SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF THIAZOLYL PENAM SULFONES

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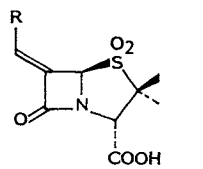
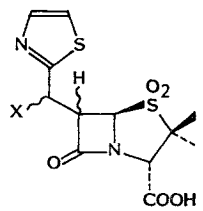
In our previous papers[1,2], we described the structure-activity relationships of 6-(heterocyclyl)-methylene penam sulfones (**1**) as a new class of β-lactamase inhibitors and proposed a mechanism of enzyme inactivation for series **1**. In continuation of these studies with series **1**, we pursued the closely related structures in series **2**, since they should follow a similar mechanistic pathway and should also act as good suicide inhibitors. Here, we report the syntheses of **2** and their synergy and β-lactamase inhibition data.

Preparation of the 6α-isomers in series **2** is illustrated in Scheme 1. Starting material, 6α-bromopenicillanate **3**, is readily prepared by esterification of the corresponding acid[3]. Transmetallation of 6α-bromopenicillanate **3** by exchange with 1 eq of methylmagnesium bromide in THF at −78°C, followed by reaction with thiazole 2-carboxyaldehyde afforded a 55%-yield of a separable 2:1 mixture of diastereomers (6α,8*R*) **4** and (6α,8*S*) **5**, respectively[2]. Compounds **4** and **5** were acylated using several reagents, *e.g.* acetic anhydride, benzoyl chloride, ethyl chloroformate, or methyl isocyanate, in the presence of pyridine in methylene chloride at room temperature to afford **6** and **7**, respectively. The (6α,8*R*) fluoro derivative **6g** was prepared in 55% yield by displacement reaction[4] of the (6α,8*S*) hydroxy compound **5** with diethylaminosulfur trifluoride in methylene chloride. Removal of the protecting group was accomplished as indicated in Scheme 1 to give the final compounds **2**.

The corresponding 6β-isomer was prepared *via* an aldol condensation as outlined in Scheme 2. 6,6-Dibromopenicillanate **8**, prepared by esterification of the corresponding acid[3], reacted with 1 eq of methylmagnesium bromide, followed by reaction with thiazole 2-carboxyaldehyde, gave a mixture of hydroxy adducts. Acetylation of this mixture, followed by stereoselective reduction with tin hydride[5], afforded a 95:5 mixture of diastereomers (6β,8*R*) **9** and (6β,8*S*) **10**. The major isomer **9** was isolated in 50% yield, starting from **8**, as crystals, mp 185°C (dec), by recrystallization from ether. Hydrogenation of **9** gave a 78%-yield of **2c**. The (6β,8*R*) hydroxy compound **2i** was obtained using a similar procedure as described in Scheme 2.



1



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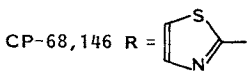
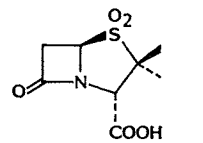
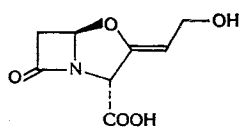


