Synthesis, Structural Characterization and Antibacterial Activity of Novel 7β-{[3-(substituted phenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-cefalosporins

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Abstract: A series of 3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-cefalosporins with various 3-phenyl-2-propenoyl substituted groups at the 7β-position were synthesized, structurally characterized and evaluated for antibacterial activity *in vitro*. To prepare these derivatives by the Vilsmeier's reagent method, it was necessary to carefully control the reaction conditions in order to avoid the formation of the biologically inactive α epimer. The NMR studies showed that the 3-phenyl-2-propenoyl moiety has little effect on chemical shifts of cephem nucleus protons and carbon atoms. Some of these cephalosporin derivatives showed good *in vitro* activity against methicillin sensible strains of *Staphylococcus aureus* (MSSA) and coagulase negative *Staphylococcus* (MSCoNS). Particularly effective were the compounds carrying a 3-(2'-chlorophenyl)-2-propenoyl or 2-methyl-3-phenyl-2-propenoyl moiety at 7β-position, both with an antibacterial potency close to cefazoline and higher than cefuroxime. All the synthesized cephalosporins were inactive against methicillin resistant strains of *Staphylococcus aureus* (MRSA) and coagulase negative *Staphylococcus* (MRCoNS).

Key Words: Cephalosporin, 3-phenyl-2-propenoyl, antibacterial, MRSA, antibiotic.

INTRODUCTION

Staphylococcus aureus is a leading cause of skin and skin-structure infections, bacteremia, and respiratory infections such as pneumonia and empyema. This organism continues to be a major cause of hospital-acquired infection and have become increasingly difficult to treat due to resistance to multiple antibiotics [1].

Methicillin resistant *Staphylococcus aureus* (MRSA) is a predominant and dangerous nosocomial pathogens and currently community acquired MRSA has started to spread. The infections caused by this organism are difficult to treat as further evolution of drug resistance occurs within the pathogen. Vancomycin remains the drug of choice in current therapy [2,3].

However, treatment failures, adverse side effects and the emergence of vancomycin- intermediate or resistant staphylococci are leading to urgent requirement for alternative anti MRSA therapies. Linezolid, daptomycin, tigecycline, and quinupristin/dalfopristin have been introduced into clinical practice, each with their own clinical pros and cons. Additionally, new lipoglycopeptides (dalbavancin, telavancin, and oritavancin) are also being investigated for the treatment of complicated skin and skin-structure infections (cSSSIs) and other indications [4].

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The development of new anti-MRSA agents, including cephalosporins, has been widely reviewed in the literature [5-8].

Ceftobiprole (formerly known as BAL9141), the active component of the prodrug ceftobiprole medocaril (formerly known as BAL5788) is the first anti-MRSA cephalosporin antibiotic and is currently in phase III clinical development. Ceftobiprole is a broad-spectrum cephalosporin with demonstrated *in vitro* activity against Gram-positive cocci, including meticillin-resistant *Staphylococcus aureus* (MRSA) and meticillin-resistant *S. epidermidis*, penicillin-resistant *S. pneumoniae*, *Enterococcus faecalis*, Gram-negative bacilli including AmpC-producing *Escherichia coli* and *Pseudomonas aeruginosa*, but excluding extended-spectrum beta-lactamase-producing strains [9,10].

Ceftaroline fosamil (TAK-599), a N-phosphono prodrug of ceftaroline (T-91825), is a derivative of a fourth-generation cephalosporin, for the potential treatment of MRSA infection. Phase III clinical trials evaluating ceftaroline fosamil for community acquired pneumonia and complicated skin and skin structure infections are underway [11].

Although most of the anti-MRSA cephalosporins developed are broad spectrum derivatives carrying an alcoximino 2-aminothiazolyl moiety at 7β-position of the cephem nucleus, efforts to discover new agents has also 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 [12-22].

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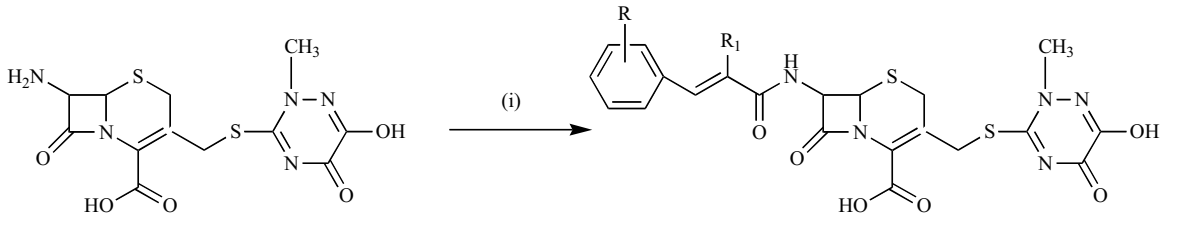
In a previous work we found a novel 7β-(3-phenyl-2-propenoyl substituted)amino-3-acetoxymethyl-cefaphalosporin that displayed a remarkable activity against MRSA strains [23]. Continuing with our research for looking anti-MRSA cephalosporins, in this paper we describe the synthesis, structural characterization and the antibacterial activity of 3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-cephalosporin derivatives with various 3-phenyl-2-propenoyl substituted groups at the 7β-position. In the literature there are no reports about the synthesis and antibacterial activity of this class of compounds.

