Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2899–2904

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and β-Lactamase Inhibitory Activity of New 6β-Cysteinesulfonamidopenicillanic Acids

Lubomir S. Changov,[a,*] Emilia D. Naydenova,[a] Galya I. Ivanova,[b] Rayna T. Gergova,[c] Emma E. Keuleyan[c] and Boris V. Aleksiev[a]

[a]*University of Chemical Technology and Metallurgy, 8 Kliment Ohridsky Blvd., 1756 Sofia, Bulgaria*
[b]*Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[c]*Chair of Microbiology, Medical University, 2 Zdrave St., 1431 Sofia, Bulgaria*

Received 13 May 1999; accepted 20 July 1999

Abstract—New 6β-cysteinesulfonamidopenicillanic acids and their sulfoxides were synthesized by sulfonylation of 6β-aminopenicillanic acid or its (*S*)-sulfoxide with (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride ethyl ester (**2a**, **1b**) and (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride benzyl ester (**2a**, **2b**). The corresponding 6β-cysteinesulfonamidopenicillanic acids sulfones **1c** and **2c** were prepared by oxidation of the sulfoxides **1b** and **2b** with potassium permanganate in aqueous medium. When combined with ampicillin some of the compounds reduced the minimal inhibitory concentrations of ampicillin against β-lactamase producing strains.

Introduction

In recent years the importance of the β-lactamase inhibitors in overcoming the resistance to β-lactam antibiotics have increased greatly. A number of natural and synthetic compounds have been found to inhibit β-lactamases—enzymes which catalyze the hydrolysis of the CO–N bond in the molecules of penicillins and cephalosporins.[1–5] Only a few of them, however, have so far found application in the clinical practice—clavulanic acid in combination with amoxicillin and ticarcillin, sulbactam in combination with ampicillin and cefoperazone, and tazobactam in combination with piperacillin.[6]

It has been assumed that the introduction of a strong electron-withdrawing group in the amino group of 6β-aminopenicillanic acid (6-APA) might increase the acidity of the 6α-hydrogen atom, leading to faster elimination across the bond between C-5 and C-6 and formation of β-aminoacrylate system.[7] The latter is a common intermediate through which proceeds inhibition of β-lactamases by sulbactam and related inhibitors.[8]

As a result of this hypothesis a number of 6β-sulfonamidoderivatives of penicillanic acid were synthesized and tested for β-lactamase inhibitory activity. It has been found that some of them exhibited good inhibitory properties against cephalosporinases.[7,9–13]

In our previous paper,[14] we described synthesis and β-lactamase inhibitory properties of a series of 6β-aryl-(alkyl)sulfonamidopenicillanic acids, some of which showed synergism when combined with ampicillin.

