Synthesis of New 5-Substituted Hydantoins and Symmetrical Twin-Drug Type Hydantoin Derivatives

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In connection with our studies on hydantoin derivatives, a conventional regioselective chemical transformation of 5-methylene hydantoins 4a–c to 5-aminomethyl-substituted hydantoins 5–10 or to 5-amino-5-methyl-disubstituted hydantoins 11–14 is described. Synthesis of bivalent twin-drug type hydantoin derivatives 19–24 and the binding property of a bivalent symmetrical hydantoin derivative 24b to sulfated glycosaminoglycans are also described.

Key words hydantoin; regioselective; antibacterial activity; isothermal titration calorimetry; bivalent twin-drug; sulfated glycosaminoglycan

The need for new antibacterial agents is largely due to the increase of bacterial infections with resistant strains, especially Gram-positive strains, in both community and hospital setting. Oxazolidinone antibacterial agents such as linezolid are a relatively new class of antibacterial agents, and the utility of this class includes activity against multidrug-resistant infections.1–3) In the early stage of invasion of bacteria or viruses, the surface glycans of organisms recognize various host cell lectins. In terms of molecular recognition, the participation of two-fold (C2) or three-fold (C3) symmetrical geometry macro-molecules is one of the common features in many biological responses,4–6) and small symmetric geometrical molecules frequently appear in many biological active compounds.7–9) From this interesting aspect of molecular symmetry, we have already reported a few examples of such types of symmetrical targeted molecules for the purpose of finding new bioactive leads or candidates.10–15)

Our previous studies on a bioisosteric replacement of the oxazolidinone ring in linezolid by a hydantoin nucleus provided a few interesting antibacterial leads.16–20) Among previously targeted hydantoin derivatives, some derivatives including a twin-drug type symmetrical hydantoin derivative20,21) showed significant antibacterial activity against Gram-positive organisms (Staphylococcus aureus). This finding of new antibacterially active molecules constructed on a hydantoin scaffold encouraged us to develop further modifications of this class of compounds.

In this article, we describe the regioselective chemical modification of 5-methylene hydantoins to 5-aminomethyl-substituted hydantoins 5–10 or to 5-amino-5-methyl-disubstituted hydantoins 11–14. Preparation of the bivalent twin-drug type hydantoin derivatives 19–24 from β-aminoalanine methyl ester (1)20) and a new carbohydrate recognition binding property of symmetrical twin-drug type hydantoin derivative 24b are also described.

Results and Discussion

In connection with our synthetic studies on new bioactive hydantoin derivatives, some molecular modifications of β-aminoalanine methyl esters (1) to bioisosteric hydantoin derivatives have been reported.16–20) As starting materials for further derivatizations in this series, 5-methylene hydantoins 4a–c were obtained from elimination (deamination) reactions of the corresponding 5-dialkylaminomethyl-hydantoins (3)17) (Chart 1) (see Experimental).

Two types of hydantoin derivatives (5–10 and 11–14) could be obtained from regioselective additions of amines to 5-methylene hydantoins 4a–c (Chart 2). Thus, reaction conditions without a solvent (neat) at room temperature (rt) (path a) resulted predominantly in the formation of 5-pyrrolidino-
methyl- or 5-benzylaminomethyl-hydantoin derivatives 5–10. In contrast, reactions in CH₂Cl₂ under rt or refluxing conditions (path b) afforded 5-amino-5-methylhydantoin derivatives 11–14 in moderate to good yields. The results are summarized in Tables 1 and 2. It is thought that the tautomeric isomer B of 5-methylene hydantoin in solution (A=⁻B)¹⁷,¹⁹) is a crucial intermediate for the formation of 5,5-disubstituted hydantoins (11–14), as shown in Chart 2. When using an excess amount of an amine, a considerable amount of ring-opened urea derivatives 15–17 was isolated as a predominant reaction product (Chart 2).

All of the structures of the above hydantoin derivatives were easily confirmed by elemental analysis and spectroscopic data. The positive FAB mass spectroscopic behaviors of these hydantoin derivatives are particularly interesting, and diagnostically useful fragmentation processes were observed. The prominent fragment iminium ion [a] for 5-dialkylaminomethyl-hydantoin derivatives 5–10 is from fission of the C(5)–C bond (α-cleavage of the molecular ion). On the other hand, in the mass spectra of 5-methyl-5-dialkylamino-hydantoin derivatives 11–14, the formation of a strong ammonium ion peak [b] resulting from C(5)–N bond cleavage of the 5-amino substituent is observed (Fig. 1).

