Natural isoquinoline alkaloids: binding aspects to functional proteins, serum albumins, hemoglobin, and lysozyme

Asma Yasmeen Khan¹ · Gopinatha Suresh Kumar¹

Received: 14 August 2015 / Accepted: 19 October 2015 / Published online: 16 November 2015

© International Union for Pure and Applied Biophysics (IUPAB) and Springer-Verlag Berlin Heidelberg 2015

Abstract The putative anticancer alkaloids berberine, palmatine, jatrorrhizine, and sanguinarine are known to bind to nucleic acids. To develop them as potential drugs for therapeutic use, their binding affinity to functional proteins and mode of transport in the circulatory system need to be clearly understood. Towards this, many studies on their binding aspects to proteins have been reported and a considerable amount of data, mostly of biophysical nature, exists in the literature. The importance of these natural isoquinoline alkaloids and the recent literature on their interaction phenomena with functional proteins, serum albumins, hemoglobin, and lysozyme are presented in this review.

Keywords Isoquinoline alkaloids · Serum albumins · Hemoglobin · Lysozyme · Interaction

Introduction

The physiological functions of proteins are known to be defined through their interaction with other molecules leading to biological recognitions. Small molecule ligand binding to proteins is a part of many fundamental cellular processes. Such association involves intermolecular forces, such as ionic, hydrogen bond, van der Waals, and other weak interactions. Ligand binding is also important in understanding the mechanisms underlying drug action and protection against

oxidation, providing valuable information for the design and development of better therapeutic drugs. Drug molecules typically achieve high binding affinity to exert their action on the proteins, and most of them bind to specific pockets on protein targets.

Studies on the binding of drugs to plasma proteins such as human serum albumin (HSA) and bovine serum albumin (BSA) are important in biological, biomedical, and pharmaceutical sciences for the discovery of new drugs. The most important physiological feature of albumin is the binding and transport of numerous ligands for effective delivery to their target sites. Tight binding of a drug can reduce the bioavailability and enhance its half-life (Kragh-Hansen et al. 2002). Simultaneous binding of two drugs, on the other hand, or displacement of one by another, may alter the therapeutic efficiency or induce adverse effects. Drug–serum albumin interaction is an intensively explored area to derive information on the delivery of drugs to tissue receptors, metabolism, distribution, and elimination from the circulatory system. This information is critical for pharmaceutical and medicinal chemists to determine and understand how drugs interact with serum proteins or other binding targets in the blood plasma and how this binding may be affected by the presence of other compounds (Svensson et al. 1986; Wainer 1993; Carter and Ho 1994).

Another important protein present in blood cells is hemoglobin (Hb), which is involved in the binding and transport of oxygen. Lysozyme (Lyz) is an antimicrobial protein found in various protective fluids of the body and functions as a protective enzyme against bacterial infections.

Isoquinoline alkaloids are a large family of phytochemicals found in a number of plants. They occur predominantly in families like Papaveraceae, Berberidaceae, and Ranunculaceae, and possess remarkable biological activities. Basically derived from phenylalanine and tyrosine, they are

✉ Gopinatha Suresh Kumar
gskumar@iicb.res.in; gsk.iicb@gmail.com

¹ Biophysical Chemistry Laboratory, Organic and Medicinal Chemistry Division, CSIR—Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700 032, India

formed from the precursor 3,4-dihydroxytyramine (dopamine) via reaction with an aldehyde or ketone. Considering the structures, this group can be divided into two major categories: simple isoquinolines, which are composed of a benzene ring fused to a pyridine ring, and benzyloisoquinolines, which contain a second aromatic ring (Fig. 1). Many other groups of alkaloids such as protoberberines, protopines, pavinines, and aporphines are structurally related to the benzyloisoquinolines.

In this article, we present, briefly, the biological importance of these alkaloids and review the various biophysical aspects of their interaction with proteins, namely BSA, HSA, Lyz, and Hb.

Importance of isoquinoline alkaloids

Isoquinoline alkaloids have remarkable medicinal relevance and are, therefore, of great interest. Many have been used extensively in folk medicine; the most important ones are berberine, palmatine, jatrorrhizine, papaverine, morphine, codeine, corydaline, emetine, sanguinarine, and chelerythrine. The structures of some of these alkaloids are presented in Fig. 1. In terms of pharmacological utilities, berberine is a digestive stimulant, gastrointestinal tonic, antimicrobial agent,

and immune stimulant, and has strong hypoglycemic and cholesterol-lowering effects (Cernáková and Kostálová 2002; Kong et al. 2004; Dong et al. 2013; Chen et al. 2014). Palmatine is a bitter digestive stimulant and is used for the treatment of jaundice. Papaverine is a smooth muscle relaxant that is also known to possess antitumoral activity. Papaverine, morphine, and codeine are analgesic, antitussive, and sedative narcotics. Sanguinarine has antimicrobial, anticholinergic, and anti-inflammatory properties, and is a potent inhibitor of NF- κ B activation (Lenfeld et al. 1981; Godowski 1989; Chaturvedi et al. 1997). It is also a putative anticancer agent (Adhami et al. 2003; Malikova et al. 2006; Hussain et al. 2007; Lu et al. 2012).

Sanguinarine can exist in the charged iminium form at low pH (<6.0) and neutral alkanolamine form at pH values >8.5 (Fig. 1) (Maiti et al. 1983, 2002); the other alkaloids are very stable against pH and temperature effects.

Binding of isoquinoline alkaloids to nucleic acids

Nucleic acids are considered as potential biotargets of isoquinoline alkaloids for exhibiting their biological activity, particularly antitumoral activity. Consequently, a large number of studies on nucleic acid binding of berberine, palmatine,

Fig. 1 Chemical structures of: **a** isoquinoline moiety, **b** benzyloisoquinoline moiety, **c** berberine, **d** palmatine, **e** jatrorrhizine, **f** sanguinarine (iminium form), and **g** sanguinarine (alkanolamine form)

jatrorrhizine, sanguinarine, and chelerythrine have been performed, which was reviewed in several elegant articles recently (Giri and Suresh Kumar 2010; Maiti and Suresh Kumar 2007, 2009, 2010; Bhadra and Suresh Kumar 2011; Suresh Kumar 2012).

Binding to serum proteins

HSA and BSA (Fig. 2) are the most abundant multifunctional proteins in human and bovine blood, accounting for about 60 % of the plasma proteins. They serve as depot and transport proteins for a large number of endogenous and exogenous materials, including fatty acids, amino acids, steroids, metals ions, and drugs (Carter et al. 1989). There are two major ligand-binding regions in serum albumins, namely, Sudlow's site I (subdomain IIA) and site II (subdomain IIIA) (Sudlow et al. 1975, 1976). Interaction of drugs with these principal binding sites can determine and control the concentration of free and active drugs by providing a reservoir and decisively influencing the absorption, distribution, metabolism, and excretion profiles of the drug. These binding pockets enable serum albumins to increase the apparent solubility of hydrophobic drugs in plasma and modulate their delivery to cells (Shaikh et al. 2006). To understand the affinity and transport of these alkaloids, a number of studies have recently been performed.

