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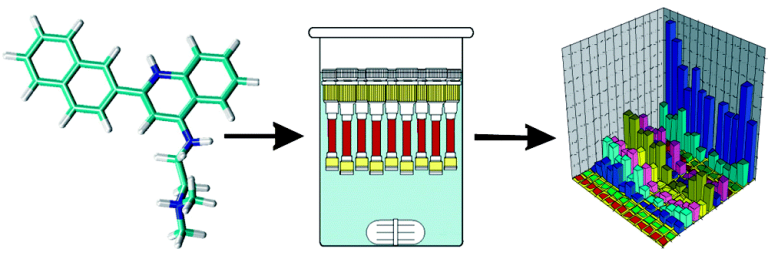
J. Am. Chem. Soc., **2003**, 125 (24), 7272-7283• DOI: 10.1021/ja034181r • Publication Date (Web): 21 May 2003



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Published on Web 05/21/2003

Triplex Selective 2-(2-Naphthyl)quinoline Compounds: Origins of Affinity and New Design Principles

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Abstract: A novel competition dialysis assay was used to investigate the structural selectivity of a series of substituted 2-(2-naphthyl)quinoline compounds designed to target triplex DNA. The interaction of 14 compounds with 13 different nucleic acid sequences and structures was studied. A striking selectivity for the triplex structure poly dA:[poly dT]$_2$ was found for the majority of compounds studied. Quantitative analysis of the competition dialysis binding data using newly developed metrics revealed that these compounds are among the most selective triplex-binding agents synthesized to date. A quantitative structure-affinity relationship (QSAR) was derived using triplex binding data for all 14 compounds used in these studies. The QSAR revealed that the primary favorable determinant of triplex binding free energy is the solvent accessible surface area. Triplex binding affinity is negatively correlated with compound electron affinity and the number of hydrogen bond donors. The QSAR provides guidelines for the design of improved triplex-binding agents.

Introduction

Triplex DNA (Figure 1) is of intense interest as a target for small molecule therapeutic agents.[1−6] Interest in triplex DNA as a target lies in two major areas. First, certain polypurine sequences within genomic DNA can form an intramolecular triplex structure called H-DNA.[2] Although the precise biological role of H-DNA is not definitively known, it could function as a regulatory signal for the control of gene expression. If so, small molecules that could selectively recognize H-DNA might be useful therapeutic agents as modulators of gene expression. Second, there is interest in the use of triplex forming oligonucleotides (TFOs) in the "antigene" therapeutic strategy.[7−12]

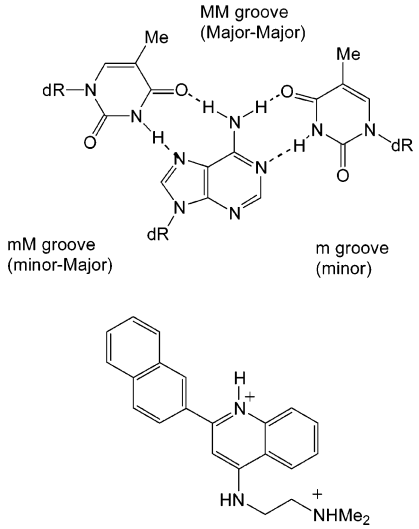

Figure 1. Structures of a dT-dA-dT triplet (top) and **LS-8** (bottom).

In this strategy, TFOs can be used to selectively target precise sequences within the genome by formation of an intermolecular triplex. Such triplex formation can interfere with biological function. Small molecules that selectively bind to such triplex

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10.1021/ja034181r CCC: $25.00

structures may serve as "enhancers" that stabilize the TFO-duplex complex, amplifying their biological effects.

Ethidum bromide, a classic intercalator, was found to bind to triplex structures, but with a lower affinity than for duplex DNA.[13,14] The Helene group first attempted the rational design of triplex-selective intercalating agents with BePI (7*H* -8 -methylbenzo [*e*] pyrido[4,3-*b*]indole),[5] and subsequently reported improved selectivity with new compounds based on that structure.[15,16] Another strategy for the design of triplex selective agents was based on naphthylquinoline compounds.[17] Three essential elements were considered in that design strategy. First, compounds should be cations to complement the high negative charge density of the triple helix. Second, compounds should have aromatic surface areas, which can optimally stack on the crescent-shaped base triplet (Figure 1). Finally, compounds should have an unfused aromatic system with torsional flexibility, since the bases within the triplet are propeller twisted. The compound designated **LS-8** (Figure 1) fulfilled these design principles, and proved to be a highly selective triplex intercalator. Extensive studies with **LS-8** suggested that it binds to triplex DNA by intercalation.[17] Both the quinoline ring nitrogen atom ($pK_a = 7.1$) and the terminal amino group of the side chain ($pK_a > 8$) are protonated in the intercalation complex,[18] yielding a dication with a favorable polyelectrolyte contribution to the binding free energy. Molecular modeling studies[17] are consistent with intercalation of the quinoline portion of **LS-8** between base-pairs of the original duplex within the triplex, stacking of the 2-naphthyl substituent with bases of the third DNA strand, and location of the aminoalkyl side chain in the minor groove.

By using footprinting experiments[19−24] and T_m measurements[17,18,24] it was shown that quinolin-4-amines containing an aryl group at position 2 and an aminoalkyl moiety at the N^4 atom bind selectively to triplex DNA in the presence of the corresponding duplex DNA. Analogues of **LS-8** with a smaller aromatic substituent than the 2-naphthyl group showed a decreased triplex/duplex binding selectivity, apparently because the smaller ring system does not stack optimally with bases of the third strand of the triplex.

The interaction of 2-arylquinolin-4-amines with nucleic acids is strongly affected by the structure of the side chain at the N^4 atom of the quinoline. In general, binding is enhanced for derivatives containing a terminal amino function, such as Me_2N in **LS-8**, that is protonated under binding conditions. The interaction is also stronger for compounds with a single alkyl group attached to the N^4 atom of the quinoline in comparison to quinolin-4-amines substituted with two alkyl groups. The primary $R-N^4H$ function, as in **LS-8**, has low steric requirements which allow for efficient conjugation of the electron pair on the N^4 atom with the quinoline system, thereby increasing basicity of ring N1 atom of the quinoline.[18] By contrast, quinolin-4-amines substituted at N^4 with two alkyl groups (that is, containing a sterically hindered secondary R_2N^4 function) show a decreased conjugation and, concomitant decreased basicity of the quinoline N1 atom.

Limited studies conducted so far with substituted quinolin-4-amines suggested that additional structural features of the side chain, such as its length, bulkiness, hydrophobicity, and the presence of heteroatoms may play an important role in the interaction of these compounds with nucleic acids. These aspects were addressed in the design of new quinolines for this work (Figure 2). Studies of the nucleic acid binding of these compounds were facilitated by the use of a newly developed competition dialysis assay that allows for a quantitative comparison of ligand interactions with 13 different nucleic acid structures and sequences. The systematic studies reported here reveal specific compounds with improved triplex binding relative to the parent **LS-8**, and further allow for a detailed quantitative structure-affinity relationship to be derived.

The competition dialysis method is now firmly established as an important tool for rapidly screening ligand-nucleic acid interactions.[25−29] In the competition dialysis method, a test ligand of interest is dialyzed against an array of nucleic acid structures. At equilibrium, the free ligand concentration is identical for all structures, and the amount bound to each structure directly and quantitatively indicates the binding affinity. The preference of the ligand for a given nucleic acid structure is unambiguously identified. Competition dialysis offers distinct advantages over thermal denaturation and footprinting methods that have been widely used in studies of structural selectivity. First and foremost, a large number of different structures and sequences may be simultaneously compared in the competition dialysis experiment, in contrast to footprinting and thermal denaturation experiments where only duplex and one other structure (triplex, for example) are typically compared. Second, competition dialysis is firmly grounded in equilibrium thermodynamics, and measures binding directly. In contrast, interpretation of thermal denaturation curves is often difficult because of the complexities of the underlying statistical mechanical mechanisms involved in nucleic acid melting reactions.[30,31] In particular, ligand redistribution can produce complex, multiphasic melting curves that are not easily interpreted. Finally, the competition dialysis method is rapid compared to both thermal denaturation and footprinting studies, and is amenable to high sample throughput. While the existing competition dialysis method provides a firm, quantitative measure of ligand structural

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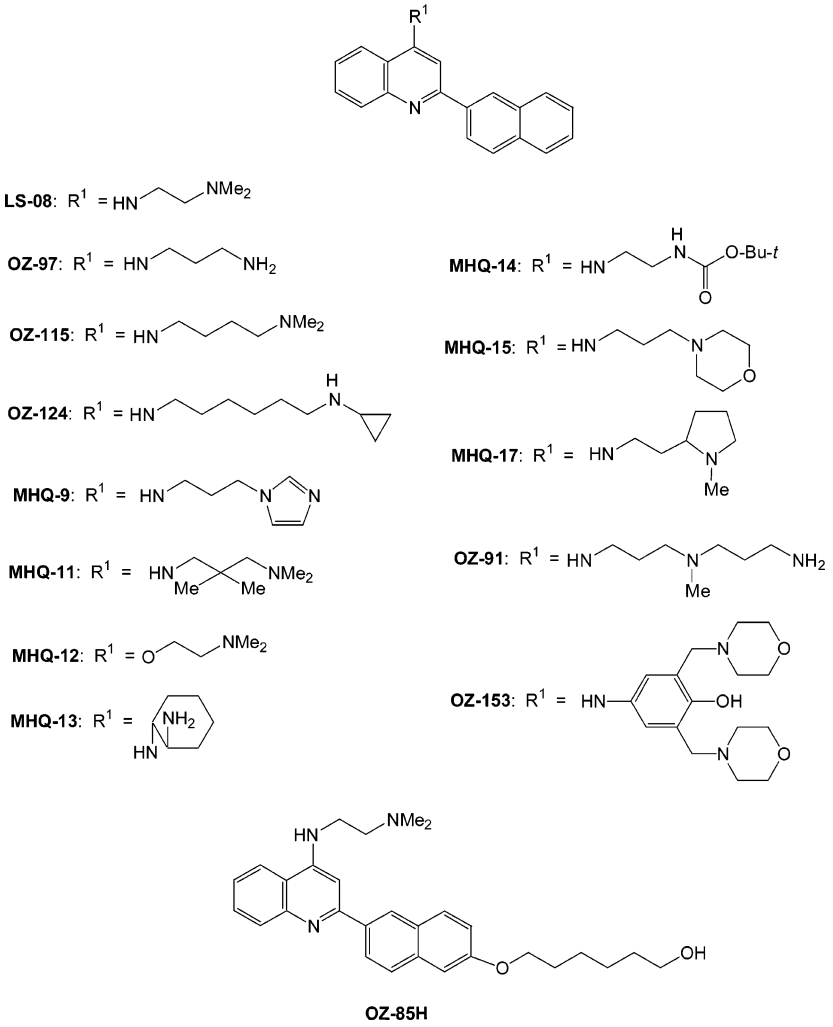

Figure 2. Structures of the naphthylquinoline compounds used in this investigation.

selectivity, we describe here new data analysis tools that make it an even more powerful and exacting measure of structural selectivity.