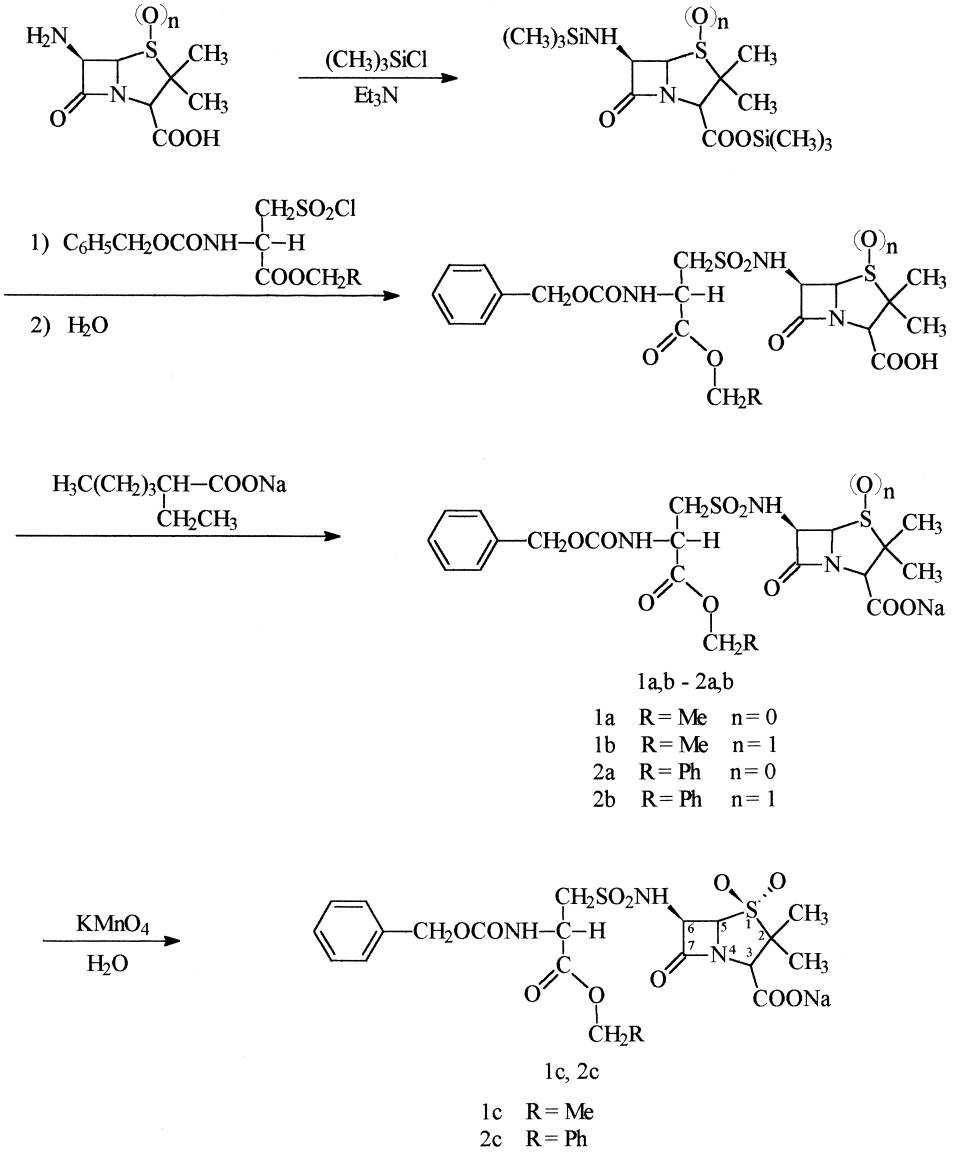
In this article, we describe the synthesis of two new (2*S*,5*R*,6*R*)-6-cysteinesulfonamido-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acids, their 4-oxides and 4,4-dioxides and the results obtained for their β-lactamase inhibitory activity.

Chemistry

The structure of the prepared compounds is shown in the Scheme 1. The choice of these structures was in accordance with previously postulated in the literature criteria which an effective inhibitor of β-lactamases must satisfy.[15] The bulky residue of the N,C-protected cysteinesulfonyl chloride at C-6 might lead to more efficient binding of the compounds with the β-lactamase while the electron-withdrawing properties of the same residue might enhance the acidity of C-6 hydrogen atom

Key words: β-Lactamase inhibitor; sulbactam; ampicillin; cysteinesulfonyl chloride; cysteinesulfonamidopenicillanic acid.
* Corresponding author. Tel.: +359-2-6254427; fax: +359-2-685488; e-mail: lschangov@hotmail.com

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00238-2



Scheme 1. Synthesis of 6β-cysteinesulfonamidopenicillanic acids, their sulfoxides and their sulfones.

and thus facilitate the elimination across the bond between C-5 and C-6. On the other hand, the oxidation of the sulfur to sulfone might provide a good leaving group at C-5 for this elimination and formation of the said β-aminoacrylate system.

The 6β-cysteinesulfonamidopenicillanic acids **1a** and **2a** were prepared by silylation of 6-APA in dichloromethane with trimethylchlorosilane in the presence of triethylamine and urea, and then reacting the silylated 6-APA with the corresponding N,C-protected cysteinesulfonyl chloride (Scheme 1). The trimethylsilyl protecting group was removed at the end of the reaction under mild conditions by adding water. The obtained compounds were isolated from the reaction mixture as sodium salts after reacting the corresponding free acids with sodium 2-ethylhexanoate.

The 6β-cysteinesulfonamidopenicillanic acids sulfoxides **1b** and **2b** were prepared in a similar way by sulfonylation of silylated 6-APA sulfoxide[14] with the corresponding cysteinesulfonyl chloride (Scheme 1).

The 6β-cysteinesulfonamidopenicillanic acids sulfones **1c** and **2c** were synthesized by a potassium permanganate oxidation[16] of the corresponding sulfoxides **1b** and **2b** using a slightly modified procedure (Scheme 1).

The chemical structures of the new compounds **1a–c** and **2a–c** were established by IR and NMR spectroscopy and the results are presented in Experimental.

