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REACTION OF 5-METHYLENE-HYDANTOINS AND ITS CHEMICAL MODIFICATION TO TWIN-DRUG TYPE SYMMETRIC MOLECULES

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Abstract – We report chemical modifications of 5-methylenehydantoin (**3a-e**) with various amine nucleophiles to 5-substitutedhydantoin and transformations of the obtained hydantoin to new symmetrical twin-drug type bivalent molecules **12** and **13**. We also report 5-methylenehydantoin derivatives (**3a**) with some symmetrical diamines such as piperidine, ethylene-1,2-diamine, and 1,2-phenylenediamine. From trials using diamines such as ethylene-1,2-diamine and 1,2-phenylenediamine, hydantoin ring-opened products **10** and **11** were isolated predominantly.

INTRODUCTION

Considering the molecular geometry of bioactive compounds, small C₂- or C₃-symmetrical molecules frequently appear in various synthetic compounds and those molecules are usually constructed on symmetrical templates.¹⁻⁴ We have already reported the synthesis of a few interesting symmetrical candidates with significant biological activities.⁵⁻¹³

As one of our projects to investigate new antibacterial active compounds,^{5, 7, 8, 11, 14} we have already reported a few symmetrical molecules. In our previous paper,⁵ we reported that treatment of 5-methylenehydantoin (**3**) without a solvent with primary or secondary aliphatic dialkylamines regioselectively gave predominantly 5-alkylaminomethylhydantoin derivatives (**4**) and that the reaction conditions with CH₂Cl₂ resulted in the formation of isomeric 5-alkylamino-5-methyl-hydantoin (**5**). Furthermore, among the reported hydantoin derivatives, we found that some twin-drug type symmetrical molecules containing two 5-substituted hydantoin nuclei in the molecules showed high levels of antibacterial activity against a Gram-positive strain (*S. aureus*). We previously reported that identical twin-drug type symmetrical molecule **A**^{5, 15} (Figure 1) shows a considerably high level of antibacterial activity, and we also confirmed

by calorimetric experiments that this bivalent molecule had an interesting binding property to sulfated glucosaminoglycans such as heparan sulfate and dermatan sulfate.¹⁶

In this article, we report regioselective chemical modification of 5-methylene-hydantoin with various amines to new 5-substituted hydantoin and preparation of new symmetrical twin-drug type bivalent molecules from the obtained hydantoin in order to find new antibacterial active leads.

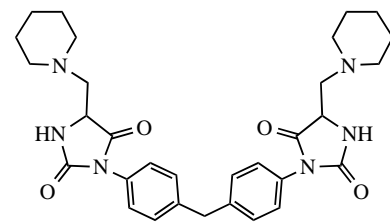
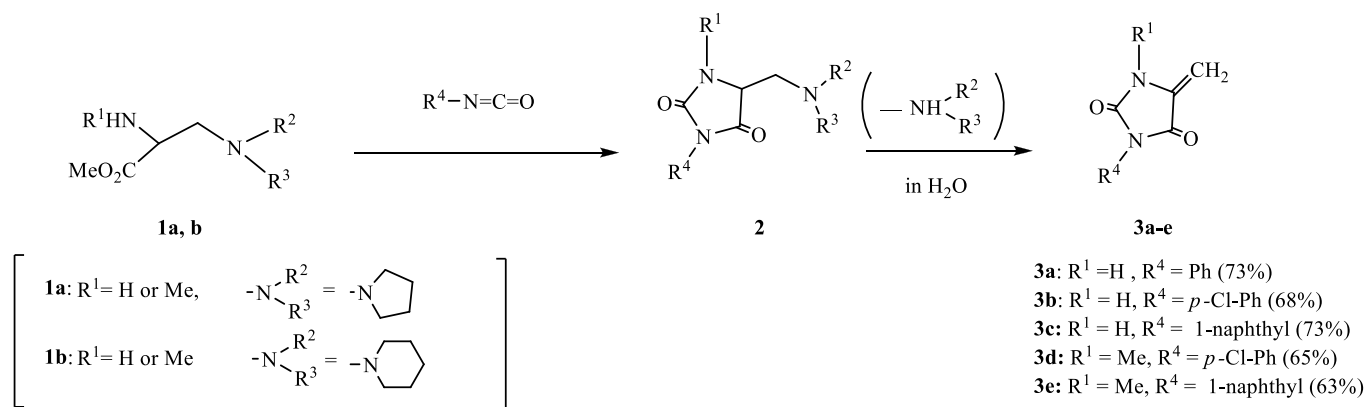


Figure 1. Structure of Compound A

RESULT AND DISCUSSION

Reaction of 5-Methylene-3-arylhydantoin (3) and Modifications of Reaction Products to Symmetrical Bivalent Molecules.

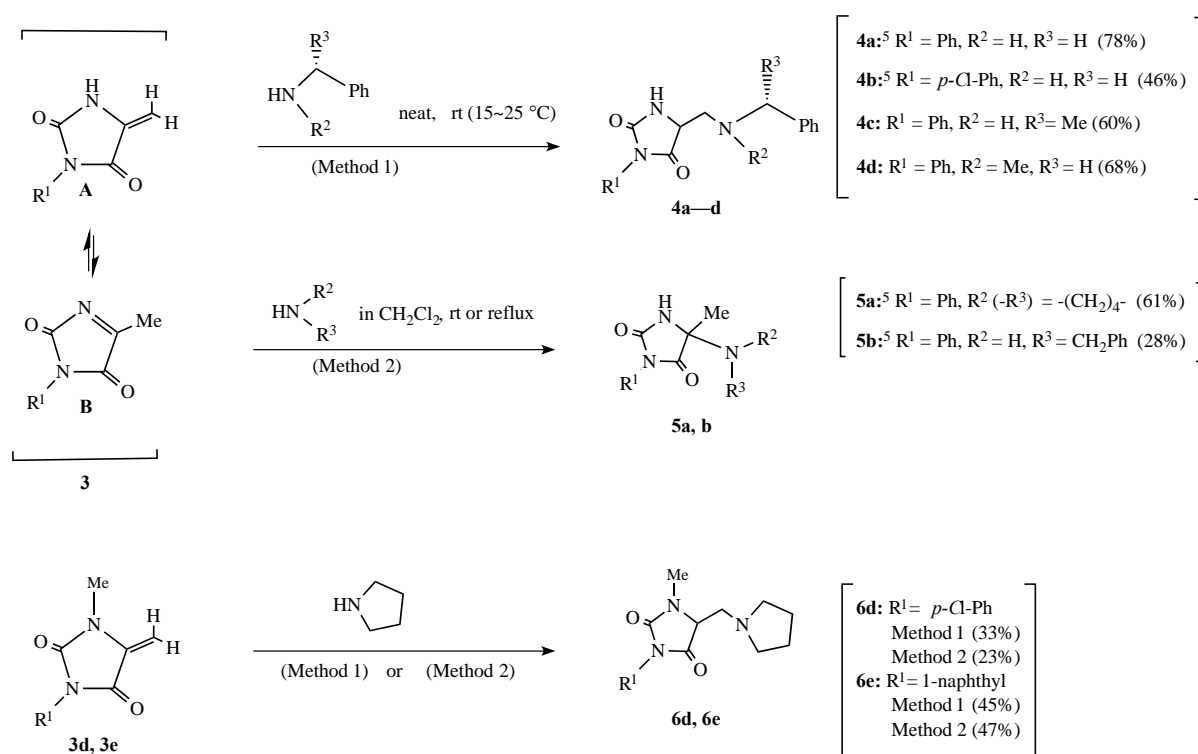
We prepared 5-methylene-3-arylhydantoin (**3a-e**) by deamination of 5-dialkylaminomethylhydantoin derivatives (**2**) readily obtained by addition of β -aminoalanines (**1**) to arylisocyanates, in a manner similar to that reported previously.^{5, 14, 17, 18} Synthesis of the compounds (**3a** and **3b**) have already been reported.⁵ Preparation of new derivatives (**3c**, **3d** and **3e**) and their physical and spectroscopic data are described in the Experimental section. The overall reaction for 5-methylene-3-arylhydantoin (**3a-e**) as starting materials^{19, 20} is shown in Scheme 1.



