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7α-METHOXY- 2-[(SUBSTITUTED)METHYLENE]CEPHEM SULFONES AS INHIBITORS OF HUMAN LEUKOCYTE ELASTASE AND THROMBIN

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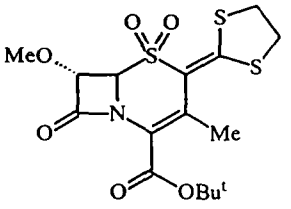
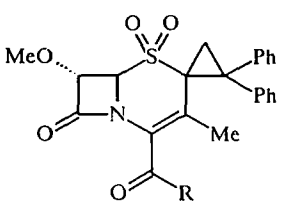
A series of 7α-methoxy-2-[(substituted)methylene]cephem sulfones was synthesized. The compounds with an acetoxy group at the C-3′ position as a leaving group were found to be potent inhibitors of HLE. Selected compounds are also found to be antithrombin agents.

Human leukocyte elastase (HLE), a serine proteinase, is reported to be capable of degrading a variety of structural proteins (including elastin, other components of connective tissue, and certain complement proteins and receptors) and is implicated in the pathogenesis of several disease states including emphysema, acute respiratory distress syndrome (ARDS), and rheumatoid arthritis. Free elastase has also been detected in the lung fluid of patients with chronic bronchitis and cystic fibrosis. Under normal conditions, the proteolytic activity of HLE in the extracellular environment is inhibited by an excess of natural inhibitors, predominantly α_1-protease inhibitor (α_1-PI) and α_2-macroglobulin (α_2-M). However, pathological conditions can arise which disrupt the elastase-antielastase balance, resulting in an uncontrolled proteolysis of structural tissue. It has been postulated that the use of synthetic, low molecular weight HLE inhibitors that can be delivered to the site of unregulated elastase activity could be an attractive approach in the treatment of such diseases. Various cephalosporin sulfones have been found to be potential elastase inhibitors [1]. We have previously reported the elastase inhibitory activity of cephalosporin sulfones having 1,3-dithiolan-2-ylidene (I) [2] and spiro(2′,2′-diphenylcyclopropane) (II) [3] moieties, respectively, at position 2.



I



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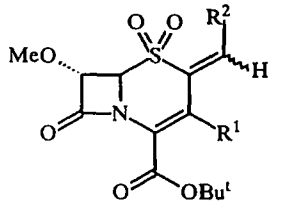

III

It is our belief that the cephem sulfones [1] share a common bioactive motif. That is, a small substituent with α-orientation at position 7 and a sulfone and a derivatized carboxylic acid (such as ester, amide, or ketone) functionality at position 4 are primarily responsible for the observed biological activity. Furthermore, we also observed that the introduction of a leaving group or a substituent at the C-3′ position does not contribute much to the biological efficacy when the position 2 is additionally substituted with a spirocyclopropyl or a 1,3-dithiolan-2-ylidene group. With the knowledge that 7α-methoxy-2-(1,3-dithiolan-2-ylidene)cephem sulfones (I) are potent elastase inhibitors, we undertook the task of establishing whether introduction of a substituted methylene at position 2 could

