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Synthesis and antimicrobial activity of coumarin 7-substituted cephalosporins and sulfones

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Abstract

Some coumarin 7-substituted cephalosporins and related sulfones were prepared and an antimicrobial assay was performed. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) carried out on cephalosporins showed a potential activity of some of the synthesized compounds against Gram-positive microorganisms. The tests performed on the corresponding sulfones showed no significant activity, neither as antimicrobial agents nor as inhibitors of β-lactamase. An association of sulfone **6a** with ampicillin was observed to inhibit Gram-positive microorganisms with a lower MIC than for ampicillin alone.

Keywords: Carbon suboxide; Cephalosporins; Sulfones; Antimicrobial activity

1. Introduction

This work on cephalosporins resumes the scheme of our previous study on the corresponding penicillins [1]. Starting from the 7-aminocephalosporanic acid (7-ACA), instead of 6-aminopenicillanic acid (6-APA), we conveniently synthesized the cephalosporins **5a–g** by carbon suboxide, thus completing the above series with the corresponding sulfones **6a–g**.

The classical method for the preparation of semisynthetic cephalosporins consists of the reaction of 7-ACA with activated acyl derivatives. Using this method, Chinese authors [2–5] prepared some of the cephalosporins **5a–g** by four reaction steps since the substituted coumarin acids needed for their synthesis were not commercially available. Thus, these acids must be synthesized according to the Knoevenagel reaction.

Instead of the above-mentioned method, we prepared the cephalosporins **5b–g** by direct reaction of carbon suboxide **4** with the Schiff bases of 7-ACA, **3b–g**; the latter were obtained from the reaction of 7-ACA **2** with the substituted 2-hydroxybenzaldehydes **1b–g**. Only the cephalosporin **5a** was prepared following the classical method, because the coumarin acid **1a** was commercially available.

The microbiological trials (minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)) on the cephalosporins were carried out using ATCC bacterial strains obtained from the Pasteur Institute of Paris.

It is known in the literature that, when conveniently modified, the sulfones of 7-aminocephalosporanic acid are potent inhibitors of the human leucocyte elastase [6–10] and are used in the treatment of pulmonary emphysema, cystic fibrosis and rheumatoid arthritis [6–10].

The sulfones **6a–g** synthesised by us have been submitted to the same tests as the corresponding cephalosporins **5a–g** and have also been analysed as β-lactamase inhibitors in a Gram-negative penicillinase-producing bacterial strain, isolated from pathological material at the Institute of Bacteriology of the University Hospital of Strasbourg.

2. Chemistry

As regards the synthesis of cephalosporin **5a** the acyl chloride route was performed. Starting from the coumarin acid **1a** the acyl chloride **1′a** was obtained by reaction with $SOCl_2$;

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the subsequent reaction with 7-ACA **2** led to the cephalosporin **5a** (Scheme 1).

For the preparation of cephalosporins **5b–g** we carried out the reaction of C_3O_2 **4** with the substituted Schiff bases **3b–g** according to a previous method [11,12]. The Schiff bases **3b–g** were obtained from the reaction of 7-ACA **2** with the substituted 2-hydroxybenzaldehydes **1b–g** (Scheme 2).

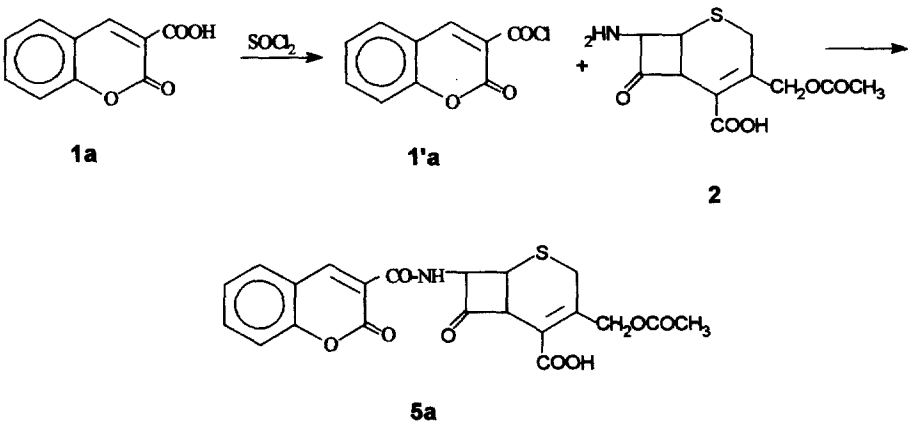
As regards sulfones **6a–g**, oxidation of the sulfur of the corresponding cephalosporins **5a–g** was carried out using two equivalents of *m*-chloroperbenzoic acid (*m*-CPBA) [6] (Scheme 3).

