The Search for Structure-Specific Nucleic Acid-Interactive Drugs: Effects of Compound Structure on RNA versus DNA Interaction Strength[†]

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ABSTRACT: The RNA genomes of a number of pathogenic RNA viruses, such as HIV-1, have extensive folded conformations with imperfect A-form duplexes that are essential for virus function and could serve as targets for structure-specific antiviral drugs. As an initial step in the discovery of such drugs, the interactions with RNA of a wide variety of compounds, which are known to bind to DNA in the minor groove, by classical or by threading intercalation, have been evaluated by thermal melting and viscometric analyses. The corresponding sequence RNA and DNA polymers, poly(A)·poly(U) and poly(dA)·poly(dT), were used as test systems for analysis of RNA binding strength and selectivity. Compounds that bind exclusively in the minor groove in AT sequences of DNA (e.g., netropsin, distamycin, and a zinc porphyrin derivative) do not have significant interactions with RNA. Compounds that bind in the minor groove in AT sequences of DNA but have other favorable interactions in GC sequences of DNA (e.q., Hoechst 33258, DAPI, and other aromatic diamidines) can have very strong RNA interactions. A group of classical intercalators and a group of intercalators with unfused aromatic ring systems contain compounds that intercalate and have strong interactions with RNA. At this time, no clear pattern of molecular structure that favors RNA over DNA interactions for intercalators has emerged. Compounds that bind to DNA by threading intercalation generally bind to RNA by the same mode, but none of the threading intercalators tested to date have shown selective interactions with RNA.

RNA viruses are responsible for a number of serious human diseases, and attempts to design drugs against these viruses are proceeding along several lines (Vaishnav & Wong-Staal, 1991; Mitsuya et al., 1990; De Clercq, 1990; Haseltine, 1989; Broder, 1992). The viral genomic RNA is folded into compact conformations with sections of A-form helix separated by single-stranded regions. The helical regions are not perfect and contain loops, bulges, and base-pair mismatches that strongly affect the local RNA conformation (Le et al., 1988; Hauber & Cullen, 1988; Ahmed et al., 1990; Feng & Holland, 1988; Karn et al., 1991; Green, 1991; Bartel et al., 1991; Dayton et al., 1992). In some cases, such as the TAR and RRE RNA sequences of HIV-1, the folded RNA conformational units are specifically recognized by regulatory proteins and are critical for viral replication or other key steps in the life cycle of the virus (Feng & Holland, 1988; Selby et al., 1989; Weeks et al., 1990; Dingwall et al., 1990; Weeks & Crothers, 1991; Karn et al., 1991). The folded RNA conformations, thus, offer attractive potential targets for drug design, and drugs that can bind specifically to such structures can inhibit viral transcription. Although there have been a large number of studies of small molecule–DNA interactions, relatively few studies have been conducted with RNA.

Our experimental approach to the design of structure-specific RNA-interactive antiviral agents involves three steps: (i) discovery of compounds that bind strongly and specifically to RNA duplexes; (ii) modification of the RNA-interactive compounds of (i) to improve their ability to interact with RNA duplexes in general and with specific RNA conformations in particular; and (iii) continued development of the agents such that they will have improved specificity for RNA conformational units, such as those that exist in TAR

or RRE. As part of this process, we have investigated the interaction with corresponding RNA and DNA duplexes of a number of very different compounds that are known to have very different interactions with DNA. The goals are to define a library of molecular structures and substituents that provide specific interactions in RNA complexes and to begin to understand how RNA conformations are selectively recognized by small molecules. Our aim at this stage is to increase the fundamental data base regarding RNA interactions. Thermal melting (T_m) methods have been used for the comparisons (cf. Figure 1) and the results are reported here.

Compound Groups. For comparison purposes, the compounds have been grouped according to their known DNA interaction modes. All compounds are cations, and their structures, arranged by group, are shown in Figures 2–6. The compounds were selected to cover the known types of binding modes and to provide the widest possible range of structural and chemical types of nucleic acid-interactive agents. Where possible, we have included compounds whose DNA complexes have been studied by X-ray and/or high-resolution NMR methods. As indicated above, high-resolution studies of RNA complexes have been much less common to date.

