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New broad-spectrum parenteral cephalosporins exhibiting potent activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Part 2: Synthesis and structure–activity relationships in the S-3578 series

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Abstract—Among the prepared novel cephalosporin derivatives related to S-3578, a series of 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(*Z*)-ethoxyiminoacetamido]-3-[1-(aminoalkyl)-1*H*-pyrazolo[4,3-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate showed potent activity against both MRSA and *Pseudomonas aeruginosa*, and displayed good water solubility.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are nosocomial pathogens associated with serious infections and considerable mortality. From the need for antibacterial agents exhibiting highly potent activity against these pathogens, we began a search for novel parenteral C-3′ quaternary ammonium cephalosporins exhibiting potent activity against both MRSA and *P. aeruginosa*.

In our previous paper, we reported that 7β-[2-(5-amino-1,2,4-thiadiazol-3-yl)-2(Z)-ethoxyiminoacetamido]-3-[1-(3-methylaminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate sulfate (S-3578, Fig. 1) showed extremely potent activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *P. aeruginosa*, and displayed good water solubility.[1]

In the course of studies of S-3578, we found that introduction of an aminoalkyl group to the 1-position of imidazo[4,5-*b*]pyridinium enhanced anti-MRSA activity (**1** and **2** in Fig. 1, and their antibacterial activity in Table 1) and led to good water solubility.[1] As an aminoalkyl group, the 3-aminopropyl group was the best match for antibacterial activity against MRSA and *P. aeruginosa*.

These findings prompted us to extend our synthetic program to various other cephalosporins in which the 1-(3-aminopropyl)-1*H*-imidazo[4,5-*b*]pyridinium group of **2** was replaced by the 2-(3-aminopropyl)-1*H*-imidazo[4,5-*b*]pyiridinium group (**3a**–**b**, Table 1) or another

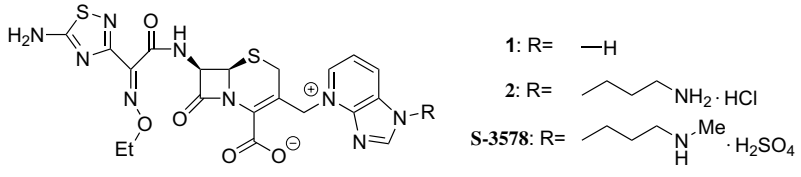

Figure 1. Structures of cephalosporin derivatives **1**, **2**, and **S-3578**.

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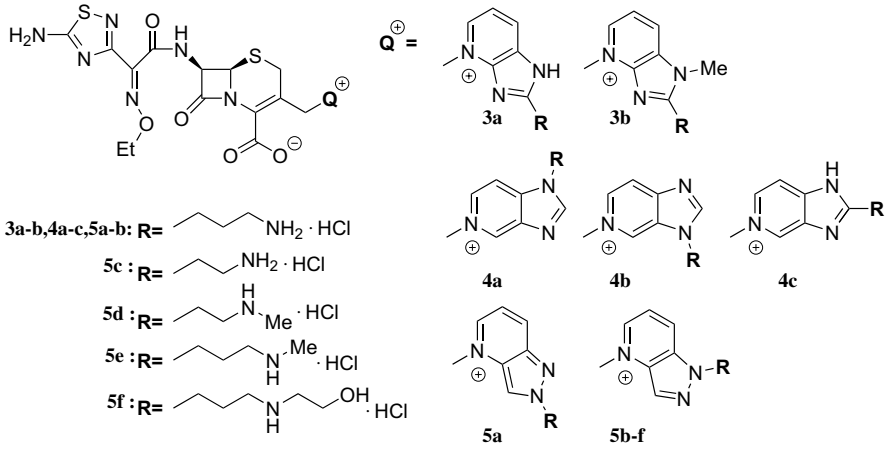
Table 1. Antibacterial activities (MIC, μg/mL) of **1-5f**, S-3578, CZOP, CFSL, and VCM



Compound	*S. a.*	MRSA 1	MRSA 2	*E. c.*	*P. a.* 1	*P. a.* 2
1	1.56	12.5	12.5	0.2	1.56	12.5
2	0.78	3.13	3.13	0.39	0.78	3.13
3a	1.56	6.25	6.25	0.2	0.78	3.13
3b	1.56	6.25	6.25	0.78	3.13	12.5
4a	3.13	25	25	0.2	0.78	3.13
4b	3.13	25	25	0.39	1.56	6.25
4c	1.56	6.25	6.25	0.05	0.78	1.56
5a	1.56	12.5	12.5	0.39	3.13	12.5
5b	0.78	6.25	6.25	0.2	0.78	3.13
5c	0.78	6.25	6.25	0.2	0.78	3.13
5d	0.78	12.5	12.5	0.39	1.56	6.25
5e	0.78	6.25	6.25	0.2	1.56	6.25
5f	0.78	6.25	6.25	0.2	1.56	6.25
S-3578	0.78	3.13	3.13	0.39	1.56	6.25
CZOP	0.78	50	50	0.05	0.39	1.56
CFSL	0.78	25	25	0.05	3.13	6.25
VCM	1.56	0.78	1.56	>100	>100	>100

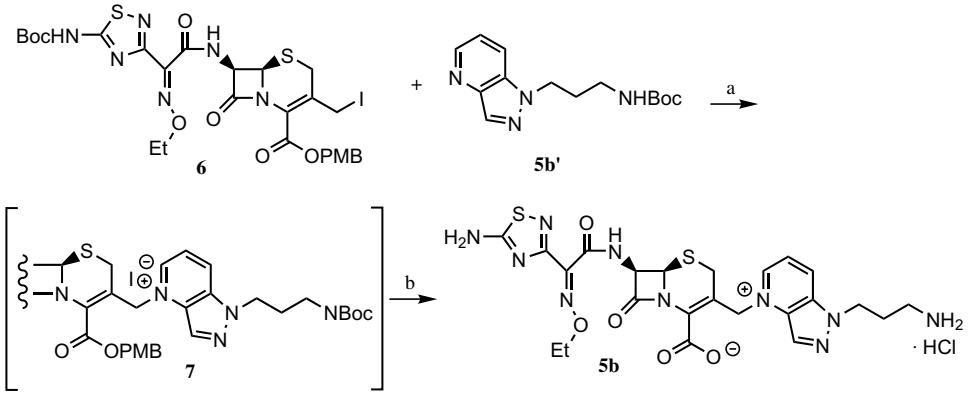
Abbreviations; *S. a.*, *Staphylococcus aureus* SMITH; MRSA 1, *S. aureus* SR3626; MRSA 2, *S. aureus* SR3637; *E. c.*, *Escherichia coli* NIHJ JC-2; *P. a.* 1, *Pseudomonas aeruginosa* SR24; *P. a. 2*, *P. aeruginosa* SR5393; CZOP, cefozopran; CFSL, cefoselis; VCM, vancomycin.

condensed-heterocyclic pyridinium derivative bearing the aminopropyl group such as the aminopropyl-imidazo[4,5-*c*]pyridinium (**4a**–**c**) or the aminopropyl-pyrazolo[4,3-*b*]pyridinium (**5a**–**b**) group. We also prepared a few other 1-(aminoalkyl)-pyrazolo[4,3-*b*]pyridinium cephalosporin derivatives (**5c**–**f**). Here we report on the synthesis and structure–activity relationships of novel cephalosporins related to S-3578.