Furthermore, in NMR spectra of 5-methyl-5-dialkylaminosubstituted hydantoin derivatives, 5-methyl and 5-carbon ring signals of the products are easily distinguished. The ¹H-NMR spectrum of these 5,5-disubstituted derivatives showed 1.53–1.57 ppm as a singlet assignable to the 5-methyl group.
and the $^{13}$C-NMR spectrum had two characteristic carbon signals at 22.9–24.0 and 73.4–75.1 ppm, easily ascribable to the substituent 5-methyl carbon and 5-position of the hydantoin ring carbon, respectively. From these data, we could easily confirm the structures of the products (see Experimental for details).

A novel N-acyl derivative $18$ was obtained from direct N-acylation of isolated 5-aminomethylhydantoin $6$ with acetic anhydride (Chart 3). This chemical modification of compound $18$ also provided chemical evidence for the validity of the structure as a secondary amine $6$ and a new promising route to 5-acylaminomethyl-hydantoins.

In addition to the above-described modifications, we also attempted to prepare twin-drug type hydantoin derivatives from $\beta$-aminoalanine methyl ester $1$ in order to find more active antibacterial leads.$^{20,21}$ The targeted bivalent twin-drug type$^{22}$ hydantoin derivatives $19$–$24$ were obtained from reactions of the corresponding diisocyanate derivatives and $\beta$-aminoalanine esters (I) (Chart 4). Details of the protocol for preparation of twin-drug type compounds are shown in Experimental. Double cyclization reactions affording bivalent hydantoin derivatives $19$–$24$ easily occurred under conditions similar to those for preparation of 5-dialkylaminomethylhydantoins described previously.$^{18}$ The results for designed twin-drug type compounds are summarized in Table 3. All of the obtained compounds exhibited very simple symmetrical $13$C-NMR spectra in DMSO-$d_{6}$, indicating little difference with respect to the signal assignable to substituted hydantoin rings and a linker moiety.$^{23}$ The linker structures applied in these twin-drug type molecules are also shown in Table 3. The yields were good to excellent and the obtained products were stable solid or crystalline materials. Through these synthetic trials, we confirmed that the above-described procedure is a conventional route to prepare new types of bivalent symmetrical twin-drug type bivalent molecules.

It is thought that sulfated sugar chains play an important role in mediating adhesion of many types of bacterial organisms to host cells or tissues. Regarding the interaction of bacterial adhesion to glycan, a few examples of binding carbohydrate specificities have been demonstrated, and some bacteria are known to bind to sulfated glycosaminoglycans such as heparan sulfate.$^{24}$ We have been interested in small molecules that interfere with such carbohydrate recognition stages in order to find new bioactive leads.$^{10,11,25,26}$ With the aim of elucidating the chemical properties of the antibacterial active symmetrical twin drug type compound $24b$, we carried out thermodynamic experiments on binding of sulfated glycosaminoglycans such as heparan sulfate and dermatan sulfate to a bivalent antibacterial active hydantoin derivative $24b$ by using isothermal titration calorimetry. Among the compounds tested, the binding reaction between twin-drug type compound $24b$ and heparan sulfate or dermatan sulfate was exothermic and the obtained thermodynamic parameters were $K=2.75\times10^{4}$1/M and $\Delta H=-9.46$ kJ/mol for heparan sulfate and $K=1.11\times10^{4}$1/M and $\Delta H=-10.9$ kJ/mol for dermatan sulfate at 298.15 K. A representative thermogram of a hydantoin derivative $24b$ titrated with heparan sulfate is shown in Fig. 2.

From the results of calorimetric experiments, we found that the twin-drug type small molecule $24b$ has an interesting binding property to sulfated glycosaminoglycans.$^{27}$ Regarding the prepared hydantoin derivatives, symmetrical twin-drug type derivatives ($24a$ and $23b$) showed significant antibacterial activity against a Gram-positive strain (Staphylococcus aureus) (MIC=0.026 nm and 0.116 nm, respectively), but these compounds were inactive against a Gram-negative strain (Escherichia coli) at a concentration of less than 0.211 nm. The difference in antibacterial activities seems to be affected by both structure of the linker and structure of the basic amine moiety in a twin-drug type molecule. Further details of an structure–activity relationship (SAR) study including other prepared hydantoin derivatives and additional thermodynamic experiments for the biological active compounds in this series.

