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# Novel Dithiocarbamate Carbapenems[1] with Anti-MRSA Activity

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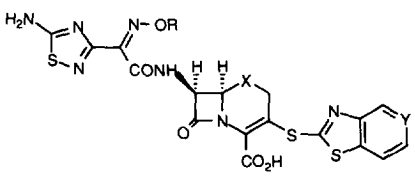
***Abstract*** : A new series of carbapenems, in which disubstituted-aminothiocarbonylthio moiety, directly attached to the C-2 position, were prepared and evaluated for their antibacterial potency. These analogs showed potent activity against high-level MRSA. Among them, **9e** and **9i** were found to exhibit good *in vivo* efficacy comparable to that of vancomycin, for mouse septicemia model with high-level MRSA.

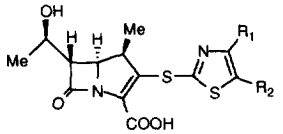

Infections caused by methicillin-resistant *staphylococcus aureus* (MRSA) have become a serious problem in the clinic because there are few anti-MRSA agents which are clinically effective. Vancomycin[2] is a potent anti-MRSA agent but its adverse effects restrict its clinical use.

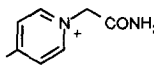
Recently β-lactam antibiotics, showing anti-MRSA activity, such as CP0467 (**1**)[3], LY-206763 (**2**)[4], SM-17466 (**3**)[5], and a Merck compound (**4**)[6] were reported. Coincidentally, these β-lactam antibiotics had a thiazolethio moiety at the C-3 position of the cephem (or carbacephem) nucleus or the C-2 position of the carbapenem nucleus, and were reported to show high affinity for the penicillin-binding protein-2' (PBP-2' or PBP-2a) of MRSA, which is essential for anti-MRSA activity.



**1** R = Me, X = S, Y = CH

**2** R = $CH_2CH_2F$, X = $CH_2$, Y = N



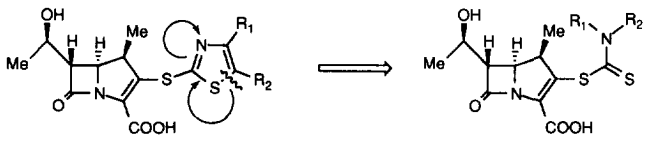
**3** $R_1$ =  $R_2$ = H

**4** $R_1$, $R_2$ = 

With thiazolo structural feature in mind, we envisaged a dithiocarbamate moiety at the C-2 side chain as in **9**, which was arrived by cleaving the 1-5 bond of the thiazole ring as shown in Figure 1. The targeted dithiocarbamate carbapenems **9**[7] were prepared *via* the coupling reaction of the carbapenem diphenylphosphates (**5** and **6**)[8] and dithiocarbamic acid salts. They were found to show potent *in vitro* and *in vivo* anti-MRSA activity, and good affinity for PBP-2'. Herein we describe the synthesis and biological properties of these novel

dithiocarbamate carbapenems.

