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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**—A series of 1β-methylcarbapenems containing variously C-2 substituted thiazol-2-ylthio groups were synthesized, and their in vitro anti-MRSA activity was examined. Among them, 1β-methyl-2-(4-arylthiazol-2-ylthio) carbapenems exhibited superior anti-MRSA activity. Introduction of a cationic moiety in the C-2 side chain not only reduced the binding to HSA but also increased the stability against DHP-I, without affecting the anti-MRSA activity. It was also found that the distance between the cationic moiety and the carbapenem skeleton was related to the strength of HSA binding and the stability against DHP-I. © 1997 Elsevier Science Ltd. All rights reserved.

## Introduction

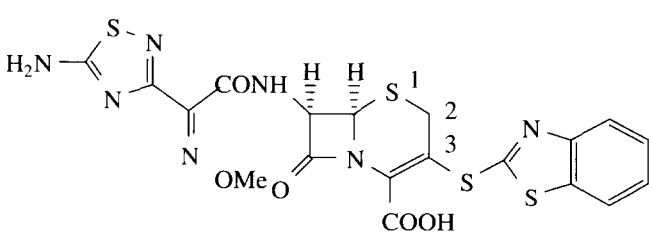
Methicillin-resistant *Staphylococcus aureus* (MRSA) was first demonstrated in 1961,[1] soon after the introduction of methicillin into clinical use. It has been shown that these strains also are resistant to many other β-lactam antibiotics.

Since the appearance of MRSA, numerous studies on MRSA have been extensively conducted.[2,3] Yokota et al.[4] demonstrated that a new penicillin-binding protein, PBP-2′, with a molecular weight of 78,000, appeared in two penicillinase-free MRSA strains and that PBP-2′ possessed low affinities for various β-lactam antibiotics. Subsequently, several studies[5] have examined PBP-2′.

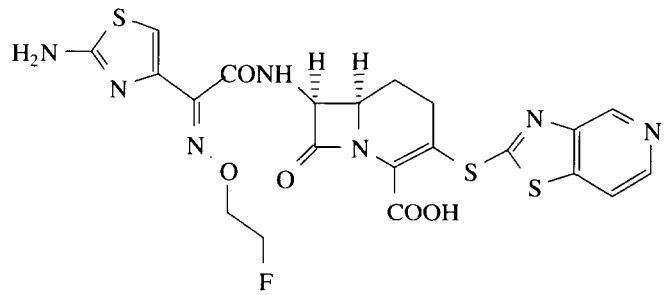
MRSA has been a major pathogen in patients suffering from nosocomial infections[2] in Japan since the introduction of third-generation cephems. The clinically used anti-MRSA agents are vancomycin[6] and arbekacin[7] which are a glycopeptide and an aminoglycoside, respectively. However, therapeutic use of these agents is relatively limited due to their side-effects. Therefore, potent anti-MRSA agents with fewer side-effects are highly desirable.

Recently, β-lactam compounds[8] have been designed as possible anti-MRSA agents, though β-lactam agents were previously considered incompatible with anti-MRSA activity because of its resistant mechanism. In 1992 and 1993, cephalosporins[9] (e.g., **CP0467**) and carbacephalosporins[10] (e.g., **LY-206763**) bearing various thiazolethio moieties at C-3 were synthesized (Figure 1), and some of these compounds exhibited both high in vitro antibacterial activity against MRSA and high affinity to PBP-2′. However, these compounds were not as effective in vivo because of their high binding affinity to human serum albumin (HSA).

We supposed that the anti-MRSA activity of these β-lactam derivatives was derived from the introduction of the benzothiazolethio side chain at the C-3 position of

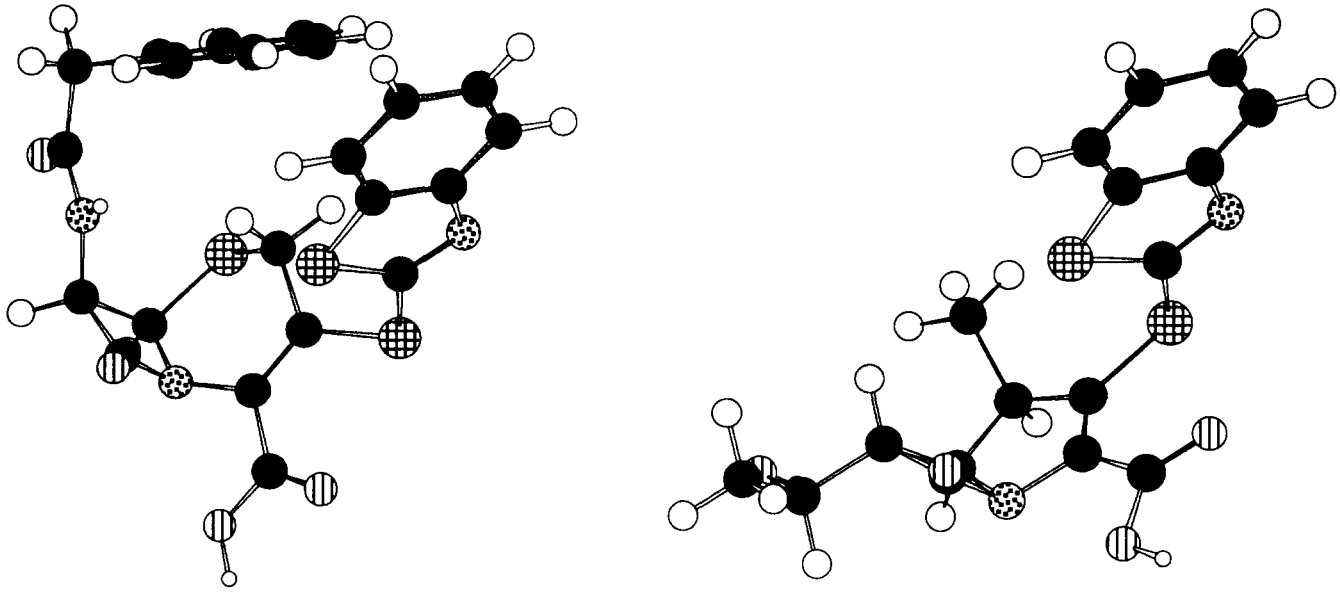


**(CP0467)**



**(LX-206763)**

**Figure 1.** The structures of **CP0467** and **LX-206763**.



cephalosporin **CP0467** carbapenem $\mathbf{d_1}$

**Figure 2.** The conformations of carbapenem $\mathbf{d_1}$ and cephalosporin **CP0467** using molecular mechanics.

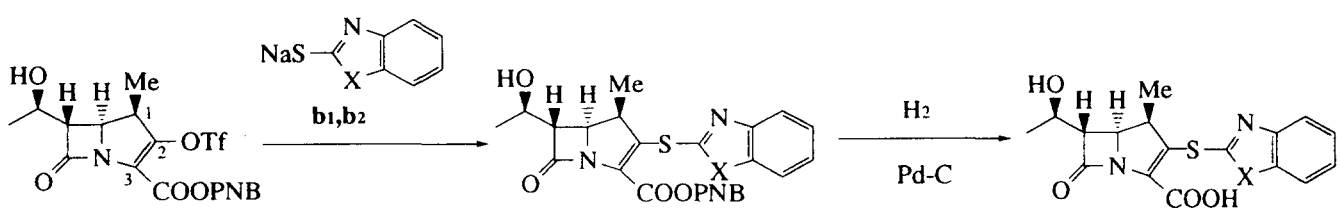
the cephem skeleton and that this may be applicable to the C-2 side-chain of the carbapenem skeleton for anti-MRSA carbapenem antibiotics. Molecular mechanics[11] were used to compare the minimized conformation of the 3-benzothiazolethio cephem with that of the 2-benzothiazolethio carbapenem, containing a 1β-methyl group important for improving the stability against dehydropeptidase-I (DHP-I). Energy-minimized conformations were generated using a 1000-step Monte Carlo conformational search with the AMBER force field by use of a Macro Model (version 3.5). The results are shown in Figure 2. Conformational analysis of carbapenem $\mathbf{d_1}$ demonstrated that both the benzothiazole from the β-lactam ring and the carboxylic acid moieties could be overlapped well with those from cephalosporin **CP0467**. This finding prompted us to synthesize a series of 1β-methyl-2-thiazolethio-carbapenems.

A preliminary account of this work has been presented previously,[12] and a similar approach has also been evaluated by another group.[13] This study discusses not only the structure–activity relationships pertaining to anti-MRSA activity but also those pertaining to HSA binding affinity and stability against DHP-I.

## Results and Discussion

### Chemistry

The introduction of the benzothiazol-2′-ylthio and the benzoxazol-2′-ylthio moieties at the C-2 position of 1β-methylcarbapenem could not be achieved similarly by the previous method[14] of reacting 2-mercaptobenzothiazole[15] $\mathbf{b_1}$ and 2-mercaptobenzoxazole $\mathbf{b_2}$ with enolphosphate **e**[14] in the presence of an amine such as diisopropylethylamine. This was due to their low nucleophilicity. Compounds $\mathbf{c_1}$ and $\mathbf{c_2}$ were finally obtained using enoltriflate **a**,[16] instead of enolphosphate **e**, and the corresponding sodium thiolate, prepared from either 2-mercaptobenzothiazole $\mathbf{b_1}$ or 2-mercaptobenzoxazole $\mathbf{b_2}$ and sodium hydride. Pd–C catalyzed hydrogenation of $\mathbf{c_1}$ and $\mathbf{c_2}$ in a mixture of

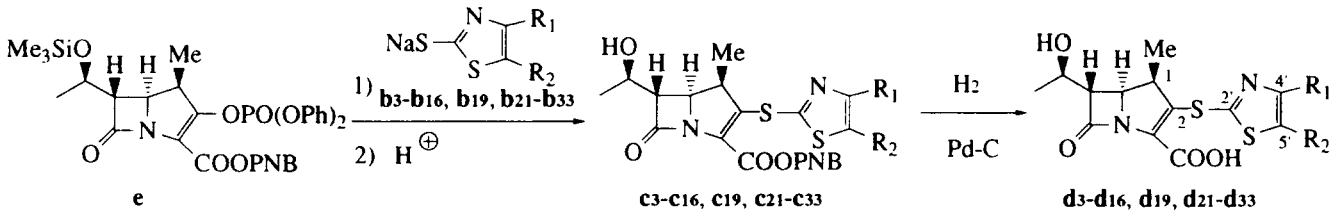



**a** PNB=*p*-nitrobenzyl Tf=trifluoromethanesulfonyl **c1,c2** **b1-d1** X=S **b2-d2** X=O **d1,d2** **(Chart 1)**

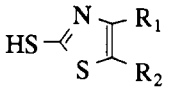
MOPS-buffer and THF provided the desired derivatives $\mathbf{d_1}$ and $\mathbf{d_2}$ (Chart 1).

Concerning 2-mercaptothiazoles $\mathbf{b_3}$–$\mathbf{b_{33}}$, the substitution reaction was achieved by treating enolphosphate **e** with the corresponding sodium thiolate to form $\mathbf{c_3}$–$\mathbf{c_{33}}$, after the treatment with dilute acid (Charts 2 and 3).

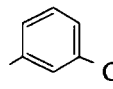
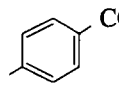
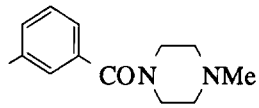
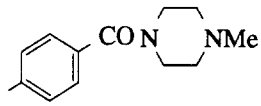
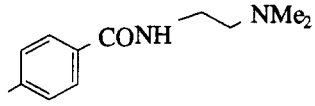
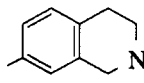
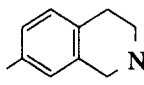
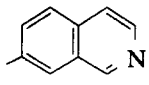
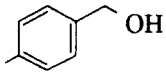
Hydrogenation removed the *p*-nitrobenzyl (PNB) group (*vide supra*). Under these conditions, the silyl ethers of $\mathbf{c_7}$, $\mathbf{c_{23}}$, $\mathbf{c_{28}}$, $\mathbf{c_{31}}$, and $\mathbf{c_{32}}$, which had not been removed in the former reaction, were removed. The reduction of $\mathbf{c_{17}}$, $\mathbf{c_{18}}$, and $\mathbf{c_{20}}$ to the corresponding amino compounds ($\mathbf{d_{17}}$, $\mathbf{d_{18}}$, and $\mathbf{d_{20}}$) was also achieved at the same time.

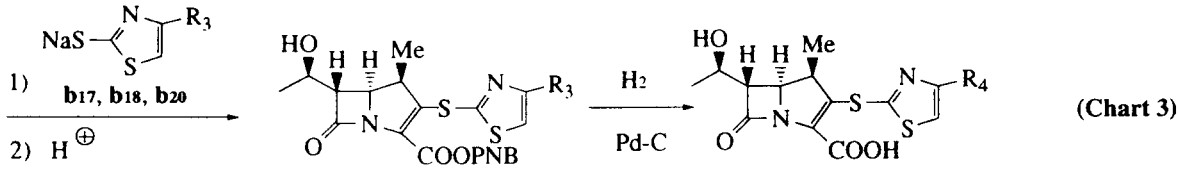



**(Chart 2)**

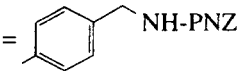


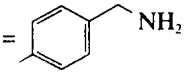

**b3-b16, b19, b21-b33**

| | R1 | R2 | | R1 | R2 |
|---|---|---|---|---|---|
| **b3** | H | H | **b23** |  | H |
| **b4** | Me | H | | | |
| **b5** | n-Pr | H | **b24** |  | H |
| **b6** | i-Pr | H | **b25** |  | H |
| **b7** | Ac | H | | | |
| **b8** | $CONMe_2$ | H | **b26** |  | H |
| **b9** | Ph | H | | | |
| **b10** | Me | Me | **b27** |  | H |
| **b11** | H | Ph | | | |
| **b12** | *o*-Cl-Ph | H | **b28** |  | H |
| **b13** | *p*-Cl-Ph | H | | | |
| **b14** | *o*-Me-Ph | H | **b29** |  | H |
| **b15** | *m*-Me-Ph | H | | | |
| **b16** | *p*-Me-Ph | H | **b30** |  | H |
| **b19** |  | H | | | |
| **b21** | 1-naphthyl | H | **b31** | 2-pyridyl | H |
| **b22** | 2-naphthyl | H | **b32** | 3-pyridyl | H |
| | | | **b33** | 4-pyridyl | H |




(Chart 3)

**b17**, **c17** : R3 = *o*-NO2-Ph
**b18**, **c18** : R3 = *p*-NO2-Ph
**b20**, **c20** : R3 = 


**d17** : R4 = *o*-NH2-Ph
**d18** : R4 = *p*-NH2-Ph
**d20** : R4 = 


PNZ=*p*-nitrobenzyloxycarbonyl

The synthesis of the quaternary ammonium derivatives $\mathbf{d_{34}}$–$\mathbf{d_{49}}$ was accomplished by quaternization of $\mathbf{c_{25}}$–$\mathbf{c_{27}}$ and $\mathbf{c_{29}}$–$\mathbf{c_{33}}$ with corresponding alkyl halides followed by deprotection. An example of the synthesis of a quaternary ammonium derivative ($\mathbf{d_{40}}$) is shown in Chart 4.

The preparation of the 2-mercaptothiazole derivatives, $\mathbf{b_9}$,[17] $\mathbf{b_{12}}$, $\mathbf{b_{13}}$,[18] $\mathbf{b_{14}}$, $\mathbf{b_{15}}$,[18] $\mathbf{b_{16}}$,[18] $\mathbf{b_{17}}$, $\mathbf{b_{18}}$,[19] $\mathbf{b_{20}}$, $\mathbf{b_{21}}$,[19] and $\mathbf{b_{22}}$,[20] and the pyridine derivatives $\mathbf{b_{31}}$–$\mathbf{b_{33}}$[21] were achieved via a simple three-step procedure: the corresponding acetophenone derivatives were brominated with bromine in the presence of a catalytic amount of $AlCl_3$, and the resulting bromomethylketones (**f**) were then treated with ammonium dithiocarbamate **g**.[22] The crude products were dehydrated upon heating to provide the 2-mercaptothiazole derivatives. As an example, the preparation of $\mathbf{b_{12}}$ is shown in Chart 5.

Benzoyl amide derivatives $\mathbf{b_{23}}$–$\mathbf{b_{27}}$ were derived from $\mathbf{h}_m$ and $\mathbf{h}_p$, which were synthesized in a manner similar to that shown in Chart 5. The corresponding benzoic acids ($\mathbf{h}_m$ or $\mathbf{h}_p$) were treated first with iPrOCOCl or EtOCOCl and then with the corresponding amines to give the desired 2-mercaptothiazoles (Chart 6). Compound $\mathbf{b_{19}}$ was obtained by $NaBH_4$ reduction of the mixed anhydride $\mathbf{i}_p$ and subsequent treatment with methylamine (Chart 7).

