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SYNTHESIS AND ANTIBACTERIAL EVALUATION OF SOME NEW 5-SUBSTITUTED HYDANTOINS AND NOVEL TWIN-DRUG TYPE DERIVATIVES

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Abstract – We report the preparation of new oxygen analogues **2** of twin-drug type bivalent mid-size lead molecule **1** and related single-drug type hydantoin derivatives **3**. The new twin-drug type oxygen analogues (**2a** and **2b**) also showed significant antibacterial activity against a Gram-positive strain (*S. aureus*). A comparison of the two types of twin-drug type mid-size compounds **1** and **2** showed that the original lead **1** had a higher level of antibacterial activity against a Gram-positive strain (*S. aureus*) than those of the compounds (**2a** and **2b**). We also report the results of antibacterial evaluation of prepared compounds and the investigated structure-activity relationships of these molecules.

C_2 - or C_3 -Symmetrical geometric molecules in the search for bioactive compounds have attracted much attention because of their promising pharmacological value for bioactive ligands for many types of receptors.¹⁻⁴ A large variety of synthetic bioactive C_2 -symmetrical bivalent molecules have been studied for many types of receptor ligands. It is generally accepted that a bivalent molecule would show enhanced affinity (activity) compared to that of the corresponding univalent molecule. Therefore, a number of studies have been carried out for the development of such symmetrical bivalent molecules.^{1,2,4} We have been interested in molecules that interfere with carbohydrate recognition stages in order to find new bioactive candidates. From the viewpoint of molecular geometry, we have already investigated a few symmetrical molecules for the purpose of finding new bioactive leads.⁵⁻¹⁰

As one of our projects to investigate antibacterial leads,⁵⁻⁷ we recently reported a representative twin-drug type symmetrical bivalent mid-size hydantoin derivative **1**¹¹ possessing a considerably high level of antibacterial activity against a Gram-positive strain (*S. aureus*) or a Gram-negative strain (*E. coli*), and

this molecule showed an interesting binding property to sulfated glycosaminoglycans such as heparan sulfate (Figure 1).^{6,7}

In this paper, we report the preparation of new oxygen analogues **2a** and **2b** of twin-drug type bivalent mid-size molecules¹² and related single-drug type hydantoin derivatives **3**. This operation of molecular modification is a well-established interchange approach of divalent atoms in a bioactive lead to obtain structural information for bioactivities.^{1,13} We also describe the results of antibacterial evaluation of prepared compounds and the investigated structure-activity relationships of these molecules.

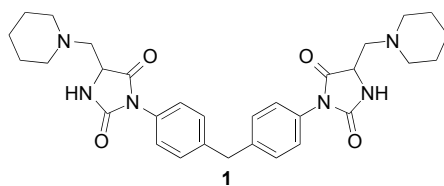
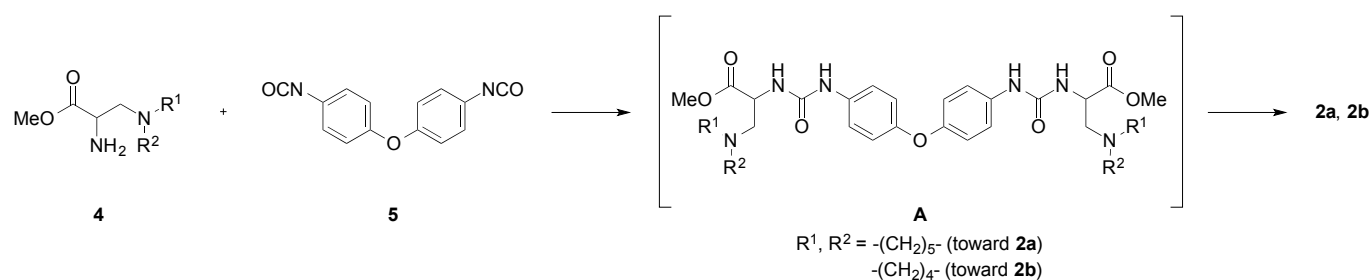