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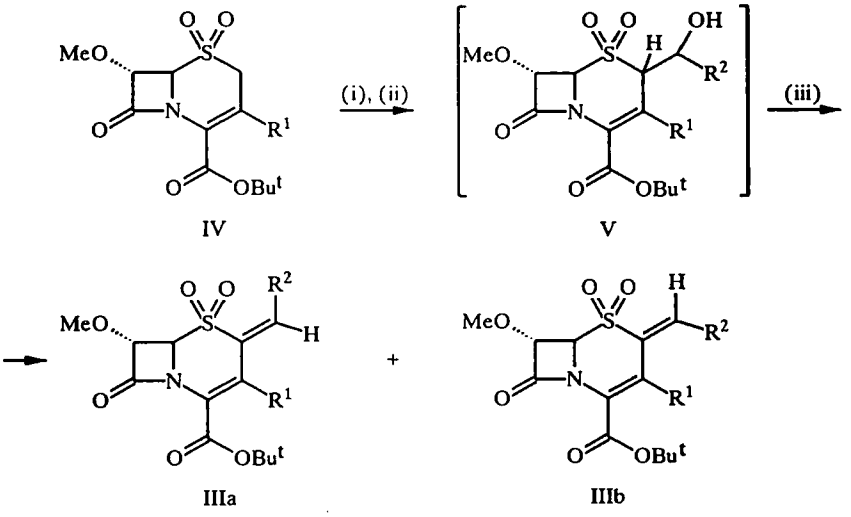
also provide compounds with potent HLE inhibitory activity. Thus, the synthesis and *in vitro* evaluation of 7α-methoxy 2-[(substituted)methylene]cephem sulfones (III) [4] is the subject of this report.

CHEMISTRY

The preparation of the compounds III is shown in the scheme. In a general procedure, 4-*tert*-butoxycarbonyl-7α-methoxy-3-methyl-3-cephem-1,1-dioxide (IV, R^1 = Me) or 4-*tert*-butoxycarbonyl-7α-methoxy-3-acetoxymethyl-3-cephem-1,1-dioxide (IV, R^1 = CH_2OAc), which was prepared based on the procedure described by Blacklock et al. [5], was treated with *n*-BuLi in the presence of diphenylamine followed by the appropriate aldehyde at -78°C.



R^1 = Me, CH_2OAc; (i) Ph_2NH, *n*-BuLi/THF, -78 °C; (ii) R^2CHO, -78 °C; (iii) Ac_2O-pyridine

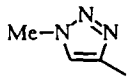
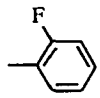
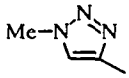
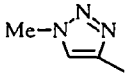
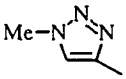
The adduct V was smoothly converted into the corresponding mixture of (*E*)- and (*Z*)-olefins, (*E*)-isomer IIIb being predominant, by treatment with acetic anhydride followed by pyridine. The olefinic compounds IIIa and IIIb were separated by column chromatography.

ENZYME ASSAY

Human leukocyte elastase was obtained from Elastin Products, Missouri, USA. Substrate: MeO-succinyl-L-alanyl-L-alanyl-L-prolyl-L-valine-*p*-nitroanilide. Reaction mixture: 10 mM phosphate buffer (pH 7.6), 500 mM NaCl, 10% DMSO, 0.35 mM substrate. The enzyme activity was determined by monitoring the increase in absorbance at 410 nm caused by the hydrolysis of chromogenic substrates. Inhibition of enzyme by the compounds described was determined after a 10 min preincubation with the enzyme in reaction mixture minus substrate. Reaction was initiated by the addition of substrate. The concentration of human leukocyte elastase used for assay was at 10 nM.

Lyophilized human plasma thrombin obtained from Boehringer, Montreal, Canada was dissolved in 0.5 M Na, K-phosphate buffer (pH 7.0) and stored at 4°C. Substrate: 0.5 mM Chromozym TH (tosyl-glycyl-prolyl-arginine-4-nitroanilide acetate) in 10% DMSO. Inhibitor: 10 μM dissolved in 10% DMSO. Buffer: 50 mM Tricine (pH 8.6), 500 mM NaCl, 0.1% PEG 8000. Temp: room temperature. A single inhibitor concentration (10 μM) was tested using a spectrophotometric end-point assay (1 ml final volume). Enzyme activity was monitored by formation of a product that absorbed light at 410 nM and an enzyme concentration which gave a rate of 0.01-0.02 AU/min was used. Enzyme and inhibitor were preincubated for 10 min in the final amounts of buffer and DMSO. The reaction was then started by the addition of substrate. After 30 min incubation, the reaction was terminated by the addition

TABLE 1. Activity of 7α-Methoxy-2-[(substituted)methylene]cephem Sulfones against Human Leukocyte Elastase (HLE)

