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Chiral discrimination in binding of enantiomers of 2-(aminoalkoxy)-substituted 4-(2-thienyl)pyrimidines and 4,6-bis(2-thienyl)pyrimidines with duplex DNA

Lucjan Strekowski,* Marek T. Cegla, Vidya Honkan, Henryk Buczak, W. Rucks Winkeljohn, Alfons L. Baumstark and W. David Wilson*

Department of Chemistry, Georgia State University, Atlanta, GA 30302-4098, USA

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Abstract—Thienylpyrimidines substituted at position 2 of the pyrimidine with a chiral aminoalkoxy group were synthesized. Upon interaction with duplex DNA, the unfused heteroaromatic system of these compounds intercalates with DNA base pairs and the protonated side chain is located in the major groove. The *S*-enantiomers bind more strongly than their *R*-counterparts with enantiomeric discrimination, as measured by a ratio of binding constants K_S/K_R, ranging from 1.2 to 2.4.

Because of the inherent chirality of DNA, its interaction with nonracemic chiral ligands gives rise to distinguishable diastereomers. Attempts have been made to exploit the chiral discrimination of DNA as a means to gain insight into the mechanisms of interactions and to understand better structural features of nucleic acid target recognitions. To date, most studies with chiral ligands have been conducted with polyamines,[1] oligopeptides,[2–6] and propeller-shaped transition metal complexes.[7–10] While the polyamines and oligopeptides are groove-binding ligands, the interaction of the metal complexes may involve groove binding and partial intercalation. In general, the metal complexes have shown limited enantiospecific discrimination power. In a similar way, a small enantiomeric discrimination has been observed for classical fused-ring intercalators substituted with a chiral cationic chain.[11] Interestingly, the natural anticancer agent (+)-daunorubicin intercalates with B-DNA, while its synthetic analog (−)-daunorubicin binds selectively to left-handed Z-DNA.[12]

Unfused bicyclic and tricyclic aromatic cations, depending on structure, can intercalate with DNA base pairs and/or bind in a DNA groove.[13] We have shown previously that the intercalation mode is enhanced with the molecules that have the unfused aromatic system polarized extensively in the direction of the side cationic chain, so that the intercalating site constitutes a positive part of the dipole.[14] In particular, this stereoelectronic effect is responsible, in part, for intercalative binding with DNA of 4-(2-thienyl)pyrimidine and 4,6-bis(2-thienyl)pyrimidine derivatives (general structures in Table 1, $R^2 = SCH_2CH_2NMe_2$ or $NHCH_2CH_2NMe_2$). The results of our previous studies are consistent with the nonclassical intercalation model in which the twisted *s*-cis thienylpyrimidine system interacts with propeller-twisted DNA base pairs and the protonated dimethylaminoethylthio or dimethylaminoethylamino group is located in the major DNA groove.[13] Intercalation of the thienylpyrimidine systems from the major groove of DNA has important ramifications for the enhancement of DNA degradation by bleomycin[15,16] and its congeners such as phleomycin and pepleomycin.[17] These antitumor antibiotics bind in the minor DNA groove, and the noncompetitive intercalation of the thienylpyrimidines from the major groove is believed to induce favorable conformational changes of the double helix, such as expansion of the minor groove, for an increased affinity of the antitumor drug toward this groove and for a favorable stereochemical fit for the drug-mediated chemistry. As part of our research directed toward finding improved potentiators of the degradation of DNA by the bleomycin family of antibiotics, we now report the interaction with DNA of thienylpyrimidines substituted with a chiral

Keywords: B-DNA; Nonclassical intercalators; Chirality.
* Corresponding authors. Tel.: +1 404 651 0999; fax: +1 404 651 1416; e-mail addresses: lucjan@gsu.edu; chewdw@langate.gsu.edu

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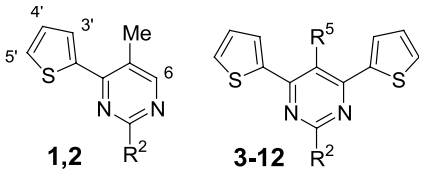
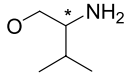
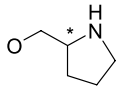
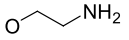
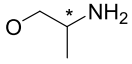
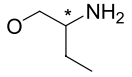
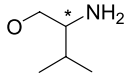
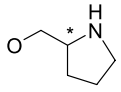
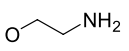
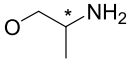
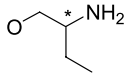
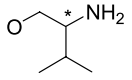
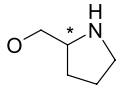
Table 1. DNA binding constants (K, M^{-1}) and binding site size (base pairs) for binding of compounds **1**–**12** with DNA[a]