CHEMISTRY

The 7β-{[3-(substituted-phenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-cefalosporins (1a-1s) were synthesized as shown in Fig. (**1**). They were prepared satisfactorily by acylation of 7β-amino-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic acid (7-ACT) with the substituted 3-phenyl-2-propenoic acids. Activation of the substituted 3-phenyl-2-propenoic acids with Vislmeier's reagent prepared from phosporyl chloride ($POCl_3$) and N,N-dimethylformamide (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 tetrahydrofuran (THF) as the solvent. The trimethylsilyl ester of the cephalosporin obtained was hydrolysed with water and the resulting cephalosporin was extracted with ethyl acetate (EtOAc). After removing the solvents, the crude product was purified by extracting the excess of substituted 3-phenyl-2-propenoic acid with diethyl ether to afford the compounds 1a-1s with high purity and yields between 46-86 % depending on the substituted 3-phenyl-2-propenoic acid employed.

In a previous work [23], we satisfactorily employed EtOAc to dissolve 7β-amino-3-acetoxymethyl-3-cephem-4-carboxylic acid (7-ACA) during the preparation of different 7β-{[3-(substituted-phenyl)-2-propenoyl]amino}-3-acetoxymethyl-cephalosporins, because 7-ACA readily dissolves in this solvent at room temperature by using a BSA/7-ACA 3:1 molar ratio. However, in the present work it was necessary to use a more polar solvent (THF) because 7-ACT does not dissolve in EtOAc although higher temperatures (65-70°C) and BSA/7-ACT molar ratios were used. By using THF as the solvent and a BSA/7-ACT 3:1 molar ratio, silylation and dissolution of 7-ACT occurred in 15 min. at 65-70 °C. However, after the acylation with substituted 3-phenyl-2-propenoic acids was effected and the isolated cephalosporins were analyzed by H^1 NMR it was detected, in addition to the doublet of doublet corresponding to the β-lactam ring 7α proton (δ 5.80-5.85 ppm), another doublet of doublet at higher fields (δ 5.52-5.55 ppm). Moreover, in the H^1 NMR spectra was also observed the presence of two additional doublets close to the doublets belonging to NH and β-lactam ring 6α protons. The additional doublet close to the typical β-lactam ring 6α proton doublet showed a 3.85 Hz coupling constant (J), it means a value smaller than the typical J for this proton (4.85 Hz). From these results and considering the integration relationship between the signals, it was possible to conclude that under the conditions employed it was obtained not only the expected β-epimer of the cephalosporin, but also the corresponding α-epimer as a by-product in a 6.5 %-17.7 % proportion depending of the substituted 3-phenyl-2-propenoic acid used. The structures of α and β epimers are presented in Fig. (**2**). In this sense, the epimerization of penicillins when are treated with BSA by 5 days at room temperature has been reported [24]. Although under analogous conditions no epimerization was observed for cephalosporins, it is possible to suppose that the higher temperatures employed in the present work during silylation of 7-ACT in THF causes the epimerization of this cephalosporanic nucleus and the further formation of both epimers during the acylation reaction. Since it is known that the cephalosporin α-epimers (compounds where



7-ACT

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'-OCH_3, 4'-OH; R_1= H
1o, R = 3'-OH, 4'-OCH_3; 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

Reagents:

(i) N,O-bis(trimethylsilyl)acetamide / THF; substituted 3-phenyl-2-propenoic acid, Vilsmeier Reagent (DMF, $POCl_3$) / THF

Fig. (1). Synthesis of the cefalosporin derivatives 1a-1s.

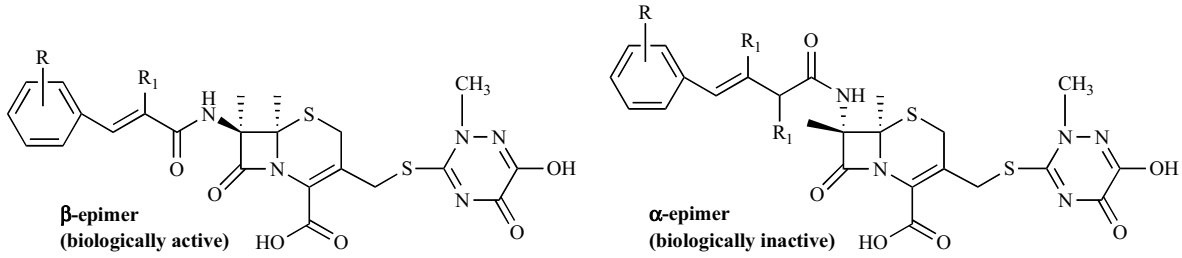


Fig. (2). α and β-Epimer of the new compounds.

the β-lactam ring proton is in the 7β-position) have not biological activity as antibiotics, then the products obtained would not be representative to measure their real antibacterial activity.