Group I: Groove-Binding Compounds. These compounds (Figure 2) generally have very high AT base-pair specificity as a result of their strong binding in the minor groove in AT-rich DNA sequences (Zimmer & Wahnert, 1986). Crystal structures have been determined for distamycin (1) (Coll et al., 1987) and pentamidine (2) (Edwards et al., 1992), minor groove complexes with DNA oligomers. The cationic steroids, 4–7, originally studied by Gabbay and co-workers (Gabbay & Glasser, 1971), provide compounds of significantly different structure for comparison. The stereochemistry at the cationic centers in the steroids was systematically varied to determine the effects of RNA interactions. The compounds of this group vary in charge from +1 to +4.

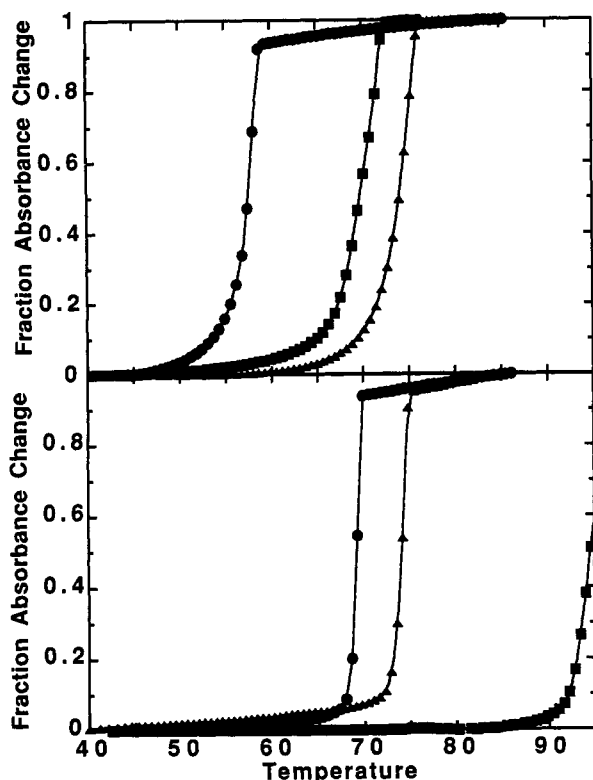* Author to whom correspondence should be addressed.

FIGURE 1: T_m curves for (top) poly(A)·poly(U) (●) and (bottom) poly(dA)·poly(dT) (●) with ethidium from group II (▲) and the diphenylfuran imidazoline from group V (■). Conditions for the experiments are given in the Materials and Methods section.
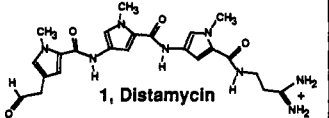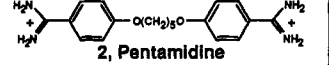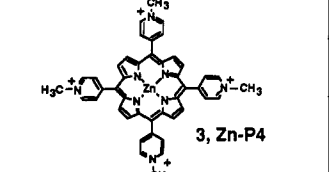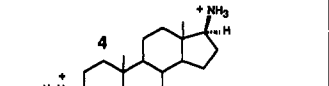
Groove-Binding Compounds
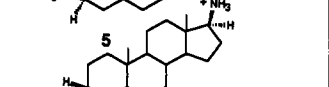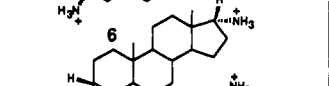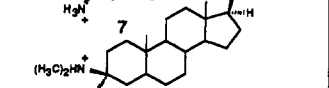
	ΔTm PolyA,U	ΔTm PolydA,dT
1, Distamycin	0.1	21.0
2, Pentamidine	0.0	12.8
3, Zn-P4	-1.5	22.0
4	2.1	13.8
5	0.9	14.4
6	2.5	7.9
7	3.1	16.5

FIGURE 2: Structures for group I compounds.

Group II: Classical Intercalators. These are fused ring aromatic heterocycles of carbocycles that have a positive charge on the ring and/or on a substituent (Figure 3). A large number of different intercalators have been discovered as natural products or have been synthesized, and many representatives are included in this group. Compounds such as ethidium (**8**), ellipticine (**12**), coralyne (**13**), acridine (**10**, **15–17**), and anthraquinone (**9**, **11**) derivatives in this group have significant biological activity against several different diseases (Waring, 1981). As is generally true, intercalators of this group do not have pronounced base-pair interaction specificity (Wilson, 1990; Waring, 1981). They vary in charge from +1 to approximately +4.