More details are reported in Section 3.

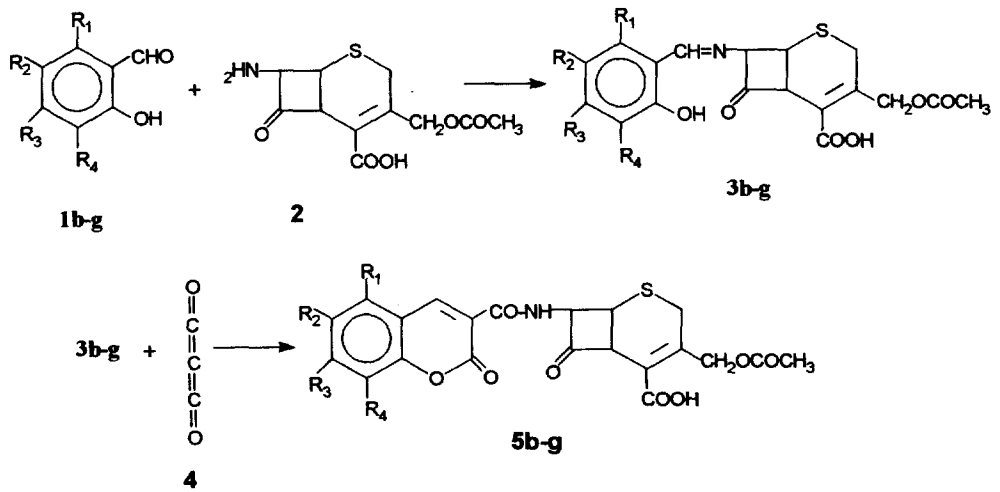
3. Experimental

3.1. Chemistry

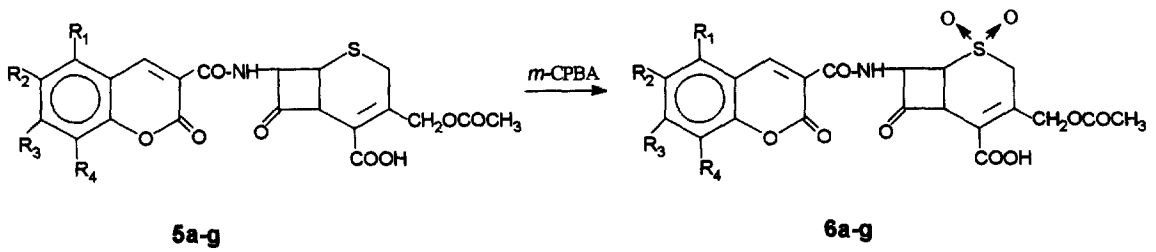
Melting points were determined on a Köfler apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer using NaCl mulls. The ^1H NMR spectra were recorded on a Varian Unity 300 instrument and the chemical shifts refer to tetramethylsilane. Elemental analyses (C, H, N) were performed on a Carlo Erba model 1106 elemental analyser and were within $\pm 0.4\%$ of theoretical values.



Scheme 1. Synthesis of cephalosporin **5a**.



Scheme 2. Preparation of cephalosporins **5b–g**.



Scheme 3. Preparation of sulfones **6a–g**.

Reagent-grade commercially available reagents and solvents were used. Carbon suboxide was prepared from pyrolisis of d-*O*-acetyltartaric anhydride [13]. Thionyl chloride was distilled (77°C, 760 mmHg) before use and technical *m*-CPBA was washed with a phosphate buffer (pH 7.5).

