Interaction of novel metal-based chemotherapeutic agents, heterotrinuclear Cu(II)/Sn$_2$(IV) complex and triphenyltin(IV) complex of N-glycoside (GATPT) with human serum albumin (HSA): Spectroscopic and molecular modeling studies.
Synthesis and characterization

Synthesis of \([\text{C}_23\text{H}_{31}\text{N}_6\text{O}_6\text{CuSn}_2\text{Cl}_5]\)

Heterotrinuclear Cu–Sn2 chemotherapeutic drug entity (Scheme 2) of the formulation \(\text{C}_23\text{H}_{31}\text{N}_6\text{O}_6\text{CuSn}_2\text{Cl}_5\) was synthesized by the procedure previously reported by us [108]. The complex exhibited novelty in executing cell death via apoptosis involving p53 mitochondrial gene mediated pathway. Because of its promising cytotoxicity profile it was obligatory to investigate its pharmacokinetic profile with HSA at the molecular level.

Synthesis of \([\text{C}_26\text{H}_{32}\text{N}_2\text{O}_5\text{Sn}]\)

The synthesis and investigation studies of novel organotin complex \([\text{C}_26\text{H}_{32}\text{N}_2\text{O}_5\text{Sn}]\), \(1-\{(2-\text{hydroxyethyl})\text{amino}\}—2-\text{amino}-1,2-\text{dideoxy-}\text{D-}\text{glucosetriphenyltin(IV)}\) (GATPT) (Scheme 2) was described previously [178].

\[ \begin{align*}
\text{(a)} & \\
\text{(b)} & \\
\end{align*} \]

Scheme 2. Structure of complex (a) \(\text{C}_23\text{H}_{31}\text{N}_6\text{O}_6\text{CuSn}_2\text{Cl}_5\) and (b) \([\text{C}_26\text{H}_{32}\text{N}_2\text{O}_5\text{Sn}]\) (GATPT).

Results and discussion

HSA is a versatile protein carrier for drug targeting. Many drugs are known to interact with HSA reversibly that can function ultimately as carriers. Herein, we have carried out interaction studies of heterotrinuclear antitumor drug entities by employing many biophysical techniques.
In vitro binding studies of complex \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) with HSA

Fluorescence spectral studies

Fluorescence spectroscopy is an effective method to explore the interaction between small molecules and bio-macromolecules. The fluorescence of HSA arises from the tryptophan, tyrosine and phenylalanine residues [179]. Phenylalanine has a very low quantum yield and the fluorescence of tyrosine is almost totally quenched if it is ionized or present near to an amino group, a carboxyl group or a tryptophan. Thus, the fluorescence of HSA is dominated by the residue Trp-214 in subdomain IIA [180,181].

![Fluorescence spectra of HSA](image)

**Figure 45.** The fluorescence spectra of HSA by different concentrations of complex \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) with the excitation wavelength at 280 nm in 5 mM Tris-HCl/50 mM NaCl buffer, pH 7.4, at room temperature: [HSA] \(\times 10^{-5}\) M; (1–5) the concentration of complex corresponding to 0, 0.33, 1.00, 1.70, 2.30, 3.00 \(\times 10^{-5}\) M, respectively. Arrow shows the intensity changes upon increasing concentration of the complex.

When small molecular substances bind to HSA, the changes of intrinsic fluorescence intensity of HSA are induced by the microenvironment of Trp residue. When HSA was subjected to excitation (\(\lambda_{ex} = 280\text{ nm}\)) in presence of complex \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\), a strong fluorescence emission peak at 350 nm due to the emission for HSA tryptophan residue (Trp–214) as well as (Trp) moiety of the complex was observed. On increasing the concentrations of complex \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) (0.33, 1.00, 1.70, 2.30 and 3.00 \(\times 10^{-5}\) M), there was an enhancement of fluorescence intensity accompanied by hypsochromic shift (blue shift) of 3–4 nm in the emission maximum (Figure 45). The fluorescence spectral changes could be attributed to binding of complex with HSA in the vicinity of tryptophan residue in IIA subdomain leading to less polar microenvironment around...
tryptophan. In order to predict the interaction between the complex and the protein, the binding constant values and number of binding sites were determined by using equations (3, 5 and 6) The binding constant values at three temperatures (298, 308 and 318 K) were found to be $3.6 \times 10^4$, $3.1 \times 10^4$ and $2.6 \times 10^4$ M$^{-1}$, respectively and are shown in Table 2.

Table 2. Binding constant ($K_b$), the number of binding sites ($n$) and thermodynamic parameters for the complex $[C_{23}H_{31}N_6O_6CuSn_2Cl_5]$–HSA system at different temperatures.