Group III: Threading Intercalators. Compounds in this group (Figure 4) have a fused aromatic ring system, as with group II; however, they have bulky and/or polar substituents placed at positions on the ring system, such that at least one of the substituents must pass between base pairs in the duplex to give a final intercalation complex with substituents in both the major and minor grooves (Wilson & Tanious, 1993). As a result, the kinetics of complex association and dissociation with DNA of compounds of this group are much slower than with classical intercalators from group II. The naphthalene diimide **22**, shown in Figure 4, is only one example of a range of compounds of this type that have been synthesized (Tanious et al., 1991; Yen et al., 1982). Crystal structures have been determined for nogalamycin–DNA oligomer complexes (Williams et al., 1990; Liaw et al., 1989).

Group IV: Unfused Aromatic Intercalators. A number of compounds of this group (Figure 5) have been synthesized by Strekowski and co-workers as amplifiers of anticancer drugs such as bleomycin, and some of the compounds have very significant anti-HIV-1 activity (Strekowski et al., 1990, 1991; Wilson et al., 1992). Even though the aromatic systems of these compounds can have a significant twist in the free state, they are able to form strong, stable intercalation complexes (Wilson et al., 1988, 1989; Strekowski et al., 1987). A structure–activity study with a series of cationic 2-arylquinoline unfused intercalators demonstrated that the RNA T_m increase produced by addition of the compounds parallels their anti-HIV-1 activity (Wilson et al., 1992).

Group V: Compounds with Mixed Binding Modes. Compounds of this group (Figure 6) display the most complex interactions with DNA. As with group I compounds, they bind strongly in the minor groove at AT sequences in DNA. Unlike group I compounds, however, most of the compounds in this group also have significant interactions with GC and mixed GC/AT sequences (Wilson et al., 1990, 1992). A switch in binding mode occurs from AT to GC complexes with these compounds since the minor groove in GC regions is wider, less negative, and more sterically hindered by the extra base-pair hydrogen bond than the minor groove in AT sequence regions (Saenger, 1984; Kopka et al, 1985a,b; Pullman & Pullman, 1981). In such sequences, intercalation can become the low-energy complex state for many group V compounds (Wilson et al., 1990, 1992a). Hoechst 33258 (**35**) also has significant binding to GC sequence regions, but apparently not as an intercalator. Although the binding mode of Hoechst 33258 in GC sequences has not been conclusively established, Clegg and co-workers (Looniens et al., 1990) have suggested that the binding mode in such sequences involves a drug dimer that binds in the DNA major groove. All other compounds of this group bind to GC and mixed sequences by intercalation.

MATERIALS AND METHODS

Materials. Poly(dA)·poly(dT) (P-L Biochemicals) and poly(A)·poly(U) (Sigma) were prepared as previously described (Tanious et al., 1992). Low-salt PIPES buffer adjusted to pH 7.0 contained 1×10^{-2} M piperazine-N,N'-bis(2-

ethanesulfonic acid) and 1×10^{-3} M EDTA. PIPES 10 buffer has 0.1 M NaCl added to the low-salt PIPES buffer with the pH adjusted to 7.0 and is the buffer for all experiments unless otherwise indicated.

Compounds. Group I: Groove-Binding Compounds. Distamycin (**1**) and Zn-P4 (**3**) were purchased from Sigma and Midcentury Chemical Company, respectively. Pentamidine was a gift from R. Tidwell of the University of North Carolina. Compounds **4-7** were prepared as previously described (Gabbay & Glasser, 1971).

Group II: Classical Intercalators. Ethidium (**8**), quinacrine (**10**), adriamycin (**11**), ellipticine (**12**), and proflavine (**15**) were purchased from Sigma. Acridine orange (**16**), acridine yellow G (**17**), toluidine blue O (**18**), methylene blue (**19**), and tilorone analog R11645DA (**20**) were purchased from Aldrich. Mitoxantrone (**9**) was a gift from Dr. K. C. Murdock, Lederle Laboratories, Pearl River, NY (Murdock et al., 1979). Coralyne (**13**) (Gough et al., 1979) and diazaphenanthrene (**14**) (Molock & Boykin, 1983) were prepared as previously described.

