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Synthesis and antimicrobial activity of new 7β-(benzo[a]dihydrocarbazolyloxyacetyl)-substituted cephalosporins

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Abstract

Selected 7β-(benzo[a]dihydrocarbazolyloxyacetyl)-substituted cephalosporins (**1a–e**) were synthesised and tested for their antimicrobial activity against Gram-positive and Gram-negative clinical pathogens. All compounds synthesised (**1a–e**) showed an in vitro antimicrobial activity similar to that of ceftriaxone and cefpirome against the Gram-positive bacteria, and superior to that of penicillin and cefaclor against pen-R *Staphylococcus aureus* species. Like all β-lactam agents, compounds **1a–e** were in an inactive Minimum Inhibitory Concentration (MIC > 32 μg/ml) against methicillin-resistant *S. aureus* species. Furthermore, as expected, no cross-resistance was observed against the erythromycin-resistant *Staphylococcus pyogenes* strain. Finally, it is worth underlining that compounds **1a** and **1e** showed a similar activity to that of ceftriaxone and superior to cefaclor against penicillin-resistant *Streptococcus pneumoniae* isolates, a key respiratory tract infection (RTI) causing pathogen difficult to treat with currently marketed antibiotics.

Keywords: Cephalosporins; 7β-(benzo[a]dihydrocarbazolyloxyacetyl)-substituted; Antimicrobial activity

1. Introduction

Since modern antibiotic therapy was started, the abuse of antibacterial agents in many fields of human medicine and of zootechnic activities has promoted the progressive development of bacterial resistance. In the case of Gram-positive bacteria, for example, some cocci, such as *Staphylococcus aureus* (*S.a.*) and *Streptococcus pneumoniae* (*S.p.*), once very sensitive to the action of penicillins (*S.a.* pen-S or *S.p.* pen-S), have become very resistant to these antibiotics (*S.a.* pen-R or *S.p.* pen-R) or, in the case of some strains of *S.a.* also to methicillin (*S.a.* met-R). Some of these cocci, once sensitive to vancomicin or to its associations with rifampicin or cephotaxime [1–5], are now resistant also to this antibiotic [6–8].

In general, at present, bacterial infection caused by pen-R Gram-positive cocci is treated with the more recent β-lactam antibiotics and/or macrolides, but it is beginning to appear evident that there is a need for new antibiotics, in order to overcome the multi-drug resistance shown by these infectious agents. In the field of cephalosporin β-lactam antibiotics of type **A**, the antimicrobial properties of the new compounds essentially depend on their ability to interact more or less strongly with the bacterial penicillin-binding proteins (PBPs), which appear to be mutated in the resistant pathogens. These interactions are essentially linked to the nature of the substituents bound to the C-3 and C-7 atoms of the cephem nucleus. In particular, as regards the C-7 position, there is good evidence that appropriate variations in the steric hindrance and/or lipophilicity of the substituent X on the amido side-chain can modify the affinity and the selectivity of new cephalosporins against the mutated PBPs of the Gram-positive pen-R and met-R strains [9–11]. In the more recent cephalosporins which possess a good activity against resistant Gram-positive cocci, the C-7 acylamido side-chain is characterised by the presence of a bulky heteroaromatic portion, in its structure which is probably able to give the drugs better molecular characteristics for their interaction with the PBPs [12]. Previous studies aiming to find new

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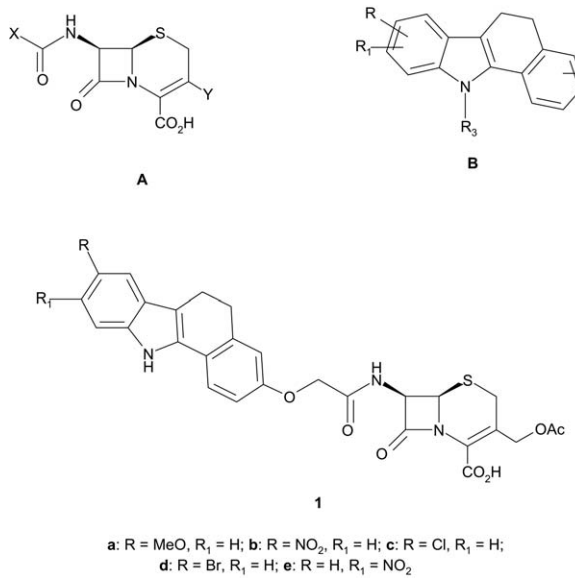


Fig. 1

pharmacologically active compounds indicated that some benzo[a]dihydrocarbazole derivatives with a **B** structure may possess different biopharmacological properties which may also be of the antimicrobial type [13,14].