Results

Synthesis of Quinoline Ligands. Readily available 4-chloro-2-(2-naphthyl)quinoline[32] (**1**) was a starting material for the preparation of quinoline derivatives containing an unsubstituted 2-naphthyl group (Scheme 1). A nucleophilic displacement of chloride from **1** by the reaction with a primary amine in the presence of a catalytic amount of $SnCl_4$ furnished all **MHQ** quinolin-4-amines, **OZ-91**, and **OZ-97**. This one-step approach could not be used for the synthesis of **OZ-115** and **OZ-124** because the corresponding amines are not readily available. These quinoline derivatives were prepared from hydroxyalkyl-amino-substituted compounds **2,3** which were transformed into chlorides **4,5** by the reaction with thionyl chloride followed by treatment of **4,5** with an amine. In the synthesis of **OZ-153**, compound **1** was treated with 4-hydroxyaniline and the resultant phenol derivative **6** was subjected to a Mannich reaction with formaldehyde and morpholine. The 4- alkoxyquinoline **MHQ-12** was synthesized by alkylation of a sodium derivative of 4-hydroxy-2-(2-naphthyl)quinoline with 2-(dimethylamino)ethyl chloride (not shown).

The synthesis of **MHQ-13**, **OZ-91**, and **OZ-97** by the reaction of **1** with a diamine was conducted with a large excess of the diamine to decrease the formation of a bis-quinoline byproduct.

A different approach was used to synthesize quinoline **OZ-85H** that contains a hydroxyalkyl-functionalized naphthyl substituent (Scheme 2). Thus, a lithium 2-(dimethylamino)-ethylamide mediated cyclization [33−35] of a Schiff base **7** derived from 2-(trifluoromethyl)aniline and 6-methoxynaphthone furnished methoxynaphthyl-substituted quinoline **8**. Demethylation [36] of **8** by the reaction with BBr_3 followed by alkylation of the resultant naphthol **9** with 1-bromo-6-(*tert*-butyldimethylsiloxy)-hexane gave **10**. Compound **OZ-85H** was obtained by desilylation of **10**. Due to high efficiencies of the first transformations **7** → **8** → **9** it was not necessary to rigorously purify the

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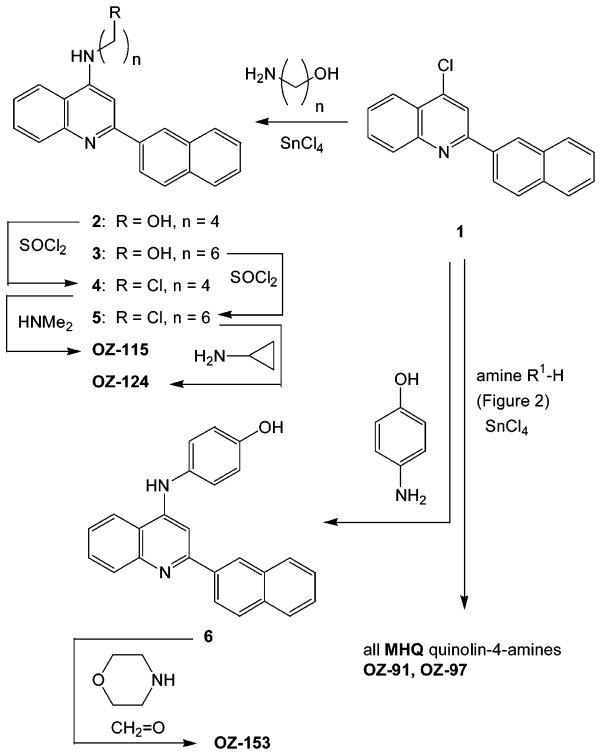
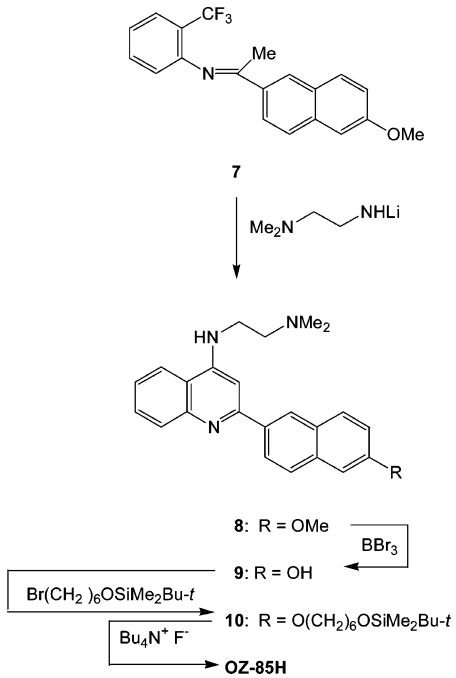
Scheme 1



Scheme 2



intermediate products **7**−**9**. On the other hand, the intermediate compound **10** and the final product **OZ-85H** were obtained in an analytically pure form and thoroughly characterized by spectral methods and elemental analysis. The structures of the remaining quinolines used in this study (Figure 2) were also fully consistent with the obtained analytical data.

DNA Binding Analysis. Figure 3A shows the results of a competition dialysis experiment using **LS-8**, the parent naphthylquinoline.[17] In the competition dialysis experiment, the different nucleic acid structures and sequences are dialyzed against a common free ligand solution. At equilibrium, more

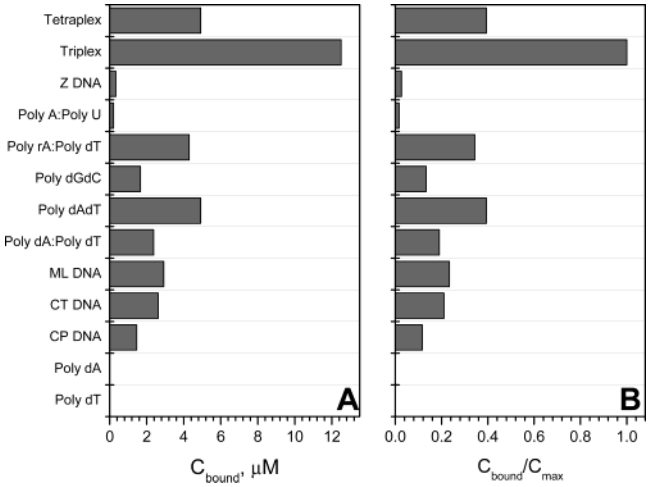


Figure 3. (A) Results of competition dialysis experiments using **LS-8**, the parent naphthylquinoline compound. (B) Normalized competition dialysis data, resulting from transformation of the data shown in (A) by dividing all C_{bound} values by C_{max}= 12.5 μM. The normalized data emphasizes the relative binding affinity for LS8 for the different nucleic acid structures and is used for calculation of the specificity sum.

Table 1. Nucleic Acid Samples Used in Competition Dialysis Experiments

conformation	nucleic acid	λ (nm)	ϵ ($M^{-1}cm^{-1}$)	monomeric unit
single-strand pyrimidine	poly dT	264	8520	nucleotide
single-strand purine	poly dA	257	8600	nucleotide
duplex DNA	*C. perfringens* (31%GC)	260	12476	base pair
	calf thymus (42%GC)	260	12824	base pair
	M. lysodeikticus (72%GC)	260	13846	base pair
	poly dA: poly dT	260	12000	base pair
	[poly (dAdT)]$_2$	262	13200	base pair
	[poly (dGdC)]$_2$	254	16800	base pair
duplex RNA	poly rA:poly rU	260	14280	base pair
DNA−RNA hybrid	poly rA: poly dT	260	12460	base pair
Z DNA	[Br-poly (dGdC)]$_2$	254	16060	base pair
triplex DNA	poly dA: (poly dT)$_2$	260	17200	triplet
tetraplex DNA	(5′T$_2$G$_{20}$T$_2$)$_4$	260	39267	tetrad

Key: λ, wavelength; ϵ, molar extinction coefficient at the wavelength λ, expressed in terms of the monomeric unit specified.

ligand accumulates in those solutions with preferred binding structures or sequences. Table 1 lists the nucleic acid structures used in this study. The data of Figure 3A show unequivocally that **LS-8** binds preferentially to the poly dA-(poly dT)$_2$ triplex structure. **LS-8** binds with lesser affinity to DNA duplex forms, to an RNA:DNA hybrid structure, and to a parallel-stranded tetraplex structure. **LS-8** shows little or no binding to single-stranded DNA, to RNA or to left-handed Z DNA. Figure 3B shows the normalized binding profile, in which the amount bound to each structure was normalized relative to the maximum amount bound to the triplex form. Figure 3B shows that the relative binding to the triplex structure is at least 2.5 times greater than to any other structure or sequence. The normalized affinity profile will be used later for a quantitative assessment of binding selectivity.

