Antibacterial Activity of Some 5-Dialkylaminomethylhydantoins and Related Derivatives

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In connection with our studies on antibacterial compounds in the class of 5-dialkylaminomethylhydantoins against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) strains, some molecular modifications were attempted. The antibacterial activities of all of the synthesized hydantoin derivatives were evaluated. Among the hydantoin derivatives designed in this study, C_2-symmetrical twin-drug type compound (7) showed the highest level of antibacterial activity against *S. aureus* strain.

Key words hydantoin; antibacterial activity; C_2-symmetrical twin-drug; *Escherichia coli*; *Staphylococcus aureus*; β-aminoalanine

Since bacteria can develop resistance to generally used antibacterial agents, resulting in the generation of various drug-resistant bacteria strains, the development of new antibacterial agents is required. In the early invasion stage of bacteria such as *Escherichia* (*E.*) *coli*, cell surface glycans of bacteria recognize host cell lectin. For such recognition by glycans, the major recognition patterns between the host and guest molecules are thought to be intermolecular hydrogen bonding interactions. This interaction process is a logical path and is thought to control biological response. We have been interested in molecules that interfere with such carbohydrate recognition stages in order to find new antibacterial leads.

In connection with our studies on antibacterial compounds, we have already reported some molecular designs, synthesis and biological evaluations of a few heterocyclic compounds or various types of functionalized symmetrical molecules.[1–5] In our previous articles on chemical modifications of linezolid, we reported synthesis and antibacterial activity of new structural isomers of oxazolidin-2-ones as a general structure (**A**)[3] or a bioisosteric replacement[6] of the oxazolidinone ring in linezolid[7] by a hydantoin nucleus (**B**) (Fig. 1). Among previously synthesized target hydantoin derivatives (**B**), we found that many 5-dialkylaminomethylhydantoin derivatives showed a wide range of remarkable antibacterial activities (minimum inhibitory concentration (MIC) values in a sub-micro molar range) against both *Staphylococcus* (*S.*) *aureus* and *E. coli* strains. This finding encouraged us to investigate further molecular modifications of this class of compounds. We therefore carried out additional synthetic investigations of newly designed 5-dialkylaminomethylhydantoin derivatives.[8]

In this article, we present results of biological evaluation for antibacterial activity of these new 5-dialkylaminomethyl-3-substituted hydantoin derivatives with *E. coli* and *S. aureus* strains.

Antibacterial Activity and Discussion Many tested hydantoin derivatives showed significant antibacterial activity

