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PREPARATION AND ANTIBACTERIAL EVALUATION OF SOME SYMMETRICAL TWIN-DRUG TYPE BIVALENT MOLECULES

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Abstract – As one of our projects to investigate new bioactive compounds, we here report the synthesis and antibacterial evaluation of a few identical linker mode twin-drug type symmetrical hydantoin derivatives. The antibacterial activity of tested twin-drug type hydantoin derivatives (**4**~**7**) and the structure-activity relationships (SARs) of these bivalent symmetrical molecules are also described.

In terms of molecular recognition, two-fold (C_2) or three-fold (C_3) symmetrical geometry of macromolecules is a common architecture involved in many biological processes, and small symmetrical molecules frequently appear in many bioactive compounds.[1-4] From this interesting aspect of molecular geometry, we have already reported some examples of such types of symmetrical molecules for the purpose of finding new bioactive leads or candidates.[5-14]

As one of our projects to investigate new antibacterial compounds, we have already reported a few five-membered antibacterial active heterocyclic compounds.[5,7,8,11,14] Among the compounds previously reported, some derivatives containing hydantoin nuclei in the molecule showed an interesting antibacterial activity against a Gram-positive (*S. aureus*) or a Gram-negative (*E. coli*) strain.[5,14,15] In our previous paper, we described a representative identical twin-drug type symmetrical molecule (**A**)[15] possessing a considerably high level of antibacterial activity, and this symmetrical bivalent molecule showed an interesting binding property to sulfated glycosaminoglycans such as heparan sulfate.[5,16]

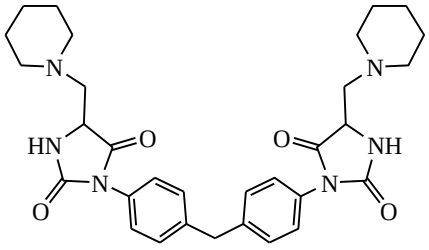
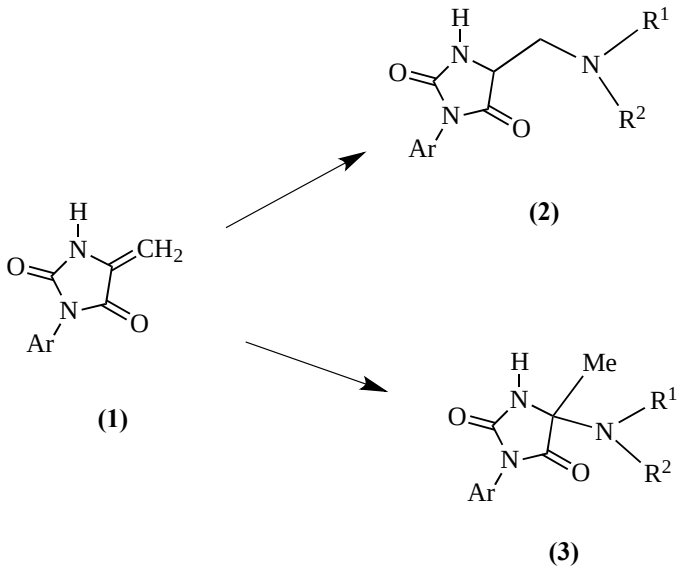


Figure 1. Structure of compound **A**

In this article, we report the preparation of some new symmetrical twin-drug type bivalent molecules. We also describe the results of antibacterial evaluation of synthesized products and the investigated structure-activity relationships of identical twin-drug type molecules.

Preparation of 5-Substituted Hydantoins and New Twin-Drug Type Symmetrical Molecules