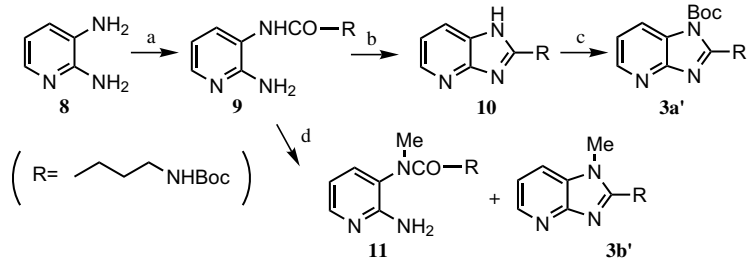
2. Chemistry

Novel C-3′ condensed-heterocyclic pyridinium cephems **3a**–**b**, **4a**–**c**, and **5a**–**f** were synthesized by a method similar to one reported.[1] Cephalosporin derivative **5b** was synthesized as shown in Scheme 1. The C-3 iodomethyl cephalosporin intermediate **6**[1] was displaced by pyrazolopyridine **5b′** to afford iodide salt **7**, which was treated with $AlCl_3$-anisole. Purification by reversed phase (HP-20) column chromatography yielded cephalosporin derivative **5b** as a hydrochloride salt. The other cephems **3a**–**b**, **4a**–**c**, **5a**, and **5c**–**f** were prepared by a method similar to that for preparing of **5b** using **6** and the corresponding condensed-heterocyclic pyridine derivatives **3a′**–**b′**, **4a′**–**c′**, **5a′**, and **5c′**–**f′**. (Structures of **3a′**–**b′**, **4a′**–**c′**, and **5a′** are shown in Schemes 2–6, and those of **5c′**–**f′** are shown in Fig. 2.)

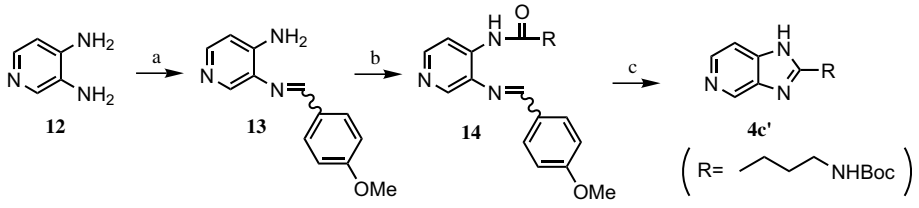
The methods of synthesizing condensed-heterocyclic pyridine derivatives are shown in Schemes 2–6. 2-(Aminoalkyl)-imidazo[4,5-*b*]pyridine derivatives **3a′** and **3b′** were synthesized as shown in Scheme 2. Compound **9** was prepared by regio-selective acylation of 2,3-diaminopyridine (**8**) with the corresponding acid using water-soluble carbodiimide hydrochloride (WSCD·HCl). The cyclization of compound **9** was performed by heating in nitrobenzene to afford compound **10** followed by treatment with di-*tert*-butyl dicarbonate (Boc_2O) in the presence of 4-(dimethylamino)pyridine (DMAP) to give compound **3a′**. On the other hand, **9** was treated with methyl iodide in the presence of sodium hydride to afford a mixture of **11** and **3b′**, which was purified by chromatography on silica gel.



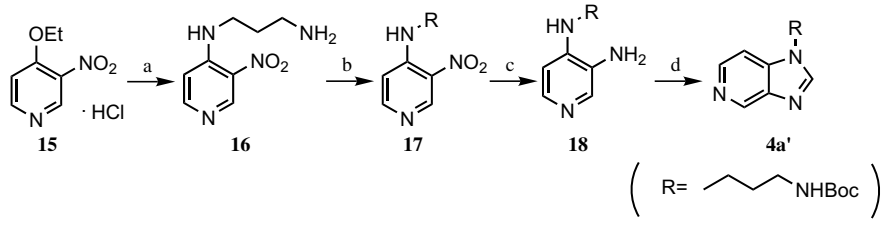
Scheme 1. Reagents and conditions: (a) DMF, RT, 3 h; (b) (i) $AlCl_3$-anisole, CH_2Cl_2, −30 to 0 °C, 1 h, (ii) purification by HP-20 chromatography.



Scheme 2. Reagents and conditions: (a) 4-*tert*-butoxycarbonylamino-butyric acid, WSCD·HCl, DMF; (b) nitrobenzene, mole sieve, 195 °C, 5 h; (c) $(Boc)_2O$, DMF, cat. DMAP; (d) NaH, MeI, DMF, 0 °C∼RT, 18 h.



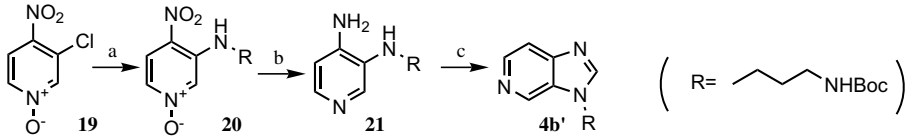
Scheme 3. Reagents and conditions: (a) *p*-anisaldehyde (1.1 equiv), 4 N NaOH (1.1 equiv), EtOH, 18 h; (b) 4-*tert*-butoxycarbonylamino-butyric acid, DMF, WSCD·HCl; (c) (i) 2 N HCl–MeOH (1:2), RT, 1 h, (ii) TFA, 100 °C, 30 min, (iii) $(Boc)_2O$, Et_3N.



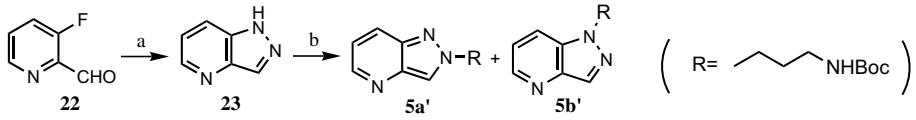
Scheme 4. Reagents and conditions: (a) 1,3-diaminopropane (1.5 equiv), EtOH, 130 °C in sealed tube, 2 h; (b) $(Boc)_2O$, THF; (c) H_2, Pd (C), MeOH; (d) $(EtO)_2CH(OAc)$, reflux, EtOH.

2-(Aminoalkyl)-imidazo[4,5-*c*]pyridine derivative **4c′** was prepared, as shown in Scheme 3. The amino group at the 3-position of diaminopyridine **12** was selectively protected as imine using *p*-anisaldehyde. The imine **13** was acylated with carboxylic acid using WSCD·HCl to give compound **14**. After hydrolysis of the imino group of **14**, the cyclization was achieved by heating in TFA and the resulting amine was protected using Boc_2O to give compound **4c′**.