On the basis of the hypothesis that the combination of the cephalosporanic nucleus with a bulky amido side-chain of a heteroaromatic type, including a molecular portion with hypothetical pharmacophoric properties, might lead to new biologically interesting cephalosporins, we planned the synthesis of compounds of type **1**, in which the 7-aminocephalosporanic portion (7-ACA) of the ''classic'' cephalosporins is acylated on the amino nitrogen with an oxyacetyl group bearing a benzo[a]dihydrocarbazolic nucleus. Therefore, a limited number of cephalosporins of type **1** were synthesised (Fig. 1), in which the R and R_1 substituents on the phenylindole portion of the benzo[a]dihydrocarbazolyl nucleus are atoms or atomic groups able to exert different electronic effects, such as chlorine or bromine atoms, or nitro or methoxy groups [15].

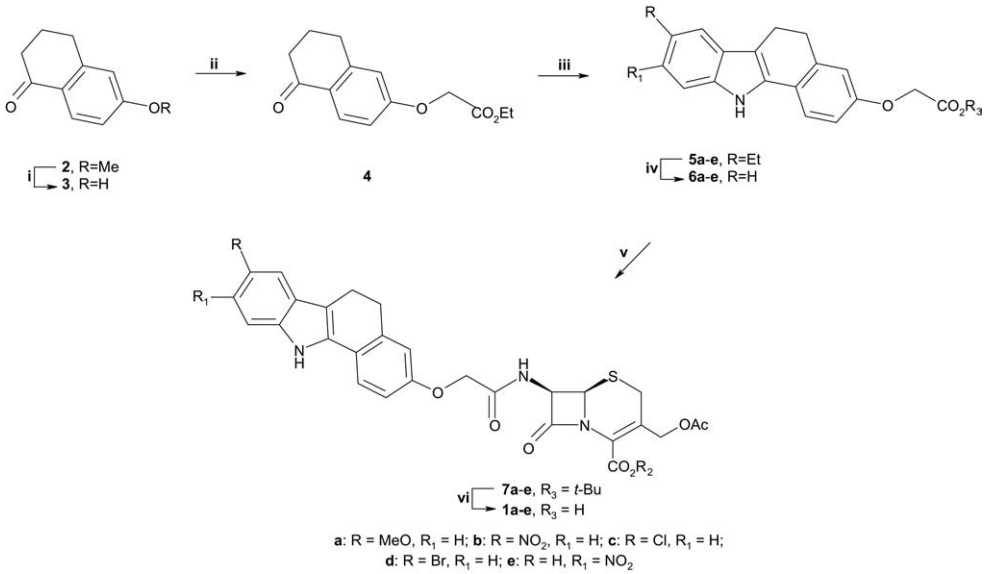
2. Chemistry

Scheme 1 shows the synthetic route to the new cephalosporins (**1a–e**) substituted on their C-7 amido side-chain with the benzo[a]dihydrocarbazolyl group. Acidic cleavage of 6-methoxy-1-tetralone **2** with 48% aqueous HBr in AcOH gave 6-hydroxy-1-tetralone **3**, which was alkylated with ethylbromoacetate in anhydrous acetone in the presence of potassium carbonate to give ether **4**. Reaction of **4** with *meta*- or *para*-substituted phenylhydrazine in refluxing AcOH gave pure C-8- or C-9-substituted ethyl 2-[(6,11-dihydro-5*H*-benzo[a]carbazol-3-yl)oxy]-acetates **5a–e**. Basic hydrolysis of ethyl esters **5a–e** by KOH in ethanol gave acids **6a–e**, which were used as acylating agents of the *tert*-butyl ester of 7-ACA (in the presence of EDCI as the condensing agent in anhydrous THF) to yield the corresponding *tert*-butyl esters **7a–e**. Acidic deprotection of **7a–e** with trifluoroacetic acid in dichloromethane in the presence of anisole gave pure cephalosporins **1a–e**.