Scheme 1

In our previous paper,⁵ we reported that treatment of 5-methylene-hydantoin (**3**) without a solvent (neat) (Method 1) with primary or secondary aliphatic dialkylamines regioselectively gave predominantly 5-alkylaminomethylhydantoin derivatives (**4**). On the other hand, the reaction conditions with CH_2Cl_2 (Method 2) resulted in the formation of 5-alkylamino-5-methylhydantoin (**5**) in moderate to good yields. For example, reactions of 5-methylenehydantoin (**3**) and various benzylamines without a solvent resulted in the formation of the corresponding 5-alkylaminomethyl or 5-dialkylaminomethylhydantoin derivatives (**4a-d**) in moderate to good yields, as shown in Scheme 2. Reactions of **3a** with primary or secondary aliphatic amines such as pyrrolidine or benzylamine in CH_2Cl_2 also gave predominantly

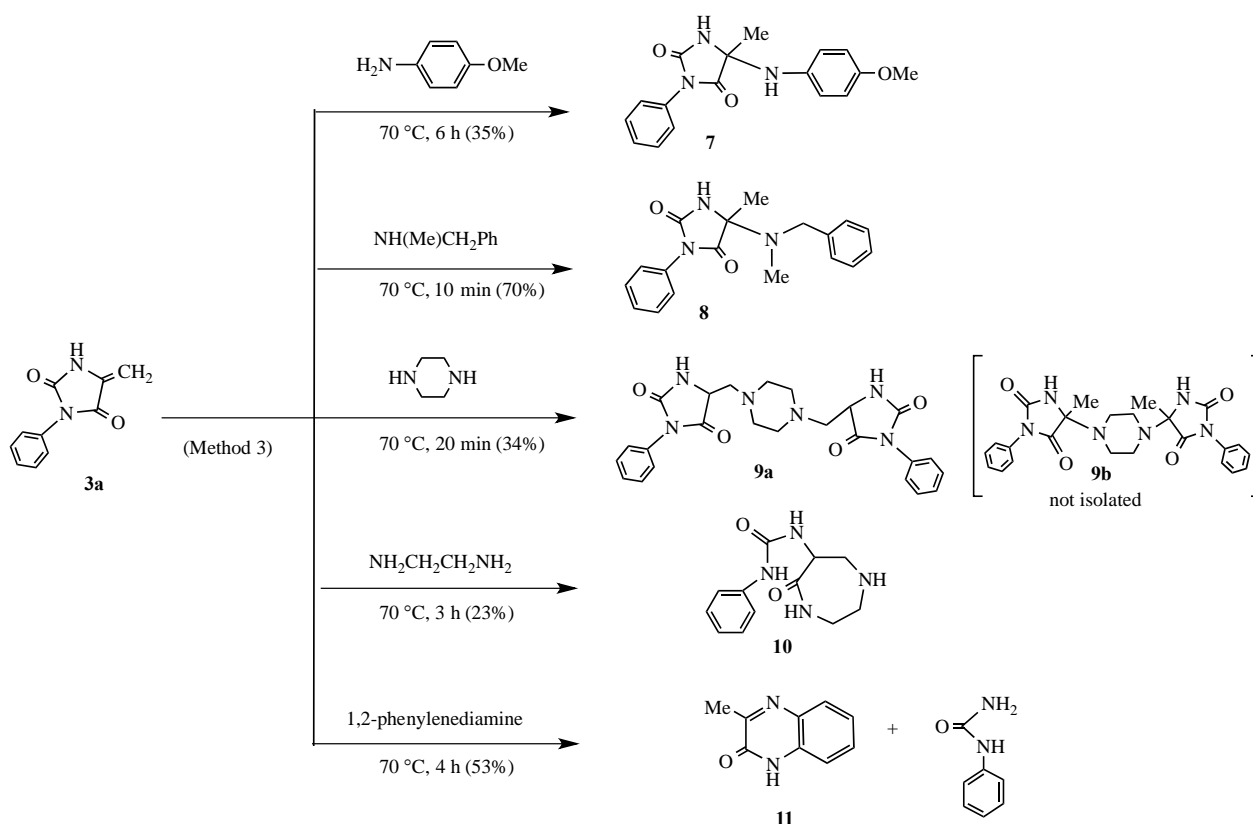
5-alkylamino-5-methylhydantoin (**5a** or **5b**) in good yield.⁵ We considered that tautomeric isomerization ($A \rightleftharpoons B$), as shown in Scheme 2, is a crucial intermediate for these regioselective additions of amine nucleophiles.¹⁸ In fact, both reactions of pyrrolidine with N(1)-methyl derivatives of 5-methylenehydantoin such as compounds **3d** and **3e**, which could not afford an imine **B**-type tautomeric isomer under the conditions of Method 1 or 2, resulted in the selective formation of 5-pyrrolidinomethylhydantoin derivatives (**6d** and **6e**) (Scheme 2). These results also reconfirm the importance of tautomeric isomerization for the orientation of nucleophilic addition of amines to the 5-methylene moiety.



Scheme 2

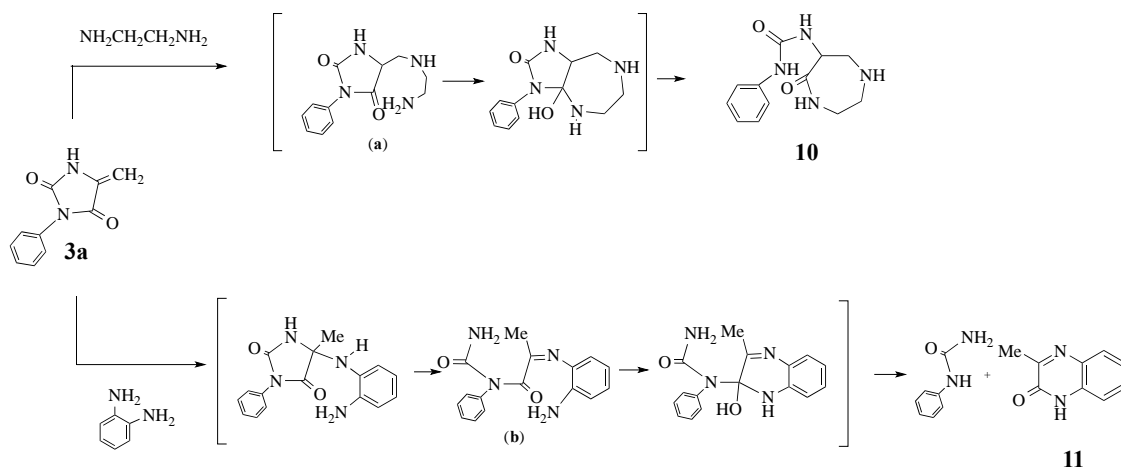
As an extension of this regioselective transformation, our previous method with CH₂Cl₂ as a reaction solvent was slightly modified. When using a solid monoamine such as anisidine, the homogenized residue obtained after evaporation of the mixture of 5-methylenehydantoin (**3**) and an amine in CH₂Cl₂ was warmed up to an appropriate temperature (70 °C) to complete the reaction (Method 3). The results obtained by this procedure were similar to those reported previously. Thus, when using a monoamine such as solid anisidine or *N*-methylbenzylamine in the above reaction conditions (Method 3), the isolated adducts were 5-amino-5-methylhydantoin derivatives (**7** and **8**) in 35% and 70% yields, respectively. In order to obtain symmetrical twin-drug type derivatives containing two 5-amino-5-methylhydantoin moieties, we further examined the reaction of 5-methylene derivative **3a** with a symmetrical diamine such as piperazine in a molar ratio of **3a**: piperazine = 2:1 under the conditions of Method 3 (see Experimental). However, this trial for obtaining twin-drug type 5-amino-5-methylhydantoin derivatives was unsuccessful

