Interaction of the putative anticancer alkaloid chelerythrine with nucleic acids: biophysical perspectives

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Abstract

Alkaloids represent an important group of molecules that have immense pharmacological potential. Benzophenanthridine alkaloids are one such class of alkaloids known for their myriad pharmacological activities that include potential anticancer activities. Chelerythrine is a premier member of the benzophenanthridine family of the isoquinoline group. This alkaloid is endowed with excellent medicinal properties and exhibits antibacterial, antimicrobial and anti-inflammatory properties. The molecular basis of its therapeutic activity is considered due to its nucleic acid binding capabilities. This review focuses on consolidating the current status on the nucleic acid binding properties of chelerythrine that is essential for the rational design and development of this alkaloid as a potential drug. This work reviews the interaction of chelerythrine with different natural and synthetic nucleic acids like double- and single-stranded DNAs, heat-denatured DNA, quadruplex DNA, double- and single-stranded RNA, tRNA and triplex and quadruplex RNA. The review emphasizes on the mode, specificity, conformational aspects and energetics of the binding that is particularly helpful for developing nucleic acid targeted therapeutics. The fundamental results discussed in this review will greatly benefit drug development for many diseases and serve as a database for the design of futuristic benzophenanthridine-based therapeutics.

Keywords Benzophenanthridine alkaloid · Chelerythrine · Nucleic acids · DNA · RNA

Introduction

The remarkable progress in the field of molecular biology and biotechnology has led to a paradigm shift towards the development of nucleic acid-based drugs for the treatment of genetic disorders (Saenger 1984; Wilson and Li 2000; Gallego and Varani 2001; Tor 2003; Vicens and Westhof 2003; Giri and Suresh Kumar 2010a, b; Bhadra and Suresh Kumar 2011). Targeting nucleic acids by small molecules has been a key strategy for many recent therapeutic interventions. Understanding the basics of such small molecule–nucleic acid interactions will facilitate specific and selective targeting of the genome, for the

treatment of many fatal genetic diseases using chemotherapeutic approaches (Waring 1981; Hurley 2002).

Chelerythrine (1,2-dimethoxy-12-methyl[1,3]-benzodioxolo[5,6-c]phenanthridin-12-ium) (Fig. 1) is a benzophenanthridine alkaloid, close to sanguinarine, isolated from the natural herb, greater celandine *Chelidonium majus* L and *Macleaya cordata*. Sanguinarine, the close analogue, has received much attention for its diverse therapeutic utility. It was shown that sanguinarine has antibacterial, antimicrobial and anti-inflammatory properties (Lenfeld et al. 1981; Godowski 1989; Obiang-Obounou et al. 2011). Sanguinarine induces apoptosis in a variety of cancer cells but does not exert an apoptotic effect on normal cells, and hence, it has the potential to be developed as an anticancer drug (Ahmad et al. 2000). Sanguinarine is a strong DNA- and RNA-interacting molecule, and its nucleic acid-binding properties have also been reviewed recently (Suresh Kumar and Hazra 2014; Suresh Kumar and Basu 2016). In the same article, the binding of chelerythrine with the RNA triplex inferred from the calorimetry result was also discussed very briefly (Suresh Kumar and Basu 2016).

Chelerythrine is a strong, selective and cell permeable protein kinase C (PKC) inhibitor, responsible for maintaining

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Fig. 1 Molecular structure of iminium form of chelerythrine

erythrocyte deformability, but it does not inhibit tyrosine protein kinases, cAMP-dependent protein kinase or calcium/calmodulin-dependent protein kinases (Herbert et al. 1990; Chmura et al. 2000; Yu et al. 2000). The antitumor, antibacterial, antimicrobial and anti-inflammatory properties of this alkaloid are also known (Niu et al. 2011; Tavares et al. 2014; Mikołajczak et al. 2015; He et al. 2018; Chmura et al. 2000). Recently, it has also been reported that G protein-activated K^+ (GIRK) current inhibition was also sensitive to the PKC inhibitor chelerythrine suggesting that following activation of CaSR and Gq, GIRK currents are modulated by PKC phosphorylation (Kienitz et al. 2019). Chelerythrine has been shown to be a promising candidate for the design of new drugs against *Dactylogyrus intermedius* which is a significant monogenean parasite on the gills of cyprinid fishes that causes severe economic losses in aquaculture and ornamental fish breeding (Li et al. 2011). Kim et al. suggested that chelerythrine can bring about heparin-binding epidermal growth factor-like growth factor (HB-EGF) shedding from the cell surface. It induced a time- and dose-dependent shedding of HB-EGF-alkaline phosphatase fusion protein expressed in MC2 rat prostate epithelial cells (Kim et al. 2005). It is also known that chelerythrine can promote Ca^{2+} -dependent calpain activation in neuronal cells in a PKC-independent manner (Saavedra et al. 2017). The anticancer potential of chelerythrine is also well documented. It is a promising antineoplastic growth inhibitor against various tumour cell lines, viz. human breast cancer (MCF-7), human uveal melanoma (OCM-1), human neuroblastoma, colon carcinoma (HCT116) cell lines, and neonatal rat cardiac myocytes (Chmura et al. 2000; Herbert et al. 1990; Weichselbaum et al. 2000; O'Neill et al. 2004; Zhang et al. 2011). More recent studies have also testified that a PKC-independent mechanism may be responsible for its anticancer activity (Yamamoto et al. 2001; Maiti and Suresh Kumar 2007). This may also be linked to its complexation with duplex and quadruplex nucleic acid structures (Cui et al. 2012; Basu et al. 2013; Basu and Suresh Kumar 2014; Bai et al. 2014). The strong nucleic acid binding, topoisomerase poisoning effect and telomerase inhibition properties of chelerythrine are often believed to be responsible for its

marked anticancer activity (Yu et al. 2000; Bai et al. 2014; Basu and Suresh Kumar 2015a). Chelerythrine is known to prevent BclXL-Bak Bcl-2 homology 3 (BH3) binding by displacing Bax binding, BH3-containing protein, from BclXL (Chan et al. 2003). It is also known that chelerythrine binds at a site different from the classic BH3 binding cleft (Zhang et al. 2006). In contrast to the earlier established pathway, chelerythrine appears to induce an alternative Bax/Bak-independent apoptotic mechanism that involved cyclosporine A-sensitive mitochondrial membrane permeability (Wan et al. 2008). Chelerythrine can downregulate the expression of VEGFA, BCL2 and KRAS by inhibiting G-quadruplex structures at their promoter regions (Jana et al. 2017). Chelerythrine has been reported to be a potential therapeutic to reduce the gastric ulceration risks in mice (Li et al. 2014). Chelerythrine is also known to improve acute cardiac allograft rejection in mice (Zhang et al. 2016).

The quaternary benzophenanthridine alkaloids, including chelerythrine, can exist as the charged quaternary form (iminium) in the pH range 2.0–6.0 and as a neutral pseudo-base form (alkanolamine) above pH 9.0 (Fig. 2), and the iminium form is established to be the exclusively DNA- and RNA-binding moiety (Maiti et al. 1983; Hossain et al. 2012; Basu et al. 2013).

Fig. 2 Equilibrium between iminium and alkanolamine forms of chelerythrine. Reprinted from Basu et al. (2013) with permission from Elsevier

Binding of chelerythrine with natural and synthetic DNAs

Su and Wei reported that the chelerythrine iminium form on binding to calf thymus (CT) DNA exhibited hypochromism and bathochromism in the absorption band, caused enhancement of fluorescence intensity and fluorescence polarization, and also perturbed the circular dichroism (CD) spectra of DNA (Su and Wei 2006). These changes were attributed to intercalation of the chelerythrine between the DNA base pairs. The fluorescence titration data was used to construct Scatchard plots which gave a binding constant value of $6.0 \times 10^5 \text{ M}^{-1}$ and a binding site size of 3.4 base pairs at 25 °C. The interaction was reported to be exothermic with a van't Hoff enthalpy of -33.7 kJ/mol . Additionally, the Gibbs energy and entropy change values were found to be -32.9 kJ/mol and -2.9 J/mol/K , respectively, and based on this, the insertion of chelerythrine into the DNA helix was reported to be enthalpy driven.

