ELSEVIER

Il Farmaco 53 (1998) 173–180

IL FARMACO

A new class of *cis*-monobactam derivatives bearing a sulfamoyloxymethyl or an *N*-alkylsulfamoyloxymethyl group at position 4: synthesis and antibacterial activity

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Received 12 November 1997; accepted 7 December 1997

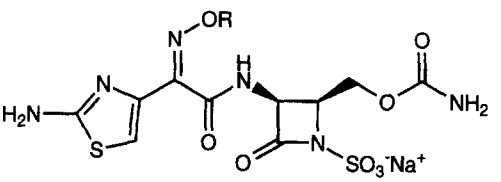
Abstract

A new series of monobactam derivatives, bearing unsubstituted or N-monosubstituted sulfamoyloxymethyl groups in position 4 was synthesized either in racemic or in optically active form. Their in vitro antibacterial activity was tested in comparison with carumonam **1a** and its methoxyimino derivative **1b**.

Keywords: Monobactams; Carumonam; β-Lactams; Antibacterial activity

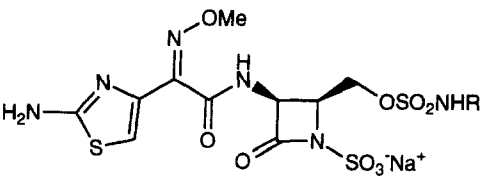
1. Introduction

The discovery in 1981 of monobactams by Squibb [1] and Takeda [2] disclosed new horizons for the clinical application of β-lactam antibiotics, since some of them, like aztreonam and carumonam **1a**, possess a specific activity against Gram-negative bacteria, together with a good β-lactamase stability. The presence of heteroatoms on the C-4 side chain of the monobactam moiety is usually responsible for an enhanced biological activity [3,4]. In this paper we report the synthesis and microbiological activity of a series of analogues of **1a** (more precisely of the corresponding methoxyimino derivative **1b** (Scheme 1)). In these new compounds the carbamoyloxymethyl group $-CH_2OCONH_2$ was replaced by a sulfamoyloxymethyl group of general formula $-CH_2OSO_2NHR$ (**2a–e**) (Scheme 1). The preparation of these products is a completion of a previously reported study, in which the synthesis of some monobactam thio- and dithiocarbamates was performed together with the evaluation of the biological properties [5].



1a R = CH_2CO_2Na

1b R = Me



2a R = H

2b R = Me

2c R = *n*-Bu

2d R = CH_2CH_2F

2e R = CH_2CH_2OMe

Scheme 1.

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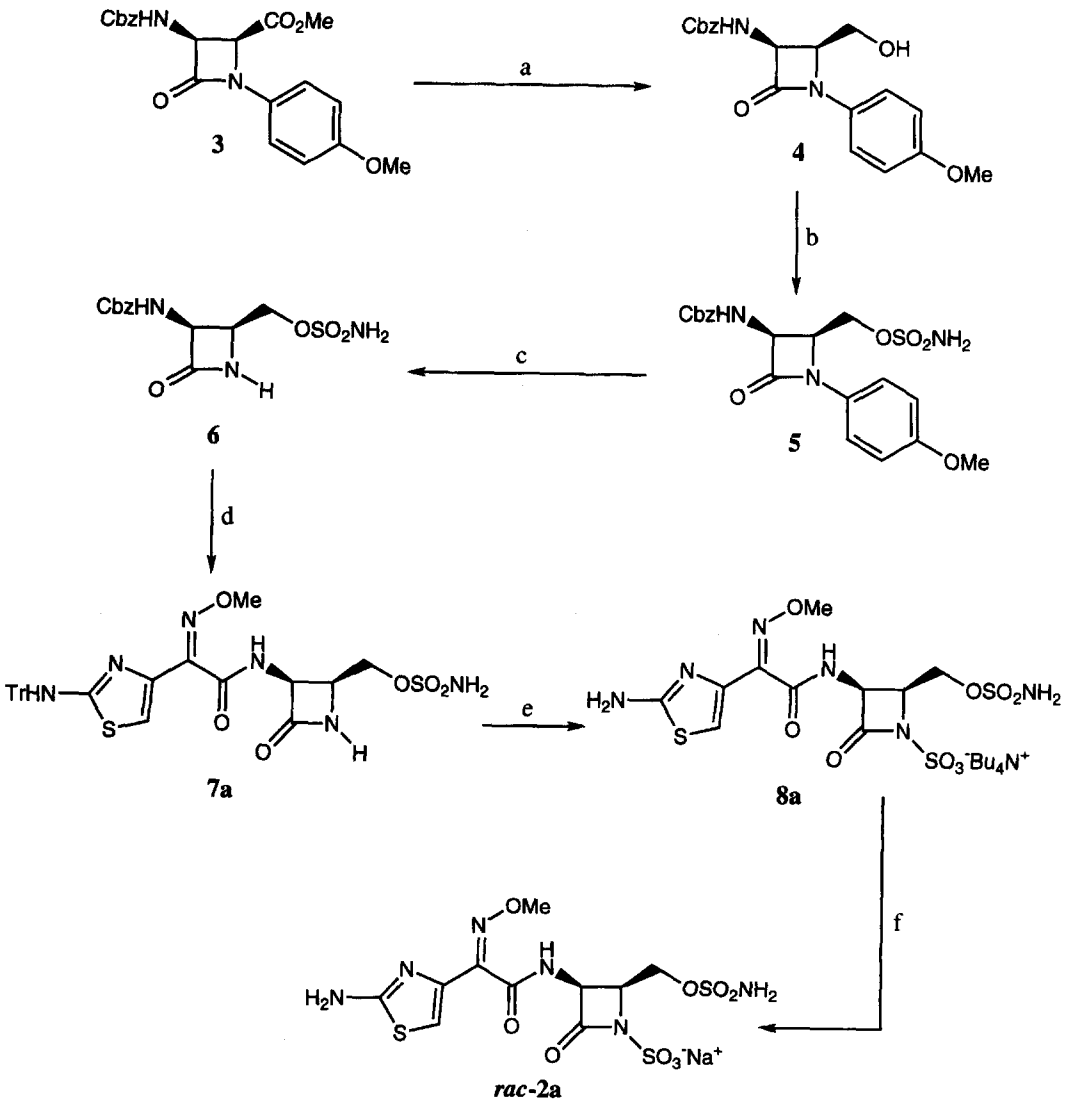
0014-827X/98/$19.00
PII S0014-827X(98)00004-4

