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Original article

# Novel cephalosporin derivatives possessing a substituted cinnamoyl moiety at the 7β-position. Synthesis, structural characterization and antibacterial activity of 3-acetoxymethyl cephalosporin derivatives

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**Abstract**

Twenty 3-acetoxymethyl cephalosporin derivatives, with various cinnamoyl (3-phenyl-2-propenoyl) substituted groups at the 7β-position, were synthesized and evaluated for antibacterial activity in vitro. Some of these cephalosporin derivatives showed good selective activity against Gram-positive bacteria. Although substitution on the aromatic ring of cinnamoyl moiety generally reduced antimicrobial activity against *Staphylococcus* sp. and *Enterococcus* sp., a hydroxy group at the *para* position, and particularly *ortho, para* di-chloro substitution, improved the activity against methicillin resistant strains of *Staphylococcus aureus* (MRSA). Substitution on the double bond α position of the cinnamoyl moiety also affected the antimicrobial activity. A cyano group attached to this position increased activity against both negative coagulase *Staphylococcus* and *Enterococcus* sp. and extended the antibacterial spectrum towards Gram-negative bacteria.


*Keywords:* Cephalosporin; Cinnamoyl; Antibacterial; MRSA

## 1. Introduction

*Staphylococcus aureus* continues to be a major pathogen among Gram-positive bacteria, affecting patients of all ages. Only shortly after the introduction of methicillin into clinical use 42 years ago, methicillin resistant strains of *S. aureus* (MRSA) were first reported. Since then, there has been a steady increase in the incidence of infections caused by this bacterium [1].

There is an increasing need for choosing appropriate agents to selectively treat infections caused by Gram-positive bacteria, in particular MRSA. Vancomycin remains the drug of choice in current therapy. However, if resistance to vancomycin becomes prevalent in MRSA, development of a new and effective agent to treat methicillin resistant staphylococcal infections will be imperative [2]. Efforts to discover new agents has led researchers to focus on antibiotics effective against specific pathogens or specific groups of bacteria, rather than the broad spectrum ones, to minimize the probability of evoking new types of resistant strains [3]. The cephalosporins of Microcide Pharmaceuticals [4–6], F. Hoffmann-La Roche [7], Bristol Myers Squibb [8–12], Eli Lilly [13], Meiji Seika [14], Takeda Chemical Industries [15–17] and Zenyaku Kogyo Co. [18] are examples that focused on Gram-positive bacteria including MRSA. Although some of these cephalosporins had shown good in vitro and in vivo activities against MRSA strains, no such drugs are available for anti-MRSA therapy yet [19]. All of these factors represent a strong incentive for the development of new and effective anti-MRSA cephalosporins.

In this paper we describe the synthesis, structural characterization and the antibacterial activity of novel 7β-(cinamoyl substituted)amino-3-acetoxymethyl-cefaphalosporins. Although the 3-acetoxymethyl cephalosporin from

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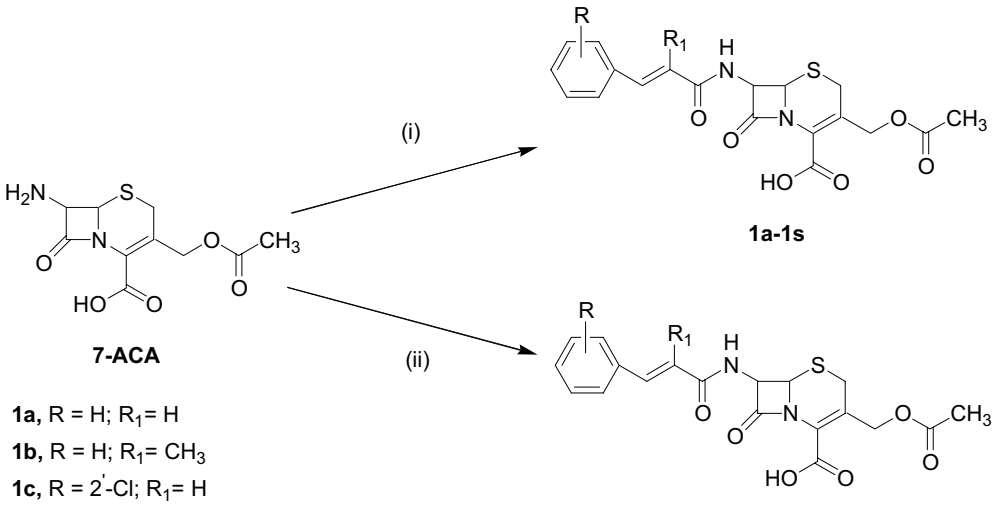
cinnamic acid was prepared some years ago in order to study the hydrolysis of the β-lactam ring [20], in the literature there are no reports about the synthesis of other 7β-(cinnamoyl substituted)amino-3-acetoxymethyl-cephalosporins and there are non available data about the antibacterial activity of this class of compounds.

From the results of the present work, it was demonstrated that compounds displayed a selective activity against Gram-positive bacteria and are potential candidates to develop anti-MRSA agents by further structural modifications.

