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Calixcephems: clustered cephalosporins analogous to calixpenams as novel potential anti-MRSA agents

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Abstract: Two novel clusters of cephalosporin based on calixarene were synthesized directly or by ring expansion of the penicillin arms of calixpenams to the corresponding cephem arms via sulfoxide intermediates. Antibiotic tests showed that calixcephems have a noteworthy increase in antibacterial activity against methicillin-resistant strains of *Staphylococcus aureus* (MRSA) with respect to parent calixpenams and their corresponding single cephems.

Key words: Calixcephem, calixpenam, calixarene, cluster, *Staphylococcus aureus*

1. Introduction

Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st century, particularly as it pertains to pathogenic organisms. The resistance of infective bacteria to present antibiotics demands research assigned to the discovery of new drugs in the antibacterial drug field.[1,2]

Staphylococcus aureus is an anaerobic gram-positive, nonspore forming spherical bacterium that belongs to the genus *Staphylococcus*. Approximately 50% of humans are carriers of this organism and it is a common cause of skin infections (e.g., boils), respiratory disease (e.g., sinusitis), and food poisoning. *S. aureus* was the first bacterium resistant to penicillin. The staphylococcal β-lactamase protein, which cleaves the β-lactam ring structure, confers resistance to penicillin. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies.[3]

The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus *Cephalosporium acremonium* in Sardinia in 1948 by Italian scientist Giuseppe Brotzu.[4] They constitute a subgroup of β-lactam antibiotics called cephems. They have a core structure, consisting of a 2-ring system that includes a β-lactam ring condensed with a dihydrothiazine ring (Figure 1). The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was derived by hydrolysis from the natural cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid (6-APA), but it was not sufficiently potent for clinical use.[5] Further work by Robert Morin led to semisynthesis of 7-amino-deacetoxycephalosporanic acid (7-ADCA) from penicillins (the Morin ring expansion), which is convenient because penicillins can be fermented with more ease than cephalosporins.[6] Chemical compounds containing this core are relatively stable to acid hydrolysis and tolerant to β-lactamases.[4] In vitro stability can be enhanced by the addition of hydrogen to positions α_1

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and α_2 of the cephalosporin structure (Figure 1). The cephalosporins like other β-lactams have the ability to kill bacteria by disrupting the transpeptidation step in the synthesis of the peptidoglycan layer of bacterial cell walls, which in the end results in osmotic lysis and death of the bacterial cell.[7] Cephalosporins are widely used antibiotics because of their clinical efficiency and desirable safety profile (due to their lower allergenic and toxicity risks as well as a board spectrum of activity).[8]

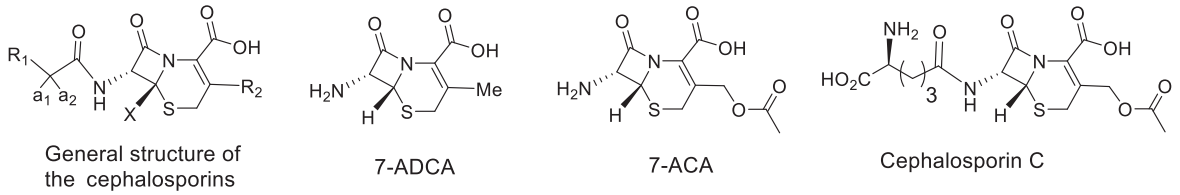


Figure 1. Chemical structure of cephalosporins.

First generation cephalosporins have good antimicrobial activity against gram-positive bacteria (*Staphylococcus*) but limited activity against gram-negative species (*E. coli*).[9] The chemical structures of the first generation cephalosporins are fairly simple. The common side groups at C-3 (R_2) for first class cephalosporins are small uncharged groups like methyl (7-ADCA core).[10]

