# Studies on Penam Sulfones

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

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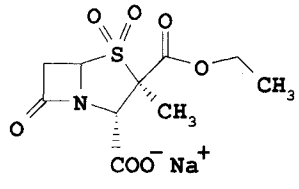
2β-Carboxamide penicillanic acid sulfones were prepared as β-lactamase inhibitors. Among all the compounds prepared, compound **2c** showed overall better *in vitro* synergy than tazobactam against strains which hyperproduce class C β-lactamases. In addition, the synergistic activity of compound **2c** 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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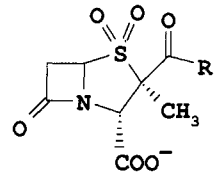

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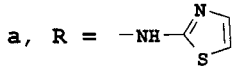
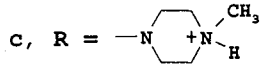
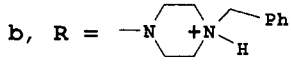
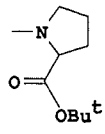
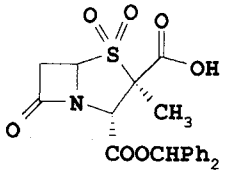
a, R = 

c, R = 

b, R = 

d, R = 



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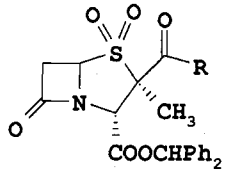

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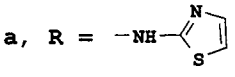
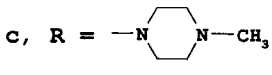
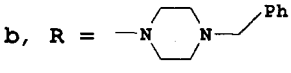
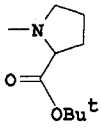
2 (a ~ d)

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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## References

1) RICHTER, H. G. F.; P. ANGEHRN, C. HUBSCHWERLEN, M. KANIA, M. G. P. PAGE, J.-L. SPECKLIN & F. K. WINKLER: Design, synthesis and evaluation of 2β-alkenyl penam sulfone acids as inhibitors of β-lactamases. J. Med. Chem. 39: 3712~3722, 1996
2) PFLIEGER, P.; P. ANGEHRN, M. BOHRINGER, C. HUBSCHWERLEN, M. G. P. PAGE, F.-P. THEIL & F. WINKLER: Bridged carbacephems as β-lactamase inhibitors: Synthesis and structure-activity relationships. 36th Intersci. Conf. on Antimicrob. Agents Chemother. Poster No. F-158, New Orleans, Sept. 15~18, 1996
3) HUBSCHWERLEN, C.; P. ANGEHRN, M. BOHRINGER, M. KANSY, M. G. P. PAGE, J.-L. SPECKLIN & R. THEN: Bridged isooxa- and iso-cephems as β-lactamase inhibitors and antibacterials: Synthesis and structure-activity relationships. 36th Intersci. Conf. on Antimicrob. Agents Chemother. Poster No. F-157, New Orleans, Sept. 15~18, 1996
4) ANGEHRN, P.; K. GUBERNATOR, E. -M. GUTKNECHT, I. H. KRAUSS, C. HUBSCHWERLEN, M. KANIA, M. G. P. PAGE & J.-L. SPECKLIN: Bridged β-lactams: A new class of β-lactamase inhibitors, synthesis and structure-activity relationships. 35th Intersci. Conf. on Antimicrob. Agents Chemother. Poster No. F-147, San Francisco, Sept. 17~20, 1995
5) SETTI, E. L.; N. A. V. REDDY, O. A. PHILLIPS, D. P. CZAJKOWSKI, H. ATWAL, R. G. MICETICH, S. N. MAITI, C. KUNUGITA & A. HYODO: Studies on penam sulfones I: Synthesis and β-lactamase inhibitory activity of 2β-alkoxycarbonyl penicillanic acid sulfones. J. Antibiotics 49: 944~946, 1996