and resulted in predominant formation of the structural isomer (**9a**). The targeted symmetrical compound (**9b**) was not isolated (Scheme 3).



Scheme 3

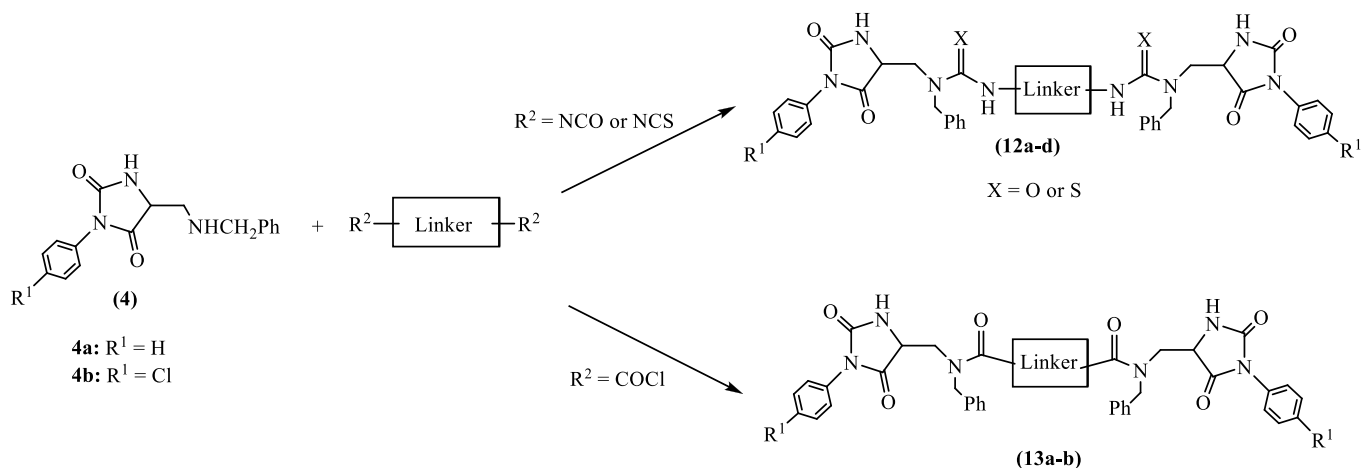
In some other attempts using the above reaction conditions (Method 3), reactions of compound **3a** with the diamines ethylene-1,2-diamine and 1,2-phenylenediamine afforded predominantly hydantoin ring-opened products (**10**) and (**11**) in 23% and 53% yields, respectively (Scheme 4). Formation of the 1,4-diazepane derivative **10** was probably initiated by nucleophilic attachment of an amine moiety to 5-exo-ethylene carbon in compound **3a** and internal ring closure of an intermediate (**a**) with cleavage of



Scheme 4

the hydantoin N3-C4 bond to afford compound **10**. In contrast, the reaction of 5-methylenehydantoin with 1,2-phenylenediamine was initiated by nucleophilic access of an amine group to the C5-hydantoin ring carbon to give a ring-opened imine (**b**) and then a similar internal ringclosure of the imine with cleavage of the hydantoin N3-C4 bond, resulting in the formation of compound **11** together with the formation of *N*-phenylurea (Scheme 4). We consider that formation of bivalent symmetrical compounds, such as compound **9b**, containing two 5-amino-5-methylhydantoin moieties in the molecules may require more defined conditions for the preparation of such compounds.

Among the above regioselective chemical transformations of 5-methylenehydantoin with amine nucleophiles, we are particularly interested in the formation of secondary amine type 5-alkylaminomethylhydantoin derivatives and in the formation of *N*-acyl derivatives by acylation reaction. In order to confirm the validity of this conventional procedure for the preparation of new bivalent 5-acylaminomethylhydantoin derivatives, we examined various diacylation reactions of primary amine adducts (**4a-b**) with aryl diisocyanates (or aryl diisothiocyanates) or with dicarboxylic acid dichloride. For targeted bivalent symmetrical molecules having two hydantoin rings in the molecule, we designed symmetrical twin-drug type molecules (a mixture of C₂-symmetrical and C_s-symmetrical compounds)²¹ shown as a general structure (**12** and **13**) starting with benzylamine adducts (**4a-b**) (Scheme 5).



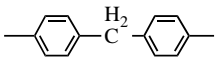
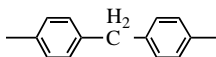
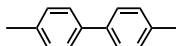


Scheme 5

In this modification, we used mainly less flexible platform linkers such as phenyl, biphenyl and diphenylmethane moieties for target bivalent symmetrical derivatives because we have already observed that flexible methylene-chain linkers [(-CH₂)_n-; n = 4~12] in previously reported examples (symmetrical molecule **A** analogs) were not preferred linker structures, resulting in no antibacterial active compounds at a concentration of less than 128 μg/mL. In order to compare the bioactivities of the twin-drug type compounds, we also prepared additional examples having flexible chain-linkers in the molecule (for example, see **13b**).

The symmetrical structures of the synthesized compounds (**12** and **13**) were easily confirmed by NMR spectroscopic analysis. All of the compounds except for compound **13b** showed magnetically equivalent spectroscopic signal patterns, indicating a symmetrical molecular feature in solution²¹ (see Experimental). The structures and yields of these new bivalent symmetrical hydantoin derivatives (**12** and **13**) are

Table 1. Chemical Structures and Yields for Symmetrical Twin-Drug Type Molecules (**12** and **13**)

| Compound No | R ¹ | X | Linker | Yield (%) |
|-------------|----------------|---|---|-----------|
| 12a | H | O |  | 52 |
| 12b | Cl | O |  | 87 |
| 12c | H | O |  | 95 |
| 12d | H | S |  | 77 |
| 13a | H | O |  | 60 |
| 13b | H | O | —(CH) ₈ — | 78 |

summarized in Table 1, and the protocol for the preparation for these new bivalent symmetrical derivatives and their spectroscopic data are shown in detail in the Experimental section.