Table 1. ^{1}**H NMR Data for Compounds 1a-s (δ, ppm; J, Hz)**

Compd.	NH[a]	H-11	H-10[d]	Aromatic Protons (range)	H-7[f]	H-6[g]	H-13[h]	H-2[i]	CH_3[j]	R
1a	9.07	c	6.76	7.34-7.64 (6H,m)[e]	5.85	5.16	4.10 and 4.39	3.59 and 3.77	3.59	
1b	9.00	7.39 (s,1H)	-	7.25-7.46 (5H,m)	5.77	5.14	4.10 and 4.38	3.63 and 3.75	3.58	2.01 (s,3H,α-CH_3)
1c	9.20	7.82[b]	6.81	7.36-7.75 (4H,m)	5.85	5.16	4.11 and 4.40	3.58 and 3.77	3.60	
1d	9.07	c	6.79	7.39-7.69 (5H,m)[e]	5.84	5.16	4.10 and 4.39	3.59 and 3.77	3.59	
1e	9.08	c	6.75	7.41-7.69 (5H,m)[e]	5.83	5.13	4.09 and 4.38	3.59 and 3.77	3.59	
1f	9.19	c	6.81	7.44-7.80 (4H,m)[e]	5.84	5.16	4.11 and 4.39	3.59 and 3.77	3.58	
1g	9.21	c	6.72	7.58-8.10 (5H,m)[e]	5.85	5.17	4.11 and 4.39	3.59 and 3.78	3.59	
1h	9.14	c	6.94	7.58-8.48 (5H,m)[e]	5.86	5.17	4.11 and 4.39	3.59 and 3.78	3.59	
1i	9.21	7.65[b]	6.92	7.76-8.38 (4H,m)	5.85	5.17	4.12 and 4.40	3.60 and 3.79	3.59	
1j	9.05	7.73[b]	6.81	6.93-7.12 (4H,m)	5.84	5.15	4.11 and 4.39	3.59 and 3.77	3.59	3.86 (s,3H,OCH_3)
1k	8.96	7.50[b]	6.77	6.91-7.34 (4H,m)	5.83	5.16	4.13 and 4.42	3.60 and 3.77	3.60	3.79 (s,3H,OCH_3)
1l	8.86	c	6.62	6.94-7.60 (5H,m)[e]	5.83	5.15	4.14 and 4.42	3.59 and 3.77	3.60	3.80 (s,3H,OCH_3)
1m	8.93	7.51[b]	6.64	6.90-7.50 (3H,m)	5.84	5.14	4.10 and 4.39	3.58 and 3.76	3.58	3.77 (s,3H,OCH_3) 3.79 (s,3H,OCH_3)
1n	8.88	7.42[b]	6.57	6.74-7.18 (3H,m)	5.83	5.13	4.10 and 4.39	3.58 and 3.76	3.58	3.79 (s,3H,OCH_3) 9.51 (br s,1H,OH)
1o	8.94	7.37[b]	6.51	6.88-7.06 (3H,m)	5.82	5.13	4.10 and 4.38	3.58 and 3.76	3.58	3.78 (s,3H,OCH_3) 9.23 (br s,1H,OH)
1p	8.88	7.44[b]	6.68	7.28-6.85 (4H,m)	5.81	5.15	4.15 and 4.43	3.60 and 3.77	3.61	
1q	8.75	c	6.55	6.65-7.50 (5H,m)[e]	5.81	5.14	4.14 and 4.43	3.59 and 3.76	3.61	
1r	9.33	8.10 (s,1H)	-	6.88-7.80 (4H,m)	5.74	5.15	4.10 and 4.30	3.62 and 3.75	3.58	
1s	8.92	c	6.71	7.15-7.57 (5H,m)[e]	5.83	5.15	4.13 and 4.42	3.60 and 3.77	3.60	2.32 (s,3H,CH_3)

a For all the compounds this signal integrates 1H and appears as a doublet with J = 8 Hz.

b This signal integrates 1H and appears as a doublet with J = 16 Hz.

c In these compounds the H-11 proton is overlapped with aromatic protons.

d For all the compounds this signal integrates 1H and appears as a doublet with J = 16 Hz.

e The H-11 proton signal is included into aromatic protons.

f For all the compounds this signal integrates 1H and appears as a doublet of doublet with J = 8 Hz and J = 5 Hz.

g For all the compounds this signal integrates 1H and appears as a doublet with J = 5 Hz.

h For all the compounds these signals integrate 2H and appear as an AB system with J = 13 Hz.

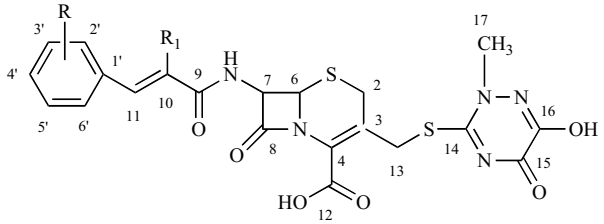
i For all the compounds these signals integrate 2H and appear as an AB system with J = 18 Hz.

j For all the compounds this signal appears as a singlet and integrates 3H.

By this cause, it was necessary to use a BSA/7-ACT 5:1 molar ratio to obtain the trimethylsilyl ester of the 7-ACT and dissolve the nucleus at room temperature to minimize the epimerization reaction. Although the time required to dissolve the 7-ACT increased (1 h), under these new reaction conditions all the cephalosporins were obtained as the pure appropriated β-epimers and were satisfactorily used to evaluate the antibacterial activity *in vitro*.

