DNA-binding studies of the natural β-carboline eudistomin U

Jennifer M. Giulietti[a], **Patrick M. Tate**[a], **Ang Cai**[b], **Bongsup Cho**[b], and **Seann P. Mulcahy**[a,*]

[a]Providence College, Department of Chemistry and Biochemistry, 1 Cunningham Square, Providence, RI 02918, USA

[b]University of Rhode Island, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, 7 Greenhouse Road, Kingston, RI 02881

Abstract

Eudistomin U is a member of the β-carboline class of heterocyclic amine-containing molecules that are capable of binding to DNA. The structure of eudistomin U is unique since it contains an indole ring at the 1-position of the pyridine ring. While simple β-carbolines are reported to intercalate DNA, an examination of the mode of binding of eudistomin U has been lacking. We report preliminary spectroscopic (UV-Vis, thermal denaturation, CD) and calorimetric (DSC) data on the binding of eudistomin U to DNA, which suggest that eudistomin U binds weakly according to a mechanism that is more complicated than other members of its class.

Graphical Abstract
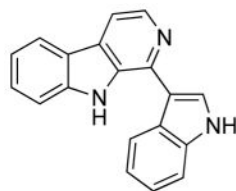
eudistomin U

Keywords

β-carboline; DNA; eudistomin U; UV-Vis; thermal denaturation; circular dichroism; differential scanning calorimetry

Targeting DNA as a macromolecular receptor is an attractive route for perturbing biological processes with small molecules.[1,2] DNA is an appealing target since its inherent chirality

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*Corresponding author: Tel.: +1-401-865-1280; fax: +1-401-865-1438; smulcahy@providence.edu.

makes possible the rational design of molecular probes. In addition to the carbon-centered chirality of the nucleotide building blocks, DNA also possesses helicity that is necessary for molecular recognition. Of the many small molecules that bind to DNA, polyamides,[2] octahedral metal complexes,[3] *cis*-platin,[4] peptide nucleic acid,[5] polyimides,[6] and natural products like mitomycin[7] and daunorubicin,[8] are the most studied. Investigations into their binding thermodynamics, binding kinetics, and sequence specificities have enhanced our understanding of ligand–DNA interactions and provide further motivation for the search of new architectural motifs with similar potential as probes for chemical biology.

β-carbolines are naturally occurring or synthetic small molecules that also bind DNA noncovalently.[9] These molecules are indolo[3,2-*b*]pyridines, which are flat, aromatic amines capable of inserting themselves in between base pairs of duplex DNA. Simple β-carbolines such as harman (**1**) and norharman (**2**, Figure 1) are widely known to intercalate into prokaryotic and eukaryotic DNA, resulting in loss of replication fidelity and impaired repair processes.[10,11] They have also been shown to inhibit DNA transcription *in vitro* and induce DNA strand breaks.[12,13] There are other more complex β-carbolines whose DNA binding properties are less well understood. For example, the marine metabolite eudistomin U (**3**) has been shown to bind strongly to GC-rich regions of DNA, but an in depth investigation of this interaction has been lacking.[8] The interaction between eudistomin U and DNA is particularly intriguing since the natural product possesses an aromatic ring attached to the 1-position of the β-carboline, which is unique in this family of molecules.

In order to evaluate eudistomin U as a novel molecular probe, we recently disclosed the synthesis of **3** in five steps through a key palladium-catalyzed Suzuki cross coupling reaction.[14] A broad cytotoxicity screen of eukaryotic and prokaryotic cells established that the natural product inhibited the growth of gram-positive bacteria at low micromolar concentrations. Given the evidence for DNA binding in the literature,[10–13] we hypothesized that binding to DNA may be responsible for the observed cytotoxicity and sought to characterize this interaction biochemically. In this Letter, we describe the DNA binding behavior of eudistomin U using spectroscopic and calorimetric techniques and further comparison to simple β-carbolines and known intercalators.

