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Synthesis and activity of cephalosporins containing an oxyiminomethylene functionality in the *ortho*-position of a phenyl- or phenoxyacetic acid C-7 side chain substituent

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Abstract

A new series of cephalosporins having in the C-7 side chain a phenyl- or a phenoxyacetamido group bearing an oxyiminomethyl function in the *ortho*-position of the aromatic ring was prepared. Their in vitro activity was tested against both Gram + and Gram − strains. © 1998 Elsevier Science S.A. All rights reserved.

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1. Introduction

As is well known, cephalosporin antibiotics exert their intrinsic activity by inhibiting the biosynthesis of cell wall of many bacteria species [1–3]. Natural or acquired resistance to cephalosporins is due to several factors, but very often can be imputed to the generation, by the microorganism, of a β-lactamase enzyme [4]. Among the structural modifications of the cephalosporanic moiety that confer good stability to β-lactamases, the introduction in the C-7 side chain of an *E*-oxyimino group and/or an aromatic or heteroaromatic substituent bearing an *ortho*-positioned polar group, are the more frequently used. Several marketed or studied cephalosporins [5] have one (e.g. for the oxime derivatives: cefuroxime [6–10], cefuroxime axetil [11,12]; e.g. for an *o*-polar group: ceforanide [13–15]) or both these features (e.g. cefixime [16–18], cefotaxime [19–22], ceftizoxime [23–26], cefmenoxime [27,28], ceftriaxone [29–32], ceftazidime [33–36], cefpirome [37,38], cefepime [39,40], etc. [41–44]).

In the present work we describe the preparation of a series of new cephalosporins **1** and **2** (Fig. 1) having the peculiar presence in the C-7 side chain of a phenyl- (**1**) or a phenoxyacetamido (**2**) group bearing an oxyiminomethyl function in the *ortho*-position of the aromatic ring. The *ortho* polar moiety could enhance the activity of these compounds, like e.g. the *ortho*-aminomethyl group does in ceforanide.

Products **1** and **2** were tested in vitro against numerous bacterial strains and their activity was comparable with many of the commercial 2-aminothiazole cephalosporins and those bearing an α-oxyiminoacetamido group at the C-7 side chain.

2. Chemistry

2.1. Preparation of cephalosporins *1* and *2*

The cephalosporins **1** and **2** can be considered to consist of a cephalosporanic nucleus **3** and a substituted phenylacetic (**4**) or phenoxyacetic (**5**) acid (Scheme 1). The condensation of these two units leads to products **1** and **2** in good yields (55–78%) and is stereoconservative, i.e. no racemization of the stereogenic centres (C-6 and C-7) occurs. The formation of the oxyiminomethyl group from the corresponding aldehyde is more conveniently performed before the condensation stage and proceeds in a stereoselective fashion affording the *E*-

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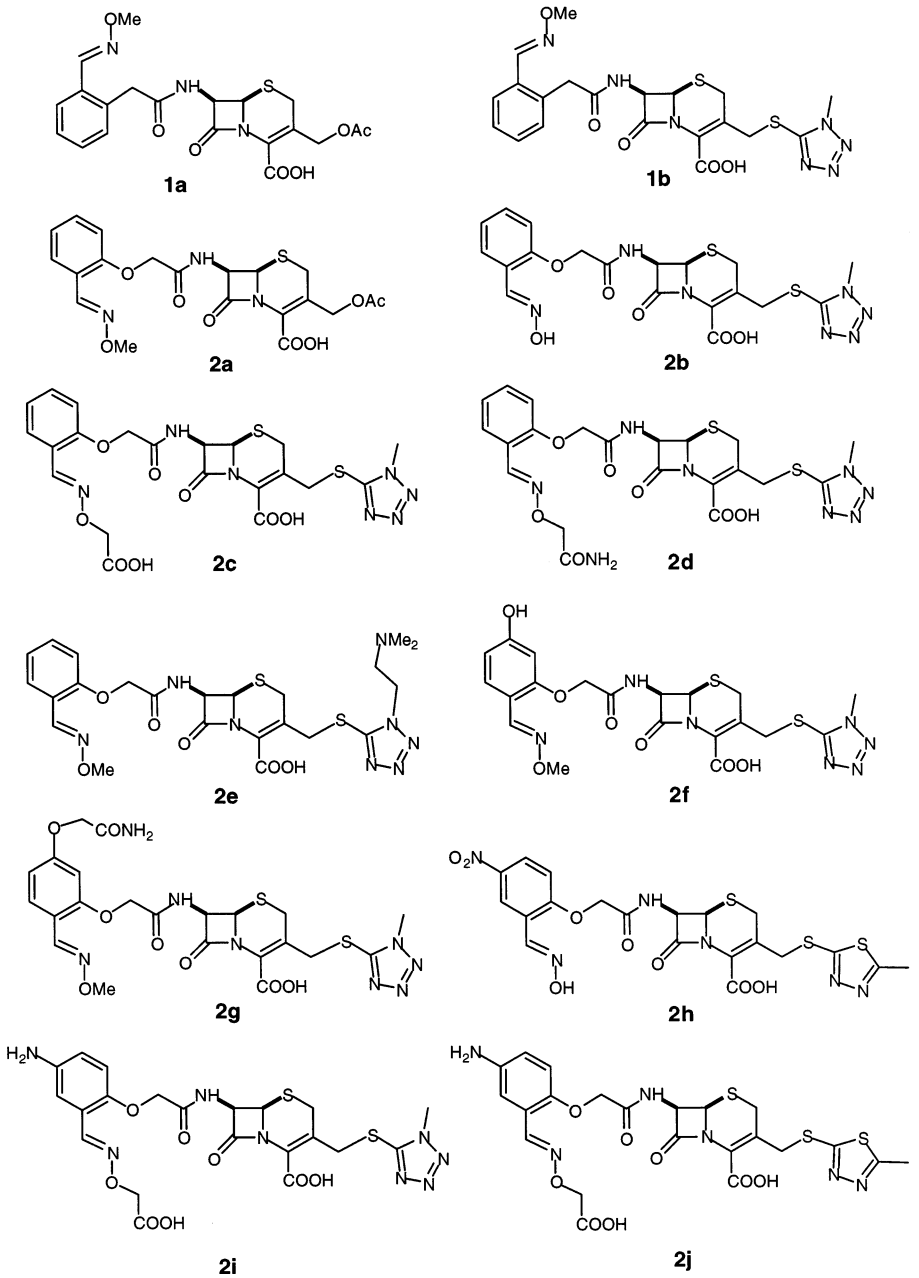
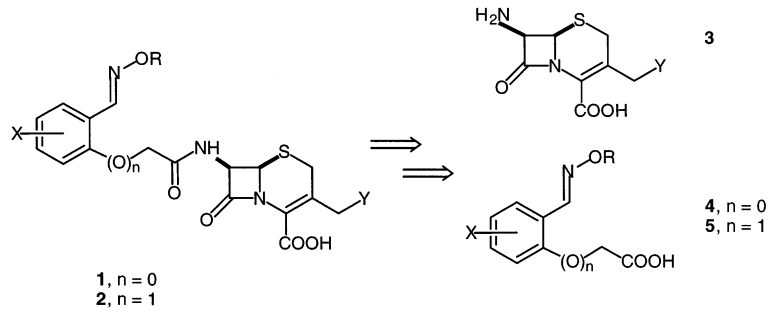