### Table 2. Preparation of 5-Amino-5-methyl-disubstituted Hydantoins 11–14 from 5-Methylene Hydantoins 4a–c

| Compd. No. | R1 | R2 | Amounts of amines$^a$ | Reaction temp. | Time | Yield (%) |
|------------|----|----|----------------------|----------------|------|-----------|
| 11         | H  | N  | 1                    | rt             | 5h   | 61        |
| 12         | H  | –NHCH$_2$Ph | 6                   | Reflux         | 5h   | 28        |
| 13         | Cl | N  | 3                    | Reflux         | 2h   | 69        |
| 14         | OMe| N  | 2                    | rt             | 1h   | 67        |

$^a$ Molar ratio of the used amine to compound 4.
Chart 3. Preparation of 5-N-Acylaminomethyl Hydantoin 18 from Compound 6

Chart 4. Synthesis of Twin-Drug Type Hydantoin Derivatives 19–24 from Compound 1

Table 3. Chemical Structures and Yields for Symmetrical Twin-Drug Type Hydantoin Derivatives 19–24

| Compd. No. | Linker         | Yield (%) |
|------------|----------------|-----------|
| 19a        | -(CH₂)₄⁻       | 67        |
| 19b        | -(CH₂)₄⁻       | 62        |
| 20a        | -(CH₂)₆⁻       | 40        |
| 20b        | -(CH₂)₆⁻       | 43        |
| 21a        | -(CH₂)₈⁻       | 71        |
| 21b        | -(CH₂)₈⁻       | 84        |
| 22a        | -(CH₂)₁₂⁻      | 78        |
| 22b        | -(CH₂)₁₂⁻      | 71        |
| 23b        |                | 40        |
| 24a        |                | 45        |
| 24b(⁹)     |                | 65        |

(⁹) The data for compound 24b were taken from ref. 19.
will be described separately.

**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 d 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 FAB-MS spectra were obtained by a JEOL JMS-HX110 mass spectrometer. Dermatan sulfate sodium salt (GAG-DS01) and heparan sulfate sodium salt (GAG-HS01) were purchased from Funakoshi Co., Ltd. All other chemicals used were of reagent grade.

**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 the bacterial growth turbidity in Muller–Hinton broth according to the Japanese Society of Chemotherapy.

**Preparation of 5-Methylene Hydantoins (4a–c)** These compounds were prepared according to the procedure reported previously. Physical and spectroscopic data of these compounds are shown below.

5-Methylene-3-phenylimidazolidine-2,4-dione (4a): Physical and spectroscopic data of this compound were reported in our previous paper.

5-Methylene-3-(4-chlorophenyl)imidazolidine-2,4-dione (4b): This compound was obtained in 68% yield; a white solid; mp >215°C. IR (KBr) cm$^{-1}$: 1775, 1728, 1671. FAB-MS (positive) $m/z$: 223 (M+H)$^+$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 4.94, 5.26 (each 1H, d, $J=2.4$ Hz, Ar H-2, H-6), 7.47 (2H, d, $J=2.1$ Hz, Ar H-3, H-5), 10.8 (1H, br, NH), $^{13}$C-NMR (DMSO-$d_6$) $\delta$: 94.9 (=CH$_2$), 128.3 (Ar C-3, C-5), 128.7 (Ar C-2, C-6), 130.6 (Ar C-4), 132.3 (Ar C-1), 134.9 (Hyd C-5), 152.6 (Hyd C-2), 161.8 (Hyd C-4).

Typical Procedure for the Preparation of Products (5–10) from 5-Methylene Hydantoins (4a–c) 3-Phenyl-5-(pyrrolidin-1-ylmethyl)imidazolidine-2,4-dione (5): A mixture of a 5-methylene hydantoin 4a (0.050 g, 0.27 mmol) and pyrrolidine (0.057 g, 0.80 mmol) was allowed to stand for 10 min at rt. Et$_3$O was added to the mixture and the precipitate was collected by filtration to give 5 (0.043 g, 62%); a white solid; mp 118–119°C; IR (KBr) cm$^{-1}$: 1774, 1723, 1721. FAB-MS (positive) $m/z$: 260 (M+H)$^+$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 1.68 (4H, brs, Pyr H-3, H-4), 2.53–2.60 (4H, m, Pyr H-2, H-5), 2.81–2.83 (1H, m, CH$_2$-Pyr), 2.91–2.95 (4H, m, CH$_2$-Pyr), 2.91–2.95 (4H, m, CH$_2$-Pyr).
5-((Benzylaminomethyl)-3-phenylimidazolidine-2,4-dione (6): A white solid; mp 151–152°C. IR (KBr) cm⁻¹: 1770, 1710. FAB-MS (positive) m/z: 296 (M+H)⁺, 120 (CH₂=NHCH₂Ph)⁺. ¹H-NMR (DMSO-d₆) δ: 2.30 (1H, br, NHCH₂Ph), 2.85–2.93 (2H, m, Hyd-CH₂), 3.73, 3.77 (each 1H, d, J = 6.5, 3.5 Hz, Hyd H-5), 7.21–7.22 (1H, m, Ar H-4 in NH-CH₂Ph), 7.29–7.32 (4H, Ar H in NH-CH₂Ph), 7.39 (2H, d, J = 8.5 Hz, Ar H in 4-chlorophenyl), 7.54 (2H, d, J = 8.5 Hz, Ar H in 4-chlorophenyl), 8.43 (1H, brs, Hyd H-1). ¹³C-NMR (DMSO-d₆) δ: 49.0 (Hyd-CH₂-NH), 52.7 (NH-CH₂Ph), 57.2 (Hyd C-5), 126.5, 127.4, 127.4, 128.0 (Ar C in NH-CH₂Ph), 128.0, 128.0, 128.6, 128.6 (Ar C in 4-chlorophenyl), 131.1 (Ar C or C-4 in 4-chlorophenyl), 131.8 (Ar C-4 or C-1 in 4-chlorophenyl), 140.4 (Ar C in NH-CH₂Ph), 155.5 (Hyd C-2), 172.2 (Hyd C-4). Anal. Calcd for C₁₇H₁₇N₃O₂Cl: C, 65.97; H, 5.77; N, 12.82. Found: C, 65.96; H, 5.95; N, 12.79.