In the IR spectra of the compounds were observed the characteristic absorption bands at 1798–1777 cm^{-1} for the β-lactam carbonyl and at 1355–1332 cm^{-1} (SO_2, asym.) and 1170–1165 cm^{-1} (SO_2, sym.) for the sulfonamide.

The spectral assignment and multiplet analysis of ^1H NMR spectra were based primary on the $^1H/^1H$ 2-D

correlated spectra (COSY) in the normal and double-quantum filtered versions.

It was observed that the oxidation of the derivatives of sulfonamidopenicillins to their sulfoxides leads to a upfield shift of the signals of H-5 by 0.17 ppm as well as the protons of β-methyl group at C-3 position by 0.31 ppm (for the numbering of the H-atoms see Scheme 1). However, the signals of the protons H-2, H-6 and $C\underline{H}_2SO_2$ from the side chain are shifted lowfield (0.08–0.18 ppm with respect to the parent compounds **1a** and **2a**). The oxidation to sulfones causes deshielding of the H-5 and protons from β-methyl group at C-3 position and shielding of H-2, H-6 and $C\underline{H}_2SO_2$ protons from the side chain with respect to the parent sulfoxides **1b** and **2b**.

Biological Activity

The obtained results are summarized in Table 1 for Gram-positive and in Table 2 for Gram-negative micro-organisms.

When tested alone, the new derivatives **1a–c** and **2a–c** showed some antimicrobial activity, but it was lower than the activity of ampicillin. When combined with ampicillin the compounds manifested synergism against Gram-posititve microbial strains of *Staphylococcus aureus* and *Enterococcus faecalis* (Table 1). Their MICs against the control strain *ATCC 29213* were 0.5 mg/L which is equal to the effect of combination ampicillin + sulbactam. The activity of the combinations of the new compounds with ampicillin was one step higher than the combined effect of ampicillin + sulbactam against *ATCC 29212*.

In the combinations of compounds **1a–c** and **2a–c** with ampicillin against strains of *Escherichia coli* and *Klebsiella pneumoniae*, elaborating ESBla, and against *Enterobacter cloacae* strains, producing natural TEM 1 β-lactamase, significant change in activity, as it was expected, was not obseved (Table 2). It is also possible that some synergism exists against *Enterobacter* strains but it is not at clinically attainable levels. Only *Moraxella (Branhamella) catarrhalis* Bla(+) strains demonstrated more interesting susceptibility—they were supressed by MICs fourfold lower than MICs of ampicillin alone.

In conclusion, some of the newly synthesized compounds showed antibacterial activity alone and synergistic activity when combined with ampicillin, but not higher than that of ampicillin alone and of the combination ampicillin + sulbactam.

Experimental

Melting points (mp) were determined on Koffler microscope and were uncorrected. Thin-layer chromatography was carried out on silica gel 60 plates F_{254} (Merck, 0.2 mm thick) using a mobile phase $PhCH_3$:EtOAc:HCOOH, 10:10:0.5 and visualisation was effected with ultraviolet light. IR spectra in KBr were recorded on a Pye Unicam SP 1000 spectrophotometer.

^{1}H NMR spectra were obtained on a Bruker Avance DRX-250 spectrometer, operating at 250.13 for ^{1}H using a dual 5 mm probe head and 0.05–0.1 M solution in DMSO-d_6. The chemical shifts are expressed relative to tetramethylsilane (TMS) but measured relative to the solvent peak at 2.49 ppm for ^{1}H. The measurements were carried out at ambient temperature (300 K). The atom numbering used for the description of the spectra is shown in Scheme 1. Typical conditions for the 1-D ^{1}H spectra were: pulse width 30°, FT size 32 K and digital resolution 0.2 Hz per point. The 2-D NMR spectra were obtained using the standard Bruker software: cosy, cosydftp (double-quantum filtered COSY) for the ^{1}H/^{1}H correlations. The 2-D homonuclear COSY experiments were typically performed with a spectral width of ca. 2000 Hz, relaxation delay 2 s, mixing pulse width 45 or 60°, number of increments 256 or 512 and FT size 1 K × 1 K. The microanalyses were performed on Perkin–Elmer elemental analyser.