3. Results and conclusions

The in vitro antibacterial activity of the synthesised cephalosporins **1a–e** against some common Gram-positive pathogens [1] is summarised in Table 1. A comparison with marketed

[1] The Gram-positive strains used in the present work were clinical isolates and belonged to the Culture Collection of GlaxoWellcome S.p.A. (Verona, Italy). Strain identification and pattern of antibiotic resistance were carded out by using the Vitek apparatus (BioMerieux, Milan, Italy). Each



Scheme 1

Table 1
In vitro antibacterial activity of cephalosporins **1a–e** and of the reference compounds penicillin G, cefaclor, ceftriaxone and cefpirome against some Gram-positive pathogens

Compound	R	R_1	MIC (μg/ml) [a]							
			S.a. [b]*63E pen-S* [f]	*S.a. 853E pen-R* [g]	*S. p.*[c]*3512 pen-S*	*S. p. 3272 pen-S*	*S. p. 4635 pen-R*	*S. p. 4636 pen-R*	*S. py.*[d] *eritro-R* [h]	*C. p.*[e]*615E*
1a	MeO	H	1	2	0.03	0.03	4	8	0.03	0.5
1b	NO_2	H	0.5	1	0.03	0.03	8	8	0.03	0.5
1c	Cl	H	2	4	012	0.12	16	32	0.12	1
1d	Br	H	1	2	0.06	0.03	8	16	0.06	0.25
1e	H	NO_2	0.5	≤0.5	0.06	0.03	4	4	0.06	0.5
Penicillin G			≤0.5	64	≤0.5	≤0.5	8	8	N.D.[i]	N.D.
Cefaclor			2	>32	0.5	1	32	32	0.5	2
Ceftriaxone			2	4	0.03	0.03	2	2	0.6	0.6
Cefpirome			2	2	0.03	0.03	1	1	0.03	0.12

[a] Minimum inhibitory concentration.
[b] *Staphylococcus aureus*.
[c] *Streptococcus pneumoniae*.
[d] *Streptococcus pyogenes*.
[e] *Clostridium perfringens*.
[f] *Penicillin* sensitive.
[g] *Penicillin* resistant.
[h] *Eritromicin* resistant.
[i] Not determined.

β-lactam antibiotics such as penicillin G, cefaclor, ceftriaxone and cefpirome is also reported [2]. Data are expressed as Minimum Inhibitory Concentration (MIC) values (μg/ml) [3]. Compounds **1a–e** were also tested against some Gram-negative bacteria [4], but they were found inactive up to a concentration of 32 μg/ml.

The activity of the tested compounds against penicillin-sensitive *S. aureus* 663E was similar to that of penicillin G and of the second-, third- and fourth-generation cephalosporins, cefaclor, ceftriaxone, and cefpirome (see Fig. 2). The activity of compounds **1a–e** against penicillin-resistant *S. aureus* 853 was similar to that of cefpirome, and significantly higher than that of penicillin G and cefaclor. As expected, like other β-lactam agents, compounds **1a–e** appeared to be completely devoid of any activity (MIC > 32 μg/ml) towards certain Methicillin-Resistant *S. aureus* (MRSA) strains (data not shown). Against *S. pneumoniae* bacteria, all new compounds **1a–e** exhibited an activity similar to that of all the reference compounds in the case of the penicillin-sensitive strains (*S.p*. 3512 and *S.p*. 3272). They also showed an antimicrobial activity similar to that of penicillin G and cefaclor, or slightly lower than that of ceftriaxone and cefpirome, against the penicillin-resistant strains (*S.p*. 4635 and *S.p*. 4636). Also in the case of the erythromycin-resistant S*treptococcus pyogenes* and the *Clostridium perfringens* 615E strains, compounds **1a–e** exhibited an activity similar to that of ceftriaxone and cefpirome and slightly better than cefaclor. No significant differences were observed in the trend of the activity of the new compounds towards the strains tested, as a function of the type or the position of the substituent R or R_1 on the benzo[a]dihydrocarbazolic system.

These data show that all the cephalosporins **1a–e**, while possessing antimicrobial properties similar to those of cephalosporins of the third and fourth generations, such as ceftriaxone and cefpirome, towards the Gram-positive bacteria tested, are, on the other hand, poorly active against selected Gram-negative pathogens (MIC > 32 μg/ml).

This result may indicate that the benzo[a]dihydrocarbazolyloxyacetic nucleus, chosen as the acyl substituent of the amino nitrogen of 7-ACA, possesses molecular characteristics suitable to interact with the Gram-positive PBPs, whereas it does not allow an effective interaction with Gram-negative β-lactam molecular targets, probably due to the extra barrier, present only in the Gram-negatives, which may

strain was maintained as a lyophilised culture and was sub-cultured twice on Blood Agar Base (BBL, Cockeysville, MD) just before use.

