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Short communication

Synthesis and biological evaluation of sodium 2α-(1,2,3-triazol-1-yl)methyl-2β-methyl-6,6-dihydropenam-3α-carboxylate-1,1-dioxide

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Summary — The synthesis and β-lactamase inhibitory activity of sodium 2α-(1,2,3-triazol-1-yl)methyl-2β-methyl-6,6-dihydropenam-3α-carboxylate-1,1-dioxide **2** (R = Na) is described. Its activity was compared with tazobactam **1** (R = Na). Compound **2** showed excellent synergism in combination with piperacillin (PIPC) and the results were either comparable or superior to tazobactam.

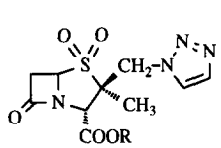
β-lactam / β-lactamase inhibitor / penam sulfone

Introduction

Though the principal direction of recent β-lactam antibiotic research has been towards the synthesis of cephalosporins with enhanced antibacterial activity and superior stability to the bacterial β-lactamases, the current 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 success of clavulanic acid [1] stimulated extensive research leading to the discovery of other β-lactamase inhibitors such as sulbactam [2] and YTR-830 [3, 4]. A number of 6-(substituted methylene)penams have been reported in the literature [5–8] as potent inhibitors of cell-free β-lactamases, but were ineffective in synergistic antibacterial tests probably because of poor penetration through the bacterial cell wall. More recently, 6-(substituted methylene)penems [9–13] have been shown to be very potent inhibitors of most bacterial β-lactamases including the class I β-lactamase, which is resistant to commercially available β-lactamase inhibitors. Although the penem derivative BRL-42715 [14–16] was discovered several years ago, there is little information about the further development of this compound. In our previous reports [3, 4] we have demonstrated that substitution of the 2β-methyl group of penicillanic acid sulfone molecule by a heterocyclic moiety (particularly by the triazole ring) provides a new class of very active β-lactamase inhibitors. One member of this family of β-lactamase inhibitors, tazobactam **1** is being developed for clinical use. In a continuation of these studies with series **1**, we pursued the closely related structure **2** since its derivatives should follow a similar mechanistic pathway and should also act as good suicide inhibitors. Here, we report a brief synthesis of **2** (scheme 1) and report on its synergy and β-lactamase inhibition data.

Chemistry

2α-(Chloromethyl)penam 1β-oxide **3** [17] was deoxygenated smoothly with phosphorus pentasulfide and pyridine [18] to give the 2α-(chloromethyl)penam **4**. The azido group at the 2α-methyl carbon was introduced by heating the corresponding 2α-(chloromethyl)penam **4** with excess sodium azide in dimethylformamide at 50–60°C for 4 h. Notably, no detectable amount of C(3)-substituted cepham was



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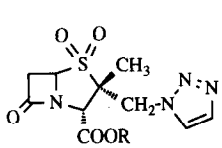

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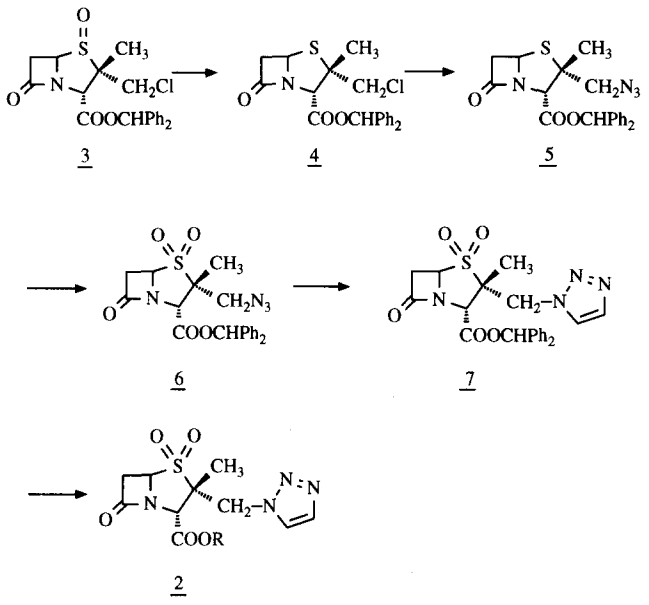

Scheme 1.

isolated; this fact is in sharp contrast to the substitution reaction of 2β-(chloromethyl)penam with sodium azide affording a mixture of 2β-(azidomethyl)penam and 3β-azido-3α-methyl cepham [19]. Oxidation of 2α-(azidomethyl)penam **5** was accomplished in 19% yield employing potassium permanganate in glacial acetic acid to provide **6**. Heating of **6** with acetylene in acetone gave the 2α-(triazolyl methyl)penam sulfone **7** as the sole product. Removal of the benzhydryl ester by hydrogenation over Pd/C afforded the 2α-(triazolylmethyl)penam-3α-carboxylic acid-1,1-dioxide **2** (R = H) which was converted to the sodium salt and purified over a HP-20 column.