No.	R^2	R^5	K (binding site size, bp)		K_S/K_R
			R-Enantiomer	*S*-Enantiomer	
1		—	6250 (6.8)	9900 (6.1)	1.6
2		—	13,000 (3.0)	18,900 (2.2)	1.4
3		H	154,000 (2.7)		
4		H	196,000 (2.2)	235,000 (2.2)	1.2
5		H	168,000 (2.6)	238,000 (2.5)	1.4
6		H	323,000 (2.3)	710,000 (2.0)	2.2
7		H	190,000 (2.0)	450,000 (2.0)	2.4
8		Me	98,000 (2.2)		
9		Me	105,000 (2.5)	160,000 (2.5)	1.5
10		Me	58,000 (2.5)	95,000 (2.5)	1.6
11		Me	301,000 (2.0)	465,000 (2.5)	1.5
12		Me	100,000 (2.0)	184,000 (2.2)	1.8

[a] The spectrophotometric binding measurements[13,22] were conducted using sonicated calf thymus DNA (800 ± 100 base pairs) in a PIPES buffer (10 mM PIPES, 1.0 mM EDTA, pH 7.00) at λ_{max} = 320 nm (**1**, **2**), 344 nm (**3**–**7**), and 341 nm (**8**–**12**).

cationic group. A substantial chiral discrimination was found for binding of the enantiomers with DNA. To our best knowledge, this is the first report on the DNA binding of chiral nonclassical intercalators.[18]

Chiral nonracemic compounds **1**, **2**, **4**–**7**, and **9**–**12** (structures in Table 1) were synthesized by the reactions of 2-chloro-5-methyl-4-(2-thienyl)pyrimidine and 2-chloro-4,6-bis(2-thienyl)pyrimidines[19] (R^2 = Cl in the general structures) with potassium alkoxides derived from nonracemic alkyl substituted 2-aminoethanols. Two achiral derivatives, **3** and **8**, were prepared in a similar way for a comparison of binding of achiral analogs with duplex DNA. In a typical run, a solution of a potassium aminoethoxide prepared by stirring a mixture of KH (35% suspension in mineral oil, 0.384 g, 3.35 mmol) and an aminoethanol (3.35 mmol) in anhydrous THF (20 mL) for 1 h under a nitrogen atmosphere was treated with a 2-chloropyrimidine derivative (3.0 mmol). The mixture was stirred under nitrogen atmosphere and heated to 40 °C for 4–10 h until a TLC analysis (silica gel, hexanes/Et_3N/EtOH, 7:2:1) showed the absence of the starting chloropyrimidine. *Caution*: heating above 40 °C causes isomerization of the initially formed alkoxypyrimidines to alkylaminopyrimidines, apparently by intramolecular rearrangement.[20] Workup included concentration on a rotary evaporator (<40 °C), addition of water (5 mL) to the

resultant residue, and extraction with ether (3 × 10 mL). Concentration of the extract gave crude product **1**–**12**, which was purified by silica gel chromatography under the conditions given above. Solid compounds were crystallized twice from ether/hexanes and oily compounds were transformed into hydrobromide salts by using a general procedure.[13] The salts were crystallized twice from EtOH/hexanes.[21]

The interaction of chiral compounds **1**, **2**, **4**–**7**, **9**–**12** and achiral analogs **3** and **8** (included for comparison) was evaluated by spectrophotometric binding as previously described[22] (Table 1). As can be seen from Table 1, the binding strength depends on the structure of the ligand. Within the series of ligands containing the same side amino chain, the weakest interaction is observed for biaromatic compounds **1**, **2**, the binding strength increases for triaromatic compounds **8**–**12** containing a methyl substituent at the pyrimidine, and the interaction is the strongest for triaromatic compounds **3**–**7** without the methyl group. The structure of the relatively small side amino chain, including absolute configuration, strongly affects the interaction strength of the ligands with duplex DNA. For example, the small molecules **1** and **2** are structural isomers with the same molecular formula, contain identical biaromatic systems, and differ only in the structure of a side chain (five carbon atoms in each case), but the ligands (*S*)-**2** and (*R*)-**2** bind twice as strongly with DNA than their respective (*S*)-**1** and (*R*)-**1** counterparts. Moreover, (*S*)-**1** binds stronger than (*R*)-**1** and (*S*)-**2** binds stronger than (*R*)-**2** with the chiral discrimination, as measured by a ratio of binding constants K_S/K_R, of 1.6 and 1.4, respectively. Importantly, the chiral discrimination, as seen with **1** and **2**, is also observed for the much stronger binding triaromatic ligands **4**–**7** and **9**–**12**. As can be seen from Table 1, the K_S/K_R, values range from 1.2 for enantiomers of **4** containing a 2-aminopropoxy chain to 2.4 for stereoisomers of **7** substituted with a 2-pyrrolidinemethoxy group.

