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New triple-helix DNA stabilizing agents

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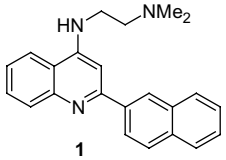
Abstract—Several substituted quinolin-4-amines and heteroaromatic analogs were synthesized and evaluated for interaction with triplex polydA·2polydT and duplex polydA·polydT by using UV-thermal melting experiments. Excellent triple-helix DNA ligands with high affinity toward T·A·T triplets and triple/duplex selectivity were designed through a rational approach.

Intermolecular triple helices are formed by the sequence-specific hydrogen bonding of a single stranded DNA in the major groove of duplex DNA. This interaction is quite weak under physiological conditions. The stabilization of triple-helical DNA is currently of immense interest in various biotechnology applications.[1] A successful strategy for increasing the interaction strength is to use triplex-specific ligands that bind strongly to triplex but only weakly to duplex DNA. Examples of selective triplex intercalators are quinolines **1**[2] and **2**[3] that have been synthesized and tested by us previously. The terminal dimethylamino group and the quinoline N1 atom in compounds **1** and **2** are protonated under physiological conditions and, due to the cationic nature, these compounds do not intercalate with the cationic C^+·GC triplets.[1–3] As a result, these compounds prefer triplex over duplex and show absolute selectivity for T·AT triplets in the presence of C^+·GC triplets. An ideal T·AT intercalator would not only bind strongly with the triplex but also would show no significant interaction with the duplex. Unfortunately, this is not the case with **1** and **2** and a number of their analogs that show significant binding with duplex DNA.[1–5] Additional triplex stabilizing agents that suffer from substantial affinity toward duplex have been developed by other groups. These are cationic derivatives of benzopyridoindoles, benzopyridiquinoxalines, dibenzophenanthrolines, coralyne, and anthraquinone, as briefly reviewed.[6]



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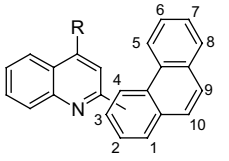

2: 2-phenanthryl, R = HN–(CH2)2–NMe2

3: 2-phenanthryl, R = N(CH2)2N–Me

4: 3-phenanthryl, R = HN–(CH2)2–NMe2

5: 9-phenanthryl, R = HN–(CH2)2–NMe2

Based on the observations described above, a series of new heteroaromatic amines were designed and synthesized. The compounds were designed to test the importance of stacking surface/planar aromatic system, rotational freedom, and charge that can complement the base triplet structure. The position of the cationic groups, which fit into the triplex grooves, was varied to find the optimum interactions for triplex-specific binding. In our search for a rapid and accurate method to evaluate triplex affinity as well as specificity of triplex over duplex binding, we have selected thermal denaturation studies of polydT·dA·dT. This DNA exists as a triplex at low temperature but as the temperature is increased, the third strand dissociates. At higher temperatures the melting temperature of the duplex can be determined in the same experiment. The interaction of a compound can thus be evaluated with both triplex and duplex in a well-controlled experiment by determining its effects on both the triplex and duplex melting temperatures. The method is rapid, accurate, and

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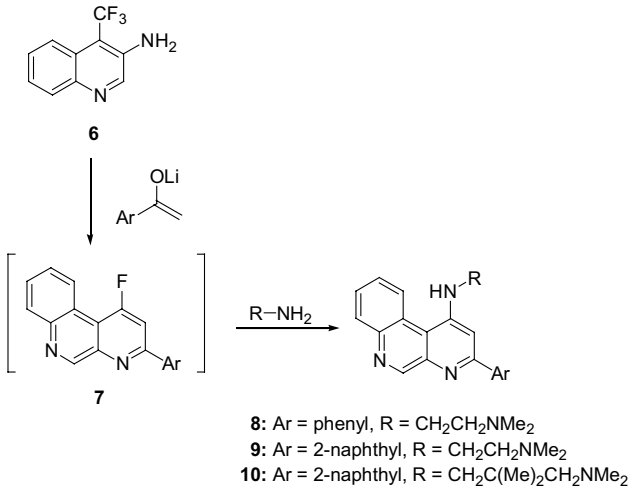
requires very little sample. A large data base of ΔT_m values, determined by this procedure, is available for comparison with new compound results. The correlation between ΔT_m values and fundamental equilibrium measurements has been shown to validate the screening method.[1–7]