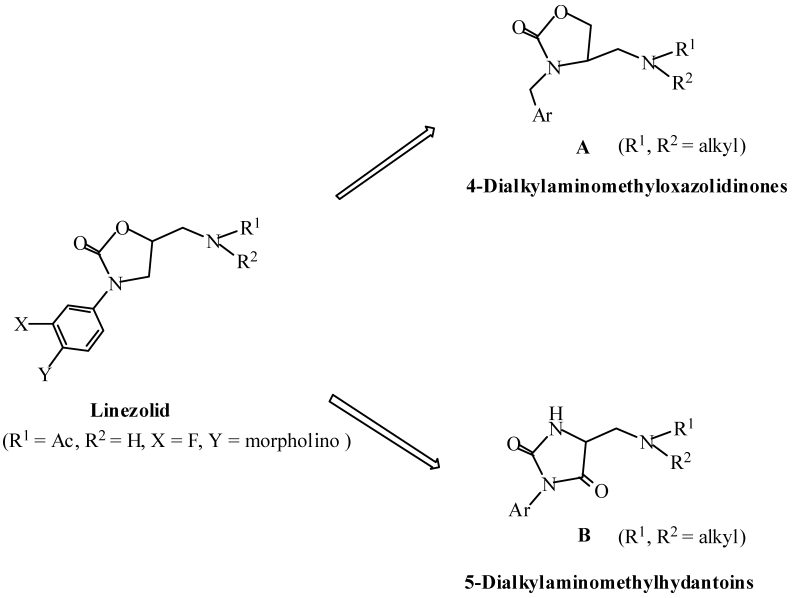

Fig. 1. Structures of **A**, **B** and Linezolid

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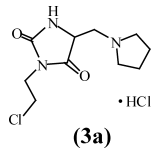
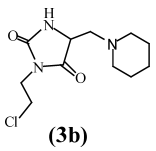
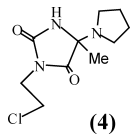
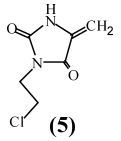
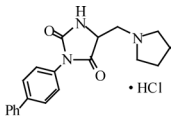
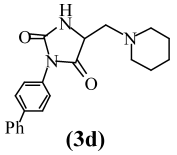
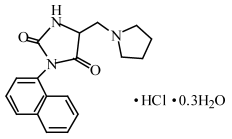
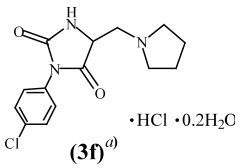
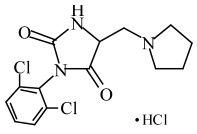
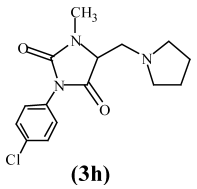
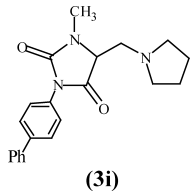
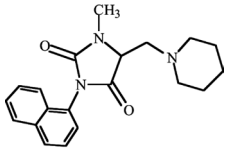
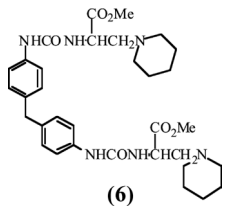
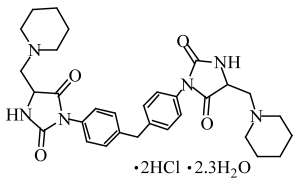
Table 1. Antibacterial Activities of Target Hydantoin Derivatives (**3**–**7**)

Compound	Antibacterial activity MIC (mM)		Compound	Antibacterial activity MIC (mM)	
	E. coli	*S. aureus*		*E. coli*	*S. aureus*
 **(3a)**	0.113	0.226	 **(3b)**	0.216	>0.432
 **(4)**	>0.513	>0.513	 **(5)**	0.367	>0.733
 **(3c)**	>0.344	>0.344	 **(3d)**	>0.332	>0.332
 **(3e)**	>0.364	0.091	 **(3f)**[a]	0.048	0.097
 **(3g)**[a]	0.044	0.088	 **(3h)**	0.104	>0.416
 **(3i)**	>0.366	>0.366	 **(3j)**	0.190	0.190
 **(6)**	>0.206	0.051	 **(7)**	0.095	0.024

a) Data were taken from ref. 6.

against both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) at concentrations of 0.024–0.226 mM.

Among the 3-(2-chloroethyl)-substituted new hydantoin derivatives (**3a**, **b**, **4**, **5**) listed in Table 1, compound (**3a**) showed the highest antibacterial activities against both of *E. coli* and *S. aureus* strains (MIC=0.113, 0.226 mM, respectively), but the levels of its activity were lower than those of 3-*p*-chlorophenyl-substituted derivatives (**3f**)[6] reported previously. 3-Chloroethyl-5-disubstituted hydantoin (**4**) did not show any remarkable antibacterial activity against either *E. coli* and *S. aureus* strains at a concentration of 0.513 mM. The deamination product (**5**) showed a weaker antibacterial activity (MIC= 0.367 mM) only against *E. coli* than those of **3a** and **3b** (see Table 1).

N(1)-Unsubstituted hydantoin derivatives (**3c**–**e**) that have a sterically bulky aryl ring such as a biphenyl or naphthyl group at the *N*(3)-position of the hydantoin ring generally showed lower levels of activity than those of 3-chlorosubstituted phenyl derivatives (**3f**, **g**) except for compound **3e**. Compound **3e** showed notable antibacterial activity against *S. aureus* (MIC= 0.091 mM). Through these structural modifications by the introduction of a sterically bulky aromatic ring, no significant

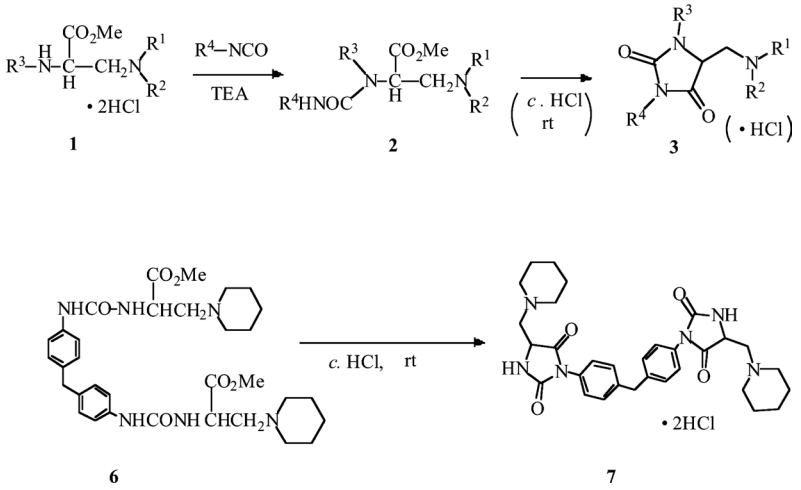


Chart 1. Synthesis of 5-Dialkylaminomethylhydantoins **3** and **7**

potentiating effect of antibacterial activity was observed.