Fig. 1. Prepared cephalosporins **1** and **2**.

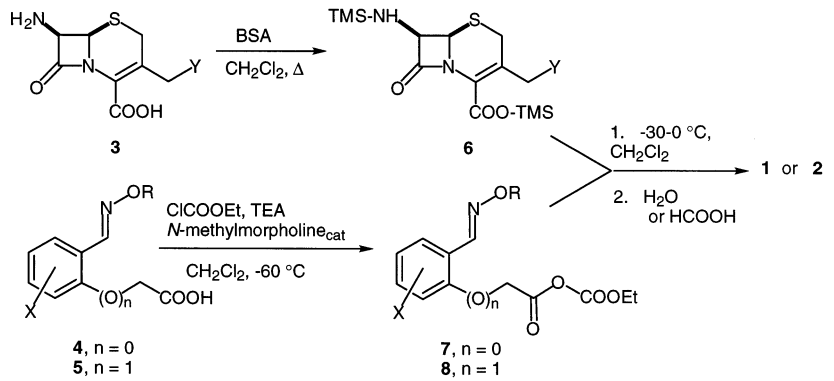
oxime. The synthesis of **1** and **2** was realized by reacting the corresponding substituted cephalosporanic acid **3** with bis-trimethylsilylacetamide (BSA) to produce the intermediate 7-silylamido-4-silyl ester (**6**) (Scheme 2). At the same time, the phenylacetic (**4**) or phenoxyacetic (**5**) acid was transformed into the corresponding mixed anhydride **7** or **8** by alkylation with ethyl chloroformate. Finally, addition of **6** to the solution containing **7** or **8**, followed by hydrolysis with water, gave the corresponding cephalosporins **1** and **2**. When R is CH_2COOBu^t, the solvolysis is conducted with formic acid to release the carboxylic acid from its *t*-butyl ester.

*2.2. Preparation of the substituted phenyl- or phenoxyacetic acid derivatives **4** and **5***

The preparations of the intermediate oxyimino containing acetic acids **4** and **5** were realized by using known synthetic procedures, both under homogeneous and phase transfer catalysis (PTC) conditions. Oximes **4a,5a** (Scheme 3) were prepared by reacting the corresponding *ortho*-formyl phenyl- or phenoxy acetic acid **9** or **10** with hydroxylamine hydrochloride. These oximes were simultaneously *O*-alkylated and esterified by reaction with an excess of methyl iodide in the presence of



Scheme 1. Structural constituents of cephalosporins **1** and **2**.



Scheme 2. Synthesis of cephalosporins **1** and **2**.

solid, anhydrous potassium carbonate under solid–liquid PTC (SL-PTC) conditions to give **11** or **12**, that were directly saponified to methoximes **4b** or **5b** with aqueous NaOH under liquid–liquid PTC (LL-PTC).

In a similar way, oximes **5c** and **5d** (Scheme 4) were generated by tandem SL-PTC bis-alkylation of **5a** with *t*-butyl chloroacetate or chloroacetamide and LL-PTC hydrolysis of the resulting intermediate **13** or **14**.

2,4-Dihydroxybenzaldehyde (**15**) was chosen for the synthesis of methoximes **5e** and **5f** (Scheme 5). The tetrahydropyranyl (THP) ether **16**, prepared by a standard procedure [45], was alkylated in good yields with ethyl bromoacetate under SL-PTC conditions and the resulting ester **17** was saponified with NaOH (LL-PTC) and *O*-deprotected to **18** by acid hydrolysis. Finally, the aldehyde **18** was transformed into the oxime **5e** with *O*-methylhydroxylamine in methanol in 42% overall yield from aldehyde **15**. The bis-alkylation of **5e** with chloroacetamide (SL-PTC) followed by saponification (LL-PTC) gave the oxime **5f**.