3-(4-Methoxyphenyl)-5-(pyrrolidin-1-ylmethyl)-imidazolidine-2,4-dione (9): A white solid; mp 98.5–99.5°C. IR (KBr) cm⁻¹: 1773, 1710. FAB-MS (positive) m/z: 290 (M+H)⁺, 84 (CH₃=Pyrr)⁺. ¹H-NMR (DMSO-d₆) δ: 1.67–1.69 (4H, brs, Pyr H-3, H-4), 2.49–2.60 (4H, m, Pyr H-2, H-5), 2.76–2.80, 2.88–2.92 (each 1H, m, CH₂Pyrr), 3.79 (3H, s, CH₃O), 4.27 (1H, dd, J = 6.5, 3.5 Hz, Hyd H-5), 7.00–7.02 (2H, m, Ar H), 7.19–7.20 (2H, m, Ar H), 8.40 (1H, br, Hyd H-1). ¹³C-NMR (DMSO-d₆) δ: 23.3 (Pyr C-3, C-4), 54.3 (Pyr C-2, C-5), 56.4 (CH₂Pyrr), 57.1 (Hyd C-5), 128.0, 128.0, 128.7, 128.7 (Ar C), 131.0 (Ar C-1 or C-4), 131.9 (Ar C-4 or Ar C-1), 155.4 (Hyd C-2), 172.2 (Hyd C-4). Anal. Calcd for C₁₅H₁₉N₃O₃: C, 62.27; H, 5.62; N, 14.52. Found: C, 62.08; H, 5.62; N, 14.47.

Typical Procedure for the Preparation of Products (11–14) from 5-Methylene Hydantoin (4a–e) 5-Methyl-3-phenyl-5-(pyrrolidin-1-yl)imidazolidine-2,4-dione (11): A solution of a 5-methylene-hydantoin 4a (0.050 g, 0.27 mmol) and pyrrolidine (0.019 g, 0.27 mmol) in CH₂Cl₂ was stirred for 5h at room temperature. After concentration of the solvent, the solid material was purified by centrifugal silica gel chromatography using AcOEt as a solvent; a white solid; mp 153–154°C. IR (KBr) cm⁻¹: 1771, 1707. FAB-MS (positive) m/z: 326 (M+H)⁺, 120 (CH₃=NHCH₂Ph)⁺. ¹H-NMR (DMSO-d₆) δ: 2.37–2.50 (1H, br, NHCH₂Ph), 2.85 (1H, dd, J = 12.5, 6.0 Hz, NHCH₂Ph), 2.91 (1H, m, Ar H-4 in NH-CH₂Ph), 2.97–2.99 (2H, m, CH₂-Pyr), 3.79 (3H, s, CH₃O), 4.25–4.27 (1H, m, Hyd H-5), 7.00–7.01 (2H, m, Ar H), 7.12–7.23 (3H, Ar H), 7.31–7.33 (4H, m, Ar H), 8.31 (1H, brs, Hyd H-1). ¹³C-NMR (DMSO-d₆) δ: 49.1 (NH=CH₂Ph), 52.7 (Hyd-CH₂-NH), 55.3 (OME), 57.1 (Hyd C-5), 113.9 (Ar C-3, C-5), 124.4 (Ar C-1), 127.8 (Ar C-2, C-6), 156.0 (Hyd C-2), 158.4 (Ar C-4), 172.2 (Hyd C-4). Anal. Calcd for C₁₇H₁₅N₄O₂: C, 76.27; H, 6.62; N, 14.52. Found: C, 76.08; H, 5.36; N, 14.17.

