Studies on Penam Sulfones

I. Synthesis and β-Lactamase Inhibitory Activity of 2β-Alkoxycarbonyl Penicillanic Acid Sulfones

EDUARDO L. SETTI, NARENDER A. V. REDDY, OLUDOTUN A. PHILLIPS, DAVID P. CZAJKOWSKI, KEVIN ATCHISON, HARNINDER ATWAL, RONALD G. MICETICH and SAMARENDRA N. MAITI*

SynPhar Laboratories Inc., #2, Taiho Alberta Center, 4290-91A Street, Edmonton, Alberta, Canada T6E 5V2

CHIEKO KUNUGITA and AKIO HYODO

Tokushima Research Institute, Taiho Pharmaceutical Co., Ltd., 224-2 Ebisuno Hiraishi, Kawauchi-cho, Tokushima 771-01, Japan

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β-Lactamase production is the main mechanism of bacterial resistance to β-lactam antibiotics. Inhibition of β-lactamase through the use of suicide inhibitors provides one strategy to overcome β-lactamase mediated reistance. Chromosomally-mediated Class I cephalosporinases that are inducible in *P. aeruginosa*, and in *Enterobacter*, *Citrobacter*, *Morganella*, *Serratia* and *Providencia* species inactivate the newly introduced broader-spectrum cephalosporins. None of the currently available β-lactamase inhibitors (clavulanate, sulbactam and tazobactam) is very effective against Class I cephalosporinases. These enzymes have found their way onto plasmids, thus paving the way for their dissemination among Gram-negative bacteria. This highlights the importance of finding β-lactamase inhibitors with activity against Class I cephalosporinases. Over the past several years, there has been a continuous effort to search for new β-lactamase inhibitors with specific activity against Class I β-lactamase[1,2]. In our previous paper[3] we reported on the modification of the 1,2,3-triazole ring of YTR-830 (tazobactam) by introducing various substituents at the C(4) carbon atom of the triazole ring with the aim of developing a cephalosporinase inhibitor. These modified tazobactam derivatives, however, did not show significant cephalosporinase inhibitory activity.

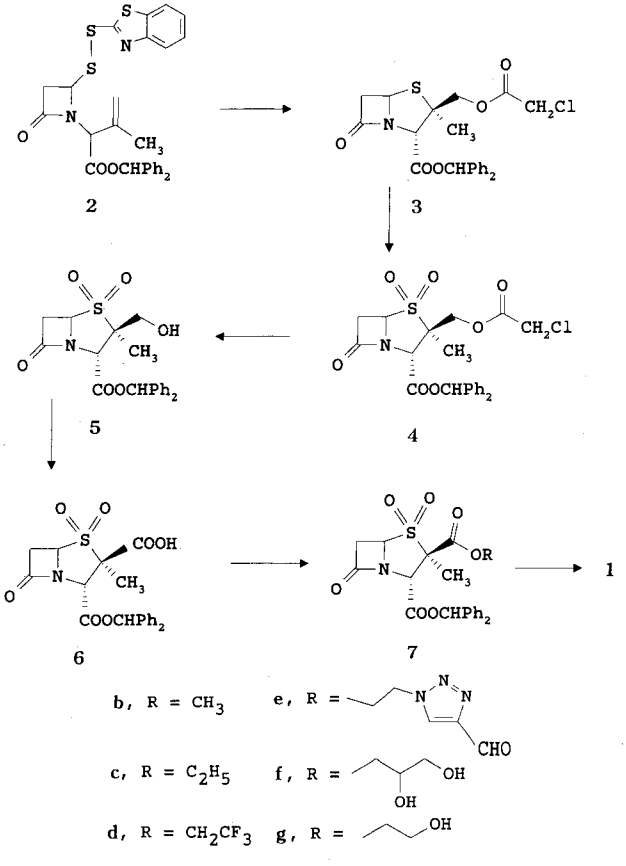
As a continuation of our search for a new broad-spectrum β-lactamase inhibitor with increased cephalosporinase activity, we modified the C(2) carbon atom of penam sulfone. In this paper, we report the synthesis of several new 2β-alkoxycarbonyl penicillanic acid sulfone derivatives **1** (**a**~**g**) and their β-lactamase inhibitory activities.

Chemistry

The synthesis of the title compounds **1** (**a**~**g**) was achieved as outlined in scheme 1. The key intermediate, 2β-hydroxymethyl penam sulfone **5** was prepared in three steps starting from unsymmetrical azetidinone disulfide **2** by using the published procedure[4] with minor modification. The compound **3** (as a mixture with the cephem by-products) was directly oxidized with $KMnO_4$ in glacial acetic acid and after column purification, the sulfone **4** was obtained in 50% yield. Heating of the sulfone **4** with thiourea in ethanol and after column purification the desired 2β-hydroxymethyl penam sulfone **5** was obtained in good yield (88%).