The Schiff bases **3b–g**, the cephalosporins **5a–g**, the sulfones **6a–g** and the intermediate **1'a** were identified by analytical and spectroscopic methods. These data are reported in Tables 1 and 2.

7-ACA was kindly supplied by Bristol Meyer Squibb (Sermoneta, Italy).

All compounds and solvents, when required, were rigorously dried before use according to standard methods [14].

3.1.1. General procedure for the preparation of the Schiff bases 3b–g

A solution of hydroxybenzaldehydes **1b–g** (9.6 mmol) in the least possible volume of DMSO, was added under stirring and at room temperature to a solution of 2.70 g (9.9 mmol) of 7-ACA **2** in 100 ml of DMSO and 1 ml of glacial acetic acid.

At completion the mixture was kept at 50°C under stirring for 24 h. At the end of the reaction, water was added until the quantitative precipitation of the Schiff bases was performed. The crude solid was filtered under vacuum, washed twice with deionized water and crystallized from acetone to yield the Schiff bases **3b–g**. Analytical and spectral data are reported in Tables 1 and 2.

3.1.2. Preparation of coumarin-3-carbonyl chloride 1'a

Coumarin-3-carboxylic acid **1a** (0.015 mol) and thionyl chloride (0.045 mol) were refluxed without a solvent and under stirring for 24 h. The excess of thionyl chloride was distilled and the crude residue washed twice with ether (20 ml) to give chloride **1'a** in an almost quantitative yield (98%). The analytical and spectral data were in accordance with the literature [1].

3.1.3. General procedure for the preparation of cephalosporin 5a

A solution of 7-ACA **2** (2.72 g, 10 mmol) in water (40 ml) containing sodium hydrogen carbonate (2.1 g, 25 mmol) and acetone (30 ml) was cooled to 0–5°C, stirred and treated with a solution of chloride **1'a** (10 mmol) in acetone (20 ml). The mixture was maintained at 0–5°C for 30 min under stirring, while the pH was kept at 7 and acidity was buffered with sodium hydrogen carbonate. The acetone was removed under reduced pressure, the aqueous layer acidified to pH 2 with 1N hydrochloric acid and twice extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was washed with diethyl ether and then crystallized from acetone to give cephalosporin **5a**. The analytical and spectral data are reported in Tables 1 and 2.

3.1.4. General procedure for the preparation of cephalosporins 5b–g

80 mmol (0.5 ml) of C_3O_2 **4**, measured at −75°C, were added at room temperature and under stirring to a suspension of the Schiff bases **3b–g** (80 mmol) in 500 ml of anhydrous acetone. The mixture was kept under stirring and at room temperature for 72 h. At the end the unreacted Schiff bases were filtered off and the resulting solution was evaporated under reduced pressure to yield a crude residue. The latter

Table 1
Physicochemical data of compounds **3b–g**, **5a–g**, **6a–g**

Comp.	M.p. (°C)	Yield (%)	Molecular formula	R_1	R_2	R_3	R_4
3b	190–192	80	$C_{16}H_{13}N_2O_6BrS$	H	Br	H	H
3c	188–190	70	$C_{16}H_{12}N_2O_6Br_2S$	H	Br	H	Br
3d	180–183	72	$C_{16}H_{13}N_2O_6ClS$	H	Cl	H	H
3e	185–187	77	$C_{16}H_{12}N_2O_6Cl_2S$	H	Cl	H	Cl
3f	165–167	68	$C_{17}H_{16}N_2O_7S$	H	H	OCH_3	H
3g	168–170	65	$C_{18}H_{18}N_2O_8S$	OCH_3	H	OCH_3	H
5a	160–161	72	$C_{20}H_{16}N_2O_8S$	H	H	H	H
5b	155–157	67	$C_{20}H_{15}N_2O_8BrS$	H	Br	H	H
5c	215–217	45	$C_{20}H_{14}N_2O_8Br_2S$	H	Br	H	Br
5d	157–158	70	$C_{20}H_{15}N_2O_8ClS$	H	Cl	H	H
5e	137–139	55	$C_{20}H_{14}N_2O_8Cl_2S$	H	Cl	H	Cl
5f	158–160	40	$C_{21}H_{18}N_2O_9S$	H	H	OCH_3	H
5g	167–169	73	$C_{22}H_{20}N_2O_{10}S$	OCH_3	H	OCH_3	H
6a	205–206	78	$C_{20}H_{16}N_2O_{10}S$	H	H	H	H
6b	225–226	69	$C_{20}H_{15}N_2O_{10}BrS$	H	Br	H	H
6c	140–142	80	$C_{20}H_{14}N_2O_{10}Br_2S$	H	Br	H	Br
6d	272–274	59	$C_{20}H_{15}N_2O_{10}ClS$	H	Cl	H	H
6e	190–192	78	$C_{20}H_{14}N_2O_{10}Cl_2S$	H	Cl	H	Cl
6f	220–221	54	$C_{21}H_{18}N_2O_{11}S$	H	H	OCH_3	H
6g	235–236	85	$C_{22}H_{20}N_2O_{12}S$	OCH_3	H	OCH_3	H