As shown in Scheme 4, 1-(aminoalkyl)-imidazo[4,5-*c*]pyridine derivative **4a′** was prepared. The ethoxy group of nitropyridine **15** was displaced by 1,3-diaminopropane to give the amine **16**, which was then treated



Scheme 5. Reagents and conditions: (a) (i) 1,3-diaminopropane (4 equiv), MeOH, reflux, (ii) dioxane–H_2O, Et_3N, Boc_2O; (b) H_2 (4 kgf/cm^2), Raney-Ni, AcOH, MeOH; (c) $HC(OEt)_3$, Ac_2O, 90 °C.



Scheme 6. Reagents and conditions: (a) hydrazine hydrate, 0 °C ~ RT (16 h), 130 °C (8 h); (b) R-OSO_2Me, NaH, DMF.

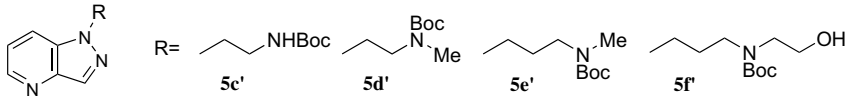


Figure 2. Structures of 1-(aminoalkyl)-pyrazolo[4,3-*b*]pyridine derivatives (**5c′**–**f′**).

with Boc_2O to give **17**. This nitro compound **17** was reduced using palladium-catalyzed hydrogenation in methanol to give compound **18**, which was treated with diethoxymethyl acetate in ethanol to give the imidazopyridine derivative **4a′**.

3-(Aminoalkyl)-imidazo[4,5-*c*]pyridine derivative **4b′** was prepared by the route shown in Scheme 5. The chloride **19**[2] was substituted by amine and the resulting amine was protected using Boc_2O to give **20**. This nitro compound **20** was reduced by Raney-Ni catalyzed hydrogenation to give compound **21**. Next, the amine **21** was treated with triethyl orthoformate in the presence of acetic anhydride to afford imidazopyridine **4b′**.

As shown in Scheme 6, 1- and 2-(aminoalkyl)-pyrazolo[4,3-*b*]pyridines, **5a′**, and **5b′**, were synthesized. Pyrazolo[4,3-*b*]pyridine **23** was prepared by treatment of 3-fluoro-pyridine-2-carbaldehyde **22**[3] with hydrazine hydrate. The alkylation of pyrazolopyridine **23** using alkyl methanesulfonate in the presence of sodium hydride gave a mixture of compound **5a′** and **5b′**, and they were purified by chromatography. Other 1-(aminoalkyl)-pyrazolopyridine derivatives **5c′**–**e′** were synthesized by a procedure similar to that described for the preparation of **5b′**. Compound **5f′** was derived by alkylation of **5b′** (see experimental section).

3. Antibacterial activities

The antibacterial activities (MICs) of **3a**–**b**, **4a**–**c**, **5a**–**f**, and reference compounds, **1**, **2**, S-3578, cefozopran (CZOP),[4] cefoselis (CFSL),[5] and vancomycin (VCM), against various selected Gram-positive and Gram-negative bacteria are shown in Table 1. MICs were determined by the standard serial two-fold agar dilution method using Mueller–Hinton agar.

Most of the cephalosporin derivatives prepared in this study showed good antibacterial activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *P. aeruginosa*, although their anti-MRSA activity was inferior to that of parent compound **2** and S-3578.

3.1. 2-(Aminopropyl)-imidazo[4,5-*b*]-pyridinium 3a–b

Comparison of **2** and **3a** indicates that the 1-substituted derivative **2** is preferred for activity against Gram-positive bacteria including MRSA over the 2-substituted derivative **3a**, and the substitution position of the aminopropyl group influences the antibacterial activity of cephalosporins. Introduction of a methyl group at the 1-position of the imidazopyridine moiety of **3a** was expected to improve antibacterial activity based on our earlier SAR studies for **S-3578** in which introduction of a methyl group at the 1-position of C-3′ imidazo[4,5-*b*]pyridine moiety enhanced antibacterial activity.[1] However, **3b** showed a decrease of activity against Gram-negative bacteria including *P. aeruginosa* without improvement of anti-MRSA activity.

3.2. Aminopropyl-imidazo[4,5-*c*]pyridinium 4a–c

Both 1-substituted derivative **4a** and 3-substituted derivative **4b** showed much less activity against MRSA than **2**, but good activity against Gram-negative bacteria. On the other hand, the 2-substituted derivative **4c** showed 4 times more potent activity against MRSA than **4a** and **4b**, and potent activity against Gram-negative bacteria including *P. aeruginosa*.

The great differences of anti-MRSA activity among **2**, **4a**, **4b**, and **4c** suggest that the manner in which the

substituted-imidazole ring fuses to the C-3′ pyridinium ring and the substitution position of the aminopropyl group are important for anti-MRSA.

3.3. Aminopropyl-pyrazolo[4,3-*b*]pyridinium 5a–b

The 2-substituted derivative **5a** showed relatively weak activity against all bacteria, especially against *P. aeruginosa*. In contrast, the 1-substituted derivative **5b** showed potent activity equal to **2** against *Staphylococcus aureus* SMITH and Gram-negative bacteria including *P. aeruginosa*, although anti-MRSA activity of **5b** was slightly inferior to **2**. The potent antibacterial activity of **5b** might be due to the structural resemblance to that of **2**.

Among of **3a**–**b**, **4a**–**c**, and **5a**–**b**, 1-(aminopropyl)-pyrazolopyridinium **5b** showed the best balanced activity against Gram-positive bacteria including MRSA and Gram-negative bacteria including *P. aeruginosa*, and displayed also good water-solubility (>50 mg/mL at neutral pH). Therefore, we prepared a few analogs of compound **5b**. We modified the length of the carbon chain of the aminopropyl group (**5c**), and introduced a substituent, such as a methyl group (**5d**, **5e**) or a hydroxyethyl group (**5f**), on the primary amine in a manner similar to our earlier SAR studies for **S-3578**.

Antibacterial activity did not improve, but **5c**, **5e**, and **5f** displayed the same activity against Gram-positive bacteria including MRSA as compound **5b** while retaining potent activity against Gram-negative bacteria including *P. aeruginosa* and acceptable water solubility (>50 mg/mL at neutral pH) for intravenous administration.

In conclusion, the described chemical modification made by changing the substitution position of the aminoalkyl function and a change of the C-3′ condensed-heterocyclic pyridinium moiety of compound **2** demonstrated that **5b**, **5c**, **5e**, and **5f** displayed potent antibacterial activity and good water solubility.

4. Experimental

MPs were determined on a Yanagimoto micro melting point apparatus and uncorrected. IR spectra were taken on JASCO IR-700. ^{1}H NMR spectra were recorded with a Varian Gemini-300 (300 MHz) or Varian Gemini-200 (200 MHz) spectrometer. Chemical shifts are reported in ppm from 2,2-dimethyl-2-silapentane-5-sulfonate (DSS in D_2O) or TMS (in $CDCl_3$ and DMSO-d_6) as internal standard. The following abbreviations are used: s singlet, d doublet, dd double doublet, t triplet, q quartet, m multiplet, ABq AB quartet, brs broad singlet. Column chromatography was carried out on Merck Kieselgel and Mitsubushi Chemical HP-20.