Binding of berberine to serum albumins

The earliest study on the interaction of berberine to BSA involved equilibrium dialysis experiments and nuclear magnetic resonance (NMR) relaxation time measurements (Tanaka et al. 1995). Amongst all biophysical methods, NMR is distinctive and can provide information about almost all aspects of protein–ligand interactions. The NMR relaxation technique is a unique method that is dependent on the fluctuations of the

nuclear interactions (such as nuclear dipoles or chemical shift anisotropies) with respect to the static magnetic field due to the overall rotational diffusion of the protein in solution as well as local fast motions in the protein. NMR relaxation times can reveal the timescale and amplitude of the fast local motion typically in the pico- to nanoseconds range, in addition to the knowledge of the overall correlation or tumbling time of the protein. The amplitudes of these fast local motions can also be used to describe the thermodynamics associated with the function of a protein. Moreover, from the ligand NMR signals, the difference in NMR relaxation properties between a small and a large molecule can be used to identify molecule binding to a larger protein. The NMR relaxation technique can also be used for the experimental validation of computational results in order to investigate the protein dynamics. From the concentration (1–10 mmol/L) dependence of the ^1H -NMR parameters of berberine to BSA, intermolecular interactions were observed. The respective signals shifted to higher magnetic fields, the spin–lattice relaxation time (T_1) decreased, and the spin–spin relaxation rate (T_2^{-1}) increased. The interaction of berberine with BSA decreased T_1 and increased T_2^{-1} with little variation on the chemical shifts. The ratio of the spin–spin relaxation rates of bound versus free berberine (T_{2b}^{-1}/T_{2f}^{-1}) suggested that the binding of berberine on BSA involved the whole berberine molecule due to the rigid ring structure of the alkaloid. The study also indicated a large number of binding sites and, hence, the binding was thought to be non-specific. The binding capacity of berberine to the protein decreased with ionic strength, which appeared to confirm the non-specific binding interaction.

Liu and colleagues reported that the binding of berberine split (and shifted) the fluorescence spectrum of BSA to form double peaks; the observed fluorescence quenching was thought to involve static quenching at the ground state with non-radiative energy transfer (Liu et al. 2003). The values of the apparent association constant at 30 °C and 37 °C were determined to be $(8.66 \pm 0.12) \times 10^4$ L/mol and $(8.72 \pm 0.20) \times 10^4$ L/mol, respectively, and the number of binding sites $3.1 \pm$

Fig. 2 Three-dimensional structure of: **a** BSA and **b** HSA

0.2. The stereo-distances (r) between berberine and tryptophan, the fluorescent amino acid residue of BSA, were found to be 3.75 nm and 3.62 nm, respectively, at the two temperatures. Accordingly, some portions of bound berberine have been suggested to be inserted into the hydrophobic pockets of BSA. The binding of berberine to BSA was proposed to be spontaneous with entropy increase and Gibbs energy decrease, with the main driving force of the interaction recognized as hydrophobic in nature (Liu et al. 2003).

The binding of berberine to BSA immobilized on a chromatographic surface was studied and the affinity was determined to be of the order of 10^4 L/mol at 30 °C (Zeng et al. 2007). The binding affinity decreased with increasing temperature, attributed to structural changes in the protein. Thermodynamic data suggested that electrostatic interaction is the main driving force in the complexation (Zeng et al. 2007). Fluorescence quenching of BSA induced by berberine was related to increased hydrophobicity in the region surrounding the Trp-212 residue of the protein (Hu et al. 2010a). Quenching constants were evaluated by Stern–Volmer and Scatchard plots to provide values of the binding affinity and the number of binding sites, which were on the orders of 10^4 L/mol and 1.0, respectively.

From the nature and magnitude of the thermodynamic parameters estimated at different temperatures, electrostatic interaction was suggested to play a major role in the association process. On the basis of results from site marker competitive experiments using warfarin and ibuprofen, which are specific binders to Sudlow's sites I and II, berberine was proposed to bind to site II. The observed energy transfer data from the donor (BSA) to the acceptor (berberine) appeared to be due to fluorescence resonance energy transfer (FRET), as the distance between them was 3.5 nm, within the threshold 2–8 nm range (Hu et al. 2010a).

The binding constant and number of binding sites of berberine on HSA was reported to be $(1.17 \pm 0.32) \times 10^5$ L/mol and 5.26, respectively, from fluorescence quenching studies (Zhao et al. 2004). A binding distance of 3.44 nm and energy transfer efficiency of 0.303 was determined following Förster's non-radiative energy transfer theory. Li et al. studied the effect of berberine on the secondary structure of HSA (Li et al. 2005a). Based on Fourier transform-infra red (FT-IR) and circular dichroism (CD) results, an alteration in the protein conformation with a reduction in α -helical content in the presence of berberine was suggested. Scatchard analysis of the binding data revealed affinity values of $(4.07 \pm 0.13) \times 10^4$, $(3.74 \pm 0.09) \times 10^4$, and $(3.45 \pm 0.11) \times 10^4$ L/mol, respectively, with one binding site. Thermodynamic analyses revealed the binding to be spontaneous, exothermic, and enthalpy-driven. The binding process was proposed to occur through hydrophobic interactions, although the role of electrostatic interaction was not excluded. Displacement experimental data

proposed that berberine is bound in the subdomain IIIA of Sudlow's site II of the protein (Li et al. 2005a).

Using electrospray ion trap mass spectrometry, the association constant and binding stoichiometry of berberine–HSA complexation were evaluated to be 1.29×10^5 L/mol and 5, respectively. The binding force was suggested to be essentially hydrophobic in nature (Cao et al. 2007).

Hu et al. proposed that fluorescence quenching of HSA in the presence of berberine (Fig. 3) was a result of the formation of a strong complex (Hu et al. 2009). The quenching constant and binding affinity values were determined using the Stern–Volmer equation and Scatchard analysis. The distance between the donor (Trp-214) and acceptor (berberine) was calculated to be 3.10 nm by FRET experiments. Estimation of thermodynamic parameters like Gibbs energy, enthalpy, and entropy at different temperatures suggested that electrostatic interactions played a major role in berberine–HSA complexation. Site marker competitive experiments identified the binding site to be in subdomain II of the protein.

Experiments with site markers were performed by Khan (2013) to probe the site of interaction of berberine with BSA and HSA. In both proteins, the binding constants sharply decreased in the presence of warfarin, but remained almost unaltered with ibuprofen and digitoxin. This result showed the binding site of the alkaloid to be in the subdomain IIA, i.e., Sudlow's site I, for both the proteins.

More recently, the surface-enhanced Raman scattering technique was used to gather information on the orientation of berberine on HSA and the site of interaction (Zhang et al. 2011). This study confirmed the site of interaction to be in the hydrophobic cavity of subdomain IIA of the protein. Detailed analysis of the signals revealed that berberine could change its

Fig. 3 Emission spectra of HSA in the presence of various concentrations of berberine. The inset shows the Stern–Volmer plot. c (HSA) = 1.0×10^{-5} M $^{-1}$; c (berberine)/(10^{-5} M $^{-1}$); curves A–M, from 0.0 to 2.4 at increments of 0.20; curve N (dashed line) shows the emission spectrum of berberine only ($T = 298$ K, $\lambda_{\text{ex}} = 295$ nm) [reprinted from Hu et al. (2009) with permission from the American Chemical Society]

orientation to bind in a vertical form in the hydrophobic cavity of HSA.

Thus, most of the spectroscopic studies reported the binding of berberine to occur in site I of subdomain IIA of BSA and HSA. These were essentially based on quenching of the fluorescence and other indirect inferences like FRET distance and site marker experimental results.