Table 2
Spectral data of compounds **3b–g**, **5a–g**, **6a–g**

Comp.	IR (nujol) (cm^{-1})	^1H NMR (DMSO-d_6) δ (ppm)
3b	3620–3150, 1780, 1740, 1630	13.74 (s, 1H, COOH, D_2O exch), 10.95 (s, 1H, OH, D_2O exch), 8.65 (s, 1H, CH=), 7.65–6.90 (m, 3H, arom), 6.00 (s, 1H, H-7), 5.22 (s, 2H, H-4,a,b), 5.02–4.98 (d, 1H, H-6), 4.96–4.64 (d, 1H, H-6), 3.69–3.48 (q, 2H, CH_2OCO), 2.46 (s, 3H, CH_3)
3c	3700–3150, 1780, 1725, 1630	13.33 (s, 1H, COOH, D_2O exch), 11.04 (s, 1H, OH, D_2O exch), 8.83 (s, 1H, CH=), 7.85–7.60 (m, 2H, arom), 5.98 (s, 1H, H-7), 5.20 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.69–4.64 (d, 1H, H-6), 3.68–3.47 (q, 2H, CH_2OCO), 2.22 (s, 3H, CH_3)
3d	3600–3100, 1780, 1725, 1630	13.19 (s, 1H, COOH, D_2O exch), 11.04 (s, 1H, OH, D_2O exch), 8.65 (s, 1H, CH=), 7.60–6.85 (m, 3H, arom), 6.00 (s, 1H, H-7), 5.23 (s, 2H, H-4,a,b), 5.03–4.98 (d, 1H, H-6), 4.70–4.65 (d, 1H, H-6), 3.76–3.49 (q, 2H, CH_2OCO), 2.47 (s, 3H, CH_3)
3e	3600–3100, 1780, 1730, 1630	13.65 (s, 1H, COOH, D_2O exch), 11.10 (s, 1H, OH, D_2O exch), 8.90 (s, 1H, CH=), 7.60–7.40 (m, 2H, arom), 6.01 (s, 1H, H-7), 5.21 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.68–4.64 (d, 1H, H-6), 3.68–3.48 (q, 2H, CH_2OCO), 2.45 (s, 3H, CH_3)
3f	3650–3130, 1780, 1740, 1625	13.40 (s, 1H, COOH, D_2O exch), 10.90 (s, 1H, OH, D_2O exch), 8.60 (s, 1H, CH=), 7.40–6.35 (m, 3H, arom), 5.98 (s, 1H, H-7), 5.20 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.69–4.64 (d, 1H, H-6), 3.70 (s, 3H, OCH_3), 3.69–3.49 (q, 2H, CH_2OCO), 2.22 (s, 3H, CH_3)
3g	3630–3150, 1780, 1735, 1630	13.00 (s, 1H, COOH, D_2O exch), 10.97 (s, 1H, OH, D_2O exch), 8.65 (s, 1H, CH=), 7.37–6.40 (m, 2H, arom), 5.98 (s, 1H, H-7), 5.21 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.68–4.64 (d, 1H, H-6), 3.93–3.85 (m, 6H, $2OCH_3$), 3.78–3.54 (q, 2H, CH_2OCO), 2.00 (s, 3H, CH_3)