Figure 4 shows the comparative binding profiles for all 14 naphthylquinoline compounds designed and synthesized for this study. Figure 4 contains a wealth of quantitative data. What is

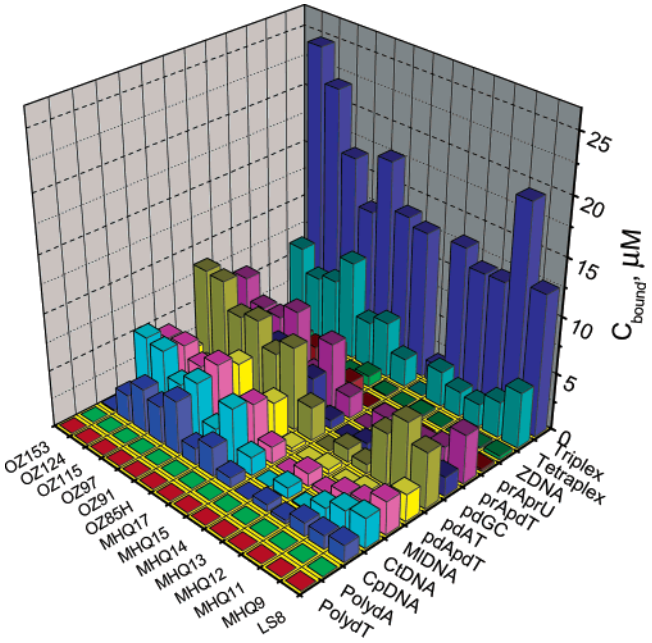


Figure 4. Comparative binding of 14 naphthylquinoline derivatives to 13 nucleic acid structures.

Table 2. Selected Binding Properties of Naphthylquinoline Compounds

compd	C_{bound}, μM	$K_{app}/10^5$, M^{-1}	$\Delta G_{Triplex}$	ΔG_{CTDNA}	SS	C_{max}/SS
LS8	12.5	2.00	−7.08	−6.09	3.06	4.09
MHQ9	19.4	3.48	−7.41	−5.98	1.97	9.83
MHQ11	11.9	1.88	−7.05	−5.68	2.11	5.64
MHQ12	11.8	1.87	−7.04	−4.18	1.32	8.93
MHQ13	13.2	2.14	−7.13	−5.49	1.77	7.49
MHQ14	1.7	0.23	−5.84	0	1.00	1.71
MHQ15	12.9	2.09	−7.11	−5.79	1.97	6.58
MHQ17	13.6	2.21	−7.14	−6.48	3.88	3.50
OZ85H	17.7	3.08	−7.34	−5.98	2.18	8.12
OZ91	12.5	2.00	−7.09	−6.52	5.33	2.35
OZ97	16.5	2.83	−7.29	−6.38	3.49	4.75
OZ115	21.8	4.10	−7.50	−6.63	3.14	6.95
OZ124	24.7	4.91	−7.61	−6.66	3.05	8.10
OZ153	0.9	0.12	−5.45	0	1.50	0.59

immediately apparent in Figure 4 is that all naphthylquinolines studied show preferential binding to the poly dA-(poly dT)$_2$ triplex structure. There are quantitative differences among these naphthylquinolines, which will be discussed in detail in later sections.

Apparent binding constants for each structure or sequence, K_{app}, may be calculated from competition dialysis data such as shown in Figures 3A and 4. [26] The simple relationship is $K_{app} = C_b/(C_f)(S_{total} - C_b)$, where C_b is the amount of ligand bound, C_f is the free ligand concentration and S_{total} is the total nucleic acid concentration. By virtue of the experimental design used in the competition dialysis experiment, $C_f = 1$ μM and $S_{total} = 75$ μM (expressed in terms of the monomeric unit of the nucleic acid, i.e., nucleotides, base pairs, triplets or tetrads). Table 2 shows the calculated K_{app} values for triplex binding, which range from 0.12×10^5 M^{-1} for **OZ-153** to 4.91×10^5 M^{-1} for **OZ-124**. We estimate, from replicate experiments, that K_{app} values contain errors in the range of 10−15%. Triplex binding free energies may be calculated by using the standard relationship $\Delta G_{Triplex} = -RT\ln K_{app}$ (Table 2).

To validate K_{app} values, a specrophotometric titration [37] study was done for the interaction of **LS-8** with triplex DNA. Upon

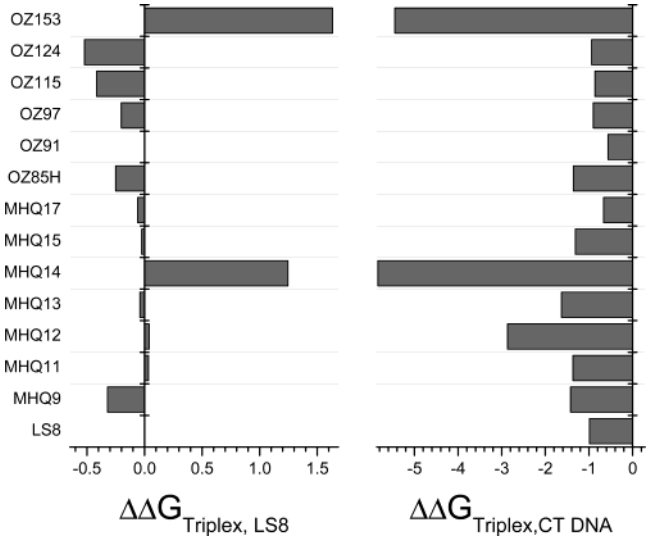


Figure 5. Comparative binding free energy difference plots. (A) The difference in triplex binding free energy ($\Delta\Delta G_{Triplex,LS8}$) relative to the binding of the parent compound LS8[25,26] for each of the naphthylquinoline derivatives. Negative value of $\Delta\Delta G_{Triplex,LS8}$ indicate *higher* triplex binding affinity relative to **LS-8**; positive values indicate *weaker* affinity. (B) The difference in triplex binding free energy ($\Delta\Delta G_{Triplex,CT\ DNA}$) relative to binding to duplex calf thymus DNA. All of the naphthylquinoline derivatives bind preferentially to the triplex structure over duplex calf thymus DNA. The magnitude of ($\Delta\Delta G_{Triplex,\ CT\ DNA}$) is a quantitative measure of the preference for triplex.

binding to triplex DNA, the absorbance spectrum of **LS-8** undergoes a red shift from a maximum near 325 nm for the free form to a maximum near 360 nm for the bound form (Figure S3A, Supporting Information). A binding isotherm was constructed by recording the absorbance of a fixed concentration of **LS-8** (5 μM) while varying the triplex concentration from 0.1 μM to 0.4 mM. Analysis of the binding isotherm [37] yielded a binding constant of 2.3 (±0.4) $\times 10^5$ M^{-1}. The value of $K_{app} = 2.0 \times 10^5$ M^{-1} obtained by competition dialysis (Table 2) is in excellent agreement with the more rigorously determined binding constant. The close agreement between K_{app} values and spectrophotometrically determined binding constants was previously demonstrated in our laboratory for the triplex binding of a cyanine dye [28] and for the binding of the indolocarbazole NB-506 to calf thymus DNA.[27]

Figure 5 shows comparative binding data for the compounds used in this study. Data are shown as differences in binding free energy relative to two different references. Figure 5A shows the difference in triplex binding free energy of each compound relative to the parent compound, **LS-8**. This difference is simply $\Delta\Delta G_{Triplex,\ LS8} = \Delta G_{Triplex} - (-7.08)$, where $\Delta G_{Triplex}$ values are listed in Table 2 and −7.08 is the binding free energy of **LS-8**. Figure 5A emphasizes those compounds with lesser or greater binding free energy relative to **LS-8**, with negative values signifying more favorable binding and positive values less favorable binding. The data of Figure 5A show that compounds **MHQ-9**, **OZ-85H**, **OZ-97**, **OZ-115**, and **OZ-124** all bind more tightly to triplex DNA than does **LS-8**. In contrast, **MHQ-14** and **OZ-153** bind less tightly.

Thermal denaturation experiments were done using selected compounds to confirm the trends shown in Table 2 and in Figure 5A (Figure S4, Supporting Information). Melting was studied

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for the poly dA:(poly dT)$_2$ triplex alone and in the presence of **LS-8**, **MHQ-9**, **MHQ-12**, **MHQ-14**, and **OZ-124**. Compounds were added at a molar ratio of 0.25 (mol compound/mol triplet), and ΔT_m was determined by difference relative to poly dA:(poly dT)$_2$ alone. In the absence of added ligand, the triplex melted at 47.0 °C, while the duplex melted at 76.3 °C. Figure S4 shows that the increase in triplex melting is linearly related to K_{app}, with $\Delta T_m = 18.1 + 4.5\ K_{app}/10^5$ ($R = 0.926$; $P = 0.023$). These data confirm the trends in triplex binding shown in Table 2 and in Figure 5A. Such confirmation is not unexpected, since we previously have directly correlated competition binding data with data from thermal denaturation studies, spectrophotometric binding studies, and enzymatic assays.[25−29]

Although $\Delta\Delta G_{Triplex,\ LS8}$ provides a quantitative measure of triplex affinity relative to the parent compound, it contains no information about selectivity toward the nucleic acid array used. To evaluate selectivity, the difference $\Delta\Delta G_{Triplex,\ CTDNA} = \Delta G_{Triplex} - \Delta G_{CTDNA}$ was calculated. This is the difference between the binding free energies of each naphthylquinoline for triplex and calf thymus DNA, where calf thymus DNA is taken to represent a standard duplex reference form. The magnitude of $\Delta\Delta G_{Triplex,\ LS8}$ provides a measure of the selectivity of triplex binding over duplex, with larger magnitudes indicating greater selectivity. Figure 5B shows the results. All of the difference values are negative, indicating that for all compounds binding to triplex is favored over binding to duplex calf thymus DNA. Compounds **MHQ-14** and **OZ-153** show the largest differences, arising from the fact that neither compound binds appreciably to duplex DNA. Note, however, that both **MHQ-14** and **OZ-153** bind less tightly to triplex DNA than does the parent compound **LS-8** (Figure 5A). For these two compounds, greater selectivity was gained at the expense of binding affinity. The combined data of Figure 5A and B provide great insight into the behavior of the naphthylquinolines. Inspection of the data shows, for example, that **MHQ-12** binds to triplex DNA essentially as well as **LS-8**, but is more selective for triplex over duplex DNA as signified by its $\Delta\Delta G_{Triplex,\ CTDNA}$ value. **MHQ-9** and **OZ-124** both bind more tightly to triplex DNA compared to **LS-8**, but their selectivity for triplex over duplex is not improved relative to the parent compound. The competition dialysis data permit quantitative conclusions to be made about relative affinity and selectivity.

Even more exacting measures of selectivity and affinity may be derived from the competition dialysis data. These are described for the first time here. Normalized competition dialysis data, such as shown in Figure 3B for **LS-8**, may be used to calculate the *specificity sum*, **SS**. **SS** is the sum of the normalized amounts bound to each nucleic acid species *i* in the assay

$$SS = \sum_i \frac{C_{b,\,i}}{C_{max}}$$

where $C_{b,\,i}$ is the amount bound and C_{max} is the maximum amount bound to any species. The index *i* ranges from 1 to 13 in the current version of the assay, corresponding to the 13 different nucleic acid structures and sequences used.