STRUCTURAL CHARACTERIZATION BY NMR

The ^{1}H NMR assignements of the new compounds are given in Table **1**, Fig. (**3**). The CH_3 protons of the heterocyclic group attached to C-3 position appear in the narrow range of δ 3.58-3.61 ppm for all the compounds. The two protons on C-2 and C-13 positions appear as AB systems with coupling constants of 18 Hz and 13 Hz respectively, indicating that these protons are not equivalent. The H-6 proton appears as a doublet at δ 5.13-5.17 ppm with a coupling constant of 5 Hz and the H-7 proton appears as a doublet of doublet located between δ 5.74-5.86 ppm. The substituents on the aromatic ring do not affect the H-7 chemical shift, but the cyano and methyl groups attached to the double bond α position (C-10) of 3-phenyl-2-propenoyl moiety produce a slight shielding of 0.07-0.08 ppm on the H-7 signal (compounds **1b** and **1r**) as compared with the corresponding α non substituted compounds (**1a** and **1q**). The signal corresponding to the amide proton (NH) appears as a doublet in the range of δ 8.75-9.33 ppm, with a coupling constant of 8 Hz. Compared with the unsubstituted compound (**1a**) the electrowithdrawing groups (excepting the chlorine atom at 3' and 4' positions) shift this signal downfield (0.07-0.14 ppm) whereas the electrodonating groups (excepting the 2'-methoxy group) shield the NH signal 0.11-0.32 ppm. In compound **1r** the influence of the α cyano group is higher than the 4'-hydroxy group and the overall effect shift the NH signal 0.26 ppm downfield. The H-10 proton, with the exception of compounds **1b** and **1r**, appears at δ 6.51-6.94 ppm as a doublet with a coupling constant of 16 Hz. This J value indicates that in all the cases the 3-phenyl-2-propenoyl moiety is the *trans* isomeric form. Generally, the electrodonating substituents on aromatic ring cause a shielding on H-10 signal (0.05-0.21 ppm) whereas the electrowithdrawing groups shift this signal downfield (highest effect in the case of nitro group at 3' and 4' positions, compounds **1h** and **1i**). In dependence of the aromatic substitution pattern, the H-11 signal appears either as a well defined doublet (J=16 Hz) in the range of δ 7.42-7.82 ppm, or it is overlapped with aromatic protons (Table **1**). In compounds **1b** and **1r** this proton resonate as a singlet due to the lack of a proton on C-10 position. It may be concluded that substitution on 3-phenyl-2-propenoyl moiety has little effect on cephem nucleus protons and the corresponding chemical shifts are typical for cephalosporins [25,26].



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'-OCH_3, 4'-OH; R_1= H
1o, R = 3'-OH, 4'-OCH_3; 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

Fig. (3). Structure of new compounds.

The ^{13}C NMR spectroscopic properties of new compounds are presented in Table **2**, Fig. (**3**). The carbonyl carbon of the amide group (C-9) resonates in the narrow range of δ 164-166 ppm with the exception of compounds **1b** and **1r**. As compared with the corresponding α non substituted compounds (**1a** and **1q**), the α cyano group produces a *ca* 2-3 ppm upfield shift on the C-9 signal (**1r**) and the α methyl group shifts the C-9 signal *ca* 4 ppm downfield. As compared with the compound **1a**, electroaceppting groups on the aromatic ring shift the C-10 signal 3-4 ppm downfield, particularly for 2' and 4' nitro derivatives (compounds **1g** and **1i**) and when a chlorine atom is located at 2' position (compounds **1c** and **1f**). Contrarily, electrodonating substituents shift this signal upfield (~ 3-4 ppm) especially when are linked to 4' position of the aromatic ring. The signal of C-11 is little influenced by electroaceppting groups (1-2 ppm upfield shift) excepting when are located on 2' position of the aromatic ring (4-5 ppm upfield shift). Electodonating substituents do not affect the resonance of C-11 signal excepting when a methoxy group is linked to 2' position (~ 5 ppm upfield shift). As compared with the α non-substituted derivatives (**1a** and **1q**), the α substitution in the double bond of 3-phenyl-2-propenoyl moiety affects strongly the chemical shifts of C-10 and C-11. The cyano group (**1r**) has little influence on C-10 signal but shifts C-11 signal 10 ppm downfield. The methyl group (**1b**) produces a shielding (~ 7 ppm) of the C-11 signal and shifts the C-10 signal *ca* 11 ppm downfield. In general the values are typical for cephalosporins [27-32], and the chemical shifts of these carbon atoms are also little affected by substitution on the 3-phenyl-2-propenoyl moiety.

BIOLOGICAL RESULTS AND DISCUSSION

The *in vitro* antibacterial activity (MIC_{50} and MIC_{90}) of the synthesized compounds (**1a-1s**), in comparison to cefazolin and cefuroxime as reference compounds, against ATCC strains (*Staphylococcus aureus* 25923 and *Escherichia coli* 25922) and various clinical isolates of Gram-positive bacteria are shown in Table **3**.

All the compounds displayed a selective activity against Gram-positive bacteria because they did not show activity (MIC > 256 μg/mL) against the Gram-negative strain taken as the reference (*Eschericchia coli* ATCC 25922).

From Table **3** it may be observed that generally, substitution on the aromatic ring of 3-phenyl-2-propenoic acid reduced or did not improve the antibacterial potency against methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin sensitive coagulase negative *Staphylococcus*

Table 2. ^{13}C NMR Data for Compounds 1a-s (δ, ppm)

