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SYNTHESIS AND BIOLOGICAL PROPERTIES OF A NEW SERIES OF ANTI-MRSA β-LACTAMS; 2-(THIAZOL-2'-YLTHIO)CARBAPENEMS

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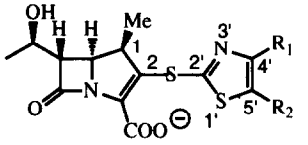
Abstract: Synthesis and biological properties of a new series of 1β-methyl carbapenems that have been variously substituted thiazol-2'-ylthio side chain on C-2 are described. Amongst synthesized compounds, 4'-arylthiazole derivatives showed potent anti-MRSA activity and the introduction of a quaternary cation was effective to reduce binding to human serum albumin without affecting the anti-MRSA activity.

Among many bacterial pathogens, methicillin resistant *Staphylococcus aureus* (MRSA)[1] still remains as an important target for the development of chemotherapuitic agents. Since only a limited number of drugs are available for treatment of MRSA infection[2], potent anti-MRSA agent with low level of side effect is highly desirable. One proposed resistant mechanism of MRSA to β-lactams is that penicillin binding protein-2' (PBP-2') produce in MRSA has a low affinity to β-lactams[3].



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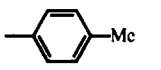
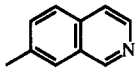
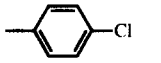
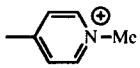
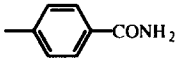
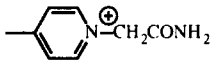
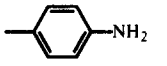
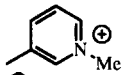
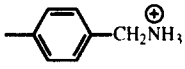
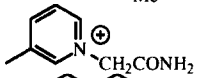
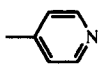
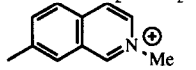
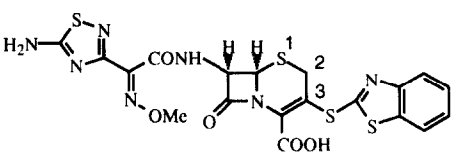
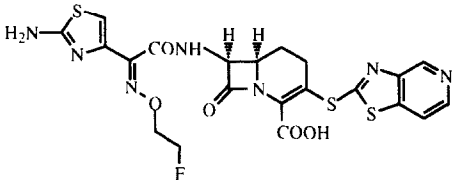
1a			**h**		H	**o**		H
b	H	H	**i**		H	**p**		H
c	Me	Me	**j**		H	**q**		H
d	Me	H	**k**		H	**r**		H
e	Pr	H	**l**		H	**s**		H
f	Ph	H	**m**		H	**t**		H
g	H	Ph	**n**		H			

Recently, cephalosporins[4] (e.g., **2**; CP0467) and carbacephalosporins[5] (e.g., **3**; LY-206763) baring various thiazolethio moieties have been synthesized and some of the compounds were found to exhibit both high potency against MRSA and high affinity to PBP-2'. In spite of the excellent *in vitro* activities, the high binding affinity (>97%) to human serum albumin (HSA) seems to prevent them from showing sufficient therapeutic efficacy. We assumed that the anti-MRSA activity of these compounds was derived from the thiazole side chain on C-3, which could be applicable to C-2 side chain of carbapenem. A preliminary conformational analysis of carbapenem **1a** using molecular mechanics[6] showed that the relative geometry of benzothiazole moiety from β-lactam ring and carboxylic acid in the most stable conformation could be well overlapped with that of a cephalosporin **2**



2 (CP0467)



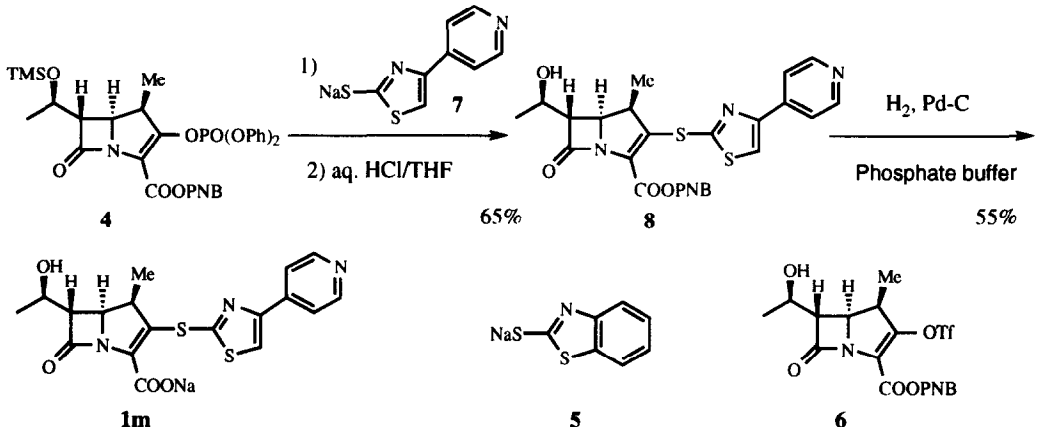
3 (LY-206763)