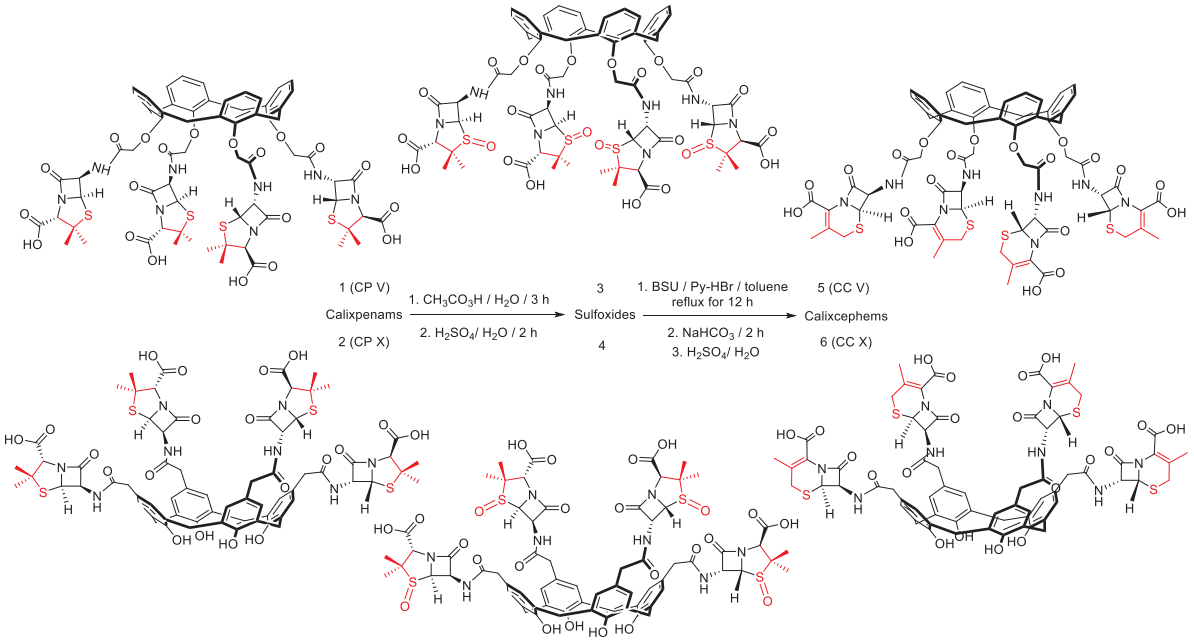
Compared to penams, cephems are less susceptible to β-lactamase (more active against penicillinase-producing bacteria such as *S. aureus*) and more stable to acid hydrolysis.[4] In addition, it is thought that antibiotic resistance is unavoidable, but medicinal chemistry can slow it down through the development of new antibiotics. There should be many strategies in order to develop new drugs, such as drug clustering.[11,12] These reasons prompted us to synthesize novel generations of cephems by using a firm molecular platform for the demonstration of the cephalosporin cluster. This idea could result in novel molecular structures with enhanced effects and antibiotic activities in comparison to single cephalosporin units (cephems V and X) and penicillin clusters (calixpenams).

According to the reasons cited for medical applications of calixarenes, these structures are suitable for the design and development of new drugs.[11,12]

In our previous work,[11] we reported the synthesis and biological evaluation of calixpenams against nonpenicillinase producing strains of *Streptococcus*, but herein we wish to report the antibacterial activities and synthesis of calixarene derivatives of cephalosporins (calixcephems) by 2 different methods: direct synthetic pathway according to a previous published method[11] and indirect pathway by Morin ring expansion. The direct synthetic strategy, like the synthesis of calixpenams, involves grafting of the 7-ADCA moieties via the formation of an amide bond between the calixarene platform and the cephem arm, but the indirect method involves converting calixpenams to calixcephems by ring expansion of the thiazolidine sulfoxide ring[11] to the dihydrothiazine ring (Figure 1) and the formation of 7-ADCA cores (cyclic sulfoxide rearrangement) in the presence of silyl protection of the carboxylate groups of 6-APA arms.