2. Chemistry

We first optimized a racemic synthesis of the simplest of these derivatives, i.e. compound **2a** (Scheme 2). As a suitable and easily accessible precursor we chose β-lactam **3**, which can be prepared on a multigram scale through a very diastereoselective cycloaddition using a known procedure [6]. In order to obtain **2a**, we first selectively reduced the ester function to the primary alcohol [7]. The sulfamoyl ester moiety was introduced by reaction of **4** with sulfamoyl chloride [8] under phase-transfer catalysis [9]. The deprotection of the azetidinone nitrogen was then realized by oxidative cleavage in the presence of ceric ammonium nitrate (CAN) [6]. In order to successfully prepare intermediate **6** in this way it was mandatory to first introduce the sulfamoyloxy group and then to deprotect the ring nitrogen. The carbobenzyloxy (Cbz) group was easily removed by hyrogenolysis followed by coupling of the amine with 2-(2-triphenylmethylamino-4-thiazolyl)-(*Z*)-2-methoxyimino acetic acid under standard conditions to give **7a** [10]. A one-pot procedure, i.e. N-sulfonation, followed by N-trityl group removal, allowed us to obtain tetrabutylammonium salt **8a**. Finally, a cation exchange over Dowex 50W X 8 (Na^+ form) resin, followed by chromatography over C-18 reverse-phase silica gel, furnished pure **2a**.

For the preparation of optically pure **2a–e**, we used intermediate **9**, prepared following the procedure reported by Thomas [11]. In this case the introduction of the $-SO_2NHR$ group was not possible under phase transfer conditions, probably due to side reactions caused by the intervention of the deprotected N–H group, which probably reacts under the basic conditions employed. We solved our problems by a new simple and economical procedure, namely, the reaction of **9** with the appropriate sulfamoyl chloride (R≠H), pre-



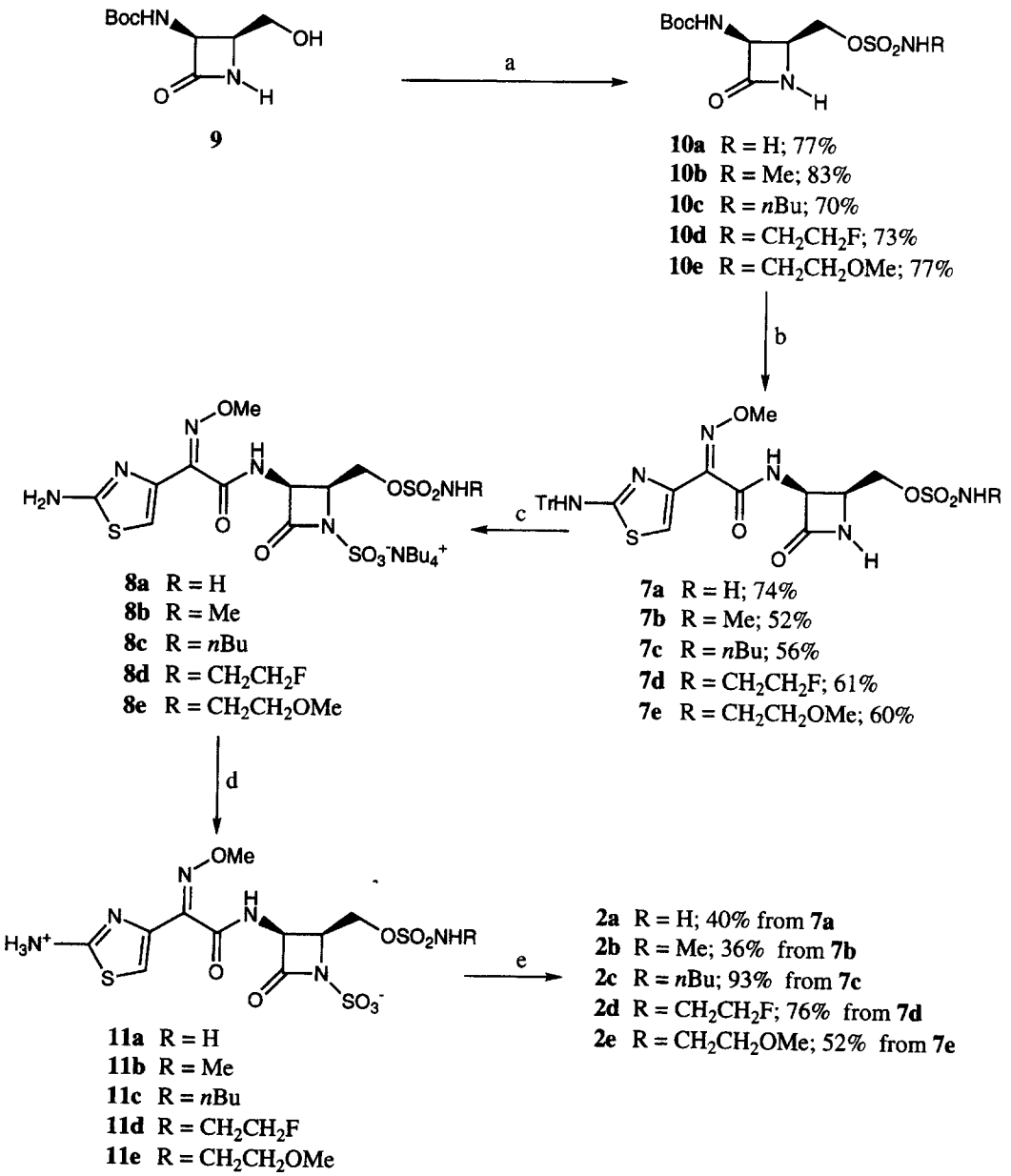
Scheme 2. Synthesis of racemic **2a**: (a) $NaBH_4$, THF/EtOH/H_2O, 0°C for 40 min, then r.t. for 1.25 h, 87%; (b) H_2NSO_2Cl, $BnEt_3N^+Cl^-$, Na_2CO_3, AcOEt, reflux, 1.5 h, 74%; (c) CAN, CH_3CN/H_2O, 0°C, 45 min, 78%; (d) 1. H_2, Pd/C, MeOH, r.t., 2.5 h; 2. 2-(2-triphenylaminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetic acid, DCC, HBT, DMF/CH_2Cl_2, 2 h at 0°C, 2 h at r.t., 71%; (e) 1. DMF·SO_3, DMF, 0°C, 1.5 h, 2. H_2O, r.t., overnight; 3. $Bu_4N^+HSO_4^-$; (f) Dowex 50W X 8 Na^+ form, chromatography on C-18 reverse-phase silica gel, 49% from **7a**.