All of the synthesized bivalent symmetrical hydantoin derivatives (see Table 1) are now under antibacterial evaluation with gram-positive (*S. aureus*) and gram-negative (*E. coli*) strains. The

results of evaluation of antibacterial activities and details of a structure-activity relationship (SAR) study will be described separately. For compounds with high levels of antibacterial activity in this article, results of thermodynamic experiments will be presented in the following papers.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were measured by a Shimadzu FT/IR-8100 spectrometer. The 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. 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 FAB-MS spectra were obtained by a JEOL JMS-HX110 mass spectrometer. The following abbreviations in parentheses were used for pyrrolidine ring (Pyr), 1-naphthyl ring (Np), hydantoin ring (Hyd), decandiamide (DD) and 1,4-diazepane ring (DAP).

5-Methylene-3-(naphthalen-1-yl)imidazolidine-2,4-dione (**3c**)

This compound was prepared from 1-naphthylisocyanate and methyl 2-amino-3-(pyrrolidin-1-yl)propanoate dihydrochloride (**1a**: R¹ = H) by the method described previously.⁵ Yield was 73%, mp 194 °C (dec). IR (KBr) cm⁻¹: 3265, 1789, 1728, 1778, 1662. FAB-MS (positive) *m/z*: 239 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 5.02 and 5.31 (each 1H, d, *J* = 1.5 Hz, =CH₂),

7.33-7.73 (5H, m, Np H), 8.03-8.12 (2H, m, Np H), 10.89 (1H, br s, Hyd H-1). $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 95.0 ($=\text{CH}_2$), 122.3, 125.5, 126.5, 127.1, 127.2 (Np C), 128.0 (Np C-8a), 128.2, 129.4 (Np C), 129.7 (Np -4a), 133.6 (Np C-1), 135.3 (Hyd C-5), 153.2 (Hyd C-2), 162.6 (Hyd C-4). Anal. Calcd for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{O}_2 \cdot 0.3 \text{H}_2\text{O}$: C, 69.01; H, 4.39; N, 11.50. Found. C, 69.23; H, 4.29; N, 11.66.

3-(4-Chlorophenyl)-1-methyl-5-methyleneimidazolidine-2,4-dione (3d)

This compound was prepared from **1a** ($\text{R}^1 = \text{Me}$) and 4-chlorophenyl isocyanate by the method described previously. ^{5, 18} Yield was 65%, mp 146-147 °C. IR (KBr) cm^{-1} : 1783, 1732, 1664. FAB-MS (positive) m/z : 237 ($\text{M}+\text{H}$)⁺. HR-FAB-MS (positive) m/z : 237.0434 (Calcd for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_2\text{Cl}$: 237.0431). $^1\text{H-NMR}$ (DMSO- d_6) δ : 3.12 (3H, s, NMe), 5.09 and 5.35 (each 1H, d, $J = 2.5$ Hz, $=\text{CH}_2$), 7.46 (2H, d, $J = 8.8$ Hz, Ar H), 7.56 (2H, d, $J = 8.8$ Hz, Ar H). $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 26.5 (NMe), 94.6 ($=\text{CH}_2$), 128.2, 128.8, (Ar C), 130.5 (Ar C-4), 132.4 (Ar C-1), 136.4 (Hyd C-5), 152.4 (Hyd C-2), 160.9 (Hyd C-4). Anal. Calcd for $\text{C}_{11}\text{H}_9\text{N}_2\text{O}_2\text{Cl}$: C, 55.83; H, 3.83; N, 11.84. Found. C, 55.71; H, 3.82; N, 11.91.

1-Methyl-5-methylene-3-(naphthalen-1-yl)imidazolidine-2,4-dione (3e)

This compound was prepared from **1b** ($\text{R}^1 = \text{Me}$) and 1-naphthyl isocyanate by the method described previously. ^{5, 18} Yield was 63%, mp 173-174 °C (dec). IR (KBr) cm^{-1} : 1774, 1729, 1661. FAB-MS (positive) m/z : 253 ($\text{M}+\text{H}$)⁺. $^1\text{H-NMR}$ (DMSO- d_6) δ : 3.19 (3H, s, NMe), 5.15 and 5.40 (each 1H, d, $J = 2.1\sim 2.4$ Hz, $=\text{CH}_2$), 7.55-7.65 (4H, m, Np H), 7.71-7.73 (1H, m, Np H), 8.04-8.09 (2H, m, Np H). $^{13}\text{C-NMR}$ (DMSO- d_6) δ : 26.6 (NMe), 94.7 ($=\text{CH}_2$), 122.5, 125.5, 126.5, 127.0, 127.1 (Np C), 128.0 (Np C-8a), 128.2, 129.5 (Np C), 129.7 (Np C-4a), 133.6 (Np C-1), 136.9 (Hyd C-5), 153.1 (Hyd C-2), 161.7 (Hyd C-4). Anal. Calcd for $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2$: C, 71.42; H, 4.79; N, 11.10. Found. C, 71.32; H, 4.93; N, 11.03.

Preparation of Compounds 4, and 6-11. [Methods 1-3 shown below were used for the Preparation of 4, and 6-11].

[Method 1] ⁵ A mixture of methylenehydantoin **3** and an appropriate molar (2~4 times molar) amount of an amine was allowed to stand at rt for several hours. The resulting reaction mixture was purified by washing with Et_2O or by column chromatography.

[Method 2] ⁵ A solution of methylenehydantoin **3** and an appropriate molar (2~5 times molar) amount of an amine in CH_2Cl_2 was stirred for several hours. After evaporation of the solvent, the resulting material was purified by washing with Et_2O or by centrifugal chromatography (silica gel).

[Method 3] A solution of methylenehydantoin **3** and an appropriate molar (2~5 times molar) amount of an amine in CH_2Cl_2 was concentrated under reduced pressure. The homogenized residue was warmed up at *ca* 70 °C in a water bath and kept for several hours. The obtained product was purified by washing with Et_2O or by centrifugal chromatography (silica gel).

3-Phenyl-5-(((R)-1-phenylethyl)amino)methyl)imidazolidine-2,4-dione (4c)

This compound was obtained by using method 1. Thus, a mixture of methylenehydantoin **3a** (55 mg, 0.29

mmol) and *R*(+)- α -methylbenzylamine (140 mg, 1.16 mmol) was kept at rt for 6 h. The resulting solid material was purified with centrifugal chromatography (silica gel) using AcOEt as a solvent to afford viscous product **4c** (54 mg, 60%). The compound **4c** was separated as a diastereomeric mixture (*ca* 2:3) as indicated by ^1H and ^{13}C -NMR data shown below. IR (KBr) cm^{-1} : 1779, 1718. FAB-MS (positive) m/z : 310 ($\text{M}+\text{H}$) $^+$, HR-FAB-MS (positive) m/z : 310.1557 (Calcd for $\text{C}_{18}\text{H}_{20}\text{N}_3\text{O}_2$; 310.1556). ^1H -NMR (DMSO- d_6) δ : 1.26 (3H, d, $J = 6.5$ Hz, $\text{NHCH}(\text{Me})\text{Ph}$), 2.1-2.3 (1H, br, $\text{NHCH}(\text{Me})\text{Ph}$), 2.68-2.80 (2H, m, $\text{Hyd-CH}_2\text{-N=}$), 3.72-3.78 (1H, m, $\text{NHCH}(\text{Me})\text{Ph}$), 4.19 (0.4H, dd, $J = 6.0, 4.0$ Hz, Hyd H-5), 4.26 (0.6H, dd, $J = 6.0, 4.0$ Hz, Hyd H-5), 7.20-7.49 (10H, m, Ar H), 8.32 (0.4H, br s, Hyd H-1), 8.36 (0.6H, br, Hyd H-1). ^{13}C -NMR (DMSO- d_6) δ : 24.0, 24.4 (Me), 47.7, 48.8 (Hyd- $\text{CH}_2\text{-NH}$), 56.95, 56.98 (Hyd C-5 or $\text{NHCH}(\text{Me})\text{Ph}$), 57.3, 58.5 ($\text{NHCH}(\text{Me})\text{Ph}$ or Hyd C-5), 126.2, 126.3, 126.4, 126.48, 126.51, 126.53, 127.51, 127.54, 128.1, 128.2, 128.57, 128.58 (Ar C), 132.2, 132.2 (Ar C-1 in Hyd-Ph), 145.5, 145.7 (Ar C-1 in $\text{NHCH}(\text{Me})\text{Ph}$), 155.82, 155.83 (Hyd C-2), 172.4, 172.5 (Hyd C-4).