Basu et al. studied in depth the interaction of the chelerythrine with CT DNA using spectroscopy, viscometry and calorimetry techniques (Basu et al. 2013). The spectroscopic properties of chelerythrine were modified on binding to DNA, and the nature of the binding was reported to be cooperative (Fig. 3). The mode of binding was established to be intercalative as revealed from hydrodynamic studies and this was further supported from fluorescence quenching, and polarization anisotropy results. The alkaloid upon binding to DNA remarkably stabilized the latter against thermal strand separation, raising the melting temperature. The alkaloid also induced conformational changes in the native B-form structure of the DNA. The bound chelerythrine molecules, which were otherwise optically inactive, were also found to acquire optical activity exhibiting induced circular dichroism peaks. The binding affinity value deduced from isothermal titration calorimetry (ITC) experiments at 20 °C was $(2.0 \times 10^6) \text{ M}^{-1}$. This value was in close agreement to those deduced from absorbance and fluorescence titration experiments (Table 1). Furthermore, from ITC studies, the binding was found to be exothermic, favoured by both negative enthalpy and positive entropy changes and showed enthalpy–entropy compensation behaviour (Fig. 4) (Table 2). The value of the heat capacity changes revealed a hydrophobic contribution to the interaction. Molecular aspects of the interaction revealed the binding to be characterized by the involvement of multiple weak non-covalent forces.

Urbanova et al. investigated the spectral, especially fluorescence characteristics, of seven selected quaternary benzo[c]phenanthridine alkaloids namely sanguinarine, chelerythrine, chelirubine, sanguirubine, chelilutine, sanguilutine and macarpine in the absence and presence of double-stranded DNA (Urbanová et al. 2009). The study revealed remarkable differences in the fluorescence behaviour

Table 1 Binding parameters for the complexation of chelerythrine with DNA evaluated from Scatchard plots analysed by the McGhee–von Hippel analysis of the absorbance and fluorescence titration data

Technique	$K \times 10^{-5} (\text{M}^{-1})^a$	n	ω	$K\omega \times 10^{-6} (\text{M}^{-1})$
Absorbance	2.19 ± 0.20	3.2	12.52	2.74
Fluorescence	2.60 ± 0.13	3.3	9.20	2.40

Average of four determinations

^a Binding constants (K) and the number of binding sites (n) refer to solution conditions of 10 mM citrate–phosphate buffer, pH 6.1, at 20 °C. ω is the cooperativity factor. Reprinted from Basu et al. (2013) with permission from Elsevier

of these alkaloids in the presence of CT DNA in comparison to the free alkaloids. The association constants for the binding of these alkaloids with CT DNA were determined (Urbanová et al. 2009).

The interaction of chelerythrine with the double-stranded, heat-denatured and single-stranded DNA was also performed in comparison to its close analogue sanguinarine (Basu and Suresh Kumar 2015a). Binding to all the three DNA conformations leads to enhancement in the fluorescence of chelerythrine. The binding was cooperative in nature with all the three types of DNA conformations. The binding affinity value with the double-stranded DNA was of the order of 10^6 M^{-1} . The binding was comparatively weak with heat-denatured DNA and significantly weaker with single-stranded DNA. The fluorescence intensity of the chelerythrine molecules bound to the double-stranded and heat-denatured DNAs was quenched significantly lesser in comparison to those bound to single-stranded DNA in the presence of the anionic quencher KI. For double-stranded and heat-denatured DNAs, the fluorescence of the bound chelerythrine molecules was polarized significantly and strong energy transfer occurred from the DNA bases to the alkaloid molecules. These fluorescence studies also revealed the binding to be intercalative. ITC studies suggested that the binding was both enthalpy and entropy driven for all the three DNA conformations. Overall, the authors concluded that sanguinarine had a stronger affinity compared to chelerythrine for all the three DNA structures (Table 3).

Bai et al. performed a comparative study on the binding aspects of three benzophenanthridine alkaloids, sanguinarine, chelerythrine and nitidine, with calf thymus (CT) DNA, poly(dG-dC).poly(dG-dC), poly(dA-dT).poly(dA-dT) and seven sequence designed double-stranded oligodeoxynucleotides to gain knowledge about their sequence selectivity for DNA binding (Bai et al. 2006). The results showed that while sanguinarine and nitidine bind preferentially to DNA sequences containing alternating GC base pairs [d(TGCGCA)₂], chelerythrine binds to DNA containing contiguous GC base pairs [5'-TGGGGA-3'/3'-ACCCCT-5'].

Table 2 Thermodynamic parameters for the association of chelerythrine with DNA from ITC studies at different temperatures

Temperature (K)	$K_a \times 10^{-6}$ (M^{-1})	N	ΔG° (kcal/mol)	ΔH° (kcal/mol)	$T\Delta S^\circ$ (kcal/mol)	ΔC_p° (cal/mol K)
283.15	3.21 ± 0.06	0.487	-8.479 ± 0.19	-2.346 ± 0.06	6.133	
293.15	2.01 ± 0.05	0.430	-8.504 ± 0.25	-4.605 ± 0.023	3.899	-142.65 ± 0.35
303.15	0.62 ± 0.01	0.354	-8.082 ± 0.31	-5.199 ± 0.045	2.883	

All the data in this table are derived from the ITC experiments conducted and are the average of four determinations. K_a denotes the binding affinity and ΔH° , the enthalpy change, were determined from ITC profiles fitting to Origin 7.0 software. The values of ΔG° , Gibbs energy change, and $T\Delta S^\circ$, the entropy contribution, were determined using the equations $\Delta G^\circ = -RT \ln K_a$ and $T\Delta S^\circ = \Delta H^\circ - \Delta G^\circ$. All the ITC profiles were fit to a model of single binding sites. Uncertainties correspond to regression standard errors. Reprinted from Basu et al. (2013) with permission from Elsevier

Interaction of chelerythrine with four synthetic sequence specific polynucleotides was studied by Basu and Suresh Kumar (2014). The binding led to strong hypochromic and bathochromic shifts in the absorption spectrum of chelerythrine, enhancement in the fluorescence with the AT and homo-GC polynucleotides and quenching with the hetero-GC polynucleotide. Cooperative binding was observed in all the cases. Intercalative binding was confirmed from fluorescence polarization anisotropy, iodide quenching and viscosity results. The binding caused thermal stabilization of the polynucleotides and moderate perturbations in the DNA

conformations. High binding affinity values of the order of $\sim 10^6 M^{-1}$ were deduced from the spectroscopic data (Table 4). The binding was exothermic and favoured by negative enthalpy and positive entropic contributions in all cases except homo-AT polynucleotide, where the binding was endothermic and purely entropy driven (Fig. 5). Salt-dependent ITC data revealed that the binding was dominated by non-polyelectrolytic forces. The negative heat capacity values established the role of hydrophobic effects in the interaction. All the thermodynamic parameters deduced from the temperature- and salt-dependent ITC studies are presented in

Fig. 3 (a) Absorption spectra of chelerythrine iminium form (2.7 μM) with increasing concentrations of CT DNA in the range 0–27 μM (curves 1–8). Inset: respective Scatchard isotherms of the binding fitted to cooperative McGhee–von Hippel analysis. (b) Absorption spectra of chelerythrine (3.5 μM) alkanolamine form with increasing concentrations of CT DNA in the range of 0–52.5 μM (curves 1–8). Inset: expanded region of spectra in the range 350–500 nm. (c) Fluorescence spectra of

chelerythrine (1.3 μM) iminium form with increasing concentration of CT DNA in the range of 0–26 μM (curves 1–9). Inset: respective Scatchard plots of binding. (d) Fluorescence spectra of chelerythrine (1.3 μM) alkanolamine form with increasing concentration of CT DNA in the range of 0–130 μM (curves 1–11). Inset: expanded spectra in the region of 500–650 nm. Reprinted from Basu et al. (2013) with permission from Elsevier

