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SYNTHESIS AND β-LACTAMASE INHIBITORY EVALUATION OF NOVEL 6α-HALO-2β-CHLOROMETHYL-2α-METHYLPENAM-3α-CARBOXYLIC ACIDS AND THEIR SULFONES AND 6α-HALO-2β-MERCAPTOBENZOTHIAZOLYLMETHYL-2α-METHYLPENAM-3α-CARBOXYLIC ACIDS

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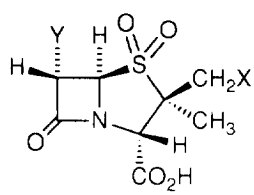
Abstract: The synthesis and the inhibitory activity against β-lactamase I from *Bacillus cereus* is described for a new series of 6α-bromo, chloro or fluoro-2β-chloromethyl-2α-methylpenam-3α-carboxylic acids (**5a-c**) and their corresponding sulfones **6a-c** and 6α-bromo, chloro or fluoro-2β-mercaptobenzothiazolylmethyl-2α-methylpenam-3α-carboxylic acids (**8a-c**). Among the novel compounds, the highest activity was expressed by 6α-chloro-2β-chloromethyl-2α-methylpenam-3α-carboxylic acid 1,1-dioxide (**6b**).

Introduction

Considerable interest has been directed towards the functionalization of the 2β-methyl group of penicillanic acid. The thermal ring opening of penicillin (1S)-sulfoxides and intermolecular trapping of the sulfenic acid intermediates with heteroaromatic thiols, led to the formation of asymmetric azetidinone disulfides. Subsequent recyclization afforded 2β-methyl substituted penicillins through the episulfonium ions.[1-4] Penam derivatives such as **1a** and **1b** are useful synthetic intermediates in the preparation of β-lactamase inhibitors. In particular, this functionalization of penicillin was applied in the synthesis of 2β-(1,2,3-triazol-1-yl)methyl-2α-methylpenam-3α-carboxylic acid 1,1-dioxide (Tazobactam) (**1c**) a broad range β-lactamase inhibitor, which has found clinical application in combination with the potent, but β-lactamase sensitive, antibiotic piperacillin.[1]



BL-P 2013	**1a**	Y = H, X = Cl
	1b	Y = H, X = N_3
Tazobactam	**1c**	Y = H, X = 1,2,3-triazole
	1d	Y = Cl, X = H
Sulbactam	**1e**	Y = H, X = H

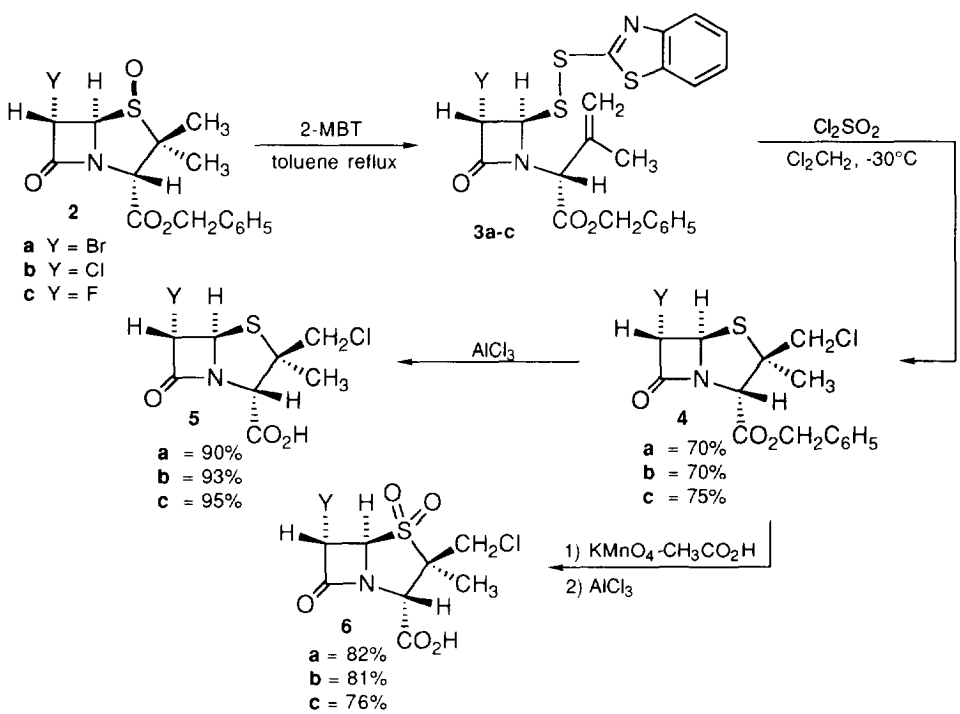
In an earlier account we described the synthesis and β-lactamase inhibitory activity of 6-fluoropenicillanic acids.[5] Herein we report further work on 6α-halo-2β-chloromethyl-2α-methylpenam-3α-