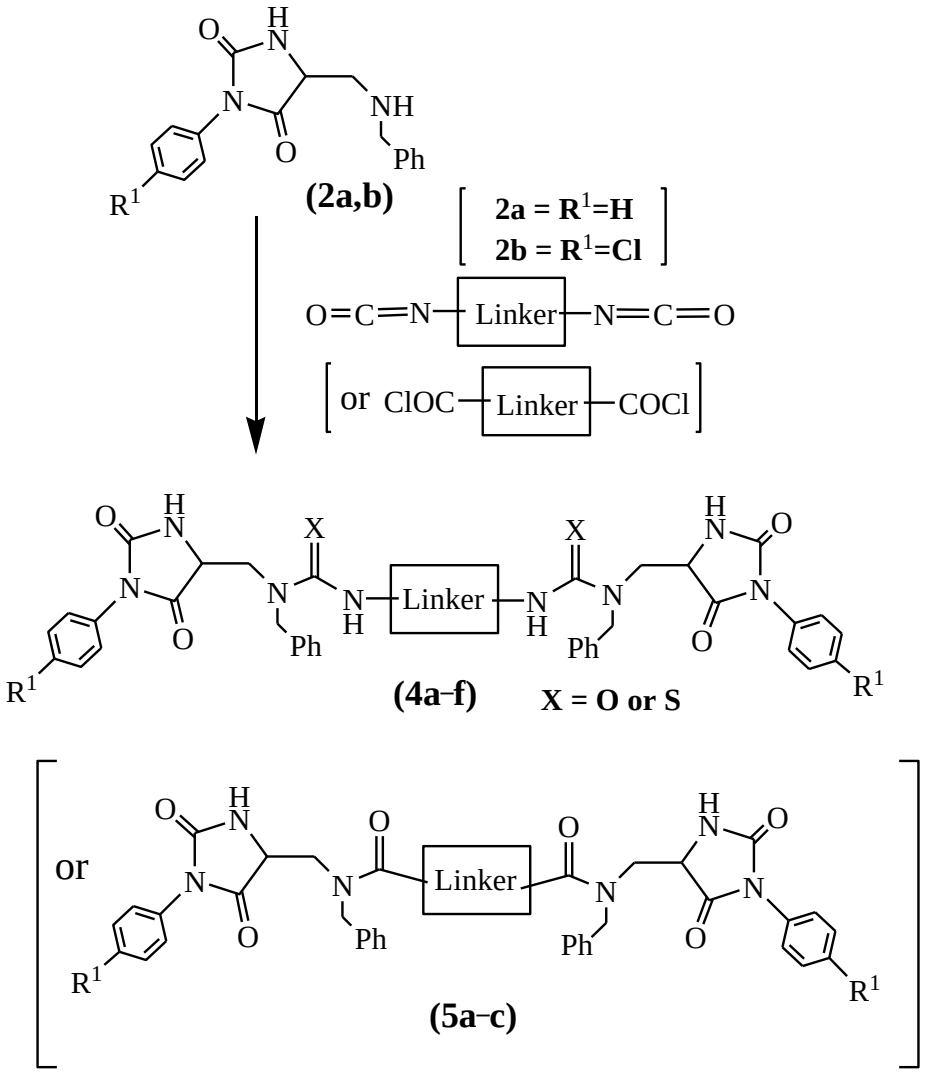
As starting materials for further derivatization described in this article, we prepared 5-methylene-3-aryl-hydantoins (**1**) by deamination of 5-dialkylaminomethylhydantoin derivatives, in a manner similar to that reported previously.[17,18] By using regioselective addition reactions of various amines to starting 5-methylene-hydantoin (**1**), we obtained two types of new 5-substituted hydantoin derivatives[5,16] [5-alkylaminomethylhydantoins (**2**) and isomeric 5-alkylamino-5-methyl-hydantoins (**3**)] (Scheme 1).



Scheme 1. Modification of 5-methylene-hydantoin (**1**) to 5-alkylaminomethylhydantoins (**2**) and isomeric 5-alkylamino-5-methyl-hydantoins (**3**)

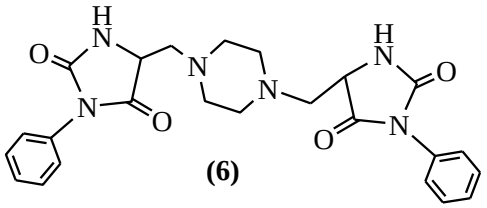
Among these new hydantoin derivatives obtained from the reactions of 5-methylene-hydantoins [**1a** (Ar=Ph) and **1b** (Ar=*p*-Cl-C_6H_4)] with amine nucleophiles, we are particularly interested in the 5-substituted hydantoin derivative having secondary amine functionality in the molecule because of its good reactivity to an acetylating agent such as acetic anhydride.[5] We examined various diacylation reactions of primary amine adducts **2a** (Ar=Ph, R^1=H, R^2=CH_2Ph) and **2b** (Ar=*p*-Cl-C_6H_4, R^1=H, R^2=CH_2Ph) with aryldiisocyanates (or aryldiisothiocyanates) to obtain twin-drug type bivalent compounds (**4** and **5**, respectively).