[2] Penicillin G, cefuroxime, cefaclor, ceftriaxone, cefpirone and amoxicillin/clavulanate combination were obtained from commercial sources.

[3] All compounds tested were daily prepared in 0.1 mM sodium phosphate buffer, pH 7.4. The Minimal Inhibitory Concentration (MIC) was determined in accordance with the technical procedure recommended by the National Committee for Clinical Laboratory Standards (1997). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, fourth ed. Approved Standards M7-A4. (National Committee for Clinical Laboratory Standards, Villanova, PA.) Serial doubling dilution, in appropriate growth medium, was prepared from each antibiotic solution. The bacterial inoculum was prepared by making a direct saline suspension of isolated colonies from an overnight agar plate. The suspension was adjusted to match the 0.5 McFarland turbidity standard and diluted in sterile broth to obtain a final inoculum of about 5×10^5 colony-forming units/ml in the microtitre plate. The plates were inoculated using a replicating device (Dynatech AM80, Molecular Devices, USA). In the case of *S. pneumoniae* and *S. pyogenes* strains Tood Hewitt media (BBL, Cockeysville, MD) were used and the Minimum Inhibitory Concentration (MIC) value was recorded after 24 h of incubation at 35 °C in 5% CO_2 atmosphere. The MIC was defined as the lowest concentration that resulted in no visible growth after 20 h of incubation at 35 °C.

[4] *Escherichia coli, Pseudomonas aeruginosa, Haemophylus influenzae* and *Bacteroides fragilis*, from the GlaxoWellcome S.p.A. (Verona, Italy) bacterial culture collection were used as the Gram-negative reference strains.

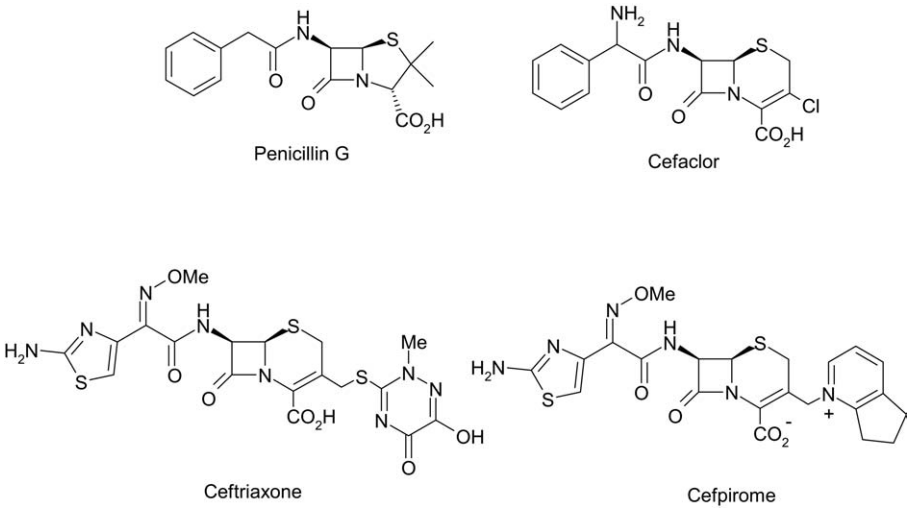


Fig. 2

limit the penetration of the molecules tested. A possible explanation of the narrow activity spectrum of compounds **1a–e** may be found in the lack of an additional substituent on the carbon adjacent to the carbonyl of the acetamido side-chain, which is usually present in the corresponding molecular portion of most of the commercially available broad-spectrum cephalosporins (see for example ceftriaxone and cefpirome of Fig. 1).

4. Experimental section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra of all compounds were obtained with a Varian Gemini 200 instrument operating at 200 MHz, in a ca. 2% solution of deuterated solvent (DMSO-d_6, CD_3OD, or $CDCl_3$). Analytical TLCs were carried out on 0.25-mm layer silica gel plates containing a fluorescent indicator; spots were detected under UV light (254 nm). Column chromatography was performed using 70–230 mesh silica gel. Mass spectra were detected with an HP-5988 A spectrometer (EI, 70 eV). Evaporation was performed in vacuo (rotating evaporator). Commercial 6-methoxy-1-tetralone **2** was purchased from Aldrich. Na_2SO_4 was always used as the drying agent. Elemental analyses were performed in our analytical laboratory and agreed with theoretical values to within ±0.4%.

