Electronic Supplementary Information

Small Molecule Induced Poly(A) Single Strand to Self-Structure Conformational Switching: Evidence for the Prominent Role of H-bonding Interactions

Sabyasachi Chatterjee and Gopinatha Suresh Kumar*

Biophysical Chemistry Laboratory, Organic and Medicinal Chemistry Division

CSIR-Indian Institute of Chemical Biology

4, Raja S.C. Mullick Road, Kolkata 700 032, India

Address for Correspondence:

Dr. G. Suresh Kumar, Ph.D

Chief Scientist, Biophysical Chemistry Laboratory

Organic and Medicinal Chemistry Division

CSIR-Indian Institute of Chemical Biology

4, Raja S.C. Mullick Road, Kolkata 700 032, India

Phone: +91 33 2472 4049/2499 5723; Fax : +91 33 2472 3967

e-mail: gskumar@iicb.res.in/gsk.iicb@gmail.com

1.1. Detailed methods

Melting studies: UV-thermal melting

Absorbance versus temperature curves (melting profiles) of poly(A) and the poly(A)–alkaloid complexes were measured on the Shimadzu Pharmaspec 1700 unit (Shimadzu Corporation, Kyoto, Japan) equipped with the Peltier controlled TMSPC-8 model cuvette accessory. In a typical experiment, poly(A) solution (10 μM) was mixed with different concentrations of the alkaloid in the degassed buffer and transferred into the eight chambered micro cuvette of 1 cm path length (100 mL) and the temperature of the microcell accessory was raised at a heating rate of 0.5 °C min^{-1}, continuously monitoring the absorbance change at 258 nm scanning in the temperature range 20 to 100 °C. The melting temperature (T_m) was taken as the midpoint of the melting transition as determined by the maxima of the first derivative plots.

Differential scanning calorimetry

Excess heat capacities as a function of temperature were measured on a MicroCal VP-differential scanning calorimeter (DSC; MicroCal, LLC). The DSC thermograms were analyzed using the Origin 7.0 software to determine the calorimetric transition enthalpy (ΔH_{cal}). This calorimetrically determined enthalpy is model-independent and unrelated to the nature of the transition. The temperature at which the excess heat capacity is maximum defines the transition temperature (T_m).

Isothermal titration calorimetry

Thermodynamics of the nucleic acid-ligand association was studied in a MicroCal VP-ITC unit (MicroCal). The titration of the poly(A) to alkaloid was automated and the data

obtained analysed by Origin 7.0 software supplied with the unit. Programmed injections of 10 μL aliquots of the poly(A) solution (1000 μM) into alkaloid solution (50 μM) kept in the calorimeter cell (1.4235 mL) were effected from the rotating injection syringe. For the control dilution experiment the poly(A) solution of the same concentration was injected to the experimental buffer in the same protocol as employed for the samples. The heat absorbed in each injection for the dilution was subtracted from the corresponding heat absorbed for nucleic acid-ligand association to finally yield the heat change for the nucleic acid-ligand binding reaction. The final data points were plotted as a function of the molar ratio (poly(A)/alkaloid), fit with a model for "one set of binding sites" and analysed using Origin software to provide thermodynamic parameters along with equilibrium binding constant (K_a) and the binding stoichiometry (N). The Gibbs energy change ($\Delta G°$) and the entropic contribution for the binding ($T\Delta S°$) were calculated by the following equations,

$$\Delta G° = -RT \ln K_a \quad (1)$$

and

$$\Delta G° = \Delta H° - T\Delta S° \quad (2)$$

Here T is the temperature in Kelvin and R is the universal gas constant (1.9872041 cal mol^{-1} K^{-1}).

Circular dichroism measurements

A Jasco J815 spectropolarimeter (Jasco International Co., Ltd., Hachioji, Japan) equipped with a Peltier controlled cuvette holder and temperature controller PFD 425

L/15 was used for monitoring the conformational changes in the poly(A) on binding of the alkaloid. A buffer baseline scan was subtracted from the averaged scan for each sample. Five scans were averaged for better spectra.