<table>
<thead>
<tr>
<th>pH</th>
<th>T (K)</th>
<th>$K_b$ ($\times 10^4$ M$^{-1}$)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>3.6</td>
<td></td>
<td>1.08</td>
</tr>
<tr>
<td>7.40</td>
<td>308</td>
<td>3.1</td>
<td>1.01</td>
</tr>
<tr>
<td>318</td>
<td>2.6</td>
<td></td>
<td>0.88</td>
</tr>
</tbody>
</table>

Absorption spectral studies

Electronic absorption spectroscopy is a reliable tool to understand the morphological changes in secondary structure of HSA via complex formation between complex $[C_{23}H_{31}N_6O_6CuSn_2Cl_5]$ and HSA. On addition of complex $[C_{23}H_{31}N_6O_6CuSn_2Cl_5]$ with incremental increase of concentrations (0.00–3.00 $\times$ 10$^{-5}$ M) to constant HSA concentration (1 M), there was a sharp increase in absorption intensity, with hyperchromism of the intraligand band at 278 nm Figure 46. This implicates that complex $[C_{23}H_{31}N_6O_6CuSn_2Cl_5]$ interacted with HSA by electrostatic mode of binding [182]. Moreover, substitution with electron–donating groups (–NH, –NH$_2$) in Tryptophan residue of complex could induce an increase in the absorption intensity and a shift in absorption spectra [183]. A small blue shift of 3 nm in the absorbance peak was observed as a result of the protein binding of complex. Generally, the absorption of a chromophore shifts, depending on the circumstance of whether it is transferred to a more hydrophilic or more hydrophobic environment. The blue shift observed could probably be due to the transition of the ligand aromatic chromophores to a less hydrophilic environment, the intrinsic binding constant ($K_b$) of complex was found to be $3.4 \times 10^4$ M$^{-1}$. Thus, from the observations of fluorescence and UV–vis spectra, it was inferred that the interaction of complex $[C_{23}H_{31}N_6O_6CuSn_2Cl_5]$ with HSA takes place via the change in microenvironment around HSA.
Figure 46. UV absorption spectra of the HSA–complex system obtained in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature: (a) HSA \(10^{-5}\) M; (b–f) complex–HSA, the complex concentrations were 0.33, 0.50, 0.70, 0.83, 1.00 \(10^{-5}\) M, respectively. Arrows show the intensity changes upon increasing concentration of the complex.

Circular dichroic studies

To ascertain the possible influence of complex binding on the secondary structure of HSA, CD measurement was performed in presence of complex at different concentrations. As shown in Figure 47, CD spectra of free HSA (line a) exhibited two negative bands in the UV–vis region at 208 and 222 nm attributable to n–\(\pi^*\) transfer for the peptide bond of \(\alpha\)-helix [184].

Figure 47. CD spectra of the HSA–complex system (a) \(1.5 \times 10^{-5}\) M [HSA]; (b) \(1.5 \times 10^{-5}\) M [HSA] + \(1.5 \times 10^{-5}\) M \([C_{23}H_{31}N_8O_6CuSn_2Cl_5]\) complex; (c) \(1.5 \times 10^{-5}\) M [HSA] + \(3.0 \times 10^{-5}\) M [complex]. pH 7.4, at room temperature.

It was observed that in presence of complex \([C_{23}H_{31}N_8O_6CuSn_2Cl_5]\), the CD signal of HSA increased. This increase of the CD signal indicates decrease of helical secondary structure content. However, the CD spectra of HSA in the presence or absence of complex was similar in shape, indicating that the structure of HSA is also
predominantly α-helical. The quantitative analysis results of the α-helix in the secondary structure of HSA were obtained from equations as described by Wang et al [185]. They differed from that of 69.5% in free HSA to 55.9% and 54.4% in the complex $[C_{23}H_{31}N_6O_{6}CuSn_2Cl_5]^{-}$–HSA system at pH 7.4 and temperature 25 °C. The α-helix gradually decreased in presence of complex, which reveals that the interaction between complex $[C_{23}H_{31}N_6O_{6}CuSn_2Cl_5]$ and HSA leads to a change of the secondary structure of protein [186].

Fourier transform infrared (FTIR) measurements

To further understand the structural alternations of HSA induced by the binding of complex to HSA, FT–IR spectroscopy were performed on HSA and complex $[C_{23}H_{31}N_6O_{6}CuSn_2Cl_5]$–HSA system. The spectrum as shown in Figure 48a was obtained by subtracting the absorption of the Tris–HCl from the spectrum of the protein solution, while the spectrum in Figure 48b was obtained by subtracting the absorption of the complex–free form from that of the complex bound form.