SS can thus range from 1 to 13, with a value of 1 indicative of absolute selectivity with compound binding to only one structure. A value of **SS** = 13, in contrast, would indicate equal binding to all structures in the assay, and a complete lack of selectivity. By way of illustration, the data for **LS-8** in Figure 3 may be used to calculate SS as follows. In Figure 3A, C_{max} is seen to be the amount bound to triplex and equals 12.4 μM. The amounts bound to the other species range from 0 to 6 μM. Normalizing the data of Figure 3A with $C_{max} = 12.4\ \mu$M yields the graph in Figure 3B. Summation of the normalized binding data in Figure 3B yield **SS** = 3.06. Values of **SS** for all compounds studied are listed in Table 2 and are shown in Figure 6A. **SS** values range from 1 to 5.33. Several compounds show improved selectivity relative to the parent compound **LS-8**, with **SS** < 3.0. For comparison, an average value of **SS** = 4.5 ± 2.0 was determined from competition dialysis data obtained for 126 compounds representing a wide variety of chemical classes and DNA binding modes (Chaires, J. B.; Ren, J., in preparation). All but one of the naphthylquinoline compounds studied here show better than average selectivity as judged by **SS** values

One limitation of the **SS** metric is that it does not contain information about compound binding affinity. This limitation may be circumvented by calculating the ratio C_{max}/**SS** where C_{max} is the maximal amount bound as defined above. This ratio embodies both affinity and selectivity. C_{max} directly measures compound affinity. If **SS** = 1, the maximal value of C_{max}/**SS** will be obtained, whereas if **SS** = 13 (no selectivity), the minimal value of the ratio will result. From results of competition dialysis studies on 126 compounds (Chaires, J. B.; Ren, J., in preparation), C_{max}/SS was found to range from 0.06 to 9.8, with an average value of 2.4 ± 2.2. Values of C_{max}/**SS** for the compounds studied here are shown in Table 2 and Figure 6B. Several compounds, notably **MHQ-9**, **MHQ-12**, **OZ-85H**, and **OZ-124**, show values of C_{max}/**SS** ≥ 8.0, indicative of both high selectivity and affinity. All of these are greatly improved over the parent compound **LS8** with C_{max}/**SS** = 4.1.

To attempt to understand the molecular determinants of triplex binding affinity for these naphthylquinoline compounds, a quantitative structure-affinity relationship (QSAR) was derived from the experimental binding constants (Table 2) and computed molecular descriptors. A full discussion of the QSAR construction is provided as Supporting Information. The best three-term QSAR to emerge was as follows

$$\log K_{app} = 0.00264(\pm 0.99965)\mathrm{SASA} - 0.693(\pm 0.125)\mathrm{EA} - 0.196(\pm 0.02)\mathrm{HB_a} + 4.66(\pm 0.44) \quad (1)$$

$$N = 14,\ R = 0.959;\ \mathrm{RMSE} = 0.130;\ F = 49.84;\ P = 0.0001$$

In this relationship, log K_{app} is the logarithm of the apparent binding constant (Table 2), SASA is the total solvent accessible surface area in Å^2, EA is electron affinity in eV, and HB_a is the number of hydrogen bond acceptors. The physical meaning of this is as follows. As SASA increases, log K_{app} increases in magnitude, indicating higher affinity for triplex DNA. Increases in the magnitudes of EA and HB_a result in decreasing binding affinity. Increasing the solvent accessible surface areas of naphthylquinoline compounds results in higher affinity for the triplex. Greater electron affinity and more hydrogen bond acceptors reduce the affinity of naphthylquinolines for triplex DNA.

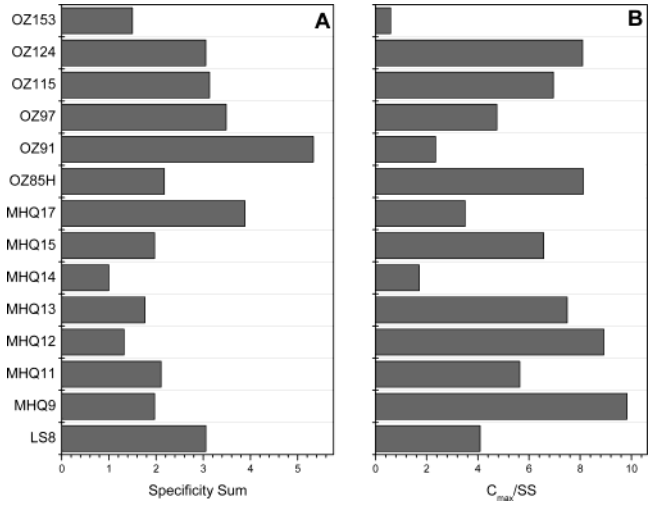

Figure 6. Values of **SS** and Cmax/**SS** for naphthylquinoline compounds.

Discussion

The discovery of small molecules that selectively bind to triplex DNA is of intense interest. A new competition dialysis assay developed in our laboratory provides the most rigorous method available for rapidly screening the structural selectivity of small molecules. New methods of data analysis are described here that make competition dialysis an even more powerful tool for measure the structural selectivity of new ligands. By using that assay, we show here that a new generation of naphthylquinoline compounds represent some of the best triplex-selective molecules synthesized to date.

The new naphthylquinolines are based on the parent compound **LS-8** (Figure 2). **LS-8** was designed as a triplex binder using three design principles.[17] First, it is a dication, and its positive charges should interact with the negatively charged triplex structure with a large, favorable polyelectrolyte contribution to its binding free energy. Second, it has a crescent shaped aromatic ring surface that should stack well on the base triplets within the triplex structure (Figure 1). Finally, its ring system is unfused, providing the flexibility to adapt to any propeller twists with the triplex structure. An unanticipated design element emerged from initial studies on **LS-8**. Molecular modeling studies revealed that the alkylamine substituent on **LS-8** fits snugly into the minor groove of the triplex, providing additional stabilizing interactions.[17] Jenkins and co-workers had long recognized the influence of side chains and groove occupancy on intercalator stability,[38−41] and subsequently demonstrated that substituted anthraquinones with substituents that occupied two triplex grooves showed improved triplex stability and selectivity.[42] The new naphthylquinoline compounds synthesized and studied here (Figure 2) were designed to explore the effects resulting from modifications of groove interactions on triplex binding.

Several of the new naphthylquinolines show greatly improved triplex affinity and selectivity. **MHQ-9**, **MHQ-12**, **MHQ-13**, **MHQ-15**, and **OZ-85H** all show significantly lower **SS** values and significantly higher C_{max}/**SS** values when compared to the parent compound **LS-8** (Figure 6). **MHQ-12** stands out as the compound with the greatest improvement in triplex selectivity, while maintaining affinity. Indeed, **MHQ-12** stands out as one of the best triplex binders synthesized to date when compared to other compounds (Table 3, Figure 7). Table 3 lists binding properties for a variety of triplex binding compounds, whose structures are shown in Figure 8. **MHQ-12** has the lowest **SS** value and the highest C_{max}/**SS** value among these compounds (Table 3), indicating combined high selectivity and affinity. Berberine and α-naphtholflavone both have **SS** values approaching that found for **MHQ-12**, but have much lower binding affinity. Coralyne has higher triplex affinity than does **MHQ-12**, but is less selective. Figure 7 shows comparative normalized competition dialysis data for the compounds listed in Table 3. **MHQ-12** clearly stands out for its nearly exclusive binding to triplex.

Table 3. Properties of Triplex-Binding Compounds

compd	SS	C_{max}/SS	ΔG_{duplex}	$\Delta G_{triplex}$
ethidium	5.05	1.93	−6.4	−6.5
BePI	6.39	1.38	−6.5	−6.8
coralyne	4.34	5.22	−6.3	−7.5
berberine	1.59	3.55	−4.7	−6.6
α-naphthoflavone	1.43	3.67	0	−6.5
2,6 anthraquinone	5.08	2.55	−6.1	−6.7
LS8	3.06	4.08	−6.1	−7.1
MHQ12	1.32	8.93	−4.2	−7.0

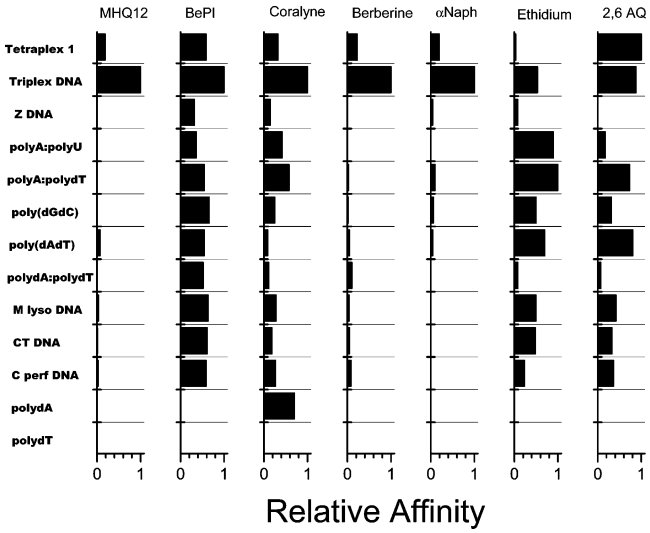

Figure 7. Comparison of the results of competition dialysis experiments on MHQ12 and several compounds reported to be selective for triplex structures.