We first investigated the binding of β-carbolines to DNA using ultraviolet-visible spectroscopy (UV-Vis). When a small molecule binds to DNA through any of a variety of mechanisms including intercalation or groove binding, spectral changes will occur that are concentration-dependent.[15] Figure 2 shows the UV-Vis spectrum of eudistomin U with increasing concentrations of calf thymus DNA. As DNA is titrated into a sample of eudistomin U at pH 7, a dramatic hyperchromic shift occurred at 257 nm, indicative of a simple interaction. Furthermore, the Soret peak around 400 nm shifted to longer wavelengths upon titration of DNA. This red-shifting of almost 20 nm is a further sign of a complexation between DNA and the natural product. The known intercalators harman and norharman displayed similar spectral changes in neutral phosphate buffer (see Supporting Information).[16] Finally, since eudistomin U possesses a basic amine functional group, we investigated the pH-dependence of this binding through changes in the UV-Vis spectrum. Similar hyperchromicity at 257 nm and red-shifting above 400 nm were observed in both basic and acidic buffer. This suggests that protonation of the pyridine had no effect on

binding, and that electrostatic interactions with the phosphate backbone of DNA were unlikely.

We next investigated the thermal denaturation of calf thymus DNA in the presence of eudistomin U. It is known that binding of a small molecule to DNA causes stabilization/ destabilization of the double helix.[17] For example, stabilization will increase the energy required to unravel the double helix into single stranded DNA. The melting temperature (T_m) is a measure of the stability of the double helix to temperature-induced denaturation and is useful in determining small molecule binding.[18–19] Table 1 shows the T_m values for harman and eudistomin U at 50 μM concentration, as well as the known intercalator ethidium bromide. These results indicate a much weaker interaction between eudistomin U and double-stranded DNA than the UV-Vis spectra suggest. Harman produced a slightly larger increase in T_m than eudistomin U. Not surprisingly, the well-known intercalator ethidium bromide **4** (Figure 1) produced strong increases in the melting temperature at much lower concentrations, which indicates strong intercalation to DNA.

Next, we investigated the ability of these small molecules to perturb the secondary structure of DNA. When small molecules bind to DNA via specific mechanisms like intercalation or groove binding, the ability of the native double helix to absorb circularly polarized light will change.[20] One can measure these changes using circular dichroism (CD). The helicity of DNA is very sensitive to small molecules and even weak binding can produce large changes in the CD spectrum. Ethidium bromide, for example, has been shown to have concentration-dependent hyperchromic effects on the helicity of DNA, but also induces ellipticity in the 300–400 nm region.

Figure 3 shows the CD spectrum of DNA upon titration with eudistomin U. Hyperchromicity and red-shifting occurred with increasing concentrations around 280 nm, indicating changes to the B-DNA structure. Induced ellipticity was observed at 349 nm, but this signal was much weaker than known intercalators like ethidium bromide. Nearly identical results were obtained under high salt conditions (0.1 M NaCl, see Supporting Information). Under basic conditions (pH = 8.9), there were no concentration-dependent changes to the CD spectrum (see Supporting Information). Under acidic conditions (pH = 5.4), increases in ellipticity were similar to neutral conditions, demonstrating a pH-dependence for this interaction.

In order to further investigate this pH-dependence, we synthesized *N*-methyl eudistomin U (**5**, Figure 1). We hypothesized that the positive formal charge on the β-carboline may more closely resemble the structure of ethidium bromide **4**. This change might direct the small molecule to DNA through stronger electrostatic interactions with the phosphate backbone, where it can then associate with DNA more easily via intercalation or groove binding. Unfortunately, we observed no induced ellipticity around 350 and only weak hyperchromicity at the B-DNA maximum. We were also surprised that both harman and norharman did not produce induced ellipticity at 350 nm, which directly challenges the conclusion that these molecules solely intercalate between the base pairs of DNA.[18] Previous CD studies of the interaction between these simple β-carbolines and bulk DNA suggested that they bind via intercalation, but the observed changes in ellipticity were

obtained only at high concentrations of ligand.[21] We were unable to reproduce this effect due to the insolubility of the ligands at these concentrations.

We also investigated the sequence-dependence of the eudistomin U-DNA interaction, which was suggested in the literature to be GC-specific. Thus, we measured the CD signal using AT- or GC-rich 16-mer nucleotides. Surprisingly, we observed no induced ellipticity nor hyperchromicity, even at concentrations as high as 200 μM. This result indicates that the interaction of DNA with eudistomin U is likely not sequence-specific.

