# SYNTHESIS AND β-LACTAMASE INHIBITORY PROPERTIES OF 2β-(THIO-SUBSTITUTED METHYL)-PENAM 1,1-DIOXIDES

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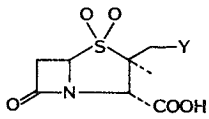
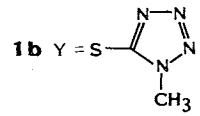
Since the discovery of the β-lactamase inhibitory properties of clavulanic acid[1], a variety of inhibitors have appeared in the literature. Among them, penicillanic acid dioxide **1** (Y=H, sulbactam[2]) and its homologues, *e.g.*, BL-P2013 (**1**, Y=Cl)[3] and YTR-830 (**1**, Y=triazolyl)[4], have attracted considerable interest as promising β-lactamase inhibitors. In order to suppress the activity of enzyme in a living bacteria cell, the inhibitor is required to penetrate to the target enzyme. Our current interest in developing a new β-lactamase inhibitor with potent permeability into the cell, enables us to investigate a modification of the C(2)-side chain of penicillanic acid dioxides **1a**~**1c**. Herein, we report novel penicillanic acid dioxides bearing thio-substituents at the C(2)-side chain which show very potent β-lactamase inhibitory properties and efficiently synergize with piperacillin against resistant strains.

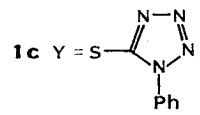
Scheme 1.



**1a** Y = SCN



**1b** Y = S



**1c** Y = S

## Chemistry

The thio-substituents at the C(2)-side chain of **1a**~**1c** were introduced by treatment of 2β-(chloromethyl)penam **3**[5], derived from 6-aminopenicillanic acid **2**, with appropriate thiolates. Reaction of **3** with potassium thiocyanate in aq $Me_2CO$ at room temp gave the 2β-(thiocyanatomethyl)penam **5a** as a sole product. On the other hand, upon treatment of **3** with 1-methyl and 1-phenyl-5-mercaptotetrazoles in aq $Me_2CO$ in the presence of potassium bicarbonate, a 2 to 1 mixture of the corresponding 2β-(thio-substituted methyl)penams **5b**, **5c** and the disulfides **7b**, **7c** were obtained. Notably, no detectable amount of C(3)-substituted cepham **8** was isolated; this fact is in sharp contrast to the substitution reaction of chlorine atom of **3** with sodium azide affording a mixture of **5** and **8** (6:4, Y=$N_3$)[5]. The oxidation of the penicillanates **5** to the dioxides **6** was performed successfully by treatment with 1.2 equiv potassium permanganate in AcOH at room temp. Finally, deprotection of the *p*-nitrobenzyl esters by hydrogenation with palladium on carbon afforded the 2β-(thio-substituted methyl)-penam-3α-carboxylic acid dioxides **1**.

## Results and Discussion

The β-lactamase inhibitory properties ($IC_{50}$) of the thio-substituted penicillanic acid dioxides **1a**~**1c** were determined by a microiodometric assay using the penicillinase from *Bacillus* sp. and benzylpenicillin as a substrate[6]. The results are summarized in Table 1. The compounds **1b** and **1c** exhibit almost the same $IC_{50}$ values as those of YTR-830[4], while the compound **1a** has a lower value.

A synergistic effect against both penicillinase and cephalosporinase producing organism was evaluated by the agar dilution method using 10 μg/ml of inhibitors in combination with piperacillin. The result of MIC of piperacillin