Group III: Threading Intercalators. Nogalamycin (**23**) was purchased from Sigma. Bisantrene (**21**) was a gift from Dr. K. C. Murdock, American Cyanamid Company. The naphthalene diimide **22** was prepared as previously described (Tanious et al., 1991).

Group IV: Unfused Aromatic Intercalators. Compounds **24** (Wilson et al., 1988), **25, 26** (Wilson et al., 1989), and **27-29** (Strekowski et al., 1991) were prepared as previously described. Compound **30** was synthesized at Georgia State University and will be reported elsewhere.

Group V: Compounds with Mixed Binding Modes. DAPI (**31**), Hoechst 33258 (**35**), and Ni-P4 (**36**) were purchased from Boehringer Mannheim Biochemicals, Aldrich, and Midcentury Chemical Company, respectively. The diphenylfuran amidine (**32**), imidazoline (**33**), and tetrahydropyrimidine (**34**), were prepared as previously described (Das & Boykin, 1977).

Methods. Thermal melting curves for DNA, RNA, and their complexes were determined as previously described (Kibler-Herzog et al., 1990) by following the absorption change at 260 nm as a function of temperature. T_m values were determined from first derivative plots. Compounds are 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 10 buffer at saturating amounts of the compound (a ratio of 0.3 mol of compound to nucleic acid bases) unless otherwise indicated.

Viscometric titrations were conducted in an Ubbelohde semimicro dilution viscometer (Cannon Series #75 viscometers) as previously described (Jones et al., 1979). One milliliter of polymer solution, approximately 1×10^{-4} M poly-(A)·poly(U) bases, was placed in the viscometer, and titrations were conducted in a constant-temperature water bath (Cannon Instrument Co.) by adding aliquots of a stock solution of the compounds. The additions were made directly into the poly-(A)·poly(U) solution by using a Hamilton syringe modified to fit into the viscometer mixing chamber (Jones et al., 1979).

RESULTS

Results will be presented by the compound groups defined above. T_m comparisons have been conducted primarily with the RNA polymer, poly(A)·poly(U), and the corresponding sequence DNA, poly(dA)·poly(dT). Typical T_m curves for the RNA and DNA polymers alone and with compounds from groups II and V are shown in Figure 1. A number of compounds were studied that had very small ΔT_m values with

both DNA and RNA in PIPES 10 buffer, and these results are not included here unless they illustrate some specific point. Frequently where a number of closely related derivatives were studied, a single example is given for illustration in the figures.

Group I: Groove-Binding Compounds. These compounds (Figure 2) have high ΔT_m values with poly(dA)·poly(dT) as expected from their known AT-specific minor-groove-binding mode with DNA. The results with the RNA polymer duplex are strikingly different, with very weak to no binding by the compounds. The slight decrease in T_m observed with the tetracationic zinc porphyrin, **3**, suggests that it may actually interact more favorably with RNA single strands than with the duplex. T_m values for the dicationic steroids, **4-7**, were determined in the low-salt PIPES buffer to keep the DNA ΔT_m values similar to those for other compounds in this group. The steroids also interact more strongly with AT sequences in DNA than with RNA.