4.1. Measurement of in vitro antibacterial activity

MIC was determined by a serial twofold dilution method with Sensitivity Disk Agar-N (Nissui Pharmaceutical, Tokyo, Japan). The overnight cultures of bacterial strains in Mueller Hinton broth (Becton Dickinson) were diluted to about 10^6 CFU/mL. Bacterial suspensions of 1 μL were spotted onto agar plates containing various concentrations of an antibiotic and incubated for 20 h at 37 °C before the MICs were scored.

4.2. [3-(2-Amino-pyridin-3-ylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester (9)

To a solution of 2,3-diaminopyridine (3.6 g, 33 mmol) in DMF (45 mL) was added 4-*tert*-butoxycarbonylaminobutyric acid (8.0 g, 40 mmol) and water-soluble carbodiimide hydrochloride (WSCD·HCl) (7.64 g, 40 mmol) under cooling in an ice-water bath. The reaction mixture was stirred at room temperature for 3 days. After evaporation of the solvent, the residue was extracted with methyl ethyl ketone and washed with brine. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and crystallization was performed from the residue with MeCN and Et_2O to obtain compound **9** (6.0 g, 65% yield); MP 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.87 (2H, m), 2.40 (2H, m), 3.28 (2H, m), 4.92 (1H, m), 5.06 (2H, brs), 6.70 (1H, dd, $J = 4.8$ Hz, 7.8 Hz), 7.84 (1H, d, $J = 7.8$ Hz), 7.91 (1H, d, $J = 4.8$ Hz), 8.54 (1H, s).

4.3. [3-(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)-propyl]-carbamic acid *tert*-butyl ester (10)

A solution of **9** (3.0 g, 10 mmol) in nitrobenzene (15 mL) was heated at 195–200 °C in the presence of molecular sieves for 5 h. After evaporation of the solvent, the residue was purified by silica-gel column chromatography to give **10** as a colorless oil (290 mg); ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 2.05 (2H, m), 3.06 (2H, m), 3.26 (2H, m), 5.08 (1H, brs), 7.20 (1H, m), 7.95 (1H, d, $J = 7.8$ Hz), 8.36 (1H, d, $J = 4.8$ Hz).

4.4. 2-(3-*tert*-Butoxycarbonylamino-propyl)-imidazo[4,5-*b*]pyridine-1-carboxylic acid *tert*-butyl ester (3a′)

To a solution of **10** (290 mg, 1.0 mmol) in DMF (3 mL) was added DMAP (10 mg) and Boc_2O (0.54 mL, 2.3 mmol), and the reaction mixture was stirred at room temperature for 3 days. After evaporation of the solvent, the residue was purified by silica-gel column chromatography to give **3a′** as a pale yellow oil (320 mg, 8.8% from **9**); ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 1.72 (9H, s), 2.14 (2H, m), 3.30 (4H, m), 4.87 (1H, brs), 7.25 (1H, m), 8.16 (1H, d, $J = 8.4$ Hz), 8.82 (1H, m).

4.5. [3-(1-Methyl-1*H*-imidazo[4,5-*b*]pyridin-2-yl)-propyl]-carbamic acid *tert*-butyl ester (3b′)

To a solution of **9** (5.88 g, 20 mmol) in DMF (59 mL) was added 60% NaH (880 mg, 22 mmol) under cooling

in an ice-water bath, and the mixture was stirred at room temperature for 1 h. To the mixture was added dropwise MeI (3.1 g, 21 mmol) under cooling in an ice-water bath. The reaction mixture was stirred at the same temperature for 2 h and then stirred at room temperature for 16 h. After addition of a mixture of brine and EtOAc, the organic layer was washed with brine, and dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica-gel column chromatography. The fraction that was eluted first with $CHCl_3$/MeOH = 9/1 was concentrated to give **11** (1.3 g, 21% yield) as a crystalline solid; MP 148–149 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s), 1.76 (2H, m), 2.12 (2H, m), 3.09 (2H, m), 3.17 (3H, m), 4.72 (1H, m), 4.74 (2H, brs), 6.71 (1H, dd, J = 4.8 Hz, 7.8 Hz), 7.28 (1H, d, J = 7.8 Hz), 8.09 (1H, d, J = 4.8 Hz).

The fraction that was eluted second with $CHCl_3$:MeOH = 9:1 was concentrated to give **3b′** (1.1 g, 18%) as a crystalline solid; MP 99–100 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.24 (2H, m), 2.12 (2H, m), 3.27 (2H, m), 3.74 (3H, s), 5.05 (1H, m), 7.15 (1H, dd, J = 4.8 Hz, 7.8 Hz), 7.58 (1H, d, J = 7.8 Hz), 8.48 (1H, d, J = 4.8 Hz).

4.6. 3-(4-Methoxy-benzylidene)-pyridine-3,4-diamine (13)

To a suspension of 2,3-diaminopyridine (21.8 g, 200 mmol) in EtOH (200 mL) was added *p*-anisaldehyde (29.9 g, 220 mmol) and 4 N NaOH (55 mL), and the mixture was stirred at room temperature for 16 h. The mixture was neutralized to pH 7 using 5 N HCl (44 mL). After addition of EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Crystallization was performed from the residue with Et_2O and hexane to give compound **13** (23.6 g, 52% yield); MP 124–125 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s), 5.91 (2H, s), 6.60 (1H, d, J = 5.7 Hz), 7.06 (2H, d, J = 8.7 Hz), 7.89 (1H, d, J = 5.4 Hz), 7.94 (2H, d, J = 8.7 Hz), 7.98 (1H, s), 8.59 (1H, s).

4.7. [3-(3-{[1-(4-Methoxy-phenyl)-methylidene]-amino}-pyridin-4-ylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester (14)

To a solution of **13** (3.0 g, 13.2 mmol) in DMF (35 mL) was added 4-*tert*-butoxycarbonylamino-butyric acid (3.68 g, 18 mmol) and WSCD·HCl (3.47 g, 18 mmol) under cooling in an ice-water bath. The reaction mixture was stirred at room temperature for 2 days. The reaction mixture was poured into H_2O. After addition of a mixture of EtOAc and $CHCl_3$, the organic layer was washed with H_2O, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo and crystallization was performed from the residue with EtOH, Et_2O and hexane to give compound **14** (5.2 g, 95% yield); MP 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 1.93 (2H, m), 2.50 (2H, m), 3.24 (2H, m), 3.90 (3H, s), 4.80 (1H, m), 7.03 (2H, d, J = 8.7 Hz), 7.89 (2H, d, J = 8.7 Hz), 8.32 (1H, 2), 8.38 (1H, s), 8.52 (1H, s), 8.62 (1H, s).