Compound No	Inhibitor	R^1	R^2	IC_{50} (nM)
1	IIIa	CH_2OAc		7.8
2	IIIa	CH_2OAc		5.8
3	IIIa	CH_2OAc		7.2
4	IIIa	CH_2OAc		6.4
5	IIIa	Me		75
6	IIIa	Me		35
7	IIIb	CH_2OAc		9.2
8	IIIb	CH_2OAc		6.0
9	IIIb	CH_2OAc		7.2
10	IIIb	CH_2OAc		6.4
11	IIIb	CH_2OAc		22
12	IIIb	Me		100

of 10% SDS (0.1 ml) and the absorbance measured. Data concerning HLE inhibition are given in Table 1. Enzyme blanks were run concurrently to account for any background absorbance by the compound. Inhibition was calculated by the net A_{410} in the presence of inhibitor compared to that in the absence of inhibitor. The data in Table 2 clearly indicates that compounds exhibit between 94 and 98% inhibition of thrombin at 10 μ.M and thus are effective thrombin inhibitors.

CONCLUSION

As can be seen from Table 1, the compounds having an acetoxy group at C(3′) as a leaving group are potent inhibitors of HLE. There is no significant difference in activity between the (*E*)- and (*Z*)-isomers. The nature of heterocycle did not influence the activity.

TABLE 2. Activity of 7α-Methoxy-2-[(substituted)methylene]cephem Sulfones against Thrombin

Compound No	% inhibition at 10 μM
2	96.8
8	98.5
10	98.9
11	94.7

EXPERIMENTAL

3-Acetoxymethyl-4-*t*-butoxycarbonyl-2-(1-methyl-1,2,3-triazol-4-yl)methylene-7α-methoxy-3-cephem-1,1-dioxide (IIIa, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 1 and IIIb, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 7). To a solution of diphenylamine (0.993 g, 5.86 mmol) in dry THF (32 ml) cooled to -10°C was added *n*-butyllithium (1.6 M in hexane, 3.7 ml, 5.89 mmol) and the solution was stirred under nitrogen at -10°C for 5 min and at room temperature for 15 min. The reaction mixture was cooled to -78°C and 4-*t*-butyloxycarbonyl-3-ester IV, R^1 = $MeCH_2OAc$ (2.03 g, 5.41 mmol) in dry THF (16 ml) was added dropwise. The mixture was stirred for 15 min and 1-methyl-1,2,3-triazol-4-carboxaldehyde (0.654 g, 5.89 mmol) in dry THF (24 ml) was added dropwise, stirred for 10 min. Acetic anhydride (1.52 ml, 16.1 mmol) in THF (8 ml) was added dropwise and stirred for 10 min at which time pyridine (0.48 ml, 5.93 mmol) was added. The reaction mixture was stirred at -78°C for 15 min and at room temperature for 1 h, ice water (25 ml) was added, the mixture was extracted with ethyl acetate (150 ml), and the aqueous layer was re-extracted with ethyl acetate (50 ml). The combined ethyl acetate layers were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give 4.15 g of crude product which was purified over a silica gel column using hexane–ethyl acetate mixture as eluent (3:2). Fast eluting components were unreacted cephem sulfone (480 mg) and triazole aldehyde (230 mg). Further elution of the column gave the (*Z*)-isomer IIIa, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 1, 360 mg) as the minor product followed by the (*E*)-isomer (IIIb, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 7, 1.08 g) as the major product.

A portion of the (*Z*)-isomer (20 mg) was crystallized from ethyl acetate–hexane, m. p. 180-182°C, decomp. ^1H NMR (200 MHz, $CDCl_3$) δ: 8.56 (1H, s); 7.57 (1H, s); 5.43 (1H, d, *J* = 13 Hz); 5.31 (1H, d, *J* = 1.5 Hz); 4.81 (1H, d, *J* = 13 Hz); 4.81 (1H, d, *J* = 1.5 Hz); 4.16 (3H, s); 3.59 (3H, s); 2.09 (3H, s); 1.58 (9H, s). Found, %: C 48.29; H 5.11; N 11.79. $C_{19}H_{24}N_4O_8S$. Calculated, %: C 48.71; H 5.16; N 11.96.

