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TWO-RING DNA MINOR-GROOVE BINDERS CONSISTING OF READILY AVAILABLE, DI-SUBSTITUTED BENZENE DERIVATIVES

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Abstract: DNA minor-groove binders consisting of two disubstituted benzene rings were prepared. Two ligands showed sequence selectivities higher than some of the best known minor-groove binders containing six-membered rings. Reversing the orientation of amide linkages in one ligand leads to another one with relaxed binding to AT sequences and greatly increased affinity toward GC sequences. © 1997 Elsevier Science Ltd.

INTRODUCTION

During the past decade, impressive progress has been made in designing DNA minor-groove recognition molecules.[1–4] Using the naturally occurring antibiotics distamycin and netropsin as paradigms, numerous minor-groove binders have been designed and synthesized. Until recently, the majority of the known minor-groove binders prefer AT rich sequences. Since the discovery of a new structural motif in which two distamycin molecules are simultaneously located in the minor groove,[5–7] ensuing results[8–14] reported during the last three years on GC and mixed sequence preference of imidazole-containing distamycin analogs have opened up the exciting possibility of recognizing a large number of these previously elusive sequences. Most of the previously reported minor-groove binders are polyamides using five-membered aromatic rings, such as N-methylpyrrole and N-methylimidazole, as building blocks. There are few examples of other types of minor-groove binders.[15,16] Except for six-membered heterocycles[17] that usually show good affinities but poor sequence-selectivities to double helical DNAs, no systematic efforts have been made to explore new types of sequence-specific minor- and major-groove binders based on readily available building blocks such as benzene, pyridine, pyrimidine and other six-membered aromatic rings. In addition to polyamides or short peptides, reports on using other types of linkages, such as urea and carbamate, in designing DNA groove binders are also rare. The discovery of new types of DNA binders should provide information on the broader picture of the molecular recognition between double helical DNA and small molecules, which parallels the efforts in designing and/or selecting DNA-binding proteins with novel sequence specificities,[18–22] and more importantly, may provide compounds with novel properties such as better bioavailability and higher selectivities toward in vivo targets.

We would like to report here one of our preliminary results from a systematic approach of designing DNA minor-groove binders based on simple building blocks and highly efficient chemistry. The ligands examined showed significantly different affinities and sequence-selectivities toward double helical DNA targets. These ligands offer several advantages over previously designed minor-groove binders consisting of five-membered rings: (1) unlike minor-groove binders consisting of five-membered rings such as pyrrole and imidazole derivatives, whose shapes (curvatures) can not be changed because there is only one possible substitution pattern for a disubstituted five-membered ring, the design described here offers the possibility of generating ligands of various shapes by combining various disubstituted six-membered rings; (2) for the type of ligands described here,

the building blocks can be connected in a number of ways by amide bonds (or other kind of linkages) of different orientations, offering the opportunity for probing the effects of subtle structural changes on binding affinities and sequence-selectivities or sequence-specificities. Ligands consisting of five-membered rings, on the other hand, are much harder to manipulate due to their susceptibility to oxidation and offer much less structural diversity; finally, six-membered aromatic rings contain a much higher degree of functional group diversity than five-membered rings. For example, ligands with new sequence selectivities may be resulted by replacing the benzene rings of a ligand with pyridine, pyrimidine and other six-membered aromatic rings, which will introduced more hydrogen bonding acceptor or donor sites into a ligand.

Various benzene derivatives have been selected as our initial choice of building blocks. Benzene derivatives are readily available, can be modified easily, and are stable under most experimental conditions. The designed two-ring ligands can not only be compared to previously reported minor-groove binders containing six-membered rings, but may also provide a simple platform for constructing future combinatorial libraries. Our design is shown below (Fig. 1).

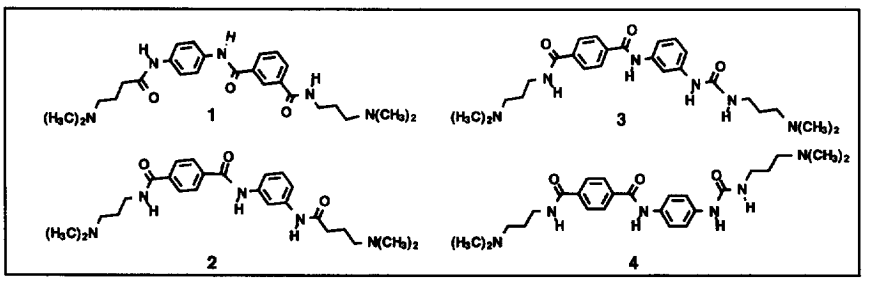

Figure 1. Design of two-ring minor-groove binders. As shown, Ligands **1**, **2**, and **3** can adopt the crescent shape that may result in their fitting snugly into the minor groove of duplex DNA. Ligand **4**, on the other hand, may not be able to adopt a similar conformation.

