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NOVEL AROMATIC UREA DERIVATIVES WITH DNA-BINDING ABILITY

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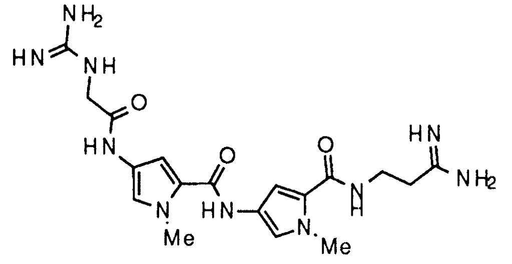
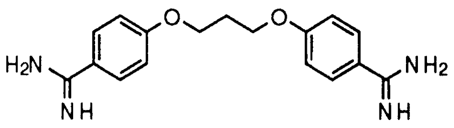
Several aromatic urea derivatives were designed and synthesized as DNA-targeting agents. *N,N'*-Dimethyl-*N,N'*-bis[(4-amidylphenyl)aminocarbonyl]-2,6-diaminopyridine (**1**) and 1,3-bis[5-(glycylamino)pyrid-2-yl]urea (**3**) showed remarkable DNA-binding abilities as determined by ultrafiltration assay using calf thymus DNA, their potencies being equal to and half that of netropsin, respectively. Compound **1** inhibited the proliferation of both L1210 cells and KB cells with similar IC_{50} values to netropsin.

KEY WORDS aromatic urea; netropsin; ultrafiltration assay; calf thymus DNA

DNA-targeting agents may have as much clinical potential as conventional protein-targeting agents.[1)] One significant class of compounds that specifically recognize DNA structures is the minor groove binders, such as netropsin / distamycin,[2)] propamidine,[3)] and so on. Structurally, these compounds consist of two terminal basic functions, (e.g. amidyl or guanidyl groups) connected to a spacer moiety (e.g. heterocyclic amide structure or ether structure) around 15 Å in length.



netropsin



propamidine

Previously, we reported unique structural properties of aromatic amides and ureas related to *N*-methylation.[4)] For example, *N,N'*-dimethyl-*N,N'*-diphenylurea has an (*E*)-conformational preference, while *N,N'*- diphenylurea always exists in a linear (*Z*)-conformation.[4b)] The conformational alteration upon *N*-methylation often affects the biological properties. Furthermore, the urea group has multiple hydrogen bonding sites which make possible specific recognition of target molecules or the formation of supramolecules. We have therefore designed and synthesized several novel DNA-binding compounds, which consist of symmetrical aromatic urea molecules having basic functions on both ends, i.e., **1**, **2**, **3**, and **4** (Fig. 1).[5)]

The DNA-binding ability of these urea derivatives (**1** – **4**) was evaluated by an ultrafiltration method[6)] using calf thymus DNA. Briefly, a test compound (100 μM) was mixed with calf thymus DNA (0.5 mM as base pairs) in 1.0 TE buffer [10 mM Tris·HCl (pH 8) – 1 mM EDTA containing 0.15 M NaCl and 10% DMSO]. The mixture was heated to 90-95°C for 60 s (to denature the DNA) and allowed to stand at room temperature overnight (for annealing). The mixture was ultrafiltered

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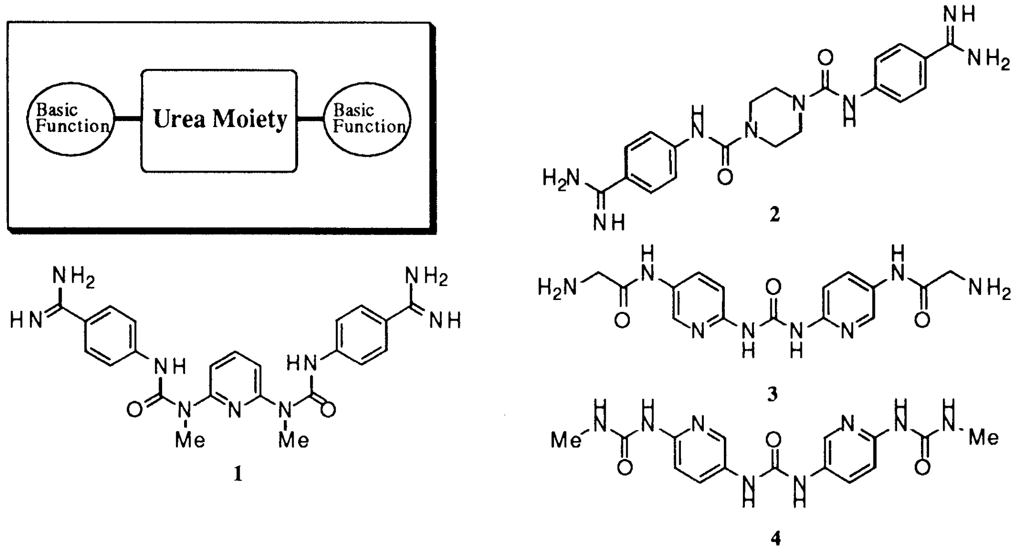