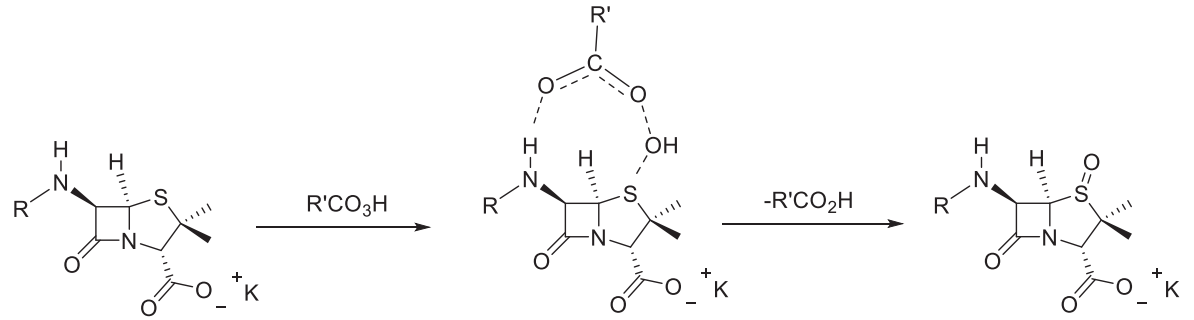
2. Results and discussion

The indirect synthetic pathway of calixcephems is depicted in Scheme 1. The target compounds were synthesized from calixpenams during 2 steps consisting of conversion of calixpenams (**1** and **2**) to their sulfoxide derivatives (**3** and **4**, respectively) followed by ring expansion to produce calixcephems (**5** and **6**).



Scheme 1. Indirect synthetic pathway to calixcephems.

For this purpose, the 6-APA moieties of the calixpenams should be oxidized to sulfoxide derivatives using peracetic acid in cold aqueous medium. The mechanism of the formation of a calixpenam sulfoxide proceeds by a nucleophilic attack of the sulfur atom on the peracid and hydrogen bonding of the peracid to the amido proton (Scheme 2). The main reaction is followed by the ring expansion process, employing N,N′-bis(trimethylsilyl)urea (BSU) and pyridine-hydrogen bromide (Py-HBr) to produce calixcephems (silyl protection method). The mechanism of the ring expansion of the 6-APA sulfoxide moieties for producing the 7-ADCA cores (cyclic sulfoxide rearrangement) is depicted in Scheme 3. As shown, the mechanism involves sulfenic acid and sulfonium ion.

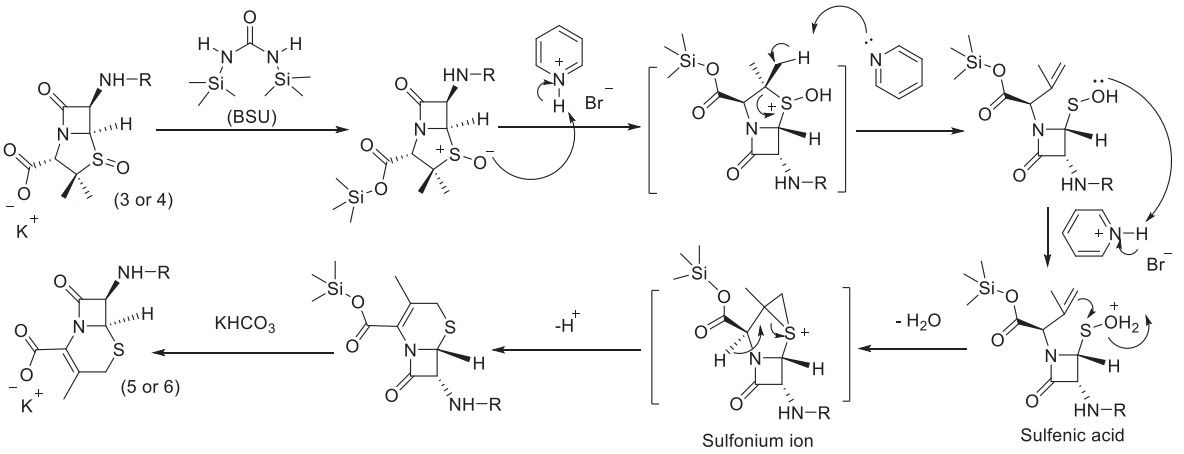


Scheme 2. The mechanism of the formation of calixpenam sulfoxide by peracid.