Results and discussion

The β-lactamase inhibitory activity of sodium 2α-(triazolylmethyl)penam-3α-carboxylate-1,1-dioxide **2** (R = Na) and tazobactam **1** (R = Na) was determined by spectrophotometrically measuring the hydrolysis of the substrate (penicillin G) in the presence and absence of the β-lactamase inhibitors using penicillinase as β-lactamase. These results are summarized in table I.

The minimum inhibitory concentrations (MIC) of piperacillin (PIPC) in combination with the triazoles (10 μg/ml) were determined against a series of β-lactamase-producing microorganisms (table II). The bacteria were cultivated in Mueller Hinton Broth

Table I. Inhibition of β-lactamase by inhibitor[a].

	1 (R = Na)	*2 (R = Na)*
IC_{50} (μM)	0.57	5.09

[a]Substrate: PCG (200 μM, sigma); enzyme: penicillinase from *B cereus* (5000 units, 30 μl, Tokyo Kasei); preincubation 30°C, 5 min; incubation 30°C, 3 min.

(Difco) and diluted to 10^7 cfu/ml and were then inoculated into the same medium containing piperacillin and the prepared triazoles 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.

Conclusion

The synergistic data (table II) indicate that the 2α-triazole (**2**, R = Na) exhibits excellent β-lactamase inhibitory activity and the results are either comparable or superior to tazobactam (**1**, R = Na) except against TEM-2 producing *E coli* and *Acinetobacter 450 L*, in which case tazobactam showed better synergism.

Experimental protocols

General procedures

Melting points were determined on a Thomas–Hoover melting point apparatus and are uncorrected. The ^{1}H-NMR spectra (δ ppm) were obtained in $CDCl_3$ with tetramethylsilane as an internal standard on a Varian EM-360 spectrometer. Infrared spectra were recorded with a Nicolet DX FTIR. Only significant maxima are listed. Analytical results for compounds followed by elemental symbols were ± 0.4% of calculated values and were determined by the Department of Chemistry, University of Alberta. All column chromatographic purifications were accomplished on silica gel 60 (E Merck, 230–400 mesh) with the appropriate solvent gradients.

Benzhydryl 2α-chloromethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate ***4***

To a solution of benzhydryl 2α-chloromethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate 1β-oxide [17] (**3**, 0.208 g, 0.0005 mol) in methylene chloride (10 ml), were added pyridine (0.16 ml) and phosphorus pentasulfide (0.111 g), and the mixture was stirred at room temperature. After 3 h, an additional portion of phosphorus pentasulfide (0.08 g) was added and the reaction mixture was stirred at room temperature for a total period of 18 h. The mixture was diluted with water, extracted with methylene chloride, washed successively with water, 0.1 N HCl, water, $NaHCO_3$ solution and finally with brine, and dried over anhydrous Na_2SO_4. The organic layer was

Table II. *In vitro* synergy of compound **2** (R = Na) with piperacillin (PIPC) against β-lactamase-producing isolates.

Test organism	*β-Lactamase*	*MIC (μg/ml)*		
		PIPC	*+ Tazobactam (R = Na)*	*+ Compound 2 (R = Na)*
E coli	SHV 1	12.5	0.78	0.78
E coli	TEM 1	50	0.78	0.78
E coli	TEM 2	> 200	0.78	100
E coli	OXA 1	25	1.78	0.78
E coli	OXA 3	3.13	0.78	0.78
K pneumoniae 366 L	SHV 1	100	6.25	3.13
K pneumoniae 101 L	TEM 1	> 200	3.13	3.13
P mirabilis 60		3.13	0.20	0.20
S marcescens 200 L	TEM + C	50	6.25	6.25
C freundii 2046 E	C	25	0.78	0.39
C freundii 962 L	TEM 2	100	3.13	1.56
E cloaceae P 99	C	200	6.25	6.25
Acinetobacter 450 L	TEM + C	> 200	0.78	12.5
P aeruginosa	PSE 2	25	12.5	12.5
P aeruginosa	PSE 3	50	6.25	3.13
P aeruginosa	PSE 4	100	12.5	12.5
S aureus 54 K		12.5	0.78	0.78
S aureus 123 K		0.78	0.39	0.78

filtered and evaporated *in vacuo* to give a brown viscous oil (0.1364 g, 67.92%). This product was directly used for the next step without further purification. ^{1}H-NMR ($CDCl_3$): 1.78 (s, 3H, CH_3); 3.08 (dd, J = 2 and 16 Hz, 1H, 6-H); 3.59 (dd, J = 4 and 16 Hz, 1H, 6-H); 3.58 (s, 2H, CH_2Cl); 4.78 (s, 1H, 3-H); 5.33 (dd, J = 2 and 4 Hz, 1H, 5-H); 7.0 (s, 1H, $COOCHPh_2$); 7.4 (broad s, 10H, aromatic).