Molecular docking study

Crystal structures of poly(A), used for the molecular docking, were obtained from the Protein Data Bank (PDB) (PDB ID: 1CVJ, 2Q66, 3GIB, 4FO2, 4HT8). The energy structure of jatrorrhizine (JAT) was minimized using the Avogadro 1.1.1 molecular editor. Water and all other bound molecules were removed from the downloaded PDB crystal structure of poly(A) by using PyMOL software. Polar hydrogen atoms and Gasteiger charges were added to the poly(A) crystal structure before starting the docking process. Nucleic acid-ligand docking was carried out with AutoDock 1.5.6 program, which utilizes the Lamarckian Genetic Algorithm (LGA). The result was analyzed by Autodock and PyMOL softwares.

Electronic absorption spectroscopy

The absorption spectral titrations were performed at 20 ± 0.5 °C on a Jasco V660 double beam double monochromator spectrophotometer equipped with a thermoelectrically controlled cell holder and temperature controller in matched quartz cuvettes of 1 cm path length. In the alkaloid-nucleic acid studies after each addition of an aliquot of the alkaloid to the poly(A) solution, the solution was thoroughly mixed and allowed to re-equilibrate for at least 5 min. before noting the absorbance values at the desired wavelength maxima.

Fluorescence spectroscopy

All the fluorescence experiments were carried out at 20 ± 0.5 °C in 10 mm quartz cuvettes using a Quanta Master 400 unit (Horiba PTI, Canada) with a 150 W Xenon lamp controlled by FelixGX spectroscopy software provided with the instrument. The temperature was controlled by the single cuvette Peltier K-155-C of the unit.

Atomic force microscopy

Atomic Force Microscopy (AFM) is the most popular scanning probe microscopy method that creates a highly magnified three dimensional image of a surface. The magnified image was generated by monitoring the motion of an atomically sharp probe as it was scanned across a surface. This enabled a direct visualization of the assembly and to measure the dimensions of the surface features. Freshly cleaved muscovite Ruby mica sheet (ASTM V1 Grade Ruby Mica (MICAFAB, Chennai, India) was used to which the sample was tightly bound. The samples for AFM were prepared by dropping 10 µl of sample solution onto the freshly cleaved mica sheet and dried under vacuum and subjected to imaging. The sample materials were visualized by AAC mode using a Pico plus 5500 AFM (Agilent Technologies, USA) with a piezoscanner at the maximum range of 9 µm.

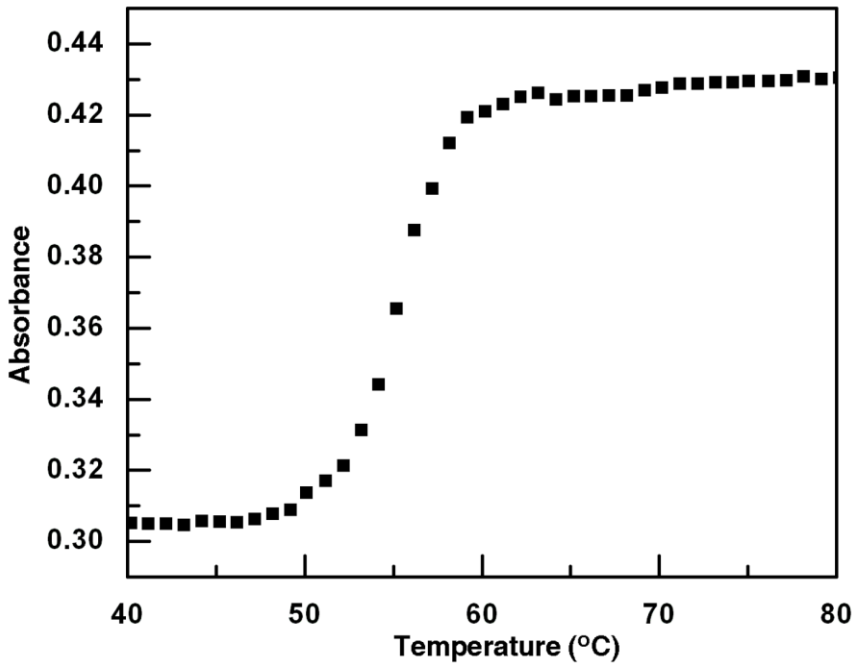

Fig. S1 UV-thermal melting profile of duplex poly(A) at pH 4.5.

