Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 24, pp. 2913-2918, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00441-2

Dicationic 2,4-Diaryl Pyrimidines as DNA Selective Binding Agents

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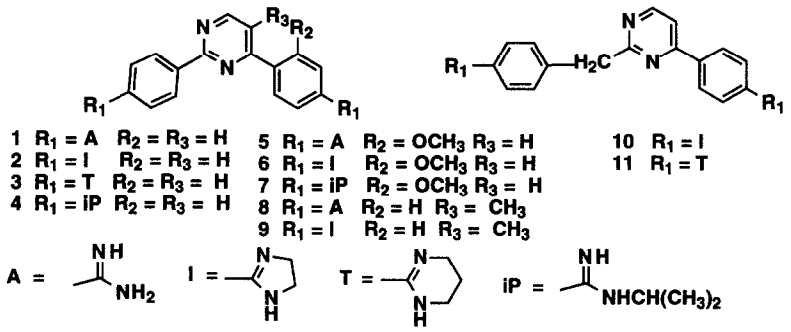
Abstract. A series of twelve 2,4-diaryl pyrimidines were synthesized. The interaction of these molecules with DNA was evaluated by study of their binding to the DNA duplex polymer poly dA•poly dT and the analogous RNA duplex polymer poly A•poly U which serves as a control for binding to non-specific nucleic acid sites.

A number of different types of small molecules are known to bind to the minor-groove of DNA at AT rich sequences.[1] Because a detailed understanding of the factors which influence the molecular basis for specific minor-groove interactions is missing, extensive studies by both theoretical[2] and experimental approaches are ongoing.[3] One important class of minor-groove binding molecules is the dicationic diarylheterocyclic systems which are of interest because of their wide range of chemotherapeutic properties including anti-tumor, anti-viral and anti-microbial activity.[4] Certain of these dicationic diaryl compounds are also of current interest as a consequence of their effectiveness against AIDS associated opportunistic diseases.[5] The minor groove of DNA has been shown to be the primary receptor for the biological action of compounds of this class, and it is essential to understand the DNA interactions in detail for the design of improved compounds.

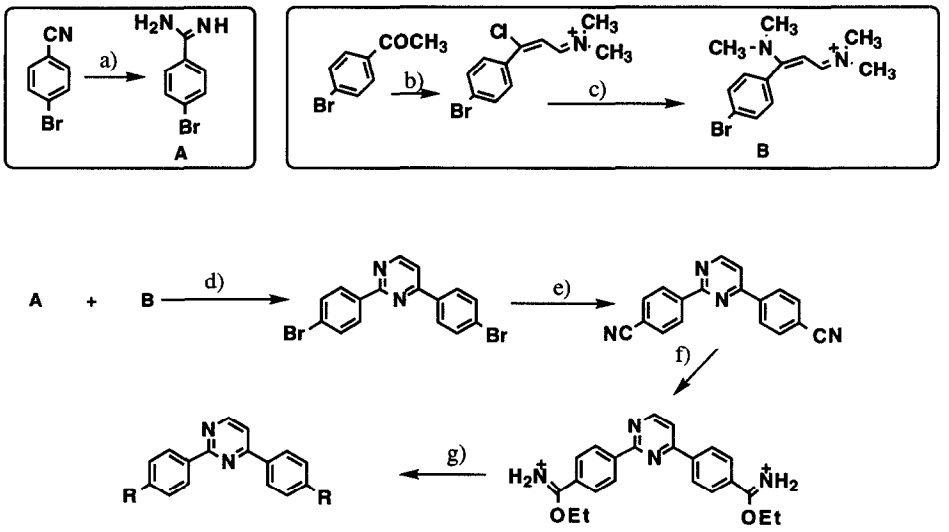
Several of the dicationic diaryl substituted heterocyclic systems have been found to bind to nucleic acids by different and often competing binding modes that include minor-groove interactions at AT rich sequences and intercalation at GC sites.[6] Previous NMR and kinetic studies have demostrated that 2,4-bis-[4-(imidazolin-2-yl)phenyl] pyrimidine (**2**) and related compounds bind in the minor-groove in AT sequences of DNA.[7] The twist of the 2,4-diaryl groups of **2** is thought to be an important factor in its greater binding affinity to poly $d(A\text{-}T)_2$ than an analogous diaryl triazine.[7,8] We have synthesized dicationic 2,4-diaryl pyrimidines which have different types of cationic centers on the 2,4-diaryl rings(**1-4**), which have *ortho*-methoxy groups on the 4-phenyl group(**5-7**) and which have a methyl group on the 5-position of the pyrimidine ring(**8-9**). Also, two dicationic pyrimidines which have a 2-(4-substituted) benzyl group have been synthesized(**10-11**). These four series of dicationic pyrimidines are predicted to exhibit different twist angles, and a comparitive study of their binding to DNA will provide information regarding the importance of conformational twist on their minor-groove binding affinity.