Benzhydryl 2α-azidomethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate 1,1-dioxide ***6***

A mixture of 0.4063 g (0.001 mol) of benzhydryl 2α-chloromethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate **4** sodium azide (0.394 g, 0.006 mol) in dimethyl formamide (8.1 ml) and water (2.6 ml) was heated at 60–65°C for 4 h. The mixture was diluted with water, extracted with chloroform, the chloroform extract was washed successively with small portions of ice-water, dried over anhydrous Na_2SO_4 and concentrated to give a brown viscous oil (0.3443 g, 83.34%) of benzhydryl 2α-azidomethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate **5**. This crude product was directly used for the next step.

Benzhydryl 2α-azidomethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate **5** (0.4971 g, 0.0012 mol) from the previous step was dissolved in a mixture of glacial acetic acid (22 ml) and water (3 ml). Potassium permanganate (0.423 g, 0.0026 mol) was added portionwise, the mixture was stirred at room temperature for 3 h. Excess potassium permanganate was decomposed by adding 30% hydrogen peroxide, the mixture was diluted with ice-cold water, the precipitated solid was filtered off and dissolved in methylene chloride, washed with water, followed by $NaHCO_3$ solution and brine. The methylene chloride layer was dried (Na_2SO_4) and evaporated *in vacuo* to yield a viscous residue (0.3735 g). The product was purified by column chromatography on silica gel (3:1, hexane/ethyl acetate) to yield 0.1016 g of **6** (18.95% yield). IR ($CHCl_3$) cm^{-1} 2114, 1802, 1745; ^{1}H-NMR ($CDCl_3$): 1.52 (s, 3H, CH_3); 3.4 (broad d, 2H, 6-H); 3.65 (broad d, 2H, CH_2N_3); 4.5 (s, 1H, 3-H); 4.68 (broad t, 1H, 5-H); 7.0 (s, 1H, $COOCHPh_2$); 7.4 (broad s, 10H, aromatic). Anal $C_{21}H_{20}N_4O_5S$ (C, H, N).

Benzhydryl 2α-(1,2,3-triazol-1-yl)methyl-2β-methyl-6,6-dihydropenam-3α-carboxylate-1,1-dioxide ***7***

A solution of benzhydryl 2α-azidomethyl-2β-methyl-6,6-dihydropenam-3α-carboxylate 1,1-dioxide **6** (0.05 g) in acetone (4 ml) was heated in a sealed steel vessel with excess of acetylene (4.0 g) at 85°C for 24 h, cooled to room temperature, evaporation of the solvent gave a pale-yellow semisolid (0.0499 g) which on crystallization from ethyl acetate/hexane gave the pure triazole **7** (0.042 g, 79.72%); mp 156–159°C; ^{1}H-NMR ($CDCl_3$): 1.58 (s, 3H, CH_3); 3.43 (broad d, 2H, 6-H); 3.68 (broad t, 1H, 5-H); 4.8 (ABq, J = 13.4 Hz, 2H, CH_2-triazole); 4.72 (s, 1H, 3-H); 7.08 (s, 1H, $COOCHPh_2$); 7.4 (broad s, 10H, ar); 7.7 (s, 2H, triazole). Anal $C_{23}H_{22}N_4O_5S$ (C, H, N).

*Sodium 2α-(1,2,3-triazol-1-yl)methyl-2β-methyl-6,6-dihydropenam-3α-carboxylate-1,1-dioxide (***2***, R = Na)*

A mixture of benzhydryl 2α-(1,2,3-triazol-1-yl)methyl-2β-methyl-6,6-dihydropenam-3α-carboxylate-1,1-dioxide (**7**, 0.012 g), sodium bicarbonate (2.3 mg), 10% Pd/C (50 mg) in ethyl acetate (10 ml) and water (5 ml) was hydrogenated at a

low pressure (2–3 kg/cm^2) at room temperature. The catalyst was removed by filtration and the aqueous layer was separated, washed with ethyl acetate, and then lyophilized to afford a white powder. The crude product was purified over MCI gel (CHP-20P, Mitsubishi Kasei Co) using water as eluent to afford a white powder of **2** (R = Na, 0.005 g, 60%); mp 235°C (decomp). ^{1}H-NMR (D_2O): 1.54 (s, 3H, CH_3); 3.40 (dd, J = 2 and 16 Hz, 1H, 6-H); 3.67 (dd, J = 4 and 16 Hz, 1H, 6-H); 4.97 (dd, J = 2 and 4 Hz, 1H, 5-H); 4.98 (s, 2H, CH_2 triazole); 5.09 (s, 1H, 3-H); 7.82 (s, 1H, triazole); 8.09 (s, 1H, triazole).

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