pared according to the literature [12], in the presence of triethylamine (Scheme 3). In this way we obtained sulfamates **10a–e**, which have been isolated in satisfactory yield by chromatography, without aqueous work-up of the crude reaction mixture. Further synthetic elaboration followed the above-described route for the synthesis of racemic **2a**, after removal of t-butoxycarbonyl (Boc) protection under acidic conditions. However, for our optimized procedure, we preferred to purify the tetrabutylammonium salts **8a–e** over silica gel. This routine allowed us to isolate the internal salts **11a–e**, sometimes together with variable amounts of purified **8a–e**. In this way the cation exchange over resin was easier and, most of all, it was possible to avoid the tedious and expensive purification over C-18 reverse-phase silica gel.

3. Experimental

3.1. Chemistry

Melting points were determined on a Büchi 535 apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer 240 instrument. Polarimetric values were determined on a Jasco DIP 181 instrument and the concentration of the solutions was expressed in g/100 ml. NMR spectra were recorded on a Varian Gemini 200 spectrometer and DMSO-d_6 was used as solvent, while the recording temperature was always specified. Coupling constants are reported in hertz. Mass spectrometric measurements were performed on a VG7070 EQHF instrument operating in the



Scheme 3. Synthesis of optically active **2a–e**: (a) $ClSO_2NHR$, Et_3N, CH_3CN, r.t. , about 1 h; (b) 1. CF_3CO_2H, CH_2Cl_2, 0°C, 30 min; 2a. triethylamine; 2b. 2-(2-tritylaminothiazol-4-yl)-(Z)-2-(methoxyimino)acetic acid, DCC, HBT, DMF/CH_2Cl_2, 2h at 0°C, 2 h at r.t.; (c) 1. DMF·SO_3, DMF, 0°C, 1.5 h, 2. H_2O, 40°C, 3 h; 3. $Bu_4N^+HSO_4^-$; (d) chromatography on silica gel, using AcOEt/MeOH mixtures as eluant; (e) Dowex 50W X 8 Na^+ form.

fast atom bombardment (FAB) mode. All reactions employing dry solvents were run under a nitrogen atmosphere. CH_2Cl_2, dimethylformamide (DMF) and acetonitrile were doubly dried over 4 Å molecular sieves. Chromatography was carried out on 220–400 mesh silica gel using the 'flash' methodology. Reverse-phase column chromatography was performed on C-18 reverse-phase silica gel 100 (Fluka). Dowex 50W X 8 resin was purchased from Fluka. Thin layer chromatography was carried out on 0.25 mm silica gel F 254 plates (Merck). Spots were detected by: (a) UV; (b) dipping into a solution of $(NH_4)_4MoO_4 \cdot 4H_2O$ (21 g) and $Ce(SO_4)_2 \cdot 4H_2O$ (1 g) in H_2SO_4 (31 ml) and H_2O (469 ml) and warming; (c) spraying with 48% HBr, warming, then dipping into a ninhydrin solution (900 mg in 300 ml n-BuOH + 9 ml AcOH) and warming. R_f values were measured after an elution of 7–9 cm in petroleum ether (PE; b.p. 40–60°C). In extractive work-up, aqueous solutions were always re-extracted thrice with the appropriate organic solvent. Organic extracts, if not otherwise indicated, were finally washed with brine, dried over Na_2SO_4 and filtered, before evaporation of the solvent under reduced pressure.

3.1.1. (±)-cis-3-[(Benzyloxycarbonyl)amino]-4-hydroxymethyl-1-(4-methoxyphenyl)-2-azetidinone ***4***

Ester **3** (5.38 g, 14.0 mmol) was dissolved in tetrahydrofuran (THF)/EtOH/H_2O (75/18/18 ml) and cooled to 0°C. Sodium borohydride (2.65 g, 70.0 mmol) was added and the mixture was stirred at 0°C for 40 min, then at room temperature (r.t.) for 1.25 h. After cooling to 0°C the reaction was cautiously quenched with saturated NH_4Cl. The biphasic system was concentrated in vacuo and then extracted with CH_2Cl_2. The combined organic layers were washed with aqueous 5% $NH_4H_2PO_4$ and, finally, with brine. After solvent removal, crude product was triturated with Et_2O to give 3.99 g of **4** as a white solid (80%). Mother liquors were chromatographed with PE/AcOEt 1:1 → PE/AcOEt/MeOH 50:50:7, giving an additional 350 mg of product (7%). R_f 0.35 (PE/Et_2O 2:8, det. A, B). ^{1}H NMR (r.t.): δ 3.67 and 3.78 (2H, AB part of ABX system, $-CH_2OH$, $J_{AB}=7.6$, J_{AX} and $J_{BX}=3.9$, 4.4 Hz), 3.73 (3H, s, $-OCH_3$), 4.32 (1H, dt, H_4, $J=4.5$, 4.8 Hz), 5.09 (2H, s, $-CH_2Ph$), 5.15 (1H, dd, H_3, $J=5.6$, 9.6 Hz), 6.94 (2H, d, aromatics ortho to $-OCH_3$, $J=9.1$ Hz), 7.30–7.40 (5H, m, aromatics of Cbz), 7.47 (2H, d, aromatics meta to $-OCH_3$, $J=8.9$), 7.79 (1H, d, $-NH$Cbz, $J=9.9$ Hz).