![Absorbance vs Wavenumber (cm⁻¹)](image)

**Figure 48.** FT–IR spectra of (a) free HSA; (b) different spectra [(HSA solution + complex solution)–complex solution] in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature in the region of 1750–1400 cm⁻¹, [HSA] × 10⁻⁵ M; $[C_{23}H_{31}N_6O_{6}CuSn_2Cl_5]$ × 10⁻⁵ M.

IR spectra of proteins exhibit a number of the amide bands, which represent different vibrations of the peptide moiety. Among these amide bands of the protein, amide I peak position occur in the range 1600–1700 cm⁻¹ (mainly C=O stretch) and amide II band in the region 1500–1600 cm⁻¹ (C–N stretch coupled with N–H bending mode).
The amide bands have a relationship with the secondary structure of protein, and amide I is more sensitive than amide II for change of secondary of protein [187]. As shown in (Figure 48b), the peak position of amide I band was shifted from 1635.71 to 1641.83 cm⁻¹, implicating that the secondary structure of the HSA protein altered due to interaction of [C₂₃H₇₁N₆O₆CuSn₂Cl₂] complex.

Three-dimensional fluorescence spectral studies

3D fluorescence spectroscopy is a modern analytical technique to investigate the conformational and structural changes of proteins by changing excitation and emission wavelength simultaneously [188]. By comparing the 3D fluorescence spectral changes of HSA in the absence and presence of complex. The 3D fluorescence spectra and contour ones of HSA and [C₂₃H₇₁N₆O₆CuSn₂Cl₂]–HSA system are shown in Figure 49 a, b, respectively, and the corresponding characteristic parameters are shown in Table 3.

![Figure 49. 3D fluorescence spectrum and corresponding contour diagrams of (a) HSA, and (b) [C₂₃H₇₁N₆O₆CuSn₂Cl₂]–HSA conjugate system. The concentration of HSA is fixed at 1.0 µM and that of complex is fixed at 10.0 µM. pH = 7.4, at room temperature.](image)

Peak a was the Rayleigh scattering peak (λₑₓ = λₑₘ). Peak b (280, 344 nm, λₑₓ, λₑₘ) mainly reflected the spectral behavior of Trp residue, and the maximum emission wavelength and the fluorescence intensity of the residue associated with its microenvironment’s polarity. Peak c was the second–ordered scattering peak (λₑₘ =
From the Figure 49, it was observed that the fluorescence intensities of peak a and b both decreased significantly and the maximum emission wavelength of the peak was changed in the presence of complex indicating the quenching of the fluorescence emission intensity of HSA.

Table 3. 3D fluorescence spectral characteristic parameters of HSA and HSA–[C_{23}H_{31}N_{6}O_{6}CuSn_{2}Cl_{5}] complex system.

<table>
<thead>
<tr>
<th></th>
<th>HSA</th>
<th>HSA–[C_{23}H_{31}N_{6}O_{6}CuSn_{2}Cl_{5}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak a</td>
<td>Peak position</td>
<td>Peak position</td>
</tr>
<tr>
<td></td>
<td>( \lambda_{ex}/\lambda_{em} )</td>
<td>( \lambda_{ex}/\lambda_{em} )</td>
</tr>
<tr>
<td></td>
<td>(nm/nm)</td>
<td>(nm/nm)</td>
</tr>
<tr>
<td>Peak a</td>
<td>280/280–360</td>
<td>280/280–360</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5.25–199.7</td>
<td>4.97–122.7</td>
</tr>
<tr>
<td>Peak b</td>
<td>288/344</td>
<td>288/348</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>186.1</td>
<td>142.3</td>
</tr>
</tbody>
</table>

These observations reflect that the interaction of [C_{23}H_{31}N_{6}O_{6}CuSn_{2}Cl_{5}] complex with HSA induced some conformational and micro-environmental changes in HSA, corroborated well with our spectroscopic results obtained from UV–vis, FT–IR and CD measurements.