## 2. Chemistry

The 7β-(cinnamoyl substituted)amino-3-acetoxymethyl-cephalosporins (**1a–1s**) were synthesized as shown in Scheme 1. They were prepared satisfactorily by acylation of 7β-amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (7-ACA) with the substituted cinnamic acids. Activation of the substituted cinnamic acids with Vislmeier reagent prepared from phosphoryl chloride ($POCl_3$) and *N,N*-dimethyl-formamide (DMF) was satisfactorily employed for the above acylation. In all cases, acylation was carried out under non aqueous conditions by trimethylsilylation using *N,O*-bis (trimethylsilyl)acetamide (BSA) and ethyl acetate (EtOAc) as the solvent. The trimethylsilyl ester of the cephalosporin obtained was hydrolyzed with water and the resulting cephalosporin was extracted with EtOAc. After removing the solvent, the crude product was purified by extracting the excess of cinnamic acid with diethyl ether to afforded the compounds **1a–1s** with high purity and yields between 65% and 84% depending on the cinnamic acid used.

Acylation of 7-ACA with 4′-*N,N*-dimethylamino cinnamic acid by the above procedure afford a mixture of 7β-(4′-*N,N*-dimethylaminocinnamoyl)amino-3-acetoxymethyl-cephalosporin (**1t**) with other impurities difficult to remove. In consequence, it was necessary to effect the acylation of 7-ACA by the acid chloride method. The acid chloride was prepared by reaction of 4′-*N,N*-dimethylamino cinnamic acid with phosphorous pentachloride ($PCl_5$) in THF and used without a further purification in the next step. Acylation was carried out under non aqueous conditions by trimethylsilylation using BSA and THF as the solvent. The final product (**1r**) was obtained with high purity by the same purification procedure employed during the synthesis of compounds **1a–1s**.




**1a,** R = H; $R_1$= H
**1b,** R = H; $R_1$= $CH_3$
**1c,** R = 2′-Cl; $R_1$= H
**1d,** R = 3′-Cl; $R_1$= H
**1e,** R = 4′-Cl; $R_1$= H
**1f,** R = 2′,4′-di-Cl; $R_1$= H
**1g,** R = 2′-$NO_2$; $R_1$= H
**1h,** R = 3′-$NO_2$; $R_1$= H
**1i,** R = 4′-$NO_2$; $R_1$= H
**1j,** R = 2′-$OCH_3$; $R_1$= H
**1k,** R = 3′-$OCH_3$; $R_1$= H
**1l,** R = 4′-$OCH_3$; $R_1$= H
**1m,** R = 3′,4′-di-$OCH_3$; $R_1$= H
**1n,** R = 3′-OH,4′-$OCH_3$; $R_1$= H
**1o,** R = 3′-$OCH_3$,4′-OH; $R_1$= H
**1p,** R = 3′-OH; $R_1$= H
**1q,** R = 4′-OH; $R_1$= H
**1r,** R = 4′-OH; $R_1$= CN
**1s,** R = 4′-$CH_3$; $R_1$= H
**1t,** R = 4′-$N(CH_3)_2$; $R_1$= H

Reagents:

(i) N,O-bis(trimethylsilyl)acetamide/EtOAc; substituted cinnamic acid, Vilsmeier Reagent (DMF, $POCl_3$)/THF
(ii) N,O-bis(trimethylsilyl)acetamide/THF; substituted cinnamic acid, $PCl_5$/THF

Scheme 1. Synthesis of the cephalosporin derivatives **1a–1t**.

## 3. Biological results and discussion

The antibacterial activity ($MIC_{50}$ and $MIC_{90}$) in vitro of the synthesized compounds (**1a–1t**), in comparison to cefazolin and cefuroxime, as reference compounds, against ATCC strains (*S. aureus* 25923 and *Escherichia coli* 25922) and various clinical isolates of Gram-positive bacteria are shown in Table 1.

Table 1
Antibacterial activity of compounds **1a–1t**. (MIC, μg/ml)