In our previous paper,[5] we reported that a few analogues of twin-drug type bivalent molecule **A** that have a flexible methylene group [-$(CH_2)_n$- ; n = 4~12] as a linker in the molecule showed no antiviral activity against a *S. aureus* or *E. coli* strain.



Scheme 2. Structures of twin-drug type hydantoin derivatives (**4** and **5**)

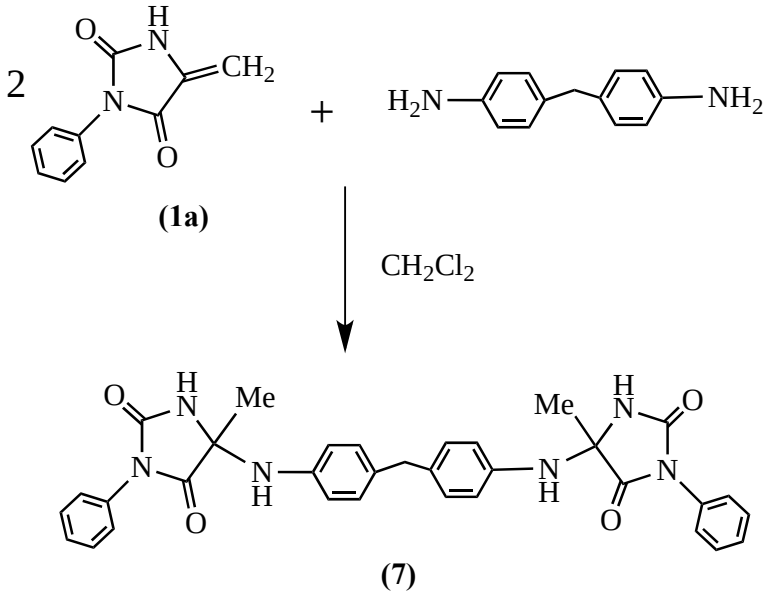
Therefore, in the modifications of these targeted bivalent symmetrical derivatives (**4a-f** and **5a**) having two hydantoin rings in the molecules, we mainly used a few less flexible linkers such as phenyl, biphenyl and diphenylmethane moieties for identical twin-drug type bivalent symmetrical derivatives. Two additional compounds **5b** and **5c** that have a flexible methylene-chain linker [-$(CH_2)_n$- : n = 8] were prepared for a comparison of antibacterial activities (see Scheme 2 and Table 1).[16] These twin-drug type derivatives were obtained in good to moderate yields from reactions of the corresponding amines (**2**) with decanedioyl dichloride in a manner similar to that reported previously.[17-19] Preparation of no-linker mode twin-drug type compound (**6**) with a piperazine ring in the molecule (see Figure 2) was also achieved previously.[16] Antibacterial evaluation of this compound showed that it had no antibacterial activity against a Gram-positive (*S. aureus*) or a Gram-negative (*E. coli*) strain (see Table 1).[20]



(6)

Figure 2. Structure of compound **6**

In addition to the above modifications, we also carried out a few trials in order to obtain different twin-drug types of bivalent symmetrical derivatives (**7**) containing two 5-amino-5-methyl-hydantoin moieties in the molecules. The reaction of 5-methylene derivative (**1a**) with a symmetrical amine such as 4,4'-diaminodiphenylmethane in a molar ratio of **1a** : 4,4'-diaminodiphenylmethane = 2/1 with a CH_2Cl_2 solvent[5,16] successfully proceeded to give the twin-drug type 5-amino-5-methyl-hydantoin derivative (**7**) as a major product (Scheme 3) (see EXPERIMENTAL for details).