5-((Benzyl(methyl)amino)methyl)-3-phenylimidazolidine-2,4-dione (**4d**)

This compound was obtained from the reaction of **3a** and *N*-methylbenzylamine in 68% yield by using method 1. Yield was 68%; mp 133-136 $^\circ\text{C}$, IR (KBr) cm^{-1} : 1772, 1710. FAB-MS (positive) m/z : 310 ($\text{M}+\text{H}$) $^+$. ^1H -NMR (DMSO- d_6) δ : 2.18 (3H, s, NMe), 2.83 (1H, dd, $J = 13.5, 3.0$ Hz, Hyd-CHH-N=), 2.91 (1H, dd, $J = 13.5, 6.0$ Hz, Hyd-CHH-N=), 3.53 and 3.69 (each 1H, d, $J = 13.5$ Hz, Ph-CH_2), 4.38-4.40 (1H, m, Hyd H-5), 7.23-7.49 (10H, m, Ar H), 8.53 (1H, s, Hyd H-1). ^{13}C -NMR (DMSO- d_6) δ : 42.4 (NMe), 56.5 (Hyd- $\text{CH}_2\text{-NH}$), 62.1 (NMe-CH}_2\text{-Ph), 126.4, 126.8, 127.5, 128.0, 128.5, 128.6 (Ar C), 132.2 (Ar C-1 in Hyd-Ph), 138.8 (Ar C-1 in NMe-CH}_2\text{-Ph), 155.8 (Hyd C-2), 172.4 (Hyd C-4). Anal. Calcd for $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_2$: C, 69.88; H, 6.19; N, 13.58. Found. C, 69.75; H, 6.26; N, 13.62.

Reaction of *N*-Methyl-5-methylene Hydantoins **3d** and **3e** with Pyrrolidine by Method 1 and Method 2.

3-(4-Chlorophenyl)-1-methyl-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione (**6d**)

[Method 1] A mixture of methylenehydantoin (**3d**) (100 mg, 0.42 mmol) and pyrrolidine (40 mg, 0.55 mmol) was allowed to stand for 5 h at rt. After addition of Et_2O to the reaction mixture, the precipitated material was filtered to give compound **6d** (43 mg, 33%). The obtained product was identical to an authentic sample.²²

[Method 2] A solution of methylenehydantoin (**3d**) (100 mg, 0.42 mmol) and pyrrolidine (40 mg, 0.55 mmol) in CH_2Cl_2 was stirred for 5 h and kept for 18 h at rt. After concentration of the solvent, the residue was purified by centrifugal chromatography (silica gel) using AcOEt to give 3-(4-chlorophenyl)-5-methyl-5-pyrrolidinylhydantoin **6d** (30 mg, 23%). The obtained product was identical to an authentic sample.²²

1-Methyl-3-(naphthalen-1-yl)-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione (**6e**)

[Method 1] A mixture of methylenehydantoin (**3e**) (30 mg, 0.12 mmol) and pyrrolidine (20 mg, 0.28 mmol) was allowed to stand for 1 h at rt. Et₂O was added to the reaction mixture and then the precipitated material was filtered to give compound **6e** (17 mg, 45%). A ratio of the two rotational isomers (major/minor) of 7:3 was indicated by the ¹H-NMR spectrum in DMSO-*d*₆ at 34.6 °C. Mp 143-145 °C. IR (KBr) cm⁻¹: 1772, 1713. FAB-MS (positive) *m/z*: 324 (M+H)⁺. HR-FAB-MS (positive) *m/z*: 324.1717 (Calcd for C₁₉H₂₁N₂O₂: 324.1712). ¹H-NMR (DMSO-*d*₆) δ: 1.71-1.81 (4H, m, Pyr H-3, H-4), 2.54-2.73 (4H, m, Pyr H-2, H-5), 3.01 (2.1H, s, NMe), 3.03 (0.9H, s, NMe), 3.00-3.12 (1H, m, Pyr-CHH), 3.20-3.23 (1H, m, Pyr-CHH), 4.23-4.33 (0.7H, m, Hyd H-5), 4.47-4.49 (0.3H, m, Hyd H-5), 7.46-7.56 (7H, m, Np H). ¹³C-NMR (DMSO-*d*₆) δ: 23.5 (Pip C-3, C-4), 27.7 (NCH₃), 52.8 (Pyr C-2, C-5), 55.2 (CH₂-Pip), 62.8 (Hyd C-5), 129.0 (Np C-8a), 130.0 (Np C-4a), 133.7 (Np C-1), 155.7 (Hyd C-2), 171.8 (Hyd C-4). These signals are ascribable to the major rotational isomer. δ: 23.45 (Pip C-3, C-4), 28.0 (NCH₃), 53.8 (Pyr C-2, C-5), 54.4 (CH₂-Pip), 61.4 (Hyd C-5), 129.0 (Np C-8a), 130.0 (Np C-4a), 133.7 (Np C-1), 155.6 (Hyd C-2), 171.9 (Hyd C-4). These signals are ascribable to the minor rotational isomer. δ: 122.7, 122.9, 125.4, 125.5, 125.5, 126.39, 126.42, 126.42, 126.7, 127.0, 128.1, 128.1, 129.1, 129.1 (Other Np C, These signals are for both rotational isomers). Anal. Calcd for C₁₉H₂₁N₃O₂ • 0.15 H₂O: C, 69.98; H, 6.58; N, 12.96. Found. C, 69.98; H, 6.62; N, 12.91.

[Method 2] A mixture of methylenehydantoin (**3e**) (30 mg, 0.12 mmol) and pyrrolidine (30 mg, 0.42 mmol) in CH₂Cl₂ was stirred for 20 h at rt. After evaporation of the solvent, Et₂O was added to the residue and then the precipitated material was filtered to give compound **6e** (18 mg, 47%). The product obtained by this method contained a small amount of the starting **3e** (by ¹H-NMR analysis).