| Organism (number of strains) | *Sa*ATCC 25923 | *Ec*ATCC 25922 | | PSSA [a] (25) | PRSA [b] (25) | MRSA [c] (20) | PSScn [d] (20) | PRScn [e] (20) | *Ent.* [f] (25) |
|---|---|---|---|---|---|---|---|---|---|
| Cefazolin | 0.25 | N.T. | R | 0.25–0.5 | 0.25–1 | 32–128 | 0.25 | 0.5–1 | 16–128 |
| | | | GM | 0.26 | 0.32 | 34 | 0.25 | 0.55 | 28.9 |
| Cefuroxime | 0.5 | N.T. | R | 0.5–8 | Feb-32 | 32–128 | 2 | 4 | 32–128 |
| | | | GM | 2.37 | 5.08 | 48.5 | 2 | 4 | 55.5 |
| **1a** | 0.5 | 256 | R | 0.5–1 | 2-Jan | 256 | 0.5–2 | 0.5–2 | 16–32 |
| | | | GM | 0.68 | 1.15 | 256 | 0.68 | 0.85 | 16.9 |
| **1b** | 1 | 256 | R | 0.25–1 | 0.5–4 | 32–256 | 0.5–2 | 0.5–2 | 256 |
| | | | GM | 0.57 | 1.41 | 119.4 | 0.72 | 0.87 | 256 |
| **1c** | 2 | 256 | R | 4-Feb | 8-Apr | 256 | 8-Apr | 8-Apr | 32 |
| | | | GM | 3.48 | 5.13 | 256 | 4.14 | 5.21 | 32 |
| **1d** | 2 | 256 | R | 4-Jan | 16-Feb | 32-128 | 8-Jan | 16-Feb | 256 |
| | | | GM | 2 | 4 | 78.8 | 3.03 | 4.29 | 256 |
| **1e** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |
| **1f** | 2 | 256 | R | 2-Jan | 4-Feb | 16-Aug | 2-Jan | 4-Feb | 64 |
| | | | GM | 1.09 | 2.23 | 14.3 | 1.2 | 2.3 | 64 |
| **1g** | 0.5 | 256 | R | 0.5–2 | 0.5–64 | 8–256 | 0.5–2 | 0.5–2 | 128–256 |
| | | | GM | 1 | 1.74 | 78.8 | 0.93 | 0.93 | 194.01 |
| **1h** | 2 | 256 | R | 4-Jan | 16–32 | 64–256 | 8-Jan | 16–64 | 256 |
| | | | GM | 1.74 | 27.9 | 157.6 | 3.03 | 29.9 | 256 |
| **1i** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |
| **1j** | 1 | 256 | R | 0.25–2 | 0.5–128 | 64–256 | 4-Jan | 16-Jan | 128–256 |
| | | | GM | 0.76 | 21.1 | 147 | 1.74 | 4.6 | 168.9 |
| **1k** | 2 | 256 | R | 0.5–2 | 16-Feb | 32–256 | 4-Jan | 8-Jan | 128–256 |
| | | | GM | 0.93 | 4.92 | 78.8 | 2.46 | 2.83 | 168.9 |
| **1l** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |
| **1m** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |
| **1n** | 64 | 256 | R | 16–32 | 64–128 | 64–256 | Aug-32 | 32 | 64–256 |
| | | | GM | 21.1 | 77.7 | 173.6 | 18.4 | 32 | 139.1 |
| **1o** | 8 | 256 | R | 16-Apr | Aug-32 | 128–256 | Aug-32 | Aug-32 | 64–256 |
| | | | GM | 9.19 | 17.1 | 168.9 | 13.9 | 14.9 | 111.43 |
| **1p** | 4 | 256 | R | 4-Jan | Feb-32 | 32–256 | 4-Feb | 4-Feb | 128–256 |
| | | | GM | 1.87 | 6.06 | 90.5 | 2.02 | 2.3 | 168.9 |
| **1q** | 4 | 256 | R | 2-Jan | Feb-32 | 32–64 | 2-Jan | 16-Aug | 64 |
| | | | GM | 1.68 | 6.35 | 44.2 | 1.92 | 11.58 | 64 |
| **1r** | 0.12 | 16 | R | 0.25–1 | Jan-64 | 32–64 | 1 | 8-Feb | 16 |
| | | | GM | 0.3 | 7.29 | 48.5 | 1 | 2.41 | 16 |
| **1s** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |
| **1t** | 256 | 256 | R | N.T. | N.T. | N.T. | N.T. | N.T. | N.T. |
| | | | GM | | | | | | |

N.T.: no tested; R: range; GM: geometric mean.
[a] Penicillin sensitive *S. aureus*.
[b] Penicillin resistant *S. aureus*.
[c] Methicillin resistant *S. aureus*.
[d] Penicillin sensitive negative coagulase *Staphylococcus*.
[e] Penicillin resistant negative coagulase *Staphylococcus*.
[f] *Enterococcus* sp.

Excepting the compounds carrying a hydroxy group on the *para* (4′) position of the cinnamoyl moiety aromatic ring (**1o–1r**) and the di-substituted derivatives **1f** (2′,4′-di-chloro) and **1n** (3′-hydroxy-4′-methoxy), the rest of the *para* substituted derivatives (**1e, 1i, 1l, 1m, 1s** and **1t**) were inactive (minimum inhibitory concentrations (MICs) > 256 μg/ml) against ATCC strains and were not tested against clinical isolates. All the active compounds displayed a selective activity against Gram-positive bacteria, but compound **1r** also showed activity (MIC = 16 μg/ml) against Gram-negative bacteria (*E. coli* ATCC 25922).

From Table 1 it may be observed that, generally, substitution on the aromatic ring of cinnamic acid reduced or did not improve the antibacterial potency against methicillin sensitive *S. aureus* (MSSA) and methicillin sensitive coagulase negative *Staphylococcus* (MSCoNS). The compounds **1a** and **1b**, synthesized from cinnamic and α-methyl cinnamic acids, were the most potent against these strains and displayed an activity close to cefazolin and higher than cefuroxime. An exception was the derivative obtained from 2′-nitro cinnamic acid (**1g**), which exhibited similar antimicrobial potency as **1a** and **1b**. The substituents that provided better antimicrobial activity on the *ortho* (2′), *meta* (3′) and *para* (4′) positions of the cinnamoyl moiety aromatic ring were the nitro, the methoxy and the hydroxy groups, respectively (compounds **1g, 1k** and **1q**). Although the di-substituted compound **1o** carries two of such groups on the most favorable positions (3′-methoxy and 4′-hydroxy), this derivative exhibited poorer antibacterial activity than the corresponding mono-substituted compounds (**1k** and **1q**). In this case, it is possible that individual effects of methoxy and hydroxy groups on antimicrobial activity become negatively affected by intra-molecular hydrogen bonding between both substituents. Probably by the same cause, the isomer of **1o** (compound **1n**) also displayed a weak antibacterial activity. The **1n** activity was poorer than **1o**, probably because this derivative has an additional structural handicap, such as the presence of a 4′-methoxy group. This substituent on the *para* position has been proved unfavorable in order to develop antibacterial activity, since compound **1l** was completely inactive.