5a	3280, 3650–3100, 1780, 1720, 1710, 1670, 1610	9.33–9.30 (d, 1H, NH, D_2O exch), 8.91 (s, 1H, CH=), 8.00–7.40 (m, 4H, arom), 6.03–5.98 (m, 1H, H-7), 5.22–5.21 (d, 2H, H-4, a,b), 5.02–498 (d, 1H. H-6), 4.69–4.65 (d, 1H, H-6), 3.69–3.49 (q, 2H, $CH_2OCO + CH_2$), 1.99 (s, 3H, CH_3)
5b	3680–3300, 3320, 1780, 1720–1700, 1660, 1640, 1600	13.74 (s, 1H, COOH, D_2O exch), 9.29–9.26 (d, 1H, NH, D_2O exch), 8.86 (s, 1H, CH=), 8.25–7.45 (m, 3H, arom), 6.00 (s, 1H, H-7), 5.22 (s, 2H, H-4 a,b), 5.02–4.98 (d, 1H, H-6), 4.69–4.64 (d, 1H, H-6), 3.69–3.48 (q, 4H, $CH_2OCO + CH_2$), 2.46 (s, 3H, CH_3)
5c	3640–3350, 3300, 1780–1700, 1660, 1610	13.33 (s, 1H, COOH, D_2O exch), 9.20–9.18 (d, 1H, NH, D_2O exch), 8.80 (s, 1H, CH=), 8.60–8.12 (m, 2H, arom), 5.98 (s, 1H, H-7), 5.20 (s, 2H, H-4, a,b), 5.01–4.97 (d, 1H H-6), 4.69–4.64 (d, 1H, H-6), 3.68–3.47 (q, 2H, CH_2OCO), 2.22 (s, 3H, CH_3)
5d	3600–3380, 3280, 1780, 1730–1700, 1650, 1600	13.19 (s, 1H, COOH, D_2O exch), 9.30–9.28 (d, 1H, NH, D_2O exch), 8.88 (s, 1H, CH=), 8.14–7.53 (m, 3H, arom), 6.00 (s, 1H, H-7), 5.23 (s, 2H, H-4,a,b), 5.03–4.98 (d, 1H, H-6), 4.70–4.65 (d, 1H, H-6), 3.76–3.49 (q, 4H, $CH_2OCO + CH_2$), 2.47 (s, 3H, CH_3)
5e	3500–3440, 3300, 1780–1700, 1660–1640, 1620	13.65 (s, 1H, COOH, D_2O exch), 9.20–9.17 (d, 1H, NH, D_2O exch), 8.81 (s, 1H, CH=), 8.09–7.96 (d, 2H, arom), 6.01 (s, 1H, H-7), 5.21 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.68–4.64 (d, 1H, H-6), 3.68–3.48 (q, 4H, CH_2OCO), 2.45 (s, 3H, CH_3)
5f	3670–3300, 3310, 1780, 1720, 1700, 1650, 1610	13.40 (s, 1H, COOH, D_2O exch), 9.29–9.25 (d, 1H, NH, D_2O exch), 8.70 (s, 1H, CH=), 7.60–7.00 (m, 3H, arom), 5.98 (s, 1H, H-7), 5.20 (s, 2H, H-4,a,b), 5.01–4.97 (d, 1H, H-6), 4.69–4.64 (d, 1H, H-6), 3.80 (s, 3H, OCH_3), 3.69–3.47 (q, 4H, CH_2OCO), 2.22 (s, 3H, CH_3)
5g	3600–3480, 3280, 1780, 1715, 1640, 1605	13.00 (s, 1H, COOH, D_2O exch), 9.21–9.18 (d, 1H, NH, D_2O exch), 8.74–8.72 (d, 1H, CH=), 6.67–6.47 (m, 2H, arom), 6.02–5.98 (d, 1H, H-7), 5.21–5.19 (d, 1H, H-6), 5.01–4.97 (d, 1H, H-2), 4.68–4.64 (d, 1H, H-2), 3.93–3.85 (m, 6H, OCH_3), 3.78–3.54 (q, 2H, CH_2OCO), 2.00 (s, 1H, CH_3)