Fig. 1. Novel DNA-Binding Aromatic Urea Derivatives

using a Centricut W-10 UF tube (Kurabou Co.) at 20°C, then the content of the test compound in the filtrate was determined by UV-absorption measurement ($\mathrm{Abs.}_{\mathrm{DNA}^+}$) and this value was taken as the concentration of free urea derivative ($[\mathrm{urea}]_{\mathrm{DNA}^+}$). The same procedure was carried out with a solution of the test compound in the absence of DNA as the control ($\mathrm{Abs.}_{\mathrm{DNA}^-}$ and $[\mathrm{urea}]_{\mathrm{DNA}^-}$). The DNA-binding ability of the test compound was defined by the following equation (Eq.1) and the results are shown in Table 1.

$$\text{DNA binding ability (\%)} = \left(1 - \frac{[\mathrm{urea}]_{\mathrm{DNA}^+}}{[\mathrm{urea}]_{\mathrm{DNA}^-}}\right) \times 100 = \left(1 - \frac{\mathrm{Abs.}_{\mathrm{DNA}^+}}{\mathrm{Abs.}_{\mathrm{DNA}^-}}\right) \times 100 \qquad \text{(Eq.1)}$$

Compounds **1**, **2**, and **3** showed 72%, 25%, and 51% DNA-binding ability, respectively, while that of netropsin is 70%. Compound **4** did not show DNA-binding ability under these conditions. When the concentration of DNA was varied in the range from 125 μM to 1000 μM as base pairs, the UV-absorption of the filtrate gradually decreased. Scatchard analyses were done for **1**, **3** and netropsin. Although linearity was rather poor for compound **3** and netropsin, the DNA-binding constant (Ka) and the number of binding sites per base pair (n) under the assay conditions were calculated as $Ka = 7.3 \times 10^3\ \mathrm{M}^{-1}$, $n = 0.4$ for **1**, $Ka = 3.6 \times 10^3\ \mathrm{M}^{-1}$, $n = 0.5$ for **3**, and $Ka = 6.5 \times 10^3\ \mathrm{M}^{-1}$, $n = 0.5$ for netropsin (Table 1).

Thus, the ultrafiltration assay showed that the DNA-binding ability of compound **1** was similar to that of netropsin. The order of DNA-binding ability of the test compounds is **1** ≥ netropsin > **3** > **2** > **4**. From the NMR spectra of **1** – **4** and the related aromatic ureas reported previously, compounds **2** and **4** are considered to exist in extended structures with the linear urea conformations.[4,7)] On the other hand, compound **1** seems to have a folded structure as a result of the specific properties of the *N*-methylated urea moiety. Detailed study on the conformations is in progress.

The *in vitro* cytotoxic activity of selected compounds (**1**, **3**, and netropsin) was examined. Compound **1** showed comparable inhibitory effects on the proliferation of L1210 cells and KB cells (IC_{50} : 37 μg/mL and 154 μg/mL, respectively) to netropsin (IC_{50} : 97 μg / mL and 91 μg / mL, respectively). Compound **3** was almost inactive in this assay (IC_{50} > 500 μg / mL on both cell lines). There is a good correlation between the estimated DNA-binding ability from ultrafiltration assay, and the *in vitro* cytotoxic activity. Thus, DNA-binding assay by ultrafiltration is a simple and useful method for the preliminary evaluation of DNA-targeting drugs.