Group II: Classical Intercalators. These molecules are intercalators with DNA, and viscometric titrations with poly(A)·poly(U) indicate that they also bind by intercalation. Representative results for RNA viscometric titrations are shown in Figure 7. Compounds of this group display the greatest variation in DNA and RNA ΔT_m ratio. The ΔT_m values for RNA range from 1 to 17 °C and for DNA from 4 to 23 °C. The ratio of $\Delta T_m(\text{RNA})/\Delta T_m(\text{DNA})$ varies from 2.4 for ethidium (**8**) to 0.09 for adriamycin (**11**) and 0.08 for coralyne (**13**). Ethidium has a significantly higher ΔT_m value for this RNA sequence than for DNA (Figures 1 and 3), while ellipticine (**12**), proflavine (**15**), and acridine orange (**16**) have similar ΔT_m values for DNA and RNA (Figure 3). All other compounds of this group have significantly more favorable interactions with DNA than with RNA. It is clear from these results that intercalators, in general, have much stronger binding to RNA than group I compounds. A number of additional acridines and carbocycles including anthraquinone derivatives were studied with results similar to the example compounds shown in Figure 3. Of the compounds in this group examined to date, ethidium has the most favorable RNA to DNA ΔT_m ratio. Quite a number of compounds in this and the other groups have ratios of $\Delta T_m(\text{RNA})/\Delta T_m$-(DNA) of 0.5 or lower (for example, in group II, mitoxantrone (**9**), quinacrine (**10**), tilorone analog **20**, and acridine yellow G (**17**) all have ratios near 0.5). We have chosen a ratio greater than 0.5 as an operational cutoff point, and only compounds with ratios above 0.5 will be considered to have significant RNA interactions that merit further study.

Group III: Threading Intercalators. Viscosity studies with this group (representative curves in Figure 7) also indicate that they intercalate with RNA as they do with DNA. Bisantrene (**21**) and the naphthalene diimide **22** of this group (Figure 4) show significantly stronger interactions with DNA than with RNA. Results with several other naphthalene diimides (not shown) were similar to those for the compound in Figure 4. Nogalamycin (**23**) ΔT_m values are also reported in the low-salt PIPES buffer since they were close to zero in PIPES 10. The compounds in this group bind to RNA in the same general range as the classical intercalators in group II.

Group IV: Unfused Aromatic Intercalators. As with intercalators in groups II and III, viscosity increases with group IV compounds indicate that they bind to RNA by intercalation as with DNA. The ΔT_m values (Figure 5) for compounds of this group are either greater for DNA than for RNA (**26** and **27-29**) or similar for DNA and RNA (**24, 25,** and **30**). No strong RNA specificity has been discovered for compounds of this group, but they show enhanced relative

Classical Intercalators. A

	ΔTm PolyA.U	ΔTm PolydA.dT
8, Ethidium	17.3	7.2
9, Mitoxantrone	11.1	22.7
10, Quinacrine	6.6	14.7
11, Adriamycin	1.2	13.1
12, Ellipticine	10.1	11.4
13, Coralyne	1.5	18.2

Classical Intercalators. B

	ΔTm PolyA.U	ΔTm PolydA.dT
14, Diazaphenanthrene	15.7	21.8
15, Proflavine	7.1	6.2
16, Acridine Orange	6.0	4.1
17, Acridine Yellow G	4.3	8.8
18, Toluidine Blue O	2.0	6.0
19, Methylene Blue	1.6	4.1
20, Tilorone Analog R11645DA	13.2	22.5

FIGURE 3: Structures for group II compounds.

Threading Intercalators

	ΔTm PolyA.U	ΔTm PolydA.dT
21, Bisantrene	10.5	25.2
22, Naphthalene Diimide	2.8	4.4
23, Nogalamycin	0.3/7.8	0.1/7.5

FIGURE 4: Structures for group III compounds.

Unfused Aromatic Intercalators

	ΔTm PolyA.U	ΔTm PolydA.dT
24	5.5	7.7
25	7.3	9.2
26	1.8	9.5
27	1.1	2.7
28	2.2	4.7
29	6.3	11.4
30	6.0	7.2

FIGURE 5: Structures for group IV compounds.

interactions with RNA when compared to all of the inter-calators in groups II and III, and it is clear that this group deserves a more detailed investigation.

Group V: Compounds with Mixed Binding Modes. The compounds of this group bind strongly in the minor groove of AT sequences of DNA, and as with group I compounds, they have high ΔT_m values with poly(dA)·poly(dT) (Figures 1 and 6). Unlike group I compounds, however, these compounds also have significant RNA interactions (Figures 1 and 6). Viscosity increases in titrations of poly(A)·poly(U) with these compounds indicate that, except for Hoechst 33258 (35), they bind to RNA by intercalation as they do with GC