1. Chemistry

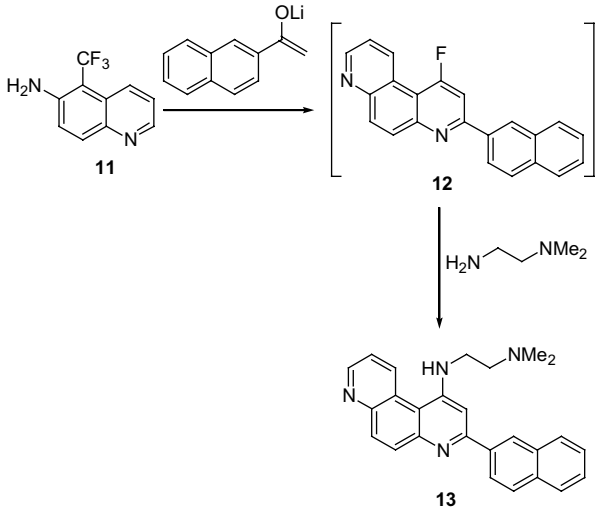
Our previous work has shown that quinoline-4-amines substituted at position 2 with an aryl and at N^4 with an aminoalkyl group, such as **1** and **2**, are good triplex intercalators. As part of this work, additional phenanthryl-substituted quinolines **3**–**5** were synthesized by using the general procedure reported for **1**[7] and **2**.[3]

Compounds **8**–**10** (Scheme 1) and **13** (Scheme 2) are substituted phenanthrolines in which the heteroaromatic subunit is larger than the quinoline in triplex intercalators synthesized by us previously. A simple approach to the synthesis of **8**–**10** is by construction of a 4,6-phenanthroline ring system by the reaction of 4-trifluoromethylquinolin-4-amine (**6**) with a lithium enolate derived from an aryl methyl ketone followed by nucleophilic displacement of fluoride from the resultant 3-aryl-1-fluoro-4,6-phenanthroline **7** by treatment with an amine.[8] A 4,7-phenanthroline derivative **13** was prepared in a similar way from 5-trifluoromethylquinolin-6-amine (**11**). In all these cases crude fluorophenanthrolines **7** and **12** were allowed to react with an appropriate amine, and the final products **8**–**10**, **13** were purified by chromatography (silica gel eluting with hexanes/EtOH/Et_3N, 18:1:1) and subsequent crystallization from hexanes/Et_2O.

Compounds **19**, **22**, **23** contain a nonplanar 5,6-dihydrobenz[*c*]acridine ring system (Scheme 3). In addition, they are at least tricationic under physiological conditions for an increased electrostatic interaction with a highly charged polyanionic triplex DNA. A polyamine-substituted unfused analog **24** (structure in Scheme 3) was also



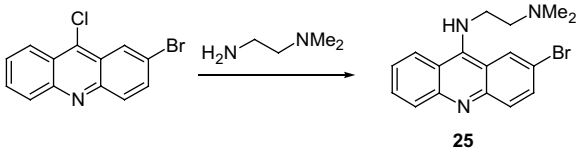
Scheme 1.



Scheme 2.

