# 6-(*E*)-ACETYLMETHYLENEPENICILLANIC ACID, A POTENT β-LACTAMASE INHIBITOR

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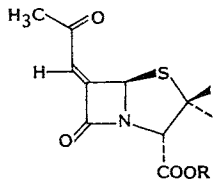
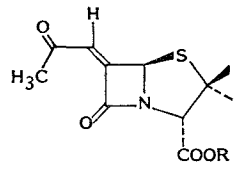
We recently demonstrated that **1a** displays potent and broad activity against β-lactamases[1,2]. We have now shown that the geometrical isomer **2a** is likewise a potent β-lactamase inhibitor, as is the corresponding pivaloyloxymethyl ester **2b** (Fig. 1). This ester was isolated as a side product in the course of synthesizing **1b**[3].

The ester **2b** (220 mg in 1 ml dimethyl sulfoxide) was incubated (0.01 M phosphate buffer, 100 ml) at pH 7 (pH-stat, 0.01 M NaOH) and 25°C for 7 hours in the presence of pig liver esterase. After extraction with diethyl ether, the aqueous phase was acidified (pH 3) at 5°C with citric acid and extracted with ethyl acetate. The resulting organic extract was dried and concentrated at reduced pressure. The acid **2a** (160 mg) could be isolated after salt formation (2 N sodium 2-ethyl caproate in ethyl acetate) as the crystalline sodium salt (150 mg): IR (KBr) $cm^{-1}$ 1744, 1713, 1664, 1610, 1400; $^1H$ NMR (270 MHz, $D_2O$) $\delta$ 1.53 and 1.58 (2×s, 2×$CCH_3$), 2.53 (s, $CH_3C$=), 4.4 (s, CHCOO), 5.83 (s, 5-CH), 6.43 (s, HC=). Like **1a** and **1b**, **2a** and **2b** show no antibacterial activity. It can be seen from Table 1 that **2a** and **2b** are potent inhibitors of the enzymes tested. Although they are less active than **1a**, their activity is comparable to that of clavulanic acid, and against *Escherichia coli* 1024 β-lactamase even superior to that of

Fig. 1.




**1a** R = H
**1b** R = $CH_2OCOC(CH_3)_3$




**2a** R = H
**2b** R = $CH_2OCOC(CH_3)_3$

Table 1. Inhibitory properties for isolated β-lactamases.

| Compound | $IC_{50}$ (μM) for the β-lactamase | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Proteus vulgaris* 1028 | | *Klebsiella pneumoniae* NCTC 418 | | *Escherichia coli* 1024 | | *Escherichia coli* TEM 1 | |
| | −E | +E | −E | +E | −E | +E | −E | +E |
| **1b** | 0.16 | 0.015 | 0.19 | 0.001 | 32 | 1.5 | 0.02 | — |
| **1a** | 0.004 | — | 0.005 | — | 1.7 | — | 0.0007 | — |
| Clavulanic acid | 0.03 | — | 0.02 | — | 85 | — | 0.04 | — |
| **2b** | 1.2 | 0.07 | 0.45 | 0.017 | 25 | 1.8 | 0.85 | 0.041 |
| **2a** | 0.048 | — | 0.024 | — | 3.6 | — | 0.0083 | — |

E: Test carried out in the presence of 10 μl of hog liver esterase.

Table 2. *In vitro* synergy with ampicillin against β-lactamase-producing isolates.

| Compound | MIC (μg/ml) inhibitor+ampicillin[a] | | | |
|---|---|---|---|---|
| | *Staphylococcus aureus* 887 | *Escherichia coli* TEM 1 | *Klebsiella pneumoniae* NCTC 418 | *Proteus vulgaris* 1028 |
| **1a** | 1.6+3.1 | 6.3+12.5 | 0.8+6.3 | 1.6+3.1 |
| **2a** | 3.1+6.3 | 12.5+12.5 | 0.8+6.3 | 3.1+3.1 |

[a] Figures presented were selected on the basis of minimal total drug concentration required for inhibition. MICs of inhibitors or ampicillin alone were in all instances greater than 50 μg/ml.

Method: Checkerboard titration.
Medium: Mueller-Hinton broth (Difco).
Inoculum: $10^5$ cfu/ml (*S. aureus*, $10^6$ cfu/ml).

Table 3. Therapeutic activity in combination with ampicillin (A) against experimental septicemias in mice ($ED_{50}$ mg/kg).

| | *Staphylococcus aureus* 887 | *Proteus vulgaris* 1028 |
|---|---|---|
| A+**1b** | 12+12 | >25+25 |
| A+**2b** | 25+25 | >25+25 |
| A | >25 | >25 |

Compounds administered subcutaneously 1 and 3 hour(s) after infection.

clavulanic acid. These compounds are also active against several β-lactamase-producing ampicillin-resistant organisms when tested in combination with ampicillin. The *in vitro* and *in vivo* properties of the test compounds are shown in Tables 2 and 3.

These results reinforce our proposal that the ketone is actively involved during the inactivation of β-lactamases[4].

## References

1) ARISAWA, M. & R. L. THEN: 6-Acetylmethylenepenicillanic acid (Ro 15-1903), a potent β-lactamase inhibitor. I. Inhibition of chromosomally and R-factor-mediated β-lactamases. J. Antibiotics 35: 1578~1583, 1982

2) ANGEHRN, P. & M. ARISAWA: 6-Acetylmethylenepenicillanic acid (Ro 15-1903), a potent β-lactamase inhibitor. II. Antibacterial properties. J. Antibiotics 35: 1584~1589, 1982

3) ADAM, S.; W. ARNOLD & P. SCHÖNHOLZER: Sulfoxides of penicillanates with non classical substituents in the 6-position. Tetrahedron 39: 2485~2491, 1983

4) ARISAWA, M. & S. ADAM: Mechanism of inactivation of TEM-1β-lactamase by 6-acetylmethylenepenicillanic acid. Biochem. J. 211: 447~454, 1983