The highest yield for the acid-catalyzed ring-expansion process was obtained with hydrogen bromide as a catalyst in the presence of a small amount of pyridine as weak base (Py-HBr).[13]

In the ring expansion reaction, it is necessary to protect the carboxyl groups due to decarboxylation of nonprotected groups. The main advantages of silylation over other methods in the protection of carboxyl groups are its easy introduction and removability. However, silyl esters are very sensitive to cleavage by water and this is mainly caused by decarboxylation of nonprotected carboxylic groups.[13] In addition, the conversion

of a calixpenam sulfoxide to a calixcephem implies the leaving of a molecule of water (Scheme 3); therefore, trapping the water formed during the ring expansion is necessary to avoid deprotection of the silylated carboxyl groups. On the other hand, using trimethylchlorosilane as silylating agent to protect the carboxyl group in the TMS form causes a rise in the amount of HCl (Cl^- of silyl compound) during the reaction and formation of oxazolone thiazolidine (non-β-lactam) as the by-product of a side reaction (due to higher reactivity of HCl than the silylated β-lactams toward reactive intermediates).[13] To solve these problems, BSU was used as a special silylating agent with a strong silylating capacity and ability to trap the water formed during the ring expansion and did not give rise to the formation of acids during the reaction.[13] To eliminate the silyl group, hydrolysis using an aqueous solution of potassium bicarbonate was performed at elevated temperatures ($50-60$ °C).



Scheme 3. The mechanism of ring expansion (cyclic sulfoxide rearrangement).

Like the previously published method,[11] in order to increase the solubility of the final products in chloroform (recording of NMR spectra) and water (in-vitro antimicrobial susceptibility testing), their potassium salt forms were prepared. The products' structures were characterized by IR, NMR, and ESI-MS spectra and elemental analysis.

The presence of the S=O double bond in the structure of sulfoxide derivatives can be easily verified by a band at 1032 and 1035 cm^{-1} for compounds **3** and **4**, respectively, in the FT-IR spectra.

IR analysis showed the presence of intense bands at about 1780, 1720, and 1680 cm^{-1} for **CC X** and **CC V** attributed to the lactam carbonyl, carboxyl carbonyl, and amide carbonyl groups, respectively.

Like calixpenams **1** and **2**,[11] compounds **5** and **6** (calixcehems) are in a cone conformation, as assessed by the Ar–CH_2–Ar resonance signals at 31.2 and 30.9 ppm in the ^{13}C NMR spectra, respectively. It was also confirmed through the presence of an AB system at 4.47–3.38 ppm (J_{AB} = 14 Hz) for **5** and at 4.34–3.42 ppm for **6** in the ^1H NMR spectra.

It is known that compounds possessing cephalosporanic acid cores have greater antibacterial effects against β-lactamase-producing bacterial strains in comparison to compounds with penicillanic acid cores;[4] thus, in order to evaluate this property of calixcephems (**5** and **6**), we compared them with the corresponding calixpenams (**1** and **2** respectively) against β-lactamase-producing strains of *Staphylococcus aureus*. On the other hand, for showing the clustering effect of calixarene scaffold, we compared calixcephems with the corresponding single cephems **7** and **8** (Figure 2). Compounds **7** and **8** are single semisynthetic cephems (cephems V and X, respectively) that are analogous to penams V and X. To obtain the best comparison, we chose 5 strains of

the bacterium, consisting of 1 non-β-lactamase-producing strain (ATCC 25923) and 4 β-lactamase-producing strains (2 methicillin-sensitive (MSSA) strains and 2 methicillin-resistant (MRSA) strains). In detail, 1 of the 2 β-lactamase-producing methicillin-sensitive strains is penicillin-resistant (ATCC 11632), but the other is penicillin-susceptible (ATCC 29213). The MRSA strains are ATCC 43300 and ATCC 33591.