Figure 1

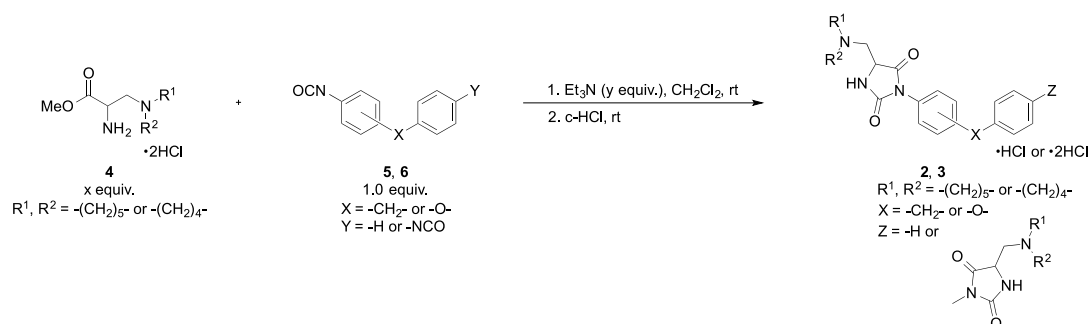
As starting materials for the derivatization to target compounds described in this article, we used β -aminoalanine derivatives (**4**) in the same manner as that reported previously.⁶ By using cyclization reactions of various aryl isocyanates (**5**, **6**) with amino acids (**4**) to hydantoin derivatives, we obtained new hydantoin derivatives including new twin-drug type mid-size compounds (**2a** and **2b**) and a few related single-drug type derivatives (**3**) (Table 1).

In the synthesis of a new oxygen analogue of the twin-drug **2** that have an ether functionality instead of a methylene group in the linker part, 4,4'-oxybis(isocyanobenzene) (**5**) was used for the starting material. Twin-drug type oxygen analogues (**2a** and **2b**) could be obtained by double cyclization of the corresponding urea intermediate (**A**) in good yield (see Figure 2).



Scheme 1

The symmetrical feature of this compound in solution was elucidated by the ¹³C-NMR spectrum (see EXPERIMENTAL).^{6,7} In this study, structural analogues of single-drug type 5-dialkylaminomethylhydantoin derivatives (**3a-3c**) were also prepared for the purpose of comparison of chemical and biological properties and also for structure-activity relationship studies of this series.

Table 1. Preparation and Antibacterial Activity of Twin-drug and Single-drug Type Hydantoin Derivatives

Entry	Amino Acid (4)	Arylisocyanate (5, 6)	x equiv.	y equiv.	Product (2, 3)	Yield (%)	MIC (mM)	
							<i>S. aureus</i>	<i>E. coli</i>
1 ^a	-	-	-	-		-	0.024	0.095
2			2.3	4.6		67	0.049	≥ 0.195
3			2.3	4.6		67	0.051	≥ 0.204
4			1.0	2.0		41	0.080	≥ 0.320
5			1.0	2.0		31	0.160	≥ 0.320
6			1.0	2.0		25	0.315	≥ 0.315
7			1.0	2.0		42	0.080	≥ 0.318

^a Data were taken from ref 6.

The new single-drug type 3-aryl-substituted hydantoins **3a-3c** obtained from the reactions of methyl ester of β -aminoalanines **4** with isocyanates **6** are also listed in the Table 1. Details of the preparation of these compounds are presented in the EXPERIMENTAL section. Regarding the antibacterial activity of single-drug type compounds that have benzyl-substituted phenyl groups, antibacterial activity against a Gram-positive strain (*S. aureus*) decreased in the order of *para*-benzyl (**3a**) (MIC=0.080 mM) > *meta*-benzyl (**3b**) (MIC=0.160 mM) > *ortho*-benzyl (**3c**) (MIC=0.315 mM)-substituted phenyl at the 3 position of the hydantoin ring. Significant antibacterial activity against a Gram-negative strain (*E. coli*)

was not observed at concentrations of 0.320-0.315 mM of all single-drug type tested compounds (**3a**, **3b**, and **3c**). We confirmed that the fragment of this *para*-benzyl-substituted phenyl moiety in the active single-drug type compound **3a** is identical to the linker structure included in the twin-drug type compound **1** (MIC=0.024 mM), indicating the superiority of *para*-methylene-junction mode over other (*ortho*- or *meta*-) junction modes. In addition, the potentiating effect by modification to a twin-drug was over 3.3 times.