Oxidation of compound **5** with $KMnO_4$ in presence of tetrabutylammonium bromide[5] gave 2β-carboxy-penam sulfone **6** (27% yield) which on treatment with ethereal solution of diazomethane gave **7b**. The compound **6** on treatment with the appropriate alcohols under standard conditions provided the 2β-alkoxy-

Scheme 1.



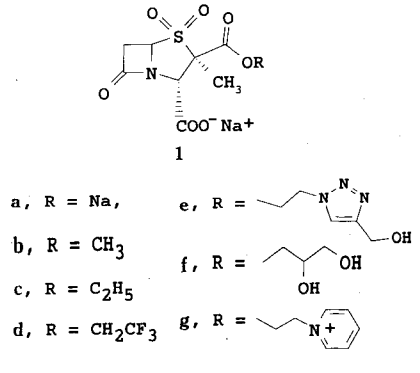
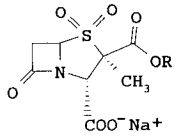
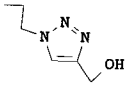
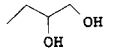
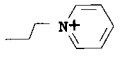


Table 1. ^{1}H NMR Data of 2β-alkoxycarbonyl penicillanic acid sulfones **1** (**a**~**g**).



1

Compound	R	δ (ppm)
1a[a]	Na	1.65 (3H, s), 3.37 (1H, dd, J=1.2 and 16.0 Hz), 3.60 (1H, dd, J=4.0 and 16.0 Hz), 4.75 (1H, s), 4.90 (1H, dd, J=1.2 and 4.0 Hz)
1b[b]	CH_3	1.62 (3H, s), 3.10 (1H, dd, J=1.3 and 16.0 Hz), 3.53 (1H, dd, J=4.0 and 16.0 Hz), 3.73 (3H, s), 4.79 (1H, s), 4.97 (1H, d, J=3.1 Hz)
1c[a]	C_2H_5	1.34 (3H, t, J=7.0 Hz), 1.76 (3H, s), 3.47 (1H, dd, J=1.5 and 16.7 Hz), 3.74 (1H, dd, J=4.2 and 16.7 Hz), 4.39 (2H, q, J=7.0 Hz), 5.11 (1H, dd, J=1.6 and 4.2 Hz), 5.22 (1H, s)
1d[a]	CH_2CF_3	1.77 (3H, s), 3.37 (1H, dd, J=4.0 and 16.7 Hz), 3.43 (1H, dd, J=1.5 and 16.7 Hz), 4.85 (2H, q, J=8.4 Hz), 5.12 (1H, dd, J=1.9 and 4.4 Hz), 5.18 (1H, s)
1e[a]		1.65 (3H, s), 3.33 (1H, dd, J=1.6 and 16.6 Hz), 3.66 (1H, dd, J=4.3 and 16.6 Hz), 4.80 (6H, m), 5.10 (1H, s), 5.20 (1H, dd, J=1.6 and 4.0 Hz), 8.02 (1H, s)
1f[a]		1.77 (3H, s), 3.43 (1H, dd, J=1.0 and 16.7 Hz), 3.55 ~ 3.80 (3H, m), 3.93~4.05 (1H, m), 4.25~4.40 (2H, m), 5.09 (1H, dd, J=1.0 and 3.0 Hz), 5.20 (1H, s)
1g[a]		1.70 (3H, s), 3.35 (1H, dd, J=1.4 and 16.6 Hz), 3.72 (1H, dd, J=4.3 and 16.6 Hz), 4.75 (1H, br d), 4.80~4.90 (2H, m), 5.03~5.10 (3H, m, overlapped with a singlet), 8.13 (2H, t, J=5.5 Hz), 8.60 (1H, t, J=5.5 Hz), 8.95 (2H, d, J=5.5 Hz)

[a] 200 MHz, D_2O, [b] 200 MHz, DMSO-d_6.

carbonyl penam sulfones **7** (**c**~**e**) and **7g**. When the 2β-(1-hydroxyethyloxycarbonyl) penam sulfone **7g** was converted to the corresponding triflate and treated with excess pyridine, the pyridinium derivative was obtained as the main product. The 2β-allyloxycarbonyl penam sulfone (prepared by the coupling of compound **6** with allyl alcohol) underwent hydroxylation[6] in presence of OsO_4 and 4-methylmorpholine *N*-oxide to afford the corresponding diol **7f**. The compounds were then treated with TFA/anisole at 0°C or hydrogenated over Pd/C to remove the protecting groups and were converted to the corresponding sodium salts. Due to the synthetic expediency the compound **1f** was tested as a mixture of optical isomers. The compound **1g** was obtained as zwitterion. During hydrogenation, the aldehyde group of compound **7e** was reduced to the alcohol and the benzhydryl group was removed simultaneously. NMR data of all the newly synthesized compounds is shown in Table 1.