C	1a	1b	1c	1d	1e	1f	1g	1h	1i	1j	1k	1l	1m	1n	1o	1p	1q	1r	1s
2	26.67	26.60	26.64	26.64	26.63	26.69	26.69	26.72	26.66	26.67	26.67	26.67	26.66	26.72	26.67	26.66	26.58	26.69	26.66
3	125.80	125.48	125.75	125.75	125.76	125.80	125.80	125.86	125.78	125.63	125.71	125.63	125.80	125.74	125.55	125.58	125.51	126.00	125.64
4	125.86	126.06	125.85	125.87	125.85	125.83	125.86	125.94	125.89	125.84	125.89	125.92	125.86	125.94	125.97	125.98	125.93	126.06	125.89
6	57.47	57.71	57.33	57.36	57.39	57.35	57.33	57.39	57.32	57.54	57.41	57.47	57.49	57.69	57.56	57.38	57.45	57.41	57.42
7	59.21	59.68	59.17	59.15	59.16	59.21	59.18	59.20	59.19	59.24	59.15	59.17	59.16	59.20	59.20	59.15	59.14	59.92	59.15
8	164.81	164.21	164.56	164.66	164.68	164.53	164.57	164.68	164.51	164.89	164.58	164.68	164.91	165.03	164.95	164.39	164.58	163.44	164.60
9	165.45	169.70	164.93	165.07	165.21	164.82	164.66	164.92	164.77	165.89	165.30	165.62	165.68	165.89	165.76	165.31	165.68	163.01	165.47
10	120.47	131.16	123.46	122.10	121.16	124.15	124.70	121.86	124.63	120.75	119.90	117.94	118.12	117.16	117.68	120.23	116.85	116.71	119.40
11	140.66	133.78	135.81	139.01	139.27	135.04	135.89	138.31	137.96	135.78	140.36	140.17	140.68	141.06	140.86	140.55	140.44	150.94	140.40
1'	134.53	135.73	133.43	136.78	133.44	131.40	129.81	136.40	140.96	122.88	135.91	127.07	127.29	126.05	127.40	135.68	125.48	122.69	131.73
2'	129.01	129.29	132.27	129.44	129.00	134.69	148.27	124.12	128.98	157.80	115.58	129.18	110.15	111.07	113.37	113.73	129.13	133.13	127.53
3'	127.74	128.46	129.99	133.65	129.39	129.49	124.97	148.29	123.83	111.76	159.25	114.35	148.86	147.83	146.74	157.52	115.62	116.36	129.41
4'	129.89	127.97	131.25	126.06	133.28	134.26	130.53	123.32	147.69	131.34	112.73	160.55	150.39	148.71	149.61	116.89	158.99	162.15	139.53
5'	127.74	128.46	127.62	130.77	129.39	128.12	133.95	130.58	123.83	120.94	129.88	114.35	111.73	115.74	112.10	129.65	115.62	116.36	129.41
6'	129.01	129.29	127.79	127.42	129.00	128.94	128.79	134.02	128.98	128.30	120.81	129.18	121.62	121.85	120.81	118.67	129.13	133.13	127.53
12	163.05	163.10	162.95	162.98	162.99	163.01	163.00	163.06	162.98	163.09	162.84	162.81	163.06	163.10	163.09	162.66	162.66	163.04	162.81
13	32.80	32.81	32,.73	32.73	32.73	32.77	32.78	32.80	32.80	32.81	32.81	32.83	32.81	32.83	32.84	32.83	32.75	32.81	32.80
14	156.23	156.22	156.14	156.15	156.27	156.20	156.20	156.23	156.17	156.25	156.02	156.00	156.16	156.25	156.26	155.87	155.87	156.25	156.01
15	160.35	160.36	160.28	160.29	160.29	160.33	160.33	160.34	160.29	160.36	160.25	160.26	160.28	160.37	160.36	160.22	160.17	160.34	160.24
16	153.73	153.72	153.66	153.67	153.68	153.72	153.70	153.75	153.67	153.75	153.59	153.56	153.70	153.75	153.75	153.47	153.47	153.75	153.58
17	42.41	42.40	42.34	42.36	42.36	42.40	42.40	42.43	42.36	42.43	42.26	42.23	42.38	42.43	42.43	42.12	42.11	42.41	42.24
18	-	14.38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	55.59	55.03	55.16	55.56	55.51	55.62	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	55.60	-	-	-	-	-	-
21	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.56	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.79

18 carbon atom of α-CH_3 group (CH_3-10 group).

19 and **20** carbon atoms of OCH_3 groups.

21 carbon atom of α-CN group (CN-10 group).

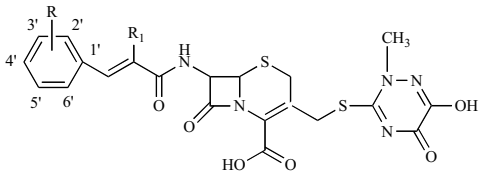
22 carbon atom of 4'-CH_3 group.

(MSCoNS). The derivatives obtained from (2'-chloro), (3'-nitro) and 3-(2'-methoxy)phenyl-2-propenoic acids (**1c**, **1h** and **1j**, respectively) were the exception because they exhibited a better antibacterial potency than the non substituted derivative (**1a**). The most effective aromatic substituted compound was the 2'-chloro derivative (**1c**) which displayed an antibacterial potency close to cefazoline and higher than cefuroxime.

With regard to the better positions for aromatic ring substitution, the most favoured was the *ortho* (2') position, followed by the *meta* (3') position. The *para* (4') position was particularly inadequate because all the 4'-mono-substituted compounds (**1e**, **1i**, **1l**, and **1s**) and also the 2',4' (**1f**) and 3',4' bi-substituted derivatives (**1m**, **1n** and **1o**) displayed a poor antimicrobial activity. This behaviour had been reported previously during the *in vitro* biological evaluation of several 7β-{[3-(substituted phenyl)-2-propenoyl]amino}-3-acetoxymethyl-cephalosporins [23].

Although the bi-substituted compound **1f**, carries a chlorine atom on the most favourable position (2'), this derivative exhibited poorer antibacterial activity than the corresponding mono-substituted compound (**1c**), probably because

Table 3. Structure-Activity Relationship of Cephalosporin Analogues 1a-1s (MIC, μg/mL)