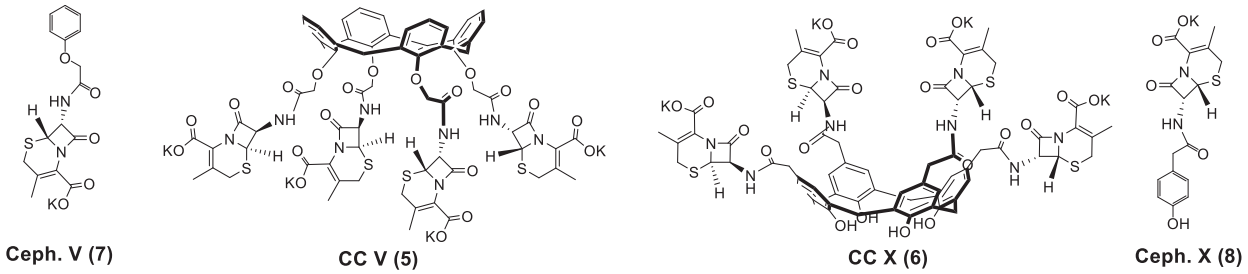


Figure 2. Structural comparison between calixcephems and their corresponding single cephems.

The in vitro antimicrobial susceptibility testing (AST) (e.g., minimum inhibition concentration (MIC) determination) of the cluster compounds was determined by broth microdilution (BMD) without 5% lysed sheep blood, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, and as described previously.[11] The results of these tests are shown in Table 1. As shown, cluster cephems **CC X** and **CC V** showed great antibiotic activities in comparison to the corresponding calixpenams **1** and **2** (cephalosporin core effect) and the corresponding single cephems V and X (calixarene clustering effect) against MRSA and penicillin-resistant MSSA (ATCC 11632) strains of *S. aureus* (these strains were completely resistant to calixpenams). As shown in Table 2, the MIC ratio of calixcephem and its corresponding monomer indicates the clustering effect of calixarene scaffold.[11,12] In the case of MSSA strains (both β-lactamase-producing ATCC 29213 and non-β-lactamase-producing ATCC 25923), the calixpenams showed more antibacterial effects with respect to calixcephems (about 4- and 10-fold, respectively). *S. aureus* ATCC 33591 is a special strain, used for drug discovery, and it is resistant to different kinds of common antibiotic such as oxacillin (MIC $>$ 64 μg/mL). ATCC 43300 is also resistant to oxacillin (MIC = 16 μg/mL). Both ATCC 33591 and ATCC 43300 are more sensitive (about 10-fold) to calixcephems in comparison to cephalosporin monomers.

Table 1. Minimal inhibitory concentration (MIC) in μg/mL for 5 strains of *S. aureus*.

MIC (μg/mL) values					
Strain / Compd.	ATCC 25923 $\beta-$lactamase (–) MSSA	ATCC 29213 $\beta-$lactamase (+) MSSA	ATCC 11632 $\beta-$lactamase (+) MSSA	ATCC 43300 $\beta-$lactamase (+) MSSA	ATCC 33591 $\beta-$lactamase (+) MSSA
6 (CC X)	0.225	0.992	0.912	1.150	1.550
5 (CC V)	0.250	1.012	0.925	1.225	1.635
2 (CP X)	0.025	0.240	$>$ 64	$>$ 64	$>$ 64
1 (CP V)	0.032	0.256	$>$ 64	$>$ 64	$>$ 64
7 (Ceph. V)	1.775	5.325	8.775	11.775	16.650
8 (Ceph. X)	1.550	5.150	8.350	10.950	16.325

Table 2. MIC ratios between calixcephems and their corresponding monomers for 5 strains of *S. aureus*.

MIC ratio \ Strain	ATCC 25923	ATCC 29213	ATCC 11632	ATCC 43300	ATCC 33591
$MIC_{Ceph.V}$ / $MIC_{CC\,V}$	7.1	5.3	9.5	9.6	10.2
$MIC_{Ceph.X}$ / $MIC_{CC\,X}$	6.9	5.2	9.1	9.5	10.5

In summary, the present work describes the first examples of calixcephems with efficient antibiotic activities against MRSA strains and penicillin-resistant MSSA strain. These compounds could be considered novel antibiotic structures with high density antibiotic surfaces.

The results of the present study demonstrate a noteworthy increase in antibacterial properties from calixpenams (**1** and **2**) to their corresponding cephalosporin derivatives (**5** and **6**) against β-lactamase-producing methicillin-resistant strains of *Staphylococcus aureus* (MRSA). This is attributed to the lower susceptibility of cephalosporin cores to β-lactamase in comparison to 6-APA core.

Moreover, the results showed that calixcephems were more active (about 10-fold) against MRSA with respect to the corresponding single cephems V and X. This is attributed to their high density antibiotic surface and the synergistic effect of cluster arms (clustering effect).