Why is **MHQ-12** a better triplex binder than **LS-8**? The possible reason is somewhat subtle. **MHQ-12** differs from **LS-8** only at one position, the terminal atom in the R1 substituent (Figure 2) where oxygen was substituted for nitrogen. That substitution lowers the energy barrier for rotation of the side chain, facilitating its reorientation within the minor groove. In vacuo, semiempirical computations using the AM1 method (not shown) show that the N → O substitution lowers the bond rotation energy barrier by 4−5 kcal mol^{-1}. One explanation for the greater triplex selectivity of **MHQ-12** relative to **LS-8**, therefore**,** is that it results from more facile reorientation of the alkylamine chain within the minor groove of the triplex. That

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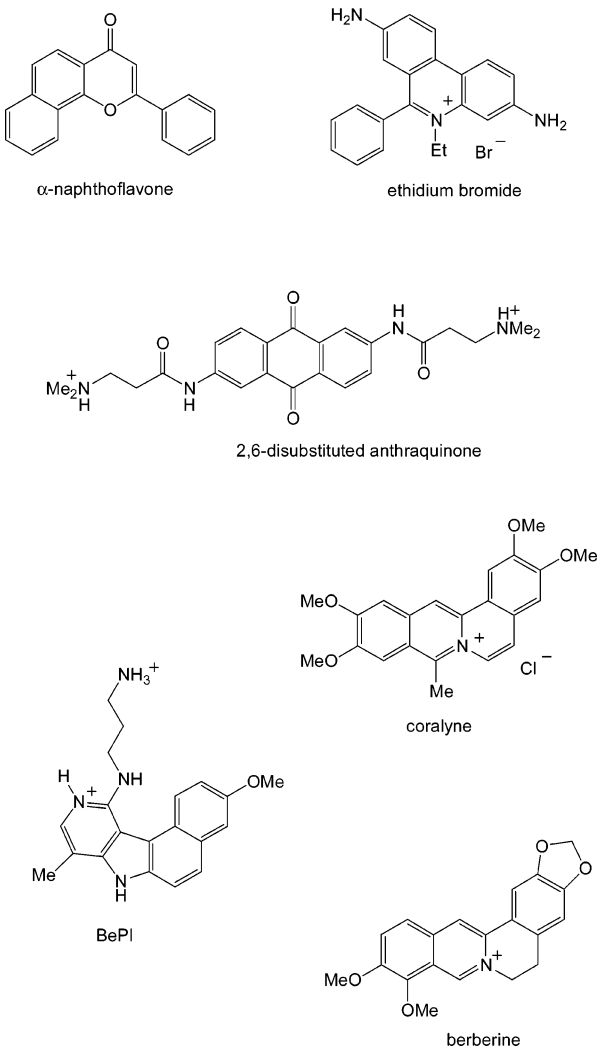

Figure 8. Structures of triplex binders.

explanation implies that ligand flexibility is an important component of triplex binding. Ligands that can more easily change conformation to adapt to elements of the triplex structure will have more favorable interactions. That conclusion suggests an important new design element for targeting triplex structures, ligand flexibility.

OZ-85H shows improved selectivity and slightly improved affinity for triplex relative to **LS-8**. **OZ-85H** is unique among the naphthylquinolines studied here in that it has a second pendant chain attached. This chain was positioned so that it might occupy a second groove within the triplex structure. It is thus similar to the 2,6-disubstituted anthraquinone compounds developed by Jenkins and colleagues as triplex selective agents.[39,42] These bind to triplexes by an intercalative "threading" mode. The addition of a second chain that can occupy an additional groove produces a molecule with distinctly improved triplex binding properties, although the improvement is not as great as is seen for molecules such as **MHQ-12** with single chain modifications. Nonetheless, **OZ-85H** represents a promising lead structure, and systematic modification of both of its pendant chains could yield greatly improved triplex binders.

MHQ-14 and **OZ-153** show dramatically reduced triplex affinity relative to **LS-8**. The reasons are different for the two compounds, but are simple in each case. **MHQ-14** was designed to have a reduced charge of +1 relative to the dicationic parent compound. It therefore will have a lower polyelectrolyte contribution to its binding free energy.[43,44] The polyelectrolyte contribution for the interaction of charged ligands with nucleic acids (ΔG_{pe}) is given by the equation $\Delta G_{pe} = RT(Z\Psi)\ln[M^+]$,[43,45,46] where R is the gas constant, T is the temperature, Z is the charge on the ligand, Ψ is the extent of counterion condensation, and M^+ is the monovalent cation concentration (0.2 M in the competition dialysis experiment). For duplex DNA, with 2 phosphates per 3.4 Å, $\Psi = 0.88$,[43,47] whereas for triplex DNA, with 3 phosphates per 3.4 Å, $\Psi = 0.923$. Loss of a +1 charge would thus reduce the polyelectrolyte contribution to the binding free energy by about 0.9 kcal mol^{-1}, which can account for most of the 1.2 kcal mol^{-1} difference in triplex binding free energy observed between **LS-8** and **MHQ-14** (Table 2, Figure 5). These data reinforce the importance of ligand charge as a design principle for targeting triplexes.

The reduced affinity of **OZ-153** arises from another source. **OZ-153** is a trication, and should otherwise have a more favorable polyelectrolyte contribution to its binding free energy than **LS-8**, yet it binds worse by 1.6 kcal mol^{-1} (Table 2, Figure 5). As is seen in Figure 2, however, **OZ153** has a bulky, branched side chain that would be difficult to fit into the minor groove of the triplex. The substantial steric hindrance presented by that side chain most probably results in greatly reduced binding affinity.

The origins of naphthylquinoline triplex affinity were further explored by construction of a QSAR, which is shown above (eq 1). Because the compounds used in this study shared a common intercalating ring structure, the derived QSAR reflects primarily the energetic contributions of the groove-binding substituents. Details of the QSAR construction are presented as Supporting Information, which also describes demonstrations of the stability and validity of the derived QSAR. The primary positive contribution to triplex binding affinity revealed by the QSAR is the total solvent accessible surface area (SASA) of the side chain. This finding is fully consistent with previous thermodynamic studies from this laboratory that show that binding of both intercalators and groove binders is driven by a large, favorable free energy contribution from the hydrophobic effect.[48−50] A substantial binding free energy contribution results from the removal of nonpolar surface area by complex formation. Such is the case here. Burial of hydrophobic side chains with greater solvent accessible surface areas within the triplex groove provides a proportionally larger free energy contribution. Unexpected properties were revealed by the QSAR that oppose triplex binding. Triplex binding affinity is negatively correlated with both electron affinity and the number of hydrogen bond acceptors. Both of these are important solvation descriptors. [51] Their opposing influence on triplex affinity indicates that the

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energetic cost of ligand desolvation is unfavorable for binding. Overall, the derived QSAR offers a powerful and quantitative description of the origin of triplex affinity of the naphthylquinolines studies here. Increases in the solvent accessible surface area of the pendant aminoalkyl chain can enhance binding, but adding substituents to the chain with electron affinity or hydrogen bond acceptor functionality will oppose binding. The derived QSAR emphasizes different properties than were discussed above to explain the triplex affinity of **MHQ-12**, **OZ-85H**, **MHQ-14**, and **OZ-153**, because we lacked appropriate descriptors to account for chain flexibility, threading potential, and steric hindrance. These issues are currently under study by use of more sophisticated 4D QSAR [52] methods that will incorporate more detailed structural and molecular dynamics information (Bishop, G. R.; Senese, C. L.; Chaires, J. B.; Hopfinger, A. J., in preparation).

The derived QSAR offers a powerful means of predicting the triplex affinity of newly designed naphthylquinoline compounds. The properties of newly designed compounds can be easily computed, and substitution of these properties into eq 1 allows prediction of their triplex binding affinity. That such an approach works is shown conclusively in the Supporting Information, Figure S2, where systematic studies were done in which each of the naphthylquinolines studied here were omitted in turn, and the remaining compounds used to construct a QSAR. In all cases, the QSAR could be used to accurately predict the known triplex binding affinity of the omitted compound.

We note that the triplex studies described here were limited to the poly dA:(poly dT)$_2$ triple helix. This is a parallel triplex that may not be representative of triplex structures in general. It is, however, widely used in studies of ligand-triplex interactions, and is one of the best characterized triple helix structures from both a structural [53] and thermodynamic [54,55] perspective. Although it is certainly desirable to investigate the interaction of the naphthylquinolines studied here with additional types of triplex structures of different strand polarity and that include guanine and cytosine, such is not possible with this version of the competition dialysis assay. Antiparallel triplex structures and triplexes containing G and C require different solution conditions (pH, divalent cations) than employed here. Although alternate triplex structures can most certainly be used in competition dialysis studies,[56] different buffer conditions are needed that require complete reformulation of the assay, with rigorous verification that all structures included are stable under the particular ionic conditions used. We intend to continue to develop the competition dialysis assay to include more structures of interest along with a range of ionic conditions. Studies of the interaction of **LS-8** with a variety of triplex structures using a several experimental techniques were previously reported [19,20,23,57,58]. These studies lend confidence that our studies with poly dA:(poly dT)$_2$ are applicable to triplexes in general. DNase I footprinting was used to show that **LS-8** facilitates formation of both parallel and antiparallel DNA triple helices.[19] Footprinting was used to investigate the effect of **LS-8** on the stringency of parallel triple helix formation of a series oligodeoxynucleotides containing all combinations of bases at a particular position.[23] Footprinting was again used to study the effect of **LS-8** on the formation of intermolecular triplexes of mixed sequence.[20] Footprinting and UV melting studies were used to investigate the effect of tethered **LS-8** on both inter- and intramolecular triplex stabilization.[21] Recently, a novel, high-throughput molecular beacon assay was used to investigate the effect of **LS-8** on the thermal denaturation of an intramolecular triplex of mixed sequence.[58] Collectively, these studies make **LS-8** one of the more extensively studied triplex binders, and indicate that our studies with poly dA:(poly dT))$_2$ are reflective of, and consistent with, its selective binding to a wide variety of triplex structures.

One reviewer expressed reservations about the utility of the specificity sum (**SS**) as a metric. The concern raised was that although the limiting value of 1 and 13 are clear indications of absolute selectivity or the complete lack of selectivity, respectively, intermediate values are ambiguous. A particular intermediate value of **SS** could arise from a number of binding distributions to the different structures. For example, a value of **SS** = 2.0 could arise from equal binding to 2 of the 13 structures, or from maximal binding to one structure, and binding to 10 others with a relative binding of 0.1. An actual example may be seen in Figure 7 for the data for ethidium (**SS** = 5.0) and the 2,6-disubstituted anthraquinone (SS=5.08). The values for **SS** are similar for these compounds, but the binding distribution is clearly different. We recognize that **SS** is a simple metric, with limited information about the detailed binding distribution, and that more sophisticated chemometric tools are needed for a more detailed description of the exact binding distributions. In our experience with over 126 compounds from diverse chemical classes that have been studied by this first-generation competition dialysis assay with 13 structures, we found an average value of **SS** = 4.5 ± 2.0 (Chaires, J. B.; Ren, J., in preparation). This average reflects that few compounds exhibit absolute structural selectivity. With reference to this average value, however, **SS** provides a clear, quantitative measure of structural preferences that is operationally useful for identifying interesting binding behavior. We freely acknowledge the simplicity and limitations of **SS**, but have found it useful and an appropriate point of departure for more sophisticated analyses.