Finally, through differential scanning calorimetry (DSC), we were able to quantify the effects on thermal denaturation of calf thymus DNA in the presence of eudistomin U (**3**), harman (**1**), and ethidium bromide (**4**). DSC is a microcalorimetry technique that measures T_m and enthalpy associated with melting and has been used successfully to screen the binding thermodynamics of nitrogen-containing small molecules.[22–24] The DSC melting curves shown in Figure 4 confirm the results obtained by optical spectroscopy. All three small molecules increased the thermal stability of calf thymus DNA as evidenced by a strong increase in T_m when compared to control. While eudistomin U (**3**) stabilized duplex DNA with a T_m of 67 °C at 5 mM, roughly equal or stronger effects were observed for harman (**1**) and ethidium bromide (**4**) at much lower concentrations. An increase in the binding enthalpy was observed for the interaction between each small molecule and calf thymus DNA, which is concentration dependent for eudistomin U (**3**).

In conclusion, we have described spectroscopic data for the interaction of DNA with the natural product eudistomin U. Our UV-Vis, thermal denaturation, and CD studies all suggest a weak binding between eudistomin U and DNA. Furthermore, the lack of any specific interaction with small oligomers of DNA indicates that a more complicated binding mechanism is involved. Finally, we have evidence that challenges literature reports that harman and norharman are intercalators, suggesting that further studies into their mechanism of binding is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Palchaudhuri R, Hergenrother P. J Curr Opin Biotechnology. 2007; 18:497.
2. Dervan PB. Bioorg Med Chem. 2001; 9:2215. [PubMed: 11553460]
3. Barton JK, Danishefsky AT, Goldberg JM. J Am Chem Soc. 1984; 106:2172.
4. Peyrone M. Annalen Chemie Pharmazie. 1844; 51:1–29.
5. Nielsen PE. Acc Chem Res. 1999; 32:624.

6. Lokey RS, Kwok Y, Guelev V, Pursell CJ, Hurley LH, Iverson BL. J Am Chem Soc. 1997; 119:7202.

7. Tomasz M. Chem Biol. 1995; 2:575. [PubMed: 9383461]

8. Wilson WD, Jones RL. Nucleic Acids Res. 1982; 10:1399. [PubMed: 7071016]

9. Cao R, Peng W, Wang Z, Xu A. Curr Med Chem. 2007; 14:479. [PubMed: 17305548]

10. de Meester C. Mutation Research. 1995; 339:139. [PubMed: 7491124]

11. Funayama Y, Nishio K, Wakabayashi K, Nagao M, Shimoi K, Ohira T, Hasegawa S, Saijo N. Mutation Research. 1996; 349:183. [PubMed: 8600349]

12. Madle E, Obe G, Hansen J, Ristow H. Mutation Research. 1981; 90:433. [PubMed: 7335109]

13. Mita S, Kamataki T, Kato R. Carcinogenesis. 1984; 5:715. [PubMed: 6426811]

14. Roggero CM, Giulietti JM, Mulcahy SP. Bioorg Med Chem Lett. 2014; 24:3549. [PubMed: 24930832]

15. Tu LC, Chen CS, Hsiao IC, Chern JW, Lin CH, Shen YC, Yeh SF. Chem Biol. 2005; 12:1317. [PubMed: 16356849]

16. Chen Z, Cao R, Shi B, Yu L, Song H, Ren Z. Chem Pharm Bull. 2010; 58:901. [PubMed: 20606334]

17. Suh D, Chaires JB. Bioorg Med Chem. 1995; 3:723. [PubMed: 7582950]

18. Hayashi K, Nagao M, Sugimura T. Nucleic Acids Res. 1977; 4:3679. [PubMed: 593881]

19. Puglisi JD, Tinoco I Jr. Methods in Enzymol. 1989; 180:304. [PubMed: 2482421]

20. Brahms J, Mommaerts WF. J Mol Biol. 1964; 10:73. [PubMed: 14222898]

21. Taira Z, Kanzawa S, Dohara C, Ishida S, Matsumoto M, Sakiya Y. Japanese Journal of Toxicology and Environmental Health. 1997; 43:83.