In vitro binding studies of [C_{26}H_{32}N_{2}O_{5}Sn] with HSA

The previously designed by our group [C_{26}H_{32}N_{2}O_{5}Sn], GATPT utilizes both biocompatibility of carbohydrates and antiproliferating property of organotin moiety [178]. The effectiveness of GATPT as antitumor chemotherapeutic drug candidate was evaluated by spectroscopic studies, DNA binding ability of GATPT and cytotoxicity activity viz. MTT assay, Hoechst 33342 DNA staining, cell cycle analysis, DNA fragmentation, Western blot analysis of whole cell lysates and mitochondrial fraction with Bcl-2 and p53 family protein and caspase-3 colorimetric assay. These mechanistic studies revealed that GATPT induced apoptosis mediated through a p53 dependent pathway. Furthermore, GATPT was found to have tumor suppression properties with no toxicity in vivo in nude mice models. However, interaction of GATPT with HSA was not studied and since HSA is the carrier protein...
involved in drug targeting therefore, binding studies of GATPT with HSA were performed as described below.

**Fluorescence studies**

The fluorescence intensity of a protein can be decreased by a variety of molecular interactions, such as excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation and collision quenching [190]. Collisional or dynamic quenching refers to a process that the fluorophore and the quencher come into contact during the lifetime of the excited-state, whereas static quenching refers to fluorophore–quencher complex formation [191]. The fluorescence spectra of HSA in the absence and presence of \([C_{26}H_{32}N_{2}O_{5}Sn]\) (GATPT) complex as a quencher at various concentration of GATPT in Tris–HCl buffer (pH 7.4) were monitored with an excitation wavelength of 295 nm is shown in Figure 50. HSA showed a strong fluorescence emission with a peak at 350 nm at \(\lambda_{ex} = 280\) nm, while GATPT has no intrinsic fluorescence under the present experiment conditions.

![Fluorescence quenching spectra](image)

**Figure 50.** Fluorescence quenching spectra of HSA (1.0 \(\times 10^{-5}\) M) by different concentrations of \([C_{26}H_{32}N_{2}O_{5}Sn]\) complex corresponding to 0.00 – 3.30 \(\times 10^{-5}\) M with the excitation wavelength at 280 nm in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature. Arrow shows the intensity changes upon increasing concentration of the quencher.

Upon increasing the concentration of GATPT, reduction in the fluorescence intensity was observed. The strong quenching of HSA fluorescence clearly indicated the binding of GATPT to HSA takes place and the interactions of HSA with GATPT changed the microenvironment around the Trp–214 residue and the tertiary structure of HSA. In order to ascertain the fluorescence quenching mechanism, the fluorescence quenching data at different temperatures (299, 308 and 318 K) were
firstly analyzed using the classical Stern–Volmer equation (7) [192]. Figure 51 shows the Stern–Volmer plots of $F_0/F$ vs. $[Q]$ at three different temperatures and the calculated $K_{sv}$ and $k_q$ values are summarized in Table 4.

\[ \text{Figure 51. Stern–Volmer plots showing HSA tryptophan quenching caused by } [C_{26}H_{32}N_{2}O_{5}Sn] \text{ complex at three different temperatures.} \]

The results revealed that the Stern–Volmer quenching constant $K_{sv}$ is inversely correlated with temperature and $k_q$ is larger than the limiting diffusion constant $K_{dif}$ of the biomolecule ($K_{dif} = 2.0 \times 10^{10}$ M$^{-1}$ s$^{-1}$) [193], which suggested that the fluorescence quenching was caused by a specific interaction between HSA and GATPT, and the quenching was not initiated by dynamic collision but arisen mainly from the HSA–GATPT complex formation.

\[ \text{Table 4. Stern–Volmer quenching constant of the } [C_{26}H_{32}N_{2}O_{5}Sn]–\text{HSA system at different temperatures.} \]

<table>
<thead>
<tr>
<th>pH</th>
<th>T (K)</th>
<th>$K_{sv}$ (10$^5$M$^{-1}$)</th>
<th>$K_{q}$ (10$^{12}$M$^{-3}$s$^{-1}$)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>299</td>
<td>2.57</td>
<td>2.57</td>
<td>0.99755</td>
</tr>
<tr>
<td>7.40</td>
<td>308</td>
<td>2.15</td>
<td>2.15</td>
<td>0.99800</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>1.86</td>
<td>1.86</td>
<td>0.99174</td>
</tr>
</tbody>
</table>

For the static quenching process, the quenching data were analyzed according to the modified Stern–Volmer equation (9) to calculate the binding constant and number of binding sites [194]. Thus, a plot of log $[(F_0–F)/F]$ versus log $[Q]$ was used to
determine $K$ as well as $n$. The binding data ($K$ and $n$) at different temperatures were presented in Figure 52 and Table 5.