RESULTS AND DISCUSSION

Four potential two-ring minor-groove binders were designed and synthesized by coupling isophthalic and terephthalic acids with derivatives of *m*- and *p*-phenylenediamines. These four compounds were chosen to study (1) the relationship between the curvature of a two-ring ligand and its binding affinities and sequence selectivities (ligands **3** and **4** in Fig. 1); and (2) the effects of different patterns of hydrogen bond donor and acceptor distribution on binding affinity and sequence selectivity.

The C_{50} values obtained from the well established ethidium displacement assay[23,24] offer a semiquantitative measurement of the structural effects of this class of compounds on DNA binding. These values can also be translated into apparent binding constants based on reported methods.[25] The results of the assays are shown in Table 1.

Viscometric analysis of DNA solutions in the presence of the four ligands, when presented (0.1 μM, 0.5 μM, 1 μM, 2 μM) in the DNA solutions, indicated no significant change in viscosity (η/η_0 = 1.01-1.03, η_0 and η are the reduced specific viscosities of DNA solution alone and in the presence of the ligand, respectively). Groove-binders cause a minimum change in viscosity ($\eta/\eta_0 \approx 1$) because they do not cause significant helix distortion. These four ligands are therefore groove binders, not intercalators.

Table 1. Ligand Binding[a] to Calf Thymus DNA, T4 Caliphage DNA, poly [d(AT)], and poly[d(GC)]

Compd	Calf thymus DNA	T4 DNA	poly(dA-dT)	poly(dG-dC)
Hoechst 33258	-	-	0.20	1.4
SN 6999	-	-	0.17	2.8
Berenil	-	-	0.90	5.7
1	47.3 ± 0.2	35.6 ± 0.2	28.4 ± 1.3	55.6 ± 0.9
2	31.9 ± 0.8	27.1 ± 0.4	1.9 ± 0.0	142.2 ± 1.9
3	43.5 ± 3.0	28.6 ± 0.5	4.2 ± 0.1	221.8 ± 1.0
4	44.8 ± 0.6	35.2 ± 0.7	23.5 ± 1.6	77.3 ± 1.8

[a]C_{50} (μM) values. [b]values from ref.17.

As shown in Figure 1, although ligands **1, 2** and **3** may all adopt a crescent conformation that may help their fitting into the minor groove of B-form DNA, they show quite different affinities and sequence selectivities toward the DNAs assayed. Ligands **1** and **2**, with one *meta*-disubstituted ring and one *para*-disubstitued ring in their molecules, can adopt a similar shape but have completely reversed orientation in their amide linkages. This difference in the patterns of hydrogen bond donors and acceptors in **1** and **2** is very likely the reason for their different affinities and sequence-selectivities toward duplex DNAs. As shown in Figure 1, ligand **2** can adopt the crescent conformation with all three amide protons pointing toward the floor of the DNA minor-groove, which favors the recognition of AT base pairs by forming hydrogen bonds with O-2 of thymine and N-3 of adenine. On the other hand, to adopt the similar crescent shape, all three carbonyl groups of ligand **1** have to be pointed to the floor of the DNA minor-groove. As shown in Table 1, ligand **2** shows a binding affinity to AT sequences that is 14 times stronger than ligand **1**. More spectacular is the much higher sequence selectivity shown by ligand **2** as compared to **1**: ligand **2** binds to AT sequences ~70 times more strongly than to GC sequences, while the binding of ligand **1** to AT sequences is significantly relaxed, which is only 1.9 time stronger than to GC sequences. One interesting phenomenon is that although the affinity of ligand **1** toward AT sequences is much lower than that of **2**, its affinity to GC sequences is obviously increased as compared to ligand **2**, another evidence supporting the binding models discussed above. The carbonyl groups of **1** can form hydrogen bonds with the protons from the C-2 amino groups of guanine on the floor of the minor-groove of GC-rich sequences. Unlike previously reported minor-groove binders with only one orientations of the amide linkages, our design makes it possible to probe different patterns of hydrogen bond acceptors and donors. As shown by **1** and **2**, changing the orientations of the backbones of minor-groove binders greatly changed their binding behavior, which may offer a new strategy for designing minor-groove binders.

Ligand **3** is similar to ligand **2** in its shape and number of protons that can act as hydrogen bond donors. The differences between **3** and **2** are that **3** has one more proton from the urea group and the dimethylaminopropyl side chain is therefore one methylene group longer. Surprisingly, as compared to **2**, **3** binds to AT sequences more weakly, and its selectivity between AT and GC sequences is also lower. Whether the weakened binding and selectivity are because of the extra hydrogen bond donor or because of the extra length of the side chain in **3** remains to be seen by further studies. Ligand **3** also binds to GC sequences more weakly than **2**, which may be explained by the additional steric hindrance introduced by the extra proton of the urea group and the C-2 amino group of guanine.

That the shape (curvature) of a ligand is important for binding is demonstrated by comparing the binding results of ligands **3** and **4**. Ligand **4** is an isomer of **3** and contains as many amide protons (hydrogen bond donors) and other groups as **3** does. However, the two *para*-substituted benzene rings may prevent **4** from adopting the conformation that is needed for effective binding, which may be the reason for its poor affinity toward AT sequences compared to ligands **2** and **3**. The sequence-selectivity of **4** is also much worse compared to ligands **2** and **3**.