The thermodynamics of the interaction of berberine with BSA and HSA was systematically investigated for the first time by the highly sensitive isothermal titration calorimetry (ITC) technique in our laboratory (Khan et al. 2013). Typical ITC profiles reported for the binding of berberine to BSA and HSA at 298.15 K are presented in Fig. 4. The binding affinity of the alkaloid to serum albumins was estimated to be of the order of 10^4 L/mol, in agreement with many of the previous reports. It was slightly higher for HSA compared to BSA. The binding was exothermic and enthalpy dominated with a small but favorable entropy contribution to the Gibbs energy change. The results from calorimetric experiments performed at various salt and temperature conditions revealed that the affinity decreased at higher salt concentrations in the range 10–100 mmol/L $[\text{Na}^+]$ and in the temperature range 10–50 °C. The heat capacity values (ΔC_p^0) suggested the binding to be specific and involving the burial of a significant portion of the non-polar surface area of the protein. Parsing of the Gibbs energy term to polyelectrolytic and non-polyelectrolytic components revealed that both forces contributed more or less equally to the binding interaction. The complete thermodynamic profile of the interaction, binding affinity values, and the number of binding sites were deduced.

Physiological transport of essential metal ions and metal-based drugs is performed by serum albumins. Blood plasma contains many metal ions at very low concentrations; these are bound to plasma proteins and are released under pathological conditions. The binding of drugs to plasma proteins may be affected in the presence of metal ions (Bal et al. 2013). Liu et al. studied the effect of Cu^{2+} , Ni^{2+} , Zn^{2+} , and Co^{2+} on the interaction of berberine and BSA (Liu et al. 2005), and revealed that the efficacy of quenching of BSA fluorescence by berberine was higher in the presence of these metal ions. In this condition, the apparent association constant and the number of binding sites decreased to the extent of 8–19 % and 25–28 %, respectively, resulting in increased concentration of the free alkaloid in the plasma. The interaction in the presence of metal ions was suggested to involve a strong quenching but a weak binding (Liu et al. 2005).

In a recent report, it was argued that the fluorescence quenching of HSA by berberine was due to inner filter effects and not due to binding (Marszalek et al. 2013). The differential absorption spectral data were interpreted to propose that the alkaloid remained in a water-like microenvironment located at the protein/water interface. The results from pulse radiolysis of hydrated electron scavenging in the HSA/alkaloid systems and steady-state absorption spectra of irradiated samples revealed that the alkaloid is not effectively protected by the protein against one-electron reduction. This result was consistent with the presence of the alkaloid at the protein–water interface and contradicted the binding at Sudlow's site I.

Overall, berberine was found to bind with a higher affinity and more favorable energetics to HSA compared to the BSA,

Fig. 4 ITC profiles for the binding of berberine to BSA and HSA at $T=298.15$ K. The upper panels represent the plot of enthalpy against time, representing the raw data for the sequential injection of **a** BSA (0.50 mM) and **b** HSA (0.60 mM) into berberine (0.05 mM) and dilution of the proteins into buffer (curves at the top are offset for clarity). The lower panels show the integrated heat data after correction of heat of dilution against the molar ratio of $[\text{Protein}]/[\text{Alkaloid}]$. The data points (closed squares) were fitted to a one-site model and the solid lines represent the best-fit results [reprinted from the Ph.D. thesis of A.Y. Khan (2013)]

although the site of binding on both proteins are identical for the alkaloids. However, the actual binding site of the alkaloid on serum albumins still remains controversial. More sophisticated techniques and detailed experimentation are required to establish the precise binding site of berberine on serum proteins.

Binding of palmatine to serum albumins

The interaction of palmatine to serum albumins was investigated by fluorescence and UV–vis absorption spectroscopy techniques initially (Wang et al. 2006). The alkaloid strongly quenched the intrinsic fluorescence of HSA, and this was suggested to result from the static quenching mechanism and non-radiative energy transfer. The affinity at 300 K was evaluated to be $(9.89 \pm 0.25) \times 10^4$ L/mol. From the thermodynamic data at different temperatures (negative enthalpy and positive entropy values), the binding was proposed to involve electrostatic interactions. The distance between HSA and palmatine was determined to be 2.693 nm according to FRET, suggesting the probability of strong energy transfer from HSA to palmatine (Wang et al. 2006). Using synchronous fluorescence spectroscopy, the binding-induced conformational changes were identified, suggesting increased polarity around the tryptophan residues and decreased hydrophobicity (Wang et al. 2006).

More recent studies of Liu and colleagues (Hu et al. 2010b) determined the binding affinity of palmatine to HSA to be $(5.44 \pm 0.30) \times 10^4$ L/mol at 298.15 K. Temperature-dependent fluorescence studies and displacement experiments were also performed to understand the binding forces and the site of binding. The results signified the involvement of electrostatic interactions to be the prominent force in palmatine–HSA interaction and proposed the binding site of the alkaloid to be site I (subdomain IIA) of HSA. A distance of 2.91 nm between palmatine and HSA was evaluated according to FRET. Structural changes involving loss of α -helical content from the initial 58 % to 33 % at a palmatine/HSA ratio of 5 were also observed.

Ou-Yang et al. (2012) suggested that the fluorescence quenching of BSA by palmatine was due to the formation of a specific complex. Binding parameters determined using the modified Stern–Volmer equation and Scatchard analysis were on the order of 10^4 L/mol. From the thermodynamic parameters evaluated through temperature-dependent study, electrostatic interactions were proposed to play a major role in the association. Site marker competitive displacement experiments proposed the binding site to be site II (subdomain IIIA). A binding distance of 3.36 nm was calculated from FRET study and conformational change was followed by visible absorption and synchronous fluorescence spectroscopy (Ou-Yang et al. 2012).

Detailed thermodynamics of the interaction of palmatine with serum proteins, in comparison with berberine, was reported by Khan et al. using ITC experiments (Khan et al. 2013). The binding affinity of palmatine was found to be on the order of 10^4 L/mol, confirming the previous spectroscopic data. The affinity to HSA was found to be higher than that to BSA. The binding was dominated by electrostatic interactions. While significant heat capacity values indicated an interplay of other forces like van der Waals interactions and H-bonding, negative heat capacity values showed the binding to be specific and involving the burial of the non-polar surface area of the protein. An enthalpy–entropy compensation phenomenon was observed in the temperature range 288.15–303.15 K. Differential scanning calorimetry (DSC) of the complexes revealed a loss in thermal stability and unfolding of the proteins on the binding of palmatine. Reduction in the α -helical content was observed from both CD and synchronous fluorescence results, corroborating the DSC results on destabilization of the helices upon alkaloid binding. Surface-enhanced Raman scattering displayed different signals for palmatine compared to berberine, and it was suggested that palmatine interacted with HSA through its C3 methoxy group (Zhang et al. 2011).

The study of Marszalek et al. (2013), however, contradicted the site of binding of palmatine on the serum albumins reported from spectroscopic studies and proposed a surface binding as in the case of berberine.

The overall results indicated that palmatine binds to both serum proteins with an affinity on the order of 10^4 L/mol. The calorimetric studies (Khan et al. 2013) suggested that palmatine binds with a slightly higher affinity to HSA than to BSA.

Binding of jatrorrhizine to serum albumins

The interaction between jatrorrhizine (Fig. 1) and HSA was studied by Li et al. using fluorescence quenching assay, CD spectroscopy, and FT-IR spectroscopy (Li et al. 2005b). The presence of a single class of binding site on HSA was suggested. The binding constants were of the order of 10^4 L/mol. The results from CD and FT-IR spectroscopy suggested a change in the secondary structure of the protein on binding. The effect of common ions on the binding constants was also investigated. Thermodynamic parameters suggested that hydrophobic and electrostatic interactions played the major role in the binding process. Using data from displacement experiments in the presence of phenylbutazone, flufenamic acid, and digitoxin, it was suggested that jatrorrhizine may be bound to Sudlow's site I in subdomain IIA of HSA. Support for binding to this site was also provided from a molecular modeling study.