The (*E*)-isomer (compd. 7) was obtained as a white foam. ^1H NMR (200 MHz, $CDCl_3$) δ: 7.78 (1H, s); 7.64 (1H, s); 5.51 (1H, d, *J* = 13.5 Hz); 5.09 (1H, d, *J* = 2.0 Hz); 5.05 (1H, d, *J* = 13.5 Hz); 4.84 (1H, d, *J* = 2.0 Hz); 4.16 (3H, s); 3.57 (3H, s); 1.95 (3H, s); 1.57 (9H, s). Found, %: C 48.37; H 5.09; N 11.82. $C_{19}H_{24}N_4O_8S$. Calculated, %: C 48.71; H 5.16; N 11.96.

3-Acetoxymethyl-4-*t*-butoxycarbonyl-2-(2-fluorophenyl)methylene-7α-methoxy-3-cephem-1,1-dioxide (IIIa, R^2 = 2-fluorophenyl, compd. 2 and IIIb, R^2 = 2-fluorophenyl, compd. 8). A solution of diphenylamine (0.498 g, 2.94 mmol) in THF (16 ml) was cooled to -10°C under nitrogen, *n*-butyllithium (1.6 M in hexane, 1.84 ml, 2.94 mmol) was added dropwise, and the reaction mixture was stirred at this temperature for 5 min and then at room temperature for 15 min. The mixture was cooled to —78°C and ester IV, R^1 = $MeCH_2OAc$ (1.0 g, 2.67 mmol) in THF (8 ml) was added dropwise. The resulting mixture was stirred for 15 min at which time 2-fluorobenzaldehyde (0.431 g, 3.47 mmol) in THF (12 ml) was added dropwise and the solution was stirred for 10 min; acetic anhydride (0.76 ml, 8 mmol) in THF (4 ml) was added dropwise and stirred for 10 min at which time pyridine (0.24 ml, 2.97 mmol) was added. The resulting mixture was stirred for 15 min and then at room temperature for 1 h; ice water (25 ml) was added dropwise and the mixture was poured into ethyl acetate (100 ml). The ethyl acetate layer was separated and the aqueous layer was extracted with ethyl acetate (50 ml). The combined ethyl acetate layers were washed with brine, dried, and concentrated. The residue was purified by column chromatography on a silica gel column using hexane—ethyl acetate mixture (1:2) as eluent. Diphenylamine and unreacted 2-fluorobenzaldehyde were eluted first followed by the minor isomer (*Z*-isomer, 0.247 g). The major isomer (*E*-isomer) was eluted next (0.656 g). The unconsumed cephem sulfone (0.27 g) was eluted last from the column. ^1H NMR (200 MHz, $CDCl_3$) of (*Z*)-isomer, compd. **2**, δ: 7.63–7.70 (1H, μ); 7.53 (1H, σ); 7.36–7.49 (1H, μ); 7.06–7.24 (2H, μ); 5.25 (1H, δ, *J* = 2.0 Hz); 4.81 (1H, d, *J* = 2.0 Hz); 4.73 and 5.48 (2H, ABq, *J* = 13 Hz); 3.51 (3H, s); 2.11 (3H, s); 1.59 (9H, s). Found, %: C 53.61; H 5.11; N 3.96. $C_{22}H_{24}FNO_8S$. Calculated, %: C 53.72; H 5.15; N 4.05.

^{1}H NMR (200 MHz, $CDCl_3$) of (*E*)-isomer, compd. **8**, δ: 7.89 (1H, s); 7.14-7.53 (4H, m); 5.19 (1H, d, *J* = 2.0 Hz); 4.93 (1H, d, *J* = 2.0 Hz); 4.46 and 4.87 (2H, ABq, *J* = 13 Hz); 3.59 (3H, s); 1.91 (3H, s); 1.56 (9H, s). Found, %: C 53.77; H 5.02; N 4.11. $C_{22}H_{24}FNO_8S$. Calculated, %: C 53.72; H 5.15; N 4.04.