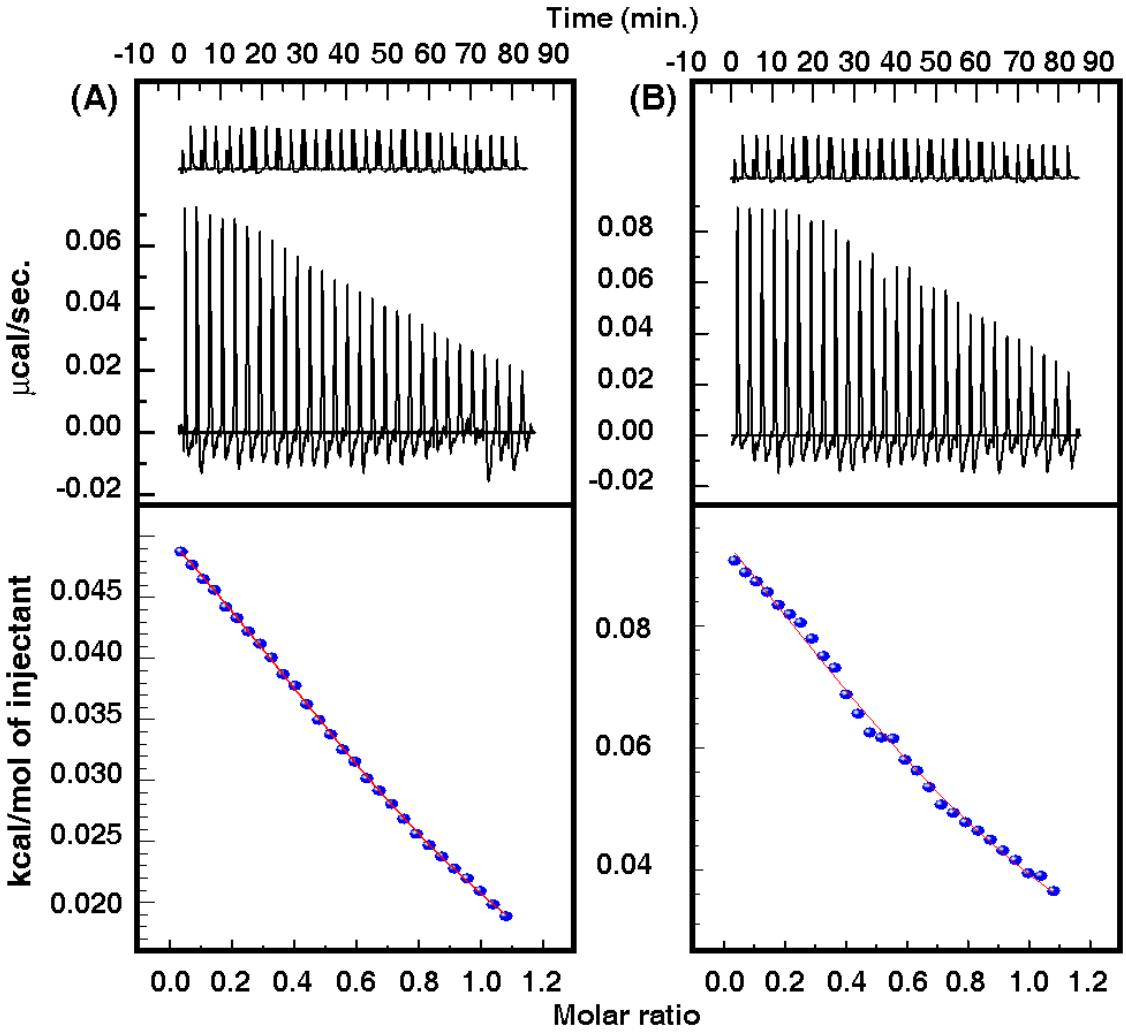

Fig. S2 ITC profiles of (A) JAT and (B) COP binding to adenosine monophosphate (AMP).