5-((Benzylationomethyl)-3-4-methoxyphenyl)-imidazolidine-2,4-dione (10): This compound was purified by centrifugal silicone gel chromatography with AcOEt as a solvent; a white solid; mp 153–154°C. IR (KBr) cm⁻¹: 1771, 1707. FAB-MS (positive) m/z: 326 (M+H)⁺, 120 (CH₃=NHCH₂Ph)⁺. ¹H-NMR (DMSO-d₆) δ: 2.37–2.50 (1H, br, NHCH₂Ph), 2.85 (1H, dd, J = 12.5, 6.0 Hz, NHCH₂Ph), 2.91 (1H, dd, J = 12.5, 4.0 Hz, NHCH₂Ph), 3.74–3.79 (2H, m, Hyd-CH₂Ph), 3.78 (3H, s, OMe), 4.25–4.27 (1H, m, Hyd H-5), 7.00–7.01 (2H, m, Ar H), 7.12–7.23 (3H, Ar H), 7.31–7.33 (4H, m, Ar H), 8.31 (1H, brs, Hyd H-1). ¹³C-NMR (DMSO-d₆) δ: 49.1 (NH=CH₂Ph), 52.7 (Hyd-CH₂-NH), 55.3 (OME), 57.1 (Hyd C-5), 113.9 (Ar C-3, C-5 in 4-methoxyphenyl), 124.8 (Ar C-1 in 4-methoxyphenyl), 126.5, 127.8, 127.8, 127.9, 127.9, 128.0, 128.0 (Ar C), 140.5 (Ar C-1 in CH₃-Ph), 156.1 (Hyd C-2), 158.3 (Ar C-4 in 4-methoxyphenyl), 172.4 (Hyd C-4). Anal. Calcd for C₁₅H₁₅N₄O₂: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.50; H, 6.00; N, 12.88.
(1H, m, Ar H-4), 7.45–7.47 (2H, m Ar H-3, H-5), 8.62 (1H, brs, Hyd H-1). 1^3C-NMR (DMSO-d_6)  δ: 23.0 (Me), 23.2 (Pyr C-3, C-4), 45.2 (Pyr C-2, C-5), 75.0 (Hyd C-5), 126.7 (Ar C-2, C-6), 127.7 (Ar C-4), 128.7 (Ar C-3, C-5), 131.8 (Ar C-1), 154.2 (Hyd C-2), 173.0 (Hyd C-4). Anal. Caled for C_{19}H_{19}N_{3}O_{3}: C, 64.85; H, 6.61; N, 16.20. Found: C, 64.78; H, 6.63; N, 16.11.

5-(Benzylation)-5-methyl-3-phenylimidazolidine-2,4-dione (12): A white solid; mp 122–124°C. [The ratio of a mixture of three solvents (AcOEt–n-hexane–MeOH) changed stepwise (50:50:0→0:100:0→0:100%)] IR (KBr) cm⁻¹: 1782, 1718. FAB-MS (positive) m/z: 296 (M+H)⁺, 108. 1^3H-NMR (DMSO-d_6) δ: 1.53 (3H, s, Me), 3.40–3.42 (1H, m, NHCHPh), 3.58 (1H, dd, J = 13.0, 6.0Hz, NHCHPhH), 3.72 (1H, dd, J = 13.0, 6.0Hz, NHCHPhH). 7.20–7.47 (10H, m, Ar H), 8.58 (1H, brs, Hyd H-1). 1^3C-NMR (DMSO-d_6) δ: 24.0 (Me), 45.9 (NH-CH₂-Ph), 73.4 (Hyd C-5), 126.5, 126.6, 127.9, 127.9, 128.5 (Ar H), 132.0 (Ar C-1 in Hyd-Ph), 140.0 (Ar C-1 in CH₂-Ph), 154.0 (Hyd C-4), 174.3 (Hyd C-4). Anal. Caled for C_{19}H_{19}N_{3}O_{3}·0.2H₂O: C, 69.14; H, 5.80; N, 14.23. Found: C, 69.08; H, 5.76; N, 14.14.

3-(4-Chlorophenyl)-5-methyl-5-(pyrroldin-1-yl)-imidazolidine-2,4-dione (13): A white solid; mp 138–140°C (AcOEt-n-hexane=7:3). IR (KBr) cm⁻¹: 1781, 1723. FAB-MS (positive) m/z: 294 (M+H)⁺, 72. 1^3H-NMR (DMSO-d_6) δ: 1.57 (3H, s, Me), 1.70–1.72 (4H, m, Pyr H-3, H-4), 2.50–2.58, 2.72–2.77 (each 2H, m, Pyr H-2, H-5), 7.40 (2H, d, J = 9.0Hz, Ar H-2, H-6 or H-3, H-5), 7.54 (2H, d, J = 9.0Hz, Ar H-3, H-5 or H-2, H-6), 8.79 (1H, brs, Hyd H-1). 1^3C-NMR (DMSO-d_6) δ: 22.9 (Me), 23.2 (Pyr C-3, C-4), 45.3 (Pyr C-2, C-5), 75.1 (Hyd C-5), 128.3 (Ar C-2, C-6 or C-3, C-5), 128.7 (Ar C-3, C-5 or C-2, C-6), 130.7 (Ar C-1 or C-4), 130.7 (C-4 or Ar C-1), 153.9 (Hyd C-2), 172.8 (Hyd C-4). Anal. Caled for C_{20}H_{21}ClN_{2}O₂: C, 64.55; H, 5.56; N, 14.13. Found: C, 65.65; H, 5.32; N, 14.13.