Thirty-four Gram-positive micro-organisms—*S. aureus, Streptococcus pyogenes, E. faecalis* and 47 Gram-negative micro-organisms—*E. coli, K. pneumoniae, E. cloacae, Acinetobacter baumannii, M. (B.) catarrhalis*, representing recent clinical isolates from the Clinical Microbiology Laboratory of the Department of Microbiology at Medical University, Sofia, Bulgaria, were studied. Control strains: *E. coli ATCC 25922, S. aureus ATCC 29213, E. faecalis ATCC 29212* were also investigated.

Table 1. Minimal inhibitory concentrations as MIC_{90} (mg/L) of six 6β-sulfonamidopenicillanic acids and their combinations with ampicillin against 34 clinical strains and two control strains of Gram-positive bacteria. Comparison with Mic_{90} (mg/L) of ampicillin and ampicillin + sulbactam[a]

		Micro-organisms[b]														
Species	n[b]	Amp	Sulb	Amp + Sulb	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	Amp + **1a**	Amp + **1b**	Amp + **1c**	Amp + **2a**	Amp + **2b**	Amp + **2c**
		MIC_{90} (mg/L)														
S. aureus	14	> 32	> 64	2	> 8	> 8	> 8	> 8	> 8	> 8	4	4	4	4	4	4
S. pyogenes	5	0.01	> 16	0.01	> 8	> 8	> 8	> 8	> 8	> 8	0.01	0.01	0.01	0.01	0.01	0.01
E. faecalis	15	1	> 16	1	> 8	> 8	> 8	> 8	> 8	> 8	0.25	0.25	0.25	0.25	0.25	0.25
S. aureus ATCC 29213		0.5	> 64	0.5	> 8	> 8	> 8	> 8	> 8	> 8	0.5	0.5	0.5	0.5	0.5	0.5
E. faecalis ATCC 29212		1	> 16	0.5	> 16	> 16	> 16	> 16	> 16	> 8	0.25	0.25	0.25	0.25	0.25	0.25

[a] Amp, ampicillin; Sulb, sulbactam; MIC_{90}, minimal inhibitory concentration that inhibits 90% of the strains; >, greater than.
[b] n, number of strains.

Table 2. Minimal inhibitory concentrations as MIC_{90} (mg/L) of six 6β-sulfonamidopenicillanic acids and their combinations with ampicillin against 47 clinical strains and 1 control strain of Gram-negative bacteria. Comparison with MIC_{90} (mg/L) of ampicillin and ampicillin + sulbactam[a]

Species	n[b]	Micro-organisms													
		Amp	Sulb	Amp + Sulb	**1a**	**1b**	**1c**	**2a**	**2b**	**2c**	Amp + **1a**	Amp + **1b**	Amp + **1c**	Amp + **2a**	Amp + **2b**	Amp + **2c**
		MIC_{90} (mg/L)														
E. coli	5	> 512	> 64	32	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
E. coli ESBla	5	> 512	> 64	16	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
E. cloacae	7	> 512	> 64	8	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
K. pneumoniae	4	> 512	> 63	4	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
K. pneumoniae ESBla	5	> 512	> 64	16	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
A. baumannii	3	> 512	8	4	> 32	> 32	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32
M. (B.) catarrhalis Bla(−)	7	0.06	16	0.06	> 8	> 8	> 8	> 8	> 8	> 8	0.06	0.06	0.06	0.06	0.06	0.06
M. (B.) catarrhalis Bla(+)	11	16	16	0.125	> 8	> 8	> 8	> 8	> 8	> 8	4	4	1	4	4	4
E. coli ATCC 25922		4	> 16	2	> 64	> 64	> 64	> 64	> 64	> 64	> 32	> 32	> 32	> 32	> 32	> 32

[a] Amp, ampicillin; Sulb, sulbactam; MIC_{90}, minimal inhibitory concentration that inhibits 90% of the strains, >, greater than; Bla, beta-lactamase; ESBla, extended spectrum beta-lactamase.
[b] n, number of strains.

Ampicillin and sulbactam were gifts from Antibiotic Co., Razgrad, Bulgaria. Susceptibility testing was performed by twofold serial dilution method according to NCCLS 1995.[17] Mueller–Hinton agar was purchased from National Institute for Infectious and Parasitic Diseases, Sofia, Bulgaria. For more fastidious micro-organisms—*S. pyogenes, M.(B.) catarrhalis*—Mueller–Hinton agar was supplemented with 5% sheep blood.