*4.1. 6-Hydroxy-3,4-dihydronaphthalen-1(2*H*)-one **3***

A solution of aqueous 48% HBr was added to a stirred solution of 6-methoxy-3,4-dihydronaphthalen-1(2*H*)-one **2** (56.7 mmol) in glacial AcOH and the mixture was refluxed at 120 °C for 12 h, and then cooled and evaporated to yield crude **3** [16] as a semisolid residue, which was purified by crystallisation from methanol/water. **3** (88%): m.p. 150–152 °C; ^{1}H NMR (DMSO-d_6) δ 1.98 (m, 2H), 2.55 (m, 2H), 2.80 (m, 2H), 5.10 (brs, 1H), 6.55 (m, 2H) and 7.68 m (m, 1H). Anal. $C_{10}H_{10}O_2$ (C, H).

*4.2. Ethyl [(5-oxo-5,6,7,8-tetrahydronaphthalen-2-yl)oxy] acetate **4***

Potassium carbonate (32.7 mmol) and ethylbromoacetate (49.0 mmol) were added sequentially to a stirred solution of tetralone **3** (32.7 mmol) in anhydrous acetone (215 ml). The stirred mixture was refluxed for 12 h and then filtered, and the resulting solution was evaporated to give the pure ester **4**. **4** (68%): m.p. 115–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz), 2.12 (m, 2H), 2.60 (t, 2H, J = 5.6 Hz), 2.90 (t, 2H, J = 6.4 Hz), 4.18 (q, 2H, J = 7.2 Hz), 4.72 (s, 2H), 6.70 (m, 2H), and 7.90 m (m, 1H). Anal. $C_{14}H_{16}O_4$ (C, H).

*4.3. General procedure for the synthesis of the 8-substituted ethyl[(6,11-dihydro-5*H*-benzo[a]carbazol-3-yl)oxy] acetate **5a–d** and ethyl[(9-nitro-6,11-dihydro-5*H*-benzo[a] carbazol-3-yl)oxy]acetate **5e***

A stirred mixture of tetralone **4** (8.1 mmol) and the appropriate 4- or 3-substituted phenylhydrazine (8.4 mmol) in glacial AcOH (11.4 ml) was refluxed for 6 h. The resulting solution was then cooled at room temperature and evaporated to give a crude solid residue which, by crystallisation from EtOH/Et_2O, yielded pure carbazole derivatives **5a–e. 5a** (69%): m.p. 167–170 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.2 Hz), 2.91 (m, 4H), 3.78 (s, 3H), 4.20 (q, 2H, J = 7.2 Hz), 4.78 (s, 2H), 6.68–7.65 (m, 6H) and 11.05 m (s, 1H). Anal. $C_{21}H_{21}NO_4$ (C, H, N). **5b** (10%): m.p. 183–184 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.2 Hz), 2.91 (m, 4H), 4.20 (q, 2H, J = 7.2 Hz), 4.77 (s, 2H), 6.70–7.66 (m, 6H) and 11.05 m (s, 1H). Anal. $C_{20}H_{18}N_2O_5$ (C, H, N). **5c** (51%): m.p. 146–149 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.2 Hz), 2.92 (m, 4H), 4.20 (q, 2H, J = 7.2 Hz), 4.79 (s, 2H), 6.85–7.65 (m, 6H) and 11.45 m (s, 1H). Anal. $C_{20}H_{18}ClNO_3$ (C, H, N). **5d** (62%): m.p. 153–155 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, 3H, J = 7.2 Hz), 2.91 (m, 4H), 4.20 (q, 2 H, J = 7.2 Hz), 4.79 (s, 2H), 6.81–7.68 (m, 6H) and 11.47 m (s, 1H). Anal. $C_{20}H_{18}BrNO_3$ (C, H, N). **5e** (65%):

m.p. 182–184 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.22 (t, 3H, J = 7.2 Hz), 3.00 (m, 4H), 4.20 (q, 2H, J = 7.2 Hz), 4.82 (s, 2H), 6.89–8.20 (m, 6H) and 11.05 m (s, 1H). Anal. $C_{20}H_{18}N_2O_5$ (C, H, N).