sequences of DNA. Detailed viscosity, NMR, and kinetics results with DAPI have clearly shown that it intercalates with the RNA polymer in a manner similar to its interactions with GC sequences in DNA (Tanious et al., 1992). The RNA viscosity decreases on addition of Hoechst 33258 (Figure 7), and it appears that this compound has an external interaction with RNA that may be similar to its interactions with GC-rich sequences in DNA (Loontiens et al., 1990). In the diphenylfuran series, 32–34, synthesized by Boykin and co-workers (Das & Boykin, 1977), the ΔT_m values for RNA are very dependent on the cationic substituent, while the DNA interactions are much more similar for the derivatives. The compounds of this group have some of the highest RNA ΔT_m values observed, but they also have high ΔT_m values with poly(dA)·poly(dT) due to their favorable AT-specific binding
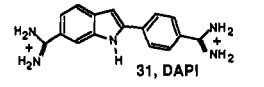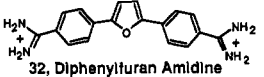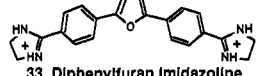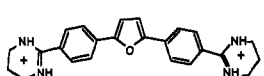
Compounds With Mixed Binding Modes
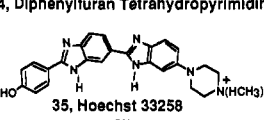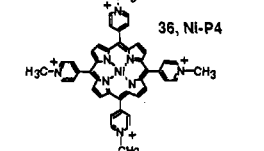
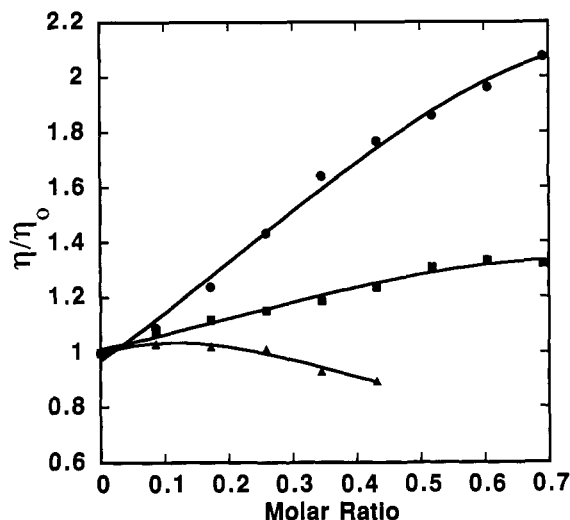|	ΔTm PolyA.U	ΔTm PolydA.dT
31, DAPI	3.9	>25
32, Diphenylfuran Amidine	5.7	24.6
33, Diphenylfuran Imidazoline	14.4	>25
34, Diphenylfuran Tetrahydropyrimidine	2.5	26.5
35, Hoechst 33258	17.5	>25
36, Ni-P4	18.2	>25

FIGURE 6: Structures for group V compounds.

FIGURE 7: Viscometric titrations of poly(A)·poly(U) with ethidium from group I (●), naphthalene diimide from group III (■), and Hoechst 33258 from group V (▲). Titrations were performed as described in the Materials and Methods section.

in the DNA minor groove. Their interactions in GC regions of DNA are much weaker (Wilson et al., 1992a).

DISCUSSION

The compounds in groups I–V are classified by their DNA-binding modes, and all either intercalate or bind in the minor groove of DNA in AT-rich regions. The nature of the minor groove in GC sequences in DNA is quite different from the AT groove (Saenger, 1984; Kopka et al., 1985a,b; Pullman & Pullman, 1981), and group I compounds do not bind significantly in GC sequences. It is clear from the ΔT_m values for group I complexes with poly(A)·poly(U) and poly-(dA)·poly(dT) that compounds which have no important interaction with DNA other than the minor-groove-binding mode do not bind significantly to RNA duplexes. Given the very different conformations and electrostatic potentials of

the grooves of A-form RNA and B-form DNA duplexes, the lack of binding of group I compounds to RNA duplexes is not surprising. The minor groove in the A-form family of RNA duplexes is shallow and wide while the major groove is narrow and deep relative to the DNA grooves. The grooves in DNA and RNA have the same chemical groups, except that the sugar 2′-H in DNA is replaced by a 2′-OH in RNA and the T-CH$_3$ group is replaced by an H in uracil (Saenger, 1984). The compounds of group I provide a high-resolution paradigm for minor groove interactions at AT sequences in DNA duplexes, but they do not provide useful information for the development of RNA groove-binding agents.