The new oxygen analogues of the twin-drug type derivative (**2a** and **2b**) also showed significant antibacterial activity (MIC=0.049~0.051 mM) against a Gram-positive strain (*S. aureus*). The corresponding single-drug type compound with a *para*-phenyloxyphenyl moiety (**3d**) showed a similar level of antibacterial activity (MIC=0.080 mM) (see Table 1). This indicates that the potentiating effect by the twin-drug approach was lower than that of compound **3a**.

A comparison of the two types of twin-drug type compounds **1** and **2** showed that the original lead **1** had a higher level of antibacterial activity (MIC=0.024 mM) against a Gram-positive strain (*S. aureus*) than those of compounds **2a** and **2b**. This result indicates that the linker including ether functionality is not effective for expressing a high level of antibacterial activity against *S. aureus*. Regarding the antibacterial activity against *S. aureus*, the results suggest the superiority of a methylene functionality as a hydrogen-bond donor (**1**) rather than two lone pairs in sp³ hybridized oxygen atoms in the center of the linker as a function for a hydrogen-bond acceptor (**2**).

In contrast, the new twin-drug type oxygen analogues (**2a** and **2b**) showed low levels of antibacterial activity against a Gram-negative strain (*E. coli*) (MIC = 0.195~0.204 mM), and all single drug type compounds (**3a**~**3d**) also showed no antibacterial activity at concentrations of 0.315~0.320 mM. The results indicate that the structural modification used is not efficient to express potentiated antibacterial activity against a Gram-negative strain (*E. coli*).

Although further detailed investigation is required for elucidation of the above phenomena, we consider that the above interesting information regarding a feature for an efficient linker structure is important for further structural modification of new twin-drug type antibacterial molecules of this series.

EXPERIMENTAL

IR spectra were measured by a Shimadzu FT/IR-8100 spectrometer. ¹H- and ¹³C-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 for ¹H-NMR and the carbon signal of the corresponding solvent [DMSO-*d*₆ (39.50 ppm)] for ¹³C-NMR. The signal assignments were confirmed by ¹H-¹H two-dimensional (2D) correlation spectroscopy (COSY), ¹H-¹³C heteronuclear multiple quantum coherence (HMQC), and ¹H-¹³C heteronuclear multiple-bond connectivity (HMBC) spectra.

High-resolution FAB-MS spectra [HRMS (FAB)] were obtained by a JEOL JMS-HX110 mass spectrometer. The following abbreviations in parentheses were used for hydantoin ring (Hyd), piperidine (Pip) and pyrrolidine (Pyr).

Preparation of Twin-drug Type Symmetrical Bivalent Hydantoin Derivative and Single-drug Type Compounds.