3-(4-Methoxyphenyl)-5-methyl-5-(pyrroldin-1-yl)imidazolidine-2,4-dione (14): A white solid; mp 128–130°C (AcOEt-n-hexane=7:3). IR (KBr) cm⁻¹: 1776, 1727. FAB-MS (positive) m/z: 290 (M+H)⁺, 72. 1^3H-NMR (DMSO-d_6) δ: 1.55 (3H, s, Me), 1.71 (4H, br, Pyr H-3, H-4), 2.50–2.57, 2.75–2.97 (each 2H, m, Pyr H-2, H-5), 3.78 (3H, s, OMe), 7.00–7.01, 7.21–7.23 (each 2H, d, J = 8.5Hz, Ar H), 8.66 (1H, br, Hyd H-1). 1^3C-NMR (DMSO-d_6) δ: 23.0 (Me), 23.2 (Pyr C-3, C-4), 45.2 (Pyr C-2, C-5), 55.3 (OMe), 74.9 (Me), 125.9, Ar C-3, C-5), 124.4 (Ar C-1), 128.1 (C-2, C-6), 154.6 (Hyd C-2), 158.6 (Ar C-4), 173.2 (Hyd C-4). Anal. Caled for C_{21}H_{21}ClN_{2}O₂: C, 69.39; H, 6.76; N, 14.08. Found: C, 69.29; H, 6.54; N, 14.13.

1-(1-Oxo-1,3-di(pyroldin-1-yl)propan-2-yl)phenyleurea (16): A mixture of a 5-methylene-hydantoin (4a) (0.10g, 0.53mmol) and pyrrolidine (0.19g, 2.68mmol) was allowed to stand for 2h at room temperature. The resulting solid material was filtered, washed with EtOAc and dried to give as white a solid (0.10g, 57%); mp 193°C. IR (KBr) cm⁻¹: 3424, 1719. FAB-MS (positive) m/z: 338 (M+H)⁺.

1(H-NMR (DMSO-d_6) δ: 2.07 (3H×0.6, s, Me), 2.18 (3H×0.4, s, Me), 3.60–3.78 (2H, m, Hyd-H-3, Hyd-H-5), 2.72–2.74 (10H, m, Ar H, Ar H), 8.51 (1H×0.6, brs, Hyd H-1). 1^3C-NMR (DMSO-d_6) δ: 21.5, 21.6 (Me), 47.2, 52.5 (CH₂-Ph), 47.2, 48.4 (Hyd-CH₃-N=), 54.6, 55.4 (Hyd C-5), 126.3 (3×), 126.5 (3×), 126.8 (3×), 127.1 (6×), 128.3 (3×), 128.6 (3×), 128.9 (3×), 131.9, 132.1 (Ar C-1 in =N=Ph), 137.4, 137.5 (Ar C-1, in CH₂-Ph), 155.5, 156.3 (Hyd C-2), 171.57, 171.64 (Hyd C-4). Anal. Caled for C_{19}H_{19}N_{3}OCl: C, 66.7; H, 6.57; N, 12.16.

Preparation of Twin-Drug Type Molecules (19–24) 3,3'-(Butane-1,4-diyli)bis(5-(pyrroldin-1-ylmethyl)imidazolidine-2,4-dione) Dihydrochloride (19a): A solution of 1,4-diisocyanatobutane (0.25g, 1.79mmol) in CH₂Cl₂ was...
added to a solution of β-aminoalanine methyl ester dihydrochloride 1a (1.00 g, 4.08 mmol) and TEA (0.41 g, 4.06 mmol) in CH₂Cl₂ (20 mL). The mixture was stirred for 3 h at rt and concentrated in vacuo. Concentrated HCl (7 mL) was added to the residue and the mixture was allowed to stand for 6 d at rt. After removal of the solvent under reduced pressure, the residue was washed with EtOH and collected by filtration to give 19a (0.59 g, 67%). An analytical sample was obtained by washing with MeOH–EtOH as a white solid; mp >260°C (dec). IR (KBr) cm⁻¹ 1769, 1716. FAB-MS (positive) m/z: 421 (M+H)^+. ^1H-NMR (DMSO-d₆) δ: 1.51 (4H, s, butane H-2, H-3), 1.90–2.01 (8H, m, Pyr H-3, H-4), 3.06–3.09 (8H, m, Pyr H-2, H-5), 3.42–3.54 (4H, m, CH₂-Pyr), 3.64 (4H, br, butane H-1, H-4), 4.62–4.63 (2H, m, Hyd H-5), 8.37 (2H, br, Hyd H-1), 10.73 (2H, br, NH^+). ^13C-NMR (DMSO-d₆) δ: 22.5 (Pyr C-3, C-4), 24.3 (btae C-2, C-3), 37.4 (butane C-1, C-4), 52.9, 54.0 (Pyr C-2, C-5), 53.5 (Hyd C-5), 55.0 (CH₂-Pyr), 156.3 (Hyd C-2), 170.9 (Hyd C-4). Anal. Calc. for C₂₂H₃₈Cl₂N₆O₄·0.5H₂O: C, 49.81; H, 7.41; N, 15.84. Found: C, 50.49; H, 7.69; N, 14.74.