Compounds $\mathbf{b_5}$[19] and $\mathbf{b_6}$[19,23] were synthesized with ammonium dithiocarbamate **g** from $\alpha$-chloroketones,[24] which were derived from butyryl chloride and isobutyryl chloride, respectively, by the treatment first with diazomethane and then with dry hydrochloride.

Compounds $\mathbf{b_3}$,[25] $\mathbf{b_4}$,[26] $\mathbf{b_7}$,[27] $\mathbf{b_{10}}$,[28] and $\mathbf{b_{11}}$[29] were prepared via the condensation of chloroacetoaldehyde, chloroacetone, 1-bromo-2,3-butadione,[30] 3-chloro-2-butanone, and 2-bromo-2-phenylacetaldehyde, respectively, with ammonium dithiocarbamate **g**.

Compound $\mathbf{b_8}$ was synthesized in seven steps from methyl pyruvate via the thiazole derivative **k** by an ester to amide functional group interconversion (Chart 8).

Among the synthesis of isoquinoline derivatives, $\mathbf{b_{28}}$ was prepared by the bromination and condensation with **g** of **n**,[31] prepared from 1,2,3,4-tetrahydroisoquinoline, and subsequent hydrochloric acid hydrolysis of **o** gave $\mathbf{b_{28}}$. Reductive alkylation of $\mathbf{b_{28}}$ gave $\mathbf{b_{29}}$ (Chart 9).

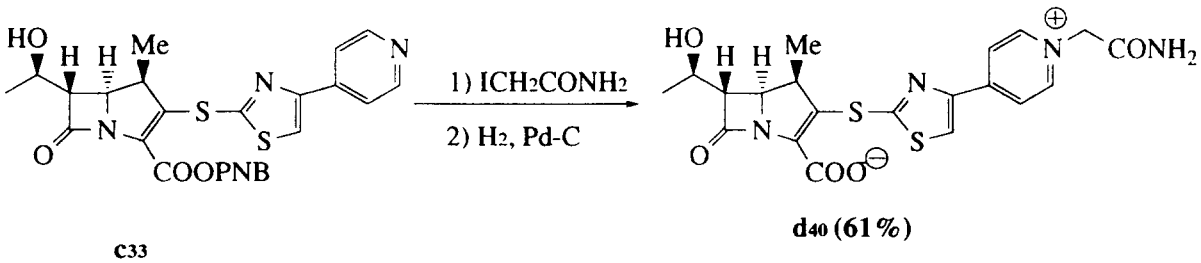
In the preparation of isoquinoline derivative $\mathbf{b_{30}}$, it was found that thiol group protection could not be carried out via the PNB derivative due to the difficulties encountered in its removal by catalytic hydrogenation. When the trityl group was used instead, the target compound $\mathbf{b_{30}}$ was easily obtained as follows: compound **q** was obtained through tritylation, followed by deamidation. The dehydrogenation and removal of the trityl group of **q** was carried out simultaneously by heating **q** at 160–180 °C in *p*-cymene in the presence of a catalytic amount of Pd–C to give $\mathbf{b_{30}}$ (Chart 10).

## Biological properties

Tables 1–5 summarize the in vitro antibacterial activities against gram-positive and gram-negative bacteria including MRSA (MS9408, low-resistance; SP-7928, high-resistance), the stability against DHP-I, and the HSA binding of newly prepared carbapenems.

As expected from the comformational analysis study, benzothiazole derivative $\mathbf{d_1}$ exhibited both good activity against MRSA and high affinity to PBP-2′ as did the corresponding benzoxazole derivative $\mathbf{d_2}$. Carbapenem $\mathbf{d_3}$, possessing a simple thiazole, also exhibited higher anti-MRSA activity in comparison with imipenem,[32] although its activity was lower than that of $\mathbf{d_1}$ (Table 1). From this finding, the thiazole moiety was considered to be the minimum structural requirement for anti-MRSA activity.

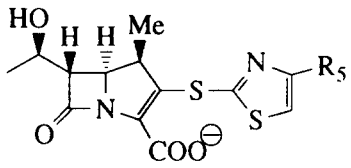
We focused on the synthesis of substituted thiazole derivatives in an attempt to improve anti-MRSA activity and to reduce HSA binding affinity by means of the substituent effect on thiazole moiety, because the synthesis of thiazole derivatives was easier than that of oxazole derivatives and the chemical modification of the benzothiazole derivatives was confined to the expansion of a variety of derivatives with limited molecular



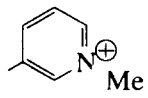
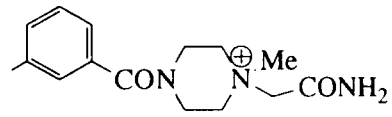
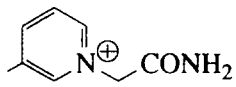
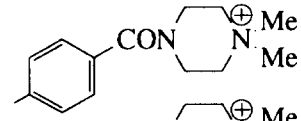
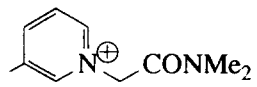
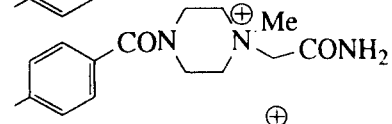
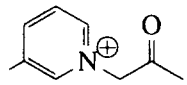
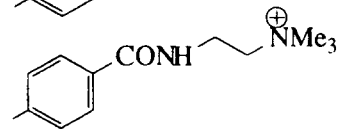
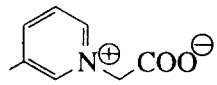
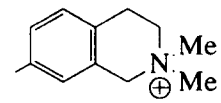
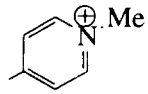
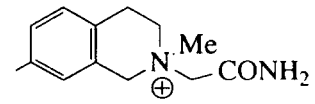
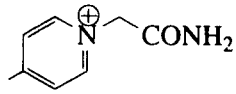
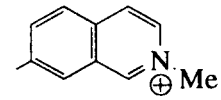
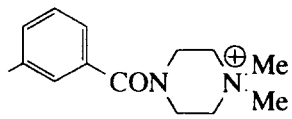
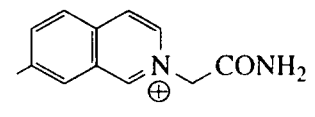

c33

d40 (61%)

**(Chart 4)**

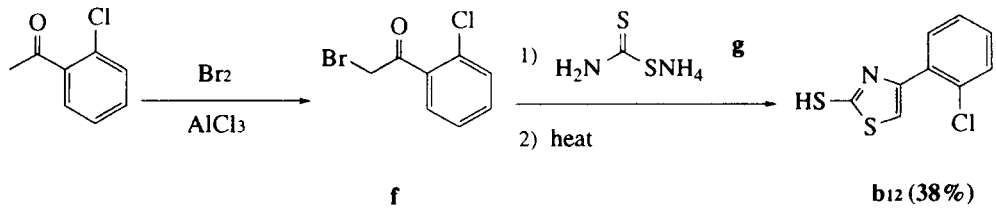


d34-d49

| R5 | | R5 | |
|---|---|---|---|
| d34 |  | d42 |  |
| d35 |  | d43 |  |
| d36 |  | d44 |  |
| d37 |  | d45 |  |
| d38 |  | d46 |  |
| d39 |  | d47 |  |
| d40 |  | d48 |  |
| d41 |  | d49 |  |

weights[33] (<500), which was important from a pharmacokinetics viewpoint, especially the excretion of the compound in vivo.

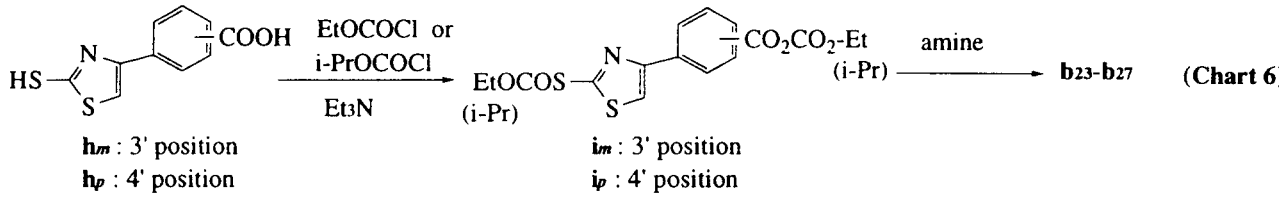
The properties of 14 carbapenems, which have a variety of substituents at C-4 and C-5 of the thiazole ring, are shown in Table 2. The anti-MRSA activity of $\mathbf{d_4}$, which had a methyl substituent at the C-4′ position, was superior to that of $\mathbf{d_3}$. However, the elongation of the alkyl chain, for example *n*-propyl ($\mathbf{d_5}$) and iso-propyl group ($\mathbf{d_6}$), did not further improve the activity. Electron-withdrawing groups, such as acetyl ($\mathbf{d_7}$) and dimethylaminocarbonyl ($\mathbf{d_8}$), at C-4′ had no effect on the anti-MRSA activity. However, the introduction of a phenyl group ($\mathbf{d_9}$) at C-4′ markedly improved the anti-MRSA activity. On the other hand, the introduction of




f

b12 (38%)

(Chart 5)

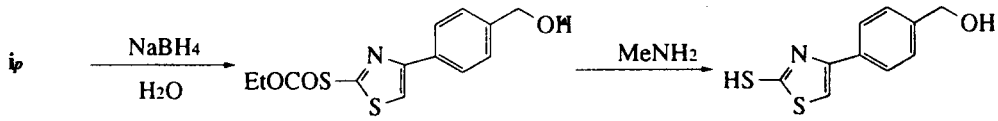



hm : 3' position
hp : 4' position

im : 3' position
ip : 4' position

b23-b27

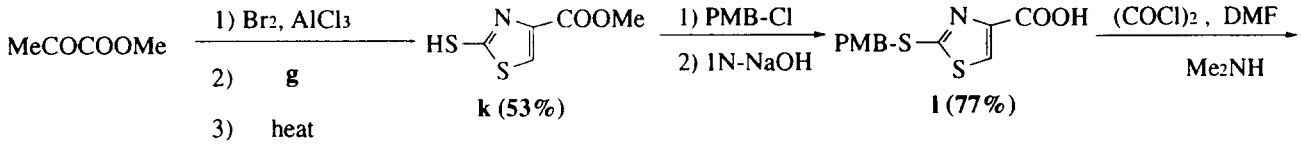
(Chart 6)




j (57%)

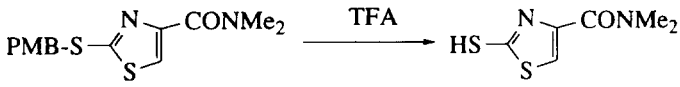
b19 (85%)

(Chart 7)




k (53%)

l (77%)

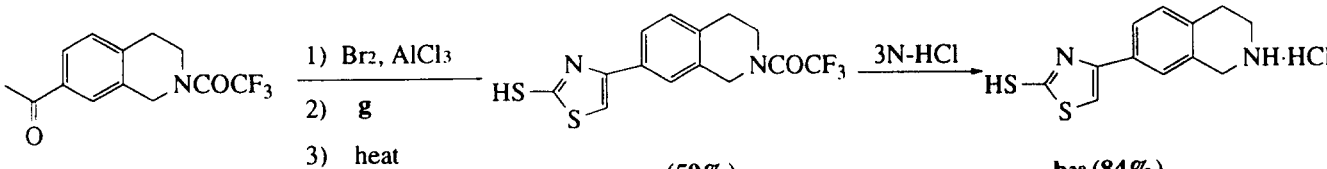



m (98%)

b8 (67%)

PMB=*p*-methoxybenzyl

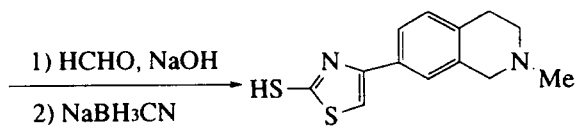
(Chart 8)




n

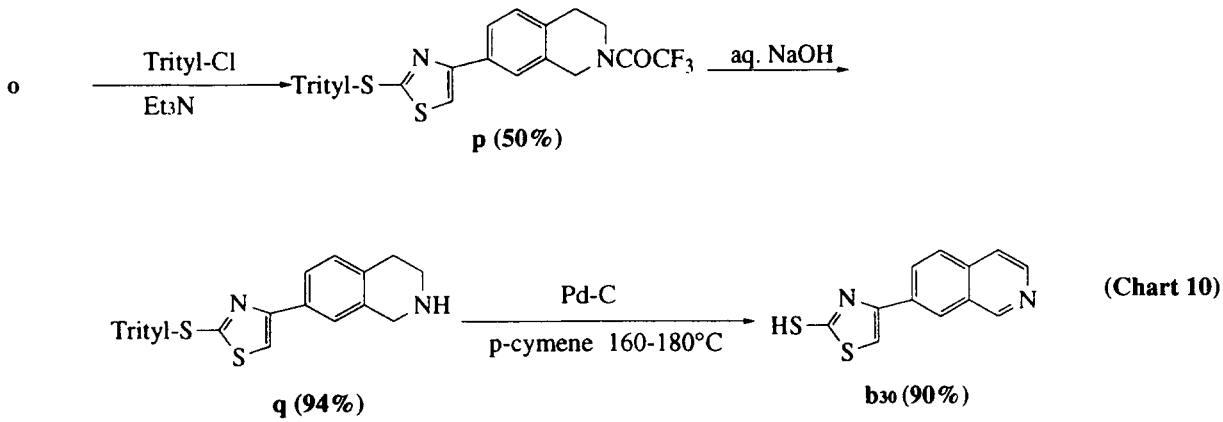
o (59%)

b28 (84%)



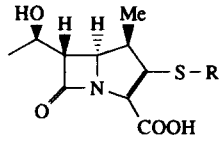

b29 (38%)

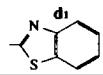
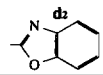
(Chart 9)




(Chart 10)

**Table 1.** Antibacterial activity, DHP-I stability, HSA binding and PBP-2′ affinity of carbapenems




| Organism | R= | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| | |  |  |  | CP0467 | Imipenem |
| *S.a.* MS9408 | | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| *S.a.* SP-7928 | | 3.13 | 6.25 | 12.5 | 0.78 | 50 |
| *S.a.* 209p | | 0.025 | <0.013 | 0.025 | 0.05 | 0.013 |
| *S.e.* IAM1296 | | 0.2 | 0.20 | 0.10 | 0.05 | 0.20 |
| *S.p.* COOK | | <0.013 | <0.013 | 0.025 | <0.013 | <0.006 |
| *K.p.* ATCC 10031 | | 0.78 | 0.20 | 0.05 | 0.025 | 0.10 |
| *E.c.* NIHJ JC-2 | | 25 | 6.25 | 0.78 | 0.39 | 0.10 |
| DHP-I stability* (min) | | n.t. | n.t. | 15 | n.t. | 12 |
| HSA binding (%) | | >94 | >94 | 69 | >94 | <10 |
| PBP-2′ affinity ($IC_{50}$) | | 6.6 | 2.6 | 58.1 | 4.6 | 124 |

*The number indicates the time of enzyme-catalyzed hydrolysis of the compound from 500–400 μM in the presence of perified 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.

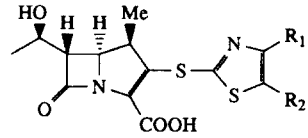
a phenyl group ($\mathbf{d_{11}}$) at C-5′ was not effective in improving the activity nor was the introduction of a methyl group ($\mathbf{d_{10}}$) at C-5′ of $\mathbf{d_4}$.

It was confirmed that the exsistence of an aromatic ring at C-4 of the thiazole ring enhanced the anti-MRSA activity, since the introduction of other aryl groups at C-4′ such as naphthalene ($\mathbf{d_{21}}$, $\mathbf{d_{22}}$), isoquinoline ($\mathbf{d_{30}}$), and pyridine ($\mathbf{d_{31}}$–$\mathbf{d_{33}}$) showed similar effects to that of the phenyl group. As for the positional effects of the pyridine ring, no significant difference among $\mathbf{d_{31}}$, $\mathbf{d_{32}}$, and $\mathbf{d_{33}}$ was observed.

Having comparable activities with **CP0467**, these C-4′ aryl derivatives, especially $\mathbf{d_9}$ and $\mathbf{d_{30}}$, had a potent anti-MRSA activity in vitro, but their HSA binding was too strong to get sufficient in vivo efficacy. Therefore, we started chemically modifying C-4′ aryl derivatives in an attempt to reduce their HSA binding without reducing the anti-MRSA activity. Thus, variously substituted phenyl derivatives of $\mathbf{d_9}$ were prepared in order to identify the substitution pattern and the functional group which would achieve this.