Among the compounds described in this paper, it is noteworthy that the introduction an *N*(1)-methyl group on the hydantoin nucleus resulted in decreases in antibacterial against both *E. coli* and *S. aureus*. For example, compound **3f** showed an MIC value of 0.048 mM against *E. coli*, but the corresponding *N*(1)-methylated derivative **3h** had decreased activity (MIC=0.104 mM). A similar decrease in activity to *S. aureus* was also observed. This peculiarity of structure indicates that the *N*(1)-H moiety in the hydantoin ring in the molecule plays an important role in the action for antibacterial activity. Compared with the structure of linezolid, we have pointed out in our previous paper that the bioisosteric hydantoin nucleus[6] has a characteristic functional group (*N*-H) for acting as a hydrogen bonding donor in the 5-membered hydantoin ring system. The results obtained for the new *N*(1)-methyl hydantoin derivatives (**3h–j**) by a bioisosteric modification seem to provide useful information regarding the importance of the presence of a functional group acting as a hydrogen bonding donor in the molecule for antibacterial activity.

Symmetrical twin-drug type molecules,[9,10] in general, are expected to produce more potent and/or selective biological activities stemming from the ligand pharmacophore. The two identical ligands could bind to the same receptor or enzyme sites simultaneously. From this well-known conception of identical twin-drugs, further molecular modifications of this class of hydantoins to twin-drug type molecules seemed to be interesting in the search for new leads. We therefore attempted synthesis and antibacterial evaluation of a twin-drug type molecule (**7**) having a diphenylmethane moiety as a linker (see Table 1). The C_2-symmetrical compound **7**[11] showed the highest level of antibacterial activity (MIC=0.024 mM) against *S. aureus* and also a high level antibacterial activity against *E. coli* (Gram-negative) (MIC=0.095 mM).

The intermediate C_2-symmetrical urea derivative **(6)** as a precursor to the compound **(7)** also showed significant antibacterial activity (MIC=0.051 mM) only against *S. aureus*.

As structural factors for antibacterial activities in this series, the results obtained suggest not only the state of an electric charge of an introduced aryl ring but also the contribution of a rotational isomer[8] about the C–N pivot bond connecting the two ring systems (see **3e** or **3j**). The results for 2-chloroethyl derivatives (**3a**, **b**) at the *N*(3) position of a hydantoin ring also seem to indicate the importance of a substituent at the *N*(3) position in the hydantoin ring together with the nature of substituents such as lipophilic properties.

At present, we have no clear evidence that these types of hydantoin derivatives have the same mode of antibacterial action as that of linezolid. However, to obtain some identical information on the mode of antibacterial activity for the target molecules as well as to confirm the usefulness of this hydantoin scaffold for antibacterial efficacy, further synthetic trials of new hydantoin derivatives including new types of C_2-symmetrical twin-drug type derivatives containing other linkers are being conducted. Regarding sugar recognition properties of highly bioactive twin-drug type compounds such as **7** and **6**, we are also planning to carry out calorimetric experiments with sugar derivatives.

Experimental

Synthesis of Target 5-Dialkylaminomethyl-3-substituted Hydantoins and Related Compounds According to the procedure reported in our previous paper,[8] we prepared target 5-dialkylaminomethyl-3-aryl-hydantoins (**3**) by cyclization of urea intermediates (**2**), which are easily obtained from the reaction of *β*-aminoalanine ester derivatives (**1**) with isocyanates. Compound (**7**) was also prepared by double cyclization of symmetrical intermediate (**6**) as shown in Chart 1. The structures of these compounds have been shown in detail in our previous paper. Compounds (**3a**, **c**, **e**, **7**) were used as hydrochlorides for antibacterial activity assay.

Assay for Antibacterial Activity We used *S. aureus* ATCC6538P and *E. coli* NBRC14237 (NIHJ) (Gram-positive and Gram-negative bacteria, respectively) as target organisms. Synthesized compounds (**3**–**7**) were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1.280 μg/mL. The minimum inhibitory concentration (MIC) of a standard strain was measured by the authentic microdilution method to monitor the bacterial growth turbidity in Muller–Hinton broth according to the Japanese Society of Chemotherapy.[12,13] The values of MIC for target compounds determined by this authentic MIC method, as well as those of previously reported compounds (**3f**, **g**) for comparison, are summarized in Table 1. The values of MIC are expressed as molar concentrations

(mM) for the discussion of structure–activity relations.

References and Notes

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11) The obtained compound **7** exhibited very simple symmetrical ^{13}C-NMR in DMSO-d_6, indicating little difference with respect to the signal assignable to substituted hydantoin rings and a linker diphenylmethane moiety. From a stereochemical viewpoint, however, product **7** can be considered to be a mixture of three twin-drug type molecules, *i.e.*, two C_2-symmetrical molecules that have the same absolute configuration (*R*,*R* or *S*,*S*) regarding two chiral hydantoin rings in the molecules and a Cs-symmetrical *meso* compound having different absolute configurations (*R*,*S*). We distinguished the presence of three predominant stereoisomers in the free base of product **7** by an HPLC method (CHIRALPAK IA®) with a mobile phase consisting of *n*-hexane–2-propanol–diethylamine=150 : 150 : 0.2 (v/v). The ratio of the three peaks (*ca.* 1 : 2 : 1) was estimated by comparison of these elution peak areas.
12) The Japanese Society of Chemotherapy (1989), *Chemotherapy*, **38**, 102–105 (1990).
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