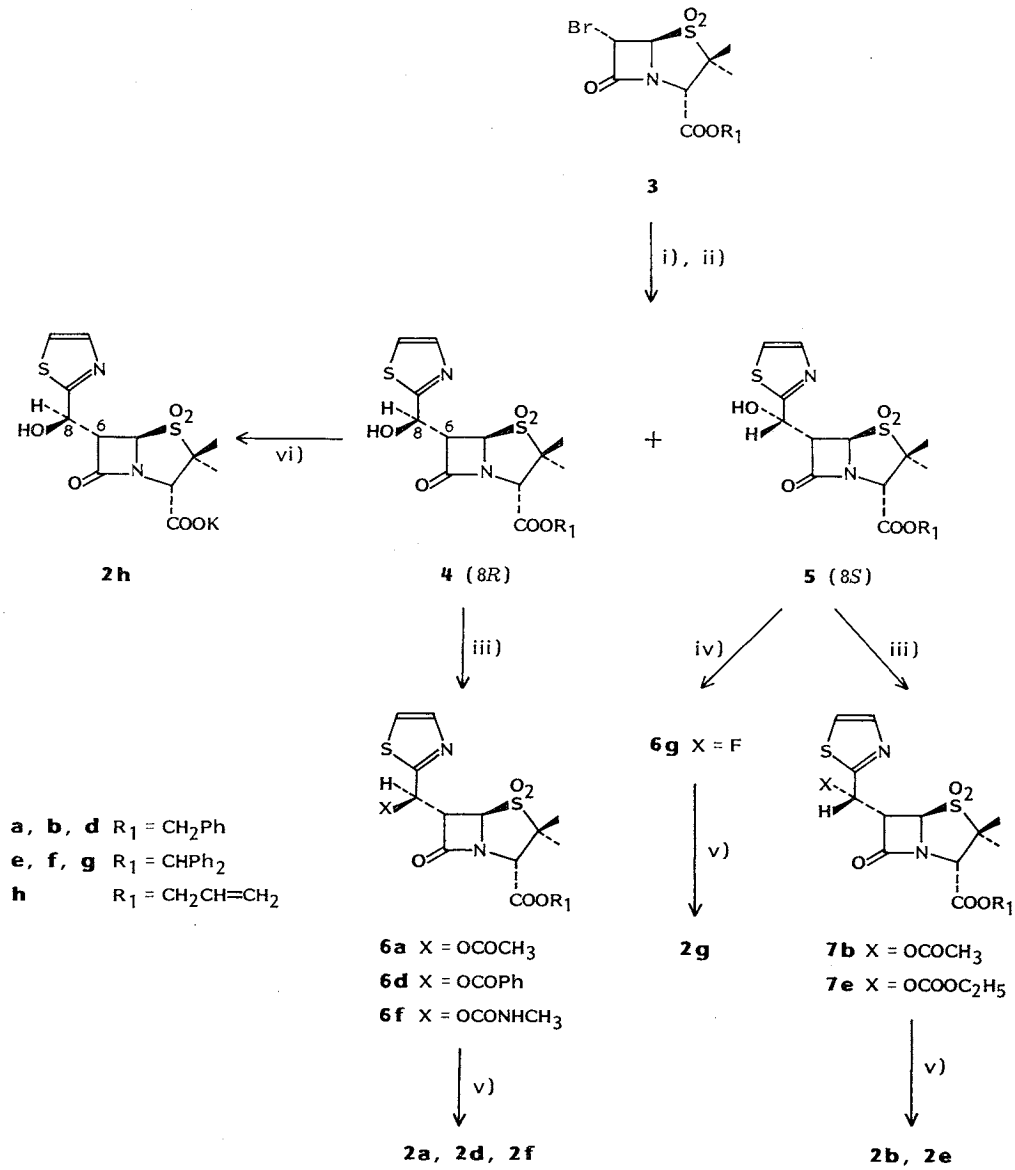


Sulbactam



Clavulanic acid

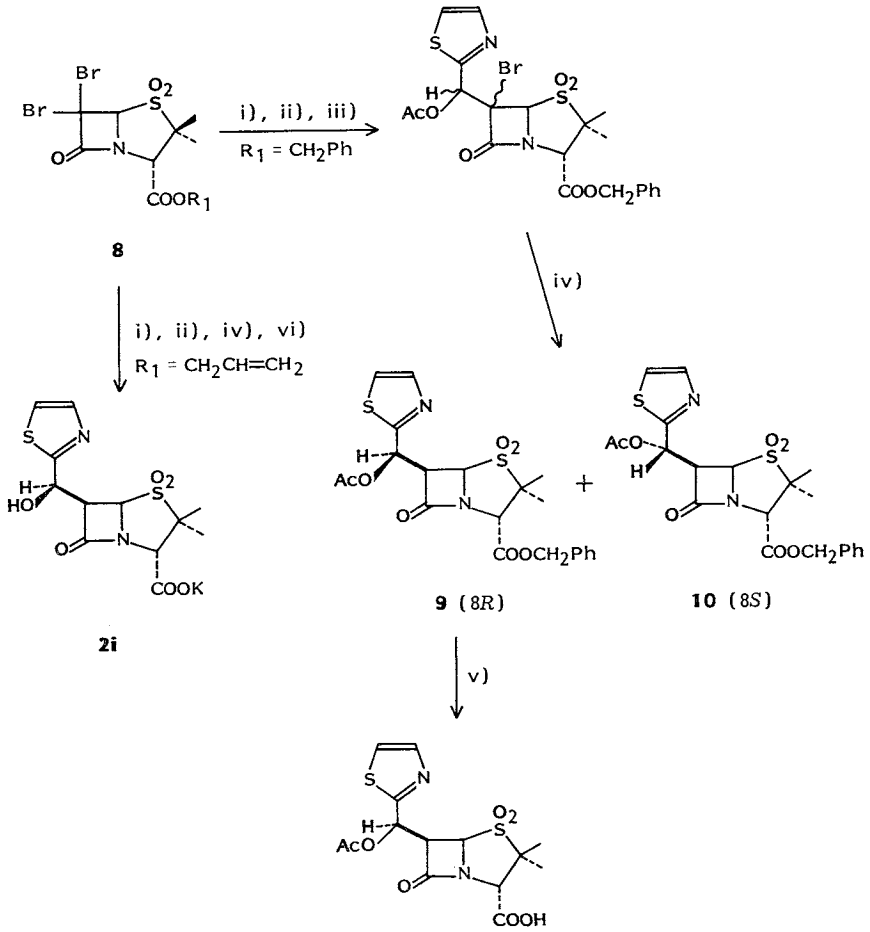
Scheme 1.