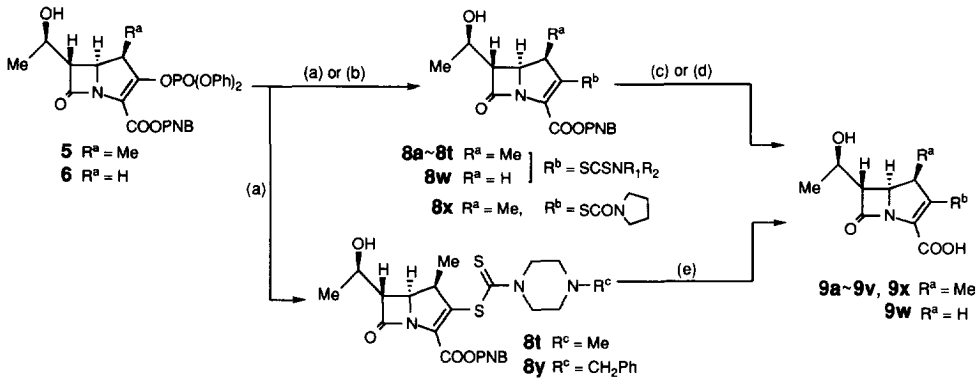
**Figure 1.**



## Synthesis

The dithiocarbamate carbapenems (**9a~9w**) were prepared as shown in Scheme 1. The coupling of dithiocarbamic acid triethylammonium salts (**7**)[7a] with the carbapenem diphenylphosphates (**5** and **6**) did not proceed in THF or DMF, probably due to the low nucleophilicity of (**7**). However we found that the reaction proceeded smoothly in THF, in the presence of lithium chloride (1.5~3 eq.) at room temperature to give the desired compounds (**8**). We speculate that *in situ* generated lithium dithiocarbamates during this reaction accelerated to give (**8**). Deprotection of (**8**) was carried out by catalytic hydrogenation or zinc reduction[9] to give the crude carbapenem, which was purified by reversed phase column chromatography affording the final carbapenem (**9a~9t**).[10] Des 1β-methyl carbapenem (**9w**) was prepared from **6** by the above method. The quaternary ammonium carbapenems (**9u** and **9v**) were prepared *via* the quaternization of **8t** and **8y** with iodomethane. Preparation of thiocarbamate derivative (**9x**) was carried out according to the literature method *via* acylation of 2-mercaptocarbapenem.[11]

**Scheme 1.**



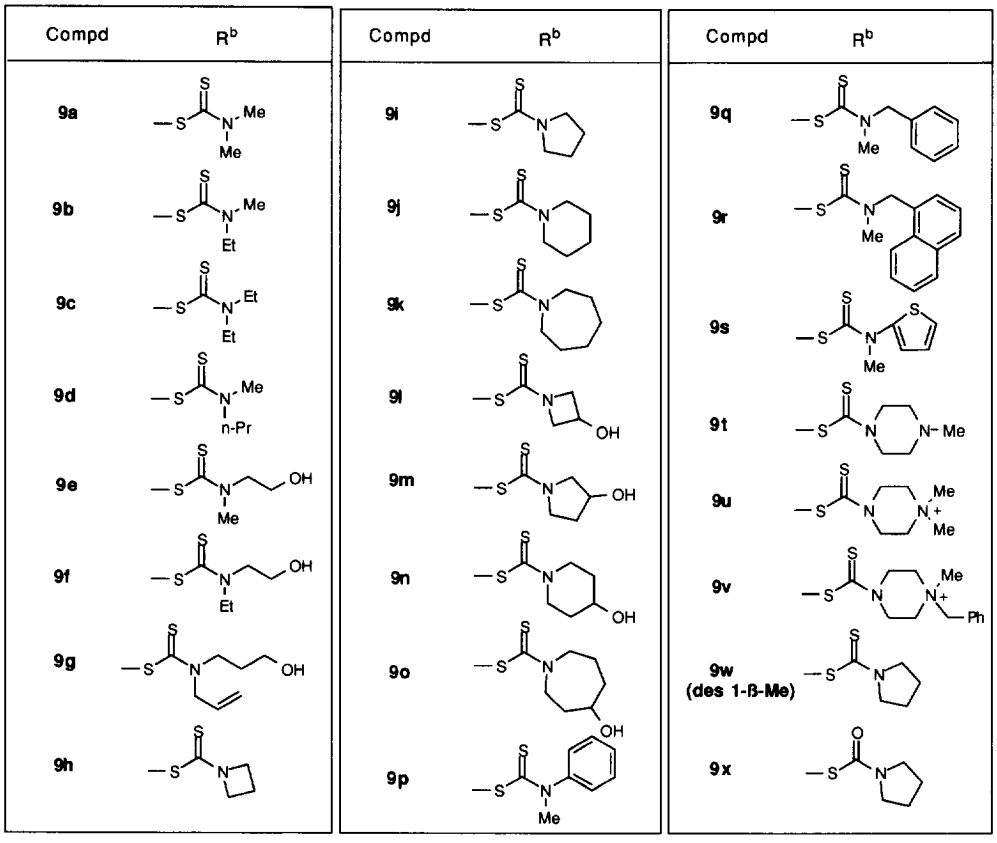
Reagents; (a) $NEt_3 \cdot HSCSNR_1R_2$ **7**, LiCl, THF, r.t., (b) $NaSH \cdot xH_2O$, DMF, -40°C then ClCO N, (c) 10% Pd-C, $H_2$, $NaHCO_3$, THF, $H_2O$, r.t., (d) or Zn, THF-phosphate buffer(pH 5.6), r.t., (e) 1) MeI, THF, r.t., 2) (c).

