ELECTRONIC SUPPLEMENTARY INFORMATIONS

Assessment of intercalative interaction of the benzophenanthridine plant alkaloid nitidine with higher ordered forms of RNA: Spectroscopic evaluation

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Table S1: Optical properties of free, triplex and duplex bound NIT

Absorbance	**Triplex**	**Duplex**
$\lambda_{max,\ free}$ (nm)	327	327
$\lambda_{max,\ bound}$ (nm)	322	327
λ_{iso} (nm)	337; 350;401	415
ε_{free} (at λ_{max}, $M^{-1}cm^{-1}$)	139360(327)	139360(327)
ε_{bound} (at λ_{max}, $M^{-1}cm^{-1}$)	107058(327)	60671(327)
$^{*}\varepsilon$ (at λ_{iso}, $M^{-1}cm^{-1}$)	65988(337),32301(350),31379(401)	13237(415)

Calculation of Binding parameters:

Sufficient stock solution of polynucleotide was added to the buffer in the cuvette to yield a concentration of 30 μM. Successive aliquots from a stock solution of NIT were added to the cuvette at regular intervals of 10 minutes. The amount of free and bound NIT was determined following the methods described by Chaires et al. earlier. [J. B. Chaires, N. Dattagupta, D. M. Crothers, Biochemistry. 1982, **21**, 3933-40] Following each addition of the NIT, the absorbance at the characteristic peak, 327 nm, and the isosbestic point, 337 nm (triplex) and 415 nm (duplex), were recorded after each equilibrium.

The total alkaloid C_T present was calculated from the relation

$$C_T = A_{iso} / \varepsilon_{iso} \text{.....(1)}$$

where ε_{iso}=65988 $M^{-1}cm^{-1}$ (at 337 nm for triplex) and 13237 $M^{-1}cm^{-1}$ (at 415 nm for triplex)

The expected absorbance at 327 is calculated as

$$A^0 = C_T \varepsilon_{f327} \text{.....(2)}$$

The difference in expected absorbance and the observed absorbance was then used to calculate the amount of bound alkaloid by the following relation

$$C_B = A / \Delta\varepsilon = (A^0 - A_{obs}) / (\varepsilon_{f327} - \varepsilon_{B327}) \text{.....(3)}$$

Where ε_{f327} is 139360 $M^{-1}cm^{-1}$ for free NIT and ε_{B327} is 107058 $M^{-1}cm^{-1}$ and 60671 $M^{-1}cm^{-1}$ for triplex bound and duplex bound NIT respectively.

ε_B was determined independently by adding a known quantity of NIT to a large excess of polynucleotide and assuming total binding.

The amount of free drug was then estimated by the difference,

$$C_f = C_T - C_B \text{.....(4)}$$

Then r is calculated by

$$r = C_B / P \text{.....(5)}$$

By calculating the r and r/C_f, Scatchard plot was plotted for the evaluation of binding constant.

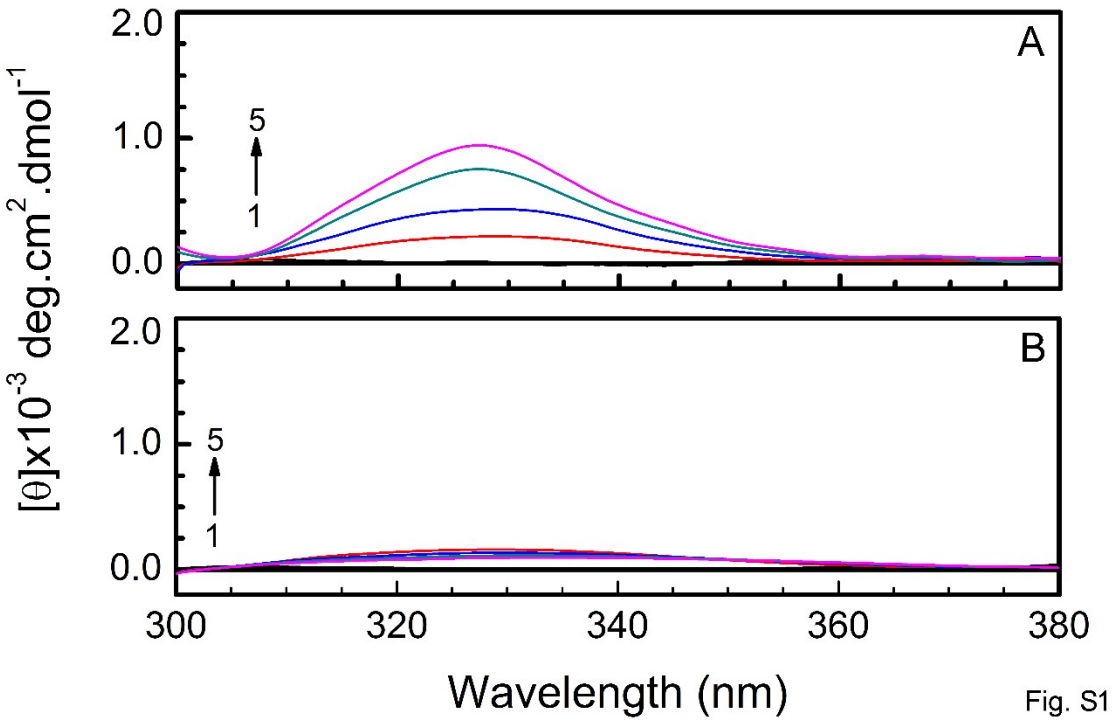

Fig. S1. Extrinsic CD spectra of NIT (20 μM) treated with U.A*U (A) and A.U (B). (A) Curves 1-5 denote 0, 20, 40, 60 and 80 μM of U.A*U in in SCH buffer at 20 °C; (B) Curves 1-5 denote 0, 20, 40, 60 and 80 μM of A.U in in SCH buffer at 20 °C.