3,3'-(Oxybis(4,1-phenylene))bis(5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione) dihydrochloride (2a). 4,4'-Oxybis(isocyanatobenzene) (211.6 mg, 0.839 mmol) was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et₃N (535.1 μ L, 3.86 mmol) in CH₂Cl₂ (2.00 mL). The mixture was stirred at rt for 1 h and washed with water, dried over Na₂SO₄, and concentrated *in vacuo*. To this mixture was added concentrated HCl (5 mL) and the mixture was allowed to stand for 5 days at rt and then the solvent was removed under reduced pressure. EtOH was added to this reaction mixture and the precipitated material was filtered to give the desired compound **2a** (357.9 mg, 67% yield) as a white solid. Mp 175-185 °C; IR (KBr) 3419 (NH), 1782 (C=O), 1720 cm⁻¹ (C=O); FAB-MS (positive) *m/z* 561 (M+H)⁺. HRMS (FAB) Calcd for C₃₀H₃₇N₆O₅⁺: *m/z* 561.2820 (M+H)⁺. Found: 561.2820; ¹H-NMR (DMSO-*d*₆) δ 1.31-1.48 (2H, m, Pip H-4 \times 2), 1.63-1.77 (2H, m, Pip H-4 \times 2), 1.77-2.05 (8H, m, Pip H-3 \times 4, H-5 \times 4), 2.88-3.13 (4H, m, Pip H-2 \times 2, H-6 \times 2), 3.40-3.49 (2H, m, Pip H-2 \times 2 or H-6 \times 2), 3.49-3.59 (4H, m, CH₂-Pip), 3.59-3.75 (2H, m, Pip H-2 \times 2 or H-6 \times 2), 4.87-5.05 (2H, m, Hyd H-5), 7.14-7.20 (4H, m, Ar H-2, H-6 or H-3, H-5), 7.38-7.44 (4H, m, Ar H-2, H-6 or H-3, H-5), 8.85 (2H, s, Hyd H-1), 10.83 (2H, s, NH⁺); ¹³C-NMR (DMSO-*d*₆) δ 21.1 (Pip C-4), 22.1 (Pip C-3 or C-5), 22.2 (Pip C-3 or C-5), 51.9 (Pip C-2 or C-6), 52.2 (Hyd C-5), 53.6 (Pip C-2 or C-6), 58.0 (CH₂-Pip), 118.8 (Ar C-2, C-6 or C-3, C-5), 127.3 (Ar C-1), 128.5 (Ar C-2, C-6 or C-3, C-5), 155.1 (Hyd C-2), 155.7 (Ar C-4), 170.1 (Hyd C-4). Anal. Calcd for C₃₀H₃₆N₆O₅ \cdot 2HCl \cdot 1.3H₂O: C, 54.84; H, 6.23; N, 12.79. Found: C, 54.81; H, 6.12; N, 12.76.

3,3'-(Oxybis(4,1-phenylene))bis(5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione) dihydrochloride (2b). A solution of 4,4'-oxybis(isocyanatobenzene) (223.7 mg, 0.887 mmol) in CH₂Cl₂ (3.00 mL) was added to a solution of methyl 2-amino-3-(pyrrolidin-1-yl)propanoate dihydrochloride (500.0 mg, 2.04 mmol) in CH₂Cl₂. To this mixture was added Et₃N (565.6 μ L, 4.08 mmol) and the mixture was stirred at rt for 2 days and then washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. Concentrated HCl was added to this mixture and the mixture was allowed to stand for several days at rt and then the solvent was removed under reduced pressure. EtOH was added to this mixture and the precipitated material was filtered to give the desired compound **2b** (361.0 mg, 67% yield) as a white solid. Mp 150-154 °C; IR (KBr) 3429 (NH), 1783 (C=O), 1722 cm⁻¹ (C=O); FAB-MS (positive) *m/z* 533 (M+H)⁺. HRMS (FAB) Calcd for C₂₈H₃₃N₆O₅⁺: *m/z* 533.2507 (M+H)⁺. Found: 533.2524; ¹H-NMR (DMSO-*d*₆) δ 1.86-2.00 (4H, m, Pyr H-3 \times 4 or H-4 \times 4 or H-3 \times 2, H-4 \times 2), 2.00-2.13 (4H, m, Pyr H-3 \times 4