Table 3 shows the assay results of $\mathbf{d_{12}}$–$\mathbf{d_{20}}$, $\mathbf{d_{23}}$, and $\mathbf{d_{24}}$, which have chloro, methyl, amino, hydroxymethyl, aminomethyl, and carbamoyl substituents at the *ortho*, *meta*, or *para*-position of the phenyl ring of $\mathbf{d_9}$. All of the substituted phenyl derivatives exhibited the same level of anti-MRSA activity as that of $\mathbf{d_9}$. Thus, it was found that the introduction of the substituent on the phenyl ring had little effect on their anti-MRSA activity.

**Table 2.** Antibacterial activity, DHP-I stability, HSA binding and PBP-2′ affinity of carbapenems




| Organism | $R_1$= $R_2$= | MIC (μg/mL) d4 Me H | d5 n-Pr H | d6 i-Pr H | d7 Ac H | d8 $CONMe_2$ H | d9 Ph H | d10 Me Me | d11 H Ph |
|---|---|---|---|---|---|---|---|---|---|
| *S.a.* MS9408 | | 0.39 | 0.39 | 0.39 | 1.56 | 1.56 | 0.10 | 0.78 | 0.39 |
| *S.a.* SP-7928 | | 3.13 | 3.13 | 6.25 | 12.5 | 12.5 | 0.78 | 6.25 | 12.5 |
| *S.a.* 209p | | 0.025 | 0.025 | 0.025 | 0.025 | 0.05 | <0.013 | 0.025 | <0.006 |
| *S.e.* IAM1296 | | 0.05 | 0.05 | 0.025 | 0.10 | 0.10 | 0.025 | 0.10 | 0.39 |
| *S.p.* COOK | | 0.025 | <0.006 | <0.006 | 0.025 | 0.025 | <0.013 | 0.025 | 0.013 |
| *K.p.* ATCC 10031 | | 0.05 | 0.20 | 0.20 | 0.05 | 0.10 | 0.10 | 0.02 | 0.39 |
| *E.c.* NIHJ JC-2 | | 3.13 | 12.5 | 12.5 | 1.56 | 3.13 | 6.25 | 3.13 | 25 |
| DHP-I stability* (min) | | 13 | 12 | 14 | 5.6 | 13 | 9.8 | 23 | 15 |
| HSA binding (%) | | 93 | 96 | 95 | 87 | 57 | >94 | >75 | >94 |
| PBP-2′ affinity ($IC_{50}$) | | 4.2 | 30.7 | 33.2 | 36.9 | 41.9 | 10.6 | 32.2 | >100 |

| Organism | $R_1$= $R_2$= | MIC (μg/mL) d21 H | d22 H | d30 H | d31 H | d32 H | d33 H |
|---|---|---|---|---|---|---|---|
| *S.a.* MS9408 | | 0.10 | 0.10 | 0.10 | 0.39 | 0.20 | 0.20 |
| *S.a.* SP-7928 | | 3.13 | 1.56 | 0.78 | 3.13 | 1.56 | 3.13 |
| *S.a.* 209p | | <0.006 | <0.013 | <0.006 | <0.013 | <0.013 | <0.013 |
| *S.e.* IAM1296 | | 0.013 | 0.025 | 0.025 | 0.05 | 0.025 | 0.5 |
| *S.p.* COOK | | <0.006 | <0.013 | <0.006 | <0.006 | <0.013 | <0.013 |
| *K.p.* ATCC 10031 | | 0.39 | 0.20 | 0.20 | 0.10 | 0.05 | 0.20 |
| *E.c.* NIHJ JC-2 | | 12.5 | 12.5 | 12.5 | 6.25 | 6.25 | 6.25 |
| DHP-I stability* (min) | | 9.2 | n.t. | 9.7 | 11 | 3.2 | 9.8 |
| HSA binding (%) | | >94 | >94 | 99 | 99 | 97 | >94 |
| PBP-2′ affinity ($IC_{50}$) | | 63.5 | 62.8 | 19.4 | n.t. | 21.0 | 10.6 |

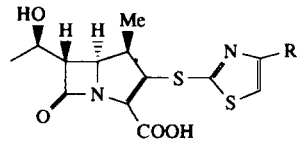
As anticipated, all these compounds showed the same high binding affinity to HSA. Among them, however, aminomethylphenyl derivative **$d_{20}$** showed significantly lower HSA binding (87%). The introduction of the basic moiety brought about the reduction of HSA binding, as demonstrated previously.[34] The antibacterial activity against *Escherichia coli* and the stability against DHP-I of **$d_{20}$** also improved. These improvements were due to the increased hydrophilicity and the introduction of the basic moiety in the C-2 side chain, respectively.[35–37] As for this series' improvement in stability against DHP-I, the stability of **$d_{18}$** also improved in spite of the weak basicity of the amine. In contrast, the stability against DHP-I of **$d_{17}$** did not improve unlike that of **$d_{18}$**. This was probably due to the difference of the relative geometry between the thiazole ring and the aminophenyl group resulting from the steric hindrance of the *ortho*-substituent.

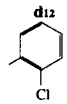
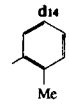
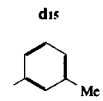
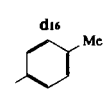
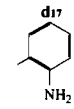
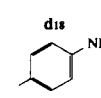
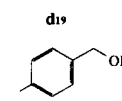
A series of carbapenem derivatives bearing a variety of amino groups on the phenyl ring were synthesized (Table 4). Though most of these compounds exhibited moderate to high anti-MRSA activity, the introduction of the amino-substituted phenyl group did not show the desired reduction in HSA binding.

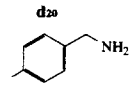
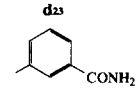
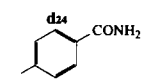
Next, we attempted to quaternize the amino moiety to further reduce the HSA binding by having a cationic moiety present in the C-2 side chain. Table 5 shows the biological data of the carbapenems, quaternized by various alkyl halides.

Compared to their parent amino compounds (**$d_{25}$-$d_{27}$**, **$d_{29}$–$d_{33}$**), the quaternization did not significantly affect the anti-MRSA activity but affected the activity against gram-negative bacteria, especially anti-*E. coli*, due to their improved permeability of the outer membrane.[37]

**Table 3.** Antibacterial activity, DHP-I stability, HSA binding and PBP-2′ affinity of carbapenems



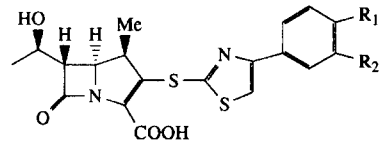
| | | MIC (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R= | $d_{12}$  | $d_{13}$  | $d_{14}$  | $d_{15}$  | $d_{16}$  | $d_{17}$  | $d_{18}$  | $d_{19}$  |
| **Organism** | | | | | | | | | |
| *S.a.* MS9408 | | 0.20 | 0.20 | 0.39 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| *S.a.* SP-7928 | | 1.56 | 1.56 | 3.13 | 1.56 | 3.13 | 3.13 | 1.56 | 1.56 |
| *S.a.* 209p | | 0.013 | <0.013 | 0.013 | <0.006 | <0.006 | 0.013 | <0.013 | <0.006 |
| *S.e.* IAM1296 | | 0.025 | 0.025 | 0.05 | 0.025 | 0.025 | 0.05 | 0.025 | 0.025 |
| *S.p.* COOK | | <0.006 | <0.013 | 0.013 | <0.006 | <0.006 | <0.006 | <0.013 | <0.006 |
| *K.p.* ATCC 10031 | | 0.20 | 0.20 | 0.39 | 0.20 | 0.39 | 0.10 | 0.05 | 0.10 |
| *E.c.* NIHJ JC-2 | | 12.5 | 12.5 | 12.5 | 12.5 | 12.5 | 6.25 | 6.25 | 6.25 |
| DHP-I stability* (min) | | 9.7 | 9.9 | n.t. | n.t. | 9.2 | 12 | 18 | 8.9 |
| HSA binding (%) | | >94 | >94 | n.t. | n.t. | >94 | 98 | 98 | 98 |
| PBP-2′ affinity ($IC_{50}$) | | 7.8 | 25.1 | 42.9 | 24.4 | 38.2 | 9.2 | 15.3 | 5.3 |

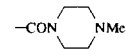
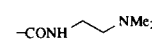
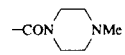
| | | MIC (μg/mL) | | |
|---|---|---|---|---|
| | R= | $d_{20}$  | $d_{23}$  | $d_{24}$  |
| **Organism** | | | | |
| *S.a.* MS9408 | | 0.20 | 0.20 | 0.20 |
| *S.a.* SP-7928 | | 1.56 | 1.56 | 1.56 |
| *S.a.* 209p | | <0.006 | <0.013 | <0.006 |
| *S.e.* IAM1296 | | 0.025 | 0.025 | 0.025 |
| *S.p.* COOK | | <0.006 | <0.013 | <0.006 |
| *K.p.* ATCC 10031 | | 0.20 | 0.20 | 0.10 |
| *E.c.* NIHJ JC-2 | | 1.56 | 12.5 | 6.25 |
| DHP-I stability* (min) | | 17 | 9.6 | 12 |
| HSA binding (%) | | 87 | 98 | 99 |
| PBP-2′ affinity ($IC_{50}$) | | 76.3 | 27.2 | 7.1 |

The quaternization, however, showed the expected, marked reduction in the HSA binding affinity in most cases. It was confirmed that not only the introduction of the cationic moiety in the C-2 side chain reduced HSA binding, but also the degree of cationic character was related to the degree of reduction in HSA binding. However, the HSA binding of $\mathbf{d_{45}}$ was not less than that of the parent compound $\mathbf{d_{27}}$. The reduction of HSA binding in $\mathbf{d_{41}}$–$\mathbf{d_{44}}$ and in $\mathbf{d_{46}}$–$\mathbf{d_{49}}$ was not significant, compared with that of the pyridine derivatives $\mathbf{d_{34}}$–$\mathbf{d_{40}}$ upon quaternization. In the highly basic amino derivatives, the amine was already protonated as ammonium ion in the measuring media. However, the difference of HSA binding affinity between the pyridinium derivatives and the isoquinolinium derivatives ($\mathbf{d_{48}}$ and $\mathbf{d_{49}}$) and the non effect of the $\mathbf{d_{45}}$ quaternization were not solely explained by the above reason. The distance between the cationic moiety and the carbapenem skeleton and their relative geometries might be correlated to the strength of HSA binding, since the HSA binding tended to be lowered by shortening this distance.

It was also found that the distance between the cationic moiety and the carbapenem skeleton influenced the stability against DHP-I. The introduction of a cationic moiety in the C-2 side chain improved the DHP-I stability,[36,37] however, the relationship between this distance and DHP-I stability has not been investigated. Among the quaternary ammonium carbapenem derivatives in Table 5, the quaternary pyridinium derivatives ($\mathbf{d_{34}}$–$\mathbf{d_{40}}$) exhibited significantly higher stability against DHP-I than those of their parent compounds. On the other hand, such significant change was not observed in the isoquinolinium derivatives ($\mathbf{d_{48}}$ and $\mathbf{d_{49}}$) as well as some other quaternary ammonium derivatives

**Table 4.** Antibacterial activity, DHP-I stability, HSA binding and PBP-2′ affinity of carbapenems




| | | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|---|
| | | $d_{25}$ | $d_{26}$ | $d_{27}$ | $d_{28}$ | $d_{29}$ |
| | $R_1$= | H |  |  |  |  |
| | $R_2$= |  | H | H | | |
| **Organism** | | | | | | |
| *S.a.* MS9408 | | 0.39 | 0.39 | 0.20 | 0.20 | 0.20 |
| *S.a.* SP-7928 | | 6.25 | 3.13 | 0.78 | 0.78 | 0.78 |
| *S.a.* 209p | | 0.025 | 0.013 | <0.006 | <0.006 | 0.013 |
| *S.e.* IAM1296 | | 0.025 | 0.025 | 0.013 | 0.025 | 0.025 |
| *S.p.* COOK | | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 |
| *K.p.* ATCC 10031 | | 0.20 | 0.10 | 0.05 | 0.10 | 0.20 |
| *E.c.* NIHJ JC-2 | | 50 | 12.5 | 6.25 | 6.25 | 6.25 |
| DHP-I stability* (min) | | 9.6 | n.t. | 8.7 | 9.9 | 12 |
| HSA binding (%) | | 85 | n.t. | 89 | 85 | 86 |
| PBP-2′ affinity ($IC_{50}$) | | 35.7 | 20.9 | 5.5 | 14.7 | 28.8 |

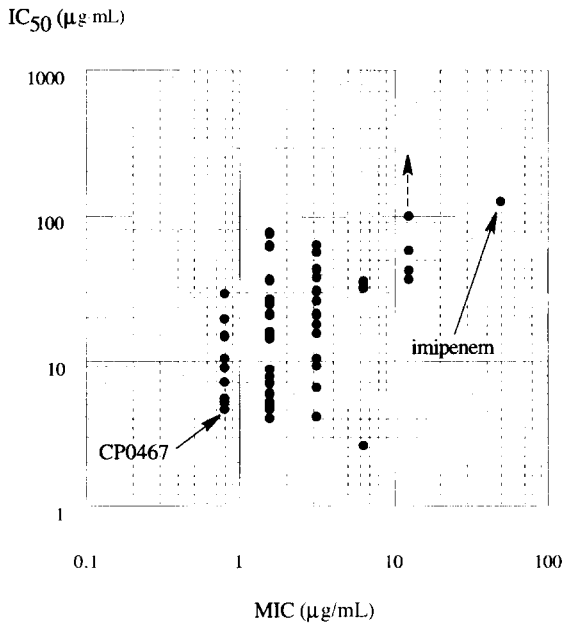
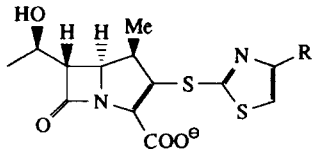



**Figure 3.** The relationship between MICs against *S. a.* SP-7928 and affinity to PBP-2′.

($d_{41}$–$d_{47}$). Furthermore, the DHP-I stabilities of the highly basic amino derivatives ($d_{25}$–$d_{29}$) in Table 4 were similar to those of the derivatives having no basic moiety in the C-2 side chain, such as the phenyl derivatives $d_9$, $d_{12}$, $d_{13}$, etc. despite the presence of the highly basic amino moiety. As discussed above, the improved DHP-I stability was also observed in the aminomethylphenyl derivative $d_{20}$ and aminophenyl derivative $d_{18}$, upon introduction of a basic moiety. These findings indicated that at least the distance between the cationic moiety and the carbapenem skeleton strongly influenced the DHP-I stability of the carbapenem compound having a cationic moiety in the C-2 side chain, though the relative geometry of the cationic moiety to carbapenem skeleton may have played an important role. The introduction of a cationic moiety into the C-2 side chain of the carbapenem enhanced the DHP-I stability only when the distance between the cationic moiety and the oxygen atom of the carboxylic acid group in the carbapenem structure was within some limited distance, around 8 Å in the case of this work.[38]

From the data listed in Tables 1–5, it is very difficult to find a clear relationship between the anti-MRSA activity and the affinity to PBP-2′ in this series of carbapenems. Compounds $d_4$, $d_{17}$, and $d_{33}$, of which affinities to PBP-2′ were fairly high ($IC_{50}$: 4.2, 9.2, and 10.6 μg/mL), exhibited only moderate anti-MRSA activities (MIC: 3.13 μg/mL against *S.a.* SP-7928), while $d_{22}$ and $d_{29}$ showed potent anti-MRSA activities (MIC: 1.56 and 0.78 μg/mL against *S.a.* SP-7928) in spite of their low affinities to PBP-2′ ($IC_{50}$: 62.8 and 28.8 μg/mL). Especially among the compounds having a relatively high affinity to PBP-2′ ($IC_{50}$: <30 μg/mL), the relationship between the anti-MRSA activity and the affinity to PBP-2′ seems ambiguous. However, a slight relationship between the anti-MRSA activity and the affinity to PBP-2′ was observed from the logarithmic graph of the MICs against *S.a.* SP-7928, which was highly resistant, versus the affinity to PBP-2′ shown in Figure 3.