4.8. [3-(1*H*-Imidazo[4,5-*b*]pyridin-2-yl)-propyl]-carbamic acid *tert*-butyl ester (4c′)

To a mixture of **14** (6.1 g, 14.7 mmol) and MeOH (30 mL) was added 2 N HCl (15 mL), and the mixture was stirred at room temperature for 1 h. After addition of 2 N NaOH (15 mL) and $CHCl_3$, the organic layer was washed with H_2O, dried over $MgSO_4$, and filtered. The filtrate was concentrated and the residue was chromatographed on silica gel column to give [3-(3-amino-pyridin-4-ylcarbamoyl)-propyl]-carbamic acid *tert*-butyl ester (2.94 g) as a crystalline solid; MP 149–151 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.87 (2H, m), 2.43 (2H, m), 3.27 (2H, m), 4.18 (2H, brs), 4.93 (1H, m), 7.72 (1H, d, J = 4.8 Hz), 8.00 (1H, d, J = 4.8 Hz), 8.11 (1H, s), 9.01 (1H, brs). This compound (2.0 g, 6.79 mmol) was dissolved in TFA (2.6 mL) and the solution was stirred at 100 °C for 30 min. After concentration in vacuo, the residue was chromatographed on HP-20 resin. The solution containing the target material was concentrated and lyophilized to give 3-(1*H*-imidazo[4,5-*c*]pyridin-2-yl)-propylamine TFA salt (2.67 g) as an amorphous powder. To a suspension of 3-(1*H*-imidazo[4,5-*c*]pyridin-2-yl)-propylamine TFA salt (1.7 g, 4.1 mmol) in DMF (15 mL) and $CHCl_3$ (15 mL) was added Et_3N (1.16 mL, 8.2 mmol) under cooling in an ice-water bath, followed by dropwise addition of Boc_2O (0.96 mL, 4.1 mmol). The reaction mixture was stirred at room temperature for 16 h. After addition of ice-cold water, sat. $NaHCO_3$ and EtOAc, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Crystallization was done using MeCN and Et_2O to give compound **4c′** (0.89 g, 50% yield from **14**); MP 114–115 °C; ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 1.94 (2H, m), 3.01 (2H, m), 3.25 (2H, m), 5.01 (1H, m), 7.51 (1H, d, J = 5.4 Hz), 8.38 (1H, d, J = 5.4 Hz), 8.96 (1H, s).

4.9. 1-(3-Nitro-pyridin-4-yl)-propane-1,3-diamine (16)

To a solution of **15** (4.2 g, 22 mmol) in EtOH (15 mL) was added 1,3-diaminopropane (2.45 g, 33 mmol) and the mixture was stirred at 130 °C in a sealed tube for 2 h. After evaporation of the solvent, the residue was diluted with THF (40 mL). The precipitate was filtered off, the filtrate was concentrated, and the residue was chromatographed on silica gel column to give **16** (4.14 g, 94% yield) as a reddish oil; ^{1}H NMR ($CDCl_3$) δ 1.87 (2H, m), 2.91 (2H, m), 3.44 (2H, m), 6.75 (1H, d, J = 6.2 Hz), 8.28 (1H, d, J = 6.2 Hz), 8.65 (1H, brs), 9.20 (1H, s).

4.10. [3-(3-Nitro-pyridin-4-ylamino)-propyl]-carbamic acid *tert*-butyl ester (17)

To a solution of **16** (4.1 g, 21 mmol) in THF (50 mL) was added dropwise Boc_2O (5.3 mL, 23 mmol) and the reaction mixture was stirred at room temperature for 16 h. After evaporation, the residue was chromatographed on silica gel column to give **17** (4.68 g, 72% yield) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.90 (2H, m), 3.28 (2H, m), 3.41 (2H, m), 4.72 (1H,

m), 6.70 (1H, d, $J = 6.3$ Hz), 8.29 (1H, d, $J = 6.3$ Hz), 9.21 (1H, s).

4.11. [3-(3-Amino-pyridin-4-ylamino)-propyl]-carbamic acid *tert*-butyl ester (18)

To a solution of **17** (4.67 g, 15.8 mmol) in MeOH (150 mL) was added 10% Pd–C (1.0 g). The mixture was stirred under hydrogen atmosphere for 90 min under the ambient pressure at room temperature. After filtration of the catalyst, the filtrate was concentrated in vacuo to give **18** (4.67 g) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ (9H, s), 1.78 (2H, m), 3.22 (2H, m), 3.27 (2H, m), 3.38 (2H, brs), 4.59 (1H, m), 4.72 (1H, m), 6.44 (1H, d, $J = 5.7$ Hz), 7.88 (1H, s), 7.94 (1H, d, 5.7 Hz).

4.12. (3-Imidazo[4,5-*c*]pyridin-1-yl-propyl)-carbamic acid *tert*-butyl ester (4a′)

A mixture of **18** (4.67 g) and acetic acid diethoxymethyl ester (13 mL) was stirred at room temperature for 16 h. After concentration of the mixture, the residue was dissolved in EtOH (15 mL) and the mixture was refluxed for 90 min, and concentrated in vacuo. The residue was chromatographed on silica gel column to give **4a′** (3.5 g, 62% yield from **17**) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 2.11 (2H, m), 3.20 (2H, m), 4.28 (2H, m), 4.82 (1H, m), 7.38 (1H, d, $J = 6.6$ Hz), 8.09 (1H, s), 8.47 (1H, d, $J = 6.6$ Hz), 9.13 (1H, s).

4.13. [3-(4-Nitro-1-oxy-pyridin-3-ylamino)-propyl]-carbamic acid *tert*-butyl ester (20)

To a solution of 3-chloro-4-nitro-pyridine 1-oxide (**19**) (3.0 g, 17.2 mmol) in MeOH (30 mL) was added a mixture of 1,3-diaminopropane (5.7 mL, 68.7 mmol) and MeOH (110 mL) and the reaction mixture was stirred at room temperature for 30 min and refluxed for 1 h. After evaporation of the solvents, the residue was dissolved in a mixture of dioxane (5 mL) and H_2O (5 mL), followed by addition of Et_3N (2.6 mL) and Boc_2O (7.4 mL, 34 mmol). The reaction was stirred for 16 h and concentrated in vacuo. The residue was chromatographed on silica gel column to give **20** (1.0 g, 18% yield) as a pale yellow oil; ^{1}H NMR (DMSO-d_6) δ 1.37 (9H, s), 1.67 (2H, m), 3.03 (2H, m), 3.37 (2H, m), 6.92 (1H, m), 7.48 (1H, d, $J = 7.2$ Hz), 8.02 (1H, d, $J = 7.2$ Hz), 8.17 (1H, s), 8.24 (1H, m).

4.14. [3-(4-Amino-pyridin-3-ylamino)-propyl]-carbamic acid *tert*-butyl ester (21)

To a solution of **20** (1.0 g, 3.2 mmol) in MeOH (60 mL) and AcOH (1.5 mL) was added Raney-Ni (1 mL, slurry in water). The mixture was stirred under hydrogen atmosphere at the pressure of 4 kgf/cm^2. After filtration of the catalyst, the filtrate was concentrated in vacuo. The residue was dissolved in H_2O, alkalized by addition of saturated NaOH and extracted with Et_2O. The organic layer was concentrated and the residue was chromatographed on silica gel column to give **21** (750 mg, 87% yield) as a pale yellow oil; ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 1.70 (2H, m), 3.02 (2H, m), 3.35 (2H, m), 4.39 (1H, m), 5.39 (2H, s), 6.41 (1H, d, $J = 5.1$ Hz), 6.87 (1H, m), 7.52 (1H, d, $J = 5.1$ Hz), 7.53 (1H, s).