*4.4. General procedure for the synthesis of the 8-substituted-[(6,11-dihydro-5*H*-benzo[a]carbazol-3-yl)oxy]acetic acids **6a–d** and [(9-nitro-6,11-dihydro-5*H*-benzo[a]carbazol-3-yl)oxy]acetic acid **6e***

A mixture of the appropriate carbazole **5a–e** (1.1 mmol) and KOH (1.2 mmol) in EtOH (37 ml) was stirred at room temperature for 48 h, and then the solvent was evaporated and the resulting residue was dissolved in H_2O. The aqueous solution was washed twice with AcOEt, acidified at pH 3 with 10% aqueous HCl and extracted three times with AcOEt. The organic extracts, washed twice with H_2O, were dried and evaporated to give pure **6a–e** as solids. **6a** (68%): m.p. 330–332 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.90 (m, 4H), 3.31 (s, 3H), 4.68 (s, 2H), 6.68–7.65 (m, 6H) and 11.05 m (s, 1H). Anal. $C_{19}H_{17}NO_4$ (C, H, N). **6b** (45%): m.p. 290–292 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.91 (m, 4H), 4.77 (s, 2H), 6.70–7.66 (m, 6H) and 11.05 m (s, 1H). Anal. $C_{18}H_{14}N_2O_5$ (C, H, N). **6c** (81%): m.p. 222–225 °C; ^{1}H NMR (CD_3OD) δ 3.01 (m, 4H), 4.77 (s, 2H), 6.85 (m, 2H), 7.57 (m, 1H) and 10.56 m (s, 1H). Anal. $C_{18}H_{14}ClNO_3$ (C, H, N). **6d** (58%): m.p. 232–235 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.91 (m, 4H), 4.69 (s, 2H), 6.92–7.68 (m, 6H) and 11.47 m (s, 1H). Anal. $C_{18}H_{14}BrNO_3$ (C, H, N). **6e** (77%): m.p. 258–259 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.80 (m, 4H), 4.72 (s, 2H), 6.99–8.20 (m, 6H) and 11.15 m (s, 1H). Anal. $C_{18}H_{14}N_2O_5$ (C, H, N).

*4.5. General procedure for the preparation of the tert-butyl esters of the 7-, 8- or 9-substituted- [(6,11-dihydro-5*H*-benzo[a]carbazol-3-yl)oxy]acetyl-cephalosporanic acids **7a–e***

EDCI (1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride) (1.2 mmol) was added to a cooled (0 °C) and stirred mixture of the appropriate acid **6a–e** (1.0 mmol) and *tert*-butyl ester of 7-ACA (1.0 mmol) in anhydrous THF (48.0 ml). The reaction was stirred continuously at room temperature until the disappearance of the starting reagents (TLC) (reaction time 18–24 h). The solvent was evaporated at r.t. and the residue was dissolved in $CHCl_3$ (25 ml), washed three times with H_2O, dried and evaporated under a vacuum to give the *tert*-butyl esters **7a–e** as pure solids. **7a** (97%): m.p. 105–107 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.53 (s, 9H), 2.06 (s, 3H), 2.96 (m, 4H), 3.25 and 3.55 (2d, 2H, J = 18 Hz), 3.84 (s, 1H), 4.56 (s, 2H), 4.76 (d, 1H, J = 12.8 Hz), 4.97 (d, 1H, J = 4,8 Hz), 5.06 (d, 1H, J = 12.8 Hz), 5.80 (dd, 1H, J = 4.8 and 7.6 Hz), 6.70–7.80 (m, 7H) and 8.18 m (s, 1H). Anal. $C_{33}H_{35}N_3O_8S$ (C, H, N). **7b** (92%): m.p. 94–97 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.54 (s, 9H), 2.07 (s, 3H), 2.96 (m, 4H), 3.25 and 3.55 (2d, 2H, J = 18 Hz), 4.57 (s, 2H), 4.73 (d, 1H, J = 12.8 Hz), 5.00 (d, 1H, J = 4.8 Hz), 5.07 (d, 1H, , J = 12.8 Hz), 5.88 (dd, 1H, J = 4.8 and 7.6 Hz), 6.70–8.19 (m, 7H) and 8.10 m (s, 1H). Anal. $C_{32}H_{32}N_4O_9S$ (C, H, N). **7c** (87%): m.p. 110–113 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.54 (s, 9H), 2.06 (s, 3H), 2.95 (m, 4H), 3.22 and 3.60 (2d, 2H, J = 18 Hz), 4.55 (d, 1H, J = 12.8 Hz), 4.99 (d, 1H, J = 4.8 Hz), 5.02 (d, 1 H, J = 12.8 Hz), 5.89 (dd, 1H, J = 4.8 and 7.2 Hz), 6.75–7.50 (m, 7H) and 8.23 m (s, 1H). Anal. $C_{32}H_{32}ClN_3O_7S$ (C, H, N). **7d** (89%): m.p. 107–110 °C; ^{1}H NMR (DMSO-d$_6$) δ 1.54 (s, 9H), 2.06 (s, 3H), 2.95 (m, 4H), 3.25 and 3.55 (2d, 2H, J = 18 Hz), 4.56 (s, 2H), 5.00 (d, 1H, J = 4.8 Hz), 5.05 (d, 1H, J = 12.8 Hz), 5.87 (dd, 1H, J = 4.8 and 7.2 Hz), 6.70–7.80 (m, 7H) and 8.18 m (s, 1H). Anal. $C_{32}H_{32}BrN_3O_7S$ (C, H, N). **7e** (75%); ^{1}H NMR (DMSO-d$_6$) δ 1.55 (s, 9H), 2.11 (s, 3H), 2.94 (m, 4H), 4.65 (s, 2H), 5.08 (d, 1H, J = 4.4 Hz), 5.87 (dd, 1H, J = 4.4 and 7.6 Hz), 6.75–8.35 (m, 7H) and 8.10 m (s, 1H). Anal. $C_{32}H_{32}N_4O_9S$ (C, H, N).