The synthesis employed for the preparation of **1- 4** is outlined in Scheme 1. The reaction to form 2,4-bis(4-bromophenyl)pyrimidine employs as the key step for pyrimidine formation, the approach used by Jutz[9] which gives good yields(86%) and uses the base promoted condensation of 4-bromobenzamidine with



1-dimethylamino-3-dimethyliminonio-1-(4-bromphenyl)propene. Conversion of 2,4-bis(4-bromophenyl) pyrimidine into the corresponding bis-nitrile and ultimately to the dicationic pyrimidines employs standard methodology which we have used extensively.[10,11]

SCHEME 1

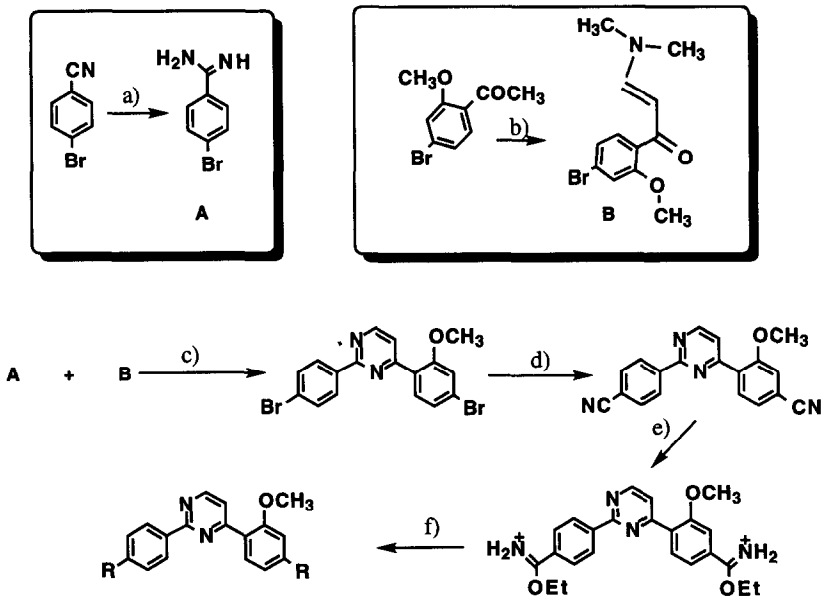


a) $C_6H_5SO_3NH_4$, 265°C,NaOH b)DMF,$POCl_3$ c) $(CH_3)_2NH$ d)NaOEt,EtOH e) CuCN,DMF
f) HCl,EtOH g) NH_3, $NH_2CH_2CH_2NH_2$, $NH_2CH_2CH_2CH_2NH_2$, or NH_2-iPr.