or H-4 \times 4 or H-3 \times 2, H-4 \times 2), 3.00-3.18 (4H, m, Pyr H-2 \times 2, H-5 \times 2), 3.58-3.68 (4H, m, CH₂-Pyr), 3.68-3.78 (4H, m, Pyr H-2 \times 2, H-5 \times 2), 4.76-4.89 (2H, m, Hyd H-5), 7.10-7.24 (4H, m, Ar H-2, H-6 or H-3, H-5), 7.35-7.53 (4H, m, Ar H-2, H-6 or H-3, H-5), 8.74 (2H, s, Hyd H-1), 11.20 (2H, s, NH⁺); ¹³C-NMR (DMSO-*d*₆) δ 22.5 (Pyr C-3 or C-4), 22.7 (Pyr C-3 or C-4), 53.3 (Pyr C-2 or C-5), 53.7 (Hyd C-5), 54.1 (Pyr C-2 or C-5), 55.2 (CH₂-Pyr), 118.8 (Ar C-2, C-6 or C-3, C-5), 127.3 (Ar C-1), 128.5 (Ar C-2, C-6 or C-3, C-5), 155.2 (Hyd C-2), 155.7 (Ar C-4), 170.0 (Hyd C-4). Anal. Calcd for C₂₈H₃₂N₆O₅ • 2HCl • 1.3H₂O: C, 53.47; H, 5.87; N, 13.36. Found: C, 53.34; H, 5.87; N, 13.36.

3-(4-Benzylphenyl)-5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione hydrochloride (3a).

1-Benzyl-4-isocyanatobenzene (403.8 mg, 1.93 mmol) was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et₃N (535.1 μ L, 3.86 mmol) in CH₂Cl₂ (2.00 mL). The mixture was stirred at rt for 1 h and washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. Concentrated HCl (5 mL) was added to this mixture and the mixture was allowed to stand for 3 days at rt and then the solvent was removed under reduced pressure. EtOH was added to this mixture and the precipitated material was filtered to give the desired compound **3a** (319.3 mg, 41% yield) as a white solid. Mp 187-191 °C; IR (KBr) 3430 (NH), 1785 (C=O), 1714 cm⁻¹ (C=O); FAB-MS (positive) *m/z* 364 (M+H)⁺. HRMS (FAB) Calcd for C₂₂H₂₆N₃O₂⁺: *m/z* 364.2020 (M+H)⁺. Found: 364.2029; ¹H-NMR (DMSO-*d*₆) δ 1.30-1.48 (1H, m, Pip H-4 \times 1), 1.64-1.76 (1H, m, Pip H-4 \times 1), 1.76-1.98 (4H, m, Pip H-3 \times 2, H-5 \times 2), 2.86-3.12 (2H, m, Pip H-2 \times 1, H-6 \times 1), 3.39-3.48 (1H, m, Pip H-2 \times 1 or H-6 \times 1), 3.48-3.57 (2H, m, CH₂-Pip), 3.57-3.72 (1H, m, Pip H-2 \times 1 or H-6 \times 1), 3.98 (2H, s, C₆H₄-CH₂-Ph), 4.84-5.00 (1H, m, Hyd H-5), 7.14-7.23 (1H, m, Ar H-4 in Ph), 7.23-7.37 (8H, m, Ar H-2, H-6, H-3, H-5 in C₆H₄, Ar H-2, H-6, H-3, H-5 in Ph), 8.79 (1H, s, Hyd H-1), 10.70 (1H, s, NH⁺); ¹³C-NMR (DMSO-*d*₆) δ 21.0 (Pip C-4), 22.1 (Pip C-3 or C-5), 22.2 (Pip C-3 or C-5), 40.6 (C₆H₄-CH₂-Ph), 51.9 (Pip C-2 or C-6), 52.2 (Hyd C-5), 53.5 (Pip C-2 or C-6), 58.0 (CH₂-Pip), 126.0, 126.6, 128.4, 128.6, 128.9 (Ar C-2, C-6 or C-3, C-5 in C₆H₄ or Ar C-2, C-6 or C-3, C-5 or C-4 in Ph), 129.7 (Ar C-1 in C₆H₄), 140.8 (Ar C-4 in C₆H₄ or Ar C-1 in Ph), 141.3 (Ar C-4 in C₆H₄ or Ar C-1 in Ph), 155.1 (Hyd C-2), 170.1 (Hyd C-4). Anal. Calcd for C₂₂H₂₅N₃O₂ • HCl: C, 66.07; H, 6.55; N, 10.51. Found: C, 66.14; H, 6.63; N, 10.43.

3-(3-Benzylphenyl)-5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione hydrochloride (3b).