## Biological properties

Table 1 shows the antibacterial activities of the novel dithiocarbamate carbapenems against *S. aureus* including MRSA, *E. coli*, and *P. aeruginosa* , and dehydropeptidase-I (DHP-I) susceptibility[12], together with those of the reference compounds. The dithiocarbamate derivatives showed good antibacterial activity against not only MSSA but against high-level MRSA as well. They also showed good to moderate activity against *S.*

*epidermidis*, *E.faecalis*, and *E.coli*, but were inactive against *P. aeruginosa*. Generally, these carbapenems were more susceptible to DHP-I than imipenem with a few exception. Compounds (**9h**, **9i**, **9j**, and **9k**) derived from the cyclic monoamines showed the highest DHP-susceptibilities while the compounds (**9u** and **9v**) carrying a quarternized ammonium moiety were the least susceptible as was reported previously.[5]

Table 2 shows the comparative activities of the pyrrolidinodithiocarbonyl derivatives (**9i**), its des 1β-methyl derivative (**9w**), and thiocarbonyl derivative (**9x**). Des-1β-methylation affected the anti-staphylococci activity greatly, by a factor of 1/2~1/4. Replacement of one sulfur atom with oxygen also reduced the activity to 1/4~1/8. These results suggested that both the 1β-methyl substituent and the dithiocarbonyl moiety of the side chain play an important role in exerting the anti-MRSA activity.




Among the dithiocarbamate derivatives, 6 compounds (**9a**, **9e**, **9i**, **9o**, **9r**, and **9v**) were further evaluated for their *in vitro* and *in vivo* anti-MRSA activities, affinities for PBP-2'[13] and serum protein binding as shown in Table 3. These compounds were 25 to 100-fold more active than imipenem against MRSA, and these results were corroborated by the good affinity for PBP-2'. In addition, the compounds (**9a**, **9e**, **9i**, and **9o**) showed good *in vivo*[14] activity comparable to that of vancomycin against MRSA. However, compound (**9r**) was not active *in vivo* due to the high serum protein binding rate. Introduction of a hydroxyl into the side chain reduced the serum protein binding in compounds (**9e** and **9o**), which appeared to enhance their *in vivo* efficacy.

Compounds (**9a**, **9e**, **9o**, and **9r**) had no epileptogenic potential at 100 μg/rathead by the rat intraventricular assay.

**Table 1.** *In vitro* antibacterial activity (MIC, μg/ml) and DHP-I susceptibility.

| Organism | MIC (μg/ml)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **9a** | **9b** | **9c** | **9d** | **9e** | **9f** | **9g** | **9h** |
| *S.aureus* 209P NIHJ JC1 | 0.012 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| *S.aureus* BB6294[a] | 3.13 | 3.13 | 3.13 | 3.13 | 3.13 | 6.25 | 3.13 | 3.13 |
| *S.aureus* CSa1009[a, b] | 6.25 | 3.13 | 3.13 | 3.13 | 6.25 | 6.25 | 3.13 | 6.25 |
| *S. epidermidis* MB5181a | 6.25 | 3.13 | 3.13 | 3.13 | 6.25 | 3.13 | 3.13 | 6.25 |
| *E. faecalis* MB4996 | 12.5 | 6.25 | 12.5 | 6.25 | 6.25 | 6.25 | 6.25 | 6.25 |
| *E.coli* NIHJ JC2 | 6.25 | 6.25 | 25 | 25 | 0.78 | 6.25 | 12.5 | 3.13 |
| *P.aeruginosa* MB5002 | 25 | >25 | 25 | 25 | >25 | >25 | >25 | >25 |
| DHP-I susceptibility[c] | 2.12 | 2.26 | 2.51 | 2.71 | 1.36 | 2.38 | 1.39 | 3.03 |