Based on the conformational study, we supposed that the carbapenems having a thiazol-2'-ylthio side chain on C-2 would exhibit good anti-MRSA activity and planed to synthesize a series of 2-(thiazol-2'-ylthio)carbapenem **1a-t** with 1β-methyl group[7], which was necessary to maintain the stability against dehydropeptidase-I (DHP-I)[8]. We describe herein the synthesis of new 1β-methyl carbapenems and their biological activities.

Synthesis

The synthesis of a series of 1β-methyl carbapenems was achieved in a similar manner to the method reported previously[9]. But it should be noted that 2'-mercaptothiazoles[10] did not react with enolphosphate **4**[9] in the presence of an amine such as diisopropylethylamine as a base because of their low neucleophilicity Sodium thiolates, prepared from most of the 2'-mercaptothiazoles and sodium hydride, were required to proceed the reaction with readily **4**. In addition, 2'-mercaptobenzothiazole did not react with enolphosphate **4** even when sodium thiolate was used. In this case, the substitution reaction was carried out using enoltriflate **6**[11]. A typical procedure is shown in the Scheme After treatment of **4** with **7**, removing the silyl group under acidic condition afforded carbapenem ester **8**. Catalytic hydrogenation of **8** over Pd-C in phosphate buffer (PH = 7.0) gave the desired carbapenem **1m**. **1m**: IR (KBr) cm^{-1} 3570 (br), 3380 (br), 1752, 1603, 1560, 1384; ^1H NMR (270 MHz, D_2O) δ 1.12 (3 H, d, J = 6 9 Hz), 1 27 (3 H, d, J = 5 9 Hz), 3.47 (2 H, m), 4.30 (2 H, m), 7.87 (2 H, d, J = 6 3 Hz), 8.23 (1 H, s), 8.61 (2 H, d, J = 6 3 Hz); UV λmax (H_2O) 305 (sh), 284. To synthesize quaternary derivatives **1p-t**, quaternization was carried out before the deprotection of *p*-nitrobenzyl group[12].

Scheme



Biological properties

In Tables 1-3, *in vitro* antibacterial activities[13] against gram-positive and gram-negative bacteria including MRSA (MS9408, low resistance; SP-7928, high resistance), stability against DHP-I[14] and binding to HSA[15] of newly prepared carbapenems are summarized.

As expected from the conformational study, benzothiazole derivative **1a** showed good activity against MRSA. It was confirmed that the thiazolethio side chain was the important for the anti-MRSA activity resulted from the antibacterial activity of **1b**. In Table 1, it is also shown that the introduction of substituent on C-4' position of thiazole ring is effective to increase the anti-MRSA activity. Phenyl substitution on C-4' seemed to be more effective than alkyl substitution, that is **1f** showed 8 to 64-fold higher anti-MRSA activity than imipenem[16]. It is evident by the PBP-2' binding assay[17] of **1f** (IC_{50} = 10.6 μg/ml) and imipenem (IC_{50} = 124 μg/ml) that the high anti-MRSA activity of the compounds prepared in this study is due to the high binding affinity to PBP-2'.

Although the anti-MRSA activity of **1f** was at a sufficiently high level, its HSA binding was too high for therapeutic use. To find out an active compound with low HSA binding, we investigated the substituents of the phenyl group in **1f**. As shown in Table 2, neither the electrostatic character, the bulkiness nor lipophilicity of the substituents on the aromatic ring largely affected the anti-MRSA activity (**1h-l**). Heteroaryl compounds **1m-o**, in which heteroaryl group was introduced instead of phenyl, also showed a high activity against MRSA. Among the compound prepared, only aminomethyl derivative **1l** showed the reduced HSA binding. Since HSA is known to be a lipophilic and cation rich protein[18], we assumed that the cationic substituent of **1l** inhibited the binding. So we prepared quaternary amine derivatives of **1m**, **1n** and **1o**. As expected, HSA bindings of the quaternary carbapenems **1p-t** decreased to less than 60% as shown in Table 3. They displayed not only a high activity against MRSA but also higher activity against gram negative bacteria (*E. coli.*) than their parent compounds. The latter could be explained by the improved permeability for the outer membrane of *E. coli.*. The quaternization also significantly improved the DHP-I stability as reported previously[12][19].