Recently, the interaction of jatrorrhizine with BSA and HSA was examined by spectroscopic and molecular modeling techniques (Mi et al. 2013). The binding parameters and thermodynamic quantities were calculated by Stern–Volmer and Scatchard methods at three temperatures, viz. 298.15, 304.15, and 310.15 K. The binding affinity was found to be on the order 10^4 L/mol, close to the values reported in the study of Li et al. (2005b). The affinity values were shown to decrease with temperature. The binding was characterized by negative enthalpy and large positive entropy changes. Electrostatic and hydrogen-bonding interactions appeared to play the major role in interaction with BSA, though electrostatic interactions were more important for HSA. Synchronous fluorescence spectral changes suggested a conformational change in HSA on the binding of jatrorrhizine. Site marker competitive displacement experiments and molecular modeling studies were used to demonstrate that jatrorrhizine is located within site II in the hydrophobic pocket of subdomain IIIA of both BSA and HSA. The model of interaction proposed by Mi et al. (2014) for the complexation of jatrorrhizine with HSA is shown in Fig. 5.

In another recent study (Li et al. 2014), jatrorrhizine binding was confirmed to occur by a static quenching mechanism from fluorescence. The binding affinity was calculated to be of the order of 10^3 L/mol with around one binding site on HSA for the alkaloid. Based on the thermodynamic parameters determined by van't Hoff analysis, electrostatic forces were implicated to play a major role in the interaction. The efficiency of energy transfer showed that jatrorrhizine was located closer to tryptophan than to tyrosine residues. Molecular modeling results suggested that the jatrorrhizine–HSA complex was stabilized by electrostatic, π – π stacking, and H-bonding interactions.

Fig. 5 Location of jatrorrhizine molecule on the HSA subdomain IIIA (site II). The structural details of the interaction between jatrorrhizine and HSA obtained by the molecular modeling method is shown in the expanded region. Jatrorrhizine molecule is shown as a cylinder model (C, gray; O, red; N, blue; H, turquoise) [reprinted from Mi et al. (2014) with permission from Elsevier B.V.]

Surface-enhanced Raman scattering results indicated that the spectral pattern of HSA interaction with jatrorrhizine was different from that of berberine and palmatine (Zhang et al. 2011). This was ascribed to a different interaction mechanism due to the presence of the hydroxyl group in the structure of jatrorrhizine.

Thus, it may be inferred from the studies of Li et al. (2005b) and Mi et al. (2013) that jatrorrhizine has almost similar binding affinity to both the proteins (10^4 L/mol), which, however, is higher than the affinity value reported by Li et al. (2014) (10^3 L/mol). However, the actual binding site of jatrorrhizine on HSA remains controversial, as different sites were proposed by Li et al. (2005b) and Mi et al. (2013), although there is consensus on the affinity values and conformational aspects of the interaction. More detailed and high-resolution structural studies are required for determining the exact location of the bound alkaloid on the serum proteins.

Binding of sanguinarine to serum albumins

Sanguinarine is a quaternary benzo[c]phenanthridine alkaloid present in the Papaveraceae family, consisting of *Sanguinaria canadensis*, *Chelidonium majus*, *Macleaya cordata*, and *Argemone mexicana* (Mahady and Beecher 1994; Vavrecková et al. 1996). It has been extensively studied for its antimicrobial and anti-inflammatory activities (Lenfeld et al. 1981; Godowski 1989), and exhibits many other biological properties, including strong anticancer activity and nucleic acid binding (Walterová et al. 1995; Malikova et al. 2006; Maiti and Suresh Kumar 2010; Bhadra and Suresh Kumar 2011). The alkaloid is used in antiplaque preparation for human use, in veterinary medicine as a bioactive component, and in animal husbandry as the feed additive “Sangrovit” (Kosina et al. 2004). The biological activity of the compound was proposed to be related to its structural equilibrium (Fig. 1) between the quaternary iminium cation and neutral alkanolamine forms (Chaturvedi et al. 1997).

The interaction of sanguinarine with BSA was first characterized by spectroscopy and calorimetric techniques in our laboratory (Hossain et al. 2011). Both iminium and alkanolamine forms were suggested to form strong complexes with BSA from spectroscopic data. The estimation of binding parameters revealed that the alkanolamine form was more strongly bound than the iminium form. Specific binding distances of 3.37 and 2.38 nm, respectively, between Trp-212 and iminium and alkanolamine forms were calculated from FRET studies. Data from competitive binding experiments using site markers were used to suggest that both forms bound to site I (subdomain IIA) of BSA. Furthermore, alteration of the protein conformation leading to a reduction of the α -helical composition was suggested to occur with both forms, leading to a partial unfolding of the protein. The binding

affinities of the alkanolamine and iminium forms to BSA were found to be $(7.94 \pm 0.15) \times 10^4$ L/mol and $(3.78 \pm 0.33) \times 10^5$ L/mol, respectively, from ITC experiments (Hossain et al. 2011). The thermodynamic parameters suggested enthalpy-driven binding for the iminium form and favorable negative enthalpy and strongly favorable entropy contributions for the binding of the alkanolamine form. Overall, the data indicated that the alkanolamine form binds to the protein more favorably than the iminium form (Hossain et al. 2011). The results revealed the involvement of different molecular forces in the complexation of the two forms of the alkaloid with BSA.

The binding of sanguinarine to HSA was studied using spectroscopy and ITC techniques in our laboratory (Hossain et al. 2012). The affinity was higher for the alkanolamine form ($K = 2.18 \pm 0.35 \times 10^5$ L/mol) compared to the iminium form ($K = 5.97 \pm 0.14 \times 10^4$ L/mol), with only one class of binding sites for both forms. As with BSA, the binding to HSA was also enthalpy-driven for the iminium form and favored by negative enthalpy and strongly favorable entropy contributions for the alkanolamine form. The thermal stability of HSA was found to decrease by 7.1 and 8.0 K, respectively, with iminium and alkanolamine forms from differential scanning calorimetric studies, indicative of a binding-induced destabilization of the protein structure.

The results suggested one order higher affinity for the alkanolamine form over the iminium form to the serum proteins. The energetics of the interaction of the two forms were also clearly different: enthalpy-driven binding for the iminium form and favorable negative enthalpy and strongly favorable entropy contributions for the binding of the alkanolamine form.

Binding of alkaloids to hemoglobin

Investigations on the interaction of alkaloids with Hb (Fig. 6) were initiated in our laboratory very recently. The binding

Fig. 6 Three-dimensional structure of Hb

aspects of berberine and palmatine to Hb was studied in considerable detail (Hazra et al. 2013) using spectroscopy and calorimetry as tools. These alkaloids quenched Hb fluorescence by a static mechanism. Only one binding site on Hb was observed, obeying the Förster distance energy transfer mechanism. Competitive fluorescence experiments with the hydrophobic probe 8-anilinonaphthalene-1-sulfonic acid (ANS) suggested that the alkaloids were not bound to the central cavity of Hb where ANS is known to bind, but at a site nearer to β -Trp 37 of the protein at the $\alpha 1\beta 2$ interface. Detailed studies using CD and synchronous fluorescence spectroscopy revealed that the binding resulted in conformational changes, leading to unfolding that exposed some hydrophobic regions of the protein. A CD spectral pattern in the far UV and Soret band region of the protein (Hazra et al. 2013) on the binding of palmatine is presented in Fig. 7.