(1a)

(7)

Scheme 3. Preparation of twin-drug type 5-amino-5-methyl-hydantoin derivative (**7**)

Antibacterial Evaluation and Discussion

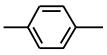
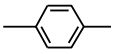
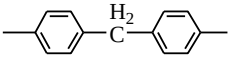
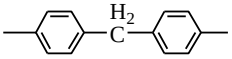
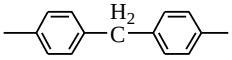
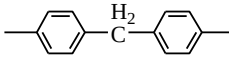
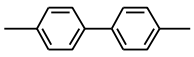
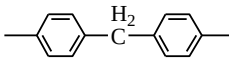
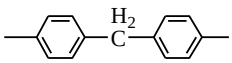
We used Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*) as target organisms for the assay of antibacterial activities of the compounds synthesized. Target compounds were dissolved in dimethyl sulfoxide (DMSO) for the bioassay, and the minimum inhibitory concentrations (MICs) of the compounds were estimated.

In our previous study,[5] we prepared a few derivatives of twin-drug type bivalent molecule **A** and evaluated antibacterial activities of the compounds. Among the previously prepared twin-drug type symmetrical compounds that have a flexible methylene group [-$(CH_2)_n$- : n = 4–12] as a linker in the

molecule **A** junction mode, none of the prepared twin-drug type symmetrical compounds showed antibacterial activity against either a Gram-positive (*S. aureus*) or Gram-negative (*E. coli*) strain (MIC => 0.193 mM).[5] In contrast, symmetrical twin-drug type compound **A**, which has the same junction mode and a less flexible linker such as a diphenylmethane moiety, showed significant antibacterial activity against both *S. aureus* (MIC = 0.026 mM) and *E. coli* (MIC = 0.095 mM).

In addition to these twin-drug type compounds, newly targeted bivalent symmetrical derivatives (**4a**–**f** and **5a**) with a different junction mode and less flexible linkers such as phenyl, biphenyl and diphenylmethane moieties were inactive against either the *E. coli* or *S. aureus* strain (MIC => 0.136~0.171 mM). Newly evaluated bivalent symmetrical derivatives (**5b** and **5c**) having a similar junction mode of two hydantoin rings in the molecules that have flexible methylene-chain linkers [-$(CH_2)_n$- : n = 8] also showed no antibacterial activity (MIC => 0.155 mM) (see Table 1).

Table 1. Antibacterial Activity of Twin-Drug Type Hydantoin Derivatives (**4**, **5**, **6** and **7**)

				MIC (mM)	
Compound No	R^1	X	Linker	*S. aureus*	*E. coli*
4a	H	O		> 0.171	> 0.171
4b	Cl	O		> 0.156	> 0.156
4c	H	O		> 0.152	> 0.152
4d	Cl	O		> 0.141	> 0.141
4e	H	S		> 0.147	> 0.147
4f	Cl	S		> 0.136	> 0.136
5a	H	O		> 0.161	> 0.161
5b	H	O	—$(CH_2)_8$—	> 0.169	> 0.169
5c	Cl	O	—$(CH_2)_8$—	> 0.155	> 0.155
6	-	-	-	> 0.277	> 0.277
7	-	-		> 0.223	> 0.223
A	-	-		0.026	0.095

In addition, the results showing no antibacterial activity of the twin-drug type 5-amino-5-methyl-hydantoin derivative (**7**) at a dose of 0.223 mM against *E. coli* or *S. aureus* suggested that the linker diphenylmethane moiety used for this linker mode is an ineffective linker for antibacterial activity. A twin-drug type bivalent molecule with a different junction mode (no-linker mode) such as compound **6** was also inactive (MIC => 0.277 mM) against both *E. coli* and *S. aureus* strains. From these experiments, we confirmed that most of the tested twin-drug type compounds showed no significant antibacterial activity against either strain. Among the many identical twin-drug type compounds that have been tested, the original lead junction mode compound **A** was reconfirmed to show the highest level of antibacterial activity.