As shown in Scheme 6, the oxime **5g** was prepared from 2-hydroxy-5-nitrobenzaldehyde (**19**) through *O*-alkylation with chloroacetic acid in the presence of aqueous NaOH followed by reaction of the resulting (2-formylaryl)oxyacetic acid (**20**) with hydroxylamine hydrochloride. Oxime **5h** was generated by reduction of **5g** with sulfidric acid in aqueous ammonia to the amino derivative **21** which was subjected to the alkylation–hydrolysis protocol previously described.

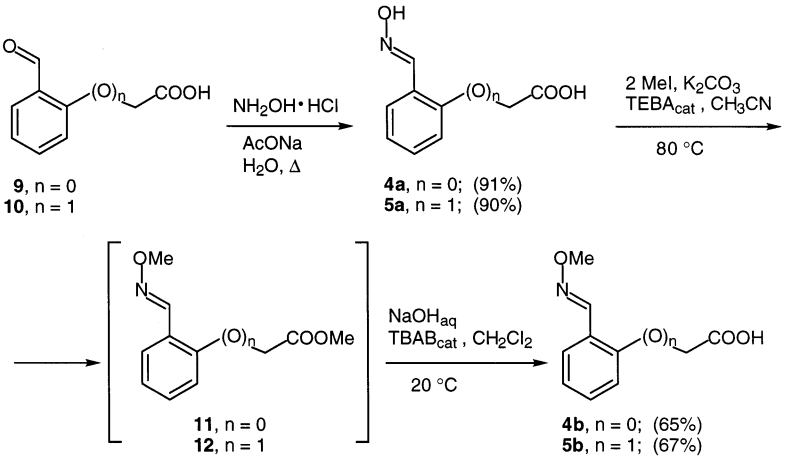
3. Experimental

3.1. Chemistry

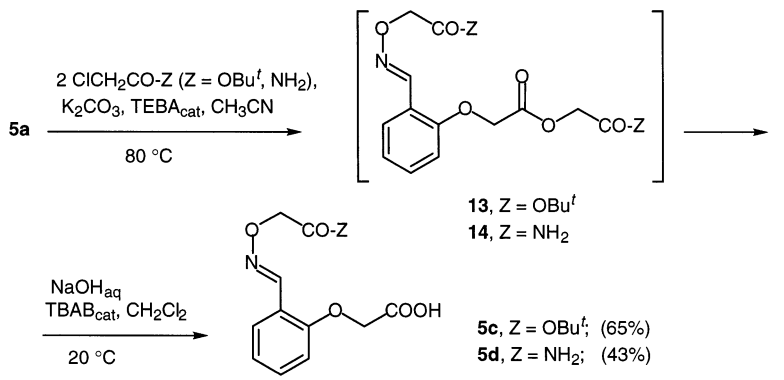
Melting points were determined on a Büchi 535 apparatus and are corrected. NMR spectra were recorded on a Bruker AC 300 spectrometer; TMS was used as external reference; δ are in ppm and J are in Hz. IR spectra were recorded on a FT-IR 1725 X Perkin–Elmer spectrometer and frequency values are in cm^{-1}. Reagent-grade commercially available reagents and solvents were used and were dried, when required, before use. All the cephalosporanic nuclei **3** are known in the literature [5].

3.1.1. Representative procedure for the synthesis of cephalosporins *1,2*. Preparation of 7-[(E)-(2-methoxyiminomethylphenoxy)acetamido]-3-acetoxy-3-cephem-4-carboxylic acid (*2a*)

(*E*)-2-(Methoxyiminomethylphenoxy)-acetic acid (**5b**) (5 g, 24 mmol) was suspended in 50 ml of dichloromethane and triethylamine (3.3 ml) was added dropwise. When the dissolution of **5b** was complete, the solution was cooled to −60°C, *N*-methylmorpholine (50 μl) was added followed by ethyl chloroformate (2.3 ml, 24 mmol). The reaction mixture was stirred at −30°C for 1 h to form the intermediate 'mixed anhydride' **8**. Separately, a mixture of 7-aminocephalosporanic acid (**3a**) (7-ACA) (8.4 g, 31 mmol) and BSA (8.3



Scheme 3. Preparation of oximes **4a,b** and **5a,b**.



Scheme 4. Preparation of oximes **5c,d** by PTC alkylation/hydrolysis of **5a**.

ml, 34 mmol) in 70 ml of anhydrous dichloromethane was refluxed until complete dissolution of 7-ACA, then it was cooled to −10°C. The solution was added dropwise to the solution of **8** at 0°C and stirred at this temperature for 3 h. The reaction mixture was then washed with ice cold water (2 × 30 ml), 1N HCl (100 ml) (to remove the unreacted 7-ACA) and water (2 × 30 ml). The organic phase was evaporated under vacuum until a solid residue was obtained that was dissolved in 200 ml of anhydrous *iso*-propanol. A solution of sodium hexanoate (4 g) in 100 ml of *iso*-propanol was added at 30°C and the mixture was left to crystallize at 30°C for 2 h, cooled with an ice/salt bath and filtered. After drying, 9.1 g (78%) of product **2a** (as sodium salt) were obtained. The same procedure was applied to the synthesis of products **1a,b** and **2b,d–h,j**. In the preparation of products **2c,i** the *t*-butyl ester function was solvolyzed in the last step of the synthesis by taking up the crude of the condensation in absolute formic acid (35 ml) instead of *iso*-propanol. The acid solution was stirred for 4 h and then diluted with toluene (100 ml) and formic acid was eliminated by azeotropic distillation under vacuum. The solid residue was dissolved in *iso*-propanol (50 ml) and sodium hexanoate (7.5 g) in *iso*-propanol (100 ml) was added. The mixture was left to crystallize overnight.