| Organism | MIC (μg/ml)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **9i** | **9j** | **9k** | **9l** | **9m** | **9n** | **9o** | **9p** | **9q** |
| *S.aureus* 209P NIHJ JC1 | 0.012 | 0.025 | 0.012 | 0.025 | 0.025 | 0.025 | 0.012 | 0.05 | 0.012 |
| *S.aureus* BB6294[a] | 3.13 | 1.56 | 1.56 | 6.25 | 6.25 | 6.25 | 3.13 | 1.56 | 1.56 |
| *S.aureus* CSa1009[a, b] | 6.25 | 3.13 | 1.56 | 6.25 | 6.25 | 6.25 | 3.13 | 3.13 | 1.56 |
| *S. epidermidis* MB5181a | 3.13 | 3.13 | 1.56 | 3.13 | 3.13 | 6.25 | 3.13 | 1.56 | 3.13 |
| *E. faecalis* MB4996 | 12.5 | 6.25 | 6.25 | 12.5 | 6.25 | 6.25 | 3.13 | 6.25 | 1.56 |
| *E.coli* NIHJ JC2 | 6.25 | 25 | >25 | 0.78 | 0.78 | 3.13 | 12.5 | >25 | 12.5 |
| *P.aeruginosa* MB5002 | >25 | >25 | >25 | >25 | >25 | >25 | >25 | >25 | >25 |
| DHP-I susceptibility[c] | 3.18 | 3.10 | 3.09 | 2.32 | 2.21 | 2.23 | 2.88 | 2.29 | 1.94 |

| Organism | MIC (μg/ml)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **9r** | **9s** | **9t** | **9u** | **9v** | Vancomycin | IPM | FK-037 |
| *S.aureus* 209P NIHJ JC1 | <0.006 | <0.006 | 0.025 | 0.012 | <0.006 | 0.39 | <0.006 | 0.20 |
| *S.aureus* BB6294[a] | 0.78 | 1.56 | 3.13 | 3.13 | 3.13 | 1.56 | 100 | 50 |
| *S.aureus* CSa1009[a, b] | 1.56 | 3.13 | 6.25 | 12.5 | 6.25 | 1.56 | 100 | 50 |
| *S. epidermidis* MB5181a | 0.39 | 0.78 | 3.13 | 3.13 | 1.56 | 1.56 | 100 | 25 |
| *E. faecalis* MB4996 | 1.56 | 3.13 | 6.25 | 1.56 | 1.56 | 1.56 | 0.78 | 25 |
| *E.coli* NIHJ JC2 | 25 | 12.5 | 1.56 | 0.1 | 1.56 | >25 | 0.10 | 0.025 |
| *P.aeruginosa* MB5002 | >25 | >25 | >25 | >25 | >25 | >25 | 1.56 | 12.5 |
| DHP-I susceptibility[c] | 4.41 | 2.05 | 1.53 | 0.11 | 0.15 | — | 1.0 | — |

* MIC was determined by an agar dilution method using Mueller-Hinton medium (Difco).

[a] Methicillin-resistant strain. [b] β-Lactamase producing strain.

[c] Relative rate of hydrolysis to imipenem, porcine renal DHP-I.

In summary we have prepared a new series of the dithiocarbamate carbapenems, which showed good *in vitro* anti-MRSA activity. Among them, compounds (**9e** and **9o**) were found to exhibit the best *in vitro* and *in vivo* activity against high-level MRSA, and possess favorable biological properties except for DHP-I stability.

**Table 2.** Comparative activities and DHP-I susceptibility of **9l** and its derivatives.

| Organism | MIC (μg/ml) | | |
|---|---|---|---|
| | **9l** | **9w** | **9x** |
| *S.aureus* 209P NIHJ JC1 | 0.012 | 0.025 | 0.05 |
| *S.aureus* BB6294[a] | 3.13 | 6.25 | 25 |
| *S.aureus* CSa1009[a, b] | 6.25 | 25 | 25 |
| *S. epidermidis* MB5181a | 3.13 | 12.5 | 25 |
| DHP-I susceptibility[c] | 3.18 | 11.7 | 0.65 |

[a] Methicillin-resistant strain. [b] β-Lactamase producing strain.
[c] Relative rate of hydrolysis to imipenem, porcine renal DHP-I.