Fig. 4 **a** Isothermal titration calorimetry profile for the titration of CT DNA with the iminium form of chelerythrine. The top panel represents raw data for the sequential injection of the alkaloid into the DNA solution. The lower panel represents the corresponding normalized heat signals versus molar ratio. The data points (■) reflect the experimental injection heat while the solid line represents the calculated fit of the data. **b** Plots of variation of thermodynamic binding parameters against temperature: $T\Delta S^\circ$ (triangles), ΔH° (circles) and ΔG° (squares) for binding of the iminium form of chelerythrine and CT DNA. **c** Plots of ΔH° (squares) and ΔG° (circles) versus $T\Delta S^\circ$ for the binding of chelerythrine with CT DNA. Reprinted from Basu et al. (2013) with permission from Elsevier

Tables 5 and 6. The binding specificity of chelerythrine followed the order homo-GC > hetero-GC > hetero-AT = homo-AT polynucleotide.

Chelerythrine–quadruplex interaction

Bai et al. studied the binding of chelerythrine to human telomeric DNA/RNA G-quadruplexes and reported that chelerythrine binds selectively and stabilizes the K^+ -form hybrid-type human telomeric DNA G-quadruplex in comparison with the Na^+ -form antiparallel-type DNA G-quadruplex (Bai et al. 2014). ESI-TOF-MS study revealed that

chelerythrine has a binding strength for DNA G-quadruplex similar to that of TMPyP4 tetrachloride. The binding stoichiometry was both 1:1 and 2:1 with DNA and RNA G-quadruplexes. The binding affinity of chelerythrine for RNA G-quadruplex is higher compared to DNA G-quadruplex. Fluorescent triazole orange displacement assay revealed that chelerythrine interacts with human telomeric RNA/DNA G-quadruplexes by the mode of end stacking. ESI-TOF-MS experiments revealed that the relative binding affinity of chelerythrine for human telomeric RNA and DNA G-quadruplexes was 6.0- and 2.5-fold, respectively, tighter than that with human telomeric double-stranded hairpin DNA. Finally, the authors concluded that chelerythrine is a

Table 3 Thermodynamic parameters for the association of sanguinarine and chelerythrine with double-stranded heat-denatured, and single-stranded DNA from isothermal titration calorimetry

Alkaloids	DNA conformation	$K_a \times 10^{-6}$ (M^{-1})	n	ΔG° (kcal/mol)	ΔH° (kcal/mol)	$T\Delta S^\circ$ (kcal/mol)
Sanguinarine	Double stranded	2.80 ± 0.05	3.6	-8.62 ± 0.02	-4.78 ± 0.01	3.84
	Heat denatured	1.70 ± 0.02	4.3	-8.37 ± 0.03	-4.58 ± 0.01	3.78
	Single stranded	0.78 ± 0.03	4.6	-7.91 ± 0.06	-4.39 ± 0.05	3.51
Chelerythrine	Double stranded	2.14 ± 0.05	3.9	-8.48 ± 0.02	-4.04 ± 0.02	4.45
	Heat denatured	0.95 ± 0.02	4.5	-8.02 ± 0.04	-4.00 ± 0.02	4.02
	Single stranded	0.54 ± 0.01	4.6	-7.70 ± 0.05	-4.07 ± 0.05	3.60

All the data in this table are derived from ITC experiments conducted and are the average of four determinations. K_a and ΔH° values were determined from ITC profiles fitting to Origin 7.0 software. The values of ΔG° were determined using the equation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$. n is the reciprocal of N , the stoichiometry. All the ITC profiles were fit to a “one set of sites” model. Reprinted from Basu and Suresh Kumar (2015a) with permission from Taylor & Francis

Table 4 Binding parameters for chelerythrine–polynucleotide complexation evaluated from Scatchard plots analysed by the cooperative McGhee–von Hippel methodology of the absorbance and fluorescence titration data

Technique	DNA	$K_i \times 10^{-5} \text{ (M}^{-1}\text{)}^a$	n	ω	$K_\omega \times 10^{-6} \text{ (M}^{-1}\text{)}$
Absorbance	Poly(dG).poly(dC)	7.53 ± 0.02	2.25	7.01	5.25
	Poly(dG-dC).poly(dG-dC)	2.96 ± 0.05	2.73	8.02	2.37
	Poly(dA).poly(dT)	2.18 ± 0.07	3.17	6.39	1.39
	Poly(dA-dT).poly(dA-dT)	1.85 ± 0.06	2.74	9.37	1.73
Fluorescence	Poly(dG).poly(dC)	7.59 ± 0.08	2.25	6.92	5.25
	Poly(dG-dC).poly(dG-dC)	2.17 ± 0.07	2.67	9.99	2.17
	Poly(dA).poly(dT)	2.17 ± 0.06	3.48	5.67	1.23
	Poly(dA-dT).poly(dA-dT)	2.84 ± 0.05	2.94	5.65	1.60

Average of four determinations

^a Binding constants (K_i) and the number of binding sites (n) refer to solution conditions of 10 mM citrate–phosphate buffer, pH 6.1, at 20 °C. ω is the cooperativity factor. K_ω , the apparent binding constant, a product of cooperative binding constant and the cooperativity factor, is comparable to K_i . Reprinted from Basu and Suresh Kumar (2014) with permission from Elsevier

promising candidate for structure-based design and development of G-quadruplex specific ligands owing to its binding selectivity for the biologically significant K^+ -form human telomeric DNA G-quadruplex over the Na^+ -form analogue, and binding specificity for human telomeric RNA G-quadruplex.

The binding of chelerythrine with the human telomeric DNA sequence (H24) was reported by Dasgupta and co-workers (Ghosh et al. 2015). The alkaloid possessed telomerase inhibitory potential as was evident from telomerase repeat amplification assay in cancer cell line extract, and the authors attributed this property to the quadruplex binding ability of chelerythrine. The molecular aspects of the interaction were characterized by spectroscopic tools like absorbance and CD

and microcalorimetric tools such as ITC and DSC. The results revealed that the alkaloid binds with μM dissociation constant and 2:1 binding stoichiometry to H24. Chelerythrine association stabilized H24, and NMR studies (^1H and ^{31}P) showed that chelerythrine binds to both the G-quartet and phosphate backbone of H24 resulting in quadruplex aggregation. Molecular dynamics simulation studies reiterated the above observations and lent insights into the mechanism of chelerythrine binding. The alkaloid showed higher specificity towards quartet binding in comparison to groove binding. MM-PBSA calculation determined the energy penalty to be -4.7 and -1.7 kcal/mol, respectively, for quartet and groove binding. It was further proposed that the chelerythrine molecule first binds to the quartet and then a second molecule binds

Table 5 ITC-derived thermodynamic profiles for the binding of chelerythrine to DNA polynucleotides at different temperatures