22. Guthrie KM, Parenty ADC, Smith LV, Cronin L, Cooper A. Biophys Chem. 2007; 126:117. [PubMed: 16750289]

23. Del Vecchio P, Esposito D, Ricchi L, Barone G. Int J Biol Macromol. 1999; 24:361. [PubMed: 10408643]

24. Paul P, Hossain M, Yadav RC, Kumar GS. Biophys Chem. 2010; 148:93. [PubMed: 20231052]
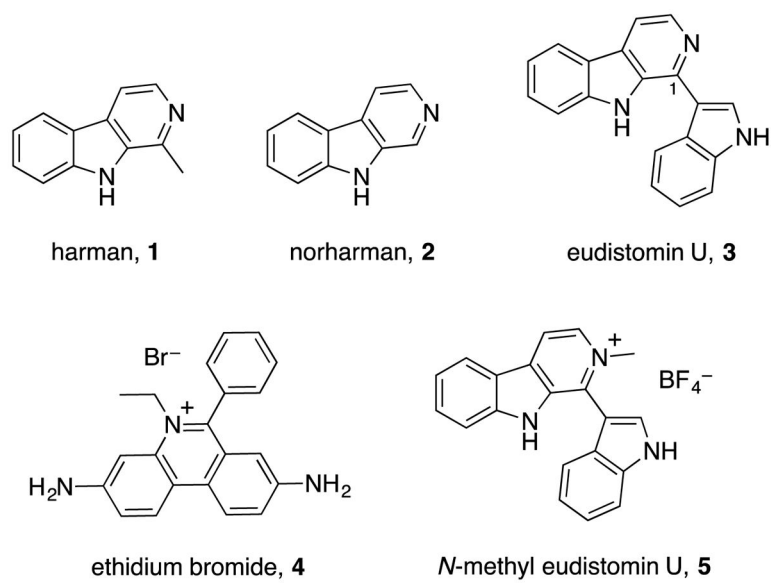
Figure 1.
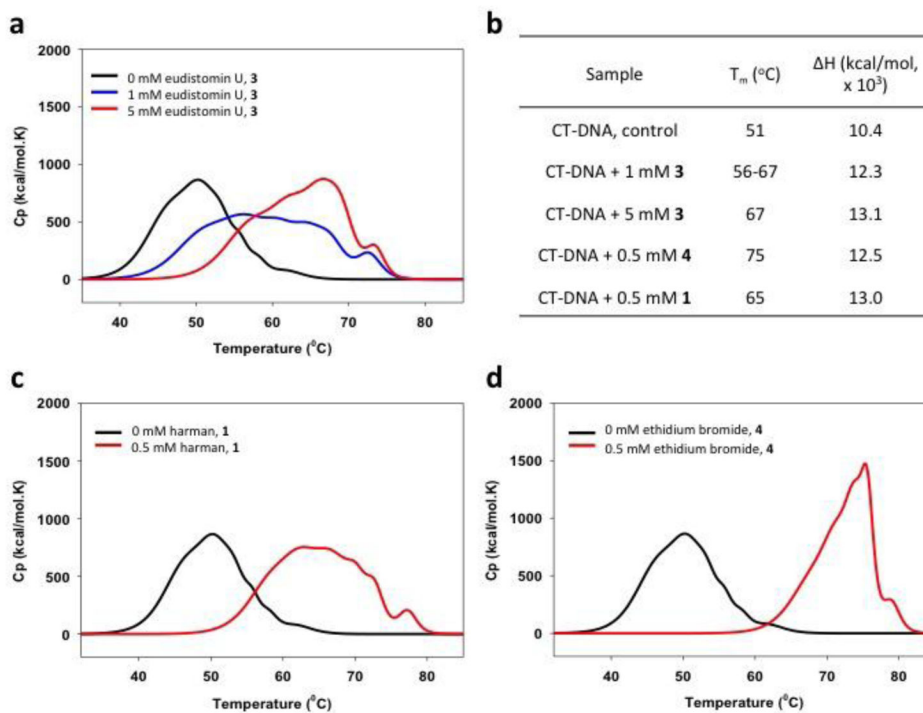Structures of natural and synthetic β-carbolines
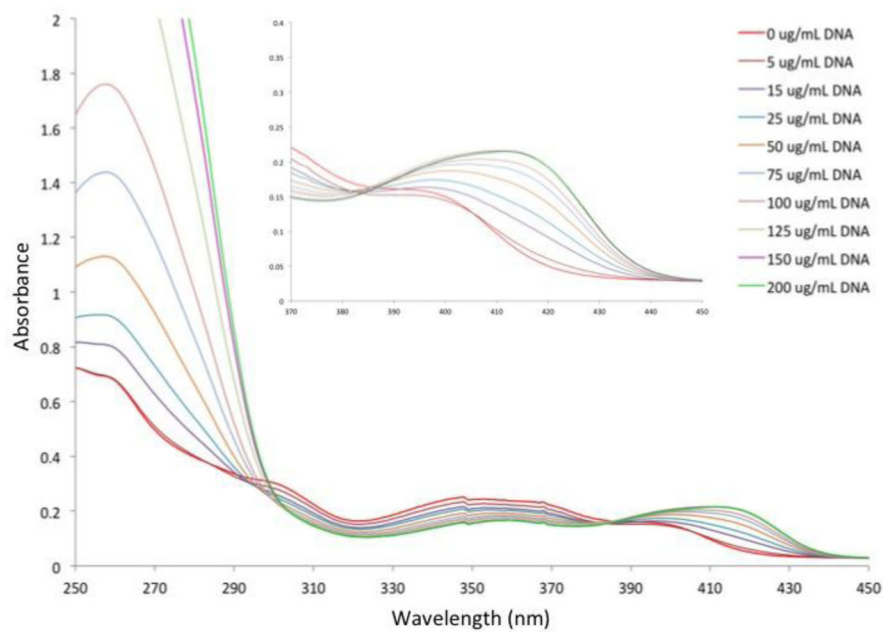
Figure 2.
UV-Vis spectra of eudistomin U and calf thymus DNA
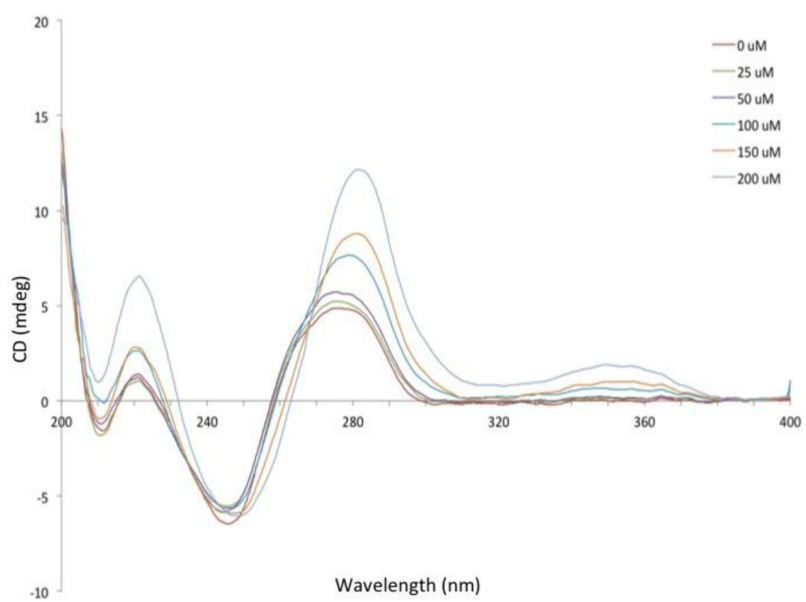
Figure 3.
Circular dichroism spectra of eudistomin U and calf thymus DNA

Figure 4.
Differential scanning calorimetry of small molecules and calf thymus DNA: a) melting profile of eudistomin U + calf thymus DNA; b) thermodynamic binding parameters: T_m and H;c) melting profile of harman + calf thymus DNA; d) melting profile of ethidium bromide + calf thymus DNA

Table 1

Thermal denaturation of calf thymus DNA

compound	T_m (°C)	change
control	66.8	--
harman, **1**[a]	70.8	4.0
eudistomin U, **3**[a]	69.1	2.3
ethidium bromide, **4**[b]	75.7	8.9

[a]50 μM;

[b]2.5 μM