*4.6. General procedure for the preparation of the 7-(((8- or 9-substituted-((6,11-dihydro-5*H*-benzo[a]carbazol-3-yl) oxy)acetyl)))-cephalosporanic acids **1a–e***

Anisole (0.62 ml) and then TFA (8 mmol) were added to a cooled (0 °C) and stirred solution of the appropriate *tert*-butyl ester of 7-ACA **7a–e** (1.0 mmol) in anhydrous CH_2Cl_2 (5.0 ml). The reaction mixture was stirred continuously at room temperature for 18–24 h until the disappearance of the starting ester (TLC). The solvent was then evaporated at r.t. and the resulting oily residue was purified by flash chromatography eluting with $CHCl_3$/MeOH, 3:1, to yield pure cephalosporin derivatives **1a–e** as solid products. **1a** (52%): m.p. 105–107 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.05 (s, 3H), 2.90 (m, 4H), 3.74 (d, 1H, J = 18.2 Hz), 3.77 (s, 3H), 4.01 (d, 1H, J = 18.2 Hz), 4.65 (s, 2H), 4.66 and 5.01 (2d, 2H, J = 13.6 Hz), 5.14 (d, 1H, J = 4.8 Hz), 5.70 (dd, 1H, J = 4.8 and 8.2 Hz), 6.65–7.76 (m, 6H), 9.04 (d, 1H, J = 8.2 Hz) and 11.06 m (s, 1H). Anal. $C_{29}H_{27}N_3O_8S$ (C, H, N). **1b** (42%): m.p. 108–111 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.04 (s, 3H), 2.75 (m, 4H), 4.68 (s, 2H), 5.06 (d, 1H, J = 4.8 Hz), 5.70 (dd, 1H, J = 4.8 and 8.2 Hz), 6.75–8.50 (m, 6H), 9.15 (d, 1H, J = 8.2 Hz) and 10.96 m (s, 1H). Anal. $C_{28}H_{24}N_4O_9S$ (C, H, N). **1c** (42%): m.p. 146–148 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.05 (s, 3H), 2.92 (m, 4H), 4.62 (s, 2H), 4.86 (d, 1H, J = 13.6 Hz), 5.12 (d, 1H, J = 4.8 Hz), 5.02 (d, 1H, J = 13.6 Hz), 5.82 (dd, 1H, J = 4.8 and 8.2 Hz), 6.75–7.50 (m, 6H), 9.10 (d, 1H, J = 8.2 Hz) and 11.49 m (s, 1H). Anal. $C_{28}H_{24}ClN_3O_7S$ (C, H, N). **1d** (54%): m.p. 134–136 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.15 (s, 3H), 3.25 (m, 4H), 4.96 (s, 2H), 5.09 (d, 1H, J = 13.6 Hz), 5.39 (d, 1H, J = 4.2 Hz), 5.44 (d, 1H, J = 4.2 Hz), 6.15 (dd, 1H, J = 4.2 and 8.2 Hz), 7.10–8.25 (m, 7H) 11.86 m (s, 1H). Anal. $C_{28}H_{24}BrN_3O_7S$ (C, H, N). **1e** (40%): m.p. 190–191 °C; ^{1}H NMR (DMSO-d$_6$) δ 2.02 (s, 3H), 2.70 (m, 4H), 4.70 (s, 2H), 5.01 (d, 1H, J = 4.4 Hz), 5.60 (dd, 1H, J = 4.4 and 9.6 Hz), 6.80–8.30 (m, 6H), 8.85 (d, 1H, J = 9.6 Hz) and 11.96 m (s, 1H). Anal. $C_{28}H_{24}N_4O_9S$ (C, H, N).