Polynucleotide	Temperature (K)	$K \times 10^{-6} \text{ (M}^{-1}\text{)}$	$\Delta G^\circ \text{ (kcal/mol)}$	$\Delta H^\circ \text{ (kcal/mol)}$	$T\Delta S^\circ \text{ (kcal/mol)}$	$\Delta Cp^\circ \text{ (cal/mol/}^\circ\text{C)}$	$\Delta G^\circ_{\text{hyd}} \text{ (kcal/mol)}$
Poly(dG).poly(dC)	283.15	6.05 ± 0.02	-8.8 ± 0.06	-4.185 ± 0.03	4.615	- 86.1	- 6.88
	293.15	5.26 ± 0.06	-9.01 ± 0.05	-5.34 ± 0.02	3.674		
	303.15	2.64 ± 0.04	-8.90 ± 0.03	-5.90 ± 0.05	2.995		
Poly(dG-dC).poly(dG-dC)	283.15	3.88 ± 0.09	-8.53 ± 0.04	-3.71 ± 0.09	4.82	- 103.0	- 8.2
	293.15	2.49 ± 0.07	-8.57 ± 0.02	-4.32 ± 0.04	4.25		
	303.15	1.07 ± 0.02	-8.36 ± 0.1	-5.76 ± 0.06	2.60		
Poly(dA).poly(dT)	283.15	2.07 ± 0.03	-8.24 ± 0.07	4.31 ± 0.08	12.55	- 138.0	- 11.4
	293.15	1.40 ± 0.04	-8.24 ± 0.03	3.34 ± 0.07	11.58		
	303.15	0.82 ± 0.02	-8.25 ± 0.06	1.55 ± 0.05	9.81		
Poly(dA-dT).poly(dA-dT)	283.15	2.84 ± 0.07	-8.41 ± 0.05	-2.23 ± 0.03	6.19	- 148.0	- 11.8
	293.15	1.70 ± 0.08	-8.34 ± 0.03	-2.42 ± 0.07	5.99		
	303.15	0.89 ± 0.02	-8.30 ± 0.01	-5.19 ± 0.06	3.11		

The data in this table are derived from ITC experiments and are averages of four determinations at different temperatures. K and ΔH° values were determined from ITC profiles fitting to Origin 7.0 software. The values of ΔG° and $T\Delta S^\circ$ were determined using equations $\Delta G^\circ = -RT\ln K$, and $T\Delta S^\circ = \Delta H^\circ - \Delta G^\circ$. All ITC profiles were fit to a model of single binding sites. Reprinted from Basu and Suresh Kumar (2014) with permission from Elsevier

Table 6 ITC-derived thermodynamic profiles for the binding of chelerythrine to DNA polynucleotides at different $[Na^+]$

Polynucleotide	$[Na^+]$	$K \times 10^{-6} (M^{-1})$	ΔG° (kcal/mol)	ΔH° (kcal/mol)	$T\Delta S^\circ$ (kcal/mol)	ΔG_t (kcal/mol $^\circ C$)	ΔG_{pe} (kcal/mol)
Poly(dG).poly(dC)	10	5.26 ± 0.06	-9.01 ± 0.05	-5.34 ± 0.02	3.67	-6.93 ± 0.08	-2.07 ± 0.07
	25	3.09 ± 0.01	-8.70 ± 0.08	-5.21 ± 0.03	3.49	-7.03 ± 0.10	-1.66 ± 0.05
	50	1.49 ± 0.01	-8.27 ± 0.07	-4.68 ± 0.08	3.60	-6.92 ± 0.07	-1.35 ± 0.01
Poly(dG-dC).poly(dG-dC)	10	2.49 ± 0.07	-8.57 ± 0.02	-4.32 ± 0.04	4.25	-6.82 ± 0.05	-1.75 ± 0.02
	25	1.08 ± 0.03	-8.09 ± 0.07	-5.37 ± 0.05	2.71	-6.68 ± 0.06	-1.41 ± 0.02
	50	0.88 ± 0.02	-7.97 ± 0.07	-4.05 ± 0.01	3.92	-6.82 ± 0.04	-1.11 ± 0.03
Poly(dA).poly(dT)	10	1.40 ± 0.04	-8.24 ± 0.03	3.34 ± 0.07	11.58	-6.70 ± 0.05	-1.54 ± 0.04
	25	0.65 ± 0.03	-7.79 ± 0.11	5.54 ± 0.06	13.33	-6.56 ± 0.09	-1.23 ± 0.05
	50	0.48 ± 0.02	-7.61 ± 0.04	2.92 ± 0.08	10.53	-6.61 ± 0.12	-1.00 ± 0.06
Poly(dA-dT).poly(dA-dT)	10	1.70 ± 0.08	-8.34 ± 0.03	-2.42 ± 0.07	5.92	-6.92 ± 0.07	-1.14 ± 0.01
	25	0.94 ± 0.02	-8.00 ± 0.06	-4.82 ± 0.03	3.20	-6.86 ± 0.09	-1.15 ± 0.02
	50	0.74 ± 0.02	-7.86 ± 0.01	-3.52 ± 0.07	4.34	-6.93 ± 0.10	-0.94 ± 0.01

All the data in this table are derived from the ITC experiments and are the average of four determinations. K_a denotes the binding affinity and ΔH° , the enthalpy change, were determined from ITC profiles fitting to Origin 7.0 software. The values of ΔG° , the Gibbs energy change, and $T\Delta S^\circ$, the entropy contribution, were determined using the equations $\Delta G^\circ = -RT \ln K_a$ and $T\Delta S^\circ = \Delta H^\circ - \Delta G^\circ$. All the ITC profiles were fit to a model of single binding sites. Uncertainties correspond to regression standard errors. Reprinted from Basu and Suresh Kumar (2014) with permission from Elsevier

to the groove. The second groove binding interaction can bring about the aggregation of H24 as was evident from the NMR results.

Ghosh and Dasgupta studied the effect of chelerythrine on G-rich NHE III1 found in the promoter region of the c-myc oncogene because guanine-rich sequences in the promoter regions of oncogenes have the ability to fold into G-quadruplex structures and thereby modulate the transcription process (Ghosh and Dasgupta 2015). The authors demonstrated through their study the ability of chelerythrine to block the

hybridization of Pu27 with its complementary strand by folding it into a quadruplex structure. Calorimetry experiments revealed that the Pu27-chelerythrine binding is primarily enthalpy driven with binding affinity of the order $\sim 10^5 M^{-1}$, but the association does not cause any major structural perturbation of Pu27. The resulting 2:1 complex has greater stability in comparison to uncomplexed Pu27. Besides, in the presence of a molecular crowding agent like Ficoll 70, the binding affinity decreased but the mode of recognition remained unaltered. Finally, the authors attributed the anticancer property of

Fig. 5 Circular dichroism spectral changes of (a) homo-GC (30 μM), (b) hetero-GC (30 μM), (c) homo-AT (30 μM) and (d) hetero-AT (30 μM) on interaction with increasing concentration of chelerythrine. Reprinted from Basu and Suresh Kumar (2014) with permission from Elsevier

chelerythrine to its potential to stabilize the quadruplex structure in the c-myc promoter region and thereby downregulate its transcription.

Jana et al. had reported that chelerythrine binds to G-quadruplexes at promoters of VEGFA, BCL2 and KRAS genes as well as downregulates their expression (Jana et al. 2017). The association of chelerythrine to G-quadruplex at the promoters of these oncogenes was monitored using spectroscopic, calorimetric, molecular dynamics simulation and quantitative RT-PCR technique. The authors suggested from the hypochromism and red shifts in the UV spectrum in conjunction with the results of ethidium bromide displacement assays, an end stacking mode of interaction of chelerythrine with the corresponding G-quadruplex structures. An enhancement in fluorescence anisotropy and CD melting temperature of the chelerythrine–quadruplex complex indicated the formation of a stable chelerythrine–quadruplex complex. ITC data confirmed that chelerythrine–quadruplex complexation is thermodynamically feasible. Quantitative RT-PCR results in conjunction with luciferase assay showed that treatment of chelerythrine to MCF7 breast cancer cells efficiently down-regulated the transcript level of all three genes which indicated that chelerythrine efficiently binds to in vivo quadruplex motifs. Molecular dynamics simulation gave the molecular picture of the interaction between chelerythrine and G-quadruplex. The work provided useful insights for the development of future therapeutics against cancer.