4.15. (3-Imidazo[4,5-*c*]pyridin-3-yl-propyl)-carbamic acid *tert*-butyl ester (4b′)

To a mixture of **21** (714 mg, 2.68 mmol) and triethyl orthoformate (5 mL) was added acetic anhydride (1 mL), and the mixture was stirred at 90 °C for 15 min and concentrated in vacuo. The residue was chromatographed on silica gel column to give **4b′** (580 mg, 88% yield) as a pale yellow oil; ^{1}H NMR (DMSO-d_6) δ 1.38 (9H, s), 1.95 (2H, m), 2.94 (2H, m), 4.35 (2H, m), 6.98 (1H, m), 7.66 (1H, d, $J = 5.6$ Hz), 8.34 (1H, d, $J = 5.6$ Hz), 8.44 (1H, s), 9.01 (1H, s).

4.16. 1*H*-Pyrazolo[4,3-*b*]pyridine (23)

3-Fluoro-pyridine-2-carbaldehyde (**22**) (6.67 g, 53 mmol) was dissolved in hydrazine monohydrate (40 mL) under cooling in an ice-water bath, and the mixture was stirred at the same temperature for 5 min. After removal of the ice-water bath, the mixture was stirred at room temperature overnight and then was heated at 130 °C for 5 h. The reaction mixture was concentrated in vacuo and to the residue was added H_2O and $NaHCO_3$ (4.45 g, 52.9 mmol). The aqueous layer was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica-gel column chromatography to give compound **23** (3.6 g, 57% yield) as a crystalline solid; MP 95–96 °C (lit.[6] MP 105–106 °C); ^{1}H NMR (DMSO-d_6) 7.36 (1H, dd, $J = 4.2$ Hz, 8.7 Hz), 8.01 (1H, d, $J = 8.7$ Hz), 8.29 (1H, s), 8.51 (1H, d, $J = 4.2$ Hz); Anal. Calcd for $C_6H_5N_3$: C, 60.5; H, 4.23; N, 35.27. Found: C, 60.75; H, 4.19; N, 35.25.

4.17. (3-Pyrazolo[4,3-*b*]pyridin-1-yl-propyl)-carbamic acid *tert*-butyl ester (5b′) and (3-pyrazolo[4,3-*b*]pyridin-2-yl-propyl)-carbamic acid *tert*-butyl ester (5a′)

To a solution of 1*H*-pyrazolo[4,3-*b*]pyridine (**23**) (357 mg, 3 mmol) in DMF (4 mL) was added 60% NaH (144 mg, 3.6 mmol) and the mixture was stirred at room temperature for 20 min. To the mixture was added a solution of 3-*tert*-butoxycarbonylaminopropyl methanesulfonate (911 mg, 3.6 mmol) in DMF (2 mL). The reaction mixture was stirred at the same temperature for 1 h. After addition of EtOAc and ice water, the organic phase was washed with water and brine, dried over Na_2SO_4, and filtered. After concentration of the filtrate, the residue was chromatographed on silica gel column. The fraction eluted with EtOAc/toluene (2/1) was concentrated to give **5b′** (330 mg, 39% yield) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 2.12 (2H, m), 3.11 (2H, m), 4.46 (2H, t, $J = 9.9$ Hz), 4.8 (1H,

brs), 7.29 (1H, m), 7.77 (1H, d, $J = 12.9$ Hz), 8.24 (1H, s), 8.58 (1H, d, $J = 6.6$ Hz).

Concentration of the fraction eluted with EtOAc/toluene (4/1) gave a regioisomer **5a′** (260 mg, 31% yield) as a pale yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 2.21 (2H, m), 3.16 (2H, m), 4.53 (2H, t, $J = 9.9$ Hz), 4.8 (1H, brs), 7.22 (1H, m), 8.03 (1H, d, $J = 14.2$ Hz), 8.24 (1H, s), 8.57 (1H, d, $J = 7.8$ Hz).

The pyrazolo[4,3-*b*]pyridine derivatives, **5c′**, **5d′** and **5e′** were prepared by the procedure similar to that described for the preparation of **5b′**. In the preparation of **5c′**, **5d′**, and **5e′**, an undesired regioisomer, 2-(substituted)-pyrazolo[4,3-*b*]pyridine was also produced.

4.18. (2-Pyrazolo[4,3-*b*]pyridin-1-yl-ethyl)-carbamic acid *tert*-butyl ester (5c′)

Compound **5c′** was obtained as a pale yellow oil in 48% yield from **23** using methanesulfonic acid 2-*tert*-butoxycarbonylamino-ethyl ester; ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 3.64 (2H, q, $J = 5.8$ Hz), 4.52 (2H, t, $J = 5.2$ Hz), 4.80 (1H, bs), 7.29 (1H, m), 7.80 (1H, d, $J = 8.8$ Hz), 8.24 (1H, s), 8.58 (1H, m).

4.19. Methyl-(2-pyrazolo[4,3-*b*]pyridin-1-yl-ethyl)-carbamic acid *tert*-butyl ester (5d′)

Compound **5d′** was obtained as a pale yellow oil in 26% yield from **23** using methanesulfonic acid 2-(*tert*-butoxycarbonyl-methyl-amino)-ethyl ester and THF (as the solvent); ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 2.38 (3H, s), 3.66 (2H, t, $J = 6.0$ Hz), 4.56 (2H, m), 7.35 (1H, m), 7.70 (1H, m), 8.26 (1H, s), 8.58 (1H, m).

4.20. Methyl-(3-pyrazolo[4,3-*b*]pyridin-1-yl-propyl)-carbamic acid *tert*-butyl ester (5e′)

Compound **5e′** was obtained as a pale yellow oil in 53% yield from **23** using methanesulfonic acid 3-(*tert*-butoxycarbonyl-methyl-amino)-propyl ester; ^{1}H NMR ($CDCl_3$) δ 1.40 (9H, s), 2.18 (2H, m), 2.88 (3H, s), 3.28 (2H, t, $J = 6.0$ Hz), 4.39 (2H, t, $J = 6.8$ Hz), 7.32 (1H, m), 7.76 (1H, d, $J = 8.8$ Hz), 8.24 (1H, s), 8.57 (1H, d, $J = 4.4$ Hz).

4.21. (2-Hydroxy-ethyl)-(3-pyrazolo[4,3-*b*]pyridin-1-yl-propyl)-carbamic acid *tert*-butyl ester (5f′)

To a solution of compound **5b′** (460 mg, 1.81 mmol) and (2-bromo-ethoxy)-triethylsilane (866 mg, 3.62 mmol) in DMF (5 mL) was added 60% NaH (145 mg, 3.62 mmol) and the mixture was stirred at room temperature for 1 h and then stirred at 40 °C for 30 min. Next, it was poured into a mixture of cold water and EtOAc. The organic layer was washed successively with water and brine, dried over Na_2SO_4, and filtered off. After evaporation, the residue was dissolved in a mixture of THF (8 mL), water (2 mL), and AcOH (1.7 mL), then this was stirred at room temperature for 18 h. The reaction mixture was poured into a mixture of ice water and EtOAc. The aqueous layer was adjusted to pH 8 with Na_2CO_3 and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel column to obtain **5f′** as a pale yellow oil in 82% yield from **5b′**; ^{1}H NMR ($CDCl_3$) δ 1.38 (9H, s), 2.22 (2H, m), 3.32 (2H, m), 3.37 (2H, m), 3.70 (2H, m), 4.41 (2H, t, $J = 6.8$ Hz), 7.30 (1H, m), 7.77 (1H, d, $J = 8.6$ Hz), 8.24 (1H, s), 8.58 (1H, m).