1-Benzyl-3-isocyanatobenzene (403.8 mg, 1.93 mmol) was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et₃N (535.1 μ L, 3.86 mmol) in CH₂Cl₂ (2.00 mL). The mixture was stirred at rt for 1 h and washed with water and brine, dried over Na₂SO₄, and concentrated *in vacuo*. The crude mixture was purified by centrifugal silica gel chromatography. The obtained urea intermediate and the targeted product without a salt of HCl were treated with concentrated HCl and HCl in MeOH, respectively, and the solvents were removed under

reduced pressure. EtOH was added to this mixture and the precipitated material was filtered to give the desired compound **3b** (241.2 mg, 31% yield) as a white solid. Mp 160-166 °C; IR (KBr) 3437 (NH), 1782 (C=O), 1710 cm^{-1} (C=O); FAB-MS (positive) m/z 364 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_3\text{O}_2^+$: m/z 364.2020 (M+H)⁺. Found: 364.2028; ¹H-NMR (DMSO-*d*₆) δ 1.32-1.46 (1H, m, Pip H-4 \times 1), 1.61-1.76 (1H, m, Pip H-4 \times 1), 1.76-1.94 (4H, m, Pip H-3 \times 2, H-5 \times 2), 2.88-3.11 (2H, m, Pip H-2 \times 1, H-6 \times 1), 3.40-3.47 (1H, m, Pip H-2 \times 1 or H-6 \times 1), 3.47-3.56 (2H, m, CH_2 -Pip), 3.56-3.70 (1H, m, Pip H-2 \times 1 or H-6 \times 1), 3.98 (2H, s, C_6H_4 - CH_2 -Ph), 4.84-4.97 (1H, m, Hyd H-5), 7.13-7.34 (8H, m, Ar), 7.34-7.47 (1H, m, Ar), 8.78 (1H, s, Hyd H-1), 10.63 (1H, s, NH^+); ¹³C-NMR (DMSO-*d*₆) δ 21.1 (Pip C-4), 22.2 (Pip C-3 or C-5), 22.3 (Pip C-3 or C-5), 40.7 (C_6H_4 - CH_2 -Ph), 52.0 (Pip C-2 or C-6), 52.2 (Hyd C-5), 53.5 (Pip C-2 or C-6), 58.0 (CH_2 -Pip), 124.4, 126.1, 126.8, 128.4, 128.7, 128.7 (Ar C-2 or C-4 or C-5 or C-6 in C_6H_4 or Ar C-2, C-6 or C-3, C-5 or C-4 in Ph),¹⁴ 131.9 (Ar C-1 in C_6H_4), 140.6 (Ar C-3 in C_6H_4 or Ar C-1 in Ph), 142.0 (Ar C-3 in C_6H_4 or Ar C-1 in Ph), 155.1 (Hyd C-2), 170.1 (Hyd C-4). Anal. Calcd for $\text{C}_{22}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{HCl}$: C, 66.07; H, 6.55; N, 10.51. Found: C, 66.10; H, 6.61; N, 10.43.

3-(2-Benzylphenyl)-5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione hydrochloride (3c).