6a	3640–3300, 3250, 1800, 1740–1700	9.7–9.66 (d, 1H, NH, D_2O exch), 8.99 (s, 1H, CH=), 8.02–7.4 (m, 4H, arom), 6.40–6.34 (m, 1H, H-7), 5.49–5.47 (d, 1H, H-6), 5.08–5.05 (d, 1H, H-2), 4.64–4.60 (d, 1H, H-2), 4.41–4.18 (q, 2H, CH_2OCO), 1.99 (s, 3H, CH_3)
6b	3600–3450, 3260, 1790, 1720, 1630	9.39–9.35 (d, 1H, NH, D_2O exch), 8.92 (s, 1H, CH=), 6.27–6.22 (m, 1H, H-7), 5.20–5.16 (d, 1H, H-2), 5.01–4.99 (d, 1H, H-6), 4.62–4.57 (d, 1H, H-2), 3.95–3.57 (q, 2H, CH_2OCO), 2 (s, 3H, CH_3)
6c	3650–3480, 3280, 1780, 1730, 1610	9.60–9.56 (d, 1H, NH, D_2O exch), 8.91 (s, 1H, CH=), 8.30–8.28 (q, 2H, arom), 6.41–6.36 (m, 1H, H-7), 5.48–5.47 (d, 1H, H-6), 5.09–5.04 (d, 1H, H-2), 4.63–4.60 (d, 1H, H-2), 4.42–4.21 (q, 2H, CH_2OCO), 2 (s, 1H, CH_3)
6d	3640–3400, 3300, 1780, 1710, 1620	13.67 (s, 1H, COOH, D_2O exch), 9.35–9.32 (d, 1H, NH, D_2O exch), 8.88 (s, 1H, CH=), 8.11–7.47 (m, 3H, arom), 6.24–6.19 (m, 1H, H-7), 5.16–5.12 (d, 1H, H-2), 4.98–4.96 (d, 1H, H-6), 4.58–4.54 (d, 1H, H-2), 3.93–3.54 (q, 2H, CH_2OCO), 1.96 (s, 3H, CH_3)
6e	3650–3480, 3250, 1790, 1730, 1615	9.58–9.55 (d, 1H, NH, D_2O exch), 8.92 (s, 1H, CH=), 8.13–8.08 (d, 2H, arom), 6.54–6.51 (m, 1H, H-7), 5.48–5.42 (d, 1H, H-2), 5.03–4.99 (d, 1H, H-6), 4.62–4.60 (d, 1H, H-2), 4.41–4.26 (q, 2H, CH_2OCO), 1.99 (s, 3H, CH_3)
6f	3680–3450, 3280, 1800, 1720, 1630	9.70–9.67 (d, 1H, NH, D_2O exch), 8.98 (s, 1H, CH=), 7.98–7.95 (d, 1H, arom), 7.16–7.06 (m, 2H, arom), 6.45–6.39 (m, 1H, H-7), 5.52–5.50 (d, 1H, H-6), 5.13–5.09 (d, 1H, H-2), 4.68–4.63 (d, 1H, H-2), 4.46–4.22 (q, 2H, CH_2OCO), 3.92 (s, 3H, OCH_3), 2.05 (s, 3H, CH_3)
6g	3650-3450, 3300, 1790, 1730, 1620	9.60–9.56 (d, 1H, NH, D_2O= exch), 8.81 (s, 1H, arom), 6.71–6.51 (m, 2H, arom), 6.39–6.34 (m, 1H, H-7), 5.47–5.45 (d, 1H, H-6), 5.08–5.04 (d, 1H, H-2), 4.63–4.58 (d, 1H, H-2), 4.41–4.17 (q, 2H, CH_2OCO), 3.93–3.85 (q, 6H, OCH_3), 2 (s, 3H,CH_3)