3-Acetoxymethyl-4-*t*-butoxycarbonyl-7α-methoxy-2-(3-pyridyl)methylene-3-cephem-1,1- dioxide (IIIa, R^2 = 3-pyridyl, compd. 3 and IIIb, R^2 = 3-pyridyl, compd. 9). To a solution of diphenylamine (0.249 g, 0.00147 mol) in dry THF (8 ml) cooled to -15°C under nitrogen was added *n*-butyllithium (0.92 ml, 0.00147 mol, 1.6 M in hexane) dropwise. The resulting mixture was stirred at -15°C for 3 min and at room temperature for 10 min. The yellowish brown solution was cooled to -78°C and stirred at this temperature for 10 min at which time ester IV, R^1 = CH_2OAc (0.592 g, 0.00133 mol) in dry THF (4 ml) was added dropwise and stirred at -78°C for 5 min. Acetic anhydride (0.38 ml, 0.00399 mol) in THF (2 ml) was added and stirred for 10 min, then pyridine (0.12 ml, 0.00133 mol) was added. The reaction mixture was stirred at -78°C for 20 min and was allowed to warm to room temperature. Ice water (10 ml) was added to the mixture and extracted with ethyl acetate. The aqueous layer was re-extracted with ethyl acetate. The combined ethyl acetate layers were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo* to give an oily residue (1.4 g) which was purified over a silica gel column using hexane–ethyl acetate mixture as eluent. The (*E*)- and (*Z*)-isomers were separated by repeated preparative TLC. ^{1}H NMR (200 MHz, $CDCl_3$) of (*Z*)-isomer, compd. **3**, δ: 8.65-8.70 (2H, m); 8.12 (1H, d, *J* = 7.8 Hz); 7.45 (1H, s); 7.45-7.55 (1H, m); 5.52 (1H, d, *J* = 13.1 Hz); 5.26 (1H, d, *J* = 1.5 Hz); 4.82 (1H, d, *J* = 1.5 Hz); 4.73 (1H, d, *J* = 13.2 Hz); 3.53 (3H, s); 2.12 (3H, s); 1.60 (9H, s). Found, %: C 53.98; H 5.12; N 5.87. $C_{21}H_{24}N_2O_8S$. Calculated, %: C 54.29; H 5.20; N 6.03.

^{1}H NMR (200 MHz, $CDCl_3$) of (*E*)-isomer, compd. **9**, δ: 8.60-8.70 (2H, m); 7.83 (1H, s); 7.68 (1H, d, *J* = 7.8 Hz); 7.33-7.48 (1H, m); 5.20 (1H, d, *J* = 2.0 Hz); 4.95 (1H, d, *J* = 2.0 Hz); 4.93 (1H, d, *J* = 13.3 Hz); 4.35 (1H, d, *J* = 13.3 Hz); 3.61 (3H, s); 1.93 (3H, s); 1.56 (9H, s). Found, %: C 53.85; H 5.17; N 5.88. $C_{21}H_{24}N_2O_8S$. Calculated, %: C 54.29; H 5.20; N 6.03.