![Lineweaver–Burk plot for the binding of HSA with GATPT complex at different temperatures.](image)

**Figure 52.** Lineweaver–Burk plot for the binding of HSA with GATPT complex at different temperatures.

The binding constant decreased with increasing temperature, which coincided with the Stern–Volmer quenching constant. As static quenching, the quenching constant can be interpreted as the association constant of the complexation reaction since static quenching occurs from the formation of a ground state complex which is nonfluorescent or weakly fluorescent between fluorophore and quencher.

**Table 5.** Binding constant ($K_b$) and the number of binding sites ($n$) for the $[\text{C}_{26}\text{H}_{32}\text{N}_{245}\text{Sn}]$–HSA system at different temperatures.

<table>
<thead>
<tr>
<th>pH</th>
<th>T (K)</th>
<th>$K_b (10^4 \text{M}^{-1})$</th>
<th>$n$</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>299</td>
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<td>0.9774</td>
<td>0.99517</td>
<td></td>
</tr>
<tr>
<td>7.40</td>
<td>308</td>
<td>0.962</td>
<td>0.9976</td>
<td>0.99997</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>0.777</td>
<td>0.9968</td>
<td>0.98756</td>
</tr>
</tbody>
</table>

The equilibrium between free and bound molecules is given by the equation (10). The binding constants $K$ and binding sites "$n$" were calculated by the slopes of the static quenching equation ($\log (F_0-F)/F$ vs. $\log [Q]$) curves as shown in Figure 53 and the results have been summarized in Table 5. The binding constants decreased with increasing temperature, which coincided with the Lineweaver–Burk, Stern–Volmer quenching constants.
Figure 53. Logarithmic plot of the fluorescence quenching of HSA at different temperatures.

Determination of thermodynamic parameters

The interacting forces between complex \([C_{26}H_{32}N_2O_5Sn]\) (GATPT) and HSA was composed of weak interactions such as electrostatic forces, hydrophobic interaction, hydrogen–bond formation and van der Waals, etc [195]. The thermodynamic parameters, enthalpy change (\(\Delta H\)), entropy change (\(\Delta S\)) and free energy change (\(\Delta G\)) are the main evidence for confirming binding modes. From the thermodynamic standpoint, \(\Delta H > 0\) and \(\Delta S > 0\) implies a hydrophobic interaction; \(\Delta H < 0\) and \(\Delta S < 0\) reflects the van der Waals force or hydrogen bond formation; and \(\Delta H=0\) and \(\Delta S > 0\) suggests an electrostatic force. The temperature–dependence of the binding constants was studied at three different temperatures (299, 308 and 318 K) so that HSA does not undergo any structural degradation (Figure 54). The negative \(\Delta H\) value observed cannot be mainly attributed to electrostatic interactions since for electrostatic interactions \(\Delta H\) is very small, almost zero. A negative \(\Delta H\) value is observed whenever there is hydrogen bond on the binding site [196]. It was not possible to account for the thermodynamic parameters of the GATPT–HSA coordination complex on the basis of a single intermolecular force.
Figure 54. Van't Hoff plot for the interaction of complex \([C_{26}H_{32}N_2O_5Sn] and HSA.

Consequently, the negative \(\Delta H\) (−3.003 KJ/mole) and positive \(\Delta S\) (65.68 J/mole K) values suggested that hydrophobic and hydrogen bond interactions play major role in the complex \([C_{26}H_{32}N_2O_5Sn]–HSA\) binding reaction and contribute to the stability of the complex \([C_{26}H_{32}N_2O_5Sn]\). The negative value of \(\Delta G\) reveal that the interaction process is spontaneous as shown in Table 6.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>(\Delta G) (kJ/mole)</th>
<th>R</th>
<th>(\Delta H) (kJ/mole)</th>
<th>(\Delta S) (J/mole K)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>−23.430</td>
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<tr>
<td>308</td>
<td>−23.485</td>
<td>0.9988</td>
<td>−3.003</td>
<td>+65.68</td>
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<tr>
<td>318</td>
<td>−23.683</td>
<td>0.9950</td>
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</table>

Fluorescence resonance energy transfer (FRET) studies

The excited molecules in the high–energy–level release their energy via radiative and/or non–radiative process. Literature reveals that fluorescence resonance energy transfer occurs whenever the emission spectrum of a fluorophore (donor) overlaps with the absorption spectrum of another molecule (acceptor) [197]. The overlap of the absorption spectrum of the complex \([C_{26}H_{32}N_2O_5Sn]\) (GATPT) with the fluorescence emission spectra of free HSA is shown in Figure 55. The rate of energy transfer depends on; (i) the extent of overlapping between fluorescence emission spectrum of donor and the absorption spectrum of acceptor, (ii) the relative orientation of the donor and acceptor dipoles, and (iii) the distance between the donor and the acceptor.
Here the donor and acceptor were HSA and the complex GATPT, respectively (Figure 56).