Table 1 Antibacterial activity, DHP-I stability and HSA of 2-(thiazol-2'-ylthio)carbapenems

	MIC (μg/ml)							
Organism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	Imipenem
S a 209p	0.025	0 025	0.025	0 025	0.025	<0 013	<0 006	0 013
S a MS9408	0 78	0.78	0 78	0 39	0 39	0 10	0 39	0 78
S a SP-7928	3 13	12 5	6 25	3 13	3 13	0 78	12.5	50
S e IAM1296	0.20	0 10	0 10	0 05	0 05	0 025	0 39	0.20
S p COOK	<0 013	0 025	0 025	0 025	<0 006	<0 013	0.013	<0 006
K p ATCC 10031	0 78	0 05	0.2	0 05	0 20	0 10	0 39	0 10
E.c. NIHJ JC-2	25	0 78	3.13	3.13	12 5	6 25	25	0.10
DHP-I stability[a] (min)	n t.	15	23	13	12	9 8	15	12
HSA binding (%)	>94	69	>75	93	96	>94	>94	<10

Table 2 Antibacterial activity, DHP-I stability and HSA binding of 2-(4'-arylthiazol-2'-ylthio)carbapenems

	MIC (μg/ml)							
Organism	**1h**	**1i**	**1j**	**1k**	**1l**	**1m**	**1n**	**1o**
S.a 209p	<0 006	<0 013	<0.006	<0.013	<0 006	<0 013	<0 013	<0 006
S a MS9408	0 20	0 20	0 20	0.20	0 20	0 20	0 20	0 10
S a SP-7928	3 13	1 56	1 56	1 56	1 56	3 13	1 56	0 78
S e IAM1296	0 025	0 025	0 025	0 025	0 025	0 05	0 025	0 025
S p COOK	<0.006	<0 013	<0 006	<0 013	<0 006	<0 013	<0 013	<0 006
K p ATCC 10031	0 39	0 20	0 10	0 05	0 20	0 20	0 05	0 20
E.c NIHJ JC-2	12 5	12 5	6 25	6 25	1 56	6 25	6 25	12.5
DHP-I stability[a] (min)	9 2	9 9	12	18	n.t	16	3 2	9 7
HSA binding (%)	>94	>94	99	98	87	97	97	99

Table 3 Antibacterial activity, DHP-I stability and HSA binding of quaternary carbapenems

	MIC (μg/ml)				
Organism	**1p**	**1q**	**1r**	**1s**	**1t**
S a 209p	<0 006	<0 006	<0 013	<0 006	<0 006
S a MS9408	0 10	0 20	0 20	0 20	0 05
S a SP-7928	1 56	1 56	1 56	1 56	0 78
S e IAM1296	0 025	0 05	0 025	0 025	0 013
S p. COOK	<0.006	<0 006	<0 013	<0 006	<0.006
K p ATCC 10031	0 025	0.025	0 05	0 05	0 05
E c. NIHJ JC-2	0 39	0.20	0 39	0.39	3.13
DHP-I stability[a] (min)	23	21	28	17	12
HSA binding (%)	12	15	7 8	24	59

[a] The number indicates the time of enzyme-catalyzed hydrolysis of the compound from 500μM to 400μM in the presence of purified renal DHP-I of swine

Abbreviations:
S a, *Staphylococcus aureus*,
S.e, *Staphylococcus epidermidis*;
S p, *Streptococcus pyogenes*;
K p, *Klebsiella pneumoniae*,
E c., *Escherichia coli*,
n t, not tested.

In this study, we have been successful in synthesizing a new series of 2-(arylthiazol-2'-ylthio)carbapenems that show a superior anti-MRSA activity. We have found that the introduction of the cationic moiety in carbapenems not only reduces the binding to HSA but also increase the stability against DHP-I without affecting the anti-MRSA activity. Further studies on these carbapenems and the detailed evaluation of **1p-t** are in progress.

Acknowledgment. We are grateful to Mr. T. Nishimura of this laboratory for the conformational analysis and helpful suggestions.

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3.59 (2 H, m), 4.27 (2 H, m), 5.51 (2 H, s), 8.38 (2 H, d, J = 7.3 Hz), 8.60 (1 H, s), 8.77 (2 H, d, J = 7.3 Hz); UV λmax (H_2O) 310, 284 (sh). a) Kim, C. U., Luh, B. Y., Misco, P. F.; Hitchcock, M. J. M., *J. Med. Chem.* **1989**, *32*, 601. b) Kim, C. U.; Misco, P. F.; Luh, B. Y.; Hitchcock, M. J. M., *J. Antibiotics* **1987**, *40*, 1707. c) Schmitt, S. M.; Salzmann, T. N.; Shih, D. H.; Cristensen, B. G., *J. Antibiotics* **1988**, *41*, 780. d) Nagao, Y.; Nagase, Y.; Kumagai, T.; Matusunaga, H.; Abe, T.; Shimada, O.; Hayashi, T.; Inoue, Y., *J. Org. Chem.* **1992**, *57*, 4243

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