The studies presented here suggest new design principles for the development of triplex selective agents. In addition to the three design principles used in the initial development of **LS-8** [17] (positive charge, aromatic surface complementary to base triplet shape, ring flexibility to accommodate propeller twist), at least two more may be added. First, pendant chains that occupy one or more of the triplex grooves are important for both selectivity and affinity. Second, these chains should be flexible and able to adapt to the triplex groove structure to optimize interactions within the groove. The studies described here reinforce the importance of groove interactions previously emphasized by Jenkins and colleagues, [39,42] and introduce the

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new concept of the importance of flexibility of the substiuents within the grooves.

Experimental Section

General. Abbreviations: AcOEt, ethyl acetate; BOP, benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; Et_3N, triethylamine; Et_2O, diethyl ether; EtOH, ethanol; MeOH, methanol; THF, tetrahydrofuran.

Synthesis of *N*-[2-(dimethylamino)ethyl]-2-(2-naphthyl)quinolin-4-amine [59] (**LS-8**), 4-chloro-2-(2-naphthyl)quinoline [32] (**1**), and 4-hydroxy-2-(2-naphthyl)quinoline *p*-toluenesulfonate [32]have been reported previously. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Melting points (Pyrex capillary) are not corrected.

4-[2-(Dimethylamino)ethoxy]-2-(2-naphthyl)quinoline (MHQ-12). A mixture of 2-chloroethyldimethylamine hydrochloride (0.22 g, 1.5 mmol), NaH (0.12 g, 5 mmol), and 4-hydroxy-2-(2-naphthyl)quinoline *p*-toluenesulfonate (0.33 g, 0.75 mmol) in anhydrous DMF (6 mL) was stirred and heated to 80 °C under a nitrogen atmosphere for 3 h. After cooling to 23 °C, a precipitate of NaCl was filtered off, washed with AcOEt (5 mL), and the organic solution was concentrated on a rotary evaporator. Silica gel chromatography eluting with pentanes/Et_2O/Et_3N (5:4:1) followed by crystallization from Et_2O/pentanes gave 0.14 g (55%) of **MHQ-12**: mp 52−54 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 6 H), 2.95 (t, J = 5.0 Hz, 2 H), 4.43 (t, J = 5.0 Hz, 2 H), 7.32 (s, 1H), 7.51 (m, 3 H), 7.71 (t, J = 8 Hz, 1 H), 7.88 (t, J = 8 Hz, 1 H), 7.97 (m, 2 H), 8.14 (d, J = 8 Hz, 1 H), 8.21 (d, J = 8 Hz, 1 H), 8.30 (d, J = 8 Hz, 1 H), 8.54 (s, 1 H); ^{13}C NMR ($CDCl_3$) _ 46.2, 58.0, 67.1, 98.7, 120.5, 121.8, 125.2, 125.4, 126.3, 126.6, 127.0, 127.7, 128.5, 128.8, 129.3, 130.0, 133.5, 133.9, 137.7, 149.4, 158.5, 162.1; high-resolution EI−MS calcd for $C_{23}H_{22}N_2O$ (M^+) *m*/*z* 342.1732, observed *m*/*z* 342.1723. Anal. Calcd for $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18. Found: C, 80.27, H, 6.63; N, 8.07.

General Procedure for MHQ-9, MHQ-11, MHQ-13, MHQ-14, MHQ-15, MHQ-17, OZ-91, and OZ-97. A mixture of 4-chloroquinoline **1** (0.29 g, 1 mmol), an amine (1.5 mL) and two drops of $SnCl_4$ was stirred and heated to 130 °C under a nitrogen atmosphere for 4 h. The resultant dark brown oil was allowed to cool to room temperature and then quenched with water (20 mL). After extraction with AcOEt, the organic layer was dried with anhydrous $MgSO_4$ and concentrated under a reduced pressure. The residue was purified by silica gel chromatography using AcOEt/pentanes (4:1) as an eluent to yield a substituted quinolin-4-amine as a pale yellow solid. The quinolinamine was crystallized from MeOH or AcOEt. In several cases the product was additionally purified by crystallization of its hydrobromide or hydrochloride salt. Thus, a solution of a quinolin-4-amine in AcOEt was stirred and treated dropwise with hydrobromic acid (47%) or hydrochloric acid (36%). The precipitated salt was crystallized twice from EtOH or AcOEt. The composition was determined by elemental analysis.

***N*-[3-(Imidazolo)propyl]-2-(2-naphthyl)quinolin-4-amine (MHQ-9).** This compound was obtained from 1-(3-aminopropyl)imidazole; yield 80%; mp 169−171 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (m, 2 H), 3.41 (q, J = 7 Hz, 2 H), 4.12 (t, J = 7 Hz, 2 H), 5.10 (bs, 1H, exchangeable with D_2O), 6.91 (s, 1H), 6.94 (m, 1 H), 7.12 (m, 1 H), 7.42 (t, J = 8 Hz, 1H), 7.50 (m, 3 H), 7.67 (t, J = 8 Hz, 2 H), 7.87 (m, 1 H), 7.96 (m, 2 H), 8.13 (d, J = 8 Hz, 1 H), 8.24 (d, J = 8 Hz, 1 H), 8.50 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 30.2, 40.3, 44.6, 96.9, 118.1, 118.8, 119.1, 124.8, 125.3, 126.2, 126.5, 126.9, 127.7, 128.3, 128.8, 129.4, 130.2, 130.5, 133.5, 133.8, 137.2, 138.1, 148.8, 149.8, 158.2; high-resolution EI-MS calcd for $C_{25}H_{22}N_4$ (M^+) *m*/*z* 378.1838, observed *m*/*z* 378.1844. Anal. Calcd for $C_{25}H_{22}N_4$: C, 79.30; H, 5.86; N, 14.81. Found: C, 79.54; H, 5.89; N, 14.78.

***N*-[2,2-Dimethyl-3-(dimethylamino)propyl]-2-(2-naphthyl)quinolin-4-amine (MHQ-11).** This compound was obtained from *N*,*N*,2,2-tetramethyl-1,3-propanediamine; yield 70%; mp 116−118 °C;^{1}H NMR ($CDCl_3$) δ 1.15 (s, 6 H), 2.44 (s, 6 H), 2.48 (s, 2 H), 3.30 (s, 2 H), 6.88 (s, 1 H), 7.39 (t, J = 8 Hz, 1 H), 7.49 (m, 2 H), 7.63 (t, J = 8 Hz, 1 H), 7.73 (d, J = 8 Hz, 1H), 7.87 (m, 1 H), 7.94 (d, J = 8 Hz, 1 H), 7.99 (m, 1 H), 8.08 (d, J = 8 Hz, 1H), 8.28 (d, J = 8 Hz, 1H), 8.45 (bs, 1 H, exchangeable with D_2O), 8.56 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.7, 34.5, 48.5, 56.1, 71.6, 95.6, 118.7, 119.9, 124.1, 125.6, 126.0, 126.2, 126.8, 127.7, 128.1, 128.8, 129.0, 130.2, 133.6, 133.7, 138.8, 148.9, 151.5, 158.4; high-resolution EI−MS calcd for $C_{26}H_{29}N_3$ (M^+) *m*/*z* 383.2361, observed *m*/*z* 383.2360. Anal. Calcd for $C_{26}H_{29}N_3 \cdot 0.5H_2O$: C, 79.55; H, 7.70; N, 10.70. Found: C, 79.45, H, 7.77; N, 10.50.

***N*-(*Trans*-2-aminocyclohexyl)-2-(2-naphthyl)quinolin-4-amine Dihydrochloride (MHQ-13·2HCl·0.5H$_2$O).** This compound was obtained from *trans*-1,2-cyclohexanediamine; yield 40%; mp 232−234 °C; ^{1}H NMR (DMSO-d_6) _ 1.35 (m, 4 H), 1.76 (m, 2 H), 2.04 (m, 1H), 2.12 (m, 1 H), 3.06 (m, 1 H), 3.72 (m, 1 H), 5.25 (bs, 2 H, exchangeable with D_2O), 6.83 (bs, 1 H, exchangeable with D_2O), 7.27 (s, 1H), 7.44 (t, J = 8 Hz, 1 H), 7.57 (m, 2 H), 7.67 (t, J = 8 Hz, 1 H), 7.91 (d, J = 8 Hz, 1H), 7.98 (m, 1H), 8.03 (d, J = 8 Hz, 1 H), 8.12 (m, 1 H), 8.34 (d, J = 8 Hz, 1 H), 8.41 (d, J = 8 Hz, 1H), 8.74 (s, 1 H); ^{13}C NMR (DMSO-d_6) δ 24.3, 30.8, 32.2, 38.5, 53.5, 55.9, 95.4, 118.4, 121.9, 123.5, 125.1, 126.2, 126.4, 127.4, 127.7, 128.5, 129.1, 129.2, 132.9, 133.2, 133.8, 137.5, 148.5, 150.0, 156.6; high-resolution EI-MS calcd for $C_{25}H_{25}N_2$ (M^+) *m*/*z* 367.2048, observed *m*/*z* 367.2044. Anal. Calcd for $C_{25}H_{25}N_2 \cdot 2HCl \cdot 0.5H_2O$: C, 66.80; H, 6.28; N, 9.35. Found: C, 67.10, H, 5.83; N, 9.47.

***N*-[2-(*t*-Butoxycarbonylamino)ethyl]-2-(2-naphthyl)quinolin-4-amine (MHQ-14).** This compound was obtained from *N*-BOC-ethylenediamine; yield 43%; mp 87−89 °C; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 9 H), 3.48 (t, J = 5.0 Hz, 2 H), 3.59 (t, J = 5.0 Hz, 2 H), 5.02 (bs, 1 H, exchangeable with D_2O), 6.33 (bs, 1 H, exchangeable with D_2O), 6.91 (s, 1 H), 7.41 (t, J = 8 Hz, 1 H), 7.49 (m, 2 H), 7.64 (t, J = 8 Hz, 1 H), 7.86 (m, 2 H), 7.95 (m, 2 H), 8.09 (d, J = 8 Hz, 1 H), 8.27 (d, J = 8 Hz, 1 H), 8.54 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 28.4, 39.5, 46.0, 80.4, 96.1, 118.1, 120.0, 124.5, 125.4, 126.1, 126.3, 126.8, 127.7, 128.2, 128.7, 129.3, 130.0, 133.5, 133.7, 138.4, 148.7, 150.6, 158.1, 158.2; high-resolution EI−MS calcd for $C_{26}H_{27}N_3O_2$ (M^+) *m*/*z* 413.2103, observed *m*/*z* 413.2104. Anal. Calcd for $C_{26}H_{27}N_3O_2 \cdot 0.5H_2O$: C, 73.91; H, 6.68; N, 9.94. Found: C, 74.41, H, 6.92; N, 9.79.