Ligands **2** and **3** bind to AT sequences more weakly than the extensively studied minor-groove binders, such as Hoechst 33258, SN 6999, and berenil that contain six-membered rings. However, both **2** and **3** show very high sequence-selectivities: ligand **2** binds to AT sequences ~70 times more strongly than to GC sequences and the same ratio for ligand **3** is about 50. As shown in Table 1, these values are much higher than the values[17] of Hoechst 33258 (7 times), SN 6999 (4 times), and berenil (6 times). Since calf thymus DNA contains random sequences and therefore fewer AT sites than poly(dA-dT), the selectivities of **2** and **3** are further demonstrated by their much weaker binding to calf thymus DNA compared to poly(dA-dT), which indicates a more stringent sequence requirement of the two ligands than previously reported ligands with six-membered rings. The high sequence-selectivities of **2** and **3** demonstrate the superiority of our design to previous approaches. Most of the reported approaches involving six-membered aromatic rings addressed the affinity problems quite well by attaching charged groups, such as guanidinium, amidinium and quaternary ammonium groups, to designed ligands, while struggling to match the sequence-selectivities of the natural products netropsin and distamysin.[17] The ligands reported here can be easily modified to incorporate any of the above charged groups, which may result in ligands of higher affinities and at the same, high sequence-selectivities.

As shown in Table 1, the comparable binding affinities (C_{50} values) of these ligands for T4 caliphage DNA and calf thymus DNA suggest their minor-groove selectivity, since the major groove of T4 caliphage DNA is blocked by α-glycosylation of the 5-(hydroxymethyl)cytidine residue.[26]

The target DNA sequences of the above ligands are being studies by footprinting experiments. Techniques such as 2-D NMR, calorimetry, CD and UV melting are also being employed to study the binding mechanisms of these new minor groove binders.

EXPERIMENTAL SECTION

Synthesis of ligands

The ligands were synthesized by acylating the amide- or urea-containing anilines with the monobenzyl ester of isophthalic acid or terephthalic acid, removing the benzyl group through hydrogenation, and coupling the monoamide acid with *N,N*-dimethyl-1,3-propanediamine. All of the coupling reactions were carried out in DMF in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 1-hydroxybenzotriazole (HOBt), with yield >90% for each coupling step. The urea-containing anilines were obtained by first treating the corresponding nitrophenyl isocyanates with *N,N*-dimethyl-1,3-propanediamine, followed by reducing the nitro groups by catalytic hydrogenation. The amide-containing amines were prepared by acylation of *m*- or *p*-nitroanilines with 4-dimethylaminobutyric acid, followed by the reduction of the nitro groups through hydrogenation. The final products were all easily purified by precipitation with the addition of water. All new compounds were successfully characterized by ^{1}H and ^{13}C NMR, and mass spectrometry.

DNA binding assays

A buffer solution (pH 6.0) containing Na_2HPO_4-HCl (50 mM), NaCl (25 mM), EDTA(1%) was prepared with distilled deionized water. All DNA solutions for the ethidium displacement assays were made in this buffer. Calf thymus DNA, poly[d(GC)], and poly[d(AT)] were purchased from Pharmacia. T4 caliphage DNA was purchase from Sigma. The stock solutions of the ligands were made in DMSO. The amount of DMSO introduced into the assaying solution was shown not to affect the results by a control experiment.

DNA binding studies were based on the well established and highly sensitive ethidium displacement assay.[23–25] Fluorescence was measured on a Perkin-Elmer Luminescence Spectrometer (LS50B) at room temperature. The binding was measured as the concentration (C_{50} value) of the ligand leading to a 50% reduction in the fluorescence intensity of bound ethidium (excitation at 546 nm, emission at 600 nm) at pH 6.0. The assay was performed by the addition of 10 μL of DNA (A_{260} = 2.0) solution to 2 mL of ethidium bromide (1.26 μM) buffer, and the maximum fluorescence was measured at room temperature. A stock solution (~10 mM) of the ligand to be assayed was then added as microliter aliquots (5 μL) into the above buffer, and the fluorescence was measured after each addition. If binding to DNA happened, the fluorescence readings would decrease after each addition of the stock solution. The addition was continued until a 50% reduction of fluorescence had occurred. If the 10 mM stock solution of ligands lowered the percent fluorescence too quickly, the solution can be further diluted to 1 mM before the titration and vice versa. Readings were taken at least three times for each ligand concentration.

Viscometric titrations

Viscometric titration of a calf thymus DNA solution (1 μM/base pair. Prepared by adding 5 μL of stock solution (A_{260} = 2.0) to 1 mL of pH 6.0 buffer) in the presence and absence of four different concentrations (0.5 μM, 1 μM, 1.5 μM and 2 μM) of a ligand was carried out on a Brookfield Model DV-I+ viscometer at room temperature.

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