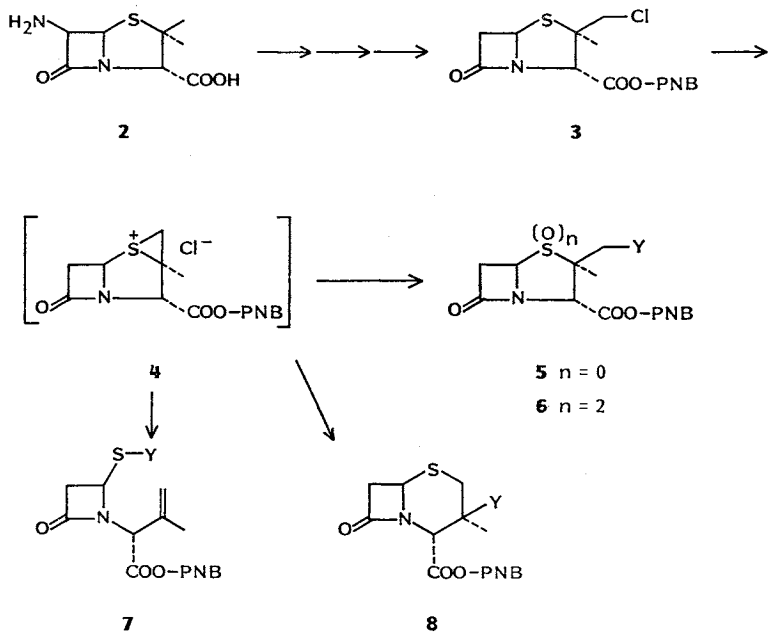
Table 1. Inhibition of β-lactamase by inhibitors.

| | YTR-830 | **1a** | **1b** | **1c** |
|---|---|---|---|---|
| $IC_{50}$ (μM) | 0.73 | 9.8 | 1.0 | 0.87 |

Table 2. *In vitro* synergy with piperacillin (PIPC) against β-lactamase-producing isolates.

| Test organisms | β-Lactamases | MIC (μg/ml) | | | | |
|---|---|---|---|---|---|---|
| | | PIPC | +YTR-830 | +1a | +1b | +1c |
| *Escherichia coli* | SHV 1 | 6.25 | 0.39 | 0.39 | 0.39 | 1.56 |
| *E. coli* | TEM 1 | 25 | 0.39 | 0.78 | 0.39 | 3.13 |
| *E. coli* | TEM 2 | 200 | 0.39 | 50 | 25 | 50 |
| *E. coli* | OXA 1 | 12.5 | 0.78 | 0.78 | 1.56 | 6.25 |
| *Klebsiella pneumoniae* 366 L | SHV 1 | 50 | 1.56 | 3.13 | 1.56 | 25 |
| *K. pneumoniae* 101 L | TEM 1 | 100 | 0.39 | 0.78 | 0.78 | 6.25 |
| *Morganella morganii* 119 | | 12.5 | 0.10 | 0.10 | 0.39 | 6.25 |
| *Proteus mirabilis* 60 | | 1.56 | 0.10 | 0.20 | 0.10 | 0.20 |
| *P. vulgaris* 20 | | 100 | 0.20 | 0.20 | 0.78 | 100 |
| *Serratia marcescens* 200 L | TEM+C | 25 | 0.39 | 3.13 | 0.39 | 50 |
| *Citrobacter* 2046 E | C | 6.25 | 0.39 | 0.39 | 0.39 | 0.39 |
| *Citrobacter* 962 L | TEM 2 | 50 | 1.56 | 1.56 | 1.56 | 25 |
| *Enterobacter cloacae* P 99 | C | 50 | 6.25 | 6.25 | 12.5 | 50 |
| *Acinetobacter* 450 L | TEM+C | 200 | 0.10 | 12.5 | 6.25 | 50 |
| *Pseudomonas aeruginosa* PSE 4 | | 100 | 3.13 | 6.25 | 25 | 50 |
| *Staphylococcus aureus* 54 K | | 3.13 | 0.39 | 0.39 | 0.39 | 0.39 |

Scheme 2.