3. Experimental

3.1. General

The melting points of all compounds were recorded on a Philip Harris C4954718 apparatus without calibration. IR spectra were determined on a Thermo Nicolet 610 Nexus FT-IR spectrometer with KBr disks. Ultraviolet spectra were recorded on a Shimadzu UV-2401/PC spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) measurements were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ using TMS as the internal reference. Elemental analyses were obtained on a PerkinElmer 240c analyzer. Mass spectra were recorded on a JEOL-JMS 600 (FAB MS) instrument. Thin layer chromatography (TLC) analyses were carried out on silica gel plates. All chemicals were purchased from Merck (Tehran, Iran) and used as received by standard procedures.

3.2. Sulfoxidation: procedure for the synthesis of compounds 3 and 4

Compound **1** or **2** (800 mg, 0.5 mmol) in the tetra potassium salt form[11] was dissolved in bidistilled water (5 mL) and then the solution was cooled to 0–5 °C, and 40% peracetic acid (5 mL, 0.9 mol) was added to the solution followed by stirring for 3 h. Then a sufficient amount of 10% (w/w) sulfuric acid solution was added dropwise until pH 2 was achieved. At constant pH and temperature, compound **3** or **4** crystals appeared. The mixture was stirred at pH 2 for 2 h, filtered, and washed with distilled water (20 mL) to obtain the pure target sulfoxide.

3.2.1. 25,26,27,28-Tetrakis(6-aminopenicillanic acid sulfoxide carbonylmethoxy)calix[4]arene (3)

Yield (640 mg, 85%), mp: 218–220 °C. IR (KBr, ν, cm^{-1}): 3389 (O–H), 1792 (C=O), 1733 (C=O), 1676 (C=O), 1032 (S=O). The expanded structure of 6-APA sulfoxide is shown in Figure 3. ^{1}H NMR (400 MHz, $CDCl_3$, in the form of potassium salt) δ 8.22 (d, J = 9.8 Hz, 4H, N–H), 7.12 (d, J = 7.6 Hz, 8H, Ar-H_m), 6.68 (t, J = 7.6 Hz, 4H, Ar-H_p), 5.91 (dd, J = 4.5 and 9.8 Hz, 4H, H-6 of APA), 4.95 (d, J = 4.5 Hz, 4H, H-5 of APA), 4.61 (s, 4H, H-3 of APA), 4.56 (d, J = 14.0 Hz, 4H, $ArCH_2Ar$ H_{ax}), 3.92 (s, 8H, ArO–CH_2),

3.45 (d, J = 14.0 Hz, 4H, $ArCH_2Ar\ H_{eq}$), 1.67 (s, 12H, CH_3), 1.25 (s, 12H, CH_3). FAB $^+$ MS m/z: 1512.39 (M^+).

3.2.2. 5,11,17,23-Tetrakis(6-aminopenicillanic acid sulfoxide carbonylmethyl)calix[4]arene-25,26,27,28-tetrol (4)

Yield (665 mg, 88%), mp: 226–227 °C. IR (KBr, ν, cm^{-1}): 3372 (O–H), 1789 (C=O), 1731 (C=O), 1670 (C=O), 1035 (S=O). The expanded structure of 6-APA sulfoxide is shown in Figure 3. ^{1}H NMR (400 MHz, $CDCl_3$, in the form of potassium salt) δ 9.68 (s, 4H, OH), 8.33 (d, J = 10.0 Hz, 4H, N–H), 7.06 (s, 8H, Ar-H), 5.99 (dd, J = 5.0 and 10.0 Hz, 4H, H-6 of APA), 5.05 (d, J = 5.0 Hz, 4H, H-5 of APA), 4.47 (s, 4H, H-3 of APA), 4.32 (bd, 4H, $ArCH_2Ar\ H_{ax}$), 3.72 (s, 8H, CH_2CO_2), 3.38 (bd, 4H, $ArCH_2Ar\ H_{eq}$), 1.72 (s, 12H, CH_3), 1.23 (s, 12H, CH_3). FAB $^+$ MS m/z: 1512.30 (M^+).