1-Benzyl-2-isocyanatobenzene (403.8 mg, 1.93 mmol) was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et₃N (535.1 μL , 3.86 mmol) in CH_2Cl_2 (2.00 mL). The mixture was stirred at rt for 1 h and washed with water, dried over Na_2SO_4 , and concentrated *in vacuo*. Concentrated HCl (5 mL) was added to this mixture and the mixture was allowed to stand for 5 days at rt and then the solvent was removed under reduced pressure. EtOH was added to this mixture and the precipitated material was filtered to give the desired compound **3c** (194.5 mg, 25% yield) as a white solid. In ¹³C-NMR spectroscopic data, it is well known that some bulky 3-substituted and 5-substituted hydantoin derivatives have two diastereomeric rotational isomers.¹⁵ In the ¹³C-NMR spectrum of compound **3c**, there are two sets of ¹³C-resonance signal patterns ascribable to diastereomeric rotational isomers as shown below. Mp 173-182 °C; IR (KBr) 3166 (NH), 1783 (C=O), 1721 cm^{-1} (C=O); FAB-MS (positive) m/z 364 (M+H)⁺. HRMS (FAB) Calcd for $\text{C}_{22}\text{H}_{26}\text{N}_3\text{O}_2^+$: m/z 364.2020 (M+H)⁺. Found: 364.2030; ¹H-NMR (DMSO-*d*₆) δ 1.30-1.48 (1H, m, Pip H-4 \times 1), 1.64-1.75 (1H, m, Pip H-4 \times 1), 1.75-2.00 (4H, m, Pip H-3 \times 2, H-5 \times 2), 2.85-3.15 (2H, m, Pip H-2 \times 1, H-6 \times 1), 3.22-3.75 (4H, m, Pip H-2 \times 1, H-6 \times 1, CH_2 -Pip), 3.84 (2H, s, C_6H_4 - CH_2 -Ph), 4.88-5.00 (1H, m, Hyd H-5), 7.02-7.14 (2H, m, Ar), 7.14-7.32 (5H, m, Ar), 7.32-7.45 (2H, m, Ar), 8.82, 8.84 (1H, s, Hyd H-1), 10.82 (1H, s, NH^+); ¹³C-NMR (DMSO-*d*₆) δ 21.0, 21.1 (Pip C-4), 22.1, 22.1 (Pip C-3 or C-5), 22.2, 22.2 (Pip C-3 or C-5), 36.6, 36.6 (C_6H_4 - CH_2 -Ph), 51.7, 51.9 (Pip C-2 or C-6), 52.2, 52.7 (Hyd C-5), 53.4, 53.7 (Pip C-2 or C-6), 57.9, 58.1 (CH_2 -Pip), 126.1, 126.1, 127.0, 127.1, 128.3, 128.3, 128.6, 128.7, 129.3, 129.3, 130.5, 130.6 (Ar C-3 or C-4 or C-5 or C-6 in C_6H_4 or Ar C-2, C-6 or C-3, C-5 or C-4 in Ph), 130.4, 130.4 (Ar C-1 in C_6H_4), 139.0, 139.0 (Ar C-2 in C_6H_4 or Ar C-1 in Ph), 139.4, 139.5 (Ar C-2 in C_6H_4 or Ar C-1

in Ph), 154.9, 155.0 (Hyd C-2), 170.0, 170.2 (Hyd C-4). Anal. Calcd for $C_{22}H_{25}N_3O_2 \cdot HCl \cdot 0.35H_2O$: C, 65.05; H, 6.62; N, 10.34. Found: C, 64.94; H, 6.71; N, 10.29.

3-(4-Phenoxyphenyl)-5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione hydrochloride (3d). A solution of 1-isocyanato-4-phenoxybenzene (407.6 mg, 1.93 mmol) in CH_2Cl_2 was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et_3N (535.1 μL , 3.86 mmol) in CH_2Cl_2 . The mixture was stirred at rt for 1 day and washed with water and brine, dried over Na_2SO_4 , and concentrated *in vacuo*. Concentrated HCl (5 mL) was added to this mixture and the mixture was allowed to stand for 3 days at rt and then the solvent was removed under reduced pressure. EtOH was added to this mixture and the precipitated material was filtered to give the desired compound **3d** (327.0 mg, 42% yield) as a white solid. Mp 178-185 °C; IR (KBr) 3454 (NH), 1778 (C=O), 1704 cm^{-1} (C=O); FAB-MS (positive) m/z 366 (M+H)⁺. HRMS (FAB) Calcd for $C_{21}H_{24}N_3O_3^+$: m/z 366.1812 (M+H)⁺. Found: 366.1826; ¹H-NMR (DMSO-*d*₆) δ 1.30-2.00 (6H, m, Pip H-3 \times 2, H-4 \times 2, H-5 \times 2), 2.70-3.90 (6H, m, Pip H-2 \times 2, H-6 \times 2, \underline{CH}_2 -Pip), 4.88-4.98 (1H, m, Hyd H-5), 7.02-7.15 (4H, m, Ar), 7.15-7.22 (1H, m, Ar), 7.40-7.50 (2H, m, Ar), 7.40-7.50 (2H, m, Ar), 8.83 (1H, s, Hyd H-1), 10.77 (1H, s, NH^+); ¹³C-NMR (DMSO-*d*₆) δ 21.1 (Pip C-4), 22.3 (Pip C-3, C-5), 22.3 (Pip C-3, C-5), 52.1 (Pip C-2 or C-6), 52.3 (Hyd C-5), 53.6 (Pip C-2 or C-6), 58.1 (\underline{CH}_2 -Pip), 118.4, 119.0, 123.9, 128.4, 130.1 (Ar C-2, C-6 or C-3, C-5 in \underline{C}_6H_4 or Ar C-2, C-6 or C-3, C-5 or C-4 in Ph), 126.8 (Ar C-1 in \underline{C}_6H_4), 155.2 (Hyd C-2), 156.1 (Ar C-4 in \underline{C}_6H_4 or Ar C-1 in \underline{Ph}), 156.3 (Ar C-4 in \underline{C}_6H_4 or Ar C-1 in \underline{Ph}), 170.2 (Hyd C-4). Anal. Calcd for $C_{21}H_{23}N_3O_3 \cdot HCl$: C, 62.76; H, 6.02; N, 10.46. Found: C, 62.56; H, 5.97; N, 10.41.

