SYNTHESIS AND BIOLOGICAL EVALUATION OF SODIUM 2β-[(2,5-DIHYDRO-6-HYDROXY-2-METHYL-5-OXO-1,2,4-TRIAZIN-3-YL)SULFONYLMETHYL]-2α-METHYLPENAM-3α-CARBOXYLATE 1,1-DIOXIDE

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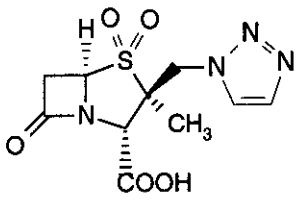
Abstract - A new β-lactamase inhibitor, sodium 2β-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)sulfonylmethyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (3), was synthesized. The compound (3) had excellent in vitro inhibitory activity except against cephalosporinase. In combination with ampicillin, piperacillin and ceftazidime, its synergistic effects was inferior to tazobactam, thus suggesting poor penetration through the bacterial cell wall.

β-Lactamases are enzymes responsible for many failures of antimicrobial therapy because of the hydrolysis of β-lactam antibiotics to inert and ineffective agents. The successful approach to overcoming the bacterial resistance to β-lactam antibiotics caused by β-lactamase production is to develop agents that can inhibit the action of the β-lactamase.

The introduction of a heterocyclic moiety at 2β-methyl group in the penicillanic acid sulfone molecule has provided compounds (e.g. 1 and 2) which display potent β-lactamase inhibitory activity against a broad range of β-lactamase producing microorganisms.[1-3] One member of this family of β-lactamase inhibitors, tazobactam (1), is being developed for clinical use. In an effort to further define the structure-activity parameters of this class of compounds, we initiated a program directed at the synthesis and biological evaluation of 2β-[(2,5-dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)sulfonylmethyl]-2α-methylpenam-3α-carboxylate 1,1-dioxide (3). Herein, we describe a brief synthesis of 3 and report on

its β-lactamase inhibitory activity.



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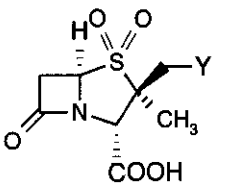

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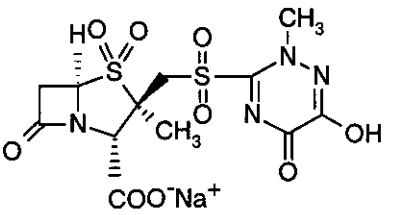

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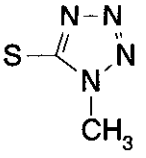
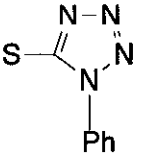
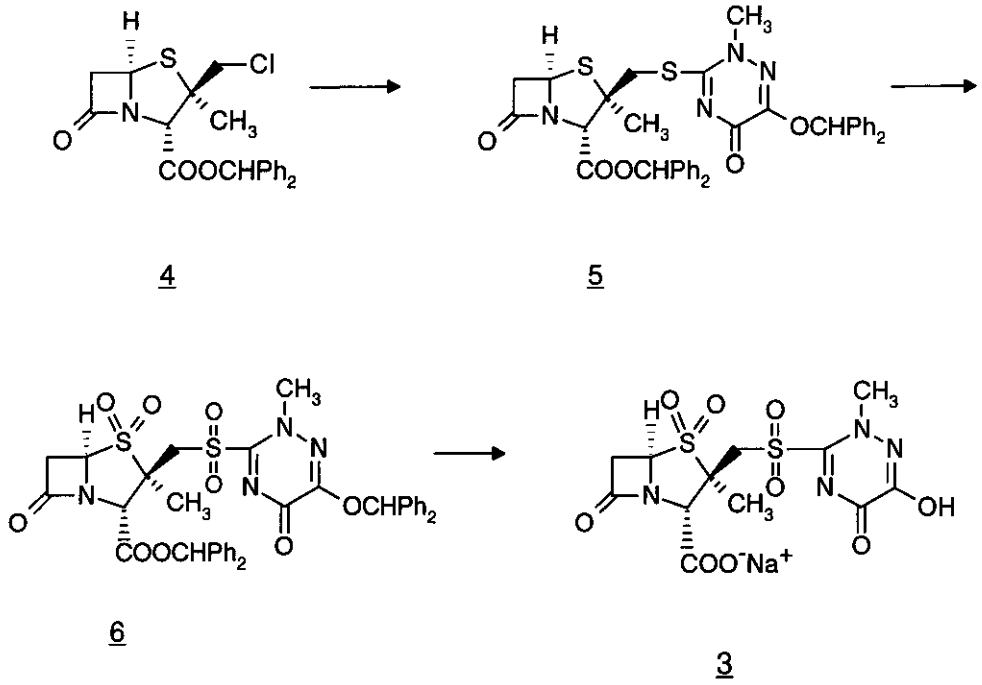
Y=  , 

The strategy for the preparation of the title compound (3) involves initial construction of an appropriately functionalized β-lactam nucleus - in the present case, 2β-chloromethylpenam (4). Treatment of 2β-chloromethylpenam (4) with 2,5-dihydro-6-diphenylmethyloxy-3-mercapto-2-methyl-5-oxo-1,2,4-triazin in a mixture of acetone-water containing potassium bicarbonate afforded 5, in low yield (38%) which was purified by silica gel column chromatography. Oxidation of 5 was accomplished in 39% yield employing potassium permanganate to provide 6. Removal of the protecting groups by trifluoroacetic acid in the presence of anisole at 0° C followed by conversion to its sodium salt afforded 3 which was purified further on a reverse phase preparative tlc.

