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.

C₂- or C₃-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 C₂-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 1¹¹ 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).\textsuperscript{6,7}

In this paper, we report the preparation of new oxygen analogues 2\textsubscript{a} and 2\textsubscript{b} of twin-drug type bivalent mid-size molecules\textsuperscript{12} 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.\textsuperscript{1,13} We also describe the results of antibacterial evaluation of prepared compounds and the investigated structure-activity relationships of these molecules.

![Figure 1](image-url)

As starting materials for the derivatization to target compounds described in this article, we used \(\beta\)-aminoalanine derivatives (4) in the same manner as that reported previously.\textsuperscript{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 (2\textsubscript{a} and 2\textsubscript{b}) 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 (2\textsubscript{a} and 2\textsubscript{b}) could be obtained by double cyclization of the corresponding urea intermediate (A) in good yield (see Figure 2).

![Scheme 1](image-url)

The symmetrical feature of this compound in solution was elucidated by the \(^{13}\)C-NMR spectrum (see EXPERIMENTAL).\textsuperscript{6,7} In this study, structural analogues of single-drug type 5-dialkylaminomethylhydantoin derivatives (3\textsubscript{a}-3\textsubscript{c}) 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

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-d₆ (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 Et$_3$N (535.1 µL, 3.86 mmol) in CH$_2$Cl$_2$ (2.00 mL). The mixture was stirred at rt for 1 h and washed with water, dried over Na$_2$SO$_4$, 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 C$_{30}$H$_{37}$N$_6$O$_5$+$^+$: $m/z$ 561.2820 (M+H)$^+$. Found: 561.2820; $^{1}$H-NMR (DMSO-$_d$$_6$) $\delta$ 1.31-1.48 (2H, m, Pip H-4 × 2), 1.63-1.77 (2H, m, Pip H-4 × 2), 2.88-3.13 (4H, m, Pip H-2 × 2, H-6 × 2), 3.40-3.49 (2H, m, Pip H-2 × 2 or H-6 × 2), 3.49-3.59 (4H, m, CH$_2$-Pip), 3.59-3.75 (2H, m, Pip H-2 × 2 or H-6 × 2), 4.87-5.05 (2H, m, Hyd H-5), 7.14-7.20 (4H, m, Ar H-2, H-6 or H-3, H-5), 7.38-7.44 (4H, m, Ar H-2, H-6 or H-3, H-5), 8.85 (2H, s, Hyd H-1), 10.83 (2H, s, NH$^+$); $^{13}$C-NMR (DMSO-$_d$$_6$) $\delta$ 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 (CH$_2$-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 C$_{30}$H$_{36}$N$_6$O$_5$ • 2HCl • 1.3H$_2$O: 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 CH$_2$Cl$_2$ (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 CH$_2$Cl$_2$. To this mixture was added Et$_3$N (565.6 µL, 4.08 mmol) and the mixture was stirred at rt for 2 days and then washed with water and brine, dried over Na$_2$SO$_4$, 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 C$_{28}$H$_{33}$N$_6$O$_5$+$^+$: $m/z$ 533.2507 (M+H)$^+$. Found: 533.2524; $^{1}$H-NMR (DMSO-$_d$$_6$) $\delta$ 1.86-2.00 (4H, m, Pyr H-3 × 4 or H-4 × 4 or H-3 × 2, H-4 × 2), 2.00-2.13 (4H, m, Pyr H-3 × 4 or H-4 × 4 or H-3 × 2, H-4 × 2), 2.22-2.35 (4H, m, Pyr H-2 × 2, H-6 × 2), 2.88-3.13 (4H, m, Pip H-2 × 2, H-6 × 2), 3.40-3.49 (2H, m, Pip H-2 × 2 or H-6 × 2), 3.49-3.65 (4H, m, Pip H-2 × 2 or H-6 × 2), 4.13-4.32 (2H, m, Hyd H-5), 7.10-7.20 (4H, m, Ar H-2, H-6 or H-3, H-5), 7.37-7.43 (4H, m, Ar H-2, H-6 or H-3, H-5), 8.85 (2H, s, Hyd H-1), 10.81 (2H, s, NH$^+$); $^{13}$C-NMR (DMSO-$_d$$_6$) $\delta$ 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 (CH$_2$-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 C$_{28}$H$_{36}$N$_6$O$_5$ • 2HCl • 1.3H$_2$O: C, 54.84; H, 6.23; N, 12.79. Found: C, 54.81; H, 6.12; N, 12.76.