	Organism (No. of strains)		*Sa*ATCC 25923	*Ec*ATCC 25922	MSSA (20)			MRSA (20)			MSCoNS (20)			MRCoNS (20)		
Comp.	**R**	**R_1**			**Range**	**MIC_{50}**	**MIC_{90}**	**Range**	**MIC_{50}**	**MIC_{90}**	**Range**	**MIC_{50}**	**MIC_{90}**	**Range**	**MIC_{50}**	**MIC_{90}**
CFZ	-	-	0.25	N.T.	0.25-1	0.25	0.5	32-128	64	64	0.25-1	0.25	0.5	16-128	32	32
CFU	-	-	0.5	N.T.	0.5-32	2	4	32-128	64	64	2-4	2	4	32-128	32	64
1a	H	H	1	>256	1-4	1.5	4	256	256	256	1-4	2	4	256	256	256
1b	H	CH_3	0.25	>256	0.25-1	0.38	1	128-256	192	256	0.25-1	0.63	1	128-256	192	256
1c	2'-Cl	H	1	>256	0.5-1	0.5	1	256	256	256	0.5-2	0.75	2	256	256	256
1d	3'-Cl	H	1	>256	1-4	1.5	4	256	256	256	1-4	2	4	256	256	256
1e	4'-Cl	H	8	>256	8-64	12	64	128	128	128	8-32	12	32	128-256	192	256
1f	2',4'-Cl	H	4	>256	2-8	3	8	256	256	256	2-32	8	32	256	256	256
1g	2'-NO_2	H	1	>256	1-8	1	8	256	256	256	1-4	1.5	4	256	256	256
1h	3'-NO_2	H	0.5	>256	0.5-2	1	2	128-256	192	256	1-4	1	4	128-256	192	256
1i	4'-NO_2	H	2	>256	2-16	4	16	64	64	64	2-16	4	6	32-256	144	256
1j	2'-OCH_3	H	0.5	>256	0.5-2	1	2	256	128	256	1-4	1	4	128-256	192	256
1k	3'-OCH_3	H	2	>256	1-8	2	8	256	256	256	4-8	4	8	128-256	192	256
1l	4'-OCH_3	H	8	>256	2-16	4	16	128	128	128	4-16	10	16	128-256	192	256
1m	3',4'-OCH_3	H	16	>256	8-32	24	32	256	256	256	16-64	24	64	256	256	256
1n	3'-OCH_3, 4'-OH	H	128	>256	4-8	4	8	256	256	256	4-16	6	16	64-256	160	256
1o	3'-OH, 4'-OCH_3	H	128	>256	32-256	48	256	256	256	256	16-128	48	128	256	256	256
1p	3'-OH	H	4	>256	1-4	2	4	128-256	192	256	1-4	2	4	128-256	192	256
1q	4'-OH	H	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T	N.T
1r	4'-OH	CN	4	>256	1-8	2	8	128	128	128	1-4	3	4	256	256	256
1s	4'-CH_3	H	8	>256	16	16	16	256	256	256	8-256	96	256	256	256	256

CFZ: cefazolin; CFU: cefuroxime; *Sa*ATCC: *Staphylococcus aureus* ATCC; *Ec*ATCC: *Eschericchia coli* ATCC; MSSA: Meticillin sensitive *Staphylococcus aureus*; MRSA: Methicillin resistant *Staphylococcus aureus*; MSCoNS: Meticillin sensitive negative coagulase *Staphylococcus*; MRCoNS: Meticillin resistant negative coagulase *Staphylococcus*; N.T: not tested.

it has the presence of the second chlorine atom at 4' position. This substituent on the *para* position has been proved unfavourable in order to develop antibacterial activity, since compound **1e** was almost inactive.

An analogous but more critical behaviour can be observed for bi-substituted compounds **1m** and **1o**, because in both cases the antibacterial activity was dramatically reduced as comparing with the corresponding 3' and 4' monosubti-tuted derivatives. In the case of **1o**, although the hydroxy group at *meta* (3') position of the aromatic ring provides an acceptable antibacterial activity (compound **1p**), the presence of an additional 4'-methoxy group reduced drastically the activity, as compared with the 3'-hydroxy monosubstituted derivative. However, this effect can not only be

explained considering the presence of a substituent in the less favourable position of the aromatic ring, because the antimicrobial potency of compound **1o** was also poorer than the 4'-metoxy mono-substituted derivative **1l**. It's possible that the individual effect of the 3'-hydroxy group on antimicrobial activity is 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, but higher than **1o**. This behaviour can be explained because **1n** has a 4'-hydroxy group but not the additional structural handicap that represents the presence of a methoxy group on the *para* position.

With regard to compound **1m**, the lack of antibacterial activity could be explained by the presence of two electrodonating groups in the aromatic ring, since in a previous work it had been demonstrated that this kind of substituents strongly reduces the activity as compared with electrowithdrawing groups [23]. The presence of a methyl group attached to the α position of the double bond of the 3- phenyl-2-propenoyl moiety (**1b**) increases notably the antibacterial activity against MSSA and MSCoNS (as compared with **1a**) and provides the most potent of the cephalosporins synthesized with MIC values very close to cefazoline and higher than cefuroxime. The effect of the cyano group at such position (compound **1r**) could not be evaluated because the needed compound for the comparison (**1q**), with a hydroxy group on the *para* position of the aromatic ring, was not tested during the present work. In order to obtain conclusive criteria about the importance of this class of substitution on antibacterial activity it is necessary to synthesize new derivatives carrying different groups at the α position of the double bond of the 3-phenyl-2-propenoyl moiety.

All the compounds were ineffective against MRSA and methicillin resistant coagulase negative *Staphylococcus* (MRCoNS). In our previous work [23] we found that the 3-acetoxymethyl cephalosporin carrying the 3-(2',4'-dichlorophenyl)-2-propenoyl moiety at 7β position of the cephem nucleus displayed a remarkable activity against these multirresistant strains (MIC_{90} = 16 μg/mL). However, from the results obtained during the present work it can be concluded that the analogous derivative synthesized from 7-ACT (**1f)** was completely inactive. A possible explanation of this phenomena would be that the [(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl group attached at C-3 position of the cephem nucleus negatively affects the penetration across the bacterial cell wall and/or the coupling of the cephalosporin to the penicillin binding proteins receptor (PBP 2A) typical of these microorganisms.