The binding affinity values of berberine and palmatine to Hb were evaluated from ITC to be $(6.49 \pm 0.24) \times 10^4$ and $(0.87 \pm 0.04) \times 10^5$ L/mol, respectively. The binding was favored by changes in both enthalpy and entropy, the former being the dominant contributor to the Gibbs energy change. From the variation of enthalpy change at different temperatures, the changes in heat capacity values were evaluated to be -178 and -174 cal mol/K for the binding of berberine and palmatine to Hb. The variation of thermodynamic parameters viz. Gibbs energy change and enthalpy change against entropy contribution for berberine and palmatine binding to Hb is presented in Fig. 8.

The fluorescence quenching mechanism of palmatine–bovine Hb complexation was suggested to follow a static process, in which electrostatic forces played an important role. The binding constant was of the order of 10^4 L/mol and the number of binding sites was ~ 1 . The binding distance r was evaluated to be ~ 2.44 nm and the primary binding site was suggested to be located at β -37 Trp moiety in the hydrophobic cavity of the protein. In addition, the values of the Hill's coefficients were determined to be ~ 1 . On the basis of the results from synchronous fluorescence and CD spectroscopy, the microenvironment and the conformation of BHb were suggested to undergo change on binding.

The binding of sanguinarine to Hb was recently investigated by us employing spectroscopic and calorimetric studies (Hazra and Suresh Kumar 2014). Both the iminium and alkanolamine forms were found to participate in the binding process. From absorbance studies, it was initially inferred that the binding affinity of the iminium form was higher than that of the alkanolamine form, the K_{BH} value (from the Benesi-Hildebrand analysis) of the former being higher by one order of magnitude. From calorimetric experiments also, the iminium form was confirmed to have one order of higher affinity over the alkanolamine form. The alkaloid was found to affect quenching of the protein fluorescence by complex formation at the ground state. Detailed studies revealed the

Fig. 7 **a** The intrinsic circular dichroism (far UV CD) spectral changes of Hb (1 μ M) on interaction with 0, 2.23, 4.46, 6.66, 8.86, 11.05, 13.23, and 15.39 μ M of palmatine (curves 1–8). **b** The Soret band CD spectral

changes of Hb (5 μ M) on interaction with 0, 2.67, 5.35, 8.02, 10.67, 13.33, 15.98, and 18.63 μ M of palmatine (curves 1–8) [reprinted from Hazra et al. (2013) with permission from the Royal Society of Chemistry]

binding of the iminium form to be exothermic and that of the alkanolamine form to be endothermic. The results of this study and analysis based on the nature and magnitude of the thermodynamic parameters identified that the binding of the iminium form involved electrostatic and H-bonding interactions, while that of the alkanolamine structure was predominantly hydrophobic in nature. The distances of iminium and alkanolamine forms from the putative binding site β -Trp-37 on the protein at the $\alpha\beta$ interface were deduced by FRET to be 2.96 and 3.03 nm, respectively. Conformational changes induced on binding were inferred from the CD, synchronous fluorescence, and 3D fluorescence results; more changes were brought about by the iminium form than the alkanolamine form. The displacement of ANS was affected more by the iminium form than the alkanolamine form, and the results were interpreted as due to more effective binding of the former to the hydrophobic regions near the central cavity of the

protein. Thus, the iminium form of the alkaloid, which showed weaker binding to serum proteins, was found to bind more strongly to Hb.

The iminium form of sanguinarine exhibited the highest binding to Hb among the alkaloids studied. A higher affinity of palmatine over berberine was apparent. Energetically, both berberine and palmatine binding was exothermic and favored by negative enthalpy and positive entropy changes. For the iminium form exothermic binding and for alkanolamine endothermic binding was observed. The results from Table 1 suggest that the binding affinity of the alkaloids followed the trend sanguinarine iminium > sanguinarine alkanolamine > palmatine > berberine.

Binding of alkaloids to lysozyme

The interaction of berberine to Lyz (Fig. 9) was investigated by Cheng et al. using spectroscopic methods (Cheng et al. 2012). Based on the fluorescence quenching data, it was suggested that the binding of berberine changed the environment of the Trp residues of Lyz due to the formation of a new complex. Static quenching was suggested to be the main mechanism, and only one binding site on Lyz was proposed for the alkaloid. The binding force between berberine and the protein was deduced to be essentially hydrophobic in nature. The interaction between berberine and Lyz under photoexcitation was studied by the laser flash photolysis method and the results demonstrated that berberine neutral radicals [BBR(-H)] reacted with the Trp residues ($K = 3.40 \pm 0.65 \times 10^9$ L/mol sec) via electron transfer to give the radical cation (Trp/NH^+) and neutral radical of Trp ($\text{TrpN}^\bullet$). Additionally, by comparing the transient absorption spectra of their reaction products, berberine was shown to selectively oxidize the Trp residues of Lyz. Through thermodynamic calculations, the reaction mechanism between berberine and Trp of Lyz was determined to be an electron transfer process.

Fig. 8 Plot of ΔG° (filled symbols) and ΔH° (open symbols) versus $T\Delta S^\circ$ for the binding of berberine (squares) and palmatine (triangles) with Hb [reprinted from Hazra et al. (2013) with permission from the Royal Society of Chemistry]

Table 1 Various binding and thermodynamic parameters for the interaction of alkaloids with Hb

Protein	Alkaloid	r (nm) ^a (FRET)	K^b (L/mol)	Stoichiometry ^b (N)	ΔH^{0b} (kcal/mol)	$T\Delta S^{0b}$ (kcal/mol)	ΔG^{0b} (kcal/mol)	ΔC_p^{0b} (cal/ mol·K)
Hemoglobin	Berberine	2.79	$(6.49 \pm 0.24) \times 10^4$	1.16	-3.04 ± 0.02	3.61	-6.65 ± 0.03	-177.50
	Palmatine	2.64	$(0.87 \pm 0.04) \times 10^5$	1.17	-2.86 ± 0.02	3.88	-6.73 ± 0.02	-174.00
	Sanguinarine iminium	2.96	$(1.18 \pm 0.08) \times 10^6$	0.38	-7.23 ± 0.98	1.05	-6.11 ± 0.98	-107.6
	Sanguinarine alkanolamine	3.03	$(1.04 \pm 0.08) \times 10^5$	0.51	1.59 ± 0.06	8.43	-6.83 ± 0.06	-297.6

^a r is the distance between the Trp residue of Hb and the bound alkaloid

^b Data compiled from Hazra et al. (2013) and Hazra and Suresh Kumar (2014)

The binding of berberine and palmatine to Lyz was recently studied in considerable detail in the authors' laboratory using a number of spectroscopic and calorimetric techniques (Jash and Suresh Kumar 2014). Binding constants were evaluated from spectroscopic data and compared to those derived from calorimetric data. Both the alkaloids were shown to induce fluorescence quenching; detailed analysis showed that the binding essentially was close to a site near the Trp-62 residue in the cleft region of the protein. From decrease in quenching constants and binding affinities with temperature, it was proposed that binding resulted in a static quenching mechanism due to complex formation at the ground state. This was also supported by the observation of energy transfer to the alkaloids from the protein due to the close proximity (within the Förster distance) of the alkaloids, which were deduced to be 3.30 nm and 3.09 nm, respectively, for berberine and

palmatine. Furthermore, conformational changes induced on binding were shown to result in a decrease of the α -helical content (as observed from CD data) and in changes in the 3D spectra of the protein.