**Table 5.** Antibacterial activity, DHP-I stability, HSA binding and PBP-2′ affinity of carbapenems



| Organism | R= | MIC (μg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | d34 | d35 | d36 | d37 | d38 | d39 | d40 |
| *S.a.* MS9408 | | 0.20 | 0.20 | 0.39 | 0.20 | 0.78 | 0.10 | 0.20 |
| *S.a.* SP-7928 | | 1.56 | 1.56 | 3.13 | 1.56 | 6.25 | 1.56 | 1.56 |
| *S.a.* 209p | | <0.013 | <0.006 | <0.006 | 0.013 | 0.025 | <0.006 | <0.006 |
| *S.e.* IAM1296 | | 0.025 | 0.025 | 0.05 | 0.025 | 0.10 | 0.025 | 0.05 |
| *S.p.* COOK | | <0.013 | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 |
| *K.p.* ATCC 10031 | | 0.05 | 0.05 | 0.05 | 0.025 | 0.025 | 0.025 | 0.025 |
| *E.c.* NIHJ JC-2 | | 0.39 | 0.39 | 0.78 | 0.78 | 0.39 | 0.39 | 0.20 |
| DHP-I stability* (min) | | 28 | 17 | 16 | 17 | 18 | 23 | 21 |
| HSA binding (%) | | 7.8 | 24 | 9.5 | 25 | 32 | 12 | 15 |
| PBP-2′ affinity ($IC_{50}$) | | 4.0 | 14.0 | 26.0 | 36.1 | 34.6 | 4.6 | 5.9 |

| Organism | MIC (μg/mL) | | | |
|---|---|---|---|---|
| | d41 | d42 | d43 | d44 |
| *S.a.* MS9408 | 0.20 | 0.39 | 0.20 | 0.39 |
| *S.a.* SP-7928 | 1.56 | 3.13 | 3.13 | 3.13 |
| *S.a.* 209p | 0.013 | 0.013 | <0.006 | 0.013 |
| *S.e.* IAM1296 | 0.013 | 0.025 | 0.025 | 0.025 |
| *S.p.* COOK | <0.006 | <0.006 | <0.006 | 0.013 |
| *K.p.* ATCC 10031 | 0.05 | 0.05 | 0.05 | 0.05 |
| *E.c.* NIHJ JC-2 | 12.5 | 12.5 | 3.13 | 3.13 |
| DHP-I stability* (min) | 8.5 | 9.2 | 11 | 10 |
| HSA binding (%) | 34 | 46 | 64 | 64 |
| PBP-2′ affinity ($IC_{50}$) | 14.0 | 56.5 | 15.6 | 18.2 |

| Organism | MIC (μg/mL) | | | | |
|---|---|---|---|---|---|
| | d45 | d46 | d47 | d48 | d49 |
| *S.a.* MS9408 | 0.20 | 0.20 | 0.10 | 0.05 | 0.05 |
| *S.a.* SP-7928 | 1.56 | 1.56 | 0.78 | 0.78 | 0.78 |
| *S.a.* 209p | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 |
| *S.e.* IAM1296 | 0.025 | 0.025 | <0.006 | 0.013 | 0.013 |
| *S.p.* COOK | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 |
| *K.p.* ATCC 10031 | 0.05 | 0.20 | 0.05 | 0.05 | 0.025 |
| *E.c.* NIHJ JC-2 | 3.13 | 50 | 6.25 | 3.13 | 1.56 |
| DHP-I stability* (min) | 8 | 9 | 7.9 | 12 | 11 |
| HSA binding (%) | 88 | 63 | 69 | 59 | 74 |
| PBP-2′ affinity ($IC_{50}$) | 4.8 | 8.8 | 5.3 | 7.2 | 9.0 |

In conclusion, we found that the introduction of a mercaptothiazole group in the carbapenem C-2 side chain enhanced anti-MRSA activity. Finally, we synthesized potent anti-MRSA agents such as **$d_{40}$**, which exhibited not only high anti-MRSA activity but also a well-balanced in vitro antibacterial spectrum against gram-positive and gram-negative bacteria. The potent anti-MRSA activity of **$d_{40}$** was also confirmed in vivo.[39] We also found that the distance between the cationic amino moiety on the C-2 side chain and the carbapenem skeleton was related to the strength of HSA binding and the stability against DHP-I. We hope that these findings will be helpful in developing a future generation of carbapenem antibiotics.

## Experimental

**General analytical methods**. Melting points were recorded on a Thomas–Hoover capillary apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1600 FTIR spectrophotometer. $^1$H NMR spectra were taken with a JEOL JNM-GX270 (270 MHz) FT spectometer, in the designated solvent, using tetramethylsilane or residual DOH (δ 4.81) as an internal reference. UV spectra were recorded on a Hitachi 330 UV-vis spectophotometer. Mass spectra were obtained on Hitachi DF/GC/MS M-80B and DPS M-0101 (3 kV) spectrometers. High-resolution mass spectra were recorded on a JEOL JMS-AX-505. Optical rotations were determined on a Jasco DIS-370 digital polarimeter. Column chromatograghy was carried out on Silica gel 60 (70–230 mesh, E. Merck).

**4-(2-Chlorophenyl)-2-thiazolyl thiol ($b_{12}$)**. Bromine (0.44 mL, 8.56 mmol) was added dropwise to a mixture of 2′-chloroacetophenone (1 mL, 8.56 mmol) and aluminium chloride (10 mg) in dry ethyl ether (5 mL) with stirring at 0 °C. Then the mixture was stood up to rt and continued to stir for 3 h. The reaction mixture was diluted with toluene, then extracted with 2.5% $KH_2PO_4$ and brine. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo to give 2-bromo-2′-chloroacetophenone.

Ammonium dithiocarbamate (**g**) (943 mg, 8.56 mmol) was added to a suspension of 2-bromo-2′-chloroacetophenone (8.56 mmol) in ethanol (30 mL) and the mixture was stirred for 20 h at rt and then heated at 70–80 °C for 30 min. The reaction mixture was cooled to room temperature, and the crystal was filtered, washed with ethanol and dried in vacuo to give **$b_{12}$** as a white crystal (739 mg, 38%): mp 163 °C. $^1$H NMR (270 MHz, $CDCl_3$) δ 6.77 (1H, s), 7.35–7.53 (4H, m), 10.99 (1H, brs); IR (KBr) cm$^{-1}$ 1464, 1332, 1286, 1253, 1066; MS *m/z* 227 (M, $C_9H_6NS_2Cl$). Anal. calcd for $C_9H_6NS_2Cl$: C 47.47, H 2.66, N 6.15. Found: C 47.44, H 2.63, N 6.24.

**4-(2-Mercaptothiazol-4-yl)benzoic acid ($h_p$)**. Bromine (0.31 mL, 6.09 mmol) was added dropwise to a mixture of 4-acetyl benzoic acid (1 g, 6.09 mmol) and aluminum chloride (10 mg) in dry ethyl ether (10 mL) with stirring at 0 °C. Then the mixture was stood up to rt and continued to stir for 3 h. The precipitate was filtered and washed with ethyl ether and water, then dried in vacuo to give 4-bromoacetyl benzoic acid (1.047 g, 71%).

Ammonium dithiocarbamate (**g**) (7.637 g, 69.3 mmol) was added to a suspension of 4-bromoacetyl benzoic acid (13.320 g, 54.8 mmol) in ethanol (100 mL) and the mixture was stirred for 20 h at rt and then heated at 70–80 °C for 30 min. The reaction mixture was cooled to rt, and the crystal was filtered and washed with ethanol and dried in vacuo to give **$h_p$** as a white crystal (9.712 g, 75 %): mp >300 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 7.44 (1H, s), 7.88 (2H, d, $J$ = 8.6 Hz), 7.97 (2H, d, $J$ =8.6 Hz); IR (KBr) cm$^{-1}$ 1684, 1608, 1469, 1422, 1301; MS *m/z* 237 (M, $C_{10}H_7NO_2S_2$).

The following compounds (**$h_m$**, **$b_{14}$**, **$b_{17}$**, and **$b_{20}$**) were prepared from the corresponding acetophenone derivatives as described for the preparation of **$b_{12}$** or **$h_p$**, respectively.

**$h_m$**. Mp >300 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 7.42 (1H, s), 7.58 (1H, t, $J$ = 7.8 Hz), 7.97 (2H, m), 8.31 (1H, m); IR (KBr) cm$^{-1}$ 1687, 1478, 1432, 1395, 1349, 1278, 1255; MS *m/z* 237 (M, $C_{10}H_7NO_2S_2$). Anal. calcd for $C_{10}H_7NO_2S_2$: C 50.61, H 2.97, N 5.90. Found: C 50.23, H 3.09, N 5.61.

**$b_{14}$**. Mp 158 °C. $^1$H NMR (270 MHz, $CDCl_3$) δ 2.40 (3H, s), 6.48 (1H, s), 7.27–7.40 (4H, m), 10.80 (1H, brs); IR (KBr) cm$^{-1}$ 1482, 1449, 1277, 1257, 1067; MS *m/z* 207 (M, $C_{10}H_9NS_2$). Anal. calcd for $C_{10}H_9NS_2$: C 57.93, H 4.38, N 6.76. Found: C 57.57, H 4.37, N 6.73.

**$b_{17}$**. Mp 172–173 °C. $^1$H NMR (270 MHz, $CDCl_3$) δ 6.58 (1H, s), 7.55 (1H, dd, $J$ = 7.3 Hz and 1.7 Hz), 7.64–7.78 (2H, m), 8.10 (1H, dd, $J$ = 1.7 Hz and 7.9 Hz); IR (KBr) cm$^{-1}$ 1519, 1461, 1434, 1351, 1259, 1069; MS *m/z* 237 (M, $C_{10}H_7NO_2S_2$). Anal. calcd for $C_{10}H_7NO_2S_2$: C 45.36, H 2.54, N 11.76. Found: C 45.09, H 2.55, N 11.65.

**$b_{20}$**. Mp 148–153 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 4.29 (2H, m), 5.20 (2H, s), 7.2–7.5 (2H, m), 7.62 (2H, d, $J$ = 8.3 Hz), 7.71 (1H, m), 7.91–8.09 (2H, m), 8.25 (2H, d, $J$ = 8.3 Hz); IR (KBr) cm$^{-1}$ 1697, 1521, 1348, 1264, 1061; MS *m/z* 401 (M, $C_{18}H_{15}N_3O_4S_2$).

**4-Methyl-1-(4-(2-mercaptothiazol-4-yl)phenylcarbonyl)-piperazine ($b_{26}$)**. To a solution of 4-(2-mercaptothiazol-4-yl)benzoic acid (**$h_p$**) (1 g, 4.21 mmol) and triethylamine (938 mg, 9.27 mmol) in dry THF (10 mL) was added dropwise isopropyl chloroformate (1.136 g, 9.27 mmol) at −78 °C. The mixture was gradually stood up to −30 °C and then 1-methyl piperazine (843 mg, 8.42 mmol) was added dropwise. The reaction mixture was gradually stood up to 0 °C and then stirred for 1 h. The reaction mixture was diluted with EtOAc, then washed with aqueous $NaHCO_3$ (pH 8.0). The organic layer was dried

($Na_2SO_4$) and concentrated in vacuo. The residue was washed with ethyl ether to give **$b_{26}$** as a crude amorphous (134 mg, 10 %). This crude sample was used in the next process without further purification: $^1$H NMR (270 MHz, DMSO-$d_6$) δ 1.77 (2H, m), 2.20, 2.23 (totally 3H, s respectively), 2.29 (2H, m), 3.34 (2H, m), 3.61 (2H, m), 7.44 (3H, m), 7.86 (2H, d, $J$ = 7.9 Hz); IR (neat) cm$^{-1}$ 1651, 1458, 1435, 1271.

The following compounds (**$b_{23}$**, **$b_{24}$**, **$b_{25}$**, and **$b_{27}$**) were prepared from **$h_m$** or **$h_p$** as described for the preparation of **$b_{26}$**, respectively.

**$b_{23}$**. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 7.39 (1H, s), 7.51 (1H, br), 7.59 (1H, t, $J$ = 7.6 Hz), 7.87 (2H, brd, $J$ = 7.6 Hz), 7.97 (1H, br), 8.23 (1H, t, $J$ = 1.7 Hz), 13.67 (1H, s).

**$b_{24}$**. Mp 175 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 7.96 (2H, d, $J$ = 8.6 Hz), 8.03 (2H, d, $J$ = 8.6 Hz), 8.52 (1H, s).

**$b_{25}$**. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 2.24, 2.25 (totally 3H, s respectively), 2.34 (2H, t, $J$ = 5.1 Hz), 7.37–7.42 (2H, m), 7.52 (1H, t, $J$ = 7.8 Hz), 7.78–7.86 (2H, m); IR (neat) cm$^{-1}$ 1693, 1633, 1434, 1291.

**$b_{27}$**. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 2.23 (6H, s), 2.47 (2H, t, $J$ = 6.4 Hz), 3.37 (2H, t, $J$ = 6.4 Hz), 7.91 (2H, d, $J$ = 8.6 Hz), 8.00 (2H, d, $J$ = 8.6 Hz), 8.27 (1H, s); IR (KBr) cm$^{-1}$ 3424 (br), 1654, 1559, 1542, 1289.

**2-(Ethoxycarbonyl)thio-4-(4-hydroxymethylphenyl)thiazole (j)**. To a solution of 4-(2-mercaptothiazol-4-yl)benzoic acid (**$h_p$**) (5 g, 21.1 mmol) and triethylamine (6.47 mL, 46.4 mmol) in dry THF (35 mL) and dry DMF (35 mL) was added dropwise ethyl chloroformate (4.44 mL, 46.4 mmol) under the nitrogen atmosphere at −78 °C. The mixture was gradually stood up to −20 °C and then sodium borohydride (3.193 g, 84.4 mmol) with water (6 mL) was gradually added. The reaction mixture was stood up to 0 °C and then stirred for 1 h. The reaction mixture was acidified (pH 6–7) with 1 N HCl and extracted with EtOAc and washed with brine. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene/EtOAc) to give **j** (3.532 g, 57%): $^1$H NMR (270 MHz, $CDCl_3$) δ 0.98 (3H, t, $J$ = 7.2 Hz), 4.01 (2H, q, $J$ = 7.2 Hz), 4.33 (2H, s), 7.02 (2H, d, $J$ = 7.9 Hz), 7.26 (1H, s), 7.48 (2H, d, $J$ = 7.9 Hz).

**4-(4-Hydroxymethylphenyl)-2-thiazolyl thiol (b$_{19}$)**. To a solution of 2-(ethoxycarbonyl)thio-4-(4-hydroxymethylphenyl) thiazole (**j**) (3.525 g, 11.9 mmol) in MeOH (80 mL) was added 30% methylamine (6.17 g, 59.7 mmol) under the nitrogen atmosphere at 0 °C. The mixture was stood up to rt and stirred for 1 h. The mixture was concentrated in vacuo and the solid residue was triturated in ethanol to give **$b_{19}$** as a colorless crystal (2.248 g, 85%): mp 161–168 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 4.54 (2H, s), 6.66 (1H, s), 7.28 (2H, d, $J$ = 8.4 Hz), 7.44 (2H, d, $J$ = 8.4 Hz). IR (KBr) cm$^{-1}$ 3000 (br), 1369, 1025.

**4-(2-Trifluoroacetyl-1,2,3,4-tetrahydroisoquinol-7-yl)-2-thiazolyl thiol (o)**. Bromine (0.84 mL, 16.2 mmol) was added dropwise to a mixture of 7-acetyl-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (**n**) (4.400 g, 16.2 mmol) and aluminum chloride (44 mg) in dry ethyl ether with stirring at 0 °C. The mixture was stood up to rt and was continued to stir for 3 h. The precipitate was filtered and washed with ethyl ether and water, then dried in vacuo to give 7-bromoacetyl-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (3.529 g, 62 %): $^1$H NMR (270 MHz, $CDCl_3$) δ 3.04 (2H, m), 3.91 (2H, m), 4.42 (2H, s), 4.87 (2H, s), 7.32 (1H, m), 7.78–7.87 (2H, m).

To a suspension of 7-bromoacetyl-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinoline (3.529 g, 10.1 mmol) in ethanol (20 mL) was added ammonium dithiocarbamate (**g**) (1.224 g, 11.1 mmol) and the reaction mixture was stirred for 20 h and then heated at 70-80 °C for 1 h. The reaction mixture was cooled to room temperature, and the precipitate was filtered and washed with ethanol and dried in vacuo to give **o** as a white crystal (3.294 g, 95%): mp 232–235 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 2.95 (2H, m), 3.84 (2H, t, $J$ = 5.9 Hz), 4.83 (2H, s), 7.25–7.30 (2H, m), 7.57–7.69 (2H, m); IR (KBr) cm$^{-1}$ 1683, 1597, 1507, 1460, 1438, 1201, 1182, 1148; MS $m/z$ 344 (M, $C_{14}H_{11}N_2OS_2F_3$). Anal. calcd for $C_{14}H_{11}N_2OS_2F_3$: C 48.83, H 3.22, N 8.13. Found: C 48.67, H 3.30, N 7.93.