It is interesting that no classes of small molecules have been defined that bind strongly in the minor groove of RNA or in the major groove of either RNA or DNA. The question of what types of small molecules bind in the grooves of RNA is extremely important for the design of RNA-interactive drugs. There are no outstanding paradigms at this point to suggest design directions for RNA groove-binding drugs. Barton and co-workers (Mei & Barton, 1988) have proposed that tris-(tetramethylphenanthroline)ruthenium(II) has a surface interaction mode in the A-form duplex minor groove, but the binding is weak and modifications that can increase the binding strength while maintaining the specificity of such compounds are not clear. The deep, narrow major groove of RNA may serve as a good drug-binding site if appropriate molecules can be found to match the groove shape and chemical groups. We are using molecular modeling methods in efforts to design such molecules for synthesis.

As with group I, group V compounds bind in the DNA minor groove at AT sequences; however, these compounds have significant alternative binding modes in GC or mixed AT/GC sequences. DAPI (31), the diphenylfurans 32–34, Ni-P4, (36), and related molecules bind to DNA by an intercalation mode in GC-containing sequences (Wilson, 1990). Evidence now indicates that these compounds bind to AU, and probably all RNA sequences, by intercalation, the binding mode at GC sequences in DNA (Tanious et al., 1992). These observations agree with our developing understanding of the sequence dependence of groove structure and interactions in nucleic acids. As discussed above, the minor groove in AT regions in DNA has the appropriate width and chemical and physical characteristics to allow strong interactions with group I and group V compounds. The minor groove in GC regions of DNA and in all RNA sequences, however, does not have properties appropriate for forming strong complexes with these compounds (Saenger, 1984; Kopka et al., 1985a,b; Pullman & Pullman, 1981). Group I molecules do not have other strong modes available and have no significant binding to RNA, while the group V compounds discussed above can switch to an intercalation binding mode in RNA and, thus, can have high ΔT_m values in RNA complexes.

The intercalation binding mode with RNA is clearly more sensitive to substituent changes in group V compounds than the DNA minor-groove-binding mode, as can be seen by comparing ΔT_m changes for the diphenylfuran derivatives. The reasons for the improved binding of the imidazoline diphenylfuran 33 are not clear, but we are synthesizing other imidazoline derivatives to determine whether they show strong RNA interactions. We are also pursuing a strategy to improve the RNA selectivity of these molecules by attaching substituents to the inner face of the molecule that contacts the floor of the minor groove in AT sequences. Such substituents may prevent the strong minor groove interactions in DNA, but

need not interfere with, and could enhance, the formation of an intercalation complex in RNA.

Hoechst 33258 (**35**) in group V also forms a strong minor groove complex with AT sequences in DNA and has a high ΔT_m with RNA; however, the extended structure of this compound makes it unlikely that it binds strongly to DNA or RNA sequences by intercalation. It has been suggested that Hoechst 33258 forms a self-associated complex in the major groove of GC sequences in DNA (Loontiens et al., 1990), and a Hoechst 33258 complex may bind in a similar manner to RNA or alternatively could form a surface complex in the wide minor groove of RNA. Wemmer and co-workers [Pelton and Wemmer (1990) and references therein] have shown that distamycin (**1**) and related compounds can form a dimer complexes in the minor groove of extended AT sequences in DNA, but the vastly different structures of the DNA and RNA grooves indicate that a different complex would be necessary to bind strongly in the A-form RNA duplex. We are synthesizing analogs of Hoechst 33258 to investigate the RNA interactions of this compound in more detail.