Scheme



Compound (3) was tested against cell free β-lactamase preparations and the IC_{50} values are shown in Table I. It demonstrated excellent in vitro activity against penicillinase, cefotaximase and plasmid-mediated class III TEM enzyme and the activity was better than tazobactam. By contrast,(3) was relatively ineffective against cephalosporinase.

Table 1. β-Lactamase inhibitory activity of compound (3)

	IC_{50} (μM)			
Inhibitor	penicillinase *(B. cereus)*	R-TEM *(E. coli)*	cephalosporinase *(E. cloacae)*	cefotaximase *(K. pneumoniae)*
3	0.07	0.005	>20	0.001
Tazobactam	0.36	0.025	2.6	0.0028

The synergistic effects of compound (3) with various antibiotics such as ampicillin (ABPC), piperacillin (PIPC), and ceftazidime (CAZ) are shown in Table II. The bacteria cultivated in Mueller Hinton Broth (Difco) and diluted to 10^7 cfu/ml were inoculated into the same medium containing the antibiotics and tazobactam (TAZ) or compound (3) in a specific concentration and incubated at 37° C for 20 h. The growth of the microorganisms was observed to determine the minimal inhibitory concentration (MIC) for rendering the inoculated medium free from turbidity.

Table II. In vitro synergy of compound 3 with ampicillin (ABPC), piperacillin (PIPC) and ceftazidime (CAZ)

Organism	MIC (μg/ml)								
	ABPC	+TAZ [a]	+3	PIPC	+TAZ [a]	+3	CAZ	+TAZ [a]	+3
S.a. 54K	3.13	0.2	0.39	3.13	0.39	0.78	12.5	12.5	12.5
S.a. 80K	3.13	<0.1	0.2	6.25	0.39	0.78	12.5	3.13	6.25
E.c. TEM-1	>200	1.56	25	100	0.39	0.39	<0.1	<0.1	<0.1
E.c. TEM-3	>200	3.13	25	200	1.56	1.56	25	0.39	0.39
E.c. TEM-7	>200	3.13	25	200	0.39	0.78	12.5	0.2	0.39
E.c. OXA-1	>200	50	200	25	3.13	12.5	0.2	0.2	<0.1
E.c. OXA-3	50	1.56	0.78	3.13	0.39	0.39	0.2	<0.1	<0.1
E.c. SHV-1	>200	3.13	12.5	50	1.56	1.56	0.2	0.2	0.2
E.c. SHV-5	>200	1.56	---	>200	<0.1	---	>200	0.39	---
K.p. 336 L	>200	12.5	100	100	3.13	6.25	0.39	0.2	0.2
K.p. CTX-1	>200	12.5	>200	>200	6.25	50	100	0.78	6.25
S.m. 200 L	>200	50	50	100	0.78	0.78	<0.1	<0.1	<0.1
P.v. CT-106	>200	25	12.5	>200	1.56	0.39	25	0.78	0.39
C.f. 2046 E	>200	0.78	<0.1	50	0.39	<0.1	0.2	<0.1	<0.1

[a] TAZ = tazobactam; S.a., *Staphylococcus aureus*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; S.m., *Serratia marcescens*; P.v., *Proteus vulgaris*; C.f., *Citrobacter freundii*.

The synergistic data (Table II) indicate that though the compound (3) had better in vitro activity except against cephalosporinase, when compared to tazobactam; it failed to protect the antibiotics significantly. The synergistic effects were inferior to tazobactam, thus suggesting poor penetration through the bacterial cell wall.

EXPERIMENTAL

All column chromatographic purifications were accomplished on silica gel 60 (E. Merck, 230 ~ 400 mesh) with the appropriate solvent gradients. ^{1}H-Nmr spectra were determined with a Bruker AC-200-F (200 MHz) spectrometer in appropriate deuterated solvents and are expressed in ppm downfield from TMS (internal standard). β-Lactamase inhibition studies were carried out on isolated enzyme preparations by spectrophotometrically measuring the hydrolysis of the substrates (penicillin G or cephaloridine) in the presence and absence of the β-lactamase inhibitors.