***N*-[3-(Morpholino)propyl]-2-(2-naphthyl)quinolin-4-amine (MHQ-15).** This compound was obtained from *N*-(3-aminopropyl)morpholine; yield 59%; mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 2.01 (m, 2 H), 2.59 (m, 4 H), 2.65 (m, 2 H), 3.56 (m, 2 H), 3.87 (m, 4 H), 6.97 (s, 1 H), 7.02 (bs, 1 H, exchangeable with D_2O), 7.44 (t, J = 8 Hz, 1 H), 7.50 (m, 2H), 7.66 (t, J = 8 Hz, 1H), 7.89 (m, 2 H), 7.97 (m, 2 H), 8.10 (d, J = 8 Hz, 1H), 8.28 (d, J = 8 Hz, 1H), 8.55 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.8, 44.0, 54.1, 58.9, 67.0, 96.5, 118.3, 120.0, 124.2, 125.5, 126.1, 126.3, 126.9, 127.7, 128.2, 128.7, 129.2, 130.3, 133.6, 133.7, 138.5, 148.8, 150.9, 158.4; high-resolution EI−MS calcd for $C_{26}H_{27}N_3O$ (M^+) *m*/*z* 397.2154, observed *m*/*z* 397.2156. Anal. Calcd for $C_{26}H_{27}N_3O \cdot 0.5H_2O$: C, 76.81; H, 6.94; N, 10.34. Found: C, 76.77, H, 7.06; N, 10.27.

***N*-[2-(1-Methylpyrrolidin-2-yl)ethyl]-2-(2-naphthyl)quinolin-4-amine Dihydrobromide (MHQ-17·2HBr·2.5H$_2$O).** This compound was obtained from 2-(2-aminoethyl)-1-methylpyrrolidine; yield 40%; mp 280−282 °C; ^{1}H NMR (DMSO-d_6) δ 1.74 (m, 1 H), 1.94 (m, 2 H), 2.05 (s, 3 H), 2.34 (m, 2 H), 2.70 (m, 2 H), 2.84 (m, 1 H), 3.43 (m, 1 H), 3.83 (m, 2 H), 7.28 (s, 1H), 7.74 (m, 3H), 8.00 (t, J = 8 Hz, 1 H), 8.14 (m, 4 H), 8.24 (d, J = 8 Hz, 1 H), 8.65 (d, J = 8 Hz, 1 H), 8.72 (s, 1 H), 9.31 (bs, 1 H, exchangeable with D_2O), 9.74 (bs, 1 H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 20.9, 28.2, 29.0, 30.6, 38.6, 55.0, 66.1, 97.6, 116.0, 120.4, 123.1, 124.9, 126.5, 127.3, 127.8, 128.3, 128.8, 129.1, 129.6, 132.2, 133.7, 134.1, 138.3, 141.9, 152.6,

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155.3; high-resolution EI−MS calcd for $C_{26}H_{27}N_3$ (M^+) *m/z* 381.2205, observed *m/z* 381.2211. Anal. Calcd for $C_{26}H_{27}N_3 \cdot 2HBr \cdot 2.5H_2O$: C, 53.07; H, 5.74; N, 7.14. Found: C, 53.24, H, 5.30; N, 6.99.

***N*-[3-[N-(3-Aminopropyl)methylamino]propyl]-2-(2-naphthyl)-quinolin-4-amine Trihydrobromide (OZ-91·3HBr).** This compound was obtained from *N*,*N*-bis(3-aminopropyl)methylamine; yield 64%; mp 232−235 °C; ^{1}H NMR (DMSO-d_6) δ 2.02 (m, 2 H), 2.23 (m, 2 H), 2.84 (s, 3 H), 2.92 (m, 2 H), 3.28 (m, 4 H), 3.83 (m, 2 H), 7.13 (s, 1H), 7.16 (m, 2 H), 7.30 (t, J = 8 Hz, 1 H), 7.91 (bs, exchangeable with D_2O), 8.02 (t, J = 8 Hz, 1 H), 8.11 (d, J = 8 Hz, 1 H), 8.20 (m, 4 H), 8.71 (d, J = 8 Hz, 1 H), 8.81 (s, 1 H), 9.44 (bs, exchangeable with D_2O), 9.82 (bs, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 22.1, 23.1, 36.5, 40.7, 49.0, 52.8, 53.7, 116.4, 120.7, 123.4, 125.3, 127.4, 127.9, 128.3, 129.1, 129.5, 129.6, 129.7, 129.9, 132.9, 134.4, 134.6, 138.7, 153.1, 155.9. Anal. Calcd for $C_{26}H_{30}N_4 \cdot 3HBr$: C, 35.43; H, 4.11; N, 6.36. Found: C, 35.43, H, 3.99; N, 6.12.

***N*-(3-Aminopropyl)-2-(2-naphthyl)quinolin-4-amine Dihydrobromide (OZ-97·2HBr·1.5 H_2O).** This compound was obtained from 1,3-diaminopropane; yield 61%; mp 280−284 °C; ^{1}H NMR (DMSO-d_6) δ 2.13 (m, 2 H), 3.03 (m, 2 H), 3.24 (bs, exchangeable with D_2O), 3.85 (m, 2 H), 7.27 (s, 1 H), 7.70 (m, 3 H), 7.95 (bs, exchangeable with D_2O), 7.99 (t, J = 8 Hz, 1 H), 8.07 (d, J = 8 Hz, 1 H), 8.18 (m, 3 H), 8.24 (t, J = 8 Hz, 1 H), 8.71 (d, J = 8 Hz, 1 H), 8.79 (s, 1 H), 9.37; (bs, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 25.9, 36.7, 40.3, 97.5, 116.2, 120.6, 123.3, 125.1, 126.9, 127.6, 128.8, 128.7, 129.1, 129.2, 129.4, 129.7, 132.6, 134.0, 134.3, 138.5, 152.7, 155.6 (two signals overlap). Anal. Calcd for $C_{22}H_{21}N_3 \cdot 2HBr \cdot 1.5H_2O$: C, 51.17; H, 5.09; N, 8.14. Found: C, 51.19, H, 4.85; N, 7.96.

***N*-(2-Dimethylamino)ethyl]-2-[6-[6-hydroxyhexyl)oxy]-2-naphthyl]quinolin-4-amine Trihydrobromide (OZ-85H·3HBr·0.5H_2O).** Condensation of 2-(trifluoromethyl)aniline with 6-methoxy-2-naphthone and cyclization of the resultant crude Schiff base **7** to quinoline **8** by treatment with lithium [2-(dimethylamino)ethyl]amide in anhydrous THF were conducted by using general procedures.[33−35] A mixture of crude compound **8** (0.7 g, 1.9 mmol), BBr_3 (1 M in hexanes, 10 mL, 10 mmol), and toluene (10 mL) was stirred at 23 °C for 3 days and then concentrated on a rotary evaporator. The residue was stirred and treated dropwise with aqueous $NaHCO_3$ until the mixture reached pH 7. Extraction of the mixture with AcOEt (5 × 10 mL) followed by concentration of the extract to 10 mL and cooling gave crystalline compound **9** [yield 0.6 g (89%); mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 2.78 (s, 6 H), 2.65 (m, 2 H), 3.54 (m, 2 H), 6.95 (bs, 1 H, exchangeable with D_2O), 7.12 (s, 2 H), 7.16 (d, J = 8 Hz, 1 H), 7.41 (t, J = 8 Hz, 1 H), 7.63 (t, J = 8 Hz, 1 H), 7.78 (d, J = 8 Hz, 1 H), 7.88 (d, J = 8 Hz, 1 H), 7.92 (d, J = 8 Hz, 1 H), 8.16 (d, J = 8 Hz, 1 H), 8.29 (d, J = 8 Hz, 1 H), 8.60 (s, 1 H), 9.83 (s, 1 H, exchangeable with D_2O)].

A mixture of **9** (0.36 g, 1 mmol), NaH (27 mg, 1.1 mmol), 1 bromo-6-(*tert*-butyldimethylsiloxy)hexane (0.33 g, 1.1 mmol), and anhydrous DMF (6 mL) was stirred at 23 °C for 5 days, then quenched with aqueous MeOH (2 mL), and concentrated on a rotary evaporator. Silica gel chromatography of the residue eluting with AcOEt/MeOH (19:1) gave a silyl derivative **10** [an oil; yield 0.46 g (80%); high-resolution EI−MS calcd for $C_{35}H_{49}N_3O_2Si$ (M^+) *m/z* 571.3594, observed *m/z* 571.3598].

A solution of **10** (80 mg, 0.14 mmol) and $Bu_4N^+F^-$ (1 M in THF, 0.4 mL, 0.4 mmol) in THF (2 mL) was allowed to stand at 23 °C for 1 h and then was concentrated. A solution of the residue in AcOEt was treated with hydrobromic acid and the resultant precipitate of the trihydrobromide of **OZ-85H** was crystallized from EtOH/AcOEt (1:1): yield 86 mg (86%); mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ 1.41 (m, 2 H), 1.48 (m, 4 H), 1.81 (m, 2 H), 2.94 (s, 6 H), 3.42 (m, 2 H), 3.55 (m, 2 H), 4.12 (m, 2H), 4.17 (m, 2 H), 7.31 (s, 1 H), 7.34 (d, J = 8 Hz, 1 H), 7.50 (s, 1 H), 7.77 (t, J = 8 Hz, 1 H), 8.02 (t, J = 8 Hz, 1 H), 8.10 (m, 3 H), 8.18 (d, J = 7 Hz, 1 H), 8.58 (d, J = 8 Hz, 1 H), 8.67 (s, 1 H), 9.15 (bs, 1 H, exchangeable with D_2O), 9.66 (bs, 1 H, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 25.2, 25.4, 28.5, 32.3, 37.7, 42.5, 54.1, 60.5, 67.8, 97.4, 106.6, 116.1, 120.2, 120.3, 123.3, 125.3, 126.5, 126.8, 127.6, 129.0, 130.5, 133.7, 136.0, 138.2, 152.9, 155.1, 158.6 (two signals overlap). Anal. Calcd for $C_{29}H_{35}N_3O_2 \cdot 3HBr \cdot 0.5H_2O$: C, 49.09; H, 5.55; N, 5.92. Found: C, 49.14, H, 5.57; N, 5.92.