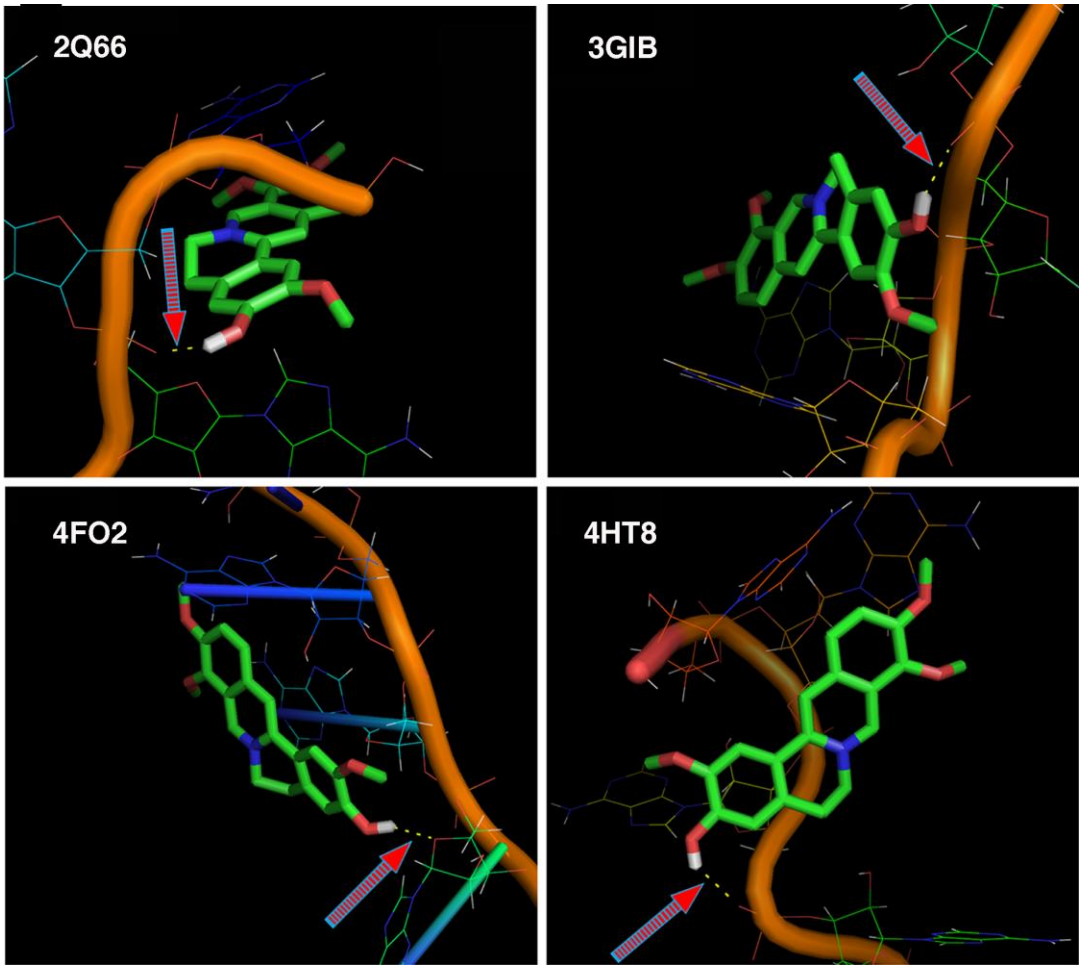

Fig. S3 Molecular docking structure for the interaction between different poly(A) structures and JAT. The arrows indicate the H- bonding interactions.