The binding properties of chelerythrine and coptisine with the human telomeric G-quadruplex were studied by spectroscopic techniques, molecular modelling and X-ray diffraction analysis in comparison to berberine and sanguinarine (Papi et al. 2017). Spectroscopic studies revealed modest, but dissimilar, rearrangements of the DNA–ligand complexes, which can be explained on the basis of particular stereochemical features for these compounds. The authors concluded that the presence of a dioxolo moiety rather than the two methoxy functional groups was responsible for the increased efficiency of coptisine and sanguinarine over berberine and chelerythrine, and the overall stability order was sanguinarine > chelerythrine $\approx$ coptisine > berberine. Accordingly, the X-ray diffraction analysis confirmed

Fig. 6 ITC profile for the titration of tRNA with chelerythrine. The top panel represents raw data for the sequential injection of the tRNA into the alkaloid solution. In the lower panel, the corresponding normalized heat signals versus molar ratio is presented. The data points (squares) are the experimental injection heats while the solid line represents the calculated fit of the data. Reprinted from Basu et al. (2016a) with permission from Elsevier

the involvement of the benzodioxolo groups in the coptisine/DNA binding by means of $\pi\cdots\pi$, $O\cdots\pi$ and $CH\cdots O$ interactions. Similar information was also provided by modelling studies,

Table 7 Thermodynamic parameters for the association of chelerythrine with tRNA from ITC studies at different salt concentrations

[Na ⁺] molarity (mM)	$K_a \times 10^{-5}$ (M ⁻¹)	N	ΔG° (kcal/mol)	ΔH° (kcal/mol)	$T\Delta S^\circ$ (kcal/mol)	ΔG°_t (kcal/mol)	ΔG°_{pe} (kcal/mol)
10	4.06 ± 0.01	0.502	-7.518 ± 0.15	-5.258 ± 0.02	2.263	-5.709 ± 0.10	1.808 ± 0.02
25	2.09 ± 0.02	0.462	-7.131 ± 0.21	-3.155 ± 0.02	3.976	-5.748 ± 0.17	1.383 ± 0.01
50	1.37 ± 0.03	0.401	-6.885 ± 0.10	-2.861 ± 0.01	4.024	-5.762 ± 0.10	1.123 ± 0.01

All the data in this table are derived from the ITC experiments conducted and are the average of four determinations. K_a is the binding affinity and ΔH° , the enthalpy change, were determined from ITC profiles fitting to Origin 7.0 software. The values of Gibbs energy change (ΔG°) and the entropy contribution ($T\Delta S^\circ$) were determined using the equations $\Delta G^\circ = -RT \ln K_a$ and $T\Delta S^\circ = \Delta H^\circ - \Delta G^\circ$. ΔG°_t and ΔG°_{pe} can be obtained from the relation, $\Delta G^\circ_t = \Delta G^\circ - \Delta G^\circ_{pe}$; n is site size which is reciprocal to N , the binding stoichiometry. All the ITC profiles were fit to a model of a single binding site. Uncertainties correspond to regression standard errors. Reprinted from Basu et al. (2016a) with permission from Elsevier

Fig. 7 Posing of chelerythrine-iminium form at tRNA; **a** classical (in black colour) and non-classical (in marine colour) H-bond interactions; **b** view showing five bases involved in the positioning of chelerythrine molecule with added hydrophobic contribution; here, black colour

represents classical H-bonds, blue non-classical carbon-mediated H-bonds, green pi-donor H-bond and magenta pi-sigma bond. Reprinted from Basu et al. (2016a) with permission from Elsevier

which provided additional support for the quadruplex versus double helix selectivity shown by these compounds. The analyses highlighted the key role of the benzodioxolo moieties in strengthening the interaction with the G4-folded human telomeric sequence and also revealed the superior G4 stabilizing properties of the benzophenanthridines compared to the protoberberines and conversely the better G4 versus double-stranded DNA selectivity profile of coptisine over the other compounds studied.

Noureini et al. studied the mechanism of telomerase inhibition by stabilization of telomeric G-quadruplex structures by several natural isoquinoline alkaloids like berberine, chelerythrine, chelidonine, sanguinarine and papaverine (Noureini et al. 2017). They observed strong inhibitory effects of chelerythrine, sanguinarine and berberine on telomerase activity and concluded it was most likely via substrate sequestration. These isoquinoline alkaloids exhibited strong interaction with telomeric sequence G-quadruplex whereas chelidonine and papaverine had no significant interaction with the telomeric quadruplex, but they strongly inhibited

telomerase at the transcription level of hTERT. Taken together, it was observed that all of the alkaloids studied exhibited varying levels and mechanisms of telomerase inhibition. Overall, a comparative study of anti-telomerase activity of the isoquinoline alkaloids of *Chelidonium majus* was undertaken and chelerythrine was the most potent inhibitor of telomerase activity by substrate sequestration through G-quadruplex stabilization. Such studies, conducted at the structural and molecular levels, are helpful in developing new and more potent drugs with fewer side effects. The authors finally concluded that isoquinoline alkaloids being the most biologically active molecules from *Chelidonium majus* are strong telomeric G-quadruplex stabilizers and telomerase inhibitors with chelerythrine being the most potent member.

Chelerythrine–chromatin interaction

Chelerythrine influences several key signal transduction pathways, and so, it is essential to study the ability of chelerythrine

Table 8 Binding parameters for the complexation of chelerythrine with RNA evaluated from Scatchard plots analysed by the McGhee–von Hippel analysis of the absorbance and fluorescence titration data

Method	System	$K_i \times 10^{-5a}$ (M^{-1})	n	ω	$K_{\omega} \times 10^{-6}$ (M^{-1})
Absorbance	Chelerythrine + poly(UAU)	1.003 ± 0.015	3.95 ± 0.012	16.88 ± 0.220	1.69 ± 0.021
	Chelerythrine + poly(AU)	0.718 ± 0.017	4.48 ± 0.013	20.14 ± 0.561	1.45 ± 0.081
	Chelerythrine + poly(U)	2.726 ± 0.062	5.43 ± 0.090	—	—
Fluorescence	Chelerythrine + poly(UAU)	0.998 ± 0.009	4.13 ± 0.008	16.84 ± 0.226	1.68 ± 0.020
	Chelerythrine + poly(AU)	0.727 ± 0.016	4.54 ± 0.010	19.68 ± 0.568	1.43 ± 0.051
	Chelerythrine + poly(U)	2.635 ± 0.051	5.42 ± 0.10	—	—

Average of four determinations. Reprinted from Basu and Suresh Kumar (2015b) with permission from the Royal Society of Chemistry

^a Binding constants (K_i) and the number of binding sites (n) refer to solution conditions of sodium cacodylate buffer, pH 6.3. ω is the cooperativity factor. The values given above are averages of four determinations

Fig. 8 **a** Stern–Volmer plots for the quenching of free chelerythrine (empty triangles) and complexes with poly(UAU) (filled squares), poly(AU) (filled circles) and poly(U) (filled triangles). **b** A plot of variation of the (η'/η) with varying molar ratio for the complexation of chelerythrine to poly(UAU) (filled squares), poly(AU) (filled circles) and poly(U) (filled triangles), and **c** variation of the relative quantum yield of chelerythrine in the presence of poly(UAU) (filled squares), poly(AU) (filled circles) and poly(U) (filled triangles). All the data in this plot are the average of four determinations. Reprinted from Basu and Suresh Kumar (2015b) with permission from the Royal Society of Chemistry

to interact with nucleoprotein complex chromatin, in eukaryotic cells. Banerjee et al. studied the association with hierarchically assembled chromatin components, that is, long chromatin, chromatosome, nucleosome, chromosomal DNA and histone H3, and also noted the resulting effect on the structure of chromatin (Banerjee et al. 2017). The authors reported that chelerythrine repressed acetylation at H3K9; the alkaloid was more target-specific in terms of gene expression alteration and comparatively less cytotoxic than sanguinarine.