We have shown previously that 4-(2-thienyl)pyrimidines and 4,6-bis(2-thienyl)pyrimidines substituted with achiral 2-(dimethylamino)ethylthio or 2-(dimethylamino)ethylamino chain intercalate with duplex DNA.[13,23,24] This conclusion was derived from NMR experiments that showed DNA-induced upfield shifts of the aromatic proton signals (0.55–0.90 ppm), compound-induced upfield shifts in the DNA imino proton signals (0.2–0.4 ppm), downfield shifts in the DNA ^{31}P NMR signal (0.4 ppm), compound-induced increases in viscosity of DNA (η/η_0 = 1.6–2.0) and compound-induced angular unwinding of closed circular DNA (14–19°). It has also been shown that these compounds under high concentration conditions, in contrast to known minor-groove intercalators, do not block the minor DNA groove from interaction with bleomycin.[15,16] Accordingly, it has been concluded that these unfused biaromatic and triaromatic compounds intercalate with duplex DNA from the major groove.

Since thienylpyrimidines **1**–**12** are close analogs of the previously investigated unfused intercalators, it can be suggested that these ligands are also intercalators that interact with DNA from its major groove. Indeed, this suggestion is fully consistent with the similarity of the binding characteristics of the achiral compounds as discussed above and the binding features of their chiral analogs. The interaction of representative compounds with DNA was analyzed by using NMR, viscosity, and T_m measurements.[24] As can be seen from Table 2, the aromatic proton signals of the selected ligands **1**, **5**, **6**, and **10** undergo large upfield shifts upon complexation with DNA, which is fully consistent with intercalation. The relatively large upfield shifts for protons of the methyl group at position 5 of the pyrimidine strongly suggests that this methyl substituent is also inserted between base pairs. All these shifts (Ar–H and Ar–Me) are larger for the more strongly binding *S*-enantiomers. On the other hand, protons of the side amino chain in these compounds are only slightly affected by DNA (upfield shifts 0.01–0.20 ppm), indicating that the side chain is not part of the intercalation complex. Fully consistent with intercalation of the unfused aromatic system are also the following compound-induced increases in viscosity of DNA (compound, η/η_0): (*S*)-**1**, 1.5; (*R*)-**1** (1.4); **3**, 2.0; (*S*)-**6**, 1.8; (*R*)-**6**, 1.7. The compound-induced thermal stabilization of duplex DNA was studied with compounds **6** and **11**. Surprisingly, the increases in T_m of DNA are quite small: (*S*)-**6**, 6.2 °C; (*R*)-**6**, 4.7 °C; (*S*)-**11**, 2.9 °C; (*R*)-**11**, 2.2 °C. Nevertheless, in agreement with other results discussed above, the increases in T_m are larger for *S*-enantiomers than for their *R*-isomers. It can be concluded that (i) the complexes of *S*- and *R*-stereoisomers with DNA are highly distorted and (ii) upon intercalation, the *S*-enantiomers cause less distortion of duplex DNA than the *R*-enantiomers.

Table 2. DNA induced upfield shifts ($\Delta\delta$) in the ^{1}H NMR spectra of selected compounds[a]

No.	Upfield shifts, $\Delta\delta$				
	H5/H6	H3′	H4′	H5′	Me-5
(*S*)-**1**	−0.75	−0.63	−0.60	−0.77	−0.40
(*R*)-**1**	−0.71	−0.62	−0.58	−0.74	−0.35
(*S*)-**5**	−0.65	−0.82	−0.63	−0.94	—
(*R*)-**5**	−0.63	−0.81	−0.61	−0.88	—
(*S*)-**6**	−0.65	−0.82	−0.63	−0.91	—
(*R*)-**6**	−0.64	−0.81	−0.60	−0.81	—
(*S*)-**10**	—	−0.63	−0.52	−0.61	−0.37
(*R*)-**10**	—	−0.58	−0.49	−0.57	−0.30

[a] Sonicated calf thymus DNA was used in the NMR experiments in D_2O (200 ± 50 base pairs) with the molar ratio 0.3 of compound per DNA base pair: 15 mM NaH_2PO_4, 0.1 mM EDTA, 10 mM NaCl, pH 7.00, 50 °C.