5-((4-Methoxyphenyl)amino)-5-methyl-3-phenylimidazolidine-2,4-dione (**7**)

This compound was prepared by using method 3. A mixture of methylenehydantoin (**3a**) (70 mg, 0.37 mmol) and *p*-anisidine (33 mg, 0.27 mmol) in CH₂Cl₂ (5 mL) was concentrated under reduced pressure. The resulting residue was warmed up to 70 °C in a water bath and kept of that temperature for 6 h. The reaction mixture was purified by centrifugal chromatography (silica gel) using AcOEt/*n*-hexane (1:1) as a solvent to give compound **7** in 35% yield (29 mg); a white solid; mp 138-140 °C. IR (KBr) cm⁻¹: 1715. FAB-MS (positive) *m/z*: 312 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 1.66 (3H, m, Hyd 5-Me), 3.27 (3H, s, OMe), 5.87 (1H, s, Ar-NH), 6.68 (2H, dd, *J* = 6.7, 2.4 Hz, Ar H-2, H-6 in anisidine), 6.78 (2H, dd, *J* = 6.7, 2.4 Hz, Ar H-3, H-5 in anisidine), 7.19-7.22 (2H, m, Ar H-2, H-6 in Hyd-Ph), 7.36-7.39 (1H, m, Ar H-4 in Hyd-Ph), 7.44-7.47 (2H, m, Ar H-3, H-5 in Hyd-Ph), 8.79 (1H, s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ: 25.3 (Hyd C(5)-Me), 55.1 (OMe), 72.2 (Hyd C-5), 114.3 (Ar C-2, C-6 in anisidine), 118.2 (Ar C-3, C-5 in anisidine), 126.5 (Ar C-2, C-6 in Hyd-Ph), 127.8 (Ar C-4 in Hyd-Ph), 128.7 (Ar C-3, C-5 in Hyd-Ph), 131.8 (Ar C-1 in Hyd-Ph), 138.1 (Ar C-1 in anisidine), 153.2 (Ar C-4 in anisidine), 153.7 (Hyd C-2), 173.7 (Hyd C-4). Anal. Calcd for C₁₇H₁₇N₃O₃: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.38; H, 5.47; N,

13.51.

5-(Benzyl(methyl)amino)-5-methyl-3-phenylimidazolidine-2,4-dione (**8**)

This compound was prepared by using method 3. From the reaction of methylenehydantoin (**3a**) (50 mg, 0.27 mmol) and *N*-methylbenzylamine (70 mg, 0.58 mmol) in CH₂Cl₂ (5 mL), compound **8** was obtained in 70% yield (57 mg) as a white solid. Mp 132-135 °C. IR (KBr) cm⁻¹: 1776, 1717. FAB-MS (positive) *m/z*: 310 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 1.67 (3H, m, Hyd C(5)-Me), 2.15 (3H, s, NMe), 3.47 and 3.71 (each 1H, d, *J* = 13.5 Hz, N-CH₂-Ph), 7.25-7.52 (10H, m, Ar H), 8.87 (1H, s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ: 22.5 (Hyd C(5)-Me), 32.1 (NMe), 54.2 (N-CH₂-Ph), 77.0 (Hyd C-5), 126.7, 126.9, 127.8, 128.1, 128.4, 128.7 (Ar C), 131.8 (Ar C-1 in Hyd-Ph), 138.9 (Ar C-1 in N(Me)CH₂-Ph), 154.2 (Hyd C-2), 173.2 (Hyd C-4). Anal. Calcd for C₁₈H₁₉N₃O₂ • 0.3 H₂O: C, 68.68; H, 6.28; N, 13.35. Found: C, 68.88; H, 6.31; N, 13.12.

5,5'(Piperazine-1,4-diylbis(methylene)bis(3-phenylimidazolidine-2,4-dione) (**9a**)

This compound was prepared by using method 3. From the reaction of methylenehydantoin (**3a**) (101 mg, 0.54 mmol) and piperazine (23 mg, 0.27 mmol) in CH₂Cl₂ (5 mL), compound **9a** was obtained in 34% yield (42 mg) as a white solid; mp 203 °C (dec). IR (KBr)cm⁻¹:1776, 1721. FAB-MS (positive) *m/z*: 463 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 2.42-2.43, 2.50-2.57 (each 4H, m, piperazine ring), 2.68-2.78 (4H, m, Hyd- CH₂- N=), 4.30 (2H, s, Hyd H-5), 7.31-7.39 (6H, m, Ar H), 7.46-7.49 (4H, m, Ar H), 8.41 (2H, s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ: 53.7 (piperazine ring), 56.2, 56.3 (Hyd C-5), 58.19, 58.22 (Hyd-CH₂-N=), 126.5, 127.6, 128.6 (Ar C), 132.2 (Ar C-1), 155.8 (Hyd C-2), 172.5 (Hyd C-4). Anal. Calcd for C₂₄H₂₆N₆O₄ • 0.5 H₂O: C, 61.13; H, 5.77; N, 17.82. Found: C, 61.23; H, 5.64; N, 17.90.

Reaction of Methylene-Hydantoin **3a** and Ethylene-1,2-diamine

By using method 3, from the reaction of methylenehydantoin (**3a**) (117 mg, 0.62 mmol) and ethylene-1,2-diamine (34 mg, 0.57 mmol) in CH₂Cl₂ (2 mL), we isolated 1-(5-oxo-1,4-diazepan-6-yl)-3-phenylurea **10** in 23% yield (33 mg) as a white solid; mp 160 °C (dec). IR (KBr) cm⁻¹: 3338, 3220, 1665, 1636. FAB-MS (positive) *m/z*: 249 (M+H)⁺, HR-FAB-MS (positive) *m/z*: 249.1351 (Calcd for C₁₂H₁₇N₄O₂; 249.1352). ¹H-NMR (DMSO-*d*₆) δ: 2.42-2.46 (2H, m, DAP H-2 and H-7), 2.50-2.92 (1H, br, DAP H-1), 2.94-2.99 (1H, m, DAP H-2), 3.01-3.09 (2H, m, DAP H-3 and H-7), 3.26-3.31 (1H, m, DAP H-3), 4.38 (1H, d, *J* = 6.7 Hz, DAP H-6), 6.53 (1H, d, *J* = 5.5 Hz, phenylurea H-1), 6.89 (1H, d, *J* = 7.0 Hz, Ar H-4), 7.21 (2H, t, *J* = 7.6 Hz, Ar H-2 and H-6), 7.38 (2H, t, *J* = 7.6 Hz, Ar H-3 and H-5), 7.93 (1H, br s, DAP H-4), 8.95 (1H, br s, phenylurea H-3). ¹³C-NMR (DMSO-*d*₆) δ: 43.8 (DAP C-3), 49.1 (DAP C-2), 50.8 (DAP C-7), 55.6 (DAP C-6), 117.4 (Ar C-2 and C-6), 120.9 (Ar C-4), 128.5 (Ar C-3 and C-5), 140.4 (Ar C-1) 154.1 (phenylurea C-2), 174.7 (DAP C-6). Anal. Calcd for C₁₂H₁₆N₄O₂ • 0.6 H₂O: C, 55.63; H, 6.69; N, 21.62. Found: C, 55.91; H, 6.55; N, 21.32.

Reaction of Methylene-Hydantoin **3a** and 1,2-Phenylenediamine

By using method 3, from the reaction of methylenehydantoin (**3a**) (117 mg, 0.62 mmol) and 1,2-phenylenediamine (29 mg, 0.27 mmol) in CH₂Cl₂ (2 mL), we obtained compound **11** (CAS 14003-34-0) in 53% yield (23 mg) as a white solid. *N*-Phenylurea was also isolated (35 mg). The obtained compound **11** showed mp 180 °C. The NMR data were identical to those of an authentic sample.²³ Anal. Calcd for C₉H₈N₂O: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.42; H, 5.01; N, 17.48.

Preparation of Bivalent Twin-Drug Type Molecules (12a-12d).