RNA binding properties of chelerythrine

Chelerythrine–tRNA binding

The interaction of chelerythrine with tRNA^{phe} (tRNA hereafter) was studied by spectroscopy, calorimetry and computational tools (Basu et al. 2016a). The charged iminium form of chelerythrine was reported to bind with tRNA cooperatively with a binding affinity of $(4.06 \pm 0.01) \times 10^5 \text{ M}^{-1}$. The neutral alkanolamine form of chelerythrine was reported not to bind with tRNA, but in the presence of a high concentration of tRNA, this form was converted to the iminium form which binds with tRNA. A partial intercalative binding mode of chelerythrine to tRNA was deduced from the steady-state anisotropy, fluorescence quenching and viscosity experiments. Chelerythrine binding caused conformational perturbations in tRNA as observed from CD spectroscopy. The strong binding of chelerythrine with the tRNA bases was independently supported by the ethidium bromide displacement assay. The binding was driven by both negative enthalpy and positive entropy contributions (Fig. 6). Although the binding was $[\text{Na}^+]$ dependent, non-electrostatic forces contributed mainly to the Gibbs energy change as is evident from the thermodynamic data presented in Table 7. The negative heat capacity change revealed the involvement of hydrophobic forces in the binding. Molecular docking studies provided insights about the chelerythrine binding pockets on tRNA and marked the interactions required for binding of chelerythrine molecule (Fig. 7). Partially intercalative binding mode was also supported by docking studies. The authors reported that the docking results corroborated well with the experiential observations.

Chelerythrine–double- and triple-stranded RNA binding

A comparative study on the interaction of chelerythrine with triplex poly(U)·poly(A)*poly(U) (hereafter UAU, · and * represents Watson–Crick and Hoogsteen base pairings, respectively), duplex poly(A)·poly(U) (hereafter AU) and single-stranded poly(U) was performed by Basu and Suresh Kumar (2015b). Chelerythrine bound to both the duplex and triplexes cooperatively with affinity of the order of 10^6 M^{-1} . A weaker non-cooperative binding ($\sim 10^5 \text{ M}^{-1}$) was observed with poly(U) (Table 8). Chelerythrine was reported to be more selective towards RNA triplex than its parent duplex. The triplex was thermally stabilized specifically without affecting the stability of the duplex. Fluorescence quenching, fluorescence polarization and energy transfer from the RNA bases to chelerythrine, along with viscosity results, provided support for an intercalative binding mode of chelerythrine to both the triplex and the duplex, and partial base stacking with poly(U) bases (Fig. 8). The conformations of both duplex and triplexes were perturbed

Fig. 9 Circular dichroism spectral changes of **a** poly(UAU) (30 μM), **b** poly(AU) (30 μM) and **c** poly(U) (30 μM) on interaction with increasing concentration of chelerythrine in the range of 0–30 μM . Reprinted from

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in the presence of chelerythrine, but the alkaloid did not affect the single-strand structure (Fig. 9). The binding of chelerythrine to all the RNAs was exothermic; to the triplex, it was entropy driven with negative enthalpy change; to the duplex, it was enthalpy driven; and to the single-stranded poly(U), it was enthalpy driven (Fig. 10). These findings provided new knowledge on the mode, mechanism, specificity and energetics of binding of chelerythrine to various RNA conformations.

Haque et al. performed a comparative study on the interaction of chelerythrine with RNA triplex poly(U).poly(A)*poly(U) and the duplex poly(A).poly(U) by using multifaceted biophysical techniques (Haque et al. 2015). The binding caused hypochromic

and bathochromic effects in the absorption spectrum of chelerythrine, an enhancement in thermal melting temperature, an increase in viscosity and perturbation of the CD spectrum. The binding constant was calculated using spectrophotometric data and was found to be greater for triplex ($30.2 \times 10^5 \text{ M}^{-1}$) compared to duplex RNA ($3.6 \times 10^5 \text{ M}^{-1}$). The authors reported that the ITC data were in agreement with the spectrophotometric data. The binding of chelerythrine to the triplex RNA was stronger compared to that of the duplex RNA. Optical melting studies indicated higher stabilization of the Hoogsteen base-paired third strand of the triplex in comparison to the Watson–Crick strands. The binding mode of chelerythrine to both the forms of RNA was intercalative as revealed from fluorescence quenching,

Fig. 10 ITC profiles for the titration of chelerythrine with **a** poly(UAU), **b** poly(AU) and **c** poly(U) at 20 $^{\circ}\text{C}$ in sodium cacodylate buffer at pH 6.3. The top panels represent the raw data resulting from the sequential injection of RNAs into polynucleotide solutions, and the lower panels represent the corresponding normalized heat signals versus molar ratio.

The data points (squares) are the experimental injection heats while the continuous line is the best fit to the experimental data. Reprinted from Basu and Suresh Kumar (2015b) with permission from the Royal Society of Chemistry

Table 9 Binding parameters for the complexation of sanguinarine and chelerythrine to single-stranded RNAs evaluated from spectroscopic data

Alkaloid	RNA	$K_i \times 10^{-5} \text{ (M}^{-1}\text{)}^a$	n^a	$K_{BH} \times 10^{-5} \text{ (M}^{-1}\text{)}^b$
Sanguinarine	Poly(rI)	12.41 ± 0.04	1.18	10.56
	Poly(rG)	3.48 ± 0.08	2.93	3.37
	Poly(rC)	1.78 ± 0.04	3.05	1.60
Chelerythrine	Poly(rI)	14.88 ± 0.04	1.97	11.64
	Poly(rG)	5.09 ± 0.02	3.15	4.37
	Poly(rC)	2.87 ± 0.01	4.06	2.40

The data presented are average of four determinants. Reprinted from Basu and Suresh Kumar (2017) with permission from Elsevier

^a K_i indicates the intrinsic binding constant to an isolated binding site, and n is the number of nucleotide phosphates excluded by the binding of one alkaloid molecule

^b K_{BH} is the apparent binding constant using Benesi–Hildebrand methodology

viscosity and sensitization of the fluorescence experiment. Thermodynamic data obtained from ITC revealed that binding was favoured by both negative enthalpy change and positive entropy changes. The results suggested that chelerythrine binds and stabilizes the triplex better than its parent duplex. Finally, the authors concluded that their results can be useful for designing efficient antigen strategies involving benzophenanthridines and RNA triplex.

Chelerythrine–single-stranded RNA binding

Basu and Suresh Kumar performed a comparative study on the interaction of single-stranded RNAs with two benzophenanthridine alkaloids, namely sanguinarine and chelerythrine (Basu and Suresh Kumar 2017). Single-stranded RNAs are potent biomolecules as they are involved in various important cellular processes. The binding aspects of the two benzophenanthridine alkaloids, sanguinarine and chelerythrine, with three single-stranded RNAs, namely poly(rI), poly(rG) and poly(rC), were studied using both spectroscopic and thermodynamic tools. The authors found that both the alkaloids bound strongly to the single-stranded RNAs and the binding affinity followed the order poly(rI) > poly(rG) > poly(rC). Chelerythrine had slightly better affinity for the RNAs in comparison to sanguinarine. Both alkaloids showed binding affinity of the order lower 10^6 M^{-1} with poly(rI), higher 10^5 order with poly(rG) and lower 10^5 order with poly(rC) (Table 9). The binding mode was found to be partially intercalative owing to the stacking interaction between the RNA bases and the alkaloids. The complexation of both the benzophenanthridines to the RNAs was primarily enthalpy driven but was also favoured by entropy changes (Fig. 11). Binding of the alkaloids caused perturbation in the RNA conformation (Fig. 12). The authors reported the fundamental binding aspects of the natural benzophenanthridine alkaloids with single-stranded RNAs and concluded that their results may help to design new-generation alkaloid-based therapeutics for targeting single-stranded RNAs.