From these experimental results, we now consider that identical twin-drug type bivalent molecules with a junction mode such as compound **A** and with less flexible linkers would be a more promising structural feature for antibacterial active molecules. In order to confirm the effectiveness of this junction mode for antibacterial activity, additional trials for preparation of these identical twin-drug type 5-substituted hydantoin derivatives are under way.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were measured by a Shimadzu FT/IR-8100 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were obtained by a JEOL JNM A-500 at 35 °C. Chemical shifts are expressed in δ ppm downfield from an internal tetramethylsilane (TMS) signal. The signal assignments were confirmed by ^{1}H - ^{1}H two-dimensional (2D) correlation spectroscopy (COSY), ^{1}H -^{13}C heteronuclear multiple quantum coherence (HMQC), and ^{1}H -^{13}C heteronuclear multiple-bond connectivity (HMBC) spectra. High-resolution FAB-MS spectra were obtained by a JEOL JMS-HX110 mass spectrometer. The following abbreviations in parentheses were used for hydantoin ring (Hyd) and decandiamide (DD).

Preparation of Starting Hydantoin Derivatives

Compounds **1a** (Ar = Ph) and **1b** (Ar = *p*-Cl-C_6H_4) were prepared by deamination of the corresponding 5-piperidinomethylhydantoins in a manner similar to that reported previously.[5,16] Compounds **2a** (Ar = Ph, R^1=H, R^2=CH_2Ph) and **2b** (Ar = *p*-Cl-C_6H_4, R^1=H, R^2=CH_2Ph) were also obtained by the same procedure as that reported previously.[5] Compounds **4a-c**, **4e**, **5a-b** and **6** were prepared by the method described in a previous paper.[16] Physical and spectroscopic data of those compounds were presented in our previous papers.[5,16]

1,1'-(Methylenebis(4,1-phenylene))bis(3-benzyl-3-((1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)-methyl)urea) (4d)

This twin-drug type compound was obtained from the reaction of 5-((benzylamino)methyl)-3-(4-chlorophenyl)imidazolidine-2,4-dione (**2b**) with

bis(4-isocyanatophenyl)methane by the same procedure as that described previously.[16] Compound **4d** was obtained in quantitative yield as a white solid. Mp 126–128 °C. The structure of compound **4d** was easily characterized by IR, ^{1}H- and ^{13}C-NMR spectroscopic data including two-dimensional (2D)-NMR methods and its elemental analysis (see below). Despite repeated operations of a high-resolution MS spectrum for this compound, however, no correct molecular ion peak was obtained, and its base peak ion (*m*/*z*) was 91 (a benzyl cation radical fragment).

IR (KBr) cm^{-1}: 1779, 1719. ^{1}H-NMR ($CDCl_3$) δ: 1.79 (2H, br, NHCON=), 3.67–3.72, 3.79–3.82 (each 2H, m, Hyd-C$\underline{H}_2$-N=), 3.74 (2H, s, Ph-C$\underline{H}_2$-Ph), 4.10–4.12 (2H, m, Hyd H-5), 4.47 and 4.55 (each 2H, d, J = 17.0 Hz, C$\underline{H}_2$-Ph), 6.94, 7.04 (each 4H, d, J = 8.5 Hz, $\underline{Ph}$-CH_2-Ph), 7.08 (2H, br s, Hyd H-1), 7.18–7.35 (18H, m, Ar H). ^{13}C-NMR ($CDCl_3$) δ: 40.6 (Ph-$\underline{C}H_2$-Ph), 49.6 (Hyd-$\underline{C}H_2$-N=), 52.6 ($\underline{C}H_2$-Ph), 57.9 (Hyd C-5), 121.1 (Ar C-2, C-6 in $\underline{Ph}$-CH_2-Ph), 126.6, 127.1, 128.2, 129.1, 129.2, 129.3, (Ar C), 130.0, (Ar C-1 or C-4 in *p*-Cl-$\underline{Ph}$), 133.9 (Ar C-4 or C-1 in *p*-Cl-$\underline{Ph}$), 136.1 (Ar C-1 in CH_2-$\underline{Ph}$), 136.4 (Ar C-1 in $\underline{Ph}$-CH_2-Ph), 137.0 (Ar C-4 in $\underline{Ph}$-CH_2-Ph), 155.7 (Hyd C-2), 156.7 (NH$\underline{C}$ON=), 171.8 (Hyd C-4). *Anal.* Calcd for $C_{49}H_{42}Cl_2N_8O_6 \cdot 0.5H_2O$: C, 64.05; H, 4.72; N, 12.20. Found: C, 64.10; H, 4.75; N, 11.96.