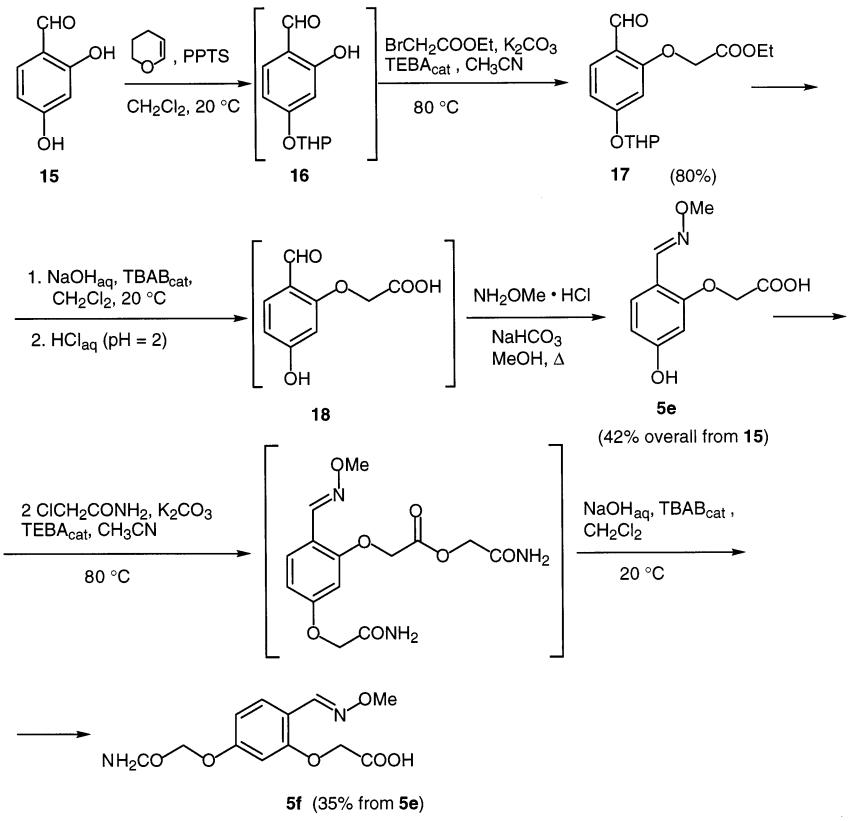
The physical and spectroscopic characteristics of products **1** and **2** are reported in Table 1.

*3.1.2. (E)-(2-Hydroxyiminomethylphenyl)-acetic acid (**4a**)*

Hydroxylamine hydrochloride (5 g, 72 mmol) and sodium acetate (20 g, 0.24 mol) were added to a solution of (2-formylphenyl)acetic acid (**9**) (5 g, 30 mmol) in 50 ml of water. The reaction mixture was refluxed for 10 min, cooled to 20°C, the pH adjusted at 2 with diluted HCl and left to crystallize. The product **4a** is filtered, washed with water and dried under vacuum at 50°C. Yield 91%. M.p. 147°C. ^{1}H NMR (DMSO-d_6) δ 13.20 (bs, 1H), 11.27 (s, 1H), 8.27 (s, 1H), 7.68–7.26 (m, 4H), 3.78 (s, 2H). IR (KBr) 3250, 1760, 1600, 1550 cm^{-1}. *Anal.* Calc. for $C_9H_9NO_3$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.25; H, 5.30; N, 7.73%.

*3.1.3. (E)-(2-Hydroxyiminomethylphenoxy)-acetic acid (**5a**)*

5a was prepared following the same procedure used for **4a**. Yield 90%. M.p. 140°C (lit. [46], 138°C).



Scheme 5. Preparation of oximes **5e,f** from 2,4-dihydroxybenzaldehyde (**15**).

*3.1.4. (E)-(2-Methoxyiminomethylphenyl)-acetic acid (**4b**)*

A solution of acid **4a** (1.8 g, 10 mmol), MeI (1.5 ml, 24 mmol) and triethylbenzylammonium chloride (TEBA) (114 mg, 0.5 mmol) in anhydrous acetonitrile (20 ml) was stirred at 80°C for 6 h in the presence of anhydrous potassium carbonate (5 g, 36 mmol). After this period the reaction mixture was cooled to 20°C and stirred overnight at this temperature. The salts were filtered and the filtrate was evaporated under vacuum. The crude methyl ester **11** was dissolved in dichloromethane (50 ml) and tetrabutylammonium bromide (200 mg, 0.6 mmol) and 15% aqueous NaOH (50 ml) were added to this solution. The heterogeneous mixture was stirred at 20°C for 12 h, then the aqueous phase was separated, acidified to pH 2 with diluted HCl and left to crystallize overnight at 4°C. The product **4b** is filtered, washed with water and dried under vacuum at 50°C. Yield 65%. M.p. 120°C. ^{1}H NMR ($CDCl_3$) δ 9.22 (bs, 1H), 8.22 (s, 1H), 7.58–7.26 (m, 4H), 3.94 (s, 3H), 3.89 (s, 2H). IR (KBr) 3000–2500, 1780, 1608, 1600 cm^{-1}. *Anal.* Calc. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.12; H, 5.91; N, 7.10%.