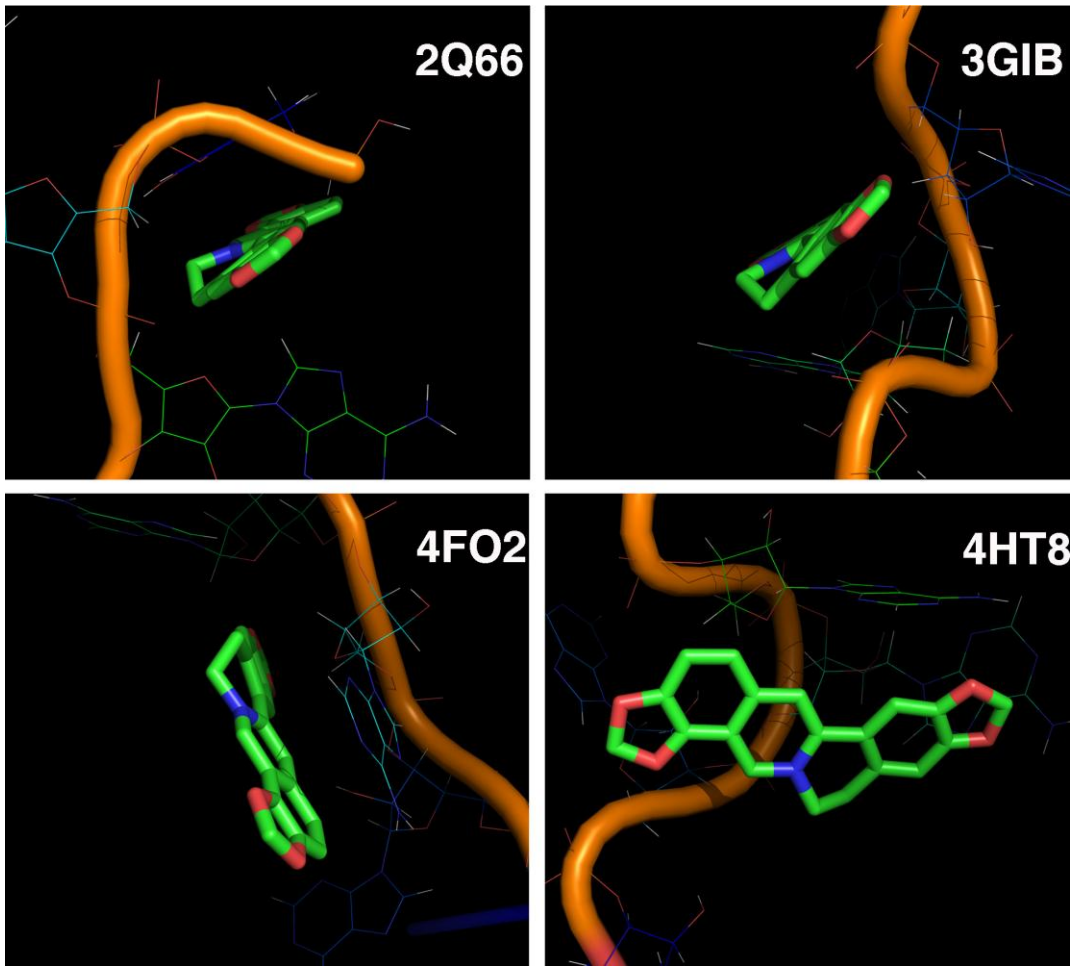

Fig. S4 Molecular docking structure for the interaction between different poly(A) structures with COP.