**4-(1,2,3,4-Tetrahydroisoquinol-7-yl)-2-thiazolyl thiol hydrochloride (b$_{28}$)**. To a mixture of $n$-butanol (26 mL) and 3 N HCl (48 mL) was added 4-(2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinol-7-yl)-2-thiazolyl thiol (**o**) (3.294 g, 9.57 mmol). And the reaction mixture was refluxed for 8 h and then concentrated in vacuo. The solid residue was triturated in ethanol to give **$b_{28}$** as a white crystal (2.288 g, 84 %): mp 230 °C (dec). $^1$H NMR (270 MHz, DMSO-$d_6$) δ 3.03 (2H, t, $J$ = 5.9 Hz), 3.39 (2H, m), 4.27 (2H, s), 7.27–7.33 (2H, m), 7.64 (2H, m); IR (KBr) cm$^{-1}$ 3422, 2946, 1672, 1505, 1458, 1041.

**4-(2-Methyl-1,2,3,4-tetrahydroisoquinol-7-yl)-2-thiazolyl thiol (b$_{29}$)**. To a solution of 4-(1,2,3,4-tetrahydroisoquinol-7-yl)-2-thiazolyl thiol hydrochloride (**$b_{28}$**) (4.655 g, 16.3 mmol) in dry THF (100 mL) were added 37 % formaldehyde (6.624 g, 81.7 mmol) and 2 N NaOH (8.2 mL). The mixture was stirred for 1 h at rt and treated with sodium cyanoborohydride (1.639 g, 26.1 mmol), then stirred for more 1 h. The reaction mixture was cooled to 0 °C and made weakly acidic with 1 N HCl and stirred for 30 min. The reaction mixture was made weakly basic with aqueous $NaHCO_3$ and extracted with dichloromethane. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo. The solid residue was purified by silica-gel column chromatography (dichloromethane/methanol) to give **$b_{29}$** (1.628 g, 38 %): $^1$H NMR (270 MHz, $CDCl_3$ containing 5% $CD_3OD$) δ 2.53 (3H, s), 2.79 (2H, t, $J$ = 5.9 Hz), 2.97 (2H, t, $J$ = 5.9 Hz), 3.64 (2H, s), 6.63 (1H, s),

7.18 (2H, m), 7.27 (1H, m); MS *m/z* 262 (M, $C_{13}H_{14}N_2S_2$).

**2-Tritylthio-4-(2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinol-7-yl) thiazole (p).** To a solution of 4-(2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinol-7-yl)-2-thiazolyl thiol (**o**) in dry dichloromethane (20 mL) and dry THF (2 mL) were added triethylamine (970 mg, 3.48 mmol) and triphenylmethyl chloride (970 mg, 3.48 mmol) under the nitrogen atmosphere at 0 °C. After stirring at the same temperature for 3 h, the reaction mixture was diluted with dichloromethane, washed with brine, dried ($Na_2SO_4$) and concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene/EtOAc) to give **p** (854 mg, 50 %): $^1$H NMR (270 MHz, $CDCl_3$) δ 2.97 (2H, m), 3.88 (2H, t, *J* = 6.4 Hz), 4.81 (2H, m), 7.1–7.6 (19H, m).

**2-Tritylthio-4-(1,2,3,4-tetrahydroisoquinol-7-yl) thiazole (q).** To a solution of 2-tritylthio-4-(2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinol-7-yl) thiazole (**p**) (754 mg, 1.29 mmol) in MeOH (10 mL) and THF (15 mL) was added 1 N NaOH (2.6 mL) at 0 °C. The mixture was stood up to rt and continued to stir for 30 min. The reaction mixture was diluted with EtOAc and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo to give **q** (594 mg, 94 %): $^1$H NMR (270 MHz, $CDCl_3$) δ 2.80 (2H, m), 3.15 (2H, t, *J* = 5.9 Hz), 4.03 (2H, s), 7.05–7.5 (19H, m).

**4-(Isoquinol-7-yl)-2-thiazolyl thiol ($b_{30}$).** To a solution of 2-tritylthio-4-(1,2,3,4-tetrahydroisoquinol-7-yl)thiazole (**q**) (352 mg, 0.71 mmol) in *p*-cymene (20 mL) was added 10 % Pd–C (0.32 g) and the mixture was heated under the nitrogen atmosphere at 160–180 °C for 7 h. The reaction mixture was cooled to rt and the catalyst was filtered off. The solution was extracted with 2 N HCl (10 mL) and the aqueous layer was alkalized (pH 9) with 2 N NaOH and washed with dichloromethane. Then the aqueous layer was neutralized (pH 6–7) with 2 N HCl and extracted with dichloromethane. The organic layer was dried ($Na_2SO_4$) and concentrated in vacuo to give **$b_{30}$** (157 mg, 90%): mp 242–258 °C. $^1$H NMR (270 MHz, DMSO-$d_6$) δ 7.78 (1H, d, *J* = 5.9 Hz), 7.96 (1H, d, *J* = 8.6 Hz), 8.15 (1H, d, *J* = 1.7 Hz), 8.18 (1H, d, *J* = 1.7 Hz), 8.26 (1H, s), 8.51 (1H, d, *J* = 5.3 Hz), 8.56 (1H, s), 9.27 (1H, s); IR (KBr) $cm^{-1}$ 3385, 1654, 1089.

**2-(4-Methoxybenzyl)thio-thiazol-4-yl-dimethylamide (m).** To a suspension of 2-(4-methoxybenzyl)thio-thiazol-4-yl-carboxylic acid (**l**) (536 mg, 2.0 mmol) in dichloromethane (10 mL) were added dropwise DMF (10 μL) and oxalyl chloride (227 μL, 2.6 mmol) under nitrogen atmosphere. After stirring for 10 min, the reaction mixture became a clear solution. After additional 15 min, the reaction mixture was concentrated in vacuo to give a red oily residue. The residue in THF (2.0 mL) was added to 50% dimethylamine (1.8 g, 20 mmol) in an ice bath under stirring. After stirring at the same temperature for 10 min, the reaction mixture was diluted with dichloromethane, washed with 0.1 M $KH_2PO_4$ buffer (pH 7.0) and dried ($MgSO_4$). Evaporation of the solvents in vacuo gave an oil, which was purified by silica-gel column chromatography (toluene/EtOAc = 2) to give **m** as a white crystal (603 mg, 98%): $^1$H NMR (270 MHz, $CDCl_3$) δ 3.10 (3H, s), 3.24 (3H, s), 3.79 (3H, s), 4.39 (2H, s), 6.83 (2H, d, *J* = 8.9 Hz), 7.28 (2H, d, *J* = 8.9 Hz), 7.74 (1H, s).

**2-Mercaptothiazol-4-yl-dimethylamide ($b_8$).** 2-(4-Methoxybenzyl)thio-thiazol-4-yl-dimethylamide (**m**) (603 mg, 1.96 mmol) in trifluoroacetic acid (20 mL) was refluxed for 10 h. The rection mixture was concentrated in vacuo and the residue was crystallized from EtOAc to give **$b_8$** as a white crystal (247 mg, 67%): mp 168–171 °C. $^1$H NMR (270 MHz, $CDCl_3$) δ 3.21 (6H, br), 6.86 (1H, s), 10.48 (1H, br); IR (KBr) $cm^{-1}$ 1605, 1437, 1411, 1313, 1187, 1060; MS *m/z* 188 (M, $C_6H_8N_2OS_2$).

**(4*R*,5*S*,6*S*,8*R*)-*p*-Nitrobenzyl-3-(4-phenylthiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate ($c_9$).** To (4*R*,5*R*,6*S*,8*R*)-*p*-nitrobenzyl-3-(trifluoromethanesulfonyloxy)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**e**) (1.0 mmol) in THF (3.0 mL) at −78 °C was added dropwise the thiolate salt solution which 4-phenyl-2-thiazolyl thiol (**$b_9$**) (207 mg, 1.0 mmol) was added to 60% NaH (41 mg, 1.0 mmol) in THF (1.5 mL) with stirring. The reaction mixture was allowed to warm to 0 °C for 4 h and continued to stir at 0 °C for 1 h. The reaction mixture was diluted with EtOAc, then extracted with 2.5% $KH_2PO_4$ and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by silica-gel column chromatography (benzene/EtOAc = 5–3) to give **$c_9$** as a light-yellow crystal (361 mg, 67%): $^1$H NMR (270 MHz, $CDCl_3$) δ 1.14 (3H, d, *J* = 7.3 Hz), 1.33 (3H, d, *J* = 6.3 Hz), 3.30 (1H, dd, *J* = 2.6 Hz and 6.6 Hz), 3.66 (1H, m), 4.30 (2H, m), 5.30 (1H, d, *J* = 13.9 Hz), 5.39 (1H, d, *J* = 13.9 Hz), 7.44 (3H, m), 7.68 (2H, d, *J* = 8.9 Hz), 7.89 (2H, d, *J* = 6.9 Hz), 8.25 (2H, d, *J* = 8.9 Hz); IR (neat) $cm^{-1}$ 3542, 1774, 1734, 1522, 1347.

The following compounds (**$c_1$**, **$c_2$**, and **$c_4$**) were prepared from **$b_1$**, **$b_2$**, and **$b_4$** as described for the preparation of **$c_9$**, respectively.

**$c_1$**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.12 (3H, d, *J* = 7.3 Hz), 1.33 (3H, d, *J* = 6.3 Hz), 3.33 (1H, dd, *J* = 6.6 and 3.0 Hz), 3.91 (1H, m), 4.29 (1H, m), 4.39 (1H, dd, *J* = 9.9 and 3.0 Hz), 5.30 (1H, d, *J* = 13.5 Hz), 5.52 (1H, d, *J* = 13.5 Hz), 7.48 (2H, m), 7.66 (2H, d, *J* = 8.9 Hz), 7.84 (1H, dd, *J* = 8.6 and 1.0 Hz), 8.01 (1H, dd, *J* = 7.9 and 1.0 Hz), 8.22 (2H, d, *J* = 8.9 Hz); IR (neat) $cm^{-1}$ 3456, 1756, 1700, 1527, 1342, 1217.

**$c_2$**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 7.3 Hz), 1.34 (3H, d, *J* = 6.3 Hz), 3.37 (1H, dd, *J* = 6.3 and 3.0 Hz), 4.00 (1H, m), 4.28 (1H, m), 4.44 (1H, dd, *J* = 10.3 and 3.0 Hz), 5.30 (1H, d, *J* = 13.5 Hz), 5.52 (1H, d,

$J$ = 13.5 Hz), 7.36 (2H, m), 7.51 (1H, m), 7.67 (3H, m), 8.21 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 3436, 1784, 1732, 1607, 1520, 1347, 1212.

**$c_4$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.09 (3H, d, $J$ = 7.3 Hz), 1.34(3H, d, $J$ = 6.3 Hz), 2.48 (3H, d, $J$ = 1.0 Hz), 3.28 (1H, dd, $J$ = 2.6 and 6.6 Hz), 3.50 (1H, m), 4.28 (2H, m), 5.28 (1H, d, $J$ = 13.9 Hz), 5.52 (1H, d, $J$ = 13.9 Hz), 7.66 (2H, d, $J$ = 8.9 Hz), 8.23 (2H, d, $J$ = 8.9 Hz); IR (KBr) $cm^{-1}$ 3449, 1809, 1705, 1527, 1327.

**(4*R*,5*S*,6*S*,8*R*)-*p*-Nitrobenzyl-3-(4-(4-nitrophenyl)thiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($c_{18}$)**. To (4*R*,5*R*, 6*S*,8*R*)-*p*-nitrobenzyl-3-(diphenylphosphoryloxy)-4-methyl-6-(1-(trimethylsilyloxy)ethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**a**) (1.33 g, 2.0 mmol) in toluene (2.0 mL) at 0 °C with stirring was added dropwise the thiolate salt solution which 4-(4-nitrophenyl)-2-thiazolyl thiol ($b_{18}$) (619 mg, 2.6 mmol) was added to 60% NaH (104 mg, 2.6 mmol) in THF (5.0 mL) with stirring. The reaction mixture was put at 5 °C for 18 h. The reaction mixture was diluted with EtOAc, then extracted with water twice and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in EtOAc (100 mL), and to the solution in an ice bath was added 1 N HCl (3.0 mL). The reaction mixture was stood up to rt and continued to stir for 30 min. The reaction mixture was washed with water twice and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was triturated in benzene to give $c_{18}$ as a light-brown crystal (454 mg, 39%): $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.16 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 3.32 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.64 (1H, m), 4.26 (1H, m), 4.34 (1H, dd, $J$ = 3.0 and 9.9 Hz), 5.30 (1H, d, $J$ = 13.9 Hz), 5.56(1H, d, $J$ = 13.9 Hz), 7.68 (2H, d, $J$ = 8.9 Hz), 7.78 (1H, s), 8.06 (2H, d, $J$ = 8.9 Hz), 8.24 (2H, d, $J$ = 8.9 Hz), 8.31 (1H, d, $J$ = 8.9 Hz); IR (KBr) $cm^{-1}$ 3448, 1778, 1702, 1510, 1344.

The following compounds ($c_3$, $c_5$–$c_{17}$, and $c_{19}$–$c_{33}$) were prepared from $b_3$, $b_5$–$b_{17}$, and $c_{19}$–$b_{33}$ as described for the preparation of $c_{18}$, respectively.

**$c_3$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.04 (3H, d, $J$ = 7.3 Hz), 1.28 (3H, d, $J$ = 6.6 Hz), 3.26 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.46 (1H, m), 4.26 (2H, m), 5.26 (1H, d, $J$ = 13.9 Hz), 5.50 (1H, d, $J$ = 13.9 Hz), 7.47 (1H, d, $J$ = 3.3 Hz), 7.64 (2H, d, $J$ = 8.6 Hz), 7.85 (1H, d, $J$ = 3.3 Hz), 8.19 (2H, d, $J$ = 8.6 Hz); IR (neat) $cm^{-1}$ 3406, 1760, 1696, 1518, 1344

**$c_5$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 0.96 (3H, t, $J$ = 7.3 Hz), 1.09 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 1.72 (2H, m), 2.77 (2H, t, $J$ = 7.5 Hz), 3.28 (1H, dd, $J$ = 2.8 and 6.8 Hz), 3.88 (1H, m), 4.26 (2H, m), 5.29 (1H, d, $J$ = 13.9 Hz), 5.53 (1H, d, $J$ = 13.9 Hz), 7.02 (1H, s), 7.67 (2H, d, $J$ = 8.9 Hz), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 1772, 1700, 1521, 1345, 1210.

**$c_6$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.09 (3H, d, $J$ = 7.6 Hz), 1.32 (9H, m), 3.13 (1H, m), 3.28 (1H, dd, $J$ = 3.0 and 6.9 Hz), 3.46 (1H, m), 4.25 (2H, m), 5.29 (1H, d, $J$ = 13.5 Hz), 5.53 (1H, d, $J$ = 13.5 Hz), 7.02 (1H, s), 7.67 (2H, d, $J$ = 8.7 Hz), 8.24 (2H, d, $J$ = 8.7 Hz); IR (neat) $cm^{-1}$ 3500 (br), 1778, 1522, 1345, 1276, 1211; FD-MS $m/z$ 503 (M, $C_{23}H_{25}N_3O_6S_2$).

**$c_7$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 0.12 (9H, s), 1.13 (3H, d, $J$ = 7.6 Hz), 1.24 (3H, d, $J$ = 5.9 Hz), 2.67 (3H, s), 3.30 (1H, dd, $J$ = 3.0 and 5.6 Hz), 3.53 (1H, m), 4.27 (2H, m), 5.31 (1H, d, $J$ = 13.7 Hz), 5.52 (1H, d, $J$ = 13.7 Hz), 7.68 (2H, d, $J$ = 8.9 Hz), 8.20 (1H, s), 8.23 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 1773, 1696, 1522, 1346, 1212.

**$c_8$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.10 (3H, d, $J$ = 6.6 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 1.86 (1H, d, $J$ = 4.6 Hz, OH), 3.12 (3H, s), 3.24 (3H, s), 3.30 (1H, dd, $J$ = 3.0 and 6.3 Hz), 3.47 (1H, m), 4.26 (2H, m), 5.30 (1H, d, $J$ = 13.9 Hz), 5.53 (1H, d, $J$ = 13.9 Hz), 7.66 (2H, d, $J$ = 8.9 Hz), 7.99 (1H, s), 8.23 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 3400, 1778, 1755, 1630, 1520, 1345.

**$c_{10}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.11 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 2.36 (3H, s), 2.37 (3H, s), 3.27 (1H, dd, $J$ = 2.6 and 6.6 Hz), 3.44 (1H, m), 4.25 (2H, m), 5.28 (1H, d, $J$ = 13.9 Hz), 5.52 (1H, d, $J$ = 13.9 Hz), 7.66 (2H, d, $J$ = 8.9 Hz), 8.23 (2H, d, $J$ = 8.9 Hz); IR (KBr) $cm^{-1}$ 3567, 1771, 1701, 1518, 1346.