4.22. 7β-[2-(5-Amino-1,2,4-thiadiazol-3-yl)-2(*Z*)-ethoxyiminoacetamido]-3-[1-(3-aminopropyl)-1*H*-pyrazolo[4,3-*b*]pyridinium-4-yl]methyl-3-cephem-4-carboxylate hydrochloride (5b)

To a solution of pyrazolopyridine (**5b′**) (330 mg, 1.19 mmol) in DMF (2 mL) was added a solution of the iodomethyl derivative (**6**) (1.18 g, 1.55 mmol) in a mixture of DMF (2 mL) and DMSO (1 mL). The reaction mixture was stirred at room temperature for 3 h, and the mixture was poured into iPr_2O. The precipitate was filtered and dried in vacuo to give pyrazolopyridinium **7** (1.3 g). To a solution of **7** (1.3 g) in CH_2Cl_2 (20 mL) was added a solution of $AlCl_3$ (1.15 g, 8.33 mmol) in anisole (5 mL) at −30 °C and the reaction mixture was stirred under cooling in an ice-water bath for 1 h. After addition of a mixture of 1 N HCl (40 mL) and H_2O (40 mL), the aqueous layer was washed with Et_2O and concentration in vacuo. The aqueous solution was chromatographed on HP-20 resin. The target product was eluted with 0.001 N HCl and the solution was concentrated in vacuo and lyophilized to give **5b** hydrochloride (120 mg, 18% yield from **5b′**) as an amorphous powder; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.19 (3H, t, $J = 7.2$ Hz), 2.22 (2H, m), 2.88 (2H, m), 3.2 and 3.6 (2H, ABq, $J = 19$ Hz), 4.13 (2H, q, $J = 7.2$ Hz), 4.78 (2H, m), 5.18 (1H, d, $J = 7.8$ Hz), 5.84 (2H, brs), 5.88 (1H, d, $J = 7.8$ Hz), 8.15 (1H, m), 8.83 (1H, s), 9.03 (1H, d, $J = 9.3$ Hz), 9.22 (1H, d, $J = 12.6$ Hz); IR (KBr) cm^{-1} 1781, 1673, 1631, 1525, 1475, 1448, 1387; Anal. Calcd for $C_{23}H_{26}N_{10}O_5S_2 \cdot 2.9HCl \cdot 5H_2O$: C, 35.3; H, 5.01; Cl, 13.14; N, 17.9; S, 8.20. Found: C, 35.2; H, 4.66; Cl, 13.05; N, 17.61; S, 7.92.

Compound **3a**: **3a** was obtained as an amorphous powder in 33% yield from **3a′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.18 (3H, t, $J = 7.2$ Hz), 2.13 (2H, m), 2.96 (2H, m), 3.11 (2H, m), 3.2–3.6 (2H, m), 4.11 (2H, 2H, q, $J = 7.2$ Hz), 5.10 (1H, d, $J = 5.1$ Hz), 5.19 and 5.66 (2H, ABq, $J = 13.8$ Hz), 5.75 (1H, d, $J = 5.1$ Hz), 8.09 1H, d, $J = 6.6$ Hz), 8.86 (1H, d, $J = 6.6$ Hz), 9.76 (1H, s); IR (KBr) cm^{-1} 1774, 1614, 1527, 1482, 1461, 1402; Anal. Calcd for $C_{23}H_{26}N_{10}O_5S_2 \cdot 1.3HCl \cdot 4.4H_2O$: C, 38.73; H, 5.10; Cl, 6.46; N, 19.64; S, 8.99. Found: C, 38.96; H, 5.05; Cl, 6.40; N, 19.65; S, 8.69.

Compound **3b**: **3b** was obtained as an amorphous powder in 26% yield from **3b′**; ^{1}H NMR (DMSO-d_6+D_2O) δ 1.20 (3H, t, $J = 7.2$ Hz), 2.21 (2H, m), 2.99 (2H, m), 3.13 (2H, m), 3.18 and 3.37 (2H, ABq,

$J = 16.8$ Hz), 3.87 (3H, s), 4.13 (2H, q, $J = 7.2$ Hz), 4.84 (1H, d, $J = 5.1$ Hz), 5.21 and 6.55 (2H, ABq, $J = 13.8$ Hz), 5.79 (1H, d, $J = 5.1$ Hz), 7.78 (1H, m), 8.73 (1H, d, $J = 8.1$ Hz), 8.90 (1H, d, $J = 6.3$ Hz); IR (KBr) cm^{-1} 1769, 1671, 1617, 1524, 1475, 1456; Anal. Calcd or $C_{24}H_{28}N_{10}O_5S_2 \cdot 1.3HCl \cdot 5.5H_2O$: C, 38.58; H, 5.44; Cl, 6.17; N, 18.75; S, 8.58. Found: C, 38.84; H, 5.51; Cl, 6.28; N, 18.97; S, 8.35.

Compound **4a**: **4a** was obtained as an amorphous powder in 36% yield from **4a′**; ^1H NMR (DMSO-d_6) δ 1.20 (3H, t, $J = 7.2$ Hz), 2.18 (2H, m), 2.85 (2H, m), 3.08 and 3.60 (2H, ABq, $J = 18.3$ Hz), 4.11 (2H, q, $J = 7.2$ Hz), 4.56 (2H, t, $J = 6.6$ Hz), 5.10 (1H, d, $J = 4.8$ Hz), 5.20 and 5.69 (2H, ABq, $J = 14.1$ Hz), 5.75 (1H, d, $J = 4.8$ Hz), 8.50 (1H, d, $J = 6.6$ Hz), 8.97 (1H, s), 9.17 (1H, d, $J = 6.6$ Hz), 9.94 (1H, s); IR (KBr) cm^{-1} 1770, 1641, 1517, 1457; HR-FABMS calcd for $C_{23}H_{27}N_{10}O_5S_2$ $[(M+H)^+]$: 587.1607, found: 587.1606.

Compound **4b**: **4b** was obtained as an amorphous powder in 33% yield from **4b′**; ^1H NMR (DMSO-d_6) δ 1.19 3H, t, $J = 7.2$ Hz), 2.29 (2H, m), 2.86 (2H, m), 3.43 and 3.55 (2H, ABq, $J = 18.9$ Hz), 4.12 (2H, q, $J = 7.2$ Hz), 4.66 (2H, m), 5.21 (1H, d, $J = 5.1$ Hz), 5.55 and 5.7 (2H, ABq, $J = 14.4$ Hz), 5.9 (1H, m), 8.16 (1H, s), 3.31 (2H, brs), 8.4 (1H, d, $J = 6.9$ Hz), 9.25 (1H, s), 9.56 (1H, d, $J = 8.4$ Hz); IR (KBr) cm^{-1} 1780, 1631, 1519, 1479, 1454, 1381; HR-FABMS calcd for $C_{23}H_{27}N_{10}O_5S_2$ $[(M+H)^+]$: 587.1607, found: 587.1609.