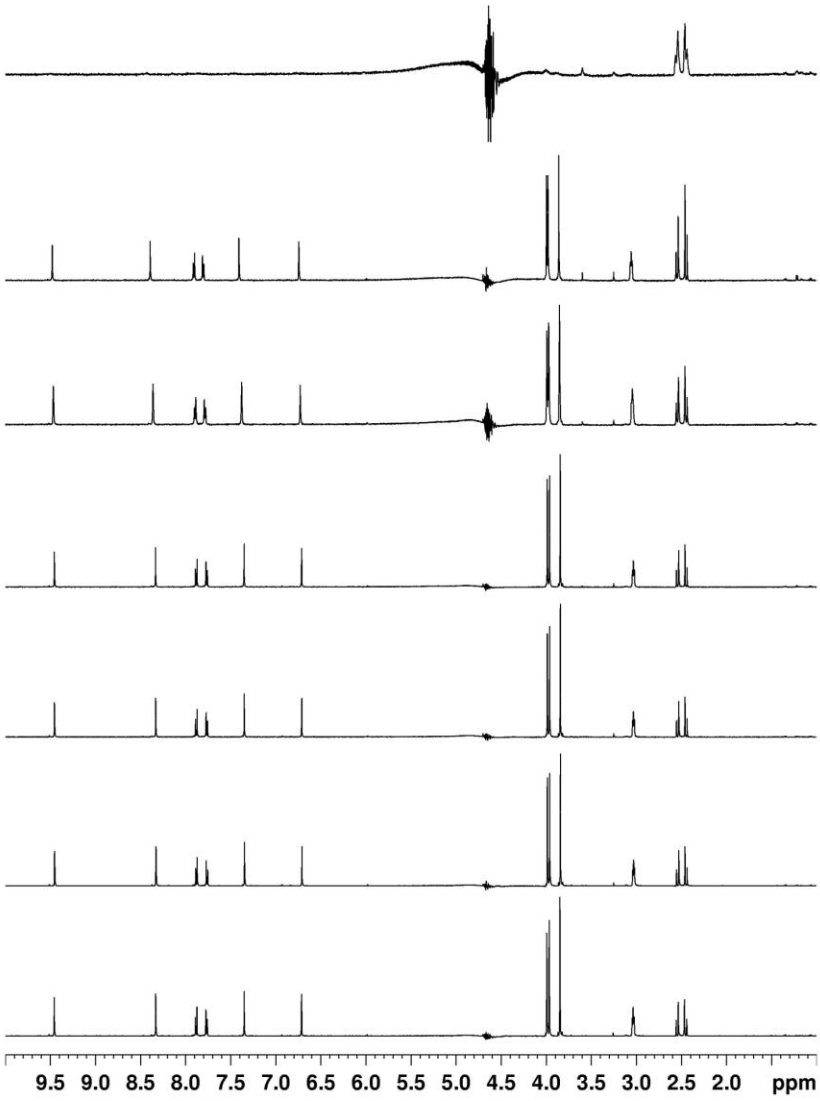

Fig. S5 ^{1}H-NMR spectral titration of JAT (400 μM) and poly(A) at 20 ^{o}C. JAT : poly(A) ratio for spectra from bottom to top are 1:0, 1:0.25, 1:0.50, 1:0.75, 1:1, 1:1.50 and 1:2, respectively.