Ampicillin, sulbactam and substances **1a–c** and **2a–c** were first dissolved in phosphate buffer pH 8, then diluted in phosphate buffer pH 6. Minimal inhibitory concentration (MIC) in mg/L was first determined for ampicillin, sulbactam and for each of the compounds **1a–c** and **2a–c** alone. Then the following combinations were prepared: ampicillin and sulbactam, ampicillin and each of the compounds **1a–c** and **2a–c**. The ratio of antimicrobial agents in all combinations was 1:1. All cultivations were at 37°C for 18–24 h.

Sodium(2*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid ethyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (1a). To a cooled to 10°C suspension of 6-aminopenicillanic acid (6-APA) (5.00 g, 0.023 mol) and urea (2.17 g, 0.036 mol) in dry dichloromethane (100 mL) was added triethylamine (8.50 mL, 6.20 g, 0.064 mol) and the mixture was stirred for 30 min at room temperature. Then trimethylchlorosilane (7.71 mL, 6.63 g, 0.061 mol) was added dropwise at 5°C and the stirring continued for another 30 min at room temperature and 1 h at 40°C.

After cooling to about 20°C (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride ethyl ester[18] (8.74 g, 0.025 mol) was added in one portion and the mixture was stirred for 1 h at room temperature and 1 h at 40°C.

The reaction mixture was cooled to 0°C and water (80 mL) was added dropwise. The two layers were separated and the water layer was extracted with dichloromethane (2×40 mL). The combined dichloromethane extracts were washed with water (40 mL) and the dichloromethane was removed under reduced pressure.

The residue was cooled to 0°C and then saturated water solution of sodium hydrogen carbonate (80 mL) was added dropwise under stirring. The resulting solution with pH about 7.5 was extracted with ethyl acetate (2×30 mL) to remove unreacted cysteinesulfonyl chloride.

The water layer was cooled to 0°C and ethyl acetate (40 mL) was added. The pH of the water layer was brought to 1.5 with 5 N sulfuric acid. The two layers were separated and the water layer was extracted with ethyl acetate (2×30 mL). The combined ethyl acetate layers were washed with saturated water solution of sodium chloride (30 mL) and dried with anhydrous sodium sulfate.

To the dried ethyl acetate solution of the 6β-cysteinesulfonamidopenicillanic acid was added at room temperature sodium 2-ethylhexanoate (3.82 g, 0.023 mol). The mixture was stirred for 15 min until a clear solution was obtained and then was concentrated under reduced pressure to about 1/5 of the initial volume.

Hexane (200 mL) was added under stirring and white precipitate appeared immediately. The mixture was stirred for 30 min and the obtained sodium salt of **1a** was filtered, suspended quickly in hexane, filtered again and dried in vacuo at 40°C. Yield 10.46 g (82%); mp 119–120°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.44.

IR (KBr) 3380 (NH), 1780 (β-lactam carbonyl), 1728 (ester carbonyl); 1630 (CH–NH), 1620, 1390 (COO^-), 1355, 1170 (SO_2NH), 755, 712 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.16 (t, 3H, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.44 (s, 3H, CH_3-3), 1.53 (s, 3H, CH_3-3), 3.47 (dd, 1H, J = 14.5 and 5.2 Hz, $CHC\underline{H_2}$b), 3.60 (dd, 1H, J = 14.5 and 3.2 Hz, $CHC\underline{H_2}$a), 4.10 (q, 2H, J = 7.0 Hz, $C\underline{H_2}CH_3$), 3.83 (s, 1H, H-2), 4.52 (ddd, 1H, J = 8.2, 5.2 and 3.2 Hz, $C\underline{H}CH_2$), 5.05 (s, 2H, $PhC\underline{H_2}OCON$), 5.09 (1H, H-6), 5.36 (d, 1H, J = 4.0 Hz, H-5), 7.36–7.29 (5H, Ar), 7.90 (d, 1H, J = 8.2 Hz, CONH), 8.3 (br, 1H, SO_2NH). Anal. calcd for $C_{21}H_{26}N_3NaO_9S_2$ (551.60): C, 45.72%; H, 4.76%; N, 7,62%; found: C, 45.43%; H, 4.69%; N, 7.42%.