carboxylic acid derivatives and their corresponding sulfones and 6α-halo-2β-mercaptobenzothiazolylmethyl-2α-methylpenam-3α-carboxylic acid derivatives, in a further attempt to obtain β-lactamase inhibitors with greater activity.

Synthetic chemistry[6]

The synthesis of 6α-bromo-, chloro- and fluoro-2β-chloromethyl-2α-methyl-penam-3α-carboxylic acids (**5a-c**) and their sulfones (**6a-c**) is shown in Scheme 1. The starting materials were benzyl 6α-bromo-, chloro- and fluoro-penicillanate (1S)-sulfoxides (**2a-c**). These are easily and quantitatively prepared by asymmetric oxidation of the corresponding 6α-halopenicillante sulfides by 2,2-dimethyldioxirane.[7] Conversion of these penicillin (1S)-sulfoxides (**2a-c**) into their corresponding unsymmetrical azetidinone disulfides **3a-c** was achieved using the Kamiya methodology[8] with 2-mercaptobenzothiazole (2-MBT) in refluxing toluene. Treatment of the azetidinone disulfides **3a-c** with sulfuryl chloride[9] in methylene chloride at -30 °C for 20 minutes afforded the benzyl 6α-halo-2β-chloromethyl-2α-methylpenam-3α-carboxylates **4a-c** in high yields (70 to 75%). Cleavage of benzyl ester group with aluminiun trichloride[10] gave the free acid analogs **5a-c**. Oxidation of **4a-c** with potassium permanganate in acetic acid afforded the corresponding benzyl penicillanate sulfones and cleavage of the benzyl ester group with aluminiun trichloride gave the free acid analogs **6a-c** in overall yields greater than 75%.

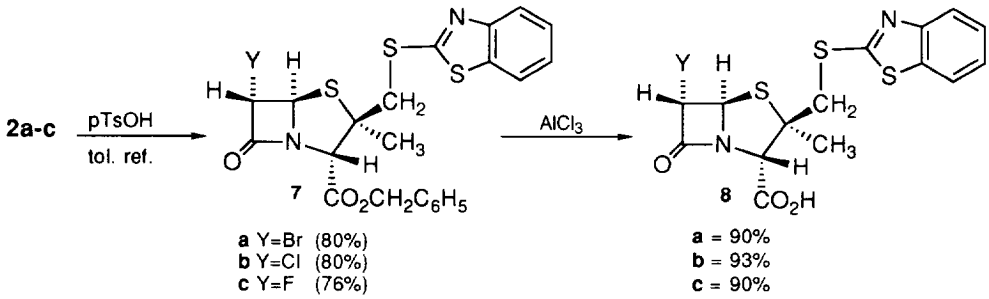
Scheme 1



When the benzyl 6α-halopenicillanate (1S)-sulfoxides **2a-c** were treated with 2-MBT in toluene at 95-100 °C in the presence of a catalytic amount of *p*-toluenesulfonic acid,[4,11] the benzyl 6α-halo-2β-

mercaptobenzothiazolylmethyl-2α-methylpenam-3α-carboxylates **7a-c** were obtained in 76 to 80% yield after silica gel column chromatography. These esters were subsequently converted to their carboxylic acids with aluminium trichloride in high yields (Scheme 2). Attempts to oxidize compounds **8a-c** gave a complex mixture of products in agreement with the finding of Singh *et al.*[12] with analogous compounds since both thioether funtionalities were susceptible to oxidation to sulfoxides and sulfones.