Compound **4c**: **4c** was obtained as an amorphous powder in 29% yield from **4c′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.18 (3H, t, $J = 7.2$ Hz), 2.15 (2H, m), 3.0 (2H, m), 3.09 (2H, m), 3.16 and 3.30 (2H, ABq, $J = 17.1$ Hz), 4.13 (2H, q, $J = 7.2$ Hz), 4.93 (1H, s, $J = 4.8$ Hz), 5.30 and 6.22 (2H, ABq, $J = 13.2$ Hz), 5.78 (1H, d, $J = 4.8$ Hz), 7.65 (1H, m), 8.52 1H, d, $J = 7.5$ Hz), 8.77 (1H, d, $J = 6.0$ Hz); IR (KBr) cm^{-1} 1773, 1614, 1526, 1474; HR-FABMS calcd for $C_{23}H_{27}N_{10}O_5S_2$ $[(M+H)^+]$: 587.1607, found: 587.1602.

Compound **5a**: **5a** was obtained as an amorphous powder in 39% yield from **5a′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.21 (3H, t, $J = 7.0$ Hz), 2.28 (2H, m), 2.95 (2H, m), 3.3 and 3.6 (2H, ABq, $J = 19$ Hz), 4.12 (2H, q, $J = 7$ Hz), 4.78 (2H, m), 5.03 (1H, d, $J = 5.2$ Hz), 5.65 and 5.82 (2H, ABq, $J = 18$ Hz), 5.76 (1H, d, $J = 5.2$ Hz), 7.98 (1H, m), 9.07 (1H, d, $J = 8.8$ Hz), 9.26 (1H, d, $J = 6$ Hz), 9.43 (1H, s); IR (KBr) cm^{-1} 1774, 1670, 1616, 1525, 1457, 1400; Anal. Calcd for $C_{23}H_{26}N_{10}O_5S_2 \cdot 1.2HCl \cdot 4H_2O$: C, 39.33; H, 5.05; Cl, 6.06; N, 19.94; S, 9.13. Found: C, 39.20; H, 5.12; Cl, 6.10; N, 10.19; S, 9.07.

Compound **5c**: **5c** was obtained as an amorphous powder in 20% yield from **5c′**; ^1H NMR (DMSO-d_6+D_2O) δ 1.20 (3H, t, $J = 7.2$ Hz), 3.2 and 3.6 (2H, ABq, $J = 19$ Hz), 3.50 (2H, m), 4.15 (2H, q, $J = 7.2$ Hz), 4.96 (2H, m), 5.19 (1H, d, $J = 5.1$ Hz), 5.89 (2H, m), 5.91 (1H, m), 8.19 1H, m), 8.88 (1H, m), 9.08 (1H, d, $J = 6.0$ Hz), 9.20 (1H, d, $J = 8.7$ Hz); IR (KBr) cm^{-1} 1781, 1673, 1631, 1525, 1475, 1448, 1387; HR-FABMS calcd for $C_{22}H_{25}N_{10}O_5S_2$ $[(M+H)^+]$: 573.1451, found: 573.1453.

Compound **5d**: **5d** was obtained as an amorphous powder in 38% yield from **5d′**; ^1H NMR (D_2O) δ 1.00 (3H, t, $J = 7.2$ Hz), 2.50 (3H, s), 2.96 and 3.26 (2H, ABq, $J = 18$ Hz), 3.46 (2H, t, $J = 5.7$ Hz), 4.04 (1H, q, $J = 7.2$ Hz), 4.72 (2H, t, $J = 5.7$ Hz), 4.90 (1H, d, $J = 5.1$ Hz), 5.47 and 5.55 (2H, ABq, $J = 14.7$ Hz), 5.58 (1H, d, $J = 5.1$ Hz), 7.77 (1H, m), 8.49 (1H, d, $J = 0.9$ Hz), 8.64 (1H, d, $J = 8.8$ Hz), 8.73 (1H, d, $J = 5.7$ Hz); IR (KBr) cm^{-1} 1779, 1663, 1621, 1524, 1470, 1390; Anal. Calcd for $C_{23}H_{26}N_{10}O_5S_2 \cdot 1.5HCl \cdot 4.8H_2O$: C, 37.96; H, 5.14; Cl, 7.31; N, 19.24; S, 8.81. Found: C, 37.7; H, 4.85; Cl, 7.39; N, 19.14; S, 8.80.

Compound **5e**: **5e** was obtained as an amorphous powder in 20% yield from **5e′**; ^1H NMR (DMSO-d_6) δ 1.20 (3H, t, $J = 7.2$ Hz), 2.3 (2H, m), 2.5 (3H, s), 2.9 (2H, m), 4.13 (2H, q, $J = 7.2$ Hz), 4.86 (2H, m), 5.19 (1H, d, $J = 7.8$ Hz), 5.87 (2H, s), 5.9 (1H, m), 8.16 (2H, s), 8.3 (1H, m), 8.87 (1H, s), 9.08 (1H, d, $J = 7.8$ Hz), 9.25 (2H, brs), 9.35 (1H, d, $J = 12.9$ Hz), 9.59 (1H, d, $J = 12.6$ Hz); IR (KBr) cm^{-1} 1779, 1673, 1621, 1524, 1473, 1391; Anal. Calcd for $C_{24}H_{28}N_{10}O_5S_2 \cdot 2.1HCl \cdot 3.8H_2O$: C, 38.66; H, 5.10; Cl, 9.98; N, 18.78; S, 8.60. Found: C, 38.75; H, 5.05; Cl, 9.81; N, 18.62; S, 8.53.

Compound **5f**: **5f** was obtained as an amorphous powder in 16% yield from **5f′**; ^1H NMR (DMSO-d_6) δ 1.20 (3H, t, $J = 7.2$ Hz), 2.3 (2H, m), 2.9–3.1 (4H, m), 3.2 and 3.6 (2H, ABq, $J = 19$ Hz), 3.67 (2H, m), 4.14 (2H, q, $J = 7.2$ Hz), 4.85 (2H, m), 5.17 (1H, d, $J = 4.8$ Hz), 5.3 (1H, m), 5.87 (2H, m), 5.9 (1H, m), 8.18 (2H, brs), 8.21 (1H, m), 8.93 (1H, s), 9.15 (1H, d, $J = 7.8$ Hz), 9.16 (2H, brs), 9.32 (1H, d, 8.7 Hz), 9.58 (1H, d, $J = 8.4$ Hz); IR (KBr) cm^{-1} 1778, 1673, 1617, 1523, 1473, 1455, 1390; Anal. Calcd for $C_{25}H_{30}N_{10}O_6S_2 \cdot 1.8HCl \cdot 4H_2O$: C, 39.08; H, 5.22; Cl, 8.31; N, 18.23; S, 8.35. Found: C, 38.91; H, 5.08; Cl, 8.03; N, 18.36; S, 8.22.

References and notes

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