*3.1.5. (E)-(2-Methoxyiminomethylphenoxy)-acetic acid (**5b**)*

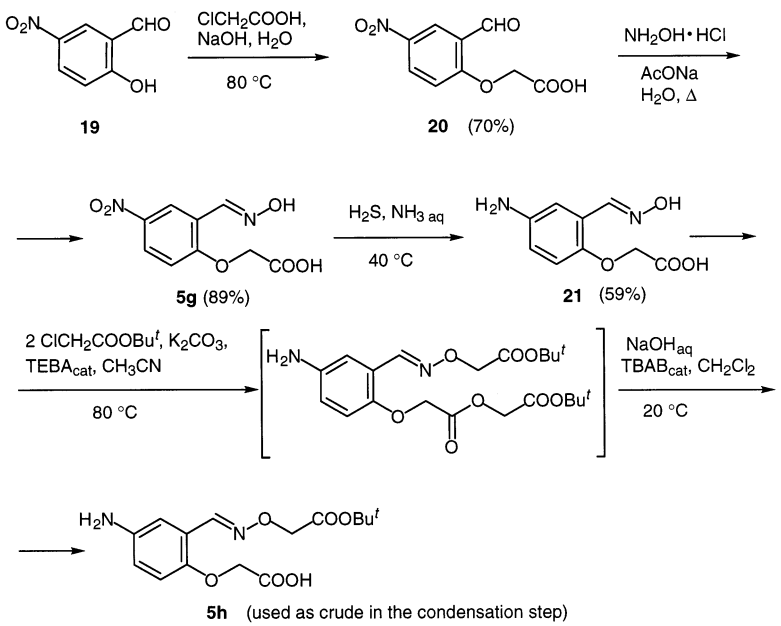
5b was prepared with the same procedure used for **4b**. Yield 67%. M.p. 122–124°C. ^{1}H NMR ($CDCl_3$) δ 9.30 (bs, 1H), 8.17 (s, 1H), 7.43–6.90 (m, 4H), 4.70 (s, 2H), 3.99 (s, 3H). IR (KBr) 3050–2577, 1969, 1740, 1713, 1609, 1601, 1495 cm^{-1}. *Anal.* Calc. for $C_9H_9NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.31; H, 5.42; N, 6.64%.

*3.1.6. (E)-(2-t-Butoxycarbonylmethyleneoxyiminomethylphenoxy)-acetic acid (**5c**)*

A heterogeneous mixture of a solution of **5a** (2.93 g, 15 mmol), *t*-butyl chloroacetate (5 ml, 35 mmol) and TEBA (228 mg, 1 mmol) in anhydrous acetonitrile (20 ml) and of anhydrous potassium carbonate (5.5 g, 4 mmol) was stirred at 80°C for 20 h. After usual work-up the ester function was hydrolyzed under LL-PTC conditions as previously described for **4b,** and **5c** was obtained after crystallization from water. Yield 65%. M.p. 128–130°C. ^{1}H NMR (DMSO-d_6) δ 13.10 (bs, 1H), 8.51 (s, 1H), 7.63–7.00 (m, 4H), 4.78 (s, 2H), 4.61 (s, 2H), 1.43 (s, 9H). IR (KBr) 3020, 2990, 2900, 1740, 1610, 1600 cm^{-1}. *Anal.* Calc. for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.09; H, 6.11; N, 4.62%.

*3.1.7. (E)-(2-Aminocarbonylmethyleneoxyiminomethylphenoxy)-acetic acid (**5d**)*

5d was prepared starting from **5a** (2.93 g, 15 mmol), chloroacetamide (3.27 g, 35 mmol), TEBA (228 mg, 1 mmol), acetonitrile (20 ml) and potassium carbonate (5.5 g, 40 mmol), with a similar procedure to that used



Scheme 6. Preparation of oximes **5g,h** from 2-hydroxy-5-nitrobenzaldehyde (**19**).

Table 1
Isolated yields, melting points and spectroscopical data of cephalosporins **1** and **2**

