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Binding interaction studies of sodium benzoate, calcium propionate and sodium propionate with bovine serum albumin using spectroscopic method and molecular docking

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Abstract
The study investigated the in vitro interaction of bovine serum albumin (BSA) with sodium benzoate (SB), calcium propionate (CP) and sodium propionate (SP) and their consequent health impact on foods and pharmaceuticals when utilized as additives. The binding interaction studies of the compounds were investigated under simulated physiological conditions using spectroscopic analysis and molecular docking with thermodynamic parameters obtained to determine the nature of binding forces. The UV spectra for the three analytes revealed significant hypochromic effect of BSA-ligand interactions compared to only BSA indicating a modification of the BSA conformation due to possible hydrophobic interactions between the aromatic rings of the amino acids and ligands and other non-covalent interactions. Thermodynamic parameters obtained shows the binding interactions are exothermic, spontaneous, and hydrogen bonding and van der Waal's forces are chiefly responsible for formation and stabilization of BSA binding with SB, CP and SP and with additional pi-alkyl interactions observed for SB binding. Docking studies depict that hydrogen bonding was observed between the cabonyl group of SB and ARG256 residue of BSA and van der Waal forces also observed between SB and nine residues within the binding pocket of BSA. CP showed multiple hydrogen bonds between its carbonyl group and GLY247, LEU249 and LEU250 residues of BSA and SP also interacted with ARG256 of the protein via hydrogen bonding, and other amino acids via distance-dependent van der Waals forces. The study explains the binding mechanisms of the analytes with BSA and could determine their resultant pharmacodynamic effect on protein function when employed as food or pharmaceutical additives.

Keywords BSA binding studies · Hydrogen bonding · Molecular docking · Protein–ligand binding · Additives

1 Introduction

The use of additives has become a common practice in the food and pharmaceutical industries, this can be in form of colorants, flavors, antioxidants, and preservatives which have been known to improve the aesthetics, organoleptic properties and consequently the quality and shelf-life of the products [1, 2]. Sodium benzoate (SB), calcium propionate (CP) and sodium propionate (SP) are widely used as preservatives in foods and pharmaceuticals because of their anti-microbial activities.

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SB because of its ability to effectively inhibit fungal and bacterial growth during storage [3, 4] has found usefulness in a range of dishes and drinks, including salads, sodas, juices, jams, and soy sauce. It is also applicable clinically in the management of numerous illnesses, including Parkinson's disease, early Alzheimer's disease, multiple sclerosis, liver disease, and urea cycle abnormalities [5, 6]. CP is a microbial inhibitor in food, tobacco and pharmaceuticals. It is used in butyl rubber to improve process ability and scotching resistance. It has been applied to prolong the shelf life of several products like bread, other baked goods, processed meat, whey and dairy products [7]. SP has similar antimicrobial activity as calcium propionate where it inhibits mold growth in food and beverages. It is a component of baked foods, nonalcoholic drinks, cheeses, puddings and fillings, gelatins, jams and jellies, meat items and soft candies [8]. Despite that these preservatives are generally recognized as safe (GRAS), with concentration limits specified by the US Food and Drug Administration (FDA) [9], these substances are mostly products of chemical syntheses which pose threat to humans and their long- term usage can have potential risk to humans and alter the configuration of DNA and transport proteins such as serum albumin in human bodies. Understanding how these additives interact with serum albumin will help reveal their impact on health. Serum albumin, the most prevalent translocator protein in blood circulation [10, 11], performs a number of vital physiological tasks, including transporting various endogenous and exogenous compounds and maintaining colloidal osmotic blood pressure [12]. In addition to being widely used in pharmaceutical and biomedical applications, bovine serum albumin (BSA) is also frequently used as a ligand-biological model to research the interactions between small molecules and globular proteins. This is because of its excellent stability, affordability, adaptability, and relevance in medicine, as well as its strong structural similarity (about 76%) with human serum albumin [13]. About 583 amino acid residues make up the single-chain, globular protein BSA, which forms 17 disulfide linkages [14]. The hydrophobic cavities found in sub-domains II-A and III-A are also known as Sudlow's sites I and II, in that order, are host specialized ligand-binding sites in BSA [15]. As a result of the contact effect, prior research has shown that chemical toxicity and durability have a significant impact on the structure of BSA [16]. Additionally, numerous investigations have demonstrated that BSA's secondary structure changes when it binds to small molecules [17–19]. Therefore, it is crucial to research how BSA interacts with compounds, especially small molecules, hence this article is aimed at investigating the interaction between BSA and additives such as sodium benzoate, calcium propionate and sodium propionate. This will provide theoretical knowledge for the physiological properties of these additives and to promote the development of safe food and pharmaceutical additives. The study involves the use of UV spectroscopy and molecular docking to ascertain BSA's structure and conformation when it binds to the additives.

2 Experimental

2.1 Materials

Potassium dihydrogen phosphate, sodium hydroxide, sodium benzoate, calcium propionate and sodium propionate were all obtained from AK Scientific California, BSA from Glentham, UK.