3.3'-((Octane-1,8-diyl)bis(5-(pyrrolidin-1-ylmethyl)-imidazolidine-2,4-dione) Dihydrochloride (21a)) This compound was obtained from the reaction of 1a and 1,8-diisocyanoctane by a method similar to that for 19a as a white solid. An analytical sample was obtained by recrystallization from MeCN–MeOH; a white solid; mp 177–182°C (dec). IR (KBr) cm⁻¹ 1766, 1706. FAB-MS (positive) m/z: 477 (M+H)^+. ^1H-NMR (DMSO-d₆) δ: 1.24 (8H, brs, octane H-3–H-6), 1.50 (4H, ddd, J=14.0, 7.0, 7.0 Hz, octane H-2, H-5), 1.88–1.91 (4H, m, Pyr H-3, H-4), 2.02–2.04 (4H, m, Pyr H-3, H-4), 2.93–3.07 (4H, m, Pyr H-2, H-5), 3.34 (4H, t, J=7.0 Hz, octane H-1, H-8), 8.45 (2H, br, Hyd H-1), 11.07 (2H, br, NH^+). ^13C-NMR (DMSO-d₆) δ: 22.4 (Pyr C-3 or C-4), 22.6 (Pyr C-4 or C-3), 25.2 (octane C-2, C-3), 38.6 (butane C-1, C-4), 52.9, (Hyd C-5), 54.1 (CH₂-Pyr), 57.9 (Pip C-2, C-6), 158.0 (Hyd C-2), 172.4 (Hyd C-4). Anal. Calc. for C₂₉H₇₁Cl₂N₂O₇·0.8H₂O: C, 51.11; H, 7.79; N, 14.90. Found: C, 51.15; H, 7.66; N, 15.00.

3.3'-((Octane-1,8-diyl)bis(5-(piperidin-1-ylmethyl)-imidazolidine-2,4-dione) Dihydrochloride (21b)) This compound was obtained from the reaction of 1b and 1,8-diisocyanoctane by a method similar to that for 19a as a white solid. An analytical sample was obtained by recrystallization from EtOH; a white solid; mp >200°C (dec). IR (KBr) cm⁻¹ 1782, 1709. FAB-MS (positive) m/z: 505 (M+H)^+. ^1H-NMR (DMSO-d₆) δ: 1.17–1.24 (8H, m, octane H-3–H-6), 1.33–1.41 (2H, m, Pip H-4), 1.49 (4H, ddd, J=14.0, 7.0, 7.0 Hz, octane H-2, H-5), 1.70 (2H, d, J=13.5Hz, Pip H-4), 1.78–1.87 (8H, m, Pip H-3, H-5), 2.90–3.09 (4H, m, Pip H₂-2, H₂-6), 3.30–3.38 (6H, m, octane H-1, H-8+CHH-Pip), 3.40–3.43 (4H, m, CHH-Pip+Pip H₂-2 or H₂-6), 3.61 (2H, d, J=11.5Hz, Pip H₂-6 or H₂-2), 4.78 (2H, d, J=9.51Hz, Hyd H-5), 8.57 (2H, brs, brd, Hyd H-1), 10.73 (2H, brs, NH^+). ^13C-NMR (DMSO-d₆) δ: 21.0 (Pip C-4), 22.0 (Pip C-3 or C-5), 22.2 (Pip C-5 or C-3), 25.8 (octane C-3, C-6), 27.2 (octane C-2, C-7), 28.2 (octane C-4, C-5), 38.0 (octane C-1, C-8), 53.1 (Pyr C-2 or C-5), 53.5 (Hyd C-5), 53.9 (Pip C-5 or C-2), 55.2 (CH₂-Pyr), 156.2 (Hyd C-2), 170.8 (Hyd C-4). Anal. Calc. for C₂₅H₆₁Cl₂N₂O₇·0.8H₂O: C, 51.11; H, 7.79; N, 14.90. Found: C, 51.15; H, 7.66; N, 15.00.