A *para* (4′) hydroxy group on the aromatic ring of the cinnamoyl moiety improved the activity against MRSA (compounds **1q** and **1r**). However, both derivatives cannot be considered effective because they showed poor $MIC_{90}$ values in vitro (64 μg/ml). Generally, *ortho* (2′) and *meta* (3′) substitution on the aromatic ring improved the activity against MRSA, although the corresponding compounds were less active than **1q** and **1r**. The best cephalosporin against MRSA strains was the 2′,4′-di-chlorocinnamoyl derivative (**1f**), which displayed a remarkable activity ($MIC_{90}$ = 16 μg/ml). From the literature [8,9], it has been demonstrated that di-chloro substitution (particularly 2′,5′-di-chloro substitution) on the aromatic ring of phenylthioacetic acid provides, by acylation of cephem nucleus, cephalosporins with excellent in vitro antimicrobial activity against MRSA. Since the compound **1f** has a spatial configuration close to 2′,5′-phenylthioacetamido cephalosporins, it would be interesting to synthesize and test the anti-MRSA properties of the corresponding 2′,5′-di-chlorocinnamoyl cephalosporin. It is possible that such compound displays a higher potency against MRSA strains than the 2′,4′-di-chloro isomer (**1f**) obtained during the present work.

The presence of a methyl group attached to the α position of the double bond of the cinnamoyl moiety (compound **1b**) did not increase the antibacterial activity against MSSA and MSCoNS, whereas a cyano group at such position (compound **1r**) improved slightly the activity against MSCoNS in comparison with compound **1q**.

All the compounds can be considered ineffective against *Enterococcus* sp. The activity of the more active compounds (**1a** and **1r**) was moderate (MICs ~ 16 μg/ml) but higher than the potency developed by cefazolin and cefuroxime against these strains. As in the case of *Staphylococcus* sp., substitution on aromatic ring of the cinnamoyl moiety generally reduced the antimicrobial activity against *Enterococcus* sp. The only substituents that provided some activity were the chlorine atom and the hydroxy group on the *ortho* (2′) and *para* (4′) positions, respectively. Substitution on the double bond α position of the cinnamoyl moiety also affected strongly the antimicrobial activity against *Enterococcus* sp. A methyl group at such position (compound **1b**) reduced dramatically the activity, whereas the presence of an α cyano group (**1r**) increased the activity in comparison with the respective, unsubstituted derivatives (**1a** and **1q**).

The presence of an α cyano group also expanded the antimicrobial spectrum towards Gram-negative bacteria, because compound **1r** displayed a moderate activity (MIC = 16 μg/ml) against *E. coli* ATCC 25922. This result could be attributed to a better penetration of compound **1r** across the Gram-negative bacteria cell wall. The observed effect may be produced by the cyano group and not by the substitution in the double bond α position, because the compound carrying a methyl group in such position (**1b**) was not active against *E. coli* ATCC. However, in order to get a definitive conclusion about this matter, it would be necessary to synthesize and test the antimicrobial activity of other α substituted derivatives.

## 4. Conclusions

The coupling of a cinnamoyl moiety to the 7-ACA cephem nucleus provides cephalosporins with selective activity against Gram-positive bacteria. The compounds obtained from cinnamic, α-methyl cinnamic and 2′-nitrocinnamic acids displayed a strong antibacterial activity against *Staphylococcus* sp. (methicillin sensible) but were inactive against MRSA and *Enterococcus* sp. The 2′,4′-di-chlorocinnamoyl was the only derivative active against MRSA and can be considered a potential candidate in order to develop new anti-MRSA agents by further structural modifications.

## 5. Experimental protocols

### 5.1. General methods

$^1$H NMR and $^{13}$C NMR spectra were measured with a Bruker AC 250F spectrometer for 250 and 62.5 MHz, respectively, in DMSO-$d_6$. TMS (0 ppm) in DMSO-$d_6$ was used as internal reference standard. The electrospray mass spectra (ESI-MS) were obtained on a Micromass Quattro II triple quadrupole spectrometer. The flow into the source was about 300 nl/min. The compounds (as sodium salts) were dissolved in MeOH at a concentration of 250 ppm.

### 5.2. Antibacterial activity in vitro

Wild-type clinical isolates used for antibacterial activity analysis were from the microorganism bank of the Center of Pharmaceutical Chemistry. MIC was determined by the dilution method on Mueller–Hinton agar plates, as recommended by the National Committee for Clinical Laboratory Standards 2000 (NCCLS). A 5 μl of cell suspension of test strains having about $10^8$ cfu/ml was inoculated and incubated at 37 °C for 24 h. The MIC was then measured.