PNB=*p*-Nitrobenzyl

alone or combined with the inhibitors was summarized in Table 2. When the compound **1a** or **1b** was combined with piperacillin, 4- to 128-folds decrease was observed in the MICs against the strains containing different plasmid-mediated β-lactamases and chromosome-encoded. The compounds **1a** and **1b** exhibit almost similar inhibitory activity as that of YTR-830[7] except for the cases against the strains of *Escherichia coli* TEM 2 and *Acinetobacter*. However, compound **1c**, which shares a similar chemical structure to compound **1b** except for its phenyl group of tetrazolyl-function, had lower activity than compound **1a** or **1b**. Although the reasons for this substituent effect are still unclear, the substitution of phenyl group on the tetrazole moiety

would affect the penetration rate to β-lactamases, lowering the rate of **1c** less than those of **1a** and **1b**.

## Experimental

MP was uncorrected. IR spectra were recorded on a Jasco IRA-1 grating spectrophotometer. $^1$H NMR spectra were taken on a Hitachi R-24 (60 MHz) and a Jeol MX-100 spectrometers (100 MHz) using TMS or sodium 3-(trimethylsilyl)propionate-$d_4$ as an internal standard. MS was obtained on a Jeol JMS-DX303 spectrometer. Microanalyses were performed in our laboratory.

*p*-Nitrobenzyl 2α-Methyl-2β-(thiocyanatomethyl)penam-3α-carboxylate (**5a**)

A solution of **3** (148 mg, 0.4 mmol) and KSCN (58 mg, 0.6 mmol) in $Me_2CO$ (3 ml) and water (1 ml) was stirred at room temp for 12 hours. The reaction mixture was diluted with EtOAc (15 ml), washed with water and brine, dried over $MgSO_4$. After evaporation of the solvents, the residue was chromatographed on $SiO_2$ with benzene - EtOAc (9 : 1) to give **5a** (150 mg, 95%): IR ($CHCl_3$) cm$^{-1}$ 2150, 1780, 1755; $^1$H NMR ($CDCl_3$) δ 1.56 (3H, s, $CH_3$), 3.21 (1H, dd, $J$=2 and 16 Hz, 6-H), 3.38 (2H, s, $CH_2SCN$), 3.67 (1H, dd, $J$=4 and 16 Hz, 6-H), 4.80 (1H, s, 3-H), 5.27 (2H, s, $OCH_2$), 5.25~5.45 (1H, m, 5-H), 7.48 (2H, d, $J$=8 Hz, aromatic H), 8.15 (2H, d, $J$=8 Hz, aromatic H).

*p*-Nitrobenzyl 2α-Methyl-2β-[(1-methyl-5-tetrazolyl)thiomethyl]penam-3α-carboxylate (**5b**)

A solution of **3** (148 mg, 0.4 mmol), 5-mercapto-1-methyltetrazole (52 mg, 0.45 mmol), and $KHCO_3$ (40 mg, 0.4 mmol) in $Me_2CO$ (6 ml) and water (2 ml) was stirred at room temp for 12 hours. The reaction mixture was diluted with EtOAc (15 ml), washed with water and brine, dried over $MgSO_4$. After evaporation of the solvents, the residue was subjected to column chromatography on $SiO_2$ with benzene - EtOAc (3 : 1) as an eluant. Initially eluted was **5b** (118 mg, 65%): MP 124~126°C; IR ($CHCl_3$) cm$^{-1}$ 1775, 1755; $^1$H NMR ($CDCl_3$) δ 1.52 (3H, s, $CH_3$), 3.11 (1H, dd, $J$=2 and 16 Hz, 6-H), 3.60~4.20 (2H, m, $CH_2S$), 3.61 (1H, dd, $J$=4 and 16 Hz, 6-H), 3.91 (3H, s, $NCH_3$), 4.81 (1H, s, 3-H), 5.26 (2H, s, $OCH_2$), 5.20~5.45 (1H, m, 5-H), 7.51 (2H, d, $J$=8 Hz, aromatic H), 8.17 (2H, d, $J$=8 Hz, aromatic H); field desorption mass spectra (FD-MS) $m/z$ 451 (M+H).