3.3'-((Hexane-1,6-diyl)bis(5-(pyrrolidin-1-ylmethyl)-imidazolidine-2,4-dione) Dihydrochloride (20a)) This compound was obtained from the reaction of 1a and 1,6-disiocyanohexane by a method similar to that for 19a as a white solid. An analytical sample was obtained by recrystallization from EtOH–MeOH (1:1); a white solid; mp >210°C (dec).
imidazolidine-2,4-dione) Dihydrochloride (22d). This compound was obtained from the reaction of 1b and 1,12-diisocyanate by a method similar to that for 19a as a white solid. An analytical sample was obtained by recrystallization from EtOH; a white solid; mp 160–163°C. IR (KBr) cm⁻¹: 3429, 2926, 1783, 1722. FAB-MS (positive) m/z: 561 (M+H)+. 1H-NMR (DMSO-d₆) δ: 1.23 (16H, brs, dodecane H-3–H-10), 1.36–1.38 (2H, m, Pip H-4), 1.49 (4H, t, J = 7.0 Hz, Hyd H-2, H-11), 1.62–1.72 (2H, m, Pip H-4), 1.80–1.87 (8H, m, Pip H-3, H-5), 2.91–3.01 (4H, m, Pip H-2, H-6), 3.31–3.38 (8H, m, dodecane H-1, H-12); 1H-NMR (CDCl₃) δ: 21.1 (Pip C-4), 22.2 (Pip C-3 or C-5), 22.5 (Pip C-5), 25.9 (dodecane C-3–C-10), 27.2 (dodecane C-2–C-11), 28.4, 28.7, 28.8 (dodecane C-4–C-9), 38.0 (dodecane C-1, C-12), 51.7 (Pip C-2 or C-6), 52.0 (Hyd C-5), 53.4 (Pip C-6 or C-2), 58.0 (CH₂-Pyr), 156.1 (Hyd C-2), 171.0 (Hyd C-4). Anal. Calcd for C₄₉H₃₄Cl₄N₆O₄·0.3H₂O: C, 55.03; H, 8.35; N, 13.80. Found: C, 55.03; H, 8.35; N, 13.80.

3,3′-(Dodecane-1,12-diyl)bis(5-(piperidin-1-ylmethyl)imidazolidine-2,4-dione) Dihydrochloride (22b): This compound was obtained from the reaction of 1b and 1,12-diisocyanatododecane by a method similar to that for 19a as a white solid. An analytical sample was obtained by recrystallization from EtOH; a white solid; mp 160–163°C. IR (KBr) cm⁻¹: 3429, 2926, 1783, 1722. FAB-MS (positive) m/z: 561 (M+H)+. 1H-NMR (DMSO-d₆) δ: 1.23 (16H, brs, dodecane H-3–H-10), 1.36–1.38 (2H, m, Pip H-4), 1.49 (4H, t, J = 7.0 Hz, Hyd H-2, H-11), 1.62–1.72 (2H, m, Pip H-4), 1.80–1.87 (8H, m, Pip H-3, H-5), 2.91–3.01 (4H, m, Pip H-2, H-6), 3.31–3.38 (8H, m, dodecane H-1, H-12); 1H-NMR (CDCl₃) δ: 21.1 (Pip C-4), 22.2 (Pip C-3 or C-5), 22.5 (Pip C-5), 25.9 (dodecane C-3–C-10), 27.2 (dodecane C-2–C-11), 28.4, 28.7, 28.8 (dodecane C-4–C-9), 38.0 (dodecane C-1, C-12), 51.7 (Pip C-2 or C-6), 52.0 (Hyd C-5), 53.4 (Pip C-6 or C-2), 58.0 (CH₂-Pyr), 156.1 (Hyd C-2), 171.0 (Hyd C-4). Anal. Calcd for C₄₉H₃₄Cl₄N₆O₄·0.3H₂O: C, 55.03; H, 8.35; N, 13.80. Found: C, 55.03; H, 8.35; N, 13.80.

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Regarding small molecule antagonists of glycosaminoglycan sulfates, it has been demonstrated that the identification of small molecule antagonists against such glycosaminoglycans may lead to the development of new pharmacological agents to treat infectious diseases that involve a glycosaminoglycan sulfate binding stage (for example, see following reference). Schuksz M., Fuster M. M., Brown J. R., Crawford B. E., Ditto D. P., Lawrence R., Glass C. A., Wang L., Tor Y., Esko J. D., Proc. Natl. Acad. Sci. U.S.A., 105, 13075–13080 (2008).