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Novel Dithiocarbamate Carbapenems[1] with Anti-MRSA Activity

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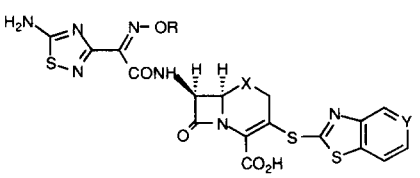
Abstract : A new series of carbapenems, in which disubstituted-aminothiocarbonylthio moiety, directly attached to the C-2 position, were prepared and evaluated for their antibacterial potency. These analogs showed potent activity against high-level MRSA. Among them, **9e** and **9i** were found to exhibit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

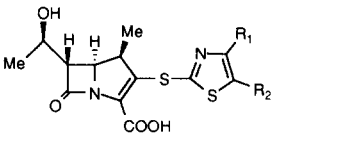
Infections caused by methicillin-resistant *staphylococcus aureus* (MRSA) have become a serious problem in the clinic because there are few anti-MRSA agents which are clinically effective. Vancomycin[2] is a potent anti-MRSA agent but its adverse effects restrict its clinical use.

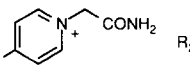
Recently β-lactam antibiotics, showing anti-MRSA activity, such as CP0467 (**1**)[3], LY-206763 (**2**)[4], SM-17466 (**3**)[5], and a Merck compound (**4**)[6] were reported. Coincidentally, these β-lactam antibiotics had a thiazolethio moiety at the C-3 position of the cephem (or carbacephem) nucleus 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



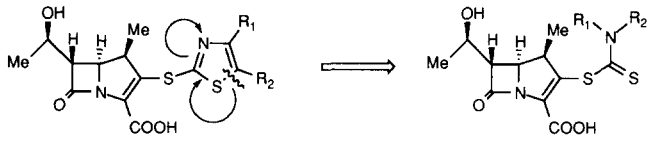
3 R_1 =  R_2 = H

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.

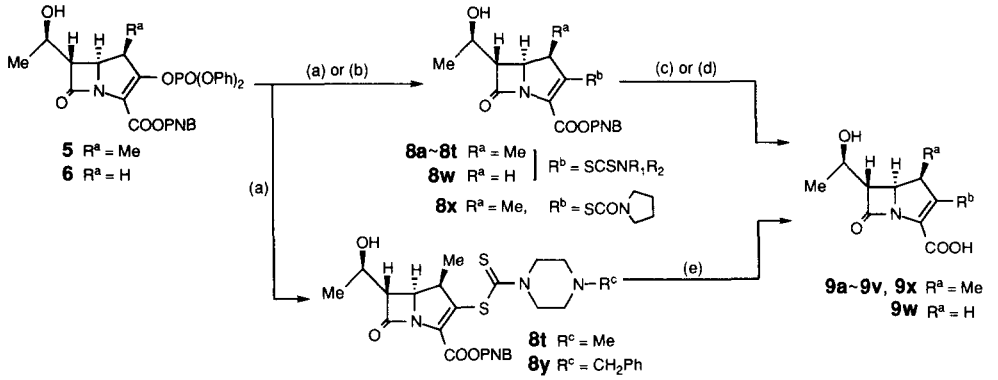
Figure 1.



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.



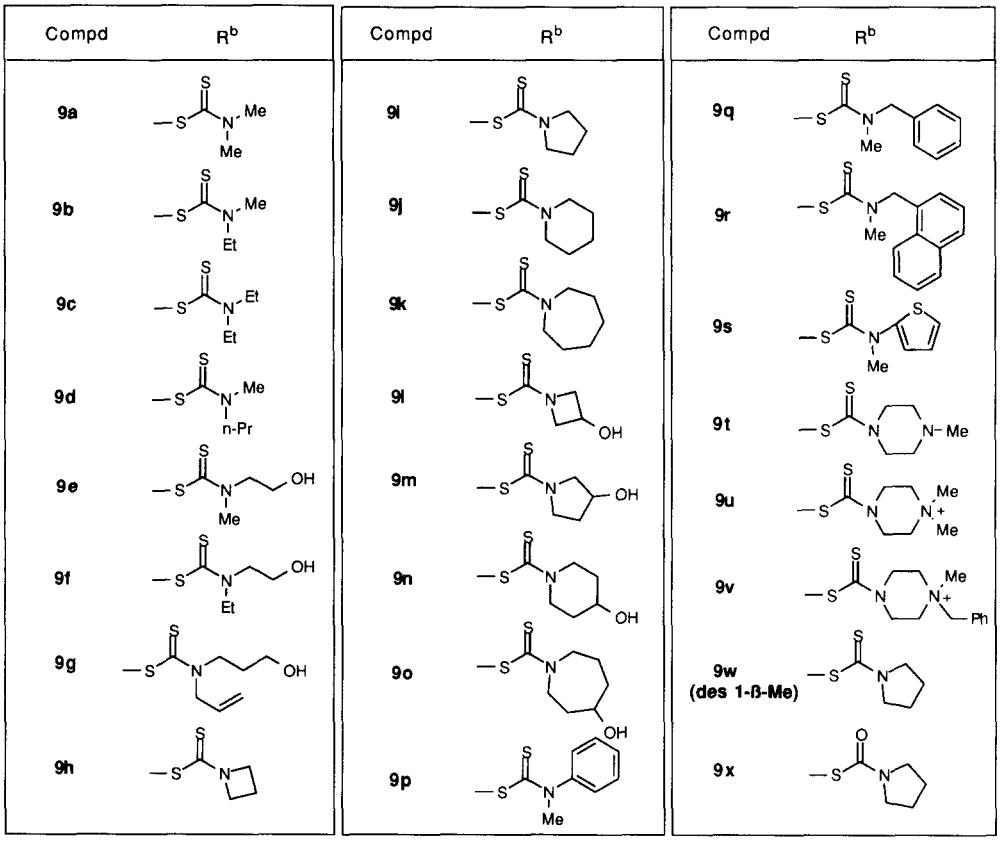
Reagents; (a) $NEt_3 \cdot HSCSNR_1R_2$ **7**, LiCl, THF, r.t., (b) $NaSH \cdot xH_2O$, DMF, -40°C then ClCO N, (c) 10% Pd-C, H_2, $NaHCO_3$, THF, H_2O, r.t., (d) or Zn, THF-phosphate buffer(pH 5.6), r.t., (e) 1) MeI, THF, r.t., 2) (c).

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 **9i** and its derivatives.

Organism	MIC (μg/ml)		
	9i	**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**	**9i**	**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 λ_{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 λ_{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 λ_{max} 280 (ε = 17,700) nm. FAB-MS *m/z* 455 $(M+2Na-H)^+$.
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12. The relative hydrolysis rate was determined by using partially purified porcine DHP-I, taking the hydrolysis rate of imipenem as 1.0.
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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