Fig. 11 Circular dichroism spectral changes of (a) poly(rI), (b) poly(rG) and (c) poly(rC) with increasing concentration of sanguinarine and (d) poly(rI), (e) poly(rG) and (f) poly(rC) with increasing concentration of

chelerythrine. Reprinted from Basu and Suresh Kumar (2017) with permission from Elsevier

Fig. 12 ITC profiles for the titration of sanguinarine with **a** poly(rI), **b** poly(rG) and **c** poly(rC) and chelerythrine with **d** poly(rI), **e** poly(rG) and **f** poly(rC). The upper panels depict the raw data resulting from the sequential injection of alkaloids into the RNA solutions, and the bottom

panels present the corresponding normalized heat signals versus molar ratio. The data points (squares) are the experimental injection heats while the continuous lines denote the best fit to the experimental data. Reprinted from Basu and Suresh Kumar (2017) with permission from Elsevier

Table 10 Thermodynamic parameters for the association of chelerythrine with single-stranded poly(A) from ITC studies at different salt concentrations at 293.15 K temperature and at a pressure of 101.1 kPa

$[\text{Na}^+]^a$ molarity/ (mM)	$K \times 10^{-6}/$ (M^{-1})	N	$\Delta_{\text{bind}}G^\circ/$ (kJ mol^{-1})	$\Delta_{\text{bind}}H^\circ/$ (kJ mol^{-1})	$T\Delta_{\text{bind}}S^\circ/$ (kJ mol^{-1})	$\Delta_i G^\circ/$ (kJ mol^{-1})	$\Delta_{\text{pe}}G^\circ/$ (kJ mol^{-1})
10 ± 0.01	9.01 ± 0.05	0.483 ± 0.05	-39.09 ± 0.25	-19.59 ± 0.03	19.50 ± 0.02	-35.04 ± 0.29	-4.05 ± 0.02
25 ± 0.01	10.10 ± 0.05	0.487 ± 0.06	-39.28 ± 0.10	-15.32 ± 0.07	24.07 ± 0.03	-36.11 ± 0.19	-3.17 ± 0.03
50 ± 0.01	14.10 ± 0.04	0.491 ± 0.05	-40.09 ± 0.07	-13.59 ± 0.05	26.49 ± 0.02	-37.52 ± 0.17	-2.57 ± 0.01
100 ± 0.01	20.10 ± 0.03	0.495 ± 0.06	-40.96 ± 0.05	-11.05 ± 0.03	29.92 ± 0.04	-38.98 ± 0.14	-1.98 ± 0.04

All the data in this table are derived from the ITC experiments: K , the binding affinity; N , the stoichiometry constant; and $\Delta_{\text{bind}}H^\circ$, the standard molar enthalpy change were determined from ITC profiles fitting to Origin 7.0 software. The values of $\Delta_{\text{bind}}G^\circ$, the standard molar Gibbs free energy change, and $T\Delta_{\text{bind}}S^\circ$, the entropy contribution, were determined using the equations $\Delta_{\text{bind}}G^\circ = -RT \ln K$, and $T\Delta_{\text{bind}}S^\circ = \Delta_{\text{bind}}H^\circ - \Delta_{\text{bind}}G^\circ$. The R is the gas constant. $\Delta_i G^\circ$ and $\Delta_{\text{pe}}G^\circ$ are the standard molar Gibbs free energy contributions from the non-polyelectrolytic and polyelectrolytic forces, respectively. All the ITC profiles were fit to a model of single binding sites. Standard uncertainties u are $u(T) = 0.01$ K and $u(P) = 1.0$ kPa. Standard uncertainty equals to one standard deviation. Reprinted from Basu and Suresh Kumar (2015c) with permission from Elsevier

^a $[\text{Na}^+]$ denotes the sodium ion concentration in the experimental buffer

Fig. 13 Circular dichroism spectra of single-stranded poly(A) (60 μM) treated with 0, 6, 12, 24, 30 36, 48 and 60 μM of chelerythrine (curves 1 to 8). The expressed molar ellipticity (θ) values are based on poly(A) concentration. Measurements were performed in a rectangular quartz cuvette of 10 mm path length. Reprinted from Basu and Suresh Kumar (2015c) with permission from Elsevier

Fig. 14 **a** Optical thermal melting profiles of poly(A) (empty circles) and (poly(A) + chelerythrine) complex (filled circles) monitored at 257 nm. **b** Circular dichroism melting profiles of poly(A) (empty triangles) and (poly(A) + chelerythrine) complex (filled circles) monitored at 257 nm. Reprinted from Basu and Suresh Kumar (2015c) with permission from Elsevier

Chelerythrine–polyriboadenylic acid (poly (A)) binding

Pradhan et al. and Basu and Suresh Kumar reported the strong binding of chelerythrine to single-stranded poly(A). The affinity was reported by both groups to be $\sim 10^7 \text{ M}^{-1}$ (Pradhan et al. 2014; Basu and Suresh Kumar 2015c). Pradhan et al. investigated the binding by different spectroscopic and viscometric techniques. These authors reported that chelerythrine induced self-structure formation in single-stranded poly(A) and the binding mode was intercalative in nature. Inference for the binding mode was deduced from fluorescence quenching, energy transfer and viscosity experiments (Pradhan et al. 2014). Analysis of the binding data was done through the non-cooperative model of McGhee and von Hippel. From temperature dependence of the binding constants in the range 15–35 $^{\circ}\text{C}$ Pradhan et al. deduced the thermodynamics of the interaction. It was proposed that the binding was driven by a large negative enthalpy change and opposing negative entropy change (Pradhan et al. 2014).

A more detailed and systematic investigation on the interaction between chelerythrine and single-stranded poly(A) was performed by Basu and Suresh Kumar using multifaceted spectroscopic and sensitive calorimetric techniques (Basu and Suresh Kumar 2015c). The binding constant was found to increase within the 10- to 100-mM Na^+ concentration range (Table 10). CD spectra confirmed a strong conformational change in single-stranded poly(A) that apparently led to the entropy increase (Fig. 13). Optical and CD melting profiles revealed that chelerythrine induced self-assembled duplex structure formation in single-stranded poly(A) as was seen from cooperative melting profiles. This was further supported from differential scanning calorimetry (DSC) data (Fig. 14). The intercalative binding of chelerythrine was proposed to involve energy transfer from the poly(A) bases to the chelerythrine molecules. Most importantly, a cooperative binding mode for chelerythrine–single-stranded poly(A) complexation was advanced for the first time from these studies in contrast to the non-cooperative binding proposed by Pradhan et al. (2014). It was concluded that the unusually high entropy-driven binding of chelerythrine led to spontaneous self-assembled structure formation in single-stranded poly(A) and binding affinity of $\sim 10^7 \text{ M}^{-1}$ was the highest reported till then for a small molecule–single-stranded poly(A) association (Basu and Suresh Kumar 2015c). Furthermore, from ITC results, the authors observed strong positive entropy as well as favourable enthalpy favouring the binding which was in contrast to the negative entropy change reported by Pradhan et al. (2014).