### 5.3. Preparation of 7β-(cinnamoyl substituted)amino-3-acetoxymethyl-cephalosporins (**1a–1s**)

#### 5.3.1. (6R,7R)-7-(cinnamoyl)amino-3-acetoxymethyl-3-cephem4-carboxylic acid (**1a**)

To a solution of DMF (0.92 ml, 12 mmol) in dry THF (14 ml) $POCl_3$ (1.1 ml, 12 mmol) was added dropwise at 0–5 °C under stirring and the mixture was stirred at this temperature for 30 min to prepare the Vilsmeier reagent. To the above mixture, cinnamic acid (11 mmol) was added under ice cooling and the reaction mixture was stirred at the same temperature for 1 h to produce an activated acid solution of the cinnamic acid. To a solution of 7-ACA (2.72 g, 10 mmol) and BSA (7.4 ml, 30 mmol) in EtOAc (30 ml) the above activated acid solution at –20 °C was added, and the reaction mixture was stirred at –20 °C for 1 h. A mixture of EtOAc (50 ml) and water (100 ml) was added to the reaction mixture and the EtOAc layer was separated. The organic layer was washed with water (3 × 10 ml), with brine (10 ml) and dried over anhydrous $Na_2SO_4$. The solvent was evaporated under vacuum and the residue was stirred with diethyl ether (30 ml) for 1 h. The resulting solid was filtered, washed with diethyl ether (3 × 10 ml) and dried to afford **1a**. (Yield: 3.17 g, 79%). Anal. calcd. for $C_{19}H_{18}N_2O_6S$: C, 56.71; H, 4.51; N, 6.96; S, 7.97; found: C, 56.64; H, 4.65; N, 6.90; S, 8.00; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and 3.65 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.17 (1H, d, $J$ = 5 Hz), 5.86 (1H, dd, $J$ = 5, 8 Hz), 6.78 (1H, d, $J$ = 16 Hz), 7.36–7.63 (6H, m), 9.08 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.51, 25.57, 57.46, 59.20, 62.66, 120.40, 123.53, 126.29, 127.69, 128.95, 129.84, 134.48, 140.61, 162.82, 164.85, 165.42, 170.16; ESI-MS *m/z* 425.4 [(M + Na)$^+$].

Preparation of compounds **1b–1s** was carried out by a method similar to that described for **1a**.

#### 5.3.2. (6R,7R)-7β-(α-methylcinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1b**)

(Yield: 2.09 g, 50.1%). Anal. calcd. for $C_{20}H_{20}N_2O_6S$: C, 57.68; H, 4.84; N, 6.73; S, 7.70; found: C, 57.63; H, 4.80; N, 6.81; S, 7.74; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (6H,s), 3.65 and 3.50 (2H, ABq, $J$ = 18 Hz), 4.70 and 4.99 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.78 (1H, dd, $J$ = 5, 8 Hz), 7.30 (1H, s), 7.37–7.47 (5H, m), 9.03 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 14.33, 20.52, 25.46, 57.67, 59.68, 62.69, 122.85, 126.66, 127.92, 128.40, 129.27, 131.09, 133.77, 135.67, 162.87, 164.24, 169.63, 170.16.; ESI-MS *m/z* 439.4 [(M + Na)$^+$].

#### 5.3.3. (6R,7R)-7β-(2′-chlorocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1c**)

(Yield: 3.53 g, 81%) Anal. calcd. for $C_{19}H_{17}ClN_2O_6S$: C, 52.24; H, 3.92; Cl, 8.12; N, 6.41; S, 7.34; found: C, 52.40; H, 3.87; Cl, 8.31; N, 6.36; S, 7.42; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.51 and 3.66 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.17 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5.8 Hz), 6.77 (1H, d, $J$ = 16 Hz), 7.44–7.67 (4H, m), 9.10 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.50, 25.58, 57.37, 59.22, 62.64, 123.46, 123.62, 126.26, 127.63, 127.78, 129.99, 131.24, 132.28, 133.45, 135.81, 162.80, 164.66, 164.97, 170.15; ESI-MS *m/z* 459.9 [(M + Na)$^+$].

#### 5.3.4. (6R,7R)-7β-(3′-chlorocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1d**)

(Yield: 2.73 g, 62.5%). Anal. calcd. for $C_{19}H_{17}ClN_2O_6S$: C, 52.24; H, 3.92; Cl, 8.12; N, 6.41; S, 7.34; found: C, 52.32; H, 3.85; Cl, 8.22; N, 6.38; S, 7.40; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.02 (3H, s), 3.49 and 3.66 (2H, ABq, $J$ = 18 Hz), 4.69 and 5.01 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.81 (1H, d, $J$ = 16 Hz), 7.36–7.72 (4H, m), 9.08 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.52, 25.55, 57.38, 59.15, 62.65, 122.07, 123.55, 126.07, 126.28, 127.43, 129.44, 130.78, 133.67, 136.77, 139.00, 162.81, 164.75, 165.08, 170.16; ESI-MS *m/z* 459.8 [(M + Na)$^+$].

#### 5.3.5. (6R,7R)-7-(4′-chlorocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1e**)

(Yield: 3.66 g, 84%). Anal. calcd. for $C_{19}H_{17}ClN_2O_6S$: C, 52.24; H, 3.92; Cl, 8.12; N, 6.41; S, 7.34; found: C, 52.33; H, 3.89; Cl, 8.04; N, 6.52; S, 7.45; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.51 and 3.67 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.18 (1H, d, $J$ = 5 Hz), 5.86 (1H, dd, $J$ = 5, 8 Hz), 6.83 (1H, d, $J$ = 16 Hz), 7.36–7.75 (5H, m), 7.82 (1H, d, $J$ = 16 Hz), 9.20 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.55, 25.57, 57.43, 59.19, 62.68, 121.16, 123.49, 126.32, 129.03, 129.41, 133.45, 134.31, 139.27, 162.84, 164.80, 165.23, 170.19; ESI-MS *m/z* 459.7 [(M + Na)$^+$].