Groups II–IV contain compounds that intercalate with DNA, but have significantly different complex geometries for compounds in the different groups. Classical intercalators in group II have 3–4 fused aromatic rings that stack with nucleic acid base pairs in the intercalation complex (Wilson, 1990). Compounds such as ethidium (**8**), quinacrine (**10**), adriamycin (**11**), and mitoxantrone (**9**) have bulky side chains that can extend into either of the DNA grooves. Some of these compounds have strong RNA interactions, but no clear pattern that defines specific groups that provide strong RNA interactions has emerged. Both ethidium and proflavine, which have amino groups on the long axis of the aromatic system that can interact with the nucleic acid backbone, have selectivity in binding to poly(A)·poly(U). Acridine orange (**16**), which has the amino groups methylated, also shows some RNA selectivity while acridine yellow G (**17**) with amino and methyl substituents on the aromatic ring long axis has stronger binding to DNA than to RNA. Ellipticine (**12**) and mitoxantrone (**9**) have similar binding to RNA, but mitoxantrone binds much more strongly to DNA. Helene and co-workers (Mergny et al., 1992) have recently shown that a pyridoindole analog of ellipticine binds selectively to a triple-helical nucleic acid. Since the triple helix has some A-form-like properties (Arnott & Selsing, 1974), members of this class of compounds may show general A-form recognition capability in RNA and in some DNA systems.

Ethidium and the diazaphenanthrene **14** in group II also bind similarly to RNA, but the diazaphenanthrene has much stronger binding to DNA. The diazaphenanthrene has an approximately +4 charge in the PIPES buffer, and much of its binding energy comes from electrostatic interactions, while ethidium has a +1 charge and much more of its RNA interaction energy comes from other sources such as van der Waals, hydrogen-bonding, and hydrophobic interactions. Phenanthridinium derivatives related to ethidium are clearly of interest for the development of RNA-specific antiviral drugs. It is significant that ethidium has shown antiviral activity against murine sarcoma virus (Roa & Bose, 1974), avian sarcoma virus (Guntaka et al., 1975), avian myeoloblastosis virus (Sarih et al., 1980), and C-type virus (Avery & Levy, 1979). We have tested a number of anthraquinone derivatives related to adriamycin (**11**) and mitoxantrone (**9**), and all show significantly better binding to DNA than to RNA.

Threading intercalators in group III intercalate with RNA as with DNA, but of the compounds that we have tested, none show strong RNA-selective interactions. It is interesting that mitoxantrone and bisantrene (**21**), which bind strongly to DNA in different complex geometries, have very similar interaction strengths with RNA. Mitoxantrone has its two charged side chains in the same groove in its nucleic acid complexes (Lown & Hanstock, 1985), while bisantrene has its charged side chains in opposite grooves (Elliott et al., 1989). We had hoped that one of these geometries would provide enhanced RNA interactions, but both of these compounds bind preferentially to DNA. A number of naphthalene diimide threading intercalators have also been tested in group III, and all have stronger binding to DNA than to RNA. It would seem that threading intercalators could favorably exploit the difference in DNA and RNA dynamics and/or groove characteristics to give enhanced RNA interactions, but the agents now in group III do not meet this goal.

Unfused aromatic intercalators in group IV are of particular interest for development as anti-HIV-1 agents for several reasons: (i) initial compounds have shown very promising anti-HIV-1 activity; (ii) in a series of 2-arylquinolines, the RNA ΔT_m is strongly correlated with the anti HIV-1 activity; and (iii) Strekowski and co-workers have devised methods for the facile synthesis of a broad range of unfused aromatic intercalators (Strekowski et al., 1990, 1991; Wilson et al., 1992b). Compounds **24**, **25**, and **30** in group IV, which have two cationic substituents on the outer edge of the aromatic system, have approximately equal RNA and DNA ΔT_m values. Compounds **26–29** have the charged groups on the molecular short axis and generally have DNA ΔT_m values that are about twice the RNA ΔT_m values. Additional derivatives related to **24**, **25**, and **30** are being prepared in efforts to enhance further the RNA interaction specificity of this class of compounds.

The results reported here significantly increase our knowledge of small-molecule interactions with RNA and lead directly to several important conclusions. First, no small molecules have yet been identified that have as strong and selective interactions in either of the RNA grooves as group I compounds have in the DNA minor groove. Second, fused ring, threading, and unfused ring DNA intercalators all intercalate with the A-form RNA duplex. Some of these compounds show a moderate preference for binding RNA over DNA, but to increase the selectivity, more complex compounds will be necessary. Third, some compounds that have different binding modes to AT and GC sequences in DNA display very promising RNA interactions. If the strong AT-binding interactions of these compounds can be reduced while the strong RNA interactions are maintained, a high level of RNA selectivity can be achieved. Syntheses of compounds to test these ideas are in progress.

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