References

[1] N.M. Clark, E. Hershberger, M.J. Zervosc, J.P. Lynch III, Antimicrobial resistance among Gram-positive organisms in the intensive care unit, Curr. Opin. Crit. Care 9 (2003) 403–412.

[2] B. Hoen, Special issues in the management of infective endocarditis caused by Gram-positive cocci, Infect. Dis. Clin. N. Am. 16 (2002) 437–452.

[3] G.C. Schito, Is antimicrobial resistance also subject to globalization? Clin. Microbiol. Infect. 8 (2002) 33–35.

[4] N. Woodford, Novel agents for the treatment of resistant Gram-positive infections, Expert. Opin. Inv. Drugs 12 (2003) 117–137.

[5] M. Bodi, C. Ardanuy, J. Rello, Impact of Gram-positive resistance on outcome of nosocomial pneumonia, Crit. Care. Med. 29 (2001) 82–86.

[6] M. Bassetti, G. Melica, G. Cenderello, R. Rosso, A. Di Biagio, D. Bassetti, Gram-positive bacterial resistance. A challenge for the next millennium, Panminerva Med. 44 (2002) 179–184.

[7] T. Nishino, Recent trend for the development of new antibacterial agents, Nion Rinsho. 60 (2002) 2216–2224.

[8] R.N. Jones, Resistance patterns among nosocomial pathogens: trends over the past few years, Chest 119 (2001) 397S–404S.

[9] O.K. Kim, Y. Ueda, M.M. Mansuri, J.W. Russell, V.W. Bidwell, Synthesis and structure–activity relationship of C-3 benzoyloxymethyl cephalosporins exhibiting anti-MRSA activities, Bioorg. Med. Chem. Lett. 7 (1997) 1945–1950.

[10] D. Lim, N.C. Strynadka, Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*, Nat. Struct. Biol. 9 (2002) 870–876.

[11] B.M. Grail, J.W. Payne, Conformational analysis of bacterial cell wall peptides indicates how particular conformations have influenced the evolution of penicillin-binding proteins, beta-lactam antibiotics and antibiotic resistance mechanisms, J. Mol. Recognit. 15 (2002) 113–125.

[12] A. Balsamo, S. Barontini, F. Calvani, D. Gentili, M. Macchia, A. Rossello, et al., New *N*-substituted 7-aminocephalosporanic acid derivatives as potential agents against streptococcus pneumoniae. Synthesis and in vitro activity, Bioorg. Med. Chem. Lett. 9 (1999) 1035–1040.

[13] M. Macchia, C. Manera, S. Nencetti, G. Broccali, D. Limonta, A. Rossello, Synthesis and antimicrobial activity of benzo[a]dihydrocarbazole and benzotetrahydrocyclohept[1,2-b]indole derivatives, Il Farmaco 51 (1996) 75.

[14] D. Gentili, E. Micali, S. Nencetti, A. Rossello, E. Di Modugno, A. Felici, XIV Convegno Nazionale Divisione di Chimica Farmaceutica, Società Chimica Italiana, Nuovi derivati benzo[a]diidrocarbazolici ad attività antibatterica 1 (224) (1998) Salsomaggiore, Pr.

[15] C.G. Wermuth, In the Practice of Medicinal Chemistry, C.G. Wermuth, Academic Press Inc, San Diego, CA, 1996, pp. 311–344.

[16] A. Miyake, K. Itoh, N. Tada, M. Tanabe, M. Hirata, Y. Oka, Synthesis of 2-(*N*-substituted amino)-6-hydroxy-1,2,3,4-tetrahydro-naph-thalen-1-ol derivatives, Chem. Pharm. Bull. 31 (1983) 2329–2348.