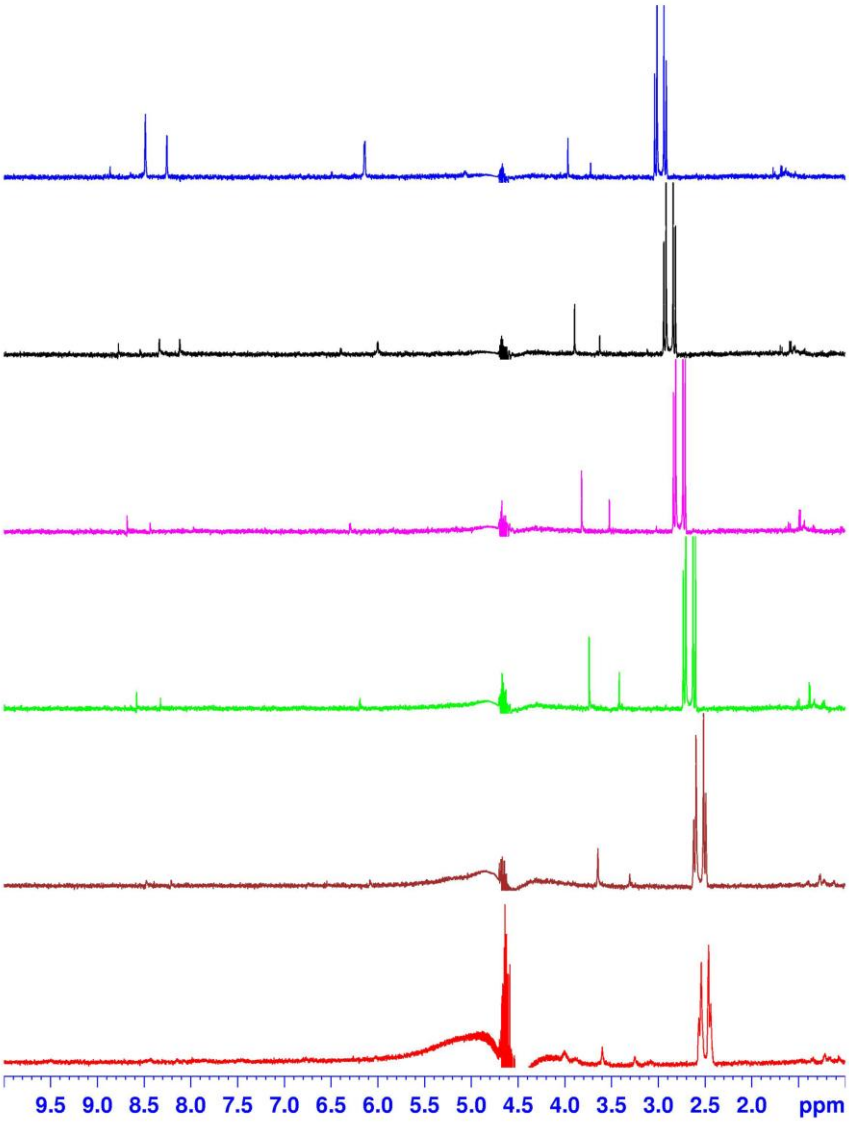

Fig. S6 Temperature dependent ^{1}H-NMR spectra on the binding of JAT to poly(A) at 1:2 ratio; from bottom upwards the spectra indicate temperatures from 20 ^{o}C to 70 ^{o}C at 10 ^{o}C interval.

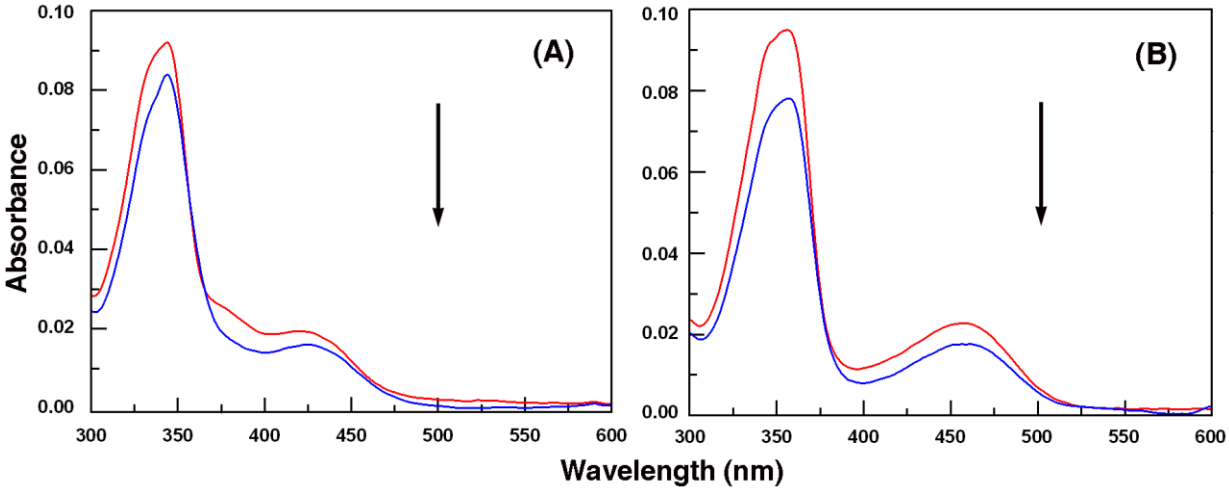

Fig. S7 Absorbance spectral changes of JAT (A) and COP (B) in the absence (red curve) and in the presence (blue curve) of poly(A). The alkaloid and poly(A) concentrations were 3 and 20 μM, respectively