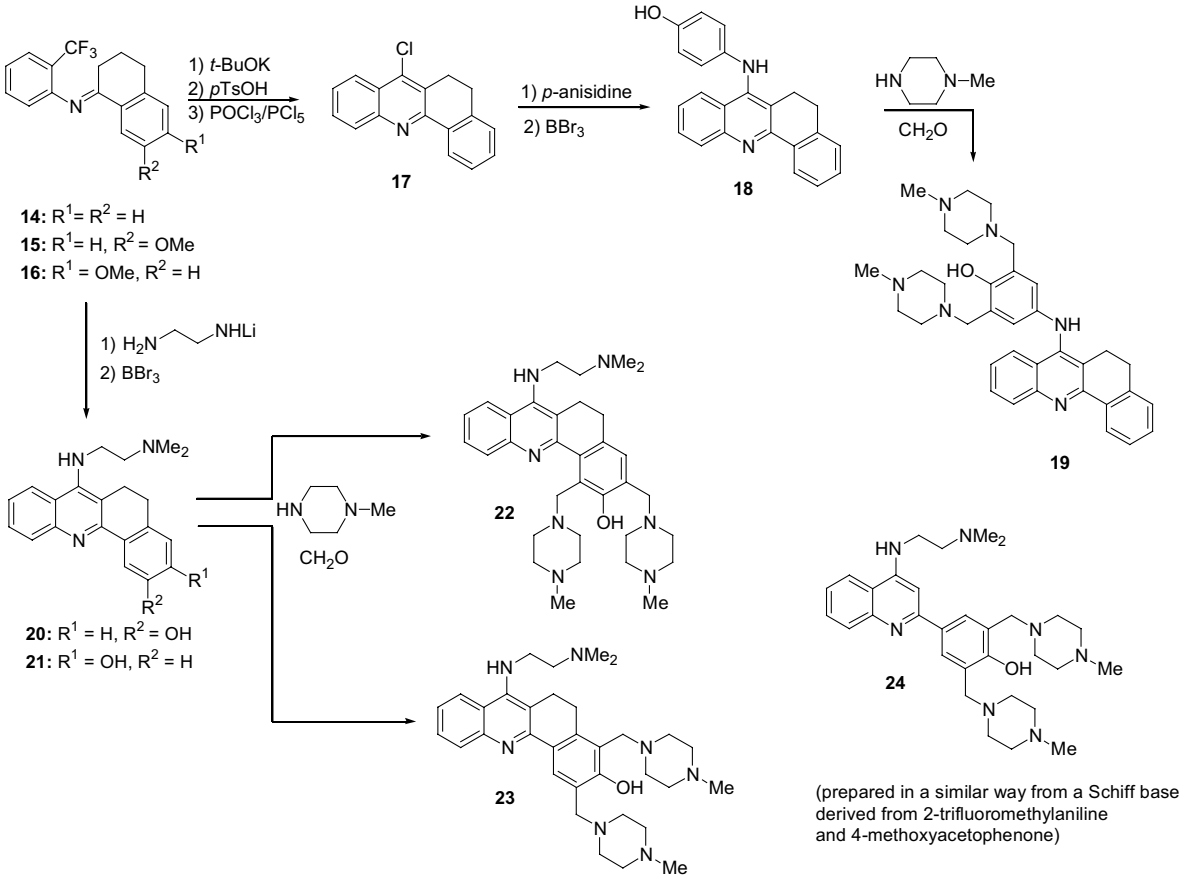
synthesized for comparison. Compound **19** was synthesized starting with Schiff base **14** derived from 2-trifluoromethylaniline and α-tetralone.[9] The intermediate products **17** and **18** were obtained by using well-developed general methodologies.[10,11] A final Mannich reaction of **18** furnished the desired product **19**. A similar strategy was used to prepare compounds **22** and **23** starting with appropriate Schiff bases **15** and **16** derived from 2-trifluoromethylaniline and methoxy-substituted α-tetralones. A Mannich reaction of the intermediate products **20** and **21** yielded the respective products **22** and **23**. The unfused analog **24** was synthesized in a similar way starting with Schiff base obtained by the reaction of 2-trifluoromethylaniline and 4-methoxyacetophenone.

Finally, a simple acridine **25** was prepared as shown in Eq. 1. Since acridines can intercalate efficiently with duplex DNA,[12] it was thought that the presence of a bulky bromine atom in the molecule **25** would inhibit this interaction.



(1)

All products **19**, **22**–**25** were isolated by chromatography as mentioned above, then transformed into hydrobromide salts,[13] and the salts were crystallized from 95% EtOH. All final compounds described in this report gave satisfactory results of elemental analysis, and their structures were fully consistent with MS, ^{1}H NMR, and ^{13}C NMR data. The compositions and mp's (°C) of the analytically pure samples, as used in DNA binding studies, are given as follows: **3**·2HBr·3H_2O, 135–137; **4**·2HBr·3H_2O, 120–122; **5**·2HBr·3H_2O, 110–111; **8**, 142–143; **9**, 169–170; **10**, 139–140; **13**, 160–161; **19**·5HBr·3H_2O, 241–243; **22**·4HBr·2H_2O, 247–248; **23**·6HBr·H_2O, 222–224; **24**·6HBr·3H_2O, 244–245 (dec); **25**·2HBr·1/2H_2O, 250–252.



Scheme 3.

