Studies on Penam Sulfones

II. Synthesis and β-Lactamase Inhibitory Activity of 2β-Carboxamide Penicillanic Acid Sulfones

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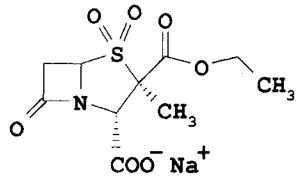
2β-Carboxamide penicillanic acid sulfones were prepared as β-lactamase inhibitors. Among all the compounds prepared, compound **2c** showed overall better *in vitro* synergy than tazobactam against strains which hyperproduce class C β-lactamases. In addition, the synergistic activity of compound **2c** in combination with ceftazidime or piperacillin was similar to that of tazobactam against TEM, OXA, and SHV enzyme producing microorganisms.

The recent occurrence and spread of chromosomally-mediated class C enzyme (cephalosporinase) that causes resistance to the newly introduced cephalosporins has been viewed with alarm by the medical community. None of the currently available β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) is very effective against class C enzymes. It appears that class C β-lactamases 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 the class C enzymes. Recently, there has been a continuous effort to search for new β-lactamase inhibitors with specific activity against class C enzymes[1~4].

During the course of our β-lactamase inhibitor research in the penam sulfone area, we discovered a series of 2β-alkoxycarbonyl penicillanic acid sulfones. One compound from this series, such as 2β-(ethoxycarbonyl)-6,6-dihydropenicillanate 1,1-dioxide (**1**) in combination



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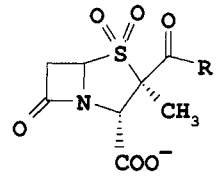

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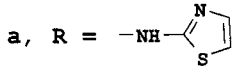
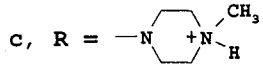
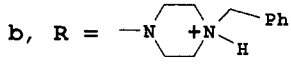
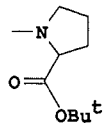
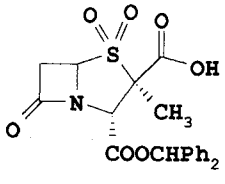
a, R = 

c, R = 

b, R = 

d, R = 



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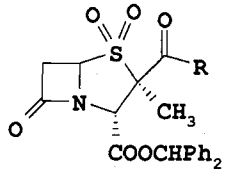

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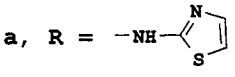
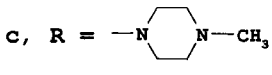
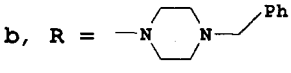
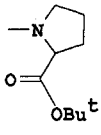
2 (a ~ d)

a, R = 

c, R = 

b, R = 

d, R = 

with ceftazidime showed good synergistic activity against chromosomally-mediated class C enzyme producing microorganisms[5]. As a continuation of our search for a new broad-spectrum β-lactamase inhibitor with improved activity against class C enzymes, we modified further the 2β-methyl group of penam sulfone leading to the discovery of a series of 2β-carboxamide penicillanic acid sulfones. Here, we report the synthesis of several 2β-carboxamide penicillanic acid sulfone derivatives (**2**) and their *in vitro* evaluation as β-lactamase inhibitors.

Chemistry

The starting material for the preparation of the title compounds **2** (**a**~**d**) was the 2β-carboxy penam sulfone **3**, which was prepared by our reported procedure[5]. Coupling of the sulfone **3** with 2-aminothiazole in presence of 1-hydroxybenzotriazole and DCC gave the compound **4a**. Reaction of the 2β-carboxy penam sulfone **3** with oxalyl chloride in presence of DMF gave the corresponding acid chloride, which on treatment with *N*-benzyl piperazine and *N*-methyl piperazine gave the compounds **4b** and **4c**, respectively, while the reaction of the acid chloride with 2-(*S*)-*t*-butoxycarbonyl pyrrolidine in presence of triethylamine gave the compound **4d**. The ester protecting group was removed by catalytic hydrogenation over Pd/C and the acid thus obtained was converted to the corresponding sodium salt by treatment with $NaHCO_3$. The compounds **2b** and **2c** were obtained as zweitterions.