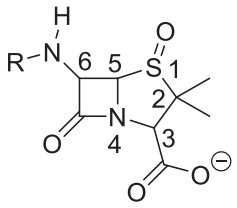


Figure 3. The numbering system for ^{1}H NMR spectrum of 6-APA sulfoxide.

3.2.3. Cyclic sulfoxide rearrangement: procedure for the synthesis of compounds 5 and 6

N,N′-bis(trimethylsilyl)urea (500 mg, 2.4 mmol) was dissolved in toluene (20 mL), and then compound **3** or **4** (500 mg, 0.3 mmol) in the tetra potassium salt form was poured into the solution. The solution was stirred at 70 °C for 1 h. After that, pyridine-HBr (100 mg, 0.62 mmol) was added, and the solution was refluxed in argon atmosphere overnight. $NaHCO_3$ 4.5% (w/v) solution (50 mL) was poured into the solution at 70 °C, and the pH of the solution was adjusted to 8. The solution was stirred for 2 h. After that, sulfuric acid 10% (w/w) solution was added to the solution dropwise to initiate the crystallization at 50 °C and the pH was adjusted to 2. The solution was cooled to 0–5 °C gently with stirring and then it was filtered. The solid product was washed with distilled water (50 mL) to obtain the pure target compound **5** or **6**.

25,26,27,28-Tetrakis(7-aminodeacotoxycephalosporanic acid carbonylmethoxy)calix[4]arene (5)

Yield (400 mg, 93%), mp: 192–194 °C. IR (KBr, ν, cm^{-1}): 3396 (O–H), 1781 (C=O), 1722 (C=O), 1683 (C=O). The expanded structure of 7-ADCA is shown in Figure 4. ^{1}H NMR (400 MHz, $CDCl_3$, in the form of potassium salt) δ 8.52 (d, J = 9.5 Hz, 4H, N–H), 7.17 (d, J = 7.0 Hz, 8H, Ar-H_m), 6.72 (t, J = 7.0 Hz, 4H, Ar-H_p), 5.89 (dd, J = 4.8 and 9.5 Hz, 4H, H-7 of ADCA), 5.03 (d, J = 4.8 Hz, 4H, H-6 of ADCA), 4.57 (s, 8H, ArO–CH_2), 4.47 (d, J = 14.0 Hz, 4H, $ArCH_2Ar\ H_{ax}$), 3.38 (d, J = 14.0 Hz, 4H, $ArCH_2Ar\ H_{eq}$), 3.25 (AB, q, J = 18.4 Hz, 8H, H-2 of ADCA) 2.08 (s, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, in the form of potassium salt) δ 169.6 (CONH), 164.8 (C-8 of ADCA), 160.2 (COO), 156.8 (ArC–O), 137.6 ($C_{(o)}$ of Ar), 129.0 ($C_{(m)}$ of Ar), 125.7 (C-4 of ADCA), 122.9 ($C_{(p)}$ of Ar), 120.2 (C-3 of ADCA), 74.9 (ArO–CH_2), 66.7 (C-6 of ADCA), 58.2 (C-7 of ADCA), 45.2 (C-2 of ACDA), 31.2 ($ArCH_2Ar$), 20.1 (C of Me). Anal. Calcd for

$C_{68}H_{64}N_8O_{20}S_4$: C, 56.66; H, 4.48; N, 7.77; S, 8.90. Found: C, 56.74; H, 4.43; N, 7.83; S, 8.87. FAB $^+$ MS m/z: 1440.36 (M^+).

3.2.4. 5,11,17,23-Tetrakis(7-aminodeacotoxycephalosporanic acid carbonylmethyl)calix[4]arene-25, 26,27,28-tetrol (6)