3.1.2. (±)-cis-3-[(Benzyloxycarbonyl)amino]-1-(4-methoxyphenyl)-4-sulfamoyloxymethyl-2-azetidinone ***5***

A solution of **4** (503 mg, 1.41 mmol) in dry AcOEt (15 ml) was treated with anhydrous Na_2CO_3 (420 mg, 3.96 mmol), benzyl triethylammonium chloride (32 mg, 141 μmol) and freshly prepared sulfamoyl chloride (489 mg, 4.23 mmol) and heated at reflux for 1.5 h. The inorganic salts were filtered off and washed with MeOH; the solution was concentrated in vacuo and directly purified by chromatography using PE/acetone 8:2 → 3:7. Compound **5** was obtained as a pale-yellow oil in 74% yield (455 mg). R_f 0.31 (PE/Et_2O 2:8, det. A, B). MS-FAB: m/z 436 ($M+1$). ^{1}H NMR (r.t.): δ 3.75 (3H, s, $-OCH_3$), 4.32 and 4.36 (2H, AB part of ABX system, $-CH_2OSO_2NH_2$, $J_{AB}=11.1$, J_{AX} and $J_{BX}=5.1$, 5.9 Hz), 4.60 (1H, dt, H_4, $J=5.4$, 5.7 Hz), 5.08 and 5.13 (2H, AB system, $-CH_2Ph$, $J=12.5$ Hz), 5.26 (1H, dd, H_3, $J=5.2$, 9.7 Hz), 6.96 (2H, dt, aromatics ortho to $-OCH_3$, $J=1.4$, 9.1 Hz), 7.30–7.43 (7H, m, aromatics of Cbz and aromatics meta to $-OCH_3$), 7.68 (2H, s, $-SO_2NH_2$), 8.18 (1H, d, $-NH$Cbz, $J=9.6$ Hz).

3.1.3. (±)-cis-3-[(Benzyloxycarbonyl)amino]-4-sulfamoyloxymethyl-2-azetidinone ***6***

A solution of **5** (1.04 g, 2.39 mmol) in 30 ml of CH_3CN/H_2O (2:1) was cooled to 0°C and treated with a solution of ceric ammonium nitrate (2.49 g, 7.14 mmol) in 10 ml of water. After 15 min the solution was saturated with NaCl and the extraction was performed with AcOEt. The combined organic layers were neutralized with 5% aqueous $NaHCO_3$, then washed with 10% Na_2SO_3 solution and brine. After solvent removal, crude **6** was purified by chromatography using PE/acetone 7:3 → 3:7 as eluant. 609 mg of azetidinone **6** were obtained as a pale-brown solid (78% yield). R_f 0.25 (AcOEt, det. A, B). MS-FAB: m/z 330 ($M+1$). ^{1}H NMR (r.t.): δ 3.94 (1H, dt, H_4, $J=5.6$, 6.8 Hz), 4.11 and 4.15 (2H, AB part of ABX system, $-CH_2OSO_2NH_2$, $J_{AB}=9.6$, J_{AX} and $J_{BX}=5.2$, 9.0 Hz), 4.98–5.08 (1H, signal partly overlapped with AB system, exact chemical shift not measurable, H_3), 5.04 and 5.10 (2H, AB system, $-CH_2Ph$, $J=11.4$ Hz), 7.32–7.40 (5H, m, aromatics of Cbz), 7.58 (2H, s, $-SO_2NH_2$), 8.12 (1H, d, $-NH$Cbz, $J=9.5$ Hz), 8.59 (1H, s, $-NH$ of β-lactam).

3.1.4. (±)-cis-3-{(Z)-2-(Methoxyimino)-2-[2-(triphenylmethyl)aminothiazol-4-yl]acetamido}-4-sulfamoyloxymethyl-2-azetidinone ***7a***

(a) Removal of the Cbz group: a solution of **5** (865 mg, 2.63 mmol) in 30 ml of MeOH was treated with 100 mg of Pd/C (10%) and hydrogenated at r.t. for 2 h. The catalyst was filtered and the solution concentrated in vacuo. R_f 0.45 (AcOEt/MeOH 95:5, det. B). (b) Acylation reaction: crude amine was dissolved in 10 ml of DMF/CH_2Cl_2 (3:1) and cooled to 0°C. Dicyclohexylcarbodiimide (DCC) (543 mg, 2.63 mmol), *N*-hydroxybenzotriazole (HBT) (355 mg, 2.63 mmol) and 2-(2-triphenylmethylaminothiazol-4-yl)-(*Z*)-2-(methoxyimino)acetic acid (1.17 g, 2.63 mmol) were added and the mixture was stirred at 0°C for 3 h and at r.t. for an additional 2 h. The reaction was concentrated in vacuo and the solid was filtered. The residue was diluted with water (200 ml) and extracted with AcOEt. The combined organic layers were washed with water, 5% saturated $NaHCO_3$, and brine and they were finally concentrated. Chromatography with PE/AcOEt 1:1 → AcOEt gave 1.16 g of **7a** as a pale-yellow solid (71%), which was then crystallized from AcOEt/Et_2O. R_f 0.73 (AcOEt, det. A, B, C). *Anal.* Found: C 55.71, H 5.06, N 13.12. Calc. for $C_{29}H_{28}N_6O_6S_2$: C 56.12,

H 4.55, N 13.54%. ^{1}H NMR (45°C): δ 3.83 (3H, s, –OCH_3), 3.98–4.23 (3H, m, H_4 + –CH_2OSO$_2$NH$_2$), 5.19 (1H, dd, H_3, J = 4.0, 8.5 Hz), 6.72 (1H, s, H_5 of thiazole), 7.31–7.48 (15H, m, aromatics of trityl), 7.50 (2H, s, –SO$_2$NH_2), 8.61 and 8.69 (2H, 2 s, –NH of β-lactam and –NHTr), 9.21 (1H, d, –NHCO–, J = 8.5 Hz).