or H-4 × 4 or H-3 × 2, H-4 × 2), 3.00-3.18 (4H, m, Pyr H-2 × 2, H-5 × 2), 3.58-3.68 (4H, m, CH2-Pyr), 3.68-3.78 (4H, m, Pyr H-2 × 2, H-5 × 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-d6) 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 µL, 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-d6) δ 1.30-1.48 (1H, m, Pip H-4 × 1), 1.64-1.76 (1H, m, Pip H-4 × 1), 1.76-1.98 (4H, m, Pip H-3 × 2, H-5 × 2), 2.86-3.12 (2H, m, Pip H-2 × 1, H-6 × 1), 3.39-3.48 (1H, m, Pip H-2 × 1 or H-6 × 1), 3.48-3.57 (2H, m, CH2-Pip), 3.57-3.72 (1H, m, Pip H-2 × 1 or H-6 × 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-d6) 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 C22H26N3O2 • 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 µL, 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 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; \(^1\)H-NMR (DMSO-\(\text{d}_6\)) \(\delta\) 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\(_6\)H\(_4\)-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\(^+\)); \(^{13}\)C-NMR (DMSO-\(\text{d}_6\)) \(\delta\) 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\(_6\)H\(_4\)-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\(_6\)H\(_4\) 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\(_6\)H\(_4\)), 139.0, 139.0 (Ar C-2 in C\(_6\)H\(_4\) or Ar C-1 in Ph), 139.4, 139.5 (Ar C-2 in C\(_6\)H\(_4\) or Ar C-1 in C\(_6\)H\(_4\)), 140.6 (Ar C-3 in C\(_6\)H\(_4\) or Ar C-1 in Ph), 142.0 (Ar C-3 in C\(_6\)H\(_4\) or Ar C-1 in Ph), 155.1 (Hyd C-2), 170.1 (Hyd C-4). Anal. Calcd for \(\text{C}_{22}\text{H}_{25}\text{N}_{3}\text{O}_{2}\) • 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 Et\(_3\)N (535.1 \(\mu\)L, 3.86 mmol) in CH\(_2\)Cl\(_2\) (2.00 mL). The mixture was stirred at rt for 1 h and washed with water, dried over Na\(_2\)SO\(_4\), 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 \(^{13}\)C-NMR spectroscopic data, it is well known that some bulky 3-substituted and 5-substituted hydantoin derivatives have two diastereomeric rotational isomers. In the \(^{13}\)C-NMR spectrum of compound \(3c\), there are two sets of \(^{13}\)C-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; \(^1\)H-NMR (DMSO-\(\text{d}_6\)) \(\delta\) 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\(_6\)H\(_4\)-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\(^+\)); \(^{13}\)C-NMR (DMSO-\(\text{d}_6\)) \(\delta\) 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\(_6\)H\(_4\)-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\(_6\)H\(_4\) 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\(_6\)H\(_4\)), 139.0, 139.0 (Ar C-2 in C\(_6\)H\(_4\) or Ar C-1 in Ph), 139.4, 139.5 (Ar C-2 in C\(_6\)H\(_4\) or Ar C-1 in C\(_6\)H\(_4\)).
in Ph), 154.9, 155.0 (Hyd C-2), 170.0, 170.2 (Hyd C-4). Anal. Calcd for C_{22}H_{25}N_{3}O_{2} • HCl • 0.35H_{2}O: 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_{2}Cl_{2} was added to a solution of methyl 2-amino-3-(piperidin-1-yl)propanoate dihydrochloride (500.3 mg, 1.93 mmol) and Et_{3}N (535.1 µL, 3.86 mmol) in CH_{2}Cl_{2}. The mixture was stirred at rt for 1 day and washed with water and brine, dried over Na_{2}SO_{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_{3}O_{3}^{+}: m/z 366.1812 (M+H)^{+}. Found: 366.1826; ^{1}H-NMR (DMSO-d_{6}) δ 1.30-2.00 (6H, m, Pip H-3 ×2, H-4 ×2, H-5 ×2), 2.70-3.90 (6H, m, Pip H-2 ×2, H-6 ×2, 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^{+}); ^{13}C-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 (CH_{2}-Pip), 118.4, 119.0, 123.9, 128.4, 130.1 (Ar C-2, C-6 or C-3, C-5 in C_{6}H_{4} or Ar C-2, C-6 or C-3, C-5 or C-4 in Ph), 126.8 (Ar C-1 in C_{6}H_{4}), 155.2 (Hyd C-2), 156.1 (Ar C-4 in C_{6}H_{4} or Ar C-1 in Ph), 156.3 (Ar C-4 in C_{6}H_{4} or Ar C-1 in Ph), 170.2 (Hyd C-4). Anal. Calcd for C_{21}H_{23}N_{3}O_{3} • 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 µg/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.\textsuperscript{16,17} The values of MIC are expressed as molar concentrations (mM) for discussion of structure-activity relations.

REFERENCES AND NOTES
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).

The obtained twin-drug type compounds 2 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, 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 $Cs$-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. We used isomeric mixtures for biological prescreening (antibacterial activity) (see $^{13}$C-NMR data of compounds 2 in EXPERIMENTAL).

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.