1,1'-(Methylenebis(4,1-phenylene))bis(3-benzyl-3-((1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)-methyl)thiourea) (4f)

This twin-drug type compound was obtained from the reaction of 5-((benzylamino)methyl)-3-(4-chlorophenyl)imidazolidine-2,4-dione (**2b**) with bis(4-isothiocyanatophenyl)methane in the same manner as that described previously.[16] Compound **4f** was obtained in 58% yield as a white solid. Mp 119–141 °C. IR (KBr) cm^{-1}: 1777, 1719. FAB-MS (positive) *m/z*: 941 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 3.88 (2H, br s, Ph-C$\underline{H}_2$-Ph), 4.12 (2H, dd, J = 14.5, 7.5 Hz, Hyd-C$\underline{H}$H-N=), 4.26–4.30 (2H, m, Hyd-CH$\underline{H}$-N=), 4.81 (2H, t, J = 17.0 Hz, Hyd H-5), 5.10, 5.18 (ech 2H, d, J = 17.0 Hz, C$\underline{H}_2$-Ph), 7.10–7.57 (26H, m, Ar H), 8.65 (2H, br s, Hyd H-1), 9.22 (2H, br s, N$\underline{H}$CSN=). ^{13}C-NMR (DMSO-*d*) δ: 40.0 (Ph-$\underline{C}H_2$-Ph), 52.0 (Hyd-$\underline{C}H_2$-N=), 54.0 ($\underline{C}H_2$-Ph), 54.8 (Hyd C-5), 126.4, 126.6, 127.0, 127.3, 128.2, 128.4, 128.5 (Ar C), 131.0, (Ar C-4 in *p*-Cl-C_6H_4), 131.9 (Ar C-1 in *p*-Cl-C_6H_4), 136.7 (Ar C-1 in CH_2-$\underline{Ph}$), 138.0 (Ar C-4 in $\underline{Ph}$-CH_2-Ph), 138.5 (Ar C-1 in $\underline{Ph}$-CH_2-Ph), 155.4 (Hyd C-2), 171.5 (Hyd C-4). 182.2 (NH$\underline{C}$SN=). *Anal.* Calcd for $C_{49}H_{42}Cl_2N_8O_4S_2$: C, 62.48; H, 4.49; N, 11.90. Found: C, 62.68; H, 4.67; N, 11.84.

***N*^{1},*N*^{10}-Dibenzyl-*N*^{1}-((1-(3-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)methyl)-*N*^{10}-((1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)methyl)decanediamide (5c)**[20]

This twin-drug type compound was obtained from the reaction of 5-((benzylamino)methyl)-3-(4-chlorophenyl)imidazolidine-2,4-dione (**2b**) with decanedioyl dichloride in the same manner as that described previously.[16] Compound **5c** was obtained as a white solid in 98% yield. Mp 76–87 °C. IR (KBr) cm^{-1}: 1781, 1721. FAB-MS (positive) *m/z*: 825 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ:

1.08–1.53 (12H, m, DD H-3, H-4, H-5), 2.25–2.29, 2.40–2.46 (each 2H, m, DD H-2), 3.63–3.77 (4H, m, Hyd-CH_2-N=), 4.47–4.73 (6H, m, Hyd H-5 + CH_2-Ph), 7.13–7.57 (18H, m, Ar H), 8.55 (2H x 0.65, br s, Hyd H-1), 8.72 (2H x 0.35, br s, Hyd H-1). ^{13}C-NMR (DMSO-d_6) δ: 24.5, 24.8 (DD C-3), 28.5, 28.6, 28.6, 28.7 (DD C-4, C-5), 32.2, 32.3 (DD C-2), 47.5, 47.5 (Hyd-CH_2-N=), 51.9, 47.4 (CH_2-Ph), 54.7, 55.5 (Hyd C-5), 126.1, 127.0, 127.2, 127.2, 128.1, 128.1, 128.3, 128.5, 128.6, 128.6 (Ar C), 131.1, 130.9 (Ar C-4 in *p*-Cl-C_6H_4), 131.9, 132.0 (Ar C-1 in *p*-Cl-C_6H_4), 137.6, 137.7 (Ar C-1 in CH_2-Ph), 155.3, 155.3 (Hyd C-2), 171.5, 171.4 (Hyd C-4), 173.4, 172.8 (CH_2CON). *Anal.* Calcd for $C_{44}H_{46}Cl_2N_6O_6 \cdot H_2O$: C, 62.63; H, 5.73; N, 9.96. Found: C, 62.74; H, 5.96; N, 9.81.