![Graph](image)

**Figure 55.** The overlap of UV absorption spectra of \([C_{26}H_{32}N_{2}O_{5}Sn]\) (GATPT) with the fluorescence emission spectra of HSA. (a) The fluorescence emission spectrum of HSA \((1.0 \times 10^{-5} \text{M})\). (b) The UV absorption spectrum of complex \([C_{26}H_{32}N_{2}O_{5}Sn]\) (GATPT) \((3.0 \times 10^{-5} \text{M})\).

From equations (13) and (14), the value of \(E, R_0, r\) and \(J\) were calculated and found to be 0.145, 1.14 nm, 3.58 nm and \(2.78 \times 10^{14} \text{M}^{-1} \text{cm}^3\), respectively. The donor to acceptor distance \(r < 7 \text{ nm}\) indicated that the energy transfer from tryptophan residue in HSA to GATPT complex occurred with high probability [198] which was also in accordance with the conditions of FRET, indicating again the static quenching interaction between \([C_{26}H_{32}N_{2}O_{5}Sn]\) (GATPT) and HSA.

![Diagram](image)

**Figure 56.** Fluorescence resonance energy transfer (FRET) mechanism.
Circular dichroic studies

To ascertain the possible influence of complex \([C_{26}H_{32}N_2O_5Sn]\) (GATPT) on the conformational transitions of HSA, CD measurement was performed in the presence and absence of complex GATPT at different concentrations (Figure 57). CD spectra of free HSA (line a) exhibit two negative bands in the ultraviolet region at 208 and 222 nm are contributed to \(n \rightarrow \pi^*\) transfer for the peptide bond of \(\alpha\)-helix [199]. It was observed that in presence of complex GATPT the CD signal of HSA increased. The increase in the CD signal showed decrease in the helical secondary structure content [200].

![CD Spectra of the HSA\-[C_{26}H_{32}N_2O_5Sn] system](image)

Figure 57. CD Spectra of the HSA—\([C_{26}H_{32}N_2O_5Sn]\) system. (a) 1.5 \(\times\) 10\(^{-3}\) M HSA; (b) 1.0 \(\times\) 10\(^{-3}\) M HSA + 3.0 \(\times\) 10\(^{-3}\) M \([C_{26}H_{32}N_2O_5Sn]\) complex. pH 7.40, at room temperature.

However, the CD spectra of HSA in the presence or absence of complex GATPT is similar in shape, which indicates that the structure of HSA was also predominantly \(\alpha\)-helical. From Equations (16) and (17), the quantitative analysis results of the \(\alpha\)-helix in the secondary structure of HSA were obtained. They differed from that of 56.37% in free HSA to 47.29% in the GATPT—HSA system at pH 7.4 and temperature 25 °C. The \(\alpha\)-helix gradually decreases in presence of GATPT complex, which reveals that the interaction between GATPT complex and HSA leads to a change of the protein's secondary structure [201].

Fourier transform infrared (FTIR) measurements

To further understand the structural alternations of HSA induced by the binding of complex \([C_{26}H_{32}N_2O_5Sn]\) (GATPT) to HSA, FT–IR spectroscopy were performed on HSA and complex GATPT–HSA system Figure 58 a, b. The spectrum in Figure 58a
was obtained by subtracting the absorption of the Tris–HCl from the spectrum of the protein solution.

Figure 58. FT–IR spectra of (a) free HSA; (b) different spectra [(HSA solution + [C_{26}H_{32}N_{2}O_{5}Sn] solution)−([C_{26}H_{32}N_{2}O_{5}Sn] solution)] in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4, at room temperature in the region of 1750–1400 cm⁻¹, [HSA], 1.5 × 10⁻⁵ M; [C_{26}H_{32}N_{2}O_{5}Sn], 3.0 × 10⁻⁵ M.

The spectrum was obtained by subtracting the absorption of the GATPT–free form from that of the complex GATPT–bound form. Infrared spectra of proteins exhibit a number of the amide bands, which represent different vibrations of the peptide moiety [202]. As shown in Figure 58, the peak position of amide I band was shifted from 1647.22 cm⁻¹ to 1652.37 cm⁻¹, indicated that the secondary structure of the HSA protein has changed due to interaction of complex GATPT [203].