Table 1. DNA-Binding Abilities and Cytotoxic Effects of Aromatic Urea Compounds **1** – **4** and Netropsin

	λmax (nm)	Abs.$_{DNA^-}$[a]	Abs.$_{DNA^+}$[a]	DNA -binding ability [b] (%)	Scatchard analyses			Cytotoxic activity [c] (IC_{50}, μg/mL)	
					Binding constant (*Ka*) (10^3 M^{-1})	Binding sites (*n*) (per bp)	Correlation coefficient (*R*)	L1210	KB
Netropsin	299	0.307	0.093	70	6.5	0.5	0.69	97	91
1	286	0.429	0.121	72	7.3	0.4	0.83	37	154
2	284	0.474	0.358	25	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]
3	267	0.539	0.272	51	3.6	0.5	0.64	> 500	> 500
4	265	0.352	0.351	0	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]	N.T.[d]

a) The UV-absorption was measured at λmax after × 5 dilution with 1.0 TE buffer. b) DNA-binding ability was calculated by use of Eq.1. c) Murine lymphoma L1210 or human epidermoid carcinoma KB cells (1×10^4 cells / mL) were cultured in RPMI 1640 / 10% FBS containing the test compound for 72 h in a CO_2 incubator at 37°C. The viability was estimated by use of the MTT assay.[8)] d) N.T.: not tested.

In conclusion, the novel aromatic urea derivatives, **1**, **2**, and **3**, were designed and synthesized as candidate DNA-targeting agents. Compounds **1**, **2**, and **3** possess significant DNA-binding abilities. In particular, compound **1** has a remarkable DNA-binding constant (7.3×10^3 M^{-1}) and an *in vitro* cytotoxic activity comparable to that of netropsin. At present, the structural requirements of these urea derivatives for activity are unclear. We regard the basic amidyl and amino groups connected to the ends of the molecule as anchor functions, and the urea moiety as a spacer and a hydrogen-bonding site for recognizing DNA base pairs. Further research on the structure-activity relationship of these urea derivatives seems worthwhile.

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5) Compound **1**: mp >300°C (dec.); ^1H NMR (DMSO-d_6) δ 3.46 (s, 6H), 7.15 (d, 2H, *J* = 8.4 Hz), 7.72 (d, 4H, *J* = 8.8 Hz), 7.75 (d, 4H, *J* = 8.8 Hz), 7.91 (t, 1H, *J* = 8.4 Hz), 8.71 (s, 4H), 9.10 (s, 4H), 10.54 (s, 2H); HRMS (FAB) for $C_{23}H_{26}N_9O_2$ (M+H) calcd. 460.2209, found 460.2268. Compound **2**: mp >300°C; ^1H-NMR (DMSO-d_6) δ 3.58 (s, 8H), 7.72-7.79 (m, 8H), 8.85 (brs, 4H), 9.13 (brs, 4H), 9.21 (s, 2H); HRMS (FAB) for $C_{20}H_{25}N_8O_2$ (M+H) calcd. 409.2100, found 409.2138. Compound **3**: mp >300°C (dec.); ^1H NMR (DMSO-d_6) δ 3.81 (d, 4H, *J* = 5.4 Hz), 7.75 (d, 2H, *J* = 8.8 Hz), 7.98 (dd, 2H, *J* = 2.4 Hz, 8.8 Hz), 8.18 (brs, 6H), 8.56 (d, 2H, *J* = 2.4 Hz), 10.40 (s, 2H), 10.77 (s, 2H); HRMS (FAB) for $C_{15}H_{19}N_8O_3$ (M+H) calcd. 359.1580, found 359.1570. Compound **4**: mp >300°C; ^1H NMR (DMSO-d_6) δ 9.05 (s, 1H), 8.62 (s, 1H), 8.25 (d, 1H, *J* = 2.6 Hz), 7.81 (brs, 1H), 7.73 (dd, 1H, *J* = 2.8 Hz, 9.0 Hz), 7.29 (d, 1H, *J* = 9 Hz), 2.71 (d, 3H, *J* = 4.4 Hz); HRMS (FAB) for $C_{15}H_{19}N_8O_3$ (M+H) calcd. 359.1580, found 359.1585.
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