*5.3.6. (6R,7R)-7-(2′,4′-di-chlorocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1f**)*

(Yield: 3.26 g, 69.3%). Anal. calcd. for $C_{19}H_{16}Cl_2N_2O_6S$: C, 48.42; H, 3.42; Cl, 15.04; N, 5.94; S, 6.80; found: C, 48.50; H, 3.38; Cl, 15.11; N, 5.96; S, 6.88; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.51 and 3.67 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.18 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5.8 Hz), 6.83 (1H, d, $J$ = 16 Hz), 7.47–7.81 (4H, m), 9.22 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.58, 25.61, 57.39, 59.26, 62.71, 123.56, 124.15, 126.38, 128.15, 128.86, 129.52, 131.41, 134.29, 134.67, 134.90, 162.87, 164.63, 164.85, 170.21; ESI-MS *m/z* 494.2 [(M + Na)$^+$].

*5.3.7. (6R,7R)-7-(2′-nitrocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1g**)*

(Yield: 3.27 g, 73%). Anal. calcd. for $C_{19}H_{17}N_3O_8S$: C, 51.00; H, 3.83; N, 9.39; S, 7.17; found: C, 51.08; H, 3.89; N, 9.37; S, 7.24; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.51 and 3.67 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.18 (1H, d, $J$ = 5 Hz), 5.87 (1H, dd, $J$ = 5, 8 Hz), 6.74 (1H, d, $J$ = 16 Hz), 7.58–8.12 (5H, m), 9.24 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.52, 25.56, 57.33, 59.15, 62.65, 123.51, 124.67, 124.90, 126.30, 128.77, 129.78, 130.50, 133.91, 135.89, 148.24, 162.81, 164.62, 164.65, 170.16; ESI-MS *m/z* 470.4 [(M + Na)$^+$].

*5.3.8. (6R,7R)-7-(3′-nitrocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1h**)*

(Yield: 3.00 g, 67%). Anal. calcd. for $C_{19}H_{17}N_3O_8S$: C, 51.00; H, 3.83; N, 9.39; S, 7.17; found: C, 50.92; H, 3.80; N, 9.33; S, 7.25; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and 3.67 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.18 (1H, d, $J$ = 5 Hz), 5.87 (1H, dd, $J$ = 5, 8 Hz), 6.96 (1H, d, $J$ = 16 Hz), 7.55–8.49 (5H, m), 9.15 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.53, 25.58, 57.35, 59.17, 62.67, 121.78, 123.26, 123.64, 124.07, 126.30, 130.51, 133.98, 136.35, 138.25, 148.24, 162.83, 164.72, 164.89, 170.18; ESI-MS *m/z* 470.3 [(M + Na)$^+$].

*5.3.9. (6R,7R)-7-(4′-nitrocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1i**)*

(Yield: 3.4 g, 76%). Anal. calcd. for $C_{19}H_{17}N_3O_8S$: C, 51.00; H, 3.83; N, 9.39; S, 7.17; found: C, 51.12; H, 3.86; N, 9.31; S, 7.28; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04 (3H, s), 3.50 and 3.66 (2H, ABq, $J$ = 18 Hz), 4.74 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.19 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.96 (1H, d, $J$ = 16 Hz), 7.65 (1H, d, $J$ = 16 Hz), 7.76–8.34 (4H, m), 9.11 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.49, 25.58, 57.35, 59.23, 62.64, 123.54, 123.85, 124.07, 126.27, 128.74, 138.22, 141.25, 147.68, 162.79, 164.60, 164.78, 170.14; ESI-MS *m/z* 470.4 [(M + Na)$^+$].

*5.3.10. (6R,7R)-7-(2′-methoxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1j**)*

(Yield: 3.26 g, 75.5%). Anal. calcd. for $C_{20}H_{20}N_2O_7S$: C, 55.55; H, 4.66; N, 6.48; S, 7.41; found: C, 55.47; H, 4.73; N, 6.39; S, 7.47; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.49 and 3.66 (2H, ABq, $J$ = 18 Hz), 3.86 (3H, s), 4.71 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.83 (1H, d, $J$ = 16 Hz), 6.92–7.69 (5H, m), 7.74 (1H, d, $J$ = 16 Hz), 9.06 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.52, 25.55, 55.51, 57.50, 59.20, 62.65, 111.69, 120.69, 120.85, 122.81, 123.43, 126.31, 128.25, 131.25, 135.71, 157.73, 162.84, 164.92, 165.84, 170.16; ESI-MS *m/z* 455.4 [(M + Na)$^+$].

*5.3.11. (6R,7R)-7-(3′-methoxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1k**)*

(Yield: 3.33 g, 77.1%). Anal. calcd. for $C_{20}H_{20}N_2O_7S$: C, 55.55; H, 4.66; N, 6.48; S, 7.41; found: C, 55.60; H, 4.62; N, 6.51; S, 7.35; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and 3.66 (2H, ABq, $J$ = 18 Hz), 3.86 (3H, s), 4.71 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.17 (1H, d, $J$ = 5 Hz), 5.86 (1H, dd, $J$ = 5, 8 Hz), 6.78 (1H, d, $J$ = 16 Hz), 6.92–7.42 (5H, m), 7.51 (1H, d, $J$ = 16 Hz), 9.05 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.52, 25.55, 55.06, 57.44, 59.17, 62.65, 112.77, 115.77, 119.97, 120.73, 123.55, 126.28, 130.01, 135.91, 140.50, 159.53, 162.83, 164.86, 165.37, 170.16; ESI-MS *m/z* 455.3 [(M + Na)$^+$].