Assays for Antibacterial Activity

We used *Staphylococcus aureus* ATCC6538P and *Escherichia coli* NBRC14237 (NIHJ) (Gram-positive and Gram-negative bacteria, respectively) as target organisms. Synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 1.280 $\mu g/mL$. The minimum inhibitory concentration (MIC) of a standard strain was measured by the authentic microdilution method to monitor bacterial growth turbidity in Muller-Hinton broth according to the Japanese Society of Chemotherapy.^{16,17} The values of MIC are expressed as molar concentrations (mM) for discussion of structure-activity relations.

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11. In the field of peptide biomedicine, much attention is being paid to interesting substances known as middle-size molecules (mid-size molecules) having molecular weights of ca. 500~50000. New hydantoin derivatives of targeted symmetrical molecules have considerably large molecular weights over 500; however, the mid-size compounds (**1** and **2**) that we have synthesized belong to a new class of non-peptide compounds. Mid-size molecules that show lectin-like carbohydrate recognition properties might have interesting functions as new ligands or drug candidates. Considering the formation of strong drug-host interaction for host sugar recognition by multivalent molecules, attempts to synthesize such non-peptide mid-size molecules are thought to be valid in the search for bioactive new leads (see also references 6 and 8).
12. The obtained twin-drug type compounds **2** exhibited very simple symmetrical ¹³C-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, the obtained twin-drug type products **2** as well as **1** can be considered to be a mixture of three twin-drug type bivalent molecules, i.e., two *C*₂-symmetrical compounds having the same absolute configurations (*R,R* and *S,S*) of two chiral hydantoin rings in the molecules and a *C*_s-symmetrical *meso* compound having different absolute configurations (*R,S*). We previously reported the presence of three stereoisomers in the free base of compound **1** found by the HPLC method.⁶ We used isomeric mixtures for biological prescreening (antibacterial activity) (see ¹³C-NMR data of compounds **2** in EXPERIMENTAL).
13. To the best of our knowledge, nitrogen- and sulfur-substituted bisisocyanate analogues of compound **1** are not currently available as commercial reagents. Therefore, we first wished to obtain some new information on structure-activity relationships from the development of new twin-drug type oxygen analogues.

14. In the ^{13}C -NMR spectrum of compound **3b**, only six carbon signals were observed and one additional aromatic C-H carbon appeared to be overlapped with one of these six signals. Despite our careful spectroscopic analysis, however, we could not identify a particular overlapped signal.
15. For example, see the following reference: F. Fujisaki, M. Egami, K. Toyofuku, and K. Sumoto, *Heterocycles*, 2013, **87**, 133 and related references cited therein.
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