Results and Discussion

Compounds **1** (**a**~**g**) were tested against cell free β-lactamase preparations and the IC_{50} values are shown in Table 2.

In *in vitro* synergy studies in combination with piperacillin (PIPC), many of these compounds showed good overall synergistic activity against TEM, OXA and SHV producing microorganisms and the values were comparable to tazobactam. However, none of these

Table 2. Inhibitory properties of 2β-alkoxycarbonyl penicillanic acid sulfones **1** (**a**~**g**).

Compound	IC_{50} (μM)		
	TEM-1 (*E. coli*)	CTX-1 (*K. pneumoniae*)	Cephase (*P. aeruginosa*)
1a	>10	0.9	>10
1b	0.4	0.1	1.0
1c	0.2	0.01	1.7
1d	0.07	0.01	0.5
1e	0.1	0.01	6.0
1f	0.07	0.02	1.0
1g	0.2	0.09	>10

compounds showed synergy against *E. coli* TEM-2, *C. freundii* CT 76 against which tazobactam showed better synergy (Table 3). Like tazobactam, these compounds also had no effect on *P. aeruginosa*. In combination with ceftazidime (CAZ) some of these derivatives, particularly the compound **1c**, was significantly better than tazobactam against cephalosporinase producing microorganisms such as *E. cloacae* P99, *E. cloacae* 40011, *M. morganii* 36010 and *E. aerogenes* 41004 (Table 4).

Modification of the C (2) carbon atom of the penam sulfone skeleton provided a series of 2β-alkoxycarbonyl penam sulfone. One compound from this series, sodium

Table 3. *In vitro* synergy of compounds **1** (**a**~**g**) with piperacillin (PIPC) against β-lactamase producing isolates.

Test organisms	MIC (μg/ml)								
	PIPC alone	+Tazobactam	+**1a**	+**1b**	+**1c**	+**1d**	+**1e**	+**1f**	+**1g**
E. coli TEM-1	200	0.39	100	1.56	3.13	3.13	6.25	0.39	0.39
E. coli TEM-2	>400	3.13	>400	>400	200	400	200	200	100
E. coli TEM-3	100	1.56	6.25	1.56	1.56	1.56	1.56	0.78	0.39
E. coli TEM-7	100	0.78	25	0.39	0.39	0.78	0.78	≤0.2	≤0.2
E. coli OXA-1	25	3.13	25	3.13	3.13	6.25	0.78	1.56	3.13
E. coli OXA-3	3.13	0.39	3.13	0.78	0.39	0.78	0.78	0.78	1.56
E. coli SHV-1	200	1.56	50	1.56	1.56	1.56	6.25	1.56	0.78
S. marcescens 200 L	100	1.56	25	1.56	1.56	1.56	12.5	1.56	0.78
P. vulgaris CT 106	400	1.56	25	12.5	6.25	25	12.5	6.25	6.25
C. freundii 2046E	>400	0.78	1.56	6.25	3.13	1.56	6.25	6.25	1.56
C. freundii CT 76	>400	12.5	>400	400	200	400	200	400	200
M. morganii 36014	100	≤0.2	50	0.78	0.39	1.56	1.56	1.56	6.25
P. aeruginosa 46220	1.56	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.78

Table 4. *In vitro* synergy of compounds **1** (**a**~**g**) with CAZ against selected beta-lactamase producing strains.

Test organisms	MIC (μg/ml)								
	CAZ alone	+Tazobactam	+**1a**	+**1b**	+**1c**	+**1d**	+**1e**	+**1f**	+**1g**
E. coli TEM-3	25	0.39	1.56	0.39	0.39	0.78	0.39	0.39	0.39
E. coli TEM-7	12.5	<0.2	3.13	0.39	0.39	0.78	0.39	<0.20	0.39
K. pneumoniae CTX-1	100	0.78	6.25	1.56	1.56	3.13	1.56	0.78	0.78
P. vulgaris CT-106	12.5	0.39	1.56	0.78	0.39	0.78	3.13	0.39	1.56
C. freundii CT 76	50	12.5	12.5	50	25	12.5	25	50	25
E. cloacae P 99	50	12.5	25	1.56	3.13	12.5	12.5	6.25	6.25
E. cloacae 40011	25	1.56	6.25	3.13	1.56	6.25	3.13	1.56	1.56
E. aerogenes 41004	25	12.5	6.25	3.13	1.56	6.25	6.25	3.13	6.25
P. aeruginosa 46220	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56
M. morganii 36010	400	6.25	200	6.25	3.13	12.5	25	12.5	200
M. morganii 36014	25	<0.20	25	<0.20	<0.20	0.39	0.39	0.39	3.13

2β-(ethoxycarbonyl)-6,6-dihydropenicillanate 1,1-dioxide **1c** showed slightly improved synergy compared to tazobactam, when tested in combination with ceftazidime (CAZ) and the activity against TEM, OXA and SHV producing microorganisms were comparable to tazobactam.

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