*5.3.12. (6R,7R)-7-(4′-methoxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1l**)*

(Yield: 3.26 g, 75.5%). Anal. calcd. for $C_{20}H_{20}N_2O_7S$: C, 55.55; H, 4.66; N, 6.48; S, 7.41; found: C, 55.43; H, 4.70; N, 6.37; S, 7.43; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and 3.66 (2H, ABq, $J$ = 18 Hz), 3.79 (3H, s), 4.71 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.63 (1H, d, $J$ = 16 Hz), 6.92–7.60 (5H, m), 8.96 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.54, 25.57, 55.25, 57.53, 59.19, 62.69, 114.43, 117.83, 123.44, 126.33, 127.06, 129.37, 140.36, 160.63, 162.86, 165.03, 165.71, 170.20; ESI-MS *m/z* 455.3 [(M + Na)$^+$].

*5.3.13. (6R,7R)-7-(3′,4′-dimethoxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1m**)*

(Yield: 3.65 g, 79%). Anal. calcd. for $C_{21}H_{22}N_2O_8S$: C, 54.54; H, 4.79; N, 6.06; S, 6.93; found: C, 54.49; H, 4.66; N, 6.10; S, 7.00; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04 (3H, s), 3.49 and 3.65 (2H, ABq, $J$ = 18 Hz), 3.80 (3H, s), 3.81 (3H, s), 4.74 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.84 (1H, dd, $J$ = 5, 8 Hz), 6.67 (1H, d, $J$ = 16 Hz), 6.93–7.23 (3H, m), 7.48 (1H, s), 8.84 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.54, 25.59, 55.33, 55.52, 57.53, 59.19, 62.69, 110.09, 111.71, 118.07, 121.65, 123.56, 126.31, 127.27, 140.67, 148.45, 150.41, 162.87, 165.05, 165.71, 170.20; ESI-MS *m/z* 485.5 [(M + Na)$^+$].

*5.3.14. (6R,7R)-7-(3′-hydroxy-4′-methoxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1n**)*

(Yield: 2.72 g, 60.7%). Anal. calcd. for $C_{20}H_{20}N_2O_8S$: C, 53.57; H, 4.50; N, 6.25; S, 7.15; found: C, 53.59; H, 4.40; N, 6.21; S, 7.12; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and

3.66 (2H, ABq, $J$ = 18 Hz), 3.80 (s, 3H, $OCH_3$), 4.70 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.15 (1H, d, $J$ = 5 Hz), 5.84 (1H, dd, $J$ = 5, 8 Hz), 6.54 (1H, d, $J$ = 16 Hz), 6.91–7.04 (3H, m), 7.39 (1H, d, $J$ = 16 Hz), 8.94 (1H, d, $J$ = 8 Hz); 9.23 (br s, 1H, OH); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.58, 25.59, 55.60, 57.57, 59.23, 62.75, 112.09, 113.36, 117.65, 120.82, 123.37, 126.43, 127.39, 140.85, 146.74, 149.60, 162.92, 165.05, 165.77, 170.24; ESI-MS *m/z* 471.3 [(M + Na)$^+$].

*5.3.15. (6*R*,7*R*)-7-(3′-methoxy-4-hydroxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1o**)*

(Yield: 2.44 g, 54.5%). Anal. calcd. for $C_{20}H_{20}N_2O_8S$: C, 53.57; H, 4.50; N, 6.25; S, 7.15; found: C, 53.61; H, 4.42; N, 6.28; S, 7.08; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04 (3H, s), 3.51 and 3.66 (2H, ABq, $J$ = 18 Hz), 3.82 (3H, s), 4.72 and 5.03 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.86 (1H, dd, $J$ = 5, 8 Hz), 6.60 (1H, d, $J$ = 16 Hz), 6.74–7.22 (5H, m), 7.45 (1H, d, $J$ = 16 Hz), 8.91(1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.56, 25.63, 55.51, 57.60, 59.21, 62.73, 111.02, 115.72, 117.13, 121.86, 123.50, 126.03, 126.41, 141.04, 147.83, 148.73, 162.89, 165.10, 165.88, 170.21; ESI-MS *m/z* 471.4 [(M + Na)$^+$].

*5.3.16. (6*R*,7*R*)-7-(3′-hydroxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1p**)*

(Yield: 2.18 g, 52.1%). Anal. calcd. for $C_{19}H_{18}N_2O_7S$: C, 54.54; H, 4.34; N, 6.70; S, 7.66; found: C, 54.51; H, 4.38; N, 6.79; S, 7.72; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 3.50 and 3.67 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.01 (2H, ABq, $J$ = 13 Hz), 5.17 (1H, d, $J$ = 5 Hz), 5.86 (1H, dd, $J$ = 5, 8 Hz), 6.71 (1H, d, $J$ = 16 Hz), 6.87–7.32 (5H, m), 7.45 (1H, d, $J$ = 16 Hz), 9.05 (1H, d, $J$ = 8 Hz); 9.64 (1H, br, s); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.54, 25.56, 57.46, 59.18, 62.69, 113.75, 117.13, 119.00, 120.15, 123.36, 126.39, 129.99, 135.96, 140.83, 157.69, 162.88, 164.86, 165.44, 170.19; ESI-MS *m/z* 441.3 [(M + Na)$^+$].