Compound	Yield (%)	M.p. (°C)	^{1}H NMR δ (ppm), J (Hz)[a]; IR (C = O, β-lactam) (cm^{-1})[b]
1a	77	180 (dec.)	12.40 (bs, 1H, COOH), 8.68 (d, 1H, $J = 8$), 8.52 (s, 1H), 7.70–7.20 (m, 4H), 5.53 (dd, 1H, $J = 8$, 5), 5.00 (d, 1H, $J = 5$), 4.95 and 4.72 (ABq, 2H, $J = 15$), 3.90 (s, 3H), 3.75 (s, 2H), 3.51 and 3.27 (ABq, 2H, $J = 13$), 2.00 (s, 3H); 1767; acid form
1b	75	120 (dec.)	9.10 (d, 1H, $J = 8$), 8.45 (s, 1H), 7.70–7.20 (m, 4H), 3.85 (s, 3H), 3.70 (s, 2H), 3.60 and 3.30 (ABq, 2H, $J = 13$); 1759; sodium salt
2a	78	175 (dec.)	9.00 (d, 1H, $J = 8$), 8.45 (s, 1H), 7.70–7.00 (m, 4H), 5.55 (dd, 1H, $J = 8$, 5), 5.00 (d, 1H, $J = 5$), 5.00–4.75 (ABq, 2H, $J = 15$), 4.72 (s, 2H), 3.90 (s, 3H), 3.45 and 3.15 (ABq, 2H, $J = 13$), 2.00 (s, 2H); 1767; sodium salt
2b	75	147 (dec.)	11.27 (s, 1H), 9.10 (d, 1H, $J = 8$), 8.27 (s, 1H), 7.68–7.26 (m, 4H), 5.65 (dd, 1H, $J = 8$, 5), 5.05 (d, 1H, $J = 5$), 4.70 (s, 2H), 4.35 and 4.25 (ABq, 2H, $J = 13$), 3.90 (s, 3H), 3.60 and 3.30 (ABq, 2H, $J = 14$); 1756; sodium salt
2c	59	205	9.20 (d, 1H, $J = 8$), 8.50 (s, 1H), 7.65–7.00 (m, 4H), 5.71 (dd, 1H, $J = 8$, 5), 5.12 (d, 1H, $J = 5$), 4.78 (s, 2H), 4.65 (s, 2H), 4.42 and 4.31 (ABq, 2H, $J = 13$), 3.90 (s, 3H), 3.75 and 3.60 (ABq, 2H, $J = 15$); 1760; disodium salt
2d	61	110 (dec.)	12.50 (bs, 1H), 9.20 (d, 1H, $J = 8$), 8.16 (s, 1H), 7.65–7.30 (m, 4H), 7.00–6.90 (m, 2H), 5.72 (dd, 1H, $J = 8$, 5), 5.13 (d, 1H, $J = 5$), 4.70 (s, 2H), 4.45 (s, 2H), 4.41 and 4.34 (ABq, 2H, $J = 14$), 3.95 (s, 3H), 3.83 and 3.62 (ABq, 2H, $J = 15$); 1770; acid form
2e	62	110 (dec.)	8.95 (d, 1H, $J = 8$), 8.45 (s, 1H), 7.72–6.95 (m, 4H), 5.50 (dd, 1H, $J = 8$, 5), 4.95 (d, 1H, $J = 5$), 4.70 (s, 2H), 4.35 (t, 2H, $J = 6$), 4.41 and 4.20 (ABq, 2H, $J = 13$), 3.91 (s, 3H), 3.90 and 3.35 (ABq, 2H, $J = 15$), 2.71 (t, 2H, $J = 6$), 2.15 (s, 6H); 1763; sodium salt
2f	76	189	13.10 (bs, 1H), 9.92 (s, 1H), 9.23 (d, 1H, $J = 9$), 8.33 (s, 1H), 7.52–6.31 (m, 3H), 5.70 (dd, 1H, $J = 9$, 5), 5.15 (d, 1H, $J = 5$), 4.67 (s, 2H), 4.42 and 4.23 (ABq, 2H, $J = 13$), 3.90 (s, 3H), 3.83 (s, 3H), 3.81 and 3.64 (ABq, 2H, $J = 15$); 1761; acid form
2g	60	136 (dec.)	9.17 (d, 1H, $J = 8$), 8.32 (s, 1H), 7.51–6.30 (m, 3H + 2H), 5.32 (dd, 1H, $J = 8$, 5), 5.11 (d, 1H, $J = 5$), 4.73 (s, 2H), 4.50 (s, 2H), 4.40 and 4.20 (ABq, 2H, $J = 14$), 3.92 (s, 3H), 3.82 (s, 3H), 3.81 and 3.63 (ABq, 2H, $J = 12$); 1755; sodium salt
2h	71	195	13.21 (bs, 1H), 11.70 (s, 1H), 9.05 (d, 1H, $J = 8$), 8.46–8.21 (m, 3H), 7.24 (d, 1H, $J = 6$), 5.61 (dd, 1H, $J = 8$, 5), 5.14 (d, 1H, $J = 5$), 4.96 (s, 2H), 4.45 and 4.26 (ABq, 2H, $J = 15$), 3.51 and 3.37 (ABq, 2H, $J = 13$), 2.65 (s, 3H); 1758; acid form
2i	57	180	13.00 (bs, 1H), 9.00 (d, 1H, $J = 9$), 8.17 (s, 1H), 7.22–6.70 (m, 3H), 5.70 (dd, 1H, $J = 9$, 5), 5.15 (d, 1H, $J = 5$), 4.73 (s, 2H), 4.64 (s, 2H), 4.52 and 4.40 (ABq, 2H, $J = 15$), 3.95 (s, 3H), 3.50 (bs, 3H), 3.72 and 3.64 (ABq, 2H, $J = 13$); 1701; inner salt
2j	55	115	13.02 (bs, 1H), 9.00 (d, 1H, $J = 8$), 8.17 (s, 1H), 7.22–6.67 (m, 3H), 5.70 (dd, 1H, $J = 8$, 5), 5.17 (d, 1H, $J = 5$), 4.70 (s, 2H), 4.51 and 4.43 (ABq, 2H, $J = 15$), 3.92 (s, 3H), 3.72 and 3.64 (ABq, 2H, $J = 13$), 3.45 (bs, 3H), 2.72 (s, 3H); 1765; inner salt

[a] In DMSO-d_6.
[b] KBr pellets.

Table 2
In vitro MICs of cephalosporins **1b,2b–j** against some Gram+ strains

Compound	MICs (μg/ml)[a]				
	S. a. ATCC 6538	*S. a.* F2 ISF 3	*S. e.* HCF B.	*S. e.* CHPL A2	*S. f.* LEP Br
1b	0.031	32	0.125	2	32
2b	0.063	32	0.25	2	128
2c	0.25	128	1	8	128
2d	0.063	32	0.125	128	0.402
2e	0.063	120	0.25	4	120
2f	0.031	8	0.031	1	0.25
2g	0.016	2	0.031	0.5	0.102
2h	0.031	16	0.063	1	32
2i	0.25	8	0.25	0.5	0.25
2j	0.016	8	0.031	8	0.25

[a] Determined by a standard dilution method. MICs, minimal inhibitory concentrations; *S. a.*, *Staphylococcus aureus*; *S. e.*, *Staphylococcus epidermidis*; *S. f.*, *Streptococcus fecalis*.