Three–dimensional fluorescence spectral studies

To obtain more information on the binding of complex [C_{26}H_{32}N_{2}O_{5}Sn] (GATPT) to HSA, three dimensional fluorescence spectroscopy was employed to investigate the conformational changes of HSA [204]. 3D spectra and contour ones of HSA and complex GATPT–HSA system were indicated in Figure 59 and the corresponding parameters are summarized in Table 7. As can be seen from Figure 59, peak a (λ<sub>ex</sub> =λ<sub>em</sub>) is the Rayleigh scattering peak and peak b (λ<sub>ex</sub> = 280, λ<sub>em</sub> = 344 nm) is mainly reflected to the fluorescence spectral behavior of HSA’s polypeptide backbone structure [205]. Peak c was the second ordered scattering peak (λ<sub>em</sub> = 2λ<sub>ex</sub>) [206]. As shown in Figure 59, it was observed that the fluorescence intensities of both peaks a
and b decreased significantly and the maximum emission wavelength of the peak changed in the presence of GATPT complex indicating some micro-environmental and conformational changes in HSA due to the interaction of GATPT.

Figure 59. 3D fluorescence spectrum and corresponding contour diagrams of (a) HSA, and (b) [C_{26}H_{32}N_2O_5Sn]—HSA conjugate system. The concentration of HSA is fixed at 1.0 μM and that of complex is fixed at 10.0 μM. pH = 7.4, at room temperature.

Table 7: 3D fluorescence spectral characteristic parameters of HSA and HSA-[C_{26}H_{32}N_2O_5Sn](GATPT).

<table>
<thead>
<tr>
<th></th>
<th>HSA</th>
<th>HSA-[C_{26}H_{32}N_2O_5Sn]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak position</td>
<td>λ_ex/λ_em (nm/nm)</td>
<td>Δλ</td>
</tr>
<tr>
<td>Peak a</td>
<td>280/280–360/360</td>
<td>0</td>
</tr>
<tr>
<td>Peak b</td>
<td>280/344</td>
<td>64</td>
</tr>
</tbody>
</table>
Molecular docking studies

Molecular modeling studies were undertaken to identify at 3D level the driving forces governing the interaction between HSA and \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) complex. From the 3D structure of crystalline albumin, it is known that HSA comprises three homologous domains (denoted I, II, and III): I (residues 1–195), II (196–383) and III (384–585); each domain has two subdomains (A and B) that assemble to form a heart shaped molecule (Figure 60) [207].

![Figure 60. Modeling of X-ray crystallographic structure of HSA (PDB ID: 1h9z). The domains and subdomains were displayed with different color, the every subdomain and classical binding site were marked in the corresponding location.](image)

The principal region of complex binding sites of HSA is located in hydrophobic cavities in subdomains IIA and IIIA, corresponding to sites I and II, respectively, and the tryptophan residue (Trp-214) of HSA in subdomain IIA [208,209]. The modeling showed (Figure 61) that \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) complex was located within the binding pocket of subdomain IIA of the protein, where Ile-142, Ala-143, Arg-146, Phe-157, Tyr-161 can make hydrophobic interaction with aromatic ring of \(L\)-tryptophan of complex. The interaction between \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) complex and HSA is not exclusively hydrophobic in nature since there are several ionic (Arg-146, Glu-153, Arg-186, Lys-190) and polar residues (Asn109, Arg-145, Lys-190, Ser193) in the proximity of the bound ligand playing important role in stabilizing complex \(via\) H–bonds and electrostatic interactions. For instance, Ser193 is in suitable position to be involved in making H–bonds with amino group of complex. Moreover,
there are hydrogen bond interactions between Arg-145, Glu-153, Arg-186, Lys-190 and complex.

**Figure 61.** (a) Molecular modeling model of \([\text{C}_{23}\text{H}_{31}\text{N}_{6}\text{O}_{6}\text{Cu}_{2}\text{Sn}_{2}\text{Cl}_{5}]\) complex (sticks representation) located within the hydrophobic pocket in subdomain IIA of HSA; (b) the interaction mode between \([\text{C}_{23}\text{H}_{31}\text{N}_{6}\text{O}_{6}\text{Cu}_{2}\text{Sn}_{2}\text{Cl}_{5}]\) complex and HSA (salmon color) represented in a cartoon form and the yellow dashed line showing hydrogen bond interaction between them.

These results suggest that formation of hydrogen bonds decreased the hydrophilicity and increased the hydrophobicity to keep the complex–HSA system stable. The calculated value for the Gibbs free energy of binding \([\text{C}_{23}\text{H}_{31}\text{N}_{6}\text{O}_{6}\text{Cu}_{2}\text{Sn}_{2}\text{Cl}_{5}]\) complex to HSA was \(-13.87 \text{ KJmol}^{-1}\). The results obtained from molecular modeling indicated that the interaction between \([\text{C}_{23}\text{H}_{31}\text{N}_{6}\text{O}_{6}\text{Cu}_{2}\text{Sn}_{2}\text{Cl}_{5}]\) complex and HSA was dominated by electrostatic and ionic interactions, which was in accord with the binding mode study.