***N*-(4-(Dimethylamino)butyl]-2-(2-naphthyl)quinolin-4-amine Dihydrobromide (OZ-115·2HBr·2H_2O).** The treatment of **1** with 4-hydroxybutylamine followed by workup as described above gave a hydroxybutyl derivative **2** [yield 70%, mp 132−133 °C (from AcOEt)]. A mixture of **2** (0.2 g, 0.6 mmol), $SOCl_2$ (2 mL), and benzene (5 mL) was heated under reflux for 4 h and then concentrated on a rotary evaporator. The residue was treated with aqueous NaOH (5%, 5 mL), and the mixture was extracted with AcOEt (3 × 10 mL). The extract was dried over anhydrous $MgSO_4$ and concentrated to give an oily residue of a chlorobutyl derivative **4**. Compound **4** was purified by crystallization of its hydrobromide **4·HBr** from EtOH/AcOEt (1:1) [yield 0.17 g (64%), mp 192−195 °C].

A mixture of **4·HBr** (0.17 g, 0.4 mmol), dimethylamine hydrochloride (0.50 g, 61 mmol), Na_2CO_3 (0.75 g, 77 mmol), and DMF (10 mL) was stirred and heated to 50 °C for 10 h. Concentration on a rotary evaporator followed by treatment of the oily residue with MeOH (5 mL) and then addition of hydrobromic acid to the resultant solution gave a crystalline salt of **OZ-115**. This salt was purified by three crystallizations from EtOH; yield 0.11 g (61%); mp > 250 °C; ^{1}H NMR (DMSO-d_6) δ 1.82 (m, 4 H), 2.78 (s, 6 H), 3.16 (m, 2 H), 3.76 (m, 2 H), 7.24 (s, 1 H), 7.72 (m, 3 H), 8.00 (t, J = 8 Hz, 1 H), 8.14 (m, 4 H), 8.23 (d, J = 8 Hz, 1 H), 8.64 (d, J = 8 Hz, 1 H), 8.70 (s, 1 H), 9.29 (bs, exchangeable with D_2O), 9.42 (bs, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 21.3, 24.7, 42.2, 42.3, 56.3, 97.4, 116.0, 120.3, 123.1, 124.8, 126.6, 127.4, 127.8, 128.4, 128.9, 129.1, 129.6, 132.4, 133.7, 134.1, 138.4, 152.4, 155.4 (two signals overlap). Anal. Calcd for $C_{24}H_{27}N_3 \cdot 2HBr \cdot H_2O$: C, 52.91; H, 5.87; N, 7.41. Found: C, 52.56, H, 5.76; N, 7.32.

***N*-[6-(Cyclopropylamino)hexyl]-2-(2-naphthyl)quinolin-4-amine, Salt with Phosphoric Acid (OZ-124·2H_3PO_4·4H_2O).** The treatment of **1** with 6-hydroxyhexylamine followed by workup as described above gave a hydroxyhexyl derivative **3** [yield 59%, mp 122−131 °C (from MeOH)]. A subsequent treatment of **3** with $SOCl_2$ as described above, gave a chlorohexyl derivative **5** as an oil. Compound **5** was purified by crystallization of its hydrobromide **5·HBr** from EtOH (yield 50%, mp 190−195 °C).

A mixture of **5·HBr** (0.22 g, 0.56 mmol), cyclopropylamine (0.39 mL, 5.6 mmol), Na_2CO_3 (0.53 g, 5.0 mmol), and DMF (5 mL) was stirred and heated to 40 °C for 12 h. After concentration on a rotary evaporator, the residue of crude **OZ-124** was purified by silica gel chromatography eluting with Et_2O/AcOEt (2:1). A solution of the oily product in MeOH (10 mL) was stirred and treated with H_3PO_4 (85%, 1 mL). After cooling the resultant precipitate was filtered and crystallized from MeOH; yield 0.19 g (49%) of **OZ-124·2H_3PO_4·4H_2O**, mp 150−158 °C; ^{1}H NMR (DMSO-d_6) δ 0.15 (m, 2 H), 0.29 (m, 2 H), 1.41 (m, 6 H), 1.77 (m, 2 H), 2.00 (m, 1 H), 2.56 (m, 2 H), 3.45 (m, 2 H), 7.13 (s, 1 H), 7.16 (bs, exchangeable with D_2O), 7.42 (t, J = 8 Hz, 1 H), 7.56 (m, 2 H), 7.65 (t, J = 8 Hz, 1 H), 7.90 (d, J = 8 Hz, 1 H), 7.97 (m, 1 H), 8.03 (d, J = 8 Hz, 1 H), 8.10 (m, 1 H), 8.27 (d, J = 8 Hz, 1 H), 8.41 (d, J = 8 Hz, 1 H), 8.72 (s, 1 H), ^{13}C NMR (DMSO-d_6) δ 5.8, 26.6, 27.9, 29.5, 30.1, 42.4, 48.9, 95.1, 118.1, 121.5, 123.6, 125.0, 126.0, 126.1, 126.4, 127.4, 127.7, 128.5, 129.0, 129.2, 132.9, 133.1, 137.5, 148.3, 150.8, 156.3 (two signals overlap); high-resolution EI−MS calcd for $C_{28}H_{31}N_3$ (M^+) *m/z* 409.2518, observed *m/z* 409.2493. Anal. Calcd for $C_{28}H_{31}N_3 \cdot 2H_3PO_4 \cdot 4H_2O$: C, 49.62; H, 6.71; N, 6.20. Found: C, 49.58, H, 6.41; N, 6.18.

***N*-[4-Hydroxy-3,5-bis(morpholinomethyl)phenyl]-2-(2-naphthyl)-quinolin-4-amine Trihydrochloride (OZ-153·3HCl·2.5H_2O).** A mixture of **1** (0.46 g, 1 mmol), 4-aminophenol (0.52 g, 4.8 mmol), and phenol (0.5 g) was heated to 100 °C for 2 h under a nitrogen atmosphere

and then cooled and treated with Et_2O (25 mL). The solution was applied to silica gel column (30 g). Elution with Et_2O/Et_3N (9:1) gave compound **6** [0.46 g (79%), mp > 300 °C].

A solution of **6** (0.40 g, 1.1 mmol), morpholine (0.25 mL, 2.8 mmol), and aqueous formaldehyde (13.3 M, 0.45 mL, 6 mmol) in EtOH (15 mL) was heated under reflux for 24 h. Concentration on a rotary evaporator was followed by silica gel chromatography of the residue eluting with Et_2O/Et_3N (9:1). The product **OZ-153** was dissolved in EtOH (10 mL) and the solution was treated with concentrated hydrochloric acid (2 mL). The resultant precipitate was filtered and crystallized from EtOH to give 0.30 g (40%) of **OZ-153·3HCl·2.5H$_2$O**; mp 224−226 °C; ^{1}H NMR (DMSO-d_6) δ 3.27 (m, 8 H), 3.50 (m, 8 H), 4.48 (s, 4 H), 7.14 (s, 1 H), 7.65 (m, 2 H), 7.79 (t, J = 8 Hz, 1 H), 7.93 (s, 2 H), 8.03 (d, J = 8 Hz, 1 H), 8.09 (s, exchangeable with D_2O), 8.12 (m, 4 H), 8.39 (d, J = 8 Hz, 1 H), 8.82 (m, 1 H), 8.94 (d, J = 8 Hz, 1 H), 10.38 (bs, exchangeable with D_2O), 11.37 (bs, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6) δ 50.7, 53.8, 63.2, 99.4, 116.2, 119.7, 120.6, 123.5, 125.0, 126.9, 127.1, 127.7, 128.3, 128.6, 128.9, 129.5, 129.6, 132.2, 134.0, 139.0, 152.9, 154.4, 155.3 (three signals overlap); Anal. Calcd for $C_{35}H_{36}N_4O_3$·3HCl·2.5H_2O: C, 58.77; H, 6.21; N, 7.83. Found: C, 59.03; H, 6.30; N, 7.70.

Competition Dialysis. Competition dialysis experiments were done exactly as previously described.[25,26] The nucleic acids used in the test array are listed in Table 1. Competition dialysis studies were done in BPES buffer, consisting of 6 mM Na_2HPO_4, 2mM NaH_2PO_4, 1mM Na_2EDTA, and 185 mM NaCl, pH 7.0.

Computational Methods. The energy barrier for side chain rotation in **LS8** and **MHQ12** was determined by computational studies using semiempirical methods. AM1 semiempirical calculations were performed using the software package HyperChem 5.01 distributed by Hypercube, Inc. Starting structures were drawn using the molecular builder in HyperChem. Initial structure geometries were optimized semiempirically from different starting conformations selected manually. Subsequent rotational studies were carried out using the atom positions of the lowest energy structure selected. Semiempirical single-point energy and geometry optimization calculations were performed on **LS8** and **MHQ12** with each assigned a singlet spin multiplicity and a total charge of +2. For the rotational study, each system was first optimized, then the dihedral angle between the quinoline and the side chain was frozen at different angles, while the rest of the system was allowed to reoptimize. Increments of 45° were used starting from 0.0°. Following optimization a single-point energy was calculated.

Acknowledgment. This work was supported by Grant No. CA35635 from the National Cancer Institute (J.B.C.). We thank Dr. David Wilson (Georgia State University) for helpful comments on the manuscript, Dr. David Magers (Mississippi College) for advice on the semiempirical calculations, and Dr. Anton Hopfinger (University of Illinois-Chicago) for advice on QSAR construction.

Supporting Information Available: Details of the construction of a Quantitative Structure-Affinity Relationship (QSAR) and results from binding and thermal denaturation studies. (11 pages with 5 tables and 4 figures). This material is available free of charge via the Internet at http://pubs.acs.org.

JA034181R