3-Acetoxymethyl-4-(*t*-butoxycarbonyl)-7α-methoxy-2-(3-thienyl)methylene-3-cephem-1,1-dioxide (IIIa, R^2 = 3-thienyl, compd. 4 and IIIb, R^2 = 3-thienyl, compd. 10). These compounds were prepared in the same manner as described above for the other compounds. ^{1}H NMR (200 MHz, $CDCl_3$) of (*Z*)-isomer: δ: 7.89 (1H, br, s); 7.45 (1H, d, *J* = 5.2 Hz); 7.30-7.40 (1H, m); 7.33 (1H, s); 5.29 (1H, d, *J* = 1.8 Hz); 4.75 (1H, d, *J* = 1.8 Hz); 4.85 and 5.45 (2H, ABq, *J* = 13 Hz); 3.55 (3H, s); 2.09 (3H, s); 1.58 (9H, s). Found, %: C 50.80; H 4.77; N 2.71. $C_{20}H_{23}NO_8S_2$. Calculated, %: C 51.16; H 4.93; N 2.98. ^{1}H NMR (200 MHz, $CDCl_3$) of (*E*)-isomer, δ: 7.74 (1H, s); 7.56 (1H, br, s); 7.39-7.43 (1H, m); 7.11 (1H, d); 5.10 (1H, d, *J* = 1.84 Hz); 4.86 (1H, d, *J* = 1.8 Hz); 4.59 and 5.14 (2H, ABq, *J* = 13.4 Hz); 3.57 (3H, s); 1.95 (3H, s); 1.57 (9H, s). Found, %: C 50.91; H 4.84; N 2.78. $C_{20}H_{23}NO_8S_2$. Calculated, %: C 51.16; H 4.93; N 2.98.

3-Acetoxymethyl-4-(*t*-butoxycarbonyl)-2-(2-furyl)methylene-7α-methoxy-3-cephem-1,1-dioxide (IIIb, R^2 = 2-furyl, compd. 11). This compound was also prepared in the same manner as described before and purified by column chromatography on a silica gel column using hexane–ethyl acetate mixture (2:1) as eluent. The minor isomer (*Z*-isomer, 15 mg) was eluted first followed by the major isomer (*E*-isomer, 350 mg). ^{1}H NMR (200 MHz, $CDCl_3$) of the (*Z*)-isomer, δ: 7.65 (1H, d, *J* = 1.5 Hz); 7.51 (1H, d, *J* = 3.5 Hz); 7.12 (1H, s); 6.60 (1H, dd, *J* = 1.5 and 3.5 Hz); 5.32 (1H, d, *J* = 2.0 Hz); 4.77 and 5.42 (2H, ABq, *J* = 13 Hz); 4.76 (1H, d, *J* = 2.0 Hz); 3.58 (3H, s); 2.09 (3H, s); 1.58 (9H, s).

^{1}H NMR (200 MHz, $CDCl_3$) of the (*E*)-isomer, δ: 7.64 (1H, d, *J* = 1.5 Hz); 7.46 (1H, s); 6.90 (1H, d, *J* = 3.5 Hz); 6.59 (1H, dd, *J* = 1.5 and 3.5 Hz); 5.08 and 5.43 (2H, ABq, *J* = 13 Hz); 5.04 (1H, d, *J* = 2.0 Hz); 4.79 (1H, d, *J* = 2.0 Hz); 3.57 (3H, s); 1.93 (3H, s); 1.58 (9H, s). Found, %: C 52.69; H 5.09; N 2.98. $C_{20}H_{23}NO_9S$. Calculated, %: C 52.97; H 5.11; N 3.08.

4-(*t*-Butoxycarbonyl)-7α-methoxy-3-methyl-2-(1-methyl-1,2,3-triazol-4-yl)methylene-3-cephem-1,1-dioxide (IIIa, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 5 and IIIb, R^2 = 1-methyl-1,2,3-triazol-4-yl, compd. 12). To a solution of diphenylamine (0.533 g, 0.00312 mol) in dry THF (15 ml) cooled to -10 °C under nitrogen was added *n*-butyllithium (1.949 ml, 0.00312 mol, 1.6 M in hexane). The resulting mixture was stirred at -10°C for 3 min and at room temperature for 10 min. The light yellowish brown solution was further cooled to -78°C and stirred for 10 min during which time ester IV, R^1 = Me (0.90 g, 0.00284 mol) in dry THF (10 ml) was added dropwise. The reaction mixture was stirred at -78°C for 10 min and a solution of 1-methyl-1,2,3-triazol-4-yl carboxaldehyde (0.315 g, 0.00284 mol) in dry THF (10 ml) was added dropwise. The mixture was stirred at -78°C for 5 min; acetic anhydride (1 ml) in THF (5 ml) was added. The yellowish brown solution was stirred at -78°C for 40 min, dry ice–acetone bath was removed, and pyridine (0.8 ml) dissolved in THF (12 ml) was added. After stirring at room temperature for 20 min ethyl acetate (150 ml) was added followed by brine (30 ml). The organic layer was separated, dried (Na_2SO_4), and