The synthesis of the methoxysubstitued pyrimidines **5-7** is outlined in Scheme 2. The requisite 2-methoxy-4-bromoacetophenone was prepared in three steps from 3-bromophenol by acetylation, followed by Fries rearrangement, and finally, methylation of the resultant 4-bromo-2-hydroxyacetophenone. In this case

pyrimidine formation is achieved by base promoted condensation between 4-bromobenzamidine and 3-dimethylamino-1-(4-bromo-2-methoxyphenyl)propenone. The conversion of 2-(4-bromophenyl)-4-(2-methoxy-4-bromophenyl)pyrimidine into the dicationic target compounds **5-7** was achieved using the approach we have described previously for this type conversion.[10] The synthesis of the 5-methyl-2,4-diarylpyrimidines **8** and **9** was accomplished by using an approach analogous to that outlined in Scheme 2, except 4-bromopropiophenone was used instead of 2-methoxy-4-bromoacetophenone. The two substituted benzyl pyrimidines **10** and **11** were prepared using the approach described in Scheme 1, except 4-bromophenylacetamidine was employed instead of 4-bromobenzamidine.

SCHEME 2

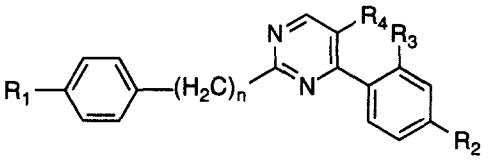


a) $C_6H_5SO_3NH_4$, 265°C,NaOH b) $(CH_3O)_2CHN(CH_3)_2$ c) NaOEt,EtOH d) CuCN,DMF
e) HCl,EtOH f) NH_3,$NH_2CH_2CH_2NH_2$, or NH_2-iPr.

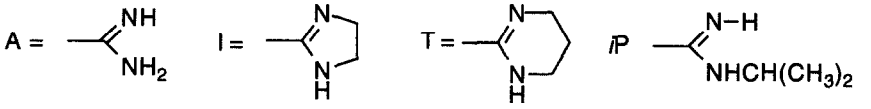
Table 1 contains the results obtained from study of the thermal melting of the pyrimidines **1-12** with an RNA model poly A•poly U and with a DNA model of corresponding sequence poly dA•poly dT. The increase in melting temperatures (ΔTm) on complexation with the various pyrimidines are related to the binding affinities of these molecules with nucleic acids.[12] It is apparent that two cationic centers are essential in order to observe significant nucleic acid binding by the diarylpyrimidines since compound **12**, a monocation, gives a negligibily small Tm increase with the nucleic acids. Elimination of one of the charged groups also results in reduction of the

number of potential hydrogen bond donors by half and consequently, the reduction in the ΔT_m value can be attributed to elimination of charge and hydrogen bonding interactions.

Table1. Nucleic Acid Binding Results for 2,4-Diaryl Pyrimidines



Compound No.	n	R_1	R_2	R_3	R_4	ΔTm(R)[a]	ΔTm(D)[b]
1	0	A	A	H	H	4.5	21.5
2	0	I	I	H	H	12.8	22.7
3	0	T	T	H	H	1.1	>25
4	0	*i*P	*i*P	H	H	-	-
5	0	A	A	OCH_3	H	2.2	14.7
6	0	I	I	OCH_3	H	2.2	18.9
7	0	*i*P	*i*P	OCH_3	H	0 9	13.9
8	0	A	A	H	CH_3	1.2	13.5
9	0	I	I	H	CH_3	6.7	14.9
10	1	I	I	H	H	0	5.1
11	1	T	T	H	H	0	7.5
12	0	H	I	H	H	1.3	0.6



a) PolyA.polyU, an A-form RNA model system.[3] b) PólydA.polydT, a B-form DNA model system.[3]