Upon binding with DNA the achiral compounds **3**, **8** and all individual enantiomers **4**–**7**, **9**–**12** give similar CD signals at λ_{max} 372.2 ± 2.2 nm for their propeller-twisted thienylpyrimidine systems (not shown). The similarity of the CD spectra strongly indicates that the unfused aromatic system of all these compounds, including *S*- and *R*-enantiomers, are twisted with the same sense of induced chirality in their intercalation complexes. Additionally, these results demonstrate that the inherent propeller twist of base pairs in native duplex DNA is retained in the intercalated DNA.

In order to gain an insight into the observed chiral discrimination in binding of enantiomers with DNA, molecular modeling with achiral ligand **3** (a reference compound) and enantiomers of **6** was conducted as previously described.[25] The lowest-energy model for the interaction of the three compounds was constructed in which the propeller-twisted thienylpyrimidine system is inserted between propeller-twisted base pairs, the side chain is located in the DNA major groove, and the protonated amino group of the side chain interacts electrostatically with a DNA phosphate. The computed total energies of the complexes are as follows: **3**, −4448 kJ/mol; (*R*)-**6**, −4467 kJ/mol; (*S*)-**6**, −4688 kJ/mol. These energies parallel the respective DNA binding constants (Table 1) and are consistent with the observed chiral discrimination between (*R*)-**6** and (*S*)-**6**. Inspection of the obtained DNA intercalation models for (*R*)-**6** and (*S*)-**6** revealed the only difference in positioning of the chiral substituent relative to the DNA major groove. Thus, the hydrophobic isopropyl group of the stronger interacting *S*-enantiomer is located on the hydrophobic surface of the groove, while this hydrophobic group of the *R*-enantiomer faces a hydrophilic aqueous environment.

In conclusion, it appears that the observed chiral discrimination is due entirely to the difference in van der Waals interaction with DNA of the side chain of the enantiomers. The difference in binding affinity between enantiomeric intercalators is not subtle.

Clearly, the structure of a small cationic substituent of the small intercalating system can affect the DNA interaction of a ligand to a greater extent than previously realized.

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20. The O → N rearrangement is not a problem in NMR and thermal melting experiments of DNA in aqueous buffer, pH = 7.00, due to protonation of the side amino chain which renders the amino group nonnucleophilic.
21. All compounds gave satisfactory results of elemental analysis (C, ±0.3; H, ±0.2; N, ±0.2). The composition, yield, mp, and specific rotation (c = 0.002, MeOH) data: (*S*)-**1**•2HBr, 54%, 217–219 °C, +26.0°; (*R*)-**1**•2HBr, 55%, 218–219 °C, −25.5°; (*S*)-**2**•1.5HBr, 58%, 174–175 °C, +17.0°; (*R*)-**2**•1.5HBr, 66%, 172–174 °C, −16.9°; **3**•HBr•H_2O, 30%, 250–252 °C, —; (*S*)-**4**, 50%, 124–127 °C, +13.8°; (*R*)-**4**, 51%, 121–125 °C, −12.6°; (*S*)-**5**, 58%, 106–108 °C, −30.0°; (*R*)-**5**, 51%, 107–109 °C, −30.4°; (*S*)-**6**, 52%, 75–77 °C, +28.1°; (*R*)-**6**, 45%, 75–78 °C, −28.0°; (*S*)-**7**•HBr, 32%, 235–238 °C, +16.2°; (*R*)-**7**•HBr, 35%, 237–239 °C, 16.1°; **8**•HBr, 30%, 212–214 °C, —; (*S*)-**9**HBr•0.5H_2O, 35%, 155–159 °C, +13.8°; (*R*)-**9**•HBr•0.5H_2O, 34%, 154–159 °C, 14.5°;(*S*)-**10**, 55%, 94–95 °C, +21.5°; (*R*)-**10**, 50%, 95–96 °C, −18.3°; (*S*)-**11**, 40%, 83–85 °C, +29.1°; (*R*)-**11**, 41%, 84–85 °C, −26.6°; (*S*)-**12**•HBr, 30%, 219–220 °C, +18.4°; (*R*)-**12**•HBr, 28%, 220–221 °C, −17.0°; ^{1}H NMR (phosphate buffer, pH 7.0, 50 °C): **1** and **2** ($\delta \pm 0.01$ ppm) 1.85 (C5-Me), 6.49 (H4′), 6.80 (H5′), 7.15 (H3′), 7.51 (H6); **3**–**7** ($\delta \pm 0.01$ ppm) 7.31 (H4′), 7.78 (H5′), 7.83 (H5), 8.01 (H3′); **8**–**12** ($\delta \pm 0.01$ ppm) 1.90 (C5-Me), 7.33 (H4′), 7.78 (H5′), 7.79 (H3′).
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