the solvent was removed to give an oily mass (1.05 g) which was purified over a silica gel column using a mixture of hexane—ethyl acetate (3:2) as eluent. The (*Z*)-isomer (compd. **5**) was eluted first (180 mg) followed by the (*E*)-isomer (compd. **12**, 440 mg). ^{1}H NMR (200 MHz, $CDCl_3$) of the (*Z*)-isomer, δ: 8.57 (1H, s); 7.46 (1H, s); 5.30 (1H, br, s); 4.77 (1H, br, s); 4.16 (3H, s); 3.59 (3H, s); 2.23 (3H, s); 1.57 (9H, s). Found, %: C 49.82; H 5.32; N 13.57. $C_{17}H_{22}N_4O_6S$. Calculated, %: C 49.74; H 5.40; N 13.65. ^{1}H NMR (200 MHz, $CDCl_3$) of the (*E*)-isomer: δ 7.85 (1H, s); 7.64 (1H, s); 5.03 (1H, d, J = 1.5 Hz); 4.81 (1H, d, J = 1.5 Hz); 4.18 (3H, s); 3.57 (3H, s); 2.30 (3H, s); 1.57 (9H, s). Found, %: C 49.88; H 5.29; N 13.56. $C_{17}H_{22}N_4O_6S$. Calculated, %: C 49.74; H 5.40; N 13.65.

***t*-Butyl-7α-methoxy-3-methyl-(2*Z*)-(3-pyridyl)methylene-3-cephem-4-carboxylate-1,1-dioxide (IIIa, R^2 = 3-pyridyl, compd. 6).** A solution of diphenylamine (0.474 g, 2.77 mmol) in THF (12 ml) was cooled to –10°C under nitrogen, *n*-butyllithium (1.6 M in hexane, 1.74 ml, 2.77 mmol) was added dropwise and the reaction mixture was stirred at –10°C for 5 min and then at room temperature for 10 min. The mixture was cooled to –78°C and *t*-butyl-3-methyl-3-cephem-7α-methoxy-4-carboxylate-1,1-dioxide (0.8 g, 2.52 mmol) in THF (8 ml) was added dropwise. The resulting mixture was stirred for 15 min at which time pyridine-3-carboxaldehyde (0.27 g, 2.52 mmol) in THF (4 ml) was added dropwise and the solution was stirred at –78°C for 10 min. Acetic anhydride (0.4 ml) in THF (4 ml) was added and stirred for 40 min at –78°C. The resulting mixture was removed from dry ice–acetone bath, pyridine (0.3 ml) was added, and it was stirred for 20 min at room temperature. Ethyl acetate (150 ml) was added followed by brine (30 ml). The organic layer was separated out, washed, dried, and concentrated to give a viscous oil which was purified by column chromatography on a silica gel column using hexane–ethyl acetate mixture (2:8) as eluent. ^{1}H NMR (200 MHz, $CDCl_3$) of the minor isomer (Z-isomer, 40 mg, compound. no. 6), δ: 7.80-8.80 (5H, m); 5.15 (1H, s); 5.03 (1H, s); 3.58 (3H, s); 1.88 (3H, s); 1.56 (9H, s). Found, %: C 55.88; H 5.38; N 6.69. $C_{19}H_{22}N_2O_6S$. Calculated, %: C 56.13; H 5.45; N 6.89. The major isomer (*E*-isomer) was always contaminated with the minor isomer and other impurities. It was impossible to get it in the pure form.

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