 ml) and DMF (2 ml). The mixture was stirred at room temp for 1 hour and oxalyl chloride (1.5 ml, 0.010 mol) was then dropped at 5°C.

The mixture was stirred at room temp for 1 hour; the solvent was removed and the residue, dissolved in THF (20 ml), was dropped to a solution prepared refluxing for 1 hour a mixture of 7-aminocephalosporanic acid (1.63 g, 0.006 mol) and hexamethyldisilazane (1.25 ml, 0.006 mol) in THF (25 ml)

and adding after cooling, triethylamine (0.84 ml, 0.006 mol). The resulting mixture was stirred at room temp for 3 hours, evaporated and poured into a cold 5% aq $NaHCO_3$ (50 ml). The aqueous layer was washed with ethyl acetate (100 ml), acidified with 10% HCl to pH 3 and extracted with ethyl acetate (2×50 ml). The organic layer was washed with water, dried and evaporated. The residue was triturated with *n*-pentane (50 ml) to give a practically pure solid **11a** (1.71 g, 68%, dec point 70~72°C).

Compounds **10a**, **10b**, **11b**~**11d**, **12a** and **12c** were similarly prepared.

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