Molecular mechanics calculations[8] predicts that the torsion angles between the 4-aryl group and the pyrimidine ring of **1-4** are approximately 27 degrees. It was suggested that such a twist complements that of the DNA minor-groove and contributes to the greater binding affinity of **2** in comparison to an analogous triazine for which the analogous twist angle is near zero.[7] The twist angles for **5-7** are estimated to be approximately 50 degrees which may account for the smaller ΔTm values on binding with poly dA·dT. The magnitude of the decline in ΔTm values for **5** and **6** compared to **1** and **2** is 6.8 and 8.0, respectively. The MM2 estimated torsion angles for the 5-methylpyrimidines **8** and **9** are approximately 53 degrees. The ΔTm values for **8** and **9** are about 8 degrees lower than those of **1** and **2**. The twist angles for the methoxyarylpyrimidines **5-7** and the 5-

methylpyrimidines **8-9** are essentially equivalent as are the binding affinities of these compounds for DNA. Analysis of all DNA binding results for minor-groove interactions of the dicationic 2,4-diarylpyrimidines suggests that there is an optimum twist between the aryl and 6-membered-heterocyclic rings of 20-30 degrees for maximizing the compound-minor groove contacts. Compounds with lower or higher twist angles have reduced ΔTm values for DNA binding.

The 2-benzyl-4-arylpyrimidines **10** and **11** present different geometry in comparison to their 2,4-diaryl analogs. From the MM2 minimized structure of **10** it is apparent that the 4-aryl ring and the pyrimidine ring are approximately coplanar, however, the benzylimidazoline system is almost perpendicular to the approximate plane of the other two rings. As a consequence of their unfavorable geometry, which leads to clash of the benzylimidazoline with the walls of the minor-groove binding site in DNA, a greatly reduced fit for **10** and **11** on binding to the minor-groove of DNA is expected. On inspection of the minimized structure, it is apparent that the binding interactions of **10** and **11** with DNA are not as favorable as those for the 2,4-diaryl analogs. Despite the fact that the radius of curvature for **10** and **11** is approximately 10A°, in the range believed to allow favorable binding interactions with the minor-groove of DNA,[13] the ΔTm values for these two compounds are significantly reduced, 5.1 and 7.5 degrees respectively, and support the conclusions drawn from the modeling studies.

As indicated by the ΔTm values the bis-imidazoline pyrimidine **2** binds strongly to RNA as well as DNA, other related imidazoline compounds are suggested to bind to RNA by intercalation.[14] The binding results for the compounds in Table 1 which are structural modifications of **2** should give insight into the RNA binding mode for these type dications. The compounds in Table 1 which exhibit low ΔTm values (e.g. **3,5,6,7,8**) on binding to RNA all have unfavorable steric interactions with the intercalation cavity; usually a result of deviations from planarity between the pyrimidine and the 2- and/or 4- aryl rings or from lack of planarity of the cationic groups. The dicationic pyrimidines which exhibit the largest ΔTm values with RNA are the imidazoline compounds **2** and **9** and they appear to more favorably fit the intercalation cavity. The lower ΔTm value for **9** is consistent with the larger twist angle between the pyrimidine and the 4-aryl ring. Interestingly, the analogous *ortho*-methoxy compound **6** has a calculated twist angle approximately the same as that of **9** yet the ΔTm value for **6** is even smaller(ca. 2 degrees). The lower ΔTm value is consistent with the observation that the methoxy group protrudes from the plane of the 4-aryl ring and would unfavorably clash with the aromatic rings of the base pairs in the intercalation cavity. Collectively, the RNA binding results listed in Table 1 for the variously modified 2,4-diarylpyrimidines are consistent with the intercalation binding mode.

This work provides the foundation of an understanding of the interplay between torsion angle and substituent steric requirements for dicationic diaryl heterocycles which allow such molecules to recognize the DNA minor-groove. Further, these results demonstrate that design of dicationic molecules which bind to the minor-groove of DNA without significant RNA binding can be achieved by providing groups which clash with the bases of the RNA intercalation cavity but can be accomodated in the DNA minor-groove.

Acknowledgment

This work was supported by NIH Grants NIAID AI-27196 and AI-33363. The Chemical Instrumental Program of NSF (CHE 8409599) provided partial support for acquisition of the Varian VXR400 spectrometer.

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(*Received in USA* 3 *October* 1994; *accepted* 14 *November* 1994)