Jash et al. recently reported the binding of the iminium and alkanolamine forms of sanguinarine to Lyz by fluorescence, UV–vis absorbance, and CD spectroscopy, and by in silico molecular docking approaches (Jash et al. 2014). Detailed thermodynamics of the binding was studied by microcalorimetry. Both forms of sanguinarine were revealed to quench the intrinsic fluorescence of Lyz, but the quenching efficiencies due to the binding varied. The equilibrium binding constants at 25 ± 1.0 °C were evaluated to be 1.17×10^5 and 3.32×10^5 L/mol, respectively, for the iminium and alkanolamine forms, with around one binding site on the protein for both forms. Conformational change in the protein on binding of the alkaloid was confirmed by absorbance, CD, 3D fluorescence, and synchronous fluorescence spectroscopy. Microcalorimetric data revealed the binding of the charged iminium form to be endothermic and predominantly involving electrostatic and hydrophobic interactions, while for the neutral alkanolamine form, it was exothermic and dominated by hydrogen-bonding interactions. The molecular distances from Lyz to the iminium and alkanolamine forms, calculated according to Förster's theory, were 3.27 and 3.04 nm, respectively. Overall, the structural data suggested both forms to bind near Trp-62/Trp-63 residues of Lyz at the cleft region of the protein. Stronger binding of the alkanolamine over the iminium form was proposed from both spectroscopic and thermodynamic results. Molecular docking studies revealed the putative binding of the alkaloid analogs to occur at the catalytic site. The docking results corroborated the results from spectroscopic and thermodynamic data that validated the stronger binding of the neutral form over the charged form. The interaction of the two forms of sanguinarine to Lyz as revealed from the docking study is reproduced in Fig. 10. It was identified that the alkaloid binding site was located near the deep crevice of the protein surface, and this was close to several crucial amino acid residues like Asp-52, Glu-35, Trp-62, and Trp-63.

Fig. 9 Three-dimensional representation of Lyz

Fig. 10 Hydrogen-bonding network and positioning of cleavage point residues (Glu-35–Asp-52) with docked sanguinarine alkanolamine (**a**) and iminium forms (**b**). The *black dotted lines* represent H bonds;

important residues are colored *purple* [reprinted from Jash et al. (2014) with permission from the American Chemical Society]

The jatrorrhizine–Lyz complex was characterized by Ying using fluorescence and FT-IR spectral studies (Ying 2010). The binding constants at different temperatures were calculated according to Stern–Volmer’s equation. The protein conformation was suggested to be altered with increase of the α -helical content and concomitant decrease of the β -turn structure. The distance from the tryptophan residue (donor) of Lyz to the bound jatrorrhizine (acceptor) was calculated as 5.39 nm by the FRET theory.

Table 2 depicts comprehensive data presenting various binding parameters in the Lyz–alkaloid interaction. Overall, the data suggest that the highest binding affinity of sanguinarine alkanolamine was followed by sanguinarine iminium, berberine, and palmatine, in that order.

Cytotoxicity of isoquinoline alkaloids

Isoquinoline alkaloids display interesting cytotoxic activities against a large number of tumor cell lines *in vitro*, and strong indications of anticancer activities *in vivo* have been reported in many cases. Cytotoxicity data can provide valuable information in identifying compounds posing health risks and aid

in the development of new drugs. Furthermore, the mechanisms involved in cytotoxicity can provide insights into the biological processes that control cell growth, proliferation, and death. Although it is beyond the scope of this article to review the cytotoxic activities of these alkaloids, several reports that highlight significant information are presented.

Various cytotoxic assays have been performed on a wide range of tumor cell lines *in vitro* and in various animal models. Some of the earlier studies on berberine, palmatine, and sanguinarine have been reviewed by Maiti and Suresh Kumar (2010). More recently, a large body of evidence presents anticancer effects of these alkaloids, being able to block the proliferation of cancer cells, poison the topoisomerases, etc. A number of mechanisms have been proposed that includes *inter alia* upregulation of reactive oxygen species (ROS) production, a mitochondria/caspases-mediated pathway, inhibition of COX-2, PGE2, and PGE2 receptors, induction of oxidative stress and generation of free radicals, decrease of mitochondrial membrane potential, p53 and p21 over-expression, the inhibition of Bcl-2 expression, etc. (Tillhon et al. 2012).

More recently, the anticancer activity of many berberine analogs have been reported to be by inhibiting the enzyme

Table 2 Various binding and thermodynamic parameters for the interaction of alkaloids with Lyz

Protein	Alkaloid	r (nm) ^a (FRET)	K^b (L/mol)	Stoichiometry ^b (N)	ΔH^{0b} (kcal/mol)	$T\Delta S^{0b}$ (kcal/mol)	ΔG^{0b} (kcal/mol)	ΔC_p^{0b} (cal/mol·K)
Lysozyme	Berberine	3.30	$(5.95 \pm 0.24) \times 10^4$	0.96	0.58 ± 0.03	7.09	-6.51 ± 0.03	−8.0
	Palmatine	3.09	$(4.30 \pm 0.40) \times 10^4$	1.25	2.37 ± 0.03	8.67	-6.30 ± 0.02	−25.0
	Sanguinarine iminium	3.27	$(1.17 \pm 0.08) \times 10^5$	0.84	1.13 ± 0.98	8.05	-6.92 ± 0.98	−6.5
	Sanguinarine alkanolamine	3.04	$(3.32 \pm 0.08) \times 10^5$	1.12	-1.38 ± 0.06	6.14	-7.52 ± 0.06	−28.5

^a r is the distance between the Trp residue of Lyz and the bound alkaloid

^b Data compiled from Jash and Suresh Kumar (2014) and Jash et al. (2014)

topoisomerase which is involved in DNA replication and by inducing apoptosis and expression of the p53 gene (Vrba et al. 2009; Park et al. 2010).

Sanguinarine has been shown to cause concentration-dependent necrosis and apoptosis of HL-60 cells. Sanguinarine-induced apoptotic signaling pathways, modulated the activity of Bcl-2 and the inhibitor of apoptosis protein (IAP) family of proteins, and led to mitochondrial dysfunction, the activation of caspases, the induction of Egr-1, and caspase 9 and 3 activation. ROS are also suggested to be critical regulators of the sanguinarine-induced apoptotic events. The potency of the lipophilic alkanolamine form (pseudo base) has been suggested to be higher than that of the cationic iminium form due to the higher uptake of the former. A detailed understanding of the signaling cascade by which sanguinarine induces apoptotic cell death is not yet available. The reader is referred to the vast data on the cytotoxicity of isoquinoline alkaloids in the literature.

Knowing the binding aspects of these alkaloids to functional proteins and elucidating their mechanisms of action in controlling the proliferation of cells would help in formulating better drugs in drug discovery programs.