Secondarily eluted was **7b** (60 mg, 34%): IR ($CHCl_3$) cm$^{-1}$ 1770, 1745; $^1$H NMR ($CDCl_3$) δ 1.86 (3H, s, $CH_3$), 3.36~3.55 (2H, m, 3-H), 4.01 (3H, s, $NCH_3$), 4.78, 4.96 (2H, two s, C=$CH_2$), 5.09 (1H, br s, CHCOO), 5.23 (2H, s, $OCH_2$), 5.16~5.40 (1H, m, 4-H), 7.46 (2H, d, $J$=8 Hz, aromatic H), 8.15 (2H, d, $J$=8 Hz, aromatic H).

*p*-Nitrobenzyl 2α-Methyl-2β-[(1-phenyl-5-tetrazolyl)thiomethyl]penam-3α-carboxylate (**5c**)

A solution of **3** (148 mg, 0.4 mmol), 5-mercapto-1-phenyltetrazole (79 mg, 0.44 mmol), and $KHCO_3$ (40 mg, 0.4 mmol) in $Me_2CO$ (6 ml) and water (2 ml) was stirred at room temp for 12 hours. In a similar manner as described above, workup of the reaction mixture gave **5c** (133 mg, 65%) and **7c** (62 mg, 32%).

**5c**: IR ($CHCl_3$) cm$^{-1}$ 1775, 1750; $^1$H NMR ($CDCl_3$) δ 1.52 (3H, s, $CH_3$), 3.08 (1H, dd, $J$=2 and 16 Hz, 6-H), 3.54 (1H, dd, $J$=4 and 16 Hz, 6-H), 3.92, 3.97 (2H, ABq, $J$=12 Hz, $CH_2S$), 4.84 (1H, s, 3-H), 5.23 (2H, s, $OCH_2$), 5.15~5.40 (1H, m, 5-H), 7.30~7.65 (7H, m, aromatic H), 8.15 (2H, d, $J$=8 Hz, aromatic H).

**7c**: IR ($CHCl_3$) cm$^{-1}$ 1770, 1745; $^1$H NMR ($CDCl_3$) δ 1.85 (3H, s, $CH_3$), 3.30~3.55 (2H, m, 3-H), 4.83, 4.98 (2H, two s, C=$CH_2$), 5.11 (1H, br s, CHCOO), 5.18 (2H, s, $OCH_2$), 5.15~5.35 (1H, m, 4-H), 7.44 (2H, d, $J$=8 Hz, aromatic H), 7.50 (5H, s, aromatic H), 8.10 (2H, d, $J$=8 Hz, aromatic H).

Oxidation of *p*-Nitrobenzyl 2β-(Thio-substituted methyl)penam-3α-carboxylates (**5**)

To a solution of **5a** (145 mg, 0.37 mmol) in glacial AcOH (5.5 ml) and water (0.9 ml), was added portionwise potassium permanganate (70 mg, 0.44 mmol) during 30 minutes. The mixture was stirred at room temp for 4 hours. The excess of permanganate was decomposed by the dropwise addition of hydrogen peroxide and the mixture was poured into ice-cold water. The mixture was extracted with $CHCl_3$, washed with water, aq $NaHCO_3$, and brine, and dried over $MgSO_4$. Evaporation of the solvents followed by recrystallization afforded **6a** (154 mg, 98%): MP 149~150°C from benzene; IR (Nujol) cm$^{-1}$ 2150, 1790, 1755; $^1$H NMR ($CDCl_3$) δ 1.58 (3H, s, $CH_3$), 3.30~3.70 (2H, m, 6-H), 3.63 (2H, s, $CH_2SCN$), 4.60~4.80 (1H,

m, 5-H), 4.72 (1H, s, 3-H), 5.33 (2H, s, $OCH_2$), 7.53 (2H, d, $J$=8 Hz, aromatic H), 8.18 (2H, d, $J$=8 Hz, aromatic H).

*Anal* Calcd for $C_{16}H_{15}N_3O_7S_2$:
C 45.17, H 3.55, N 9.87.
Found: C 45.37, H 3.70, N 9.56.

Dioxides **6b** and **6c** were also obtained from **5b** and **5c** by the method described for **6a**.