Table 3
In vitro MICs of cephalosporins **1b,2b–j** against some Gram− strains

Compound	MICs (μg/ml)[a]					
	E. c. ATCC 8739	*E. c.* ISF 432	*E. c.* R ISF 10	*P. v.* CNUR 6	*K. p.* ATCC 10031	*S. e.*
1b	32	4	100	100	4	>128
2b	4	4	100	100	2	128
2c	16	4	100	100	4	6
2d	4	4	100	100	4	128
2e	2	4	100	100	4	128
2f	2	2	100	100	2	16
2g	0.25	0.5	32	100	0.25	16
2h	8	16	100	100	8	>128
2i	2	4	16	100	2	2
2j	4	4	32	100	6	6

[a] Determined by a standard dilution method. MICs, minimal inhibitory concentrations; *E. c.*, *Escherichia coli*; *E. c.* R, *Escherichia coli* R+TEM; *P. v.*, *Proteus vulgaris*; *K. p.*, *Klebsiella pneumoniae*; *S. e.*, *Shighella enteridis*.

for **5c**. Acid **5d** was isolated as a solid after crystallization. Yield 43%. M.p. 179–182°C. ^{1}H NMR (DMSO-d_6) δ 13.10 (bs, 1H), 8.55 (s, 1H), 7.65–7.37 (m, 2H), 7.35 (bs, 1H), 7.27 (bs, 1H), 7.25–7.00 (m, 2H), 4.78 (s, 2H), 4.56 (s, 2H). IR (KBr) 3990, 3250, 2900–2600, 1710, 1650, 1585 cm^{-1}. *Anal.* Calc. for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.80; N, 11.11. Found: C, 52.25; H, 4.88; N, 11.00%.

3.1.8. Preparation of the tetrahydropyranyl ether i.e. *17*

A mixture of 2,4-dihydroxybenzaldehyde (**15**) (138 mg, 1.01 mmol), 3,4-dihydro-2*H*-pyran (340 mg, 4 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (25 mg) in dichloromethane (2 ml) was stirred under anhydrous atmosphere at 20°C for 24 h. The solvent was evaporated under vacuum, the crude of the reaction was dissolved in anhydrous acetonitrile (1 ml) and ethyl bromoacetate (184 mg, 1.1 mmol), TEBA (23 mg, 0.1 mmol) and potassium carbonate (158 mg, 1.1 mmol) were added. This mixture was stirred at 80°C for 2 h, cooled at 20°C, diluted with 50 ml of ethyl ether and filtered through Celite. The filtrate was evaporated under vacuum and the crude was flash chromatographed (silica gel 230–400 mesh) using ethyl ether/petroleum ether (1:2) as eluant. Yield 80%. M.p. 65°C. ^{1}H NMR ($CDCl_3$), δ 10.39 (s, 1H), 7.83 (d, 1H, $J=9$), 6.76 (dd, 1H, $J=9$, 3) 6.50 (d, 1H, $J=3$), 5.48 (t, 1H, $J=3$), 4.70 (s, 2H), 4.26 (q, 2H, $J=7$), 3.87–3.75 (m, 1H), 3.65–3.52 (m, 1H), 1.95–1.56 (m, 6H), 1.19 (t, 3H, $J=7$). *Anal.* Calc. for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54. Found: C, 62.30; H, 6.48%.

3.1.9. (E)-(4-Hydroxy-2-methoxyiminomethylphenoxy)-acetic acid (*5e*)

The ethyl ester **17** (244 mg, 0.80 mmol) was hydrolyzed through the LL-PTC procedure used for **4b**. The crystallization at pH 2 hydrolyzed the tetrahy-

dropyranyl ether function and gave the crude (2-formyl-4-hydroxyphenoxy)acetic acid **18** (150 mg, 0.76 mmol). To a heterogeneous mixture of **18** and sodium hydrogencarbonate (305 mg, 3.0 mmol) in MeOH (2 ml) *O*-methylhydroxylamine hydrochloride (30% aqueous solution) (115 μl, 1.52 mmol) was added dropwise, keeping the pH in the range 3–4. After decoloration with charcoal, the product was crystallized twice with water in order to obtain the desired pure *E*-isomer **5e** as white crystals. Yield 42% (overall from **15**). M.p. 120°C. ^{1}H NMR (DMSO-d_6) δ 13.07 (bs, 1H), 9.96 (s, 1H), 8.28 (s, 1H), 7.59 (d, 1H, $J = 9$), 6.41 (dd, 1H, $J = 9$, 3), 6.32 (d, 1H, $J = 3$), 4.67 (s, 2H), 3.80 (s, 3H). IR (KBr) 3300–3200, 1705, 1620, 1600 cm^{-1}. *Anal.* Calc. for $C_{10}H_{11}NO_5$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.25; H, 4.81; N, 6.15%.

3.1.10. (E)-(5-Aminocarbonylmethyleneoxy-2-methoxy-iminomethylphenoxy)-acetic acid (5f)

The product **5f** was prepared from **5e** by alkylation (SL-PTC)–hydrolysis (LL-PTC) as reported for the synthesis of **5d** (Section 3.1.7). Yield 35%. M.p. 183–185°C. ^{1}H NMR (DMSO-d_6) δ 13.10 (bs, 1H), 8.27 (s, 1H), 7.51 (d, 1H, $J = 9$), 7.35–7.28 (bs, 2H), 6.42 (dd, 1H, $J = 9$, 3), 6.33 (d, 1H, $J = 3$), 4.70 (s, 2H), 3.80 (s, 3H). IR (KBr) 3300–3200, 1705, 1620, 1600 cm^{-1}. *Anal.* Calc. for $C_{12}H_{14}N_2O_6$: C, 51.06; H, 5.00; N, 9.93. Found: C, 51.21; H, 5.15; N, 9.86%.