Scheme 2



Inhibitory Activity against β-lactamase I

The 6α-halo-2β-chloromethyl-2α-methylpenam-3α-carboxylic acids and their corresponding sulfones and 6α-halo-2β-mercaptobenzothiazolyl-2α-methylpenam carboxylic acids were evaluated for their ability to inhibit β-lactamase I of *Bacillus cereus*, a class A enzyme.[13] The inhibitory activity for these derivatives is listed in the Table below and is expressed in terms of their IC_{50} values. The activity for the previously known β-lactamase inhibitors BL-P 2013 (**1a**), 6α-chloropenicillanic acid sulfone (**1d**) and penicillanic acid sulfone (**1e**) are given for comparison.

Table.

Compound	IC_{50} (mM)[a] Without Preincubation	With 10 min. Preincubation
1a	0.25 ± 0.01	0.3 ± 0.07
1d	1.5 ± 0.2	0.2 ± 0.04
1e	0.025 ± 0.001	0.025 ± 0.002
5a	>5	1 ± 0.2
5b	>5	>5
5c	>5	N.D.[b]
6a	3 ± 0.8	1 ± 0.3
6b	0.45 ± 0.12	0.07 ± 0.01
6c	>3	>3
8a	>5	>5
8b	>5	>5
8c	>5	>5

[a]For methodology, see Enzymatic methods in Ref. 5. The IC_{50} and standard error values were estimated by non-linear least squares regression fitting the inhibition values obtained at different [I] to the equation: **Inhibition = Maximal inhibition [I] / (IC_{50} + [I])**. [b]Not Determined.

Among the novel compounds synthesized, the highest activity was expressed by compound **6b**.When this compound was incubated for 10 minutes with the enzyme the IC_{50} value obtained was six to seven times smaller than that obtained without preincubation. This result suggests that **6b** would behave as a time-dependent inhibitor. The IC_{50} value obtained with preincubation also indicates that **6b** is three to four-fold more active than BL-P 2013 (**1a**) and 6α-chloropenicillanic acid sulfone (**1d**). Without preincubation, **6b** exhibited activity comparable to that of **1a** and was more active than **1d**. When **1a** and **1d** are compared with penicillanic acid sulfone (**1e**), it can be concluded that substitution by a chlorine atom of either the hydrogen in C-6α, as in **1d**, or the hydrogen in C-2β-methyl group, as in **1a**, produces a decrease in the inhibitory activity. Substitution of both hydrogen atoms, as in **6b**, results in a smaller decrease in inhibitory activity. Therefore the combination of both detrimental substitutions do not produce a further decrease in activity but, conversely, it yields a compound, **6b**, more active than **1a** and **1d**.

The inhibition of β-lactamase I in this series of 6α-halo-2β-chloromethylpenam sulfones (**6a-c**) were shown to depend on the halogen atom, where the 6α-chloro derivative **6b** was the most active. The sulfones **6a-c** were more potent than their corresponding sulfides **5a-c**. The IC_{50} values in the 6α-halo-2β-mercaptobenzothiazolylmethyl-2α-methyl penam series indicate that these compounds are rather poor inhibitors of β-lactamase I.

Conclusion

Replacement of the 6α-hydrogen atom for a chlorine atom in **1a** or introduction of a chlorine atom in the 2β-methyl group in 6α-chloropenicillanic acid sulfone (**1d**) led to the identification of the novel and potent β-lactamase I inhibitor, 6α-chloro-2β-chloromethyl-2α-methylpenam-3α-carboxylic acid 1,1-dioxide (**6b**).

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