CONCLUSIONS

To prepare these derivatives by the Vilsmeier's reagent method, it was necessary to use THF as the solvent and a BSA/7-ACT 5:1 molar ratio in order to prevent the formation of the biologically inactive α-epimer. The NMR studies showed that the 3-phenyl-2-propenoyl moiety has little effect on chemical shifts of cephem nucleus protons and carbon atoms. The coupling of a 3-phenyl-2-propenoyl moiety to the 7-ACT cephem nucleus provides cephalosporins with selective activity against Gram-positive bacteria. The compounds obtained from 3-(2'-chlorophenyl) and 2-methyl-3-phenyl-2-propenoic acids displayed a strong antibacterial activity (close to cefazolin and higher than cefuroxime) against methicillin sensible strains of *Staphylococcus sp.* (MSSA and MSCoNS). All the synthesized cephalosporins were inactive against methicillin resistant strains of *Staphylococcus sp.* (MRSA and MRCoNS).

EXPERIMENTAL PROTOCOLS

General Methods

^{1}H NMR and ^{13}C NMR spectra were measured with a Bruker Avance DPX-300 spectrometer for 300 MHz and 75 MHz respectively in DMSO-d_6 and using TMS as internal reference standard. The NMR assignments were based on 2D COSY, HMQC and HMBC NMR spectra.

The electrospray mass spectra (ESI-MS) were obtained on a spectrometer Waters-Micromass ZQ. 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.

Antibacterial Activity *In Vitro*

Evaluation of antibacterial activity was conducted by agar dilution method as recommended by the National Committee for Clinical Laboratory Standards 2000 (NCCLS) [33]. Wild-type clinical isolates used for antibacterial activity analysis were from the microorganism bank of the Center of Pharmaceutical Chemistry. The antibacterial activity of the synthesized compounds was screened against the following bacterial strains: methicillin sensitive *Staphylococcus aureus* (MSSA), methicillin sensitive coagulase negative *Staphylococcus* (MSCoNS), methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative *Staphylococcus* (MRCoNS).

The solutions with concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 μg/mL were prepared from the solution (10 mg / mL) of the interested cephalosporin. A 50 μL of cell suspension of test strains was added to sterile plates of 96 wells having about 10^8 cfu/mL. All bacteria were grown on Muller-Hinton Agar (Hi-media) plates (37°C, 24 h). The plates were visually examined and the last hole with no bacterial growth was determined. The MIC was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculums. The MIC of the test compounds was compared with the reference drug cefazolin and cefuroxime.

Synthesis of 7β-{[3-(substituted phenyl)-2-propenoyl] amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-cefalosporins (1a-1s)

(6R,7R)-7β-{[3-phenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-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's reagent. To the above mixture, 3-phenyl-2-propenoic acid (11 mmol) was added under ice cooling and the reaction mixture was

stirred at the same temperature for 1h to produce an activated acid solution of the 3-phenyl-2-propenoic acid. To a solution of 7β-amino-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic acid (7-ACT) (3.71 g, 10 mmol) and BSA (12.24 mL, 50 mmol) in THF (40 mL) the above activated acid solution was added at – 20°C, and the reaction mixture was stirred at – 20°C for 1h. To the reaction mixture a mixture of EtOAc (50 mL) and water (100 mL) was added, and the organic layer was separated. The organic layer was washed with water (3 x 10 mL), with brine (10 mL) and dried over anhydrous Na_2SO_4. The solvents were evaporated under vacuum and the residue was stirred with diethyl ether (30 mL) for 1h. The resulting solid was filtered, washed with diethyl ether (3 x 10 mL) and dried to afford **1a**. (Yield: 3.36 g, 67 %). Anal. calcd. for $C_{21}H_{19}N_5O_6S_2$: C, 50.29; H, 3.82; N, 13.96; S, 12.79; found: C, 50.22; H, 3.84; N, 13.90; S, 12.77; ESI-MS m/z 524.4 $[(M + Na)^+]$.

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

(6R,7R)-7β-{[2-methyl-3-phenyl-2-propenoyl]amino}-3-[(2, 5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1b)

(Yield: 4.45 g, 86.3 %). Anal. calcd. for $C_{22}H_{21}N_5O_6S_2$: C, 51.25; H, 4.11; N, 13.58; S, 12.44; found: C, 51.13; H, 4,13; N, 13.58; S, 12.39; ESI-MS m / z 538.4 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(2-chlorophenyl)-2-propenoyl]amino}-3-[(2, 5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1c)

(Yield: 2.94 g, 55 %). Anal. calcd. for $C_{21}H_{18}ClN_5O_6S_2$: C, 47.06; H, 3.38; Cl, 6.61; N, 13.07; S, 11.97; found: C, 47.12; H, 3.35; Cl, 6.65; N, 13.07; S, 11.92; ESI-MS m / z 558.9 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3-chlorophenyl)-2-propenoyl]amino}-3-[(2, 5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1d)

(Yield: 4.46 g, 83.3 %). Anal. calcd. for $C_{21}H_{18}ClN_5O_6S_2$: C, 47.06; H, 3.38; Cl, 6.61; N, 13.07; S, 11.97; found: C, 47.00; H, 3.41; Cl, 6.57; N, 13.01; S, 12.01; ESI-MS m / z 558.7 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-chlorophenyl)-2-propenoyl]amino}-3-[(2, 5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1e)