3.1.11. (E)-(2-Formyl-4-nitrophenoxy)-acetic acid (20)

A solution of 2-hydroxy-5-nitrobenzaldehyde (**19**) (2.5 g, 15 mmol), chloroacetic acid (1.41 g, 15 mmol), NaOH (1.2 g, 30 mmol) in H_2O (40 ml) was refluxed under stirring for 3 h. After cooling the reaction mixture was acidified to pH 3 with 32% HCl and left to crystallize overnight at 20°C. Yield 70%. M.p. 190°C (lit. [47] 190–192°C).

3.1.12. (E)-(2-Hydroxyiminomethyl-4-nitrophenoxy)-acetic acid (5g)

The oxime **5g** was prepared starting from aldehyde **20** (2.36 g, 10.4 mmol), hydroxylamine hydrochloride (1.67 g, 24.1 mmol) and sodium acetate (6.9 g, 84 mmol) in 20 ml of water by using the procedure reported for **4a** (Section 3.1.2) and was isolated after crystallization from water. Yield 89%. M.p. 193°C. ^{1}H NMR (DMSO-d_6) δ 13.40 (bs, 1H), 11.72 (s, 1H), 8.46 (d, 1H, $J = 3$), 8.34 (s, 1H), 8.22 (dd, 1H, $J = 9$, 3), 7.42 (d, 1H, $J = 9$), 4.97 (s, 2H). IR (KBr) 3600, 3490, 3250–2900, 1720, 1610, 1508 cm^{-1}. *Anal.* Calc. for $C_9H_8N_2O_6$: C, 45.01; H, 3.36; N, 11.66. Found: C, 44.92; H, 3.44; N, 11.50%.

3.1.13. (E)-(4-Amino-2-hydroxyiminomethylphenoxy)-acetic acid (21)

A solution of **5g** (1.32 g, 5.5 mmol) and hydrogen sulfide (0.6 g, 17.6 mmol) in 6N ammonia (6.5 ml, 39 mmol) was stirred at 40°C for 5 h. The excess hydrogen sulfide was removed under vacuum at 50°C. The reaction mixture was cooled at 20°C, the sulfur produced during the reduction crystallized and was filtered off. The pH was adjusted to 4 to crystallize the product **21** that was filtered, redissolved in an aqueous solution buffered at pH 8, decolorized over charcoal and recrystallized with hydrochloric acid at pH 3. The product **21** was isolated as a clear crystalline powder. Yield 59%. M.p. 187°C. ^{1}H NMR ($CDCl_3$) δ 11.70 (bs, 1H), 8.17 (s, 1H), 7.10–6.68 (m, 3H), 4.70 (s, 2H), 3.50 (bs, 3H). IR (KBr) 3500–3050, 1850, 1650, 1505 cm^{-1}. *Anal.* Calc. for $C_9H_{10}N_2O_4$: C, 51.43; H, 4.80; N, 13.33. Found: C, 51.30; H, 4.90; N, 13.21%.

3.1.14. (E)-(4-Amino-2-t-butoxycarbonylmethyleneoxy-iminomethylphenoxy)-acetic acid (5h)

Acid **5h** was prepared according to the procedure described for the synthesis of **5c** starting from **21** (420 mg, 2 mmol), *t*-butyl chloroacetate (0.7 ml, 5 mmol), TEBA (20 mg), anhydrous potassium carbonate (1 g, 7.2 mmol) and anhydrous acetonitrile (2 ml). After usual work-up the ester function was hydrolyzed under LL-PTC conditions as in **5c**. Crude **5h** was obtained as a solid after crystallization from water and was used as such in the condensation reactions with the cephalosporanic nuclei.

3.2. Microbiology

Cephalosporins **1b,2b–j** were tested for in vitro antibacterial activity against some Gram + (*Staphylococcus aureus* ATCC 6538, *S. aureus* F2 ISF 3, *Staphylococcus epidermidis* HCF BersetC, *S. epidermidis* CHPL A2, *Streptococcus fecalis* LEP Br) and some Gram − (*Escherichia coli* ATCC 8739, *E. coli* ISF 432, *E. coli* R + TEM ISF 10, *Proteus vulgaris* CNUR 6, *Klebsiella pneumoniae* ATCC 10031, *Shighella enteridis*) bacterial strains. Minimal inhibitory concentrations (MICs) were determined by an Agar dilution technique using Müller–Hinton 2 agar (bioMerieux). The inocolum used was 10^4 CFU per spot. The cephalosporins **1** and **2** were diluted in the test medium in the range 128–0.016 mg/ml.

4. Results and discussion

Even if the MICs values found for the cephalosporins **1** and **2** (Tables 2 and 3) represent an initial screening, they give an indication of the potentiality of these new β-lactam antibiotics.

In general, these products showed a good activity against Gram + microorganism (Table 2) and part of the Gram − tested (Table 3), whereas they resulted less or no active against *E. coli* R + TEM, *P. vulgaris* and

S. enteridis. Among the tested products, cephalosporin **2g**, that contains an oxyiminomethyl and an oxyacetamido group in the C-7 side chain benzene ring, together with a (1-methyl-1,2,3,4-tetrazol-5-yl)thiomethyl group at the C-3 position, seems to be the more active against both Gram + and Gram − strains.

In conclusion, the introduction of an oxyiminomethylene function at the *ortho*-position of the aromatic ring generated a new class of cephalosporins with an interesting antibacterial activity, that could stimulate the search for further useful structural modifications.

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