Diphenylmethyl 2β-[(2,5-Dihydro-6-diphenylmethyloxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)thiomethyll-2α-methyl penam-3α-carboxylate 1,1-dioxide (5)

2β-Chloromethyl penam (4, 2.0 g, 0.005 mol) was dissolved in 30 ml of acetone; 1.625 g (0.005 mol) of 2,5-dihydro-6-diphenylmethyloxy-3-mercapto-2-methyl-5-oxo-1,2,4-triazin was added followed by 0.5 g (0.005 mol) of potassium bicarbonate. To this mixture water (10 ml) was added dropwise. Within 30 min, some white solid separated out; additional volume (15 ml) of acetone was added to make the mixture homogeneous. The mixture was stirred overnight at room temperature. Acetone was removed under reduced pressure. The residue was taken in methylene chloride, washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated (3.4 g). The product was purified over a silica gel column using benzene-ethyl acetate mixture (3:1) as eluant. The pure compound was obtained as a white foam (1.3 g, 38%). Nmr ($CDCl_3$) : δ 1.30 (s, 3H, CH_3); 3.18 (dd, 1H, J = 1.7 and 16.0 Hz); 3.54 (dd, 1H, J = 4.0 and 16.0 Hz); 3.60 (s, 3H, *N*-CH_3); 3.62 (d, 1H, J = 14.0 Hz, CH_2S); 3.92 (d, 1H, J = 14.0 Hz, CH_2S); 4.83 (s, 1H, H-3); 5.32 (dd, 1H, J = 1.5 and 3.8 Hz, H-5); 6.75 (s, 1H); 6.92 (s, 1H); 7.22-7.50 (m, 20 H, aromatic). Anal. Calcd for $C_{38}H_{35}N_4O_5S_2$: C, 66.01; H, 4.96; N, 8.11. Found: C, 65.90; H, 4.86; N, 7.64.

Diphenylmethyl 2β-[(2,5-Dihydro-6-diphenylmethyloxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)sulfonyl-methyl)]-2α-methylpenam-3α-carboxylate 1,1-dioxide (6)

930 mg (1.35 mmol) of the sulfide (5) was dissolved in 15 ml of glacial acetic acid, 2 ml of water was added dropwise. To this mixture 426 mg (2.70 mmol) of $KMnO_4$ was added and the mixture was stirred at room temperature for 4 h; cooled in an ice-bath, 30% H_2O_2 (2 ml) was added to decompose excess $KMnO_4$. The precipitated solid was filtered off. The solid was dissolved in methylene chloride, washed with water, dilute sodium bicarbonate solution, brine, and dried (Na_2SO_4). The product was purified by silica gel column chromatography. Initially the column was eluted with methylene chloride and finally with 5% acetone in methylene chloride. The title compound was obtained as a white foam, 400 mg (39.2%). Nmr ($CDCl_3$) : δ 1.20 (s, 3H, CH_3); 3.49 (dd, 2H, J = 2.6 and 4.5 Hz); 3.56 (s, 3H, *N*-CH_3); 3.88 (d, 1H, J = 14.7 Hz, CH_2SO_2); 4.10 (d, 1H, J = 14.7 Hz, CH_2SO_2); 4.55 (dd, 1H, J = 2.3 and 3.7 Hz, H-5); 4.89 (s, 1H, H-3); 6.75 (s, 1H); 6.90 (s, 1H); 7.12-7.50 (m, 20H, aromatic). Anal. Calcd for $C_{38}H_{35}N_4O_9S_2$: C, 60.46; H, 4.54; N, 7.42. Found: C, 60.51; H, 4.40; N, 7.08.

Sodium 2β-[(2,5-Dihydro-6-hydroxy-2-methyl-5-oxo-1,2,4-triazin-3-yl)sulfonylmethyl)]-2α-methyl-penam-3α-carboxylate 1,1-dioxide (3)

The diphenylmethyl ester (6, 250 mg, 0.346 mmol) was dissolved in 400 μl of anisole, cooled to 0° C; to this solution TFA (399 μl, 5.18 mmol) was added and the mixture was stirred at 0° C for 1.5 h. To this mixture isopropyl ether was added, the precipitated solid was collected by filtration and washed thoroughly with ether. The acid (80 mg, 0.2046 mmol) was taken in 2 ml of water, 18 mg (0.214 mmol) of $NaHCO_3$ was added. After stirring at room temperature for 1 h, the mixture was filtered through a small bed of Celite, then purified by reverse phase preparative tlc using acetone-water (4:1) mixture as the developing solvent. After freeze-drying the product was obtained as a white fluffy solid, 32 mg (18.7%). Nmr (D_2O): δ 1.59 (s, 3H, CH_3); 3.40 (dd, 1H, J = 2.0 and 16.7 Hz); 3.70 (dd, 1H, J = 4.3 and 16.7 Hz); 3.72 (s, 3H, N-CH_3); 3.92 (d, 1H, J = 15.8 Hz, CH_2SO_2); 4.28 (d, 1H, J = 15.8 Hz, CH_2SO_2); 4.51 (s, 1H, H-3); 5.02 (dd, 1H, J = 2.3 and 3.7 Hz).

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