*5.3.17. (6*R*,7*R*)-7-(4′-hydroxycinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1q**)*

(Yield: 2.96 g, 70.9%). Anal. calcd. for $C_{19}H_{18}N_2O_7S$: C, 54.54; H, 4.34; N, 6.70; S, 7.66; found: C, 54.60; H, 4.30; N, 6.75; S, 7.78; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04 (3H, s), 3.51 and 3.69 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.01 (2H, ABq, $J$ = 13 Hz), 5.18 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.57 (1H, d, $J$ = 16 Hz), 6.84–7.44 (5H, m), 8.97 (1H, d, $J$ = 8 Hz); 9.90 (1H, br, s); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.51, 25.55, 57.54, 59.18, 62.66, 115.78, 116.75, 123.80, 125.50, 126.30, 129.50, 140.74, 159.22, 162.89, 165.04, 165.84, 170.20; ESI-MS *m/z* 441.4 [(M + Na)$^+$].

*5.3.18. (6*R*,7*R*)-7-(4′-hydroxy-α-cyanocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1r**)*

(Yield: 2.91 g, 65.7%). Anal. calcd. for $C_{20}H_{17}N_3O_7S$: C, 54.17; H, 3.86; N, 9.48; S, 7.23; found: C, 54.03; H, 3.92; N, 9.47; S, 7.31; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.05 (3H, s), 3.53 and 3.68 (2H, ABq, $J$ = 18 Hz), 4.73 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.21 (1H, d, $J$ = 5 Hz), 5.77 (1H, dd, $J$ = 5, 8 Hz), 6.97–7.89 (5H, m), 8.13 (1H, s), 9.36 (1H, d, $J$ = 8 Hz); (s); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.53, 25.48, 57.34, 59.93, 62.62, 100.43, 116.29, 116.64, 122.63, 123.53, 126.29, 133.06, 150.94, 162.08, 162.78, 162.91, 163.40, 170.14; ESI-MS *m/z* 466.3 [(M + Na)$^+$].

*5.3.19. (6*R*,7*R*)-7-(4′-methylcinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1s**)*

(Yield: 2.91 g, 70%). Anal. calcd. for $C_{20}H_{20}N_2O_6S$: C, 57.68; H, 4.84; N, 6.73; S, 7.70; found: C, 57.81; H, 4.73; N, 6.80; S, 7.84; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.03 (3H, s), 2.32 (3H, s), 3.50 and 3.66 (2H, ABq, $J$ = 18 Hz), 4.71 and 5.02 (2H, ABq, $J$ = 13 Hz), 5.16 (1H, d, $J$ = 5 Hz), 5.85 (1H, dd, $J$ = 5, 8 Hz), 6.72 (1H, d, $J$ = 16 Hz), 7.17–7.62 (5H, m), 9.03 (1H, d, $J$ = 8 Hz);$^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.50, 20.92, 25.57, 57.49, 59.21, 62.66, 119.36, 123.49, 126.32, 127.67, 129.54, 131.75, 139.68, 140.56, 162.82, 164.90, 165.56, 170.14; ESI-MS *m/z* 439.4 [(M + Na)$^+$].

*5.3.20. (6*R*,7*R*)-7-(4′-*N*,*N*-dimethylaminocinnamoyl)amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (**1t**)*

To an ice cooled suspension of 4′-*N,N*-dimethylamino cinnamic acid (2.1 g, 11 mmol) in THF (10 ml) $PCl_5$ (2.5 g, 12 mmol) was added and the mixture was stirred 10 min under ice cooling and then 30 min at room temperature to prepare an acid chloride solution. To a solution of 7-ACA (2.72 g, 10 mmol) and BSA (7.4 ml, 30 mmol) in THF (30 ml) the above acid chloride solution at –30 °C was added, and the reaction mixture was stirred for 2 h at –20 °C ~ –10 °C. To the reaction mixture was added a mixture of EtOAc (50 ml) and water (100 ml), and the EtOAc layer was separated. The organic layer was washed with water (3 × 10 ml), then with brine (10 ml) and was dried over anhydrous $Na_2SO_4$. The solvent was evaporated under vacuum and the residue was stirred with diethyl ether (30 ml) for 2 h. The resulting solid was filtered, washed with diethyl ether (3 × 10 ml) and dried to afford **1t**. (Yield: 2.67 g, 60%). Anal. calcd. for $C_{21}H_{23}N_3O_6S$: C, 56.62; H, 5.20; N, 9.43; S, 7.20; found: C, 56.71; H, 5.07; N, 9.39; S, 7.23; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.04 (3H, s), 2.96 (6H, s), 3.49 and 3.65 (2H, ABq, $J$ = 18 Hz), 4.73 and 5.02 (2H, ABq, $J$ = 18 Hz), 5.14 (1H, d, $J$ = 5 Hz), 5.83 (1H, dd, $J$ = 5, 8 Hz), 6.50 (1H, d, $J$ = 16 Hz), 6.65–7.50 (5H, m), 8.73 (1H, d, $J$ = 8 Hz); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 20.33, 25.47, 57.54, 59.18, 62.53, 111.82, 114.67, 121.86, 123.36, 126.27, 128.97, 140.97, 151.26, 162.65, 165.00, 166.03, 169.96; ESI-MS *m/z* 468.5 [(M + Na)$^+$].

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