**6b**: 93% yield; IR ($CHCl_3$) $cm^{-1}$ 1805, 1760; $^1H$ NMR ($CDCl_3$) $\delta$ 1.48 (3H, s, $CH_3$), 3.30~3.70 (2H, m, 6-H), 3.94 (3H, s, $NCH_3$), 4.08, 4.22 (2H, ABq, $J$=10 Hz, $CH_2S$), 4.55~4.90 (1H, m, 5-H), 4.75 (1H, s, 3-H), 5.33 (2H, s, $OCH_2$), 7.63 (2H, d, $J$=8 Hz, aromatic H), 8.20 (2H, d, $J$=8 Hz, aromatic H); FD-MS $m/z$ 483 (M+H).

**6c**: 92% yield; mp 151~153°C; IR (KBr) $cm^{-1}$ 1805, 1765; $^1H$ NMR ($CDCl_3$) $\delta$ 1.52 (3H, s, $CH_3$), 3.35~3.60 (2H, m, 6-H), 4.01, 4.31 (2H, ABq, $J$=15 Hz, $CH_2S$), 4.61 (1H, m, 5-H), 4.74 (1H, s, 3-H), 5.30 (2H, s, $OCH_2$), 7.48 (5H, s, aromatic H), 7.61 (2H, d, $J$=8 Hz, aromatic H), 8.18 (2H, d, $J$=8 Hz, aromatic H); FD-MS $m/z$ 545 (M+H).

Sodium 2$\alpha$-Methyl-2$\beta$-(thiocyanatomethyl)-penam-3$\alpha$-carboxylate 1,1-Dioxide (**1a**)

To a solution of **6a** (300 mg, 0.71 mmol) in EtOAc (5.3 ml) was added a suspension of 10% palladium on carbon (60 mg) and $NaHCO_3$ (60 mg, 0.71 mmol) in water (3.5 ml). The mixture was hydrogenated at 1~5 kg/cm². After the hydrogen uptake ceased, the catalysts were removed by filtration, and the aqueous solution was subjected to column chromatography on MCI gel CHP-20P (Mitsubisi Chemical Industries Limited) with $Me_2CO$ - water as gradient eluent. The fractions involving the sodium salt **1a** were concentrated at 30°C under reduced pressure and freeze-dried to give **1a** (13 mg, 6%): MP 140~150°C (dec); IR (KBr) $cm^{-1}$ 2152, 1785, 1628; $^1H$ NMR ($D_2O$) $\delta$ 1.69 (3H, s, $CH_3$), 3.30~3.90 (2H, m, 6-H), 3.92 (2H, s, $CH_2SCN$), 4.41 (1H, s, 3-H), 5.05~5.11 (1H, m, 5-H).

**1b** and **1c** were also obtained from **6b** and **6c** by the method described for **1a**.

**1b**: 41% yield; mp 157~158°C (dec); IR (KBr) $cm^{-1}$ 1785, 1628; $^1H$ NMR ($D_2O$) $\delta$ 1.62 (3H, s, $CH_3$), 3.39 (1H, dd, $J$=2 and 17 Hz, 6-H), 3.67 (1H, dd, $J$=4 and 17 Hz, 6-H), 4.06 (3H, s, $NCH_3$), 4.11 (2H, m, $CH_2S$), 4.75 (1H, s, 3-H), 5.02 (1H, dd, $J$=2 and 4 Hz, 5-H).

**1c**: 58% yield; mp 208~210°C (dec); IR (KBr) $cm^{-1}$ 1782, 1620; $^1H$ NMR ($D_2O$) $\delta$ 1.55 (3H, s, $CH_3$), 3.33 (1H, dd, $J$=2 and 17 Hz, 6-H), 3.68 (1H, dd, $J$=4 and 17 Hz, 6-H), 4.09, 4.17 (2H, ABq, $J$=15 Hz, $CH_2S$), 4.45 (1H, s, 3-H), 5.02 (1H, dd, $J$=2 and 4 Hz, 5-H), 7.67 (5H, s, aromatic H).

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