**Molecular docking studies of \([\text{C}_{26}\text{H}_{32}\text{N}_{2}\text{O}_{5}\text{Sn}]\)**

Molecular docking technique was employed to search the exact binding sites inside the molecular target HSA. The principal region of complex \([\text{C}_{26}\text{H}_{32}\text{N}_{2}\text{O}_{5}\text{Sn}]\) (GATPT) binding sites of HSA are located in hydrophobic cavities in subdomain IIA and IIIA, corresponding to site I and site II, respectively and tryptophan residue (Trp-214) of HSA in subdomain II A. There is a large hydrophobic cavity in subdomain IIA to accommodate the GATPT complex. The resulting docked pattern (Figure 62a) indicated that GATPT complex was located within the subdomain IIA of HSA [210], suggesting the existence of hydrophobic interaction between GATPT and HSA, correlated with the binding mode observed by thermodynamic analysis and
fluorescence quenching mechanism of HSA in presence of GATPT. Furthermore, there are also a number of specific electrostatic interactions and hydrogen bonds, because several ionic and polar residues in the proximity of the ligand play an important role in stabilizing the molecule via H–bonds and electrostatic interactions. As shown in Figure 62b, there are hydrogen bond interactions between the oxygen atoms of GATPT and Lys–199, Trp214, Arg–218, Val 343 and Leu453 residues of HSA [211]. These results suggested the formation of hydrogen bonds decreased the hydrophilicity and increased the hydrophobicity to keep the GATPT–HSA system stable. The calculated binding Gibbs free energy (ΔG°) was —39.19 kJ mol⁻¹, which is close to the experimental data (−23.430 kJ mol⁻¹) to some extent. However, the results revealed the interaction between GATPT and HSA was dominated by hydrophobic force. The results obtained from molecular docking indicated that the interaction between GATPT and HSA was dominated by hydrophobic forces as well as hydrogen bonds, which was consistent with our experimental results.

Figure 62. (a) Molecular docked model of GATPT complex (stick representation) located within the hydrophobic pocket in subdomain IIA of HSA; (b) the interaction mode between GATPT complex and HSA (green colour) represented in a cartoon form and the yellow dashed line showing hydrogen bond interaction between them.
Conclusion

In pursuit of our continued interest in metal–based compounds, our research group has previously synthesized novel antitumor chemotherapeutic agents \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) (GATPT). Preliminary DNA binding and \textit{in vitro} antitumor studies of the complexes against various human cancer cell lines exhibited remarkably good cytotoxicity activity as compared to standard drugs and antiproliferative activity with minimal side effects. As it is well–known that conformational changes of proteins induced upon interaction with drugs affect the protein’s biological function (HSA) which has profound influence on free concentration and metabolism of drug in blood plasma and drugs can reach target tissues by binding with HSA; consequently binding with HSA may influence the chemotherapeutic process. The interaction studies of both complexes \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) with HSA was investigated by employing various spectroscopic (fluorescence, UV–vis, CD, FTIR, 3D fluorescence) and molecular modeling techniques were carried out to evaluate the mechanistic approach, binding mode and pharmacological drug response. Fluorescence quenching results indicated the binding of both complexes \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) with HSA in the vicinity of tryptophan residue in IIA subdomain following the static quenching mechanism. On the basis of spectroscopic results (CD, FT–IR, 3D–fluorescence) we have concluded that the secondary structure of HSA was changed and both complexes bound to site I of protein, which was located within hydrophobic pocket of subdomain IIA. However, for complex \([C_{26}H_{32}N_2O_5Sn]\) thermodynamic parameters were calculated which showed negative \(\Delta G\) value indicating that hydrophobic and hydrogen bond interactions play major roles in the binding process. The binding distance \((r)\) was also calculated to be 3.58 nm for complex \([C_{26}H_{32}N_2O_5Sn]\). Our results are well supported by molecular modeling experiments which further validated the binding of both complexes \([C_{23}H_{31}N_6O_6CuSn_2Cl_5]\) and \([C_{26}H_{32}N_2O_5Sn]\) in subdomain IIA of HSA. These studies provide valuable information to understand the mechanistic pathway of drug delivery and pharmacological behavior of these chemotherapeutic drug entities.