3.1.5. (±)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-sulfamoyloxymethyl-2-oxoazetidine-1-sulfonic acid, sodium salt ***2a***

(a) Preparation of **8a**: 565 mg of **7a** (0.91 mmol) were dissolved in 3 ml of dry DMF and cooled to 0°C. DMF·SO$_3$ (4 ml, about 1 M in DMF) complex (prepared following Ref. [13]) was added dropwise. The resulting pink–orange solution was stirred at 0°C for 1.5 h. Then 3 ml of water were added and the mixture was stirred overnight at r.t. The reaction was diluted with KH$_2$PO$_4$ solution (0.5 N, 45 ml); pH was adjusted to 6 by addition of NaHCO$_3$ (aqueous saturated solution). The white solid (trityl alcohol) was filtered and washed with water. Tetrabutylammonium hydrogen phosphate (314 mg, 0.92 mmol) was added and an extraction with CH$_2$Cl$_2$ was performed. The combined organic layers were concentrated under reduced pressure to give 546 mg of an amorphous pale-brown solid. The structure of **8a** was confirmed by ^{1}H NMR analysis of the crude compound. (b) Cation exchange: this was realized by passing crude **8a**, dissolved in a 4:1 mixture of H$_2$O/acetone, through a column filled with Dowex 50W X 8 (3–4 g/100 mg of **8a**) resin. The eluted water solution was lyophilized and, owing to the presence of a certain amount of unexchanged **8a**, the resulting yellow powder was chromatographed on C-18 reverse-phase silica gel, using H$_2$O → H$_2$O/CH$_3$CN 9:1 as eluant. After lyophilization, 215 mg of a pure pale-yellow solid were obtained (49% yield from **7a**). M.p. 139–142°C (H$_2$O, dec.). ^{1}H NMR (65°C): δ 3.86 (3H, s, –OCH_3), 4.16–4.25 (2H, AB part of ABX system, –CH_2OSO$_2$NH$_2$, J_{AB} = 8.2, J_{AX} and J_{BX} = 5.2, 8.6 Hz), 4.59 (1H, centre of m, H_4), 5.23 (1H, d, H_3, J = 4.8 Hz), 6.76 (1H, s, H_5 of thiazole), 6.99 (2H, s, –SO$_2$NH_2), 7.35 (2H, very broad s, –NH_2), 9.10 (1H, very broad s, –NHCO–).

3.1.6. (3S,4S)-cis-3-[(t-Butoxycarbonyl)amino]-4-sulfamoyloxymethyl-2-azetidinone ***10a***

939 mg of **9** (4.34 mmol) were dissolved in dry CH$_3$CN (20 ml), cooled to 0°C and treated with Et$_3$N (1.74 ml, 12.48 mmol) and ClSO$_2$NH$_2$ (1.44 g, 12.46 mmol). As soon as the reaction began, a white solid precipitated. The resulting mixture was stirred at r.t. for 1 h. The solvent was removed in vacuo and the crude mixture was directly purified by chromatography, using AcOEt/ETP 6:4 → 100% AcOEt. The white solid (987 mg, 77% yield) was triturated with Et$_2$O. R_f 0.54 (AcOEt, det. C). M.p. 112.0–112.5°C (Et$_2$O). [α]$_D$ = +40.6° (*c* 1.08, MeOH). ^{1}H NMR (36°C): δ 1.41 (9H, s, –C(CH_3)$_3$), 3.90 (1H, dt, H_4, J = 5.3, 6.2 Hz), 4.12 (2H, d, –CH_2OSO$_2$NH$_2$, J = 6.4 Hz), 4.94 (1H, dd, H_3, J = 5.1, 9.2 Hz), 7.53 (2H, s, –SO$_2$NH_2), 7.65 (1H, d, –NHBoc, J = 9.2 Hz), 8.49 (1H, s, –NH of β-lactam).

3.1.7. (3S,4S)-cis-3-[(t-Butoxycarbonyl)amino]-4-(N-methyl)sulfamoyloxymethyl-2-azetidinone ***10b***

10b was prepared and purified following the procedure reported for **10a** using ClSO$_2$NHMe (the sulfamoyl chlorides (R ≠ H) were prepared according to Ref. [12]) (b.p. 107–110°C, p = 0.5 mbar). Yield: 83%. R_f 0.30 (AcOEt/PE 8:2, det. C). M.p. 126.5–128.0°C (Et$_2$O). [α]$_D$ = +29.3° (*c* 1.07, MeOH). ^{1}H NMR (45°C): δ 1.40 (9H, s, –C(CH_3)$_3$), 2.59 (3H, d, –NHCH_3, J = 4.9 Hz), 3.93 (1H, dt, H_4, J = 3.9, 5.2 Hz), 4.07 and 4.11 (2H, AB part of ABX system, –CH_2OSO$_2$NH–, J_{AB} = 10.3, J_{AX} and J_{BX} = 3.8, 9.2 Hz), 4.93 (1H, dd, H_3, J = 5.5, 9.2 Hz), 7.61 (1H, d, –NHBoc, J = 9.4 Hz), 7.73 (1H, q, –SO$_2$NHCH$_3$, J = 4.6 Hz), 8.43 (1H, s, –NH of β-lactam).

3.1.8. (3S,4S)-cis-3-[(t-Butoxycarbonyl)amino]-4-(N-butyl)sulfamoyloxymethyl-2-azetidinone ***10c***

10c was prepared and purified following the procedure reported for **10a** using ClSO$_2$NHnBu (the sulfamoyl chlorides (R ≠ H) were prepared according to Ref. [12]) (b.p. 125–128°C, p = 0.1 mbar). Yield: 70%. R_f 0.80 (AcOEt, det. C). M.p. 121.8–123.0°C (Et$_2$O). [α]$_D$ = +42.8° (*c* 1.11, MeOH). ^{1}H NMR (50°C): δ 0.88 (3H, t, –(CH$_2$)$_3$CH_3, J = 7.1 Hz), 1.41 (9H, s, –C(CH_3)$_3$), 1.26–1.52 (4H, m, –CH$_2$(CH_2)$_2$CH$_3$), 2.94 (2H, dt, –NHCH_2–, J = 5.9, 6.6 Hz), 3.90 (1H, dt, H_4, J = 5.1, 7.7 Hz), 4.09 and 4.11 (2H, AB part of ABX system, –CH_2OSO$_2$NH–, J_{AB} = 10.4, J_{AX} and J_{BX} = 2.1, 10.9 Hz), 4.92 (1H, dd, H_3, J = 5.3, 9.0 Hz), 7.57 (1H, broad s, –NHBoc), 7.79 (1H, t, –SO$_2$NHCH$_2$–, J = 5.3 Hz), 8.40 (1H, s, –NH of β-lactam).