1,1'-(1,4-Phenylene)bis(3-benzyl-3-((2,5-dioxo-1-phenylimidazolidin-4-yl)methyl)urea) (**12a**)

A solution of 1,4-diisocyanatebenzene (35 mg, 0.22 mmol) in CH₂Cl₂ was added to a solution of 5-((benzylamino)methyl)-3-phenylimidazolidine-2,4-dione (**4a**) (100 mg, 0.34 mmol) in CH₂Cl₂, and the resulting mixture was stirred for 1 d at rt. After concentration of the solvent, the obtained solid material was purified by centrifugal chromatography (silica gel) using AcOEt as a solvent to afford a white solid **12a** (66 mg, 52%). Mp 135-145 °C. IR (KBr) cm⁻¹: 3412, 1779, 1718, 1644. FAB-MS (positive) *m/z*: 751 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 3.73-3.74 (4H, m, Hyd-CH₂-N=), 4.52 (2H, t, *J* = 6.0 Hz, Hyd H-5), 4.68, 4.74 (each 2H, d, *J* = 17.0 Hz, CH₂-Ph), (2H, d, *J* = 17.0 Hz, CHH-Ph), 7.25-7.47 (24H, m, Ar H), 8.36 (2H, br s, NHCON=), 8.55 (2H, br s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ: 47.5 (Hyd-CH₂-N=), 50.1 (CH₂-Ph), 55.6 (Hyd C-5), 120.4 (Ar C in NH-Ph-NH), 126.5, 126.87, 126.91, 127.6, 128.4, 128.5 (Ar C), 132.1 (Ar C-1 in Hyd-Ph), 134.7 (Ar C-1 in NH-Ph-NH), 138.1 (Ar C-1 in CH₂-Ph), 155.5 (NHCON=), 155.7 (Hyd C-2), 171.9 (Hyd C-4). Anal. Calcd for C₄₂H₃₈N₈O₆ • 0.5 H₂O: C, 66.39; H, 5.17; N, 14.75. Found: C, 66.36; H, 5.43; N, 14.95.

1,1'-(1,4-Phenylene)bis(3-benzyl-3-((1-(4-chlorophenyl)-2,5-dioxoimidazolidin-4-yl)methyl)urea) (**12b**)

This compound was obtained from the reaction of **4b** and 1,4-diisocyanatebenzene in 87% yield by a method similar to that for **12a** as a white solid. Mp 236-237 °C. IR (KBr) cm⁻¹: 1771, 1717, 1648. FAB-MS (positive) *m/z*: 819 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 3.72 (4H, s, Hyd-CH₂-N=), 4.51 (2H, t, *J* = 6.0 Hz, Hyd H-5), 4.68 and 4.74 (each 2H, d, *J* = 17.0 Hz, CH₂-Ph), 7.26-7.28 (8H, m, Ar H-2, H-3, H5, H-6 in CH₂-Ph), 7.32 (4H, s, Ar H in NH-Ph-NH), 7.35-7.36 (2H, m, Ar H-4 in CH₂-Ph), 7.40, 7.50 (each 4H, d, *J* = 9.0 Hz, Ar H in *p*-Cl-Ph), 8.35 (2H, br s, NHCON=), 8.61 (2H, br s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ: 47.5 (Hyd-CH₂-N=), 50.2 (CH₂-Ph), 55.5 (Hyd C-5), 120.4 (Ar C in NH-Ph-NH), 126.8, 126.9, 128.1, 128.4, 128.5 (Ar C), 131.0 (Ar C-4 in *p*-Cl-Ph), 131.9 (Ar C-1 in *p*-Cl-Ph), 134.7 (Ar C-1 in NH-Ph-NH), 138.1 (Ar C-1 in CH₂-Ph), 155.4 (NHCON=), 155.5 (Hyd C-2), 171.7 (Hyd C-4). Anal. Calcd for C₄₂H₃₆N₈O₆Cl₂ • H₂O: C, 60.22; H, 4.57; N, 13.38. Found: C, 60.24; H, 4.39; N, 13.45.

1,1'-(Methylenebis(4,1-phenylene))bis(3-benzyl-3-((2,5-dioxo-1-phenylimidazolidin-4-yl)methyl)urea) (**12c**)

This compound was obtained from the reaction of **4a** and bis(4-isocyanatophenyl)methane in 95% yield

by a method similar to that for **12a** as a white solid. Mp 128-145 °C. IR (KBr) cm^{-1} : 1779, 1717. FAB-MS (positive) m/z : 841 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ : 3.73-3.74 (4H, d, $J = 6.0$ Hz, Hyd-CH₂-N=), 3.80 (2H, br s, Ph-CH₂-Ph), 4.51 (2H, t, $J = 6.0$ Hz, Hyd H-5), 4.68 and 4.74 (each 2H, d, $J = 17.0$ Hz, CH₂-Ph), 7.05-7.47 (28H, m, Ar H), 8.40 (2H, br s, NHCON=), 8.54 (2H, br s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ : 40.0 (Ph-CH₂-Ph), 47.5 (Hyd-CH₂-N=), 50.2 (CH₂-Ph), 55.5 (Hyd C-5), 120.1, 126.5, 126.9, 126.9, 127.1, 128.3, 128.4, 128.5 (Ar C), 132.1, (Ar C-1 in Ph-Hyd), 135.2 (Ar C-4 in Ph-CH₂-Ph) 138.0 (Ar C-1 in Ph-CH₂-Ph), 138.1 (Ar C-1 in CH₂-Ph), 155.4 (NHCON=), 155.7 (Hyd C-2), 171.9 (Hyd C-4). Anal. Calcd for C₄₉H₄₄N₈O₆ • 0.4H₂O: C, 69.39; H, 5.32; N, 13.21. Found: C, 69.40; H, 5.45; N, 12.98.

1,1'-(Methylenebis(4,1-phenylene))bis(3-benzyl-3-((2,5-dioxo-1-phenylimidazolidin-4-yl)methyl)-thiourea) (12d)

A solution of bis(4-isothiocyanatophenyl)methane (42 mg, 0.15 mmol) in CH₂Cl₂ was added to a solution of (**4a**) (100 mg, 0.34 mmol) in CH₂Cl₂, and the resulting mixture was stirred for 4 h at rt. After concentration of the solvent, the obtained solid material was washed with EtOH to give **12d** (100 mg, 77%) as a white solid. Mp 132-136 °C. IR (KBr) cm^{-1} : 1779, 1717. FAB-MS (positive) m/z : 873 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ : 3.89 (2H, br s, Ph-CH₂-Ph), 4.10-4.14 (2H, m, Hyd-CH₂-N=), 4.26-4.30 (2H, m, Hyd-CH₂-N=), 4.80 (2H, t, $J = 6.5$ Hz, Hyd H-5), 5.11 and 5.20 (each 2H, bd, $J = 17.0$ Hz, CH₂-Ph), 7.14-7.47 (28H, m, Ar H), 8.59 (2H, s, Hyd H-1), 9.23 (2H, s, NHCSN=). ¹³C-NMR (DMSO-*d*₆) δ : 40.0 (Ph-CH₂-Ph), 52.0 (Hyd-CH₂-N=), 53.9 (CH₂-Ph), 54.8 (Hyd C-5), 126.4, 126.59, 126.62, 127.0, 127.6, 128.1, 128.4, 128.5 (Ar C), 132.1, (Ar C-1 in Ph-Hyd), 136.7 (Ar C-1 in CH₂-Ph), 138.0 (Ar C-4 in Ph-CH₂-Ph), 138.5 (Ar C-1 in Ph-CH₂-Ph), 155.7 (Hyd C-2), 171.7 (Hyd C-4), 182.3 (C=S). Anal. Calcd for C₄₉H₄₄N₈O₄S₂: C, 67.41; H, 5.08; N, 12.83. Found: C, 67.17; H, 5.16; N, 12.76.