Conclusions and future perspectives

In this review, we have summarized the recent advances on the interaction of some important natural isoquinoline alkaloids to functional proteins, namely, serum albumins, hemoglobin, and lysozyme. The aim of drug discovery is to develop novel small-molecule therapeutics that can control the proliferation of diseases. These alkaloids have been found to exhibit excellent cytotoxic effects to a variety of tumor cells. Proteins are the main targets for drugs. Estimating the binding affinity of a drug candidate to functional proteins is, thus, important to understand its pharmacological and toxicological actions. To develop natural alkaloids as potential drug candidates, detailed knowledge of their interaction with proteins is essential. Common blood proteins to which drugs bind are albumin, lipoprotein, hemoglobin, etc., although the site of binding on the proteins of many drugs are not yet unequivocally delineated through high-resolution techniques. The alkaloids dealt with here bind with medium affinity to the plasma proteins and appear, therefore, to have high overall drug clearance, making them potential candidates for therapeutic utility. They have higher affinity to nucleic acids than serum proteins, which make them suitable candidates for delivery by serum proteins. Analogs of these alkaloids may be developed for better therapeutic capability. The binding data in terms of structural and energetic aspects to the biotargets, when compared with those of new analogs developed, may lead to the development of better therapeutic agents. The current knowledge on their interaction with functional proteins summarized

here will, hopefully, be guiding factors in futuristic endeavor to develop better drugs to arrest diseases.

Acknowledgments GSK gratefully acknowledges the generous support from the Council of Scientific and Industrial Research (CSIR), Govt. of India through network projects NWP0036 and BSC0123 for the studies on alkaloid macromolecule interactions. The authors thank Dr. Basudeb Achari, Ex. Emeritus Scientist, CSIR—Indian Institute of Chemical Biology for his valuable inputs and suggestions.

Compliance with ethical standards

Conflict of interest Asma Yasmeen Khan declares that she has no conflict of interest.

Gopinatha Suresh Kumar declares that he has no conflict of interest.

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

References

- Adhami VM, Aziz MH, Mukhtar H, Ahmad N (2003) Activation of prodeath Bcl-2 family proteins and mitochondrial apoptosis pathway by sanguinarine in immortalized human HaCaT keratinocytes. *Clin Cancer Res* 9:3176–3182
- Bal W, Sokółowska M, Kurowska E, Faller P (2013) Binding of transition metal ions to albumin: sites, affinities and rates. *Biochim Biophys Acta* 1830:5444–5455
- Bhadra K, Suresh Kumar G (2011) Therapeutic potential of nucleic acid-binding isoquinoline alkaloids: binding aspects and implications for drug design. *Med Res Rev* 31:821–862
- Cao Y, Han F, Chen Y (2007) Studies on the non-covalent binding between berberine and human serum albumin by electrospray ion trap mass spectrometry. *J Anal Sci* 23:389–392
- Carter DC, Ho JX (1994) Structure of serum albumin. *Adv Protein Chem* 45:153–203
- Carter DC, He XM, Munson SH, Twigg PD, Gemert KM, Broom MB, Miller TY (1989) Three-dimensional structure of human serum albumin. *Science* 244:1195–1198
- Cernáková M, Kostálová D (2002) Antimicrobial activity of berberine—a constituent of *Mahonia aquifolium*. *Folia Microbiol (Praha)* 47: 375–378
- Chaturvedi MM, Kumar A, Darnay BG, Chainy GB, Agarwal S, Aggarwal BB (1997) Sanguinarine (pseudocheilerythrine) is a potent inhibitor of NF- κ B activation, I κ B α phosphorylation, and degradation. *J Biol Chem* 272:30129–30134
- Chen C, Yu Z, Li Y, Fichna J, Storr M (2014) Effects of berberine in the gastrointestinal tract—a review of actions and therapeutic implications. *Am J Chin Med* 42:1053–1070
- Cheng LL, Wang M, Wu MH, Yao SD, Jiao Z, Wang SL (2012) Interaction mechanism between berberine and the enzyme lysozyme. *Spectrochim Acta A Mol Biomol Spectrosc* 97:209–214
- Dong H, Zhao Y, Zhao L, Lu F (2013) The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. *Planta Med* 79:437–446
- Giri P, Suresh Kumar G (2010) Isoquinoline alkaloids and their binding with polyadenylic acid: potential basis of therapeutic action. *Mini Rev Med Chem* 10:568–577
- Godowski KC (1989) Antimicrobial action of sanguinarine. *J Clin Dent* 1:96–101