2. DNA binding studies

Interaction of ligands with duplex polyA·polydT and triplex polydA·2polydT were evaluated by UV-thermal experiments under conditions identical with those reported previously for quinolines **1** and **2**, for direct comparison.[2,3] Briefly, compound stabilization of DNA samples was compared by the increase in T_m ($\Delta T_m = T_m$ of the complex $- T_m$ of the free nucleic acid) they produce in PIPES 20 buffer with 0.2 M NaCl (pH = 7.0) at saturating amounts of the compound (a ratio of 0.2 mol of compound to nucleic acid base duplets or triplets). ΔT_m values are reproducible to ±0.5 °C. The T_m of the triplex is 41 °C and of the duplex is 74 °C under these conditions. The results are shown in Table 1. Although the correlation between compound binding affinity and the increase in DNA T_m is not completely linear, the agreement is quite good within any series of compounds. For example, the high selectivity of quinoline **1** toward triplex DNA in the presence of duplex DNA, as derived from competition dialysis experiments, was confirmed by using T_m measurements. Moreover, the relative binding affinities of **1** with duplex and triplex DNA[1] parallel the corresponding T_m values given in Table 1.

As can be seen from Table 1, a 2-(2-naphthyl)quinoline **1** and a 2-(2-phenanthryl)quinoline **2** exhibit virtually identical triplex/duplex selectivities with both compounds showing a substantial stabilization of duplex DNA. The selectivity is slightly improved for the 2- and 9-phenanthryl analogs **3**, **5** of **2** albeit at the expense of a decreased triplex affinity relative to that for **2**. Surprisingly, the 2-(3-phenanthryl)quinoline **4** stabilizes duplex DNA strongly. On the other hand, the phenanthroline derivatives **8, 9**, and **13** show strong

Table 1. T_m increases for interaction of compounds **1**–**5**, **8**–**10**, **13**, **19**, **22**–**25** with triplex polydA·2polydT and duplex polydA·polydT

No	ΔT_m (°C)	
	Triplex	Duplex
1[a]	35.6	5.5
2[b]	35.3	5.2
3	18.6	0.3
4	28.1	13.6
5	15.4	0.5
8	27.5	0.0
9	23.1	0.0
10	8.2	0.0
13	24.0	0.0
19	17.8	0.0
22	10.7	0.3
23	1.6	0.7
24	3.6	0.7
25	16.6	0.0

[a] Taken from Ref. 2.
[b] Taken from Ref. 3.

interaction with triplex DNA with virtually no stabilization of duplex DNA. Although groove binding of these compounds with the duplex cannot be excluded, because of T_m measurement limitations, almost certainly they stabilize triplex DNA by intercalation. The diminished affinity toward the triplex of ligand **10** substituted with a bulky aminoalkyl group is consistent with intercalation of phenanthridine ligands from the narrow minor groove of the triplex. Following intercalation of the phenanthridine system of **10** with triplex, the bulky cationic substituent would interact poorly with the minor groove, thus lowering the complex stability, as observed.

Preliminary molecular modeling of systems related to **1** and **13** (AM1 molecular orbital, Spartan software) indicate correlated differences in twist and stereoelectronic effects in the two compounds. Steric clash between the side chain NH moiety and a proton on the pyrido group of **13** causes the side chain to twist out of the aromatic plane. A much smaller twist is observed for the quinoline system of **1**. In addition, the pyrido group and twist of **13** give it different stereoelectronic and stacking properties than **1**. All of these effects could lead to the observed decrease in triplex T_m for **13**. Clearly, a full understanding of the differences in DNA binding properties of these compounds will require more detailed studies that are in progress.

Compound **19** is also a good triplex stabilizing agent. The ring system of this compound is complementary with the T·A·T base triplet and the cationic group must fit well into a triplex groove. On the other hand, an increased charge concentration by protonation of **22**–**24** as well as unfavorable steric interactions within the triplex grooves results in an adverse effect on triplex stabilization. These polycations apparently bind weakly and externally to both duplex and triplex by electrostatic interaction.[14]

Acridines are known to intercalate with duplex DNA and triplex DNA with little selectivity.[12] In part, the excellent triplex/duplex selectivity of ligand **25** can be explained in terms of the lack of intercalation with duplex, because of the presence of a bulky bromine atom in the molecule, as already suggested.

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