Yield (415 mg, 96%), mp: 186–188 °C. IR (KBr, ν, cm^{-1}): 3380 (O–H), 1778 (C=O), 1720 (C=O), 1681 (C=O). The expanded structure of 7-ADCA is shown in Figure 4. ^{1}H NMR (400 MHz, $CDCl_3$, in the form of potassium salt) δ 9.70 (s, 4H, OH), 8.73 (d, J = 9.8 Hz, 4H, N–H), 6.97 (s, 8H, Ar-H), 6.01 (dd, J = 5.0 and 9.8 Hz, 4H, H-7 of ADCA), 4.95 (d, J = 5.0 Hz, 4H, H-6 of ADCA), 4.45 (s, 8H, CH_2CO_2), 4.34 (bd, 4H, $ArCH_2Ar$ H_{ax}), 3.42 (bd, 4H, $ArCH_2Ar$ H_{eq}), 3.30 (AB, q, J = 19.0 Hz, 8H, H-2 of ADCA), 2.13 (s, 12H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, in the form of potassium salt) δ 170.4 (CONH), 165.2 (C-8 of ADCA), 163.1 (COO), 149.8 (ArC–O), 128.2 ($C_{(o)}$ of Ar), 127.6 ($C_{(m)}$ of Ar), 127.1 (ArC*–CH2), 124.8 (C-4 of ADCA), 119.9 (C-3 of ADCA), 65.9 (C-6 of ADCA), 58.7 (C-7 of ADCA), 45.9 (C-2 of ADCA), 39.0 (*CH_2CO), 30.9 ($ArCH_2Ar$), 19.8 (C of Me). Anal. Calcd for $C_{68}H_{64}N_8O_{20}S_4$: C, 56.66; H, 4.48; N, 7.77; S, 8.90. Found: C, 56.63; H, 4.52; N, 7.80; S, 8.79. FAB $^+$ MS m/z: 1440.35 (M^+).

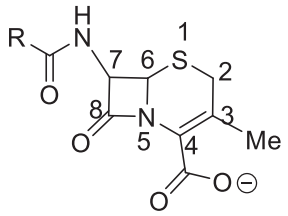


Figure 4. The numbering system for ^{1}H NMR spectra of 7-ADCA.

3.3. Bacterial strains

In the present study, microbiological tests were carried out with cluster compounds against 5 gram-positive standard strains of *Staphylococcus aureus*, consisting of 2 β-lactamase-producing methicillin-resistant (MRSA) strains ATCC 43300 and ATCC 33591 and 3 methicillin-sensitive (MSSA) strains, including 1 non-β-lactamase-producing strain ATCC 25923 and 2 β-lactamase-producing strains ATCC 29213 and ATCC 11632, the latter of which is a penicillin-resistant strain.

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References

1. Leeb, M. *Nature* **2004**, *431*, 892–893.
2. Hansmann, D.; Bullen, M. M. *Lancet* **1967**, *2*, 264–265.
3. Kluytmans, J.; Van Belkum, A.; Verbrugh, H. *Clin. Microbiol. Rev.* **1997**, *10*, 505–520.
4. Murray, B. E.; Moellering, R. C. *Annu. Rev. Med.* **1981**, *32*, 559–581.
5. Tan, Q.; Song, Q.; Wei, D. *Enzyme Microb. Tech.* **2006**, *39*, 1166–1172.

6. Morin, R. B.; Jackson, B. G.; Mueller, R. A.; Lavagnino, E. R.; Scanlon, W. B.; Andrews, S. L. *J. Am. Chem. Soc.* **1963**, *85*, 1896–1897.
7. Morin, R. B.; Gorman, M. *β-Lactam Antibiotics, Chemistry and Biology*; Academic Press: New York, NY, USA, 1981.
8. Singh, J.; Arrieta, A. C. *Semin. Pediatr. Infect. Dis.* **1999**, *10*, 14–22.
9. Kalman, D.; Barriere, S. L. *Tex. Heart. Inst. J.* **1990**, *17*, 203–215.
10. Garcia-Rodriguez, J. A.; Bellido, J. L. M.; Sanchez, J. E. G. *Int. J. Antimicrob. Agents* **1995**, *5*, 231–243.
11. Nasuhi Pur, F.; Akbari Dilmaghani, K. *Turk. J. Chem.* **2014**, *38*, 288–296.
12. Nasuhi Pur, F.; Akbari Dilmaghani, K. *J. Coord. Chem.* **2014**, *67*, 440–448.
13. de Koning, J. J.; Kooreman, H. J.; Tan, H. S.; Verweij, J. *J. Org. Chem.* **1975**, *40*, 1346–1347.