2.2 Equipment

UV/Visible spectrophotometer (double beam PC 8 scanning autocell, UVD-3200, Labomed Inc.), pH meter (Mettler Toledo, PHS-3C).

2.3 Sample preparation

Phosphate buffer solution (PBS) of pH 7.4 was prepared by using potassium dihydrogen phosphate and sodium hydroxide according to the British Pharmacopoeia [20]. A 1×10^{-6} M stock solution of BSA was prepared by adding 0.0332 g of the sample to a 500 ml volumetric flask containing 200 ml PBS. It was gently mixed to dissolve and made up to volume with PBS. Stock solutions of each of sodium benzoate, calcium propionate and sodium propionate were prepared in aqueous medium to achieve a concentration of 1 mM and the eventual working concentrations in each case were 1, 2, 4, 6 and 8 µM.

2.4 UV–Vis spectra analysis

Equal volumes of BSA and aliquot solution of the different concentrations of SB were placed in sample tubes to make a volume of 5 ml and the mixture was allowed to react together for a period of 10 min at a temperature of 25 °C. Similar procedures were carried out for CP and SP. The UV–visible spectra of BSA alone and with the samples were recorded from 200 to 400 nm while employing the corresponding ligand solution for baseline correction of the spectrophotometer. The entire procedure was carried out in triplicate and the experiment also repeated at 37 and 45 °C**.**

2.5 Molecular docking

The crystal structure of Bovine Serum Albumin (BSA) in complex with 3,5-diiodosalicylic acid was retrieved on https://www.rcsb.org/ (Protein data bank, PDB ID: 4JK4). The protein has a resolution of 2.65 Å [21]. The 3D structures of SB, CP, and SP were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Both protein and ligands were prepared on UCSF Chimera 1.16, eliminating water molecules, co-crystallized ligand, and other molecules from the protein [22]. Structures minimisation and optimisation for protein was done at 200 steepest descent steps (and 100 steepest descent steps for ligands), 0.02 steepest descent steps size (Ă), ten conjugate gradient steps, and 0.02 conjugate gradient steps size (Ă) 10 update intervals using the structure editing wizard. Charges were then added to the ligands through ANTECHAMBER. The Autodock Vina of PyRx was used to speculate the binding interactions of SB, CP, and SP on BSA [23]. The binding site of the co-crystallized ligand on the protein was pre-defined using Chimera (at < 5 Å) and fifty-five (55) amino acid residues were implicated [24]. A site-directed docking was therefore carried out using the grid dimensions: Center X:87.6383 Y:23.5980 Z:26.1382; Dimensions (Angstrom) X:44.2478 Y:33.1153 Z:43.1179. Discovery Studio 2021 Client was used for molecular visualization.

3 Results and discussion

3.1 UV Spectrophotometric analysis

UV–vis spectroscopic technique can be used to explore the structural changes of protein and also look into the conformational changes that occur in proteins during their binding interactions with small molecular substances. The binding kinetics studies were carried out at three-level temperature; 25, 37 and 45 °C depicting room temperature, body temperature and the temperature obtained as a result of processing and milling during industrial activities respectively. Figures 1, 2, 3 showed the overlaid UV absorption spectra of the interaction of BSA with SB, CP and SP respectively at varying temperatures.

The UV spectra of BSA revealed two characteristic bands; one intense large band at 217 nm with an intensity of 0.447 and one weak band at 277 nm with an intensity of 0.047. The former is associated with the π–π^* transition of C=O functional groups in the peptide and carboxylic acid moieties in the compounds (N-terminal peptides as N–CHR–COOH and C– terminal peptide as NH_2-CHR-CO) and the latter with the π–π^* transition of the aromatic ring portions of hydrophobic amino acids (tryptophan, tyrosine, phenylalanine) in the BSA structure [25].

At 25 °C**,** the strong absorption band of BSA-SB was observed around 215 nm showing a slight hypsochromic shift ($\Delta\lambda = -2$ nm) which is due to the absorbance of peptides and carboxylic moieties into the protein compound. The addition of SB (from 1 to 8 μM) to the BSA solution reduces the intensity from 0.279 to 0.2460 indicating a hypochromic effect. The weak absorption bands at 277 nm reveal a constant band across the aliquot concentrations with a decrease in the intensity from 0.026 to 0.018 indicating a hypochromic effect. A similar observation was recorded at 37 °C where a strong band was observed at 215 nm and the weak band at 276 nm indicating a slight hypsochromic shift of 1 nm. However, at 45 °C, the high intensity band BSA-SB shifted 3 nm short of the free BSA with reduce absorbance upon titration with increased concentration of SB. The weak absorption band were observed at 277 nm with an hypsochromic effect observed just like at previous temperatures recorded. When BSA was titrated with CP, UV spectra also showed similar patterns across the temperature levels considered with strong intensity band appearing between 214 and 217 nm and the weaker band at 277 nm, however with reduced absorbance value as the concentration of ligand increases. The same pattern was observed with SP save that the low intensity bands were observed at 278 nm ($\Delta\lambda = +1$ nm).