N⁴,N^{4'}-Dibenzyl-N⁴,N^{4'}-bis((2,5-dioxo-1-phenylimidazolidin-4-yl)methyl)-[1,1'-biphenyl]-4,4'-dicarboxamide (13a)

A solution of [1,1'-biphenyl]-4,4'-dicarbonyl dichloride (42 mg, 0.15 mmol) in CH₂Cl₂ was added to a solution of compound **4a** (100 mg, 0.34 mmol) and TEA (29 mg, 0.29 mmol) in CH₂Cl₂ (20 mL). The mixture was stirred for 1 h at rt and precipitated material was filtered off. The filtrate was concentrated under reduced pressure. A small amount of MeOH was added to the oily residue to give **13a** (72 mg, 60%) as a white solid. Mp 132-138 °C. IR (KBr) cm^{-1} : 1632. FAB-MS (positive) m/z : 797 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ : 3.72-3.81 (4H, m, Hyd-CH₂-N=), 4.66-4.73 (6H, m, CH₂-Ph + Hyd H-5), 7.19-7.80 (28H, m, Ar H), 8.73 (2H, br s, Hyd H-1). ¹³C-NMR (DMSO-*d*₆) δ : 46.2 (Hyd-CH₂-N=), 53.3 (Ph-CH₂), 54.3 (Hyd C-5), 126.6, 126.7, 127.1, 127.4, 127.6, 128.2, 128.5, 128.7 (Ar C), 132.1, (Ar C-1 in Ph-Hyd), 135.4 (Ar C-4 in COPh-), 136.8 (Ar C-1 in Ph-CH₂), 140.1 (Ar C-1 in COPh-), 155.8 (Hyd C-2), 171.5 (CON or Hyd C-4), 171.6 (Hyd-C-4 or CON). Anal. Calcd for C₄₈H₄₀N₆O₆ • 0.3H₂O: C,

71.86; H, 5.10; N, 10.48. Found: C, 71.87; H, 5.18; N, 10.35.

***N*¹,*N*¹⁰-Dibenzyl-*N*¹,*N*¹⁰-bis((2,5-dioxo-1-phenylimidazolidin-4-yl)methyl)decanediamide (13b)**

A solution of decanedioyl dichloride (17 mg, 0.07 mmol) in CH₂Cl₂ was added to a solution of compound **4a** (50 mg, 0.17 mmol) and TEA (15 mg, 0.15 mmol) in CH₂Cl₂ (20 mL). The mixture was stirred for 1 h at rt and concentrated *in vacuo*. The residue was washed with water to give **13b** (42 mg, 78%) as a white solid. Mp 86-90 °C. IR (KBr) cm⁻¹: 1778, 1721. FAB-MS (positive) *m/z*: 757 (M+H)⁺. ¹H-NMR (DMSO-*d*₆) δ: 1.13-1.47 (8H, m, DD H-4, H-5), 1.49-1.54 (4H, m, DD H-3), 2.27-2.29, 2.46-2.50 (each 2H, m, DD H-2), 3.62-3.77 (4H, m, Hyd-CH₂-N=), 4.48-4.74 (6H, m, CH₂-Ph + Hyd H-5), 7.23-7.56 (20H, m, Ar H), 8.49 (1.3H, br s, Hyd H-1), 8.67(0.7H, br s, Hyd H-1). ¹³C-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), 32.15, 32.22 (DD C-2), 47.4, 51.8 (CH₂-Ph), 47.5, 47.5 (Hyd-CH₂-N=), 54.7, 55.5 (Hyd C-5), 126.1, 126.49, 126.53, 126.8, 127.1, 127.2, 127.5, 127.7, 128.3, 128.5, 128.55, 128.61 (Ar C), 132.0, 132.1 (Ar C-1 in Hyd-Ph), 137.6, 137.7 (Ar C-1 in CH₂-Ph), 155.6, 155.6 (Hyd C-2), 171.6, 171.7 (Hyd C-4), 172.8, 173.3 (CH₂-CO-N). Anal. Calcd for C₄₄H₄₈N₆O₆ • 2.3H₂O: C, 66.20; H, 6.64; N, 10.53. Found: C, 66.19; H, 6.68; N, 10.83.

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16. Regarding heat of binding between the twin-drug type compound **A** and sulfated glycosaminoglycans, we previously used dermatan sulfate (GAG-DS01) and heparan sulfate (GAG-HS01) as sulfated glycosaminoglycans.⁵ Shortly afterward, we obtained an additional result of exothermic binding reaction of compound **A** with scallop heparan sulfate (GM01/01). In isothermal titration experiments, the binding reaction with GM01/01 [**Hepgm**] was also exothermic and compound **A** showed thermodynamic parameters of $K = 4.10 \times 10^4$ 1/M and $\Delta H = -9.98$ kJ/mol. Thermodynamic parameters for dermatan sulfate (GAG-DS01) [**Der**: $K = 1.11 \times 10^3$ 1/M and $\Delta H = -10.9$ kJ/mol] and heparan sulfate (GAG-HS01) [**Hep**: $K = 2.75 \times 10^4$ 1/M and $\Delta H = -9.46$ kJ/mol] were shown in our previous paper.⁵ The calculated titration fitting curves of the above three kinds of sulfated glycosaminoglycans are shown together in the Figure.

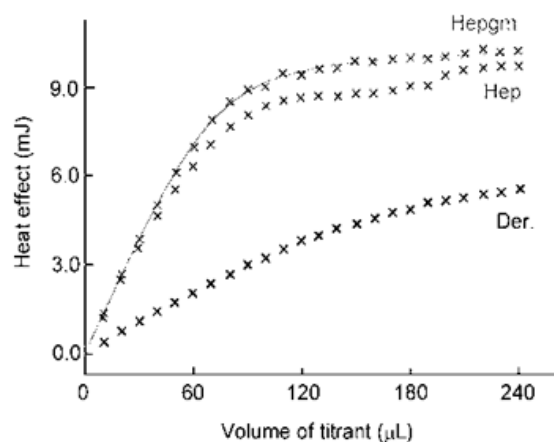


Figure. Calculated Fitting Curves for a Twin-Drug **A** Binding to Heparan Sulfates [**Hepgm** and **Hep**] and Dermatan Sulfate [**Der**].

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19. Recently, it has been reported that chemical modifications of 1,3-dibenzyl-5-methylene-hydantoin with a few reagents to 5-substituted hydantoin derivatives (see reference 20).
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21. All of the obtained compounds except for compound **13b** 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, obtained

products **12-13** can be considered to be a mixture of three twin-drug type bivalent molecules, i.e., two C₂-symmetrical molecules that have the same absolute configuration (*R,R* or *S,S*) regarding two chiral hydantoin rings in the molecules and a C_s-symmetrical *meso* compound having different absolute configurations (*R,S*). We previously found the presence of three stereoisomers in the free base of compound **A** by the HPLC method.¹⁵ In the case of compounds **13b**, we consider that the diastereomeric mixture gave rise to slightly different non-equivalent magnetic resonance patterns. We used isomeric mixtures for biological prescreening (antibacterial activity) and for calorimetric experiments.

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