- Hazra S, Hossain M, Suresh Kumar G (2013) Binding of isoquinoline alkaloids berberine, palmatine and coralyne to hemoglobin: structural and thermodynamic characterization studies. *Mol Biosyst* 9: 143–153
- Hazra S, Suresh Kumar G (2014) Structural and thermodynamic studies on the interaction of iminium and alkanolamine forms of sanguinarine with hemoglobin. *J Phys Chem B* 118:3771–3784
- Hossain M, Khan AY, Suresh Kumar G (2011) Interaction of the anticancer plant alkaloid sanguinarine with bovine serum albumin. *PLoS One* 6:e18333
- Hossain M, Khan AY, Suresh Kumar G (2012) Study on the thermodynamics of the binding of iminium and alkanolamine forms of the anticancer agent sanguinarine to human serum albumin. *J Chem Thermodyn* 47:90–99
- Hu YJ, Liu Y, Xiao XH (2009) Investigation of the interaction between Berberine and human serum albumin. *Biomacromolecules* 10:517–521
- Hu YJ, Ou-Yang Y, Dai CM, Liu Y, Xiao XH (2010a) Binding of berberine to bovine serum albumin: spectroscopic approach. *Mol Biol Rep* 37:3827–3832
- Hu YJ, Ou-Yang Y, Dai CM, Liu Y, Xiao XH (2010b) Site-selective binding of human serum albumin by palmatine: spectroscopic approach. *Biomacromolecules* 11:106–112
- Hussain AR, Al-Jomah NA, Siraj AK, Manogaran P, Al-Hussein K, Abubaker J, Plataniias LC, Al-Kuraya KS, Uddin S (2007) Sanguinarine-dependent induction of apoptosis in primary effusion lymphoma cells. *Cancer Res* 67:3888–3897
- Jash C, Suresh Kumar G (2014) Binding of alkaloids berberine, palmatine and coralyne to lysozyme: a combined structural and thermodynamic study. *RSC Adv* 4:12514–12525
- Jash C, Payghan PV, Ghoshal N, Suresh Kumar G (2014) Binding of the iminium and alkanolamine forms of sanguinarine to lysozyme: spectroscopic analysis, thermodynamics, and molecular modeling studies. *J Phys Chem B* 118:13077–13091
- Khan AY (2013) Biophysical studies on the interaction of isoquinoline alkaloids with serum albumins. Ph.D. thesis, Jadavpur University, Kolkata
- Khan AY, Hossain M, Suresh Kumar G (2013) Binding of plant alkaloids berberine and palmatine to serum albumins: a thermodynamic investigation. *Mol Biol Rep* 40:553–566
- Kong W, Wei J, Abidi P, Lin M, Inaba S, Li C, Wang Y, Wang Z, Si S, Pan H, Wang S, Wu J, Wang Y, Li Z, Liu J, Jiang JD (2004) Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nat Med* 10:1344–1351
- Kosina P, Walterová D, Ulrichová J, Lichnovský V, Stiborová M, Rýdlová H, Vácar J, Krecman V, Brabec MJ, Šimánek V (2004) Sanguinarine and chelerythrine: assessment of safety on pigs in ninety days feeding experiment. *Food Chem Toxicol* 42:85–91
- Kragh-Hansen U, Chuang VT, Otagiri M (2002) Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. *Biol Pharm Bull* 25:695–704
- Lenfeld J, Kroutil M, Marsálek E, Slavík J, Preininger V, Šimánek V (1981) Antiinflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus*. *Planta Med* 43:161–165
- Li Y, He WY, Tian J, Tang J, Hu Z, Chen X (2005a) The effect of berberine on the secondary structure of human serum albumin. *J Mol Struct* 743:79–84
- Li Y, He W, Liu J, Sheng F, Hu Z, Chen X (2005b) Binding of the bioactive component jatrorrhizine to human serum albumin. *Biochim Biophys Acta* 1722:15–21
- Li J, Li J, Jiao Y, Dong C (2014) Spectroscopic analysis and molecular modeling on the interaction of jatrorrhizine with human serum albumin (HSA). *Spectrochim Acta A Mol Biomol Spectrosc* 118:48–54
- Liu XF, Xia YM, Fang Y, Liu LL, Zou L (2003) Interaction between bovine serum albumin and berberine chloride extracted from Chinese herbs of *Coptis chinensis* Franch. *Chem J Chinese Univ* 25:2099–2103
- Liu XF, Xia YM, Fang Y (2005) Effect of metal ions on the interaction between bovine serum albumin and berberine chloride extracted from a traditional Chinese Herb *Coptis chinensis franch.* *J Inorg Biochem* 99:1449–1457
- Lu JJ, Bao JL, Chen XP, Huang M, Wang YT (2012) Alkaloids isolated from natural herbs as the anticancer agents. *Evid Based Complement Alternat Med* 2012, Article ID 485042, 12 pages
- Mahady GB, Beecher CWW (1994) Quercetin-induced benzophenanthridine alkaloid production in suspension cell cultures of *Sanguinaria canadensis*. *Planta Med* 60:553–557
- Maiti M, Suresh Kumar G (2007) Molecular aspects on the interaction of protoberberine, benzophenanthridine, and aristolochia group of alkaloids with nucleic acid structures and biological perspectives. *Med Res Rev* 27:649–695
- Maiti M, Suresh Kumar G (2009) Biophysical aspects and biological implications of the interaction of benzophenanthridine alkaloids with DNA. *Biophys Rev* 1:119–129
- Maiti M, Suresh Kumar G (2010) Polymorphic nucleic acid binding of bioactive isoquinoline alkaloids and their role in cancer. *J Nucleic Acids* 2010, Article ID 593408, 23 pages
- Maiti M, Nandi R, Chaudhuri K (1983) The effect of pH on the absorption and fluorescence spectra of sanguinarine. *Photochem Photobiol* 38:245–249
- Maiti M, Das S, Sen A, Das A, Suresh Kumar G, Nandi R (2002) Influence of DNA structures on the conversion of sanguinarine alkanolamine form to iminium form. *J Biomol Struct Dyn* 20:455–464
- Malikova J, Zdarilova A, Hlobilkova A (2006) Effects of sanguinarine and chelerythrine on the cell cycle and apoptosis. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 150:5–12
- Marszałek M, Konarska A, Szajdzinska-Pietek E, Wolszczak M (2013) Interaction of cationic protoberberine alkaloids with human serum albumin. No spectroscopic evidence on binding to Sudlow's site 1. *J Phys Chem B* 117:15987–15993
- Mi R, Li PQ, Hu YJ, Fan XY, Li HY, Yu XC, Ouyang Y (2013) Biophysical studies on the interactions of jatrorrhizine with bovine serum albumin by spectroscopic and molecular modeling methods. *Mol Biol Rep* 40:4397–4404
- Mi R, Hu YJ, Fan XY, Ouyang Y, Bai AM (2014) Exploring the site-selective binding of jatrorrhizine to human serum albumin: spectroscopic and molecular modeling approaches. *Spectrochim Acta A Mol Biomol Spectrosc* 117:163–169
- Ou-Yang Y, Li XL, Wang H, Fang M, Hu YJ (2012) Determination of the specific interaction between palmatine and bovine serum albumin. *Mol Biol Rep* 39:5495–5501
- Park H, Bergeron E, Senta H, Guillemette K, Beauvais S, Blouin R, Sirois J, Faucheux N (2010) Sanguinarine induces apoptosis of human osteosarcoma cells through the extrinsic and intrinsic pathways. *Biochem Biophys Res Commun* 399:446–451
- Shaikh SMT, Seetharamappa J, Kandagal PB, Ashoka S (2006) Binding of the bioactive component isothipendyl hydrochloride with bovine serum albumin. *J Mol Struct* 786:46–52
- Sudlow G, Birkett DJ, Wade DN (1975) The characterization of two specific drug binding sites on human serum albumin. *Mol Pharmacol* 11:824–832
- Sudlow G, Birkett DJ, Wade DN (1976) Further characterization of specific drug binding sites on human serum albumin. *Mol Pharmacol* 12:1052–1061
- Suresh Kumar G (2012) RNA targeting by small molecules: Binding of protoberberine, benzophenanthridine and aristolochia alkaloids to various RNA structures. *J Biosci* 37:539–552
- Svensson CK, Woodruff MN, Baxter JG, Lalka D (1986) Free drug concentration monitoring in clinical practice. Rationale and current status. *Clin Pharmacokinet* 11:450–469

- Tanaka M, Asahi Y, Masuda S (1995) Interaction between drugs and water-soluble polymers. VII. binding of berberine with bovine serum albumin. *J Macromol Sci Pure Appl Chem* 32:339–347
- Tillhon M, Guamán Ortiz LM, Lombardi P, Scovassi AI (2012) Berberine: new perspectives for old remedies. *Biochem Pharmacol* 84:1260–1267
- Vavrecková C, Gawlik I, Müller K (1996) Benzophenanthridine alkaloids of *Chelidonium majus*; I. Inhibition of 5- and 12-lipoxygenase by a non-redox mechanism. *Planta Med* 62:397–401
- Vrba J, Dolezel P, Vicar J, Ulrichová J (2009) Cytotoxic activity of sanguinarine and dihydrosanguinarine in human promyelocytic leukemia HL-60 cells. *Toxicol In Vitro* 23:580–588
- Wainer IW (1993) The impact of new liquid chromatography chiral stationary phase technology on the study of stereoselective pharmacokinetics. *Trends Anal Chem* 12:153–158
- Walterová D, Ulrichová J, Válka I, Vicar J, Vavrecková C, Táborská E, Harjrader RJ, Meyer DL, Cerná H, Simánek V (1995) Benzo[c]phenanthridine alkaloids sanguinarine and chelerythrine: biological activities and dental care applications. *Acta Univ Palacki Olomuc Fac Med* 139:7–16
- Wang YQ, Zhang HM, Zhang GC (2006) Studies of the interaction between palmatine hydrochloride and human serum albumin by fluorescence quenching method. *J Pharm Biomed Anal* 41:1041–1046
- Ying LI (2010) Spectral study on the interaction between jatrorrhizine to lysozyme. *Chinese J Spectrosc Lab* 5:1983–1987
- Zeng X, Lei G, Wei Y (2007) Investigation on binding interaction of berberine chloride with bovine serum albumin immobilized onto chromatographic supports by frontal chromatography. *Chin J Chromatogr* 25:348–352
- Zhang W, Zhao Y, Bai X, Wang Y, Zhao D (2011) The orientation of protoberberine alkaloids and their binding activities to human serum albumin by surface-enhanced Raman scattering. *Spectrochim Acta A Mol Biomol Spectrosc* 78:1105–1109
- Zhao CC, Zheng WF, Li MQ (2004) The interaction of berberine and human serum albumin. *Spectrosc Spect Anal* 24:111–113