3.1.9. (3S,4S)-cis-3-[(t-Butoxycarbonyl)amino]-4-[N-(2-fluoroethyl)]sulfamoyloxymethyl-2-azetidinone ***10d***

10d was prepared and purified following the procedure reported for **10a** using ClSO$_2$NHCH$_2$CH$_2$F (the sulfamoyl chlorides (R ≠ H) were prepared according to Ref. [12]) (b.p. 95–100°C, p = 0.01 mbar). Yield: 73%. R_f 0.38 (AcOEt, det. C). M.p. 129–130°C (Et$_2$O/AcOEt). [α]$_D$ = +36.4° (*c* 1.00, MeOH). ^{1}H NMR (50°C): δ 1.40 (9H, s, –C(CH_3)$_3$), 3.16–3.41 (2H, multiplicity of signal not analysable due to overlap with H$_2$O contained in DMSO-d$_6$, –NHCH_2–), 3.91 (1H, dt, H_4, J = 5.2, 7.6 Hz), 4.02–4.20 (2H, m, –CH_2OSO$_2$NH–), 4.47 (2H, dt, –CH_2F, J = 5.0, 47.3 Hz), 4.93 (1H, dd, H_3, J = 5.2, 9.4 Hz), 7.62 (1H, d, –NHBoc, J = 8.8 Hz), 8.24 (1H, t, –SO$_2$NHCH$_2$–, J = 5.3 Hz), 8.43 (1H, s, –NH of β-lactam).

3.1.10. (3S,4S)-cis-3-[(t-Butoxycarbonyl)amino]-4-[N-(2-methoxyethyl)]sulfamoyloxymethyl-2-azetidinone ***10e***

10e was prepared and purified following the procedure reported for **10a** using ClSO$_2$NHCH$_2$CH$_2$OMe (the sulfamoyl chlorides (R ≠ H) were prepared according to Ref. [12]) (b.p. 125–130°C, p = 0.5 mbar). Yield: 77%. R_f 0.61

(AcOEt, det. C). $[\alpha]_D = +33.2°$ (*c* 1.04, MeOH). ^{1}H NMR (50°C): δ 1.41 (9H, s, $-C(CH_3)_3$), 3.11 (2H, q, $-NHCH_2-$, $J = 5.8$ Hz), 3.27 (3H, s, $-OCH_3$), 3.41 (2H, t, $-CH_2OCH_3$, $J = 5.6$ Hz), 3.91 (1H, dt, H_4, $J = 5.5$, 7.0 Hz), 4.11 (2H, d, $-CH_2OSO_2NH-$, $J = 5.9$ Hz), 4.92 (1H, centre of m, H_3), 7.60 (1H, broad s, –N*H*Boc), 7.98 (1H, broad s, $-SO_2NHCH_2-$), 8.42 (1H, s, –N*H* of β-lactam).

3.1.11. (3S,4S)-cis-3-{(Z)-2-(Methoxyimino)-2-[2-(triphenylmethyl)aminothiazol-4-yl]acetamido}-4-sulfamoyloxymethyl-2-azetidinone **7a**

(a) Removal of the Boc group: 706 mg of **10a** (2.39 mmol) were suspended in dry CH_2Cl_2 (4 ml). After cooling to 0°C, trifluoroacetic acid (6 ml) was added dropwise and suddenly a solution was obtained. After 30 min the solvents were removed in vacuo. The residue was treated twice with 6 ml of toluene and evaporated to dryness. Finally, the crude product was dried at 5×10^{-2} mbar overnight. R_f 0.27 (AcOEt/MeOH 9:1, det. C). (b) Acylation reaction: a solution of the above prepared ammonium trifluoroacetate in 8 ml of dry DMF/CH_2Cl_2 2:1 was cooled to 0°C and treated with triethylamine (999 µl, 7.17 mmol). After a few minutes, dicyclohexylcarbodiimide (493 mg, 2.39 mmol), *N*-hydroxybenzotriazole (323 mg, 2.39 mmol) and 2-[2-(triphenylmethyl)aminothiazol-4-yl]-(*Z*)-2-(methoxyimino)acetic acid (1.06 g, 2.39 mmol) were added and the mixture was stirred at 0°C for 2 h and at r.t. for an additional 2 h. For the work-up the same procedure used for the preparation and purification of racemic **7a** was followed. Yield: 74%. Crystallization with AcOEt/PE gave a yellow solid. M.p.: compound decomposes before melting. $[\alpha]_D = +16.6°$ (*c* 1.16, MeOH).

3.1.12. (3S,4S)-cis-3-{(Z)-2-(Methoxyimino)-2-[2-(triphenylmethyl)aminothiazol-4-yl]acetamido}-4-(N-methyl)sulfamoyloxymethyl-2-azetidinone **7b**

The same procedure used for the preparation and purification of **7a** was followed. Yield: 52%. Crystallization with AcOEt/PE gave a pale-yellow solid. R_f 0.48 (AcOEt, det. A, B). M.p.: compound decomposes before melting. $[\alpha]_D = +68.6°$ (*c* 1.16, MeOH). ^{1}H NMR (r.t.): δ 2.55 (3H, d, $-NHCH_3$, $J = 4.8$ Hz), 3.82 (3H, s, $-OCH_3$), 3.93–4.12 (3H, m, $H_4 + -CH_2OSO_2NH-$), 5.20 (1H, dd, H_3, $J = 3.6$, 8.9 Hz), 6.73 (1H, s, H_5 of thiazole), 7.19–7.40 (15H, m, aromatics of trityl), 7.82 (1H, q, $-SO_2NH-$, $J = 4.9$ Hz), 8.73 and 8.84 (2H, 2 s, –N*H* of β-lactam and –N*H*Tr), 9.27 (1H, d, –N*H*CO–, $J = 8.8$ Hz).

3.1.13. (3S,4S)-cis-3-{(Z)-2-(Methoxyimino)-2-[2-(triphenylmethyl)aminothiazol-4-yl]acetamido}-4-(N-butyl)sulfamoyloxymethyl-2-azetidinone **7c**

The same procedure used for the preparation and purification of **7a** was followed. Yield: 56%. Trituration with Et_2O gave a pale-yellow solid. R_f 0.79 (AcOEt, det. A, B). M.p.: compound decomposes before melting. $[\alpha]_D = +18.7°$ (*c* 1.22, MeOH). ^{1}H NMR (50°C): δ 0.86 (3H, t, $-(CH_2)_3CH_3$, $J = 7.1$ Hz), 1.19–1.49 (4H, m, $-CH_2(CH_2)_2CH_3$), 2.92 (2H, dt, $-NHCH_2-$, $J = 5.9$, 6.7 Hz), 3.83 (3H, s, $-OCH_3$), 3.75–4.16 (3H, m, $H_4 + -CH_2OSO_2NH-$), 5.19 (1H, dd, H_3, $J = 3.6$, 8.6 Hz), 6.72 (1H, s, H_5 of thiazole), 7.19–7.43 (15H, m, aromatics of trityl), 7.78 (1H, t, $-SO_2NH-$, $J = 5.3$ Hz), 8.60 and 8.64 (2H, 2 s, –N*H* of β-lactam and –N*H*Tr), 9.16 (1H, d, –N*H*CO–, $J = 8.8$ Hz).