**$c_{11}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.15 (3H, d, $J$ = 7.3 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 3.30 (1H, dd, $J$ = 6.3 and 2.6 Hz), 3.59 (1H, m), 4.30 (2H, m), 5.31 (1H, d, $J$ = 13.5 Hz), 5.54 (1H, d, $J$ = 13.5 Hz), 7.17 (1H, dd, $J$ = 8.6 and 1.3 Hz), 7.41 (2H, t, $J$ = 8.6 Hz), 7.53 (2H, dd, $J$ = 8.6 and 1.3 Hz), 7.67 (2H, d, $J$ = 8.9 Hz), 8.00 (1H, s), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 3450, 1782, 1708, 1700, 1522, 1346.

**$c_{12}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.15 (3H, d, $J$ = 7.6 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.66 (1H, m), 4.21-4.33 (2H, m), 5.30 (1H, d, $J$ = 13.4 Hz), 5.54 (1H, d, $J$ = 13.4 Hz), 7.1–7.4 (2H, m), 7.49 (1H, dd, $J$ = 2.5 and 7.1 Hz), 7.68 (2H, d, $J$ = 8.9 Hz), 7.88 (1H, dd, $J$ = 2.5 and 7.1 Hz), 7.91 (1H, s), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) $cm^{-1}$ 3460 (br), 1777, 1702, 1520, 1344, 1275, 1210, 1139.

**$c_{13}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 3.29 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.61 (1H, m), 4.30 (2H, m), 5.29 (1H, d, $J$ = 13.5 Hz), 5.53 (1H, d, $J$ = 13.5 Hz), 7.41 (2H, d, $J$ = 8.6 Hz), 7.55 (1H, s), 7.67 (2H, d, $J$ = 8.6 Hz), 7.81 (2H, d, $J$ = 8.6 Hz), 8.22 (2H, d, $J$ = 8.6 Hz); IR (neat) $cm^{-1}$ 3567, 1771, 1701, 1518, 1346.

**$c_{14}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.15 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 2.42 (3H, s), 3.30 (1H, dd, $J$ = 6.3 and 3.0 Hz), 3.65 (1H, m), 4.26 (2H, m), 5.30 (1H, d, $J$ = 13.9 Hz), 5.55 (1H, d, $J$ = 13.9 Hz), 7.06–7.56 (5H, m), 7.68 (2H, d, $J$ = 8.8 Hz), 8.24 (2H, d, $J$ = 8.8 Hz); IR (neat) $cm^{-1}$ 3429 (br), 1772, 1521, 1347.

**c15**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.33 (3H, d, $J$ = 6.6 Hz), 2.43 (3H, s), 3.30 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.62 (1H, m), 4.24–4.37 (2H, m), 5.30 (1H, d, $J$ = 13.7 Hz), 5.55 (1H, d, $J$ = 13.7 Hz), 7.1–7.55 (4H, m), 7.57 (1H, s), 7.68 (2H, d, $J$ = 8.9 Hz), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3369 (br), 1771, 1702, 1520, 1346, 1210.

**c16**. $^1$H NMR(270 MHz, $CDCl_3$) δ 1.26 (3H, d, $J$ = 7.3 Hz), 1.44 (3H, d, $J$ = 6.3 Hz), 2.38 (3H, s), 2.96 (1H, m), 3.21 (1H, dd, $J$ = 7.3 and 5.3 Hz), 4.68–4.83 (2H, m), 5.27 (1H, d, $J$ = 13.5 Hz), 5.34 (1H, d, $J$ = 13.5 Hz), 7.39 (2H, d, $J$ = 8.7 Hz), 7.60 (1H, s), 7.68 (2H, d, $J$ = 8.1 Hz), 7.77 (2H, d, $J$ = 8.1 Hz), 8.01 (2H, d, $J$ = 8.7 Hz).

**c17**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.14 (3H, d, $J$ = 7.3 Hz), 1.36 (3H, d, $J$ = 6.3 Hz), 3.35 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.67 (1H, m), 4.2–4.4 (2H, m), 5.28 (1H, d, $J$ = 13.5 Hz), 5.52 (1H, d, $J$ = 13.5 Hz), 7.52 (1H, s), 7.5–7.7 (5H, m), 7.86 (1H, m), 8.23 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3429 (br), 1771, 1702, 1524, 1347, 1276, 1194.

**c19**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.13 (3H, d, $J$ = 7.6 Hz), 1.31 (3H, d, $J$ = 6.3 Hz), 3.29 (1H, dd, $J$ = 2.6 and 6.3 Hz), 3.62 (1H, m), 4.23 (2H, m), 4.73 (2H, s), 5.30 (1H, d, $J$ = 13.5 Hz), 5.52 (1H, d, $J$ = 13.5 Hz), 7.43 (2H, d, $J$ = 7.9 Hz), 7.58 (1H, s), 7.66 (2H, d, $J$ = 8.6 Hz), 7.87 (2H, d, $J$ = 7.9 Hz), 8.22 (2H, d, $J$ = 8.6 Hz); IR (neat) cm$^{-1}$ 3435, 1766, 1607, 1520, 1346, 1211.

**c20**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.14 (3H, d, $J$ = 7.3 Hz), 1.33 (3H, d, $J$ = 6.3 Hz), 3.30 (1H, dd, $J$ = 3.0 and 6.9 Hz), 3.62 (1H, m), 4.22–4.33 (2H, m), 4.44 (2H, d, $J$ = 5.9 Hz), 5.24 (2H, s), 5.30 (1H, d, $J$ = 13.9 Hz), 5.54 (1H, d, $J$ = 13.9Hz), 7.37 (2H, d, $J$ = 8.2 Hz), 7.52 (2H, d, $J$ = 8.2 Hz), 7.58 (1H, s), 7.68 (2H, d, $J$ = 8.9 Hz), 7.86 (2H, d, $J$ = 8.2 Hz), 8.20–8.26 (4H, m); IR (neat) cm$^{-1}$ 3410 (br), 1777, 1721, 1711, 1520, 1346, 1211, 1139.

**c21**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.19 (3H, d, $J$ = 7.3 Hz), 1.33 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, dd, $J$ = 6.6 and 2.6 Hz), 3.66 (1H, m), 4.26 (2H, m), 5.30 (1H, d, $J$ = 13.9 Hz), 5.54 (1H, d, $J$ = 13.9 Hz), 7.52 (3H, m), 7.57 (1H, s), 7.69 (3H, m), 7.88 (2H, m), 8.20 (3H, m).

**c22**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.17 (3H, d, $J$ = 7.3 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, dd, $J$ = 6.6 and 2.6 Hz), 3.68 (1H, m), 4.30 (2H, m), 5.32 (1H, d, $J$ = 13.9 Hz), 5.55 (1H, d, 13.9 Hz), 7.52 (2H, m), 7.70 (3H, m), 7.90 (4H, m), 8.24 (2H, d, $J$ = 8.9 Hz), 8.43 (1H, s); IR (neat) cm$^{-1}$ 3510, 1758, 1706, 1559, 1522, 1337, 1279.

**c23**. $^1$H NMR (270 MHz, $CDCl_3$) δ 0.10 (9H, s), 1.13 (3H, d, $J$ = 7.3 Hz), 1.20 (3H, d, $J$ = 5.9 Hz), 3.27 (1H, dd, $J$ = 3.0 and 5.3 Hz), 3.54 (1H, m), 4.25 (2H, m), 5.31 (1H, d, $J$ = 13.9 Hz), 5.56 (1H, d, $J$ = 13.9 Hz), 5.15 (1H, brs), 6.23 (1H, brs), 7.53 (1H, t, $J$ = 7.6 Hz), 7.65 (2H, d, $J$ = 8.6 Hz), 7.67 (1H, s), 7.80 (1H, dd, $J$ = 1.3 and 7.6 Hz), 8.05 (1H, dd, $J$ = 1.3 and 7.6 Hz), 8.23 (2H, d, $J$ = 8.6 Hz), 8.33 (1H, t, $J$ = 1.3 Hz); IR (neat) cm$^{-1}$ 3362, 1772, 1684, 1522, 1340.

**c24**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.16 (3H, d, $J$ = 7.6 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 3.32 (1H, dd, $J$ = 2.6 and 6.6 Hz), 3.64 (1H, m), 4.27–4.35 (2H, m), 5.31 (1H, d, $J$ = 13.7 Hz), 5.55 (1H, d, $J$ = 13.7 Hz), 7.68 (3H, m), 7.89 (2H, d, $J$ = 8.6 Hz), 7.98 (2H, d, $J$ = 8.6 Hz), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3366(br), 1771, 1668, 1520, 1346, 1211.

**c25**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 2.33 (3H, s), 2.37 (2H, m), 2.51 (2H, m), 3.30 (1H, dd, $J$ = 3.0 and 6.9 Hz), 3.49 (2H, m), 3.65–3.84 (3H, m), 4.21–4.35 (2H, m), 5.30 (1H, d, $J$ = 13.5 Hz), 5.54 (1H, d, $J$ = 13.5 Hz), 7.37–7.52 (2H, m), 7.62 (1H, s), 7.68 (2H, d, $J$ = 8.9 Hz), 7.90–7.97 (2H, m), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3429 (br), 1777, 1620, 1524, 1443, 1346, 1274.

**c26**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.14 (3H, d, $J$ = 7.3 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 2.33 (3H, s), 2.39 (2H, m), 2.48 (2H, m), 3.30 (1H, dd, $J$ = 2.8 and 6.6 Hz), 3.48 (2H, m), 3.64 (1H, m), 3.79 (2H, m), 4.19–4.34 (2H, m), 5.30 (1H, d, $J$ = 13.7 Hz), 5.54 (1H, d, $J$ = 13.7 Hz), 7.49 (2H, d, $J$ = 8.6 Hz), 7.64 (1H, s), 7.68 (2H, d, $J$ = 8.9 Hz), 7.93 (2H, d, $J$ = 8.6 Hz), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3381 (br), 1772, 1522, 1457, 1346, 1274.

**c27**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.37 (3H, d, $J$ = 6.3 Hz), 2.35 (6H, s), 2.65 (2H, m), 3.30 (1H, dd, $J$ = 3.0 and 6.9 Hz), 3.59 (3H, m), 4.28 (2H, m), 5.30 (1H, d, $J$ = 13.5 Hz), 5.54 (1H, d, $J$ = 13.5 Hz), 7.54 (2H, d, $J$ = 8.9 Hz), 7.68 (2H, d, $J$ = 8.9 Hz), 7.90 (3H, m), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3375, 1772, 1734, 1652, 1521, 1347.

**c28**. $^1$H NMR (270 MHz, $CDCl_3$) δ 0.11 (9H, s), 1.12 (3H, d, $J$ = 7.3 Hz), 1.21 (3H, d, $J$ = 6.3 Hz), 2.91 (2H, m), 3.2–3.3 (3H, m), 3.5–4.3 (5H, m), 5.31 (1H, d, $J$ = 13.7 Hz), 5.52 (1H, d, $J$ = 13.7 Hz), 7.0–7.6 (4H, m), 7.68 (2H, d, $J$ = 8.8 Hz), 8.23 (2H, d, $J$ = 8.8 Hz); IR (neat) cm$^{-1}$ 1774 (sh), 1754 (br), 1523, 1347, 1252, 1209, 1108.

**c29**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.12 (3H, d, $J$ = 7.3 Hz), 1.32 (3H, d, $J$ = 6.3 Hz), 2.49 (3H, s), 2.73 (2H, m), 2.95 (2H, m), 3.29 (1H, dd, $J$ = 2.6 and 6.6 Hz), 3.58 (3H, m), 4.28 (2H, m), 5.30 (1H, d, $J$ = 13.9 Hz), 5.54 (1H, d, $J$ = 13.9 Hz), 7.10–7.65 (4H, m), 7.68 (2H, d, $J$ = 8.9 Hz), 8.24 (2H, d, $J$ = 8.9 Hz); IR (neat) cm$^{-1}$ 3381, 1772, 1700, 1522, 1346; FD-MS $m/z$ 606 (M, $C_{30}H_{30}N_4O_6S_2$).

**c30**. $^1$H NMR (270 MHz, $CDCl_3$) δ 1.16 (3H, d, $J$ = 7.3 Hz), 1.31 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, dd, $J$ = 2.6 and 6.6 Hz), 3.68 (1H, m), 4.25 (1H, m), 4.34 (1H, dd, $J$ = 2.6 and 9.9 Hz), 5.30 (1H, d, $J$ = 13.5 Hz), 5.53 (1H, d, $J$ = 13.5 Hz), 7.65 (1H, d, $J$ = 5.6 Hz), 7.65 (2H, d, $J$ = 8.6 Hz), 7.74 (1H, s), 7.88 (1H, d, $J$ = 8.6 Hz), 8.16 (1H, dd, $J$ = 2.0 and 8.6 Hz), 8.20 (2H, d, $J$ = 8.6 Hz), 8.53

(1H, brs), 9.31 (1H, s); IR (neat) $cm^{-1}$ 3388, 1734, 1608, 1522, 1346.

**$c_{31}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 0.11 (9H, s), 1.13 (3H, d, $J$ = 7.3 Hz), 1.22 (3H, d, $J$ = 6.3 Hz), 3.27 (1H, dd, $J$ = 3.0 and 5.6 Hz), 3.53 (1H, m), 4.26 (2H, m), 5.32 (1H, d, $J$ = 14.0 Hz), 5.53 (1H, d, $J$ = 14.0 Hz), 7.20 (1H, m), 7.68 (2H, d, $J$ = 8.6 Hz), 7.80 (1H, m), 8.12 (1H, d, $J$ = 7.9 Hz), 8.20 (1H, s), 8.23 (2H, d, $J$ = 8.6 Hz), 8.63 (1H, d, $J$ = 5.0 Hz); IR (neat) $cm^{-1}$ 1780, 1521, 1346, 1251, 1206.

**$c_{32}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 0.10 (9H, s), 1.13 (3H, d, $J$ = 7.3 Hz), 1.20 (3H, d, $J$ = 6.3 Hz), 3.28 (1H, dd, $J$ = 3.0 and 5.6 Hz), 3.62 (1H, m), 4.26 (2H, m), 5.30 (1H, d, $J$ = 13.9 Hz), 5.50 (1H, d, $J$ = 13.9 Hz), 7.38 (1H, ddd, $J$ = 0.7, 5.0 and 7.9 Hz), 7.67 (1H, s), 7.67 (2H, d, $J$ = 8.9 Hz), 8.19 (1H, td, $J$ = 1.7 and 7.9 Hz), 8.22 (2H, d, $J$ = 8.9 Hz), 8.60 (1H, dd, $J$ = 1.7 and 5.0 Hz), 9.11 (1H, dd, $J$ = 0.7 and 1.7 Hz); IR (KBr) $cm^{-1}$ 1772, 1700, 1522, 1340.

**$c_{33}$**. $^1H$ NMR (270 MHz, $CDCl_3$) δ 1.16 (3H, d, $J$ = 7.3 Hz), 1.34 (3H, d, $J$ = 6.3 Hz), 3.32 (1H, dd, $J$ = 3.0 and 6.6 Hz), 3.65 (1H, m), 4.24–4.37 (2H, m), 5.31 (1H, d, $J$ = 13.7 Hz), 5.55 (1H, d, $J$ = 13.7 Hz), 7.68 (2H, d, $J$ = 8.9 Hz), 7.78 (2H, d, $J$ = 6.1 Hz), 8.25 (2H, d, $J$ = 8.9 Hz), 8.71 (2H, d, $J$ = 6.1 Hz); IR (neat) $cm^{-1}$ 3421 (br), 1774, 1701, 1604, 1343, 1275, 1220, 1150.

**(4*R*,5*S*,6*S*,8*R*)-3-(4-Phenylthiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid ($d_9$)**. A mixture of (4*R*,5*S*,6*S*,8*R*)-*p*-nitrobenzyl-3-(4-phenylthiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($C_9$) (650 mg, 1.21 mmol) and 10% Pd–C (650 mg) in THF (35 mL) and 0.05 M MOPS buffer (35 mL, pH 7.0) was stirred under hydrogen atmosphere for 1 h under the ambient pressure and at rt. The catalyst was filtered off and the solution was washed with dichloromethane. The separated aqueous layer was concentrated briefly to remove any residual organic solvents in vacuo and then subjected to column chromatography on Diaion CHP-20P which was successively eluted with water containing 8–16% of THF. The fractions were combined and lyophilized to give **$d_9$** as a colorless powder (98 mg, 20%): $^1H$ NMR (270 MHz, $D_2O$) δ 1.11 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.6 Hz), 3.60 (2H, m), 4.27 (1H, m), 4.37 (1H, dd, $J$ = 2.6 and 9.6 Hz), 7.46 (3H, m), 7.70 (3H, s); IR (KBr) $cm^{-1}$ 3495, 1734, 1477, 1369; UV $\lambda_{max}$ ($H_2O$) 320 (sh), 290, 247, 225 (sh).