5,5'-((Methylenebis(4,1-phenylene))bis(azanediyl))bis(5-methyl-3-phenylimidazolidine-2,4-dione) (7)

This compound (**7**) was prepared by using the method described previously.[16] A mixture of methylene-hydantoin (**1a**) (100 mg, 0.53 mmol) and 4,4'-diaminodiphenylmethane (52 mg, 0.26 mmol) in CH_2Cl_2 (5 mL) was concentrated under reduced pressure. The resulting residue was warmed up to 70 °C in a water bath and kept for 12 h. After addition of AcOEt to the reaction mixture, the precipitated material was filtered off. The filtrate was concentrated and the residue was purified by centrifugal chromatography (silica gel) using AcOEt as a solvent to give **7** as a white solid in 35% yield (53 mg). Mp 135–137 °C. IR (KBr) cm^{-1}: 1716. FAB-MS (positive) *m/z*: 575 $(M+H)^+$. ^{1}H-NMR (DMSO-d_6) δ: 1.67 (6H, s, Me), 3.64 (2H, s, Ph-CH_2-Ph), 6.21 (2H, s, NH-Ph), 6.55 (4H, d, J = 8.5 Hz, Ar H-2, H-6 in Ph-CH_2-Ph), 6.95 (4H, d, J = 8.5 Hz, Ar H-3, H-5 in Ph-CH_2-Ph), 7.24–7.27 (4H, m, Ar H in Hyd-Ph), 7.35–7.39 (2H, m, Ar H in Hyd-Ph), 7.43–7.47 (4H, m, Ar H in Hyd-Ph), 8.77 (2H, s, H-1). ^{13}C-NMR (DMSO-d_6) δ: 25.7 (Me), 39.4 (Ph-CH_2-Ph), 71.4 (Hyd C-5), 115.3 (Ar C-2, C-6 in Ph-CH_2-Ph), 126,5, 127.9, 128.7 (Ar C-2, C-3, C-4, C-5, C-6 in Hyd-Ph), 128.9 (Ar C-3, C5 in Ph-CH_2-Ph), 131.8, (Ar C-1 in Ph-Hyd), 132.1 (Ar C-4 in Ph-CH_2-Ph), 142.7 (Ar C-1 in Ph-CH_2-Ph), 153.6 (Hyd C-2), 173.7 (Hyd C-4). *Anal.* Calcd for $C_{33}H_{30}N_6O_4$: C, 68.98; H, 5.26; N, 14.63. Found: C, 68.76; H, 5.37; N, 14.38.

Assays 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 (**4**~**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.[21,22] The determined values of MIC for target compounds by this authentic MIC method are summarized in Table 1.

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20. Many of the obtained twin-drug type compounds exhibited very simple symmetrical ^{13}C-NMR spectra in DMSO-d_6, indicating little difference with respect to the signal assignable to two substituted hydantoin rings and a linker moiety. From a stereochemical viewpoint, obtained products

4, **5**, **6** and **7** can be considered to be a mixture of three twin-drug type bivalent molecules, i.e., two chiral hydantoin rings in the molecules and a Cs-symmetrical *meso* compound having different absolute configurations (*R,S*). We previously reported the presence of three stereoisomers in the free base of compound **A** found by the HPLC method.[15] In the case of compound **5c** as well as **5b**[16], we consider that the diastereomixture gave rise to slightly different non-equivalent magnetic resonance patterns (see ^{13}C-NMR data of compound **5c** in EXPERIMENTAL). We used isomeric mixtures for the biological prescreening (antibacterial activity).

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