3.1.14. (3S,4S)-cis-4-[N-(2-Fluoroethyl)]sulfamoyloxymethyl-3-{(Z)-2-(methoxyimino)-2-[2-(triphenylmethyl)-aminothiazol-4-yl]acetamido}-2-azetidinone **7d**

The same procedure used for the preparation and purification of **7a** was followed. Yield: 61%. Trituration with Et_2O gave a pale-yellow solid. R_f 0.49 (AcOEt/PE 8:2, det. A, B). M.p.: compound decomposes before melting. $[\alpha]_D = +20.4°$ (*c* 1.0, MeOH). ^{1}H NMR (50°C): δ 3.25 (2H, ddt, $-NHCH_2-$, $J = 4.8$, 5.3, 26.5 Hz), 3.84 (3H, s, $-OCH_3$), 3.94–4.20 (3H, m, $H_4 + -CH_2OSO_2NH-$), 4.49 (2H, dt, $-CH_2F$, $J = 5.0$, 66.1 Hz), 5.19 (1H, dd, H_3, $J = 4.0$, 8.6 Hz), 6.72 (1H, s, H_5 of thiazole), 7.21–7.40 (15H, m, aromatics of trityl), 8.20 (1H, broad t, $-SO_2NH-$, $J = 5.2$ Hz), 8.60 and 8.64 (2H, 2 s, –N*H* of β-lactam and –N*H*Tr), 9.16 (1H, d, –N*H*CO–, $J = 8.7$ Hz).

3.1.15. (3S,4S)-cis-4-[N-2-Methoxyethyl)]sulfamoyloxymethyl-3-{(Z)-2-(methoxyimino)-2-[2-(triphenylmethyl)aminothiazol-4-yl]acetamido}-2-azetidinone **7e**

The same procedure used for the preparation and purification of **7a** was followed. Yield: 60%. Trituration with Et_2O gave a pale-yellow solid. R_f 0.34 (AcOEt, det. A, C). M.p.: compound decomposes before melting. $[\alpha]_D = +23.1°$ (*c* 1.24, MeOH). ^{1}H NMR (50°C): δ 3.09 (2H, dt, $-NHCH_2-$, $J = 5.3$, 5.8 Hz), 3.25 (3H, s, $-CH_2OCH_3$), 3.38 (2H, t, $-CH_2CH_3$, $J = 5.7$ Hz), 3.84 (3H, s, $=NOCH_3$), 3.92–4.19 (3H, m, $H_4 + -CH_2OSO_2NH-$), 5.20 (1H, dd, H_3, $J = 3.7$, 8.8 Hz), 6.72 (1H, s, H_5 of thiazole), 7.19–7.40 (15H, m, aromatics of trityl), 7.94 (1H, broad t, $-SO_2NH-$, $J = 5.7$ Hz), 8.59 and 8.64 (2H, 2 s, –N*H* of β-lactam and –N*H*Tr), 9.14 (1H, d, –N*H*CO–, $J = 8.6$ Hz).

3.1.16. (3S,4S)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-(sulfamoyloxymethyl)-2-oxoazetidine-1-sulfonic acid, sodium salt **2a**

(a) Preparation of **8a**: the same procedure used for the synthesis of racemic **8a** was followed. However, the best results for trityl deblocking were obtained by warming the quenched solution at 40°C for 3 h instead of overnight stirring at r.t. As above, the extraction of the crude product with an organic solvent (CH_2Cl_2, if necessary in the presence of 10% MeOH) was realized on the tetrabutylammonium salt. Crude **8a** was purified by chromatography on silica gel with 100% AcOEt → AcOEt/MeOH 8:2. In this way a mixture of **8a** and **11a** (the latter usually prevailed and sometimes was the only recovered product) was isolated. (b) Cation exchange: the procedure used for racemic **8a** was followed. Usually **2a** obtained in this way was pure enough and did not need to be

purified again over C-18 reverse-phase silica gel. Yield: 40% from **7a**. $R_{f(11a)}$ 0.07 (AcOEt, det. A, C). M.p. 170–175°C (H_2O, dec., pale-yellow solid).

3.1.17. (3S,4S)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-(N-methyl)sulfamoyloxymethyl-2-oxoazetidine-1-sulfonic acid, sodium salt **2b**

The same procedure and purification described above for **2a** was followed. Yield: 36% from **7b**. $R_{f(11b)}$ 0.19 (CH_2Cl_2/MeOH 9:1, det. A, C). M.p. 135–140°C (H_2O, dec., pale-yellow solid). ^{1}H NMR (45°C): δ 2.56 (3H, s, –NHCH_3), 3.85 (3H, s, –OCH_3), 4.10–4.24 (2H, m, –CH_2OSO$_2$NH–), 4.46–4.57 (1H, m, H_4), 5.27 (1H, broad s, H_3), 6.73 (1H, s, H_5 of thiazole), 7.12 (2H, s, –NH_2 of thiazole), 7.72 (1H, broad s, –SO$_2$NH–), 9.29 (1H, d, –NHCO–, J = 4.0 Hz).