was purified and crystallized from acetone to give the cephalosporins **5b–g**. The analytical and spectral data are reported in Tables 1 and 2.

*3.1.5. General procedure for the preparation of the cephem sulfones **6a–g***

Cephalosporins **5a–g** (3 mmol) and dry acetone (50 ml) were placed in a 250 ml flask and the resulting mixture cooled under nitrogen to 0°C. *m*-CPBA (6.4 mmol, 95%, 1.1 g) dissolved in 20 ml of dry acetone was added, the cooling bath was removed and the mixture kept at room temperature under stirring for 5 h. The volume of the reaction mixture was concentrated (60%) under reduced pressure and the precipitated colourless solid was filtered off and washed with CH_2Cl_2 to give sulfones **6a–g**. The analytical and spectral data are reported in Tables 1 and 2.

3.2. Microbiology

3.2.1. Strains

Cephalosporins **5a–g** and sulfones **6a–g** were tested against a reference strain of *Staphylococcus aureus* (ATCC 25923), a reference strain of *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) obtained from the Pasteur Institute of Paris.

A strain of ampicillin resistant *S. aureus* and a strain of extended spectrum β-lactamase-producing *Klebsiella pneumoniae* were isolated from pathological material at the Institute of Bacteriology of the University Hospital of Strasbourg.

Organisms were grown overnight in Müller-Hinton broth [15] and diluted to produce a final inoculum of 1×10^7 cfu/ml.

3.2.2. Antibacterial activity

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of all synthesized compounds (cephalosporins **5a–g** and sulfones **6a–g**) were determined using the reference method of broth dilution [16].

To prepare stock solutions, all compounds were dissolved in dimethylsulfoxide.

The final concentrations tested for each compounds ranged from 0.125 to 256 μg/ml.

The inhibitory activity of sulfones **6a–g** was tested at a fixed concentration of 4 μg/ml with varying concentrations of the associated antibiotic, either cefotaxime (0.125–256 μg/ml) against an extended spectrum of β-lactamase-producing *K. pneumoniae*, or ampicillin (0.125–256 μg/ml) against a penicillinase-producing strain of *S. aureus*.

4. Results and discussion

MICs and MBCs of the different compounds tested against the reference strains are shown in Table 3.

The antimicrobial test performed on cephalosporins **5a–g** confirmed the potential activity of these compounds against Gram-positive microorganisms.

As regards the activity of the different sulfones, they showed no significant activity, neither as antimicrobial agents (MIC > 256 μg/ml) nor as inhibitors of β-lactamase when screened in association with cefotaxime.

Considering that the sulfones are inactive as β-lactamase inhibitors and that the cephalosporins are also inactive on Gram-negative microorganisms, while they are slightly active against *S. aureus*, two parallel MIC sensitivity tests were carried out on a strain of ampillicin-resistant *S. aureus*. In one, ampicillin was used as an antibiotic, while in the other an association of ampicillin + sulfone **6a** was used. This association was observed to inhibit Gram-positive microorganisms with a lower MIC than for ampicillin. The MIC of ampicillin alone against a penicillinase-producing strain of *S. aureus* was 128 μg/ml. When associated with 4 μg/ml of sulfone **6a** it fell to 64 μg/ml.

Table 3
MICs and MBCs of compounds **5a–g** and **6a–g** against the reference strains

Comp.	MIC (μg/ml)			MBC (μg/ml)		
	S. aureus	*E. coli*	*Ps. aeruginosa*	*S. aureus*	*E. coli*	*Ps. aeruginosa*
5a	16	> 100	> 100	32	> 100	> 100
5b	16	> 100	> 100	32	> 100	> 100
5c	64	> 100	> 100	> 100	> 100	> 100
5d	> 100	> 100	> 100	> 100	> 100	> 100
5e	4	> 100	> 100	8	> 100	> 100
5f	64	> 100	> 100	64	> 100	> 100
5g	64	> 100	> 100	64	> 100	> 100
6a	> 100	> 100	> 100	> 100	> 100	> 100
6b	> 100	> 100	> 100	> 100	> 100	> 100
6c	> 100	> 100	> 100	> 100	> 100	> 100
6d	> 100	> 100	> 100	> 100	> 100	> 100
6e	> 100	> 100	> 100	> 100	> 100	> 100
6f	> 100	> 100	> 100	> 100	> 100	> 100
6g	> 100	> 100	> 100	> 100	> 100	> 100

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