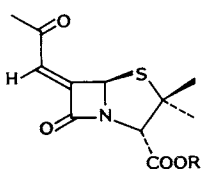
METHYLENE (2*S*,5*R*)-6-(3-FORMYLALLYLIDENE)PENICILLANATE PIVALATE, A PRODRUG OF A NEW β-LACTAMASE INHIBITOR

Sir:

In earlier papers[1~4] we described the synthesis and biological properties of the 6-acylmethylenepenicillanates, potent broad spectrum β-lactamase inhibitors. We also demonstrated[5] that the geometry of the olefin, which imparts a relative spatial constraint to the carbonyl function, plays an important role in the biological activity displayed by this class of β-lactamase inhibitors; compound **1** (*Z*-olefin) is more potent than **2** (*E*-olefin). (Fig. 1)

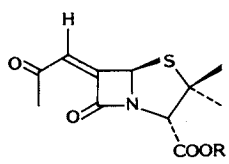
This paper reports further work carried out to investigate the impact on the biological activity of moving the carbonyl group on the side chain away from the bicyclic β-lactam system, but nevertheless still incorporated in a conjugated system as in **4**. To this end, we exploited the high reactivity[6] of the carbonyl function of the 6-acylmethylenepenicillanates. Furthermore, in order to minimize the steric hindrance of the alkyl substituent (*i.e.* methyl), we chose the 6-formylmethylenepenicillanate **3** (Fig. 2) as starting material for further elongation, being nevertheless aware that the alkyl substituent also plays a role during the inactivation process[7]. Compound **3** was then submitted to the Wittig reaction with formylmethylene triphenylphosphorane; a stirred solution of the ester **3** (2.55 g, 7.2 mmol) in 25 ml CH_2Cl_2 was treated under argon at 20°C with formylmethylene triphenylphosphorane (3.35 g, 11 mmol). After 10 minutes the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel (C_6H_{12} - EtOAc, 8 : 2) to give pivaloyloxymethyl (2*S*,5*R*)-6-[(all-*Z*)-3-formylallylidene]-penicillanate (**4**) as a light-colored oil in 55% yield (1.5 g, 3.9 mmol); IR ($CHCl_3$) cm^{-1} 2752, 1769, 1755, 1689, 1609; ^1H NMR (80 MHz, $CDCl_3$) δ 1.23 (s, $C(CH_3)_3$), 1.53 and 1.6 ($2\times$s, $2\times CCH_3$), 4.63 (s, CHCOO), 5.85 and 5.94 ($2\times$d, J_{gem}= 5.5 Hz, O–CH_2–O), 5.99 (s, H_5), 6.5 (dd, J_{trans}= 15 Hz, J=7.5 Hz, H_{10}), 6.90 (d, J_{trans}=11.5 Hz,

Fig. 1. Structures of compounds **1** and **2**.



1

R=$CH_2OCOC(CH_3)_3$



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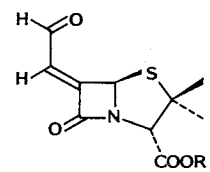
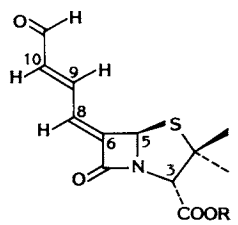


3

R=$CH_2OCOC(CH_3)_3$



4

Table 1. Inhibitory properties against isolated β-lactamases.

Compound	IC$_{50}$ (μM) for the β-lactamase					
	Proteus vulgaris 1028[a]		*Klebsiella pneumoniae* NCTC 418[b]		*E. coli* 1024[c]	
	−E	+E	−E	+E	−E	+E
CP-45899	0.11	—	2.8	—	8.0	
4	1.4	0.0065	48	1.5	>100	11

E: Test carried out in the presence of 10 μl of hog liver esterase following the procedures described in reference 1, after a 15-minute preincubation time of the β-lactamase and inhibitor.

β-Lactamase type:

[a] Inducible, chromosomally determined cephalosporinase (type Ic).

[b] Constitutive penicillinase (type II).

[c] Constitutive chromosomal cephalosporinase (type I).

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

Compound	MIC (μg/ml) inhibitor + ampicillin[a]		
	Staphylococcus aureus 887	*Proteus vulgaris* 1028	*E. coli* 1024
CP-45899	6.25+6.25	1.6+1.6	12.5+12.5
4	25+25	25+25	25+25

[a] Figures presented were selected on the basis of minimal 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).

H_8), 7.15 (dd, $J_{trans}=11.5$ Hz, $J_{trans}=15$ Hz, H_9), 9.76 (d, $J=7.5$ Hz, –CHO). Like **1** and **2**[5], **4** shows no antibacterial activity, but it can be seen from Table 1 that the acid corresponding to **4**, which is released in the presence of esterase, is a potent β-lactamase inhibitor of the enzymes tested. Its activity is comparable to that of CP-45899 (sulbactam)[8], and is even superior against *Proteus vulgaris* 1028 β-lactamase. When tested in combination with ampicillin, **4** is also active *in vitro* against several β-lactamase-producing ampicillin-resistant organisms (Table 2).

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