i) CH_3MgBr - THF, ii) thiazole 2-carboxyaldehyde, iii) for **a** and **b**, Ac_2O - pyridine; for **d**, PhCOCl - pyridine; for **e**, EtOCOCl - pyridine; for **f**, CH_3NCO - pyridine, iv) Et_2NSF_3, v) for **a**, **b** and **d**, H_2 - 5% Pd/C; for **e**, **f** and **g**, anisole, $AlCl_3$ - CH_3NO_2[6], vi) $Pd(PPh_3)_4$, PPh_3, potassium 2-ethylhexanoate[7].

The products **2a**~**2i** are inhibitors of various β-lactamases. The % inhibition data is presented in Table 1. Compounds **2a**, **2b** and **2c** were selected for IC_{50} studies and the results are shown in Table 2. The 8-acyloxy derivatives (**2a**~**2f**) and the fluoro analog **2g** showed good inhibitory activity. Among these, **2g** possesses the most potent β-lactamase inhibitory activity. In fact, its activity is equal to that of CP-68,146. Considering the possibility that elimination of **2** to CP-68,146 may occur prior to interaction with the enzyme, compounds **2a** and **2c** were chosen for stability studies in buffer pH 7.0 at 37°C. Compound **2a** was found to be stable ($T_{1/2}$>

Scheme 2.



i) CH_3MgBr - THF, ii) thiazole 2-carboxyaldehyde, iii) Ac_2O - pyridine, iv) *n*-Bu_3SnH, v) H_2 - 5% Pd/C, vi) $Pd(PPh_3)_4$, PPh_3, potassium 2-ethylhexanoate.

23 hours) under these conditions, suggesting that it does not eliminate to CP-68,146 prior to enzyme interaction. However, compound **2c** is gradually converted to CP-68,146 in buffer pH 7.0 at 37°C with a half-life of 1.5 hours (by HPLC); thus it is possible that some of the activity may actually come from the elimination product, CP-68,146. We propose that the elimination reaction for those stable compounds in series **2** (*e.g.* **2a**) occurs during the β-lactamase inactivation process, thus enzyme inactivation of **2** may provide the same acyl-enzyme inhibited form as does CP-68,146. On the other hand, the 8-hydroxy analogs (**2h** and **2i**), exhibit weaker penicillinase inhibition, perhaps because the 8-hydroxy group of these analogs is a poorer leaving group. This leads to a slower inactivation rate to form a stable acyl-enzyme intermediate according to our proposed mechanism[1].

The MIC data of a 1 : 1 combination of ampicillin plus inhibitor against several representative β-lactamase producing bacteria are listed in Table 3 using CP-68,146, sulbactam, and clavulanic acid as controls. All the derivatives **2a**～**2g** demonstrate synergistic activity comparable to the controls. The 8-hydroxy analogs (**2h** and **2i**), however, only produce a moderate synergistic effect against penicillinase producing organisms (01A400, 51A129 and 53A079), but demonstrated synergy comparable to CP-68,146

Table 1. β-Lactamase inhibitory activity.