The following compounds (**$d_1$–$d_8$, $d_{10}$–$d_{33}$**) were prepared from **$c_1$–$c_8$, $c_{10}$–$c_{33}$** as described for the preparation of **$d_9$**, respectively.

**$d_1$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.3 Hz), 3.50 (2H, m), 4.26 (1H, m), 4.36 (1H, dd, $J$ = 10.3 and 3.0 Hz), 7.49 (1H, t, $J$ = 7.6 Hz), 7.58 (1H, t, $J$ = 7.6 Hz), 7.94 (1H, d, $J$ = 8.6 Hz), 7.97 (1H, d, $J$ = 8.6 Hz); IR (KBr) $cm^{-1}$ 3426, 1772, 1560, 1457, 1420, 1274, 1144; UV $\lambda_{max}$ ($H_2O$) 282 (sh), 300, 318 (sh).

**$d_2$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.11 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 3.37 (1H, m), 3.48 (1H, dd, $J$ = 5.9 and 3.0 Hz), 4.30 (2H, m), 7.52 (2H, m), 7.92 (2H, m); IR (KBr) $cm^{-1}$ 3408, 1761, 1617, 1397, 1240; UV $\lambda_{max}$ ($H_2O$) 249, 303

**$d_3$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.3 Hz), 3.20 (1H, m), 3.47 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.24 (2H, m), 7.74 (1H, d, $J$ = 3.3 Hz), 7.88 (1H, d, $J$ = 3.3 Hz); IR (KBr) $cm^{-1}$ 3372, 1767, 1706, 1207; UV $\lambda_{max}$ (aq. 2% $NaHCO_3$) 308.

**$d_4$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.6 Hz), 2.43 (3H, d, $J$ = 1.0 Hz), 3.22 (1H, m), 3.47 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.23 (2H, m), 7.29 (1H, q, $J$ = 1.0 Hz); IR (KBr) $cm^{-1}$ 3422, 1753, 1604, 1396; UV $\lambda_{max}$ ($H_2O$) 309.

**$d_5$**. $^1H$ NMR (270 MHz, $D_2O$) δ 0.90 (3H, t, $J$ = 7.4 Hz), 1.07 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.6 Hz), 1.70 (2H, m), 2.76 (2H, t, $J$ = 7.3 Hz), 3.18 (1H, m), 3.45 (1H, dd, $J$ = 3.0 and 6.3 Hz), 4.20–4.27 (2H, m), 7.33 (1H, s); IR (KBr) $cm^{-1}$ 3379 (sh), 1752, 1604, 1560, 1395; UV $\lambda_{max}$ ($H_2O$) 333 (sh), 311, 283 (sh).

**$d_6$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 1.29 (6H, d, $J$ = 6.9 Hz), 3.16 (2H, m), 3.46 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.26 (2H, m), 7.34 (1H, s); IR (KBr) $cm^{-1}$ 3388, 1752, 1603, 1394, 1284; UV $\lambda_{max}$ ($H_2O$) 309, 267 (sh).

**$d_7$**. $^1H$ NMR (270 MHz, D2O) δ 1.09 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 2.64 (3H, s), 3.35 (1H, m), 3.51 (1H, dd, $J$ = 5.9 and 2.6 Hz), 4.28 (2H, m), 8.55 (1H, s); IR (KBr) $cm^{-1}$ 3406, 1755, 1690, 1599, 1490, 1394, 1262, 1211; UV $\lambda_{max}$ ($H_2O$) 295, 257.

**$d_8$**. $^1H$ NMR(270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.29 (3H, d, $J$ = 6.3 Hz), 3.11 (3H, s), 3.12 (3H, s), 3.30 (1H, m), 3.50 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.26 (2H, m), 8.02 (1H, s); IR (KBr) $cm^{-1}$ 3420, 1759, 1612, 1392, 1253; UV $\lambda_{max}$ ($H_2O$) 310, 250 (sh).

**$d_{10}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.6 Hz), 2.31 (3H, s), 2.38 (3H, s), 3.18 (1H, m), 3.43 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.26 (2H, m); IR (KBr) $cm^{-1}$ 3408, 1765, 1376; UV $\lambda_{max}$ ($H_2O$) 310.

**$d_{11}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 3.26 (1H, m), 3.47 (1H, dd, $J$ = 5.9 and 2.6 Hz), 4.25 (2H, m), 7.30 (1H, brd, $J$ = 6.9 Hz), 7.48 (2H, brt, $J$ = 7.3 Hz), 7.64 (2H, brd, $J$ = 7.3 Hz), 8.06 (1H, s); IR (KBr) $cm^{-1}$ 3422, 1762, 1604, 1395, 1287; UV $\lambda_{max}$ ($H_2O$) 309, 287 (sh).

**$d_{12}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.29 (3H, d, $J$ = 6.3 Hz), 3.37 (1H, m), 3.51 (1H, dd, $J$ = 5.6 and 3.3 Hz), 4.26 (2H, m), 7.49 (2H, m), 7.63

(2H, m), 7.92 (1H, s); IR (KBr) $cm^{-1}$ 3420, 1752, 1604, 1394, 1290; UV $\lambda_{max}$ ($H_2O$) 312, 255.

**$d_{13}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.09 (3H, d, $J$ = 7.3 Hz), 1.25 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, m), 3.46 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.25 (2H, m), 7.48 (2H, d, $J$ = 8.6 Hz), 7.77 (2H, d, $J$ = 8.6 Hz), 7.87 (1H, s); IR (KBr) $cm^{-1}$ 3428, 1758, 1603, 1395; UV $\lambda_{max}$ ($H_2O$) 310 (sh), 266.

**$d_{14}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.14 (3H, d, $J$ = 7.3 Hz), 1.28 (3H, d, $J$ = 6.6 Hz), 2.36 (3H, s), 3.31 (1H, m), 3.49 (1H, m), 4.26 (2H, m), 7.34–7.55 (4H, m), 7.68 (1H, m); IR (KBr) $cm^{-1}$ 3448 (br), 1772, 1617, 1395; UV $\lambda_{max}$ ($H_2O$) 311.

**$d_{15}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.11 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 2.43 (3H, s), 3.32 (1H, m), 3.48 (1H, m), 4.28 (2H, m), 7.33 (1H, d, $J$ = 7.6 Hz), 7.44 (1H, t, $J$ = 7.9 Hz), 7.67 (1H, d, $J$ = 8.6 Hz), 7.70 (1H, s), 7.88 (1H, s); IR (KBr) $cm^{-1}$ 3406, 1757, 1602, 1459, 1394, 1286; UV $\lambda_{max}$ ($H_2O$) 312, 258.

**$d_{16}$**. $^1H$ NMR (270 MHz, DMSO-$d_6$) δ 0.95 (3H, d, $J$ = 7.3 Hz), 1.12 (3H, d, $J$ = 6.3 Hz), 2.33 (3H, s), 3.09 (1H, dd, $J$ = 6.3 and 2.6 Hz), 3.30 (1H, m), 3.92 (1H, m), 4.06 (1H, dd, $J$ = 9.6 and 2.6 Hz), 4.94 (1H, d, $J$ = 5.0 Hz, OH), 7.25 (2H, d, $J$ = 7.9 Hz), 7.83 (2H, d, $J$ = 7.9 Hz), 8.02 (1H, s); IR (KBr) $cm^{-1}$ 3394, 1764, 1600, 1398, 1292.

**$d_{17}$**. $^1H$ NMR (270 MHz, $D_2O$) 1:1 mixture of two components δ 1.29 (3H, d, $J$ = 6.3 Hz), 1.54 (3/2H, d, $J$ = 6.9 Hz), 1.69 (3/2H, d, $J$ = 6.9 Hz), 3.36 (1H, m), 3.51 (1H, m), 4.30 (2H, m), 7.00 (1H, m), 7.17 (1/2H, t, $J$ = 7.3 Hz), 7.32 (1/2H, t, $J$ = 7.3 Hz), 7.52 (2H, m), 7.78 (1/2H, s), 7.80 (1/2H, s); IR (KBr) $cm^{-1}$ 3419, 1752, 1599, 1394, 1286; UV $\lambda_{max}$ ($H_2O$) 301.

**$d_{18}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.01 (3H, d, $J$ = 7.3 Hz), 1.23 (3H, d, $J$ = 6.6 Hz), 3.21 (1H, m), 3.43 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.20 (2H, m), 7.10 (2H, d, $J$ = 8.6 Hz), 7.65 (1H, s), 7.69 (2H, d, $J$ = 8.6 Hz); IR (KBr) $cm^{-1}$ 3347, 1762, 1602, 1476, 1394; UV $\lambda_{max}$ ($H_2O$) 285.

**$d_{19}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 6.9 Hz), 1.27 (3H, d, $J$ = 6.6 Hz), 3.30 (1H, m), 3.48 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.24 (2H, m), 4.70 (2H, s), 7.50 (2H, d, $J$ = 8.3 Hz), 7.85 (2H, d, $J$ = 8.3 Hz), 7.91 (1H, s); IR (KBr) $cm^{-1}$ 3434, 1752, 1598, 1394, 1284; UV $\lambda_{max}$ ($H_2O$) 316, 265.

**$d_{20}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.13 (3H, d, $J$ = 7.6 Hz), 1.29 (3H, d, $J$ = 6.6 Hz), 3.35 (1H, m), 3.50 (1H, m), 4.26 (2H, m), 7.59 (2H, d, $J$ = 8.3 Hz), 7.94 (2H, d, $J$ = 8.3 Hz), 7.99 (1H, s); IR (KBr) $cm^{-1}$ 3422 (br), 1770, 1651, 1559, 1541, 1395, 1034; UV $\lambda_{max}$ ($H_2O$) 313 (sh).

**$d_{21}$**. $^1H$ NMR (270 MHz, DMSO-$d_6$) δ 1.02 (3H, d, $J$ = 7.3 Hz), 1.13 (3H, d, $J$ = 6.3 Hz), 3.14 (1H, brd, $J$ = 6.6 Hz), 3.30 (1H, m), 3.93 (1H, m), 4.06 (1H, brd, $J$ = 9.6 Hz), 4.97 (1H, d, $J$ = 5.0 Hz), 7.58 (3H, m), 7.71 (1H, d, $J$ = 6.9 Hz), 7.96 (1H, s), 8.00 (2H, m), 8.22 (1H, m); IR (KBr) $cm^{-1}$ 3417, 1752, 1600, 1397, 1286; UV $\lambda_{max}$ ($H_2O$) 294.

**$d_{22}$**. $^1H$ NMR (270 MHz, DMSO-$d_6$) δ 0.98 (3H, d, $J$ = 7.3 Hz), 1.12 (3H, d, $J$ = 6.0 Hz), 3.12 (1H, dd, $J$ = 6.6 and 2.6 Hz), 3.35 (1H, m), 3.93 (1H, m), 4.11 (1H, dd, $J$ = 9.6 and 2.6 Hz), 4.95 (1H, d, $J$ = 5.0 Hz, OH), 7.55 (2H, m), 7.90 (4H, m), 8.46 (1H, s), 8.53 (1H, s); IR (KBr) $cm^{-1}$ 3424, 1752, 1661, 1394, 1275; UV $\lambda_{max}$ ($H_2O$) 305 (sh), 293, 282 (sh), 247.

**$d_{23}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.09 (3H, d, $J$ = 6.9 Hz), 1.26 (3H, d, $J$ = 6.6 Hz), 3.32 (1H, m), 3.47 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.25 (2H, m), 7.60 (1H, dd, $J$ = 5.9 and 7.9 Hz), 7.85 (1H, dd, $J$ = 0.7 and 5.9 Hz), 7.90 (1H, s), 7.97 (1H, d, $J$ = 7.9 Hz), 8.16 (1H, d, $J$ = 0.7 Hz); IR (KBr) $cm^{-1}$ 3425, 1752, 1670, 1603, 1394; UV $\lambda_{max}$ ($H_2O$) 318 (sh), 269 (sh), 215 (sh).

**$d_{24}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.12 (3H, d, $J$ = 7.3 Hz), 1.28 (3H, d, $J$ = 6.3 Hz), 3.36 (1H, m), 3.50 (1H, dd, $J$ = 5.9 and 3.0 Hz), 4.29 (2H, m), 7.89 (1H, d, $J$ = 8.6 Hz), 7.93 (1H, d, $J$ = 8.6 Hz), 8.02 (1H, s); IR (KBr) $cm^{-1}$ 3423, 1751, 1653, 1614, 1560, 1394; UV $\lambda_{max}$ ($H_2O$) 282.

**$d_{25}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.08 (3H, d, $J$ = 7.3 Hz), 1.25 (3H, d, $J$ = 6.6 Hz), 2.36 (3H, s), 2.53 (2H, m), 2.69 (2H, m), 3.32 (1H, m), 3.51 (3H, m), 3.81 (2H, m), 4.24 (2H, m), 7.45 (1H, dd, $J$ = 1.3 and 7.9 Hz), 7.59 (1H, t, $J$ = 7.9 Hz), 7.82 (1H, d, $J$ = 1.3 Hz), 7.93 (2H, m); IR (KBr) $cm^{-1}$ 3427, 1762, 1603, 1457, 1388, 1285; UV $\lambda_{max}$ ($H_2O$) 312 (sh), 262.

**$d_{26}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.06 (3H, d, $J$ = 6.6 Hz), 1.24 (3H, d, $J$ = 6.3 Hz), 2.78 (3H, s), 3.00–3.40 (5H, m), 3.45 (1H, m), 3.65–4.10 (4H, m), 4.26 (2H, m), 7.53 (2H, d, $J$ = 8.3 Hz), 7.88 (2H, d, $J$ = 8.3 Hz), 7.93 (1H, s); IR (KBr) $cm^{-1}$ 3424, 1758, 1654, 1457, 1387, 1284; UV $\lambda_{max}$ ($H_2O$) 332 (sh), 287.

**$d_{27}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 0.88 (3H, d, $J$ = 7.6 Hz), 1.14 (3H, d, $J$ = 6.3 Hz), 3.00 (6H, s), 3.13 (1H, m), 3.29 (1H, dd, $J$ = 3.0 and 5.9 Hz), 3.46 (2H, m), 3.83 (2H, m), 3.95 (1H, dd, $J$ = 3.0 and 9.2 Hz), 4.10 (1H, m), 7.80 (5H, m); IR (KBr) $cm^{-1}$ 3421, 1762, 1609, 1560, 1388, 1290; UV $\lambda_{max}$ ($H_2O$) 288.

**$d_{28}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 0.93 (3H, d, $J$ = 6.6 Hz), 1.13 (3H, d, $J$ = 6.6 Hz), 3.10 (3H, m), 3.36 (1H, m), 3.48 (2H, m), 4.12 (2H, m), 4.34 (2H, brs), 7.25 (1H, d, $J$ = 8.3 Hz), 7.50 (1H, s), 7.59 (1H, d, $J$ = 8.3 Hz), 7.73 (1H, s); IR (KBr) $cm^{-1}$ 3396, 1762, 1598, 1389; UV $\lambda_{max}$ ($H_2O$) 316 (sh), 263.

**$d_{29}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.01 (3H, d, $J$ = 7.6 Hz), 1.20 (3H, d, $J$ = 6.3 Hz), 2.99 (3H, s), 3.21 (3H, m), 3.41 (1H, dd, $J$ = 3.0 and 5.9 Hz), 3.51 (2H, m), 4.18 (2H, m), 4.38 (2H, brs), 7.35 (1H, d, $J$ = 7.9 Hz), 7.58 (1H, brs), 7.70 (1H, d, $J$ = 7.9 Hz), 7.83 (1H, s); IR

(KBr) $cm^{-1}$ 3422, 1759, 1600, 1472, 1387, 1274; UV $\lambda_{max}$ ($H_2O$) 320 (sh), 265.

**$d_{30}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.11 (3H, d, $J$ = 6.9 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 3.31 (1H, m), 3.49 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.26 (2H, m), 7.65 (1H, d, $J$ = 5.3 Hz), 7.78 (1H, d, $J$ = 9.9 Hz), 7.80 (1H, s), 7.89 (1H, d, $J$ = 9.9 Hz), 8.02 (1H, brs), 8.25 (1H, d, $J$ = 5.3 Hz), 8.95 (1H, s); IR (KBr) $cm^{-1}$ 3448, 1751, 1603, 1496, 1395; UV $\lambda_{max}$ ($H_2O$) 300, 291 (sh), 248 (sh), 223.