Basu et al. (2016b) first reported the binding of chelerythrine to double-stranded polyriboadenylic acid. The binding was thermodynamically characterized by negative enthalpy and positive entropy changes along with enthalpy–entropy compensation

Table 11 Thermodynamic parameters for the association of chelerythrine with double-stranded poly(A) from ITC at different temperatures

T/K	$10^{-5} K/M^{-1}$	n	$\Delta_r H^\circ/(kJ\ mol^{-1})$	$T\Delta_r S^\circ/(kJ\ mol^{-1})$	$\Delta_r G^\circ/(kJ\ mol^{-1})$	$\Delta_r C_p^\circ/(kJ\ K^{-1}\ mol^{-1})$
288.15 ± 0.01	4.43 ± 0.12	1.67 ± 0.04	-8.49 ± 0.03	22.66 ± 0.09	-31.15 ± 0.12	
293.15 ± 0.01	3.94 ± 0.11	1.70 ± 0.04	-8.97 ± 0.03	22.43 ± 0.08	-31.40 ± 0.11	-0.12 ± 0.01
298.15 ± 0.01	3.42 ± 0.09	1.71 ± 0.05	-9.29 ± 0.04	22.30 ± 0.05	-31.59 ± 0.09	
308.15 ± 0.01	2.01 ± 0.07	2.43 ± 0.09	-10.83 ± 0.05	20.46 ± 0.02	-31.29 ± 0.07	

The ITC experiments were conducted at a pressure of 101.10 kPa. T is the temperature in kelvins (K). K , the equilibrium constant; n , the site size which is the reciprocal of binding stoichiometry (N); and $\Delta_r H^\circ$, the standard molar enthalpy change, were determined from ITC profiles fitting to Origin 7.0 software as described in the text. The values of $\Delta_r G^\circ$, standard molar Gibbs energy change, and $T\Delta_r S^\circ$, the standard molar entropy contribution were determined using the equations $\Delta_r G^\circ = -RT \ln K$ and $T\Delta_r S^\circ = \Delta_r H^\circ - \Delta_r G^\circ$. R is the gas constant. $\Delta_r C_p^\circ$ is the standard molar heat capacity change. Standard uncertainties u are $u(T) = 0.01$ K and $u(P) = 1.0$ kPa. The standard uncertainties are equal to one standard deviation. The uncertainties in the values of thermodynamic parameters also include an additional 3% error due to possible errors in the values of the concentrations of the reactants. Reprinted from Basu et al. (2016b) with permission from Elsevier

behaviour. The thermodynamic quantities for the association of chelerythrine with double-stranded poly(A) are presented in Table 11. The affinity $(3.94 \pm 0.11) \times 10^5\ M^{-1}$ at 293.15 K was about two orders lower than its binding affinity to single-stranded poly(A). The binding stabilized the structure of poly(A) duplex thermally. The data from DSC for the complexation of chelerythrine with duplex poly(A) is shown in Table 12. The binding caused hypochromic and bathochromic effects in the absorption spectrum of chelerythrine and increased its fluorescence intensity (Fig. 15). The binding interaction was observed to be cooperative in nature. The binding mode was inferred to be intercalative as revealed by fluorescence quenching, anisotropy, and hydrodynamic experiments (Fig. 16). Overall the binding of chelerythrine to double-stranded poly(A) was much weaker compared to that with single-stranded poly(A).

Subsequent to the report of Basu et al. (vide supra), Pradhan et al. also probed the interaction of chelerythrine with double-stranded poly(A) using various spectroscopic techniques (Basu et al. 2016b; Pradhan et al. 2017). These authors reported a binding affinity of $(1.10 \pm 0.10) \times 10^5\ M^{-1}$ towards the double-stranded poly(A) which was deduced from the analysis of a non-cooperative Scatchard plot. The interaction also caused perturbations in the circular dichroism spectrum of double-stranded poly(A). The binding of chelerythrine to double-stranded poly(A) was also reported to be intercalative

in nature supporting and confirming the original proposal of Basu et al. (2016b). Furthermore, the thermodynamic results obtained by Pradhan et al. suggested an enthalpy-dominated binding in contrast to the entropy-dominated binding reported by Basu et al. (2016b; Pradhan et al. 2017).

Conclusions

There is increasing interest in understanding the nucleic acid binding properties of small molecules, particularly, natural products and their analogues. The aim of such investigations is to develop more effective drugs with lower toxicity and higher efficacy that can be targeted to specific DNA and RNA sequences in the cells for therapeutic intervention. The major advantage of natural products over synthetic compounds is their high abundance in nature, low toxicity and can be easily absorbed and metabolized in the body. In this regard, benzophenanthridine alkaloid chelerythrine represents a promising drug candidate. This review summarizes the major advancements in the field of chelerythrine–DNA and chelerythrine–RNA interactions. These advances have been attained through extensive biophysical investigations aimed at understanding the mode, mechanism, base-sequence specificity, structural affinity and energetics of its interaction. The

Table 12 DSC data and equilibrium constants for chelerythrine–double-stranded poly(A) complexation

	T_{fus}/K	$\Delta T_{fus}/K$	$\Delta H_{cal}/(kJ\ mol^{-1})$	$\Delta H_{VH}/(kJ\ mol^{-1})$
Poly(A)	360.41 ± 0.13	–	59.29 ± 0.11	60.10 ± 0.27
Poly(A) + chelerythrine	366.49 ± 0.17	6.08 ± 0.04	65.65 ± 0.12	715.46 ± 0.20

The DSC experiments were performed under a pressure of 206.84 kPa. T_{fus} is the melting temperature obtained from DSC. ΔH_{cal} is the calorimetric enthalpy. ΔH_{VH} is the van't Hoff enthalpy. ΔH_{cal} and ΔH_{VH} were obtained from the analysis of DSC data. Standard uncertainty u is $u(P) = 1.0$ kPa. Standard uncertainties are equal to one standard deviation. Reprinted from Basu et al. (2016b) with permission from Elsevier

Fig. 15 **a** Absorption spectral titration of chelerythrine (curve 1) with increasing concentration of double-stranded poly(A) (curves 2–7). **b** Fluorescence spectral titration of chelerythrine (curve 1) with increasing concentration of double-stranded poly(A) (curves 2–9). Reprinted from Basu et al. (2016b) with permission from Elsevier

ability of chelerythrine to intercalate with remarkably high affinity to various DNA and RNA structures and even induce spontaneously self-structure in single-stranded poly(A) is now well known. A detailed knowledge of the binding aspects with double- and single-stranded DNA and RNAs, and preference for other polymorphic and higher-order structures, has also been revealed. Knowledge gained from structural data has been further complemented by detailed thermodynamic data. A comprehensive understanding of these aspects will permit the development of better benzophenanthridine-based drug molecules and open new avenues for diagnostic studies useful for various biomedical applications.

Future perspectives

This review has focused on the naturally derived plant alkaloid chelerythrine and summarized its binding interaction with various nucleic acid structures and sequences reported till date. It highlights the molecular mechanisms underlying the interaction of chelerythrine with different nucleic acid structures that will be useful for researchers to understand and generate new selective and safer compounds with improved therapeutic applications for the prevention and treatment of cancer. On the basis of the information gathered, chelerythrine

represents an important anticancer drug of plant origin with enormous potential for further future development (although more advanced pharmacokinetic experiments and clinical trials are required). Recently, pharmaceutical companies have put emphasis on drug discovery from natural sources through combinatorial chemistry, which includes the creation of libraries containing millions of organic small molecules, in silico molecular docking, molecular modelling, pharmacophore designing and receptor-based QSAR studies. Various biotechnological companies have worked towards the identification and validation of different natural products and the development of these compounds into therapeutics (Butler 2004; Dey et al. 2019). To meet the increasing global demand, a steady and sufficient supply of anticancer drugs is necessary. It is expected that as a result of this review, there will be a greater awareness regarding the remarkable promise of chelerythrine towards anticancer therapy.

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Fig. 16 **a** Stern-Volmer plots for the quenching of chelerythrine fluorescence by KI at 298.15 K in the absence (filled circles) and in the presence (filled squares) of double-stranded poly(A). **b** Change of fluorescence anisotropy value of chelerythrine on binding with double-stranded poly(A). Reprinted from Basu et al. (2016b) with permission from Elsevier

Compliance with ethical standards

Ethical approval This article does not contain any studies with human or animal subjects performed by the authors.

Conflict of interest The authors declare that they have no competing interests.

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