Sodium (2*S*,4*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid ethyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4-oxide (1b). The sulfoxide **1b** was prepared in a similar way as the sulfide **1a**, starting from 6-APA (*S*)-sulfoxide 4-toluenesulfonate (8.7 g, 0.021 mol), urea (2.17 g, 0.036 mol) and triethylamine (8.50 mL, 6.20 g, 0.064 mol). After the addition of the trimethylchlorosilane (14.15 mL, 12.17 g, 0.112 mol) and later of the (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride ethyl ester (8.08 g, 0.023 mol), the reaction mixture were stirred all the time at room temperature instead of 40°C as in the preparation of **1a**. The sodium salt **1b** was isolated in a similar manner using 3.49 g (0.021 mol) sodium 2-ethylhexanoate. Yield 7.69 g (63%); mp 104–105°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.17. IR (KBr) 3300 (NH), 1785 (β-lactam carbonyl), 1725 (ester carbonyl), 1645 (CH–NH), 1620, 1385 (COO^-), 1355, 1170 (SO_2NH), 1028 (S–O); 750, 710 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.16 (t, 3H, J = 7.0 Hz, $CH_2\underline{CH_3}$), 1.13 (s, 3H, CH_3-3), 1.54 (s, 3H, CH_3-3), 3.55 (dd, 1H, J = 15.1 and 5.7 Hz, $CHC\underline{H_2}$b), 3.75 (dd, 1H, J = 15.1 and 2.8 Hz, $CHC\underline{H_2}$a), 4.10 (q, 2H, J = 7.0 Hz, $C\underline{H_2}CH_3$), 3.94 (s, 1H, H-2), 4.53 (ddd, 1H, J = 8.2, 5.7 and 2.8 Hz, $C\underline{H}CH_2$), 5.05 (s, 2H, $PhC\underline{H_2}OCON$), 5.19 (1H, H-6), 5.19 (1H, H-5), 7.36–7.29 (5H, Ar), 7.90 (d, 1H, J = 8.2 Hz, CONH), 8.0 (br, 1H, SO_2NH). Anal. calcd for $C_{21}H_{26}N_3NaO_{10}S_2$ (567.60): C, 44.43%; H, 4.63%; N, 7.40%; found: C, 44.13%; H, 4.54%; N, 7.26%.

Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid ethyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (1c). Sodium 6β-[(*R*)-*N*-benzyloxycarbonylcysteic acid ethyl ester *S*-amido]penicillanate sulfoxide **1b** (5.0 g, 0.009 mol) was dissolved in water (50 mL) and the solution was cooled to 0°C. A solution of potassium permanganate (2.21 g, 0.014 mol) and 85% *ortho*-phosphoric acid (0.67 mL, 1.14 g, 0.009 mol) in water (40 mL) was added dropwise at 0–5°C. The mixture was stirred for 1 h at the same temperature and then ethyl acetate (50 mL) was added. The pH of the water layer was lowered to about 2 with 5 N sulfuric acid and then 12% water solution of sodium pyrosulfite was added dropwise to destroy the excess of potassium permanganate. After that pH was adjusted to 1.5 with 5 N sulfuric acid.

The two layers were separated and the water layer was extracted with ethyl acetate (2×30 mL). The combined ethyl acetate extracts were washed with saturated water solution of sodium chloride (40 mL) and dried with anhydrous sodium sulfate.

Sodium 2-ethylhexanoate (1.50 g, 0.009 mol) was added at room temperature to the dried ethyl acetate solution of the 6β-cysteinesulfonamidopenicillanic acid sulfone. The mixture was stirred for 15 min until a clear solution was obtained and then was concentrated under reduced pressure to about 1/5 of the initial volume.

Hexane (200 mL) was added under stirring and white precipitate appeared immediately. The mixture was stirred for 30 min and the obtained sodium salt of **1c** was filtered, suspended quickly in hexane, filtered again and dried in vacuo at 40°C. Yield 4.37 g (85%); mp 110–111°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.26. IR (KBr) 3380 (NH), 1798 (β-lactam carbonyl), 1725 (ester carbonyl), 1642 (CH–NH), 1625, 1385 (COO^-), 1365, 1165 (SO_2NH), 1328, 1125 (SO_2), 752, 712 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.16 (t, 3H, J = 7.1 Hz, $CH_2\underline{CH_3}$), 1.29 (s, 3H, CH_3-3), 1.41 (s, 3H, CH_3-3), 3.40 (1H, $CHC\underline{H_2}$b), 3.65 (1H, J = 10.4 Hz, $CHC\underline{H_2}$a), 4.09 (q, 2H, J = 7.1 Hz, $C\underline{H_2}CH_3$), 3.79 (s, 1H, H-2), 4.52 (ddd, 1H, J = 8.5, 4.9 and 3.2 Hz, $C\underline{H}CH_2$), 5.04 (s, 2H, $PhC\underline{H_2}OCON$), 5.04 (dd, 1H, J = 4.6 and 9.4 Hz, H-6), 5.33 (d, 1H, J = 4.6 Hz, H-5); 7.37–7.29 (5H, Ar), 7.89 (d, 1H, J = 8.5 Hz, CONH). Anal. calcd for $C_{21}H_{26}N_3NaO_{11}S_2$ (583.60): C, 43.22%; H, 4.50%; N, 7.20%; found: C, 42.92%; H, 4.44%; N, 7.02%.

Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid benzyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate (2a). The compound was synthesized in a similar way as **1a**. For the sulfonylation (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride benzyl ester[18] (10.30 g, 0.025 mol) was used. Yield 12.20 g (86%); mp 109–110°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.50. IR (KBr) 3380 (NH), 1780 (β-lactam carbonyl), 1725 (ester carbonyl), 1635 (CH–NH), 1617, 1385 (COO^-), 1355, 1170 (SO_2NH), 750, 710 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.45 (s, 3H, CH_3-3), 1.54 (s, 3H, CH_3-3), 3.48 (dd, 1H, J = 14.5 and 5.1 Hz, $CHC\underline{H_2}$b), 3.65 (dd, 1H, J = 14.5 and 3.4 Hz, $CHC\underline{H_2}$a), 3.85 (s, 1H, H-2), 4.62 (ddd, 1H, J = 8.4, 8.1 and 3.4 Hz, $C\underline{H}CH_2$), 5.04 (s, 2H, $PhC\underline{H_2}OCON$), 5.14 (s, 2H, $PhC\underline{H_2}OCOC$), 5.1 (1H, H-6), 5.36 (d, 1H, J = 4.0 Hz, H-5), 7.34–7.32 (10H, Ar), 7.94 (d, 1H, J = 8.1 Hz, CONH), 8.4 (br, 1H, SO_2NH). Anal. calcd for $C_{26}H_{28}N_3NaO_9S_2$ (613.67): C, 50.88%; H, 4.61%; N, 6.85%; found: C, 50.60%; H, 4.59%; N, 6.73%.

Sodium (2*S*,4*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid benzyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4-oxide (2b). The compound was synthesized in a similar way as **1b**. For the sulfonylation (*R*)-*N*-benzyloxycarbonylcysteinesulfonyl chloride benzyl ester (9.47 g, 0.023 mol) was used. Yield 8.94 g (66%); mp 121–122°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.22. IR (KBr) 3300 (NH), 1777 (β-lactam carbonyl), 1722 (ester carbonyl), 1640 (CH–NH), 1622, 1385 (COO^-), 1350, 1170 (SO_2NH), 1025 (S–O); 755, 710 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.16 (s, 3H, CH_3-3), 1.54 (s, 3H, CH_3-3), 3.63 (dd, 1H, J = 14.6 and 9.5 Hz, $CHC\underline{H_2}$b); 3.83 (dd, 1H, J = 14.6 and 3.5 Hz, $CHC\underline{H_2}$a), 3.97 (s, 1H, H-2), 4.64 (ddd, 1H, J = 9.5, 8.3 and 3.5 Hz, $C\underline{H}CH_2$), 5.05 (s, 2H, $PhC\underline{H_2}OCON$), 5.15 (s, 2H, $PhC\underline{H_2}OCOC$), 5.19 (dd, 1H, J = 9.1 and 4.2 Hz, H-6), 5.08 (d, 1H, J = 4.2 Hz, H-5), 7.37–7.30 (10H, Ar), 7.97 (d, 1H, J = 8.3 Hz, CONH), 7.05 (br, 1H, SO_2NH). Anal. calcd for $C_{26}H_{28}N_3NaO_{10}S_2$ (629.67): C, 49.59%; H, 4.49%; N, 6.67%; found: C, 49.38%; H, 4.40%; N, 6.58%.