**Table 3.** Anti-MRSA activities and other biological properties of the representative dithiocarbamate carbapenems.

| Compd | **9a** | **9e** | **9l** | **9o** | **9r** | **9v** |
|---|---|---|---|---|---|---|
| *In vitro* anti-MRSA activity[a] G-mean MIC (μg/ ml) | 4.59 | 4.36 | 4.04 | 3.55 | 1.15 | 5.36 |
| MIC range | 3.13–6.25 | 3.13–6.25 | 3.13–6.25 | 3.13–6.25 | 0.78–3.13 | 3.13–12.5 |
| *In vivo* anti-MRSA-activity[b] $ED_{50}$ (μg/ml) | 5.91 | 4.80 | 6.24 | 2.88 | >50 | ca. 10 |
| Affinity to PBP-2[c] $IC_{50}$ (μg/ml) | 9.6 | 3.8 | 5.6 | 1.9 | NT | NT |
| DHP-I susceptibility | 2.12 | 1.33 | 3.18 | 2.88 | 4.41 | 0.15 |
| Serum protein binding (%)[d] | 88(69) | 47(58) | 94(95) | 85(92) | 99(99) | 60(82) |
| Epileptogenicity (100μg/rat head, n=5) | 0/5 | 0/5 | 1/5 | 0/5 | 0/5 | 3/5 |

[a] High-level MRSA (27 strains); geometric-mean MIC(μg/ml) of methicillin, imipenem, and vancomycin were 3000, 115, and 1.33, respectively.
[b] $ED_{50}$s (mg/kg) of imipenem and vancomycin were >200 and 5.56, respectively.
[c] $IC_{50}$ (μg/ml) of imipenem was 125.
[d] Binding rate for human (mouse) serum at a carbapenem concentration of 10 μg/ml.

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10. **9a**: IR (KBr) $cm^{-1}$ 1749, 1603, 1387, 1248. $^1H$ NMR (300 MHz, $D_2O$) δ 1.10 (3H, d, *J* = 7.3 Hz), 1.28 (3H, d, *J* = 6.3 Hz), 3.48 (3H, s), 3.49 (3H, s), 3.53 (1H, dd, *J* = 3.0, 6.0 Hz), 3.71 (1H, m), 4.28 (1H, m), 4.36 (1H, dd, *J* = 3.0, 12.0 Hz). UV $\lambda_{max}$ 279 (ε = 17,000) nm. FAB-HRMS *m/z* calcd for $C_{13}H_{17}N_2O_4S_2Na_2$ $(M+2Na-H)^+$: 375.0425, found 375.0430. **9e**: IR (KBr)$cm^{-1}$ 3430, 1749, 1608, 1506, 1386. $^1H$ NMR (300 MHz, $D_2O$) δ 1.16 (3H, dd, *J* = 2.3, 7.3 Hz), 1.33 (3H, d, *J* = 6.3 Hz), 3.55 (3H, d, *J* = 2.3 Hz), 3.01~3.11 (1H, m), 3.91~3.98 (2H, m), 4.09~4.15 (1H, m), 4.20~4.37 (2H, m), 4.38~4.45 (1H, m). UV $\lambda_{max}$ 277 (ε = 13,700) nm. FAB-HRMS *m/z* calcd for $C_{14}H_{19}N_2O_5S_2Na_2$ $(M+2Na-H)^+$: 405.0531, found 405.0523. **9o**: IR (KBr) $cm^{-1}$ 3400, 1745, 1691, 1612, 1390. $^1H$ NMR (300 MHz, $D_2O$) δ 1.08 (3H, d, *J* = 7.0 Hz), 1.25 (3H, d, *J* = 6.5 Hz), 1.55~2.30 (6H, m), 2.51 (1H, m), 3.71 (1H, m), 3.80~4.15 (4H, m), 4.23 (1H, m), 4.34 (1H, d, *J* = 3.0, 9.5 Hz). UV $\lambda_{max}$ 280 (ε = 17,700) nm. FAB-MS *m/z* 455 $(M+2Na-H)^+$.
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13. The PBP-2' affinity was determined by the competition assay with [$^{14}C$]benzylpenicillin using membrane isolated from MRSA BB6294 strain.
14. ICR mice (4 weeks old, male) were infected intraperitonealy with homotypic MRSA BB6221 in 5% gastric mucin. Each agent was administered subcutaneously at 1hr after infections in combination with cilastatin at a dose of 40 mg/kg.

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