3.1.18. (3S,4S)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-(N-butyl)sulfamoyloxymethyl-2-oxoazetidine-1-sulfonic acid, sodium salt **2c**

The same procedure and purification described above for **2a** was followed. Yield: 93% from **7c**. $R_{f(11c)}$ 0.27 (AcOEt/MeOH 85:15, det. A, C). M.p. 170–180°C (H_2O, dec., pale-yellow solid). ^{1}H NMR (45°C): δ 0.86 (3H, t, –(CH$_2$)$_3$CH_3, J = 7.2 Hz), 1.52–1.22 (4H, m, –CH$_2$(CH_2)$_2$CH$_3$), 2.91 (2H, t, –NHCH_2–, J = 7.0 Hz), 3.85 (3H, s, –OCH_3), 4.12 and 4.18 (2H, AB part of AB system, –CH_2OSO$_2$NH–, J_{AB} = 8.6, J_{AX} and J_{BX} = 1.1, 5.8 Hz), 4.55 (1H, centre of m, H_4), 5.26 (1H, dd, H_3, J = 5.0, 9.1 Hz), 6.72 (1H, s, H_5 of thiazole), 7.07 (2H, s, –NH_2 of thiazole), 7.77 (1H, broad s, –SO$_2$NH–), 9.23 (1H, d, –NHCO–, J = 9.1 Hz).

3.1.19. (3S,4S)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-[N-(2-fluoroethyl)]-sulfamoyloxymethyl-2-oxoazetidine-1-sulfonic acid, sodium salt **2d**

The same procedure and purification described above for **2a** was followed. Yield: 76% from **7d**. $R_{f(11d)}$ 0.50 (AcOEt/MeOH 8:2, det. A, C). M.p. 123–130°C (H_2O, dec., very pale-yellow solid). ^{1}H NMR (50°C): δ 3.15–3.33 (2H, multiplicity of signal not analysable due to overlap with H_2O contained in DMSO-d$_6$, –NHCH_2–), 3.86 (3H, s, –OCH_3), 4.16 and 4.22 (2H, AB part of AB system, –CH_2OSO$_2$NH–, J_{AB} = 8.1, J_{AX} and J_{BX} = 5.0, 10.0 Hz), 4.47–4.57 (1H, m, H_4), 4.44 (2H, dt, –CH_2F, J = 5.1, 47.3 Hz), 5.26 (1H, dd, H_3, J = 5.1, 9.0 Hz), 6.73 (1H, s, H_5 of thiazole), 7.08 (2H, s, –NH_2 of thiazole), 8.19 (1H, broad s, –SO$_2$NH–), 9.23 (1H, d, –NHCO–, J = 9.2 Hz).

3.1.20. (3S,4S)-cis-3-[2-(2-Aminothiazol-4-yl)-(Z)-2-(methoxyimino)acetamido]-4-[N-(2-methoxyethyl)]-sulfamoyloxymethyl-2-oxoazetidine-1-sulfonic acid, sodium salt **2e**

The same procedure and purification described above for **2a** was followed. Yield: 52% from **7e**. $R_{f(11e)}$ 0.14 (AcOEt/MeOH 9:1, det. A, C). M.p. 145–162°C (H_2O, dec., white solid). ^{1}H NMR (50°C): δ 3.08 (2H, t, –NHCH_2–, J = 5.9), 3.25 (3H, s, –CH$_2$OCH_3), 3.39 (2H, t, –CH_2OCH$_3$, J = 7.2 Hz), 3.85 (3H, s, =NOCH_3), 4.11–4.24 (2H, m, –CH_2OSO$_2$NH–), 4.47–4.59 (1H, m, H_4), 5.26 (1H, dd, H_3, J = 4.8, 9.2 Hz), 6.73 (1H, s, H_5 of thiazole), 7.09 (2H, s, –NH_2 of thiazole), 7.94 (1H, broad s, –SO$_2$NH–), 9.23 (1H, d, –NHCO–, J = 9.2 Hz).

3.2. Bacteriological assay

All compounds were tested for in vitro antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *E. coli* hyperpermeable strain DC2, *E. coli* β-lactamase lacking LIEGI, *Pseudomonas aeruginosa* ATCC 27853, *Ps. aeruginosa* β-lactamase lacking hyperpermeable strain VR5. Minimal inhibitory concentrations (MICs) were determined by an agar dilution technique using Müller-Hinton 2 agar (bioMerieux). The inoculum used was 10^4 CFU per spot deposited on the agar by a Denley Multipoint Inoculator. The compounds were diluted in the test medium in order to obtain the required 64–0.03 μg/ml range. MICs were noted after 18 h of incubation at 35°C.

Table 1
In vitro antibacterial activity of monobactam sulfamates **2a–e**

Compound	R	MIC (μg/ml) [a]					
		S.a.	E.c.	E.c. DC2	E.c. L.	Ps.a.	Ps.a. β-
(*rac*)-2a	H	>64	4	1	64	>64	4
2a	H	>32	4	1	8	>32	
2b	Me	>32	1	0.12	2	>32	
2c	n-Bu	>32	8	0.25	16	>32	>32
2d	CH_2CH_2F	>32	2	0.06	2	>32	8
2e	CH_2CH_2OMe	>32	4	0.06	4	>32	4
1a	CH_2CO_2H	>64	0.12	<0.06	0.06	2	<0.06
1b	Me	>64	0.06	<0.06	0.06	8	0.06

[a] The MICs were determined by a standard dilution method.
S.a. = *Staphylococcus aureus* ATCC 29213; E.c. = *Escherichia coli* ATCC 25922; E.c. DC2 = *E. coli* hyperpermeable strain; E.c. L. = *E. coli* LIEGI, β-lactamase lacking; Ps.a. = *Pseudomonas aeruginosa* ATCC 27853; Ps.a. β- = *Ps. aeruginosa* VR5, β-lactamase lacking hyperpermeable strain.

4. Biological results and conclusions

The results of the in vitro antibacterial evaluation are reported in Table 1, in comparison with the activity of carumonam **1a** and its methoxyimino derivative **1b** (compounds **1a,b** were prepared according to Ref. [10]). All optically pure compounds showed an enhanced, although very moderate, activity against *S. aureus* with respect to **1a,b**. They were all active against *E. coli*, the best being **2b** and **2d**. The results obtained with *E. coli* lacking β-lactamase and with the hyperpermeable strain indicate that these compounds are stable to β-lactamases, but that they cross the outer membrane of the bacterium with difficulty. Again, all compounds were unable to cross the membrane of *Ps. aeruginosa*, with the consequence that they are inactive. Finally, the activity against the β-lactamase hyperpermeable *Pseudomonas* strain was moderate.

In conclusion, the substitution of a carbamoyloxymethyl with a sulfamoyloxymethyl group gives a new class of monobactams with a certain antibacterial activity. Nevertheless, they turned out to be less active when compared with both **1a** and **1b**.

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