(Yield: 2.95 g, 55 %). Anal. calcd. for $C_{21}H_{18}ClN_5O_6S_2$: C, 47.06; H, 3.38; Cl, 6.61; N, 13.07; S, 11.97; found: C, 47.10; H, 3.35; Cl, 6.70; N, 13.18; S, 11.91; ESI-MS m / z 558.7 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(2,4-dichlorophenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1f)

(Yield: 3.71 g, 65.1 %). Anal. calcd. for $C_{21}H_{17}Cl_2N_5O_6S_2$: C, 44.22; H, 3.00; Cl, 12.43; N, 12.28; S, 11.24; found: C, 44.30; H, 3.05; Cl, 12.44; N, 12.36; S, 11.22; ESI-MS m / z 593.5 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(2-nitrophenyl)-2-propenoyl]amino}-3-[(2, 5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1g)

(Yield: 4.46 g, 81.6 %). Anal. calcd. for $C_{21}H_{18}N_6O_8S_2$: C, 46.15; H, 3.32; N, 15.38; S, 11.73; found: C, 46.13; H, 3.37; N, 15.29; S, 11.79; ESI-MS m / z 569.4 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3-nitrophenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1h)

(Yield: 4.48 g, 82 %). Anal. calcd. for $C_{21}H_{18}N_6O_8S_2$: C, 46.15; H, 3.32; N, 15.38; S, 11.73; found: C, 46.07; H, 3.30; N, 15.43; S, 11.63; ESI-MS m / z 569.4 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-nitrophenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1i)

(Yield: 4.1 g, 75 %). Anal. calcd. for $C_{21}H_{18}N_6O_8S_2$: C, 46.15; H, 3.32; N, 15.38; S, 11.73; found: C, 46.21; H, 3.34; N, 15.30; S, 11.64; ESI-MS m / z 569.3 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(2-methoxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1j)

(Yield: 3.72 g, 70 %). Anal. calcd. for $C_{22}H_{21}N_5O_7S_2$: C, 49.71; H, 3.98; N, 13.18; S, 12.06; found: C, 49.61; H, 4.00; N, 13.22; S, 12.01; ESI-MS m / z 554.3 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3-methoxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1k)

(Yield: 3.72 g, 70 %). Anal. calcd. for $C_{22}H_{21}N_5O_7S_2$: C, 49.71; H, 3.98; N, 13.18; S, 12.06; found: C, 49.73; H, 3.92; N, 13.27; S, 12.01; ESI-MS m / z 554.6 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-methoxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1l)

(Yield: 3.13 g, 59 %). Anal. calcd. for $C_{22}H_{21}N_5O_7S_2$: C, 49.71; H, 3.98; N, 13.18; S, 12.06; found: C, 49.81; H, 3.94; N, 13.20; S, 12.12; ESI-MS m / z 554.5 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3,4-dimethoxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1m)

(Yield: 2.6 g, 46.3 %). Anal. calcd. for $C_{23}H_{23}N_5O_8S_2$: C, 49.19; H, 4.13; N, 12.47; S, 11.42; found: C, 49.26; H, 4.05; N, 12,51; S, 11.50; ESI-MS m / z 584.5 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl] amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1n)

(Yield: 3.71 g, 68 %). Anal. calcd. for $C_{22}H_{21}N_5O_8S_2$: C, 48.26; H, 3.87; N, 12.79; S, 11.71; found: C, 48.14; H, 3.92; N, 12.81; S, 11.66; ESI-MS m / z 570.6 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3-hydroxy-4-methoxyphenyl)-2-propenoyl] amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1o)

(Yield: 3.72 g, 68 %). Anal. calcd. for $C_{22}H_{21}N_5O_8S_2$: C, 48.26; H, 3.87; N, 12.79; S, 11.71; found: C, 48.30; H, 3.80; N, 12.83; S, 11.81; ESI-MS m / z 570.5 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(3-hydroxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1p)

(Yield: 3.73 g, 72 %). Anal. calcd. for $C_{21}H_{19}N_5O_7S_2$: C, 48.74; H, 3.70; N, 13.53; S, 12.39; found: C, 48.80; H, 3.62; N, 13.64; S, 12.33; ESI-MS m / z 540.3 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-hydroxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1q)

(Yield: 3 g, 58 %). Anal. calcd. for $C_{21}H_{19}N_5O_7S_2$: C, 48.74; H, 3.70; N, 13.53; S, 12.39; found: C, 48.72; H, 3.67; N, 13.58; S, 12.35; ESI-MS m / z 540.3 $[(M + Na)^+]$.

(6R,7R)-7β-{[2-cyano-3-(4-hydroxyphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1r)

(Yield: 4.12 g, 76 %). Anal. calcd. for $C_{22}H_{18}N_6O_7S_2$: C, 48.70; H, 3.34; N, 15.49; S, 11.82; found: C, 48.73; H, 3.31; N, 15.42; S, 11.93; ESI-MS m / z 565.5 $[(M + Na)^+]$.

(6R,7R)-7β-{[3-(4-methylphenyl)-2-propenoyl]amino}-3-[(2,5-dihydro-6-hydroxy-2-methyl)-5-oxo-cis-triazin-3-yl]-thiomethyl-3-cephem-4-carboxylic Acid (1s)

(Yield: 3.35 g, 65 %). Anal. calcd. for $C_{22}H_{21}N_5O_6S_2$: C, 51.25; H, 4.11; N, 13.58; S, 12.44; found: C, 51.37; H, 4.07; N, 13.51; S, 12.45; ESI-MS m / z 538.5 $[(M + Na)^+]$.

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