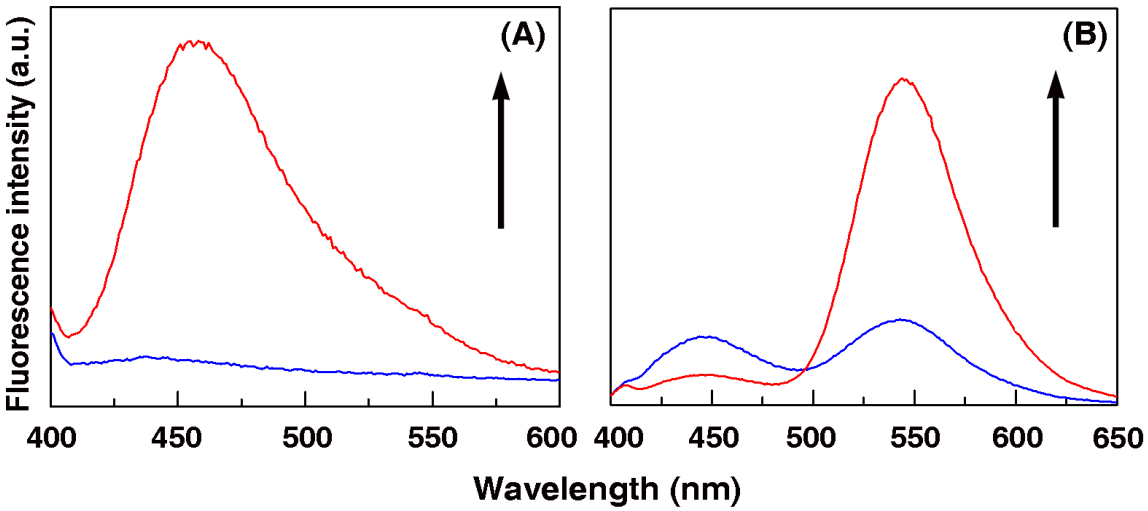

Fig. S8 Fluorescence spectra of JAT (A) and COP (B) (blue curve) and corresponding complex with poly(A) (red curve). Concentration of the alkaloids and poly(A) were 2 and 25 μM, respectively.

Table S1 ITC data of binding JAT and COP with AMP

Alkaloid	**N**	K_a **x** 10^3 **(M^{-1})**	ΔH^o **(kcal/mol)**	$T\Delta S^o$ **(kcal/mol)**	ΔG^o **(kcal/mol)**
JAT	0.96±0.02	11.40±0.11	0.07±.01	5.50	5.43±0.01
COP	0.93±0.05	8.54±0.02	0.15±.01	5.42	5.27±0.01

Table S2 Temperature dependent ITC binding data of JAT-poly(A) interaction

T	**N**	K_a **x** 10^6 (M^{-1})	ΔH^o (kcal/mol)	$T\Delta S^o$ (kcal/mol)	ΔG^o (kcal/mol)
283.15	1.84	4.06×10^6	-2.10	6.82	-8.92
293.15	2.41	1.14×10^6	-2.05	6.06	-8.11
303.15	3.86	4.87×10^5	-1.96	5.65	-7.61

Table S3 Details information of the molecular modeling results

PDB ID	JAT	COP
	H-bonding distance (Å)	H-bonding distance (Å)
1CVJ	2.5	No
2Q66	1.8	No
3GIB	1.8	No
4FO2	1.8	No
4HT8	2.2	No