Results and Discussion

Compounds **2** (**a**~**d**) were tested against cell free β-lactamase preparations and the IC_{50} are shown in Table 1. Against isolated cephalosporinase (isolated from *P. aeruginosa* 46012), none of the title compounds showed good inhibitory activity. However, in *in vitro* synergy studies in combination with piperacillin (PIPC), the compound **2c** showed good overall synergy against cephalosporinase producing organisms (Table 2), especially against *C. freundii* CT 76, *E. cloacae* P99, *E. aerogenes* 41006 and was superior to tazobactam (TAZ). On the other hand, the synergistic activity of compound **2c** was comparable to tazobactam against TEM, OXA, and SHV type enzyme producing microorganisms. Similarly, in combination with ceftazidime (CAZ), compound **2c** was the only compound which showed excellent synergy against CAZ resistant cephalosporinase producing strains, such as *C. freundii* CT 76, *E. cloacae* P99, *E. cloacae* 40011, *E. aerogenes* 41004, *E. aerogenes* 41006 (Table 3). Like tazobactam, these compounds failed to show any significant synergy against *P. aeruginosa*, either due to lack of penetration or poor affinity towards the target enzymes.

Modification of the 2β-methyl group of sulbactam led to the discovery of a series of 2β-carboxamide penicillanic acid sulfones **2** (**a**~**d**). One compound from this series,

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

Compound	IC_{50} (μM)		
	TEM-1 (*E. coli*)	CTX-1 (*K. pneumoniae*)	Cephase (*P. aeruginosa*)
2a	7.4	0.1	>10
2b	0.9	0.01	15
2c	0.2	0.01	>10
2d	17	0.16	7.6

Table 2. *In vitro* synergy of compounds **2** (**a**~**d**) with PIPC against selected β-lactamase producing strains.

Test organisms	MIC (μg/ml)					
	PIPC alone	PIPC+ TAZ	PIPC+ **2a**	PIPC+ **2b**	PIPC+ **2c**	PIPC+ **2d**
E. coli TEM-1	200	0.78	100	6.25	0.39	50
E. coli TEM-2	>400	50	>400	>400	3.13	>400
E. coli TEM-3	200	1.56	25	6.25	3.13	50
E. coli TEM-7	200	0.78	100	3.13	0.39	100
E. coli OXA-1	25	3.13	50	12.5	3.13	25
E. coli OXA-3	6.25	0.78	3.13	0.78	0.39	6.25
E. coli SHV-1	>400	1.56	200	12.5	3.13	50
K. pneumoniae CTX-1	>400	12.5	200	12.5	12.5	400
S. marcescens 200 L	200	1.56	50	12.5	0.78	25
P. vulgaris CT-106	400	1.56	200	400	200	400
C. freundii 2046 E	>400	0.78	12.5	1.56	0.78	25
C. freundii CT 76	>400	25	>400	400	12.5	>400
E. cloacae P 99	200	50	100	200	12.5	200
E. cloacae 40011	50	12.5	50	50	6.25	25
E. aerogenes 41004	25	25	25	25	12.5	12.5
E. aerogenes 41006	200	100	200	200	12.5	200
P. aeruginosa 46220	1.56	0.39	1.56	0.78	0.39	0.78
M. morganii 36014	100	0.39	50	50	6.25	50

Table 3. *In vitro* synergy of compounds **2** (**a**~**d**) with CAZ against selected β-lactamase producing strains.

Test organisms	MIC (μg/ml)					
	CAZ alone	CAZ+ TAZ	CAZ+ **2a**	CAZ+ **2b**	CAZ+ **2c**	CAZ+ **2d**
E. coli TEM-1	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
E. coli TEM-2	0.39	<0.20	0.39	<0.20	<0.20	0.39
E. coli TEM-3	25	<0.20	6.25	0.78	0.39	12.5
E. coli TEM-7	12.5	<0.20	6.25	1.56	0.39	6.25
E. coli OXA-1	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
E. coli OXA-3	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
E. coli SHV-1	0.39	<0.20	0.39	<0.20	<0.20	<0.20
K. pneumoniae CTX-1	100	0.78	25	3.13	0.78	100
S. marcescens 200 L	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
P. vulgaris CT-106	25	0.78	12.5	3.13	1.56	25
C. freundii 2046 E	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
C. freundii CT 76	50	25	50	50	3.13	50
E. cloacae P 99	100	25	100	50	12.5	100
E. cloacae 40011	25	6.25	25	12.5	1.56	25
E. aerogenes 41004	25	12.5	6.25	25	1.56	25
C. aerogenes 41006	25	25	25	25	1.56	25
P. aeruginosa CT 122	100	100	100	50	50	100
P. aeruginosa 46220	1.56	1.56	1.56	1.56	1.56	1.56
M. morganii 36014	25	<0.20	25	12.5	3.13	12.5

such as compound **2c**, showed improved synergy than tazobactam against strains which hyperproduce class C β-lactamases. In combination with ceftazidime and piperacillin, the synergistic activity of compound **2c** against TEM, OXA, and SHV enzyme producing organisms was similar to that seen with the tazobactam. Among all the compounds prepared, compound **2c** showed overall better synergy in combination with ceftazidime against CAZ resistant cephalosporinase producing strains except *P. aeruginosa*.

Acknowledgments

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