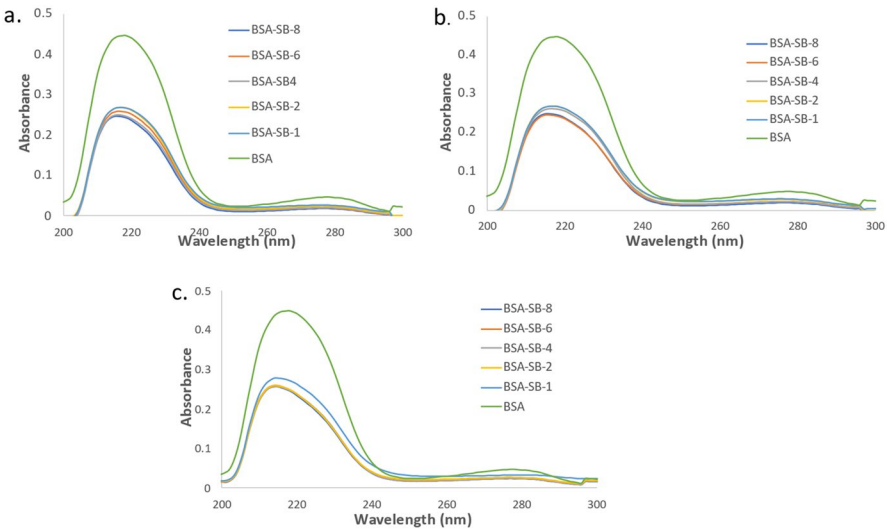

Fig. 1 Overlaid UV absorption spectra of BSA alone and BSA with varying concentrations of SB at 25 (**a**), 37 (**b**) and 45 °C (**c**)

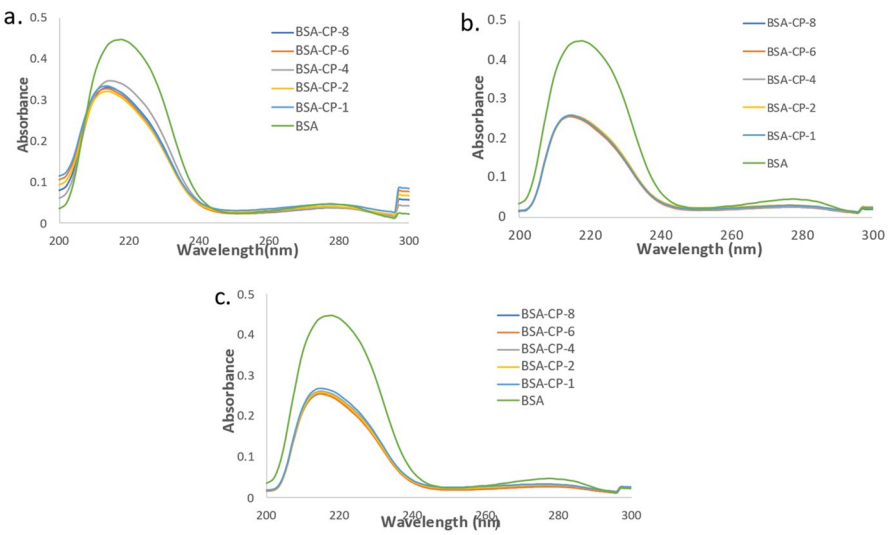

Fig. 2 Overlaid UV absorption spectra of BSA alone and BSA with varying concentrations of CP at 25 (**a**), 37 (**b**) and 45 °C (**c**)

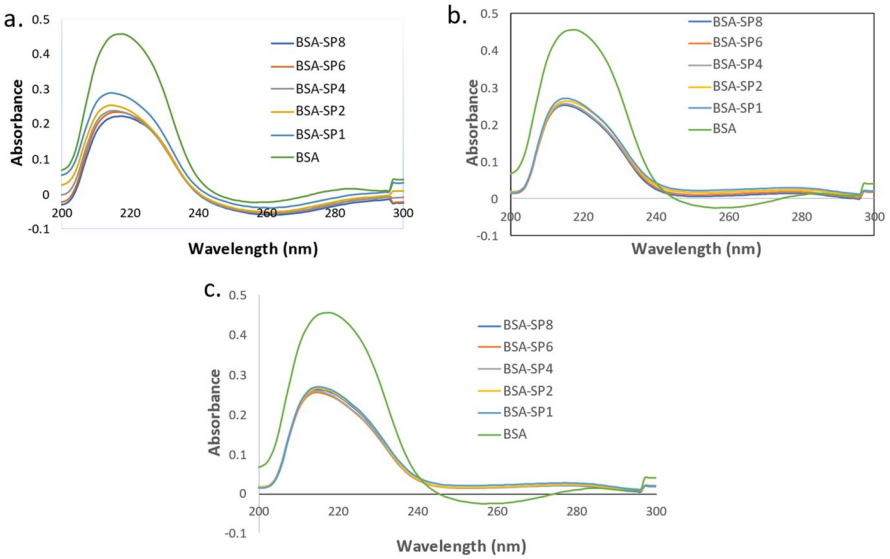

Fig. 3 Overlaid UV absorption spectra of BSA alone and BSA with varying concentrations of SP at 25 (**a**), 