**$d_{31}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.12 (3H, d, $J$ = 7.6 Hz), 1.26 (3H, d, $J$ = 6.3 Hz), 3.37 (1H, m), 3.49 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.26 (2H, m), 7.47 (1H, m), 7.99 (2H, m), 8.17 (1H, s), 8.58 (1H, d, $J$ = 5.0 Hz); IR (KBr) $cm^{-1}$ 3402 (br), 1758, 1603, 1394, 1285, 1146; UV $\lambda_{max}$ ($H_2O$) 321 (sh), 291.

**$d_{32}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.6 Hz), 3.36 (1H, m), 3.49 (1H, dd, $J$ = 3.3 and 5.9 Hz), 4.26 (2H, m), 7.70 (1H, dd, $J$ = 5.0 and 6.9 Hz), 8.06 (1H, s), 8.40 (1H, d, $J$ = 6.9 Hz), 8.58 (1H, d, $J$ = 5.0 Hz), 8.98 (1H, s); IR (KBr) $cm^{-1}$ 3422, 1752; UV $\lambda_{max}$ ($H_2O$) 315 (sh), 280, 221 (sh).

**$d_{33}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.12 (3H, d, $J$ = 6.9 Hz), 1.27 (3H, d, $J$ = 5.9 Hz), 3.3-3.55 (2H, m), 4.2–4.4 (2H, m), 7.87 (2H, d, $J$ = 6.3 Hz), 8.23 (1H, s), 8.61 (2H, d, $J$ = 6.3 Hz); IR (KBr) $cm^{-1}$ 3568 (br), 3381 (br), 1752, 1603, 1560, 1384; UV $\lambda_{max}$ ($H_2O$) 305 (sh), 284.

**(4*R*,5*S*,6*S*,8*R*)-3-(4-(1-(Aminocarbonylmethyl)pyridinio-4-yl)thiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($d_{40}$)**. A mixture of (4*R*,5*S*,6*S*,8*R*)-*p*-nitrobenzyl-3-(4-(4-pyridyl)thiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**$c_{33}$**) (48.1 g, 89.4 mmol) and 2-iodoacetamide (82.6 g, 447 mmol) was refluxed in a mixture of acetone (400 mL) and THF (800 mL) for 7 h. The reaction mixture was cooled in an ice bath and a crystal in it was filtered in vacuo and washed with acetone. The crystal was dried in vacuo to give (4*R*,5*S*,6*S*,8*R*)-*p*-nitrobenzyl-3-(4-(1-aminocarbonylmethyl-pyridinio-4-yl)thiazol-2-ylthio)-4-methyl-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate iodide (62.3 g). A mixture of this crystal (10.0 g) and 10% Pd–C (20 g) in THF (500 mL) and 0.1 M potassium phosphate buffer (500 mL, pH 7.0) was stirred under hydrogen atmosphere for 3 h under the ambient pressure and at rt. The catalyst was filtered off and the solution was washed with dichloromethane. The separated aqueous layer was concentrated briefly to remove any residual organic solvents in vacuo and then subjected to column chromatography on Diaion CHP-20P which was successively eluted with water containing 2–8% of THF. The fractions were combined and lyophilized to give **$d_{40}$** as a yellow powder (4.03 g, 61% from **$c_{33}$**): $[\alpha]_D^{30}$ +15.9° (*c* 0.113, $H_2O$); $^1H$ NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.6 Hz), 3.50 (2H, m), 4.27 (2H, m), 5.51 (2H, s), 8.38 (2H, d, $J$ = 7.3 Hz), 8.60 (1H, s), 8.77 (2H, d, $J$ = 7.3 Hz); IR (KBr) $cm^{-1}$ 3365, 1759, 1696, 1640, 1388, 1279; UV $\lambda_{max}$ ($H_2O$) 310, 282 (sh); High-resolution FABMS *m/z* calcd for $C_{20}H_{21}N_4O_5S_2$; 461.0953 ($MH^+$); found: 461.0951.

The following compounds (**$d_{34}$–$d_{39}$**, **$d_{41}$–$d_{49}$**) were prepared from **$c_{25}$–$c_{27}$**, **$c_{29}$–$c_{33}$** as described for the preparation of **$d_{40}$**, respectively.

**$d_{34}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.3 Hz), 3.39 (1H, m), 3.50 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.26 (2H, m), 4.48 (3H, s), 8.12 (1H, dd, $J$ = 5.9 and 8.3 Hz), 8.30 (1H, s), 8.77 (1H, d, $J$ = 5.9 Hz), 8.90 (1H, d, $J$ = 8.3 Hz), 9.29 (1H, s); IR (KBr) $cm^{-1}$ 3424, 1762, 1602, 1388; UV $\lambda_{max}$ ($H_2O$) 303, 264.

**$d_{35}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 7.3 Hz), 1.25 (3H, d, $J$ = 6.3 Hz), 3.39 (1H, m), 3.50 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.24 (2H, m), 5.59 (2H, s), 8.18 (1H, dd, $J$ = 6.3 and 8.3 Hz), 8.30 (1H, s), 8.78 (1H, d, $J$ = 6.3 Hz), 9.00 (1H, d, $J$ = 8.3 Hz), 9.30 (1H, s); IR (KBr) $cm^{-1}$ 3392, 1753, 1698, 1605, 1390, 1286; UV $\lambda_{max}$ ($H_2O$) 342 (sh), 300, 268.

**$d_{36}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.11 (3H, d, $J$ = 7.3 Hz), 1.26 (3H, d, $J$ = 6.3 Hz), 3.02 (3H, s), 3.18 (3H, s), 3.40 (1H, m), 3.50 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.24 (2H, m), 5.82 (2H, s), 8.19 (1H, dd, $J$ = 5.9 and 7.9 Hz), 8.32 (1H, s), 8.72 (1H, d, $J$ = 5.9 Hz), 9.01 (1H, d, $J$ = 7.9 Hz), 9.25 (1H, s); IR (KBr) $cm^{-1}$ 3421, 1758, 1662, 1601, 1386, 1262; UV $\lambda_{max}$ ($H_2O$) 292, 240 (sh).

**$d_{37}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.06 (3H, d, $J$ = 7.3 Hz), 1.24 (3H, d, $J$ = 6.3 Hz), 2.46 (3H, s), 3.39 (1H, m), 3.48 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.23 (2H, m), 4.81 (2H, s), 8.16 (1H, dd, $J$ = 6.3 and 8.3 Hz), 8.25 (1H, s), 8.63 (1H, d, $J$ = 6.3 Hz), 8.95 (1H, d, $J$ = 8.3 Hz), 9.13 (1H, s); IR (KBr) $cm^{-1}$ 3410, 1759, 1600, 1384; UV $\lambda_{max}$ ($H_2O$) 301, 271 (sh), 233(sh).

**$d_{38}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.13 (3H, d, $J$ = 7.3 Hz), 1.28 (3H, d, $J$ = 6.3 Hz), 3.42 (1H, m), 3.52 (1H, dd, $J$ = 3.0 and 5.9 Hz), 4.28 (2H, m), 5.32 (2H, s), 8.17 (1H, dd, $J$ = 5.3 and 7.9 Hz), 8.31 (1H, d, $J$ = 1.0 Hz), 8.76 (1H, dd, $J$ = 1.0 and 5.3 Hz), 8.96 (1H, d, $J$ = 7.9 Hz), 9.28 (1H, s); IR (KBr) $cm^{-1}$ 3418, 1752, 1636, 1374, 1286; UV $\lambda_{max}$ ($H_2O$) 301, 271 (sh).

**$d_{39}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.12 (3H, d, $J$ = 7.3 Hz), 1.27 (3H, d, $J$ = 6.3 Hz), 3.44 (1H, m), 3.53 (1H, dd, $J$ = 3.0 and 6.3 Hz), 4.2–4.35 (2H, m), 4.38 (3H, s), 8.40 (2H, d, $J$ = 6.9 Hz), 8.61 (1H, s), 8.78 (2H, d, $J$ = 6.9 Hz); IR (KBr) $cm^{-1}$ 3380 (br), 1762, 1636, 1605, 1388; UV $\lambda_{max}$ ($H_2O$) 312, 285 (sh).

**$d_{41}$**. $^1H$ NMR (270 MHz, $D_2O$) δ 1.04 (3H, d, $J$ = 7.3 Hz), 1.24 (3H, d, $J$ = 6.3 Hz), 3.28 (1H, m), 3.30 (6H, s), 3.44 (1H, dd, $J$ = 3.0 and 5.9 Hz), 3.53 (2H, m), 3.65 (2H, m), 3.93 (2H, m), 4.20 (4H, m), 7.48 (1H, d, $J$ = 7.6 Hz), 7.60 (1H, t, $J$ = 7.6Hz), 7.85 (1H, s), 7.90 (1H, s), 7.91 (1H, d, $J$ = 7.6 Hz); IR (KBr) $cm^{-1}$ 3424, 1752,

1602, 1457, 1382, 1280; UV $\lambda_{max}$ ($H_2O$) 315 (sh), 265 (sh).

**$d_{42}$**. $^1$H NMR (270 MHz, $D_2O$) δ 1.07 (3H, d, $J$ = 7.3 Hz), 1.25 (3H, d, $J$ = 6.3 Hz), 3.29 (1H, m), 3.49 (3H, s), 3.60–4.40 (9H, m), 4.23 (2H, m), 4.34 (2H, s), 7.50 (1H, d, $J$ = 7.6 Hz), 7.62 (1H, t, $J$ = 7.9 Hz), 7.88 (1H, s), 7.93 (1H, s), 7.96 (1H, d, $J$ = 7.9 Hz); IR (KBr) $cm^{-1}$ 3422, 1762, 1696, 1636, 1605, 1448, 1387; UV $\lambda_{max}$ ($H_2O$) 311, 290 (sh), 263 (sh).

**$d_{43}$**. $^1$H NMR (270 MHz, $D_2O$) δ 0.92 (3H, d, $J$ = 7.3 Hz), 1.19 (3H, d, $J$ = 6.3 Hz), 3.19 (1H, m), 3.30 (6H, s), 3.36 (1H, dd, $J$ = 2.6 and 5.6 Hz), 3.57 (4H, m), 3.91 (2H, m), 4.15 (4H, m), 7.50 (2H, d, $J$ = 8.3 Hz), 7.79 (2H, d, $J$ = 8.3 Hz), 7.81 (1H, s); IR (KBr) $cm^{-1}$ 3422, 1752, 1620, 1560, 1458, 1282; UV $\lambda_{max}$ ($H_2O$) 318 (sh), 278.

**$d_{44}$**. $^1$H NMR (270 MHz, $D_2O$) δ 1.10 (3H, d, $J$ = 6.9 Hz), 1.22 (3H, d, $J$ = 6.3 Hz), 3.25 (1H, m), 3.42 (1H, dd, $J$ = 2.6 and 5.6 Hz), 3.45 (3H, s), 3.60–4.15 (8H, m), 4.23 (2H, m), 4.34 (2H, s), 7.54 (2H, d, $J$ = 8.6 Hz), 7.84 (2H, d, $J$ = 8.6 Hz), 7.88 (1H, s); IR (KBr) $cm^{-1}$ 3420, 1758, 1696, 1609, 1448, 1387, 1283; UV $\lambda_{max}$ ($H_2O$) 319 (sh), 274.

**$d_{45}$**. $^1$H NMR (270 MHz, $D_2O$) δ 0.84 (3H, d, $J$ = 7.3 Hz), 1.16 (3H, d, $J$ = 6.3 Hz), 3.12 (1H, m), 3.26 (9H, s), 3.27 (1H, m), 3.62 (2H, m), 3.89 (2H, m), 4.07 (1H, dd, $J$ = 2.3 and 10.2 Hz), 4.13 (1H, m), 7.65 (5H, m); IR (KBr) $cm^{-1}$ 3419, 1762, 1654, 1608, 1560, 1388, 1290; UV $\lambda_{max}$ ($H_2O$) 285.

**$d_{46}$**. $^1$H NMR (270 MHz, $D_2O$) δ 1.04 (3H, d, $J$ = 7.3 Hz), 1.22 (3H, d, $J$ = 6.3 Hz), 3.25 (6H, s), 3.31 (3H, m), 3.43 (1H, dd, $J$ = 3.0 and 5.6 Hz), 3.76 (2H, m), 4.20 (2H, m), 4.65 (2H, brs), 7.42 (1H, d, $J$ = 7.9 Hz), 7.61 (1H, s), 7.77 (1H, d, $J$ = 7.9 Hz), 7.87 (1H, s); IR (KBr) $cm^{-1}$ 3428, 1758, 1600, 1387, 1264; UV $\lambda_{max}$ ($H_2O$) 316 (sh), 265.

**$d_{47}$**. $^1$H NMR (270 MHz, $D_2O$ -5% DMSO-$d_6$) δ 1.04 (3H, d, $J$ = 7.3 Hz), 1.23 (3H, d, $J$ = 6.6 Hz), 3.20–3.60 (4H, m), 3.45 (3H, s), 3.96 (1H, m), 4.21 (3H, m), 4.23 (2H, s), 5.00 (2H, m), 7.45 (1H, d, $J$ = 7.9 Hz), 7.66 (1H, s), 7.82 (1H, d, $J$ = 7.9 Hz), 7.92 (1H, s); IR (KBr) $cm^{-1}$ 3406, 1752, 1700, 1598, 1387, 1263; UV $\lambda_{max}$ ($H_2O$) 315 (sh), 265.

**$d_{48}$**. $^1$H NMR (270 MHz, $D_2O$) δ 1.06 (3H, d, $J$ = 7.3 Hz), 1.23 (3H, d, $J$ = 6.6 Hz), 3.34 (1H, m), 3.47 (1H, d, $J$ = 2.0 and 5.6 Hz), 4.22 (2H, m), 4.54 (3H, s), 8.15 (1H, s), 8.24 (1H, d, $J$ = 8.3 Hz), 8.37 (1H, d, $J$ = 6.6 Hz), 8.46 (2H, m), 8.65 (1H, s), 9.67 (1H, s); IR (KBr) $cm^{-1}$ 3421, 1757, 1602, 1388, 1281; UV $\lambda_{max}$ ($H_2O$) 305, 265 (sh), 253 (sh), 240.

**$d_{49}$**. $^1$H NMR (270 MHz, $D_2O$) δ 1.02 (3H, d, $J$ = 7.2 Hz), 1.22 (3H, d, $J$ = 6.3 Hz), 3.33 (1H, m), 3.46 (1H, dd, $J$ = 2.6 and 5.9 Hz), 4.21 (2H, m), 5.64 (2H, m), 7.97 (1H, s), 8.11 (1H, d, $J$ = 8.9 Hz), 8.30 (2H, m), 8.43 (2H, m), 9.62 (1H, s); IR (KBr) $cm^{-1}$ 3392, 1758, 1697, 1600, 1388, 1282; UV $\lambda_{max}$ ($H_2O$) 300, 271, 242.

### Measurement of in vitro antibacterial activity

MICs were determined by the twofold serial agar dilution method, with Müller–Hinton medium (Difco Laboratories, Detroit, Michigan, U.S.A.), supplemented with 5% defibrinated horse blood for *Streptococci* was used.

An overnight broth culture or bacterial suspension was diluted with the corresponding broth or with phosphate-buffered saline supplemented with 0.01% gelatin to give a final concentration of approximately $10^7$ CFU/mL. A portion (about 5 μL) of the dilution was plated onto a drug-containing agar surface with an inoculum apparatus (Microplanter; Sakuma Seisakusho, Tokyo, Japan). The final inoculum size was approximately $10^5$ CFU per spot. The plates were incubated at 37 °C for 20 h, except for methicillin-resistant *Staphylococci* (incubated at 35 °C for 24 h in Müller–Hinton medium supplemented with 4% NaCl). MIC was defined as the lowest antibiotic concentration that completely prevented visible growth.

The degree of homogeneity of phenotypic resistance in *S. aureus* was assessed by disk diffusion tests and population analysis.[40]

### Stability test of carbapenem compounds to DHP-I

The stability of carbapenems against renal DHP-I was determined with purified swine renal DHP-I, as reported previously.[41] The activity of DHP-I was spectrophotometrically determined by measuring the hydrolysis of glycyldehydrophenylalanine as a substrate.

### Affinity test of carbapenem compounds to PBP-2′

The binding of carbapenem to PBP-2′ was independently determined by the method of Sumita et al.[42] Membrane fractions were pretreated with various concentrations of antibiotics for 30 min at 30 °C, the final concentration of 300 μg of radioactive benzylpenicillin (diluted fivefold with nonradioactive benzylpenicillin) per mL was then added, and the fractions were incubated for another 30 min at 30 °C.

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