Sodium (2*S*,5*R*,6*R*)-6-[(*R*)-*N*-benzyloxycarbonylcysteic acid benzyl ester *S*-amido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate-4,4-dioxide (2c). The compound was synthesized in a similar way as **1c** by oxidation of the sulfoxide **2b** with potassium permanganate. Yield 4.51 g (88%); mp 100–101°C; R_f ($PhCH_3$:EtOAc:HCOOH, 10:10:0.5) = 0.31. IR (KBr) 3350 (NH), 1798 (β-lactam carbonyl), 1725 (ester carbonyl), 1645 (CH–NH), 1625, 1385 (COO^-), 1332, 1172 (SO_2NH); 1285, 1125 (SO_2), 755, 710 (Ar-) cm^{-1}. ^1H NMR (250.13 MHz, DMSO-d_6) δ 1.29 (s, 3H, CH_3-3), 1.43 (s, 3H, CH_3-3), 3.52 (dd, 1H, J = 12.9 and 8.1 Hz, $CHCH_2$b), 3.72 (dd, 1H, J = 12.9 and 6.0 Hz, $CHCH_2$a), 3.80 (s, 1H, H-2), 4.65 (ddd, 1H, J = 8.1, 8.0 and 6.0 Hz, $CHCH_2$), 5.05 (s, 2H, $PhCH_2OCON$), 5.14 (s, 2H, $PhCH_2OCOC$), 5.1 (1H, H-6), 5.33 (d, 1H, J = 4.1 Hz, H-5), 7.35–7.33 (10H, Ar), 7.92 (d, 1H, J = 8.0 Hz, CONH). Anal. calcd for $C_{26}H_{28}N_3NaO_{11}S_2$ (645.67): C, 48.36%; H, 4.38%; N, 6.51%; found: C, 48.19%; H, 4.29%; N, 6.37%.

References

1. Sutherland, R. *Progr. Drug Res.* **1993**, *41*, 95.
2. Mascaretti, O. A.; Roveri, O. A.; Danelon, G. O. In *Recent Progress in the Chemical Synthesis of Antibiotics and Related Microbial Products*; Lukacs, G., Ed.; Springer–Verlag: Berlin, 1993; Vol. 2, pp 677–749.
3. Coulton, S.; François, I. In *Progress in Medicinal Chemistry*; Ellis, G. P.; Luscombe, D. K., Eds; Elsevier Science B.V.: Amsterdam, 1994; Vol. 31, pp 297–349.
4. Mascaretti, O. A.; Boschetti, C. E.; Danelon, G. O.; Mata, E. G.; Roveri, O. A. *Curr. Med. Chem.* **1995**, *1*, 441.
5. Coleman, K. *Exp. Opin. Invest. Drugs* **1995**, *4*, 693.
6. Sutherland, R. *Infection* **1995**, *23*, 191.
7. Dmitrienko, G. I.; Copeland, C. R.; Arnold, L.; Savard, M. E.; Clarke, A. J.; Viswanatha, T. *Bioorg. Chem.* **1985**, *13*, 34.
8. Knowles, J. R. *Acc. Chem. Res.* **1985**, *18*, 97.
9. Mezes, P. S. F.; Clarke, A. J.; Dmitrienko, G. I.; Viswanatha, T. *FEBS Lett.* **1982**, *143*, 265.
10. Mezes, P. S. F.; Friesen, R. W.; Viswanatha, T.; Dmitrienko, G. I. *Heterocycles* **1982**, *19*, 207.
11. Kazimierczak, J.; Mikolajczyk, J.; Kotulowa, W.; Cieslak, J. *Acta Polon. Pharm.* **1984**, *41*, 19.
12. Davern, P.; Sheeshy, J.; Smyth, T. *J. Chem. Soc. Perkin Trans. 2* **1994**, 381.
13. Vanwetswinkel, S.; Fastrez, J.; Marchand-Brynaert, J. *J. Antibiot.* **1994**, *47*, 1041.
14. Changov, L. S.; Vassileva-Lukanova, B. K.; Angelova-Galabova, A.; Pavlova, A. V.; Spasov, S. L. *Arzneim.-Forsch.* **1994**, *44*(II), 856.
15. Fisher, J.; Belasco, J. G.; Charnas, R. L.; Khosla, S.; Knowles, J. R. *Philos. Trans. R. Soc. London* **1980**, *B289*, 309.
16. Johnson, D. A.; Panetta, C. A.; Cooper, D. E. *J. Org. Chem.* **1963**, *28*, 1927.
17. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard; 3rd ed. (Document M100-S6/M7-A3); NCCLS: Villanova, PA, 1995.
18. Aleksiev, B.; Stoev, S. *Pharmazie* **1964**, *24*, 305.