Compound	Stereoisomer	X	Enzyme inhibition (%)[a]				
			S.a. 01A400	*E.c.* 51A129	TEM-1 51A560	*E.cl.* 67B009	*P.a.* 52A104
2a	(6α,8*R*)	$OCOCH_3$	76	98		2	
2b	(6α,8*S*)	$OCOCH_3$	59	71		9	
2c	(6β,8*R*)	$OCOCH_3$	82	98	87	5	64
2d	(6α,8*R*)	OCOPh	71	47	46	8	
2e	(6α,8*S*)	OCOOEt	52	40		12	
2f	(6α,8*R*)	$OCONHCH_3$	30	26		8	
2g	(6α,8*R*)	F	90	100		68	
2h	(6α,8*R*)	OH	0	11		33	
2i	(6β,8*R*)	OH	0	5		10	
CP-68,146[b]			97	96	99	28	99
Sulbactam[b]			46	40	42	9	4
Clavulanic acid[b]			85	81			

[a] The *in vitro* % inhibitory data was determined by the microiodometric assay as described in ref 2. The % inhibition at the enzyme level was obtained at the following indicated concentrations (μM) for inhibitor [I] and substrate [S]: *Staphylococcus aureus* (*S.a.*) 01A400, [I]/[ampicillin]=8/32; *Escherichia coli* (*E.c.*) 51A129, [I]/[ampicillin]=1/32; Plasmid TEM-1 51A560, [I]/[ampicillin]=1/32; *Enterobacter cloacae* (*E.cl.*) 67B009, [I]/[benzylpenicillin]=8/32; *Pseudomonas aeruginosa* (*P.a.*) 52A104, [I]/[benzylpenicillin]=8/32.

[b] See ref 2 for the source of the material.

Table 2. Determination of IC_{50} of β-lactamase inhibitors.

Inhibitor	IC_{50} (μM) TEM-1 β-lactamase[a]	
	No preincubation[b]	15 minutes preincubation[c]
CP-68,146	0.09	0.0027
Sulbactam	3.0	0.31
Clavulanic acid	9.8	0.19
2a	410	0.47
2b	7.6	0.13
2c	2.9	0.017

[a] IC_{50} determinations were done as described in ref 2.

[b] β-Lactamase inhibitor added to substrate and enzyme at 0 time.

[c] β-Lactamase inhibitor preincubated 15 minutes with enzyme prior to addition of substrate.

Table 3. MIC data[a] of a 1 : 1 combination of an inhibitor with ampicillin.

Inhibitor	MIC (μg/ml)[b] of inhibitor+ampicillin (1 : 1)							
	S.a. 01A400	*E.c.* 51A129	*H.i.* 042	*K.p.* 53A079	*S.m.* 63A095	*E.cl.* 67B009	*M.m.* 97A001	*P.a.* 52A104
2a	0.39	100	<0.2	6.25	50	100	12.5	>100
2b	0.39	>100	<0.2	6.25	50	>100	25	>100
2c	0.39	50	<0.2	6.25	25	>100	6.25	>100
2d	1.56	100	—	12.5	25	>100	12.5	>100
2e	0.78	100	—	6.25	100	100	6.25	>100
2f	1.56	100	—	12.5	50	>100	25	>100
2g	0.78	50	—	6.25	50	100	25	>100
2h	6.25	>100	—	25	12.5	50	3.12	>100
2i	6.25	>100	—	50	12.5	100	6.25	>100
CP-68,146	0.39	50	0.39	3.12	25	100	3.12	>100
Sulbactam	0.78	100	0.78	6.25	12.5	50	6.25	100
Clavulanic acid	0.39	12.5	0.39	12.5	50	100	>100	>100
Ampicillin alone	50	>200	>200	100	>200	200	>200	>200

[a] MIC determinations were done as described in ref 2.

[b] Concentration of both components; *i.e.* MIC of 0.39 μg/ml is 0.39 μg/ml inhibitor plus 0.39 μg/ml ampicillin; test organisms: *S.a.*, *Staphylococcus aureus*; *E.c.*, *Escherichia coli*; *H.i.*, *Haemophilus influenzae*; *K.p.*, *Klebsiella pneumoniae*; *S.m.*, *Serratia marcescens*; *E.cl.*, *Enterobacter cloacae*; *M.m.*, *Morganella morganii*; *P.a.*, *Pseudomonas aeruginosa*.

—: Not tested.

and sulbactam against inducible cephalosporinase producing bacteria (63A095, 97A001 and 67B009). All the compounds in series **2** are devoid of antibacterial activity.

In conclusion, introduction of a functionality chemically equivalent to the 6-(2-thiazolyl)-methylene group of CP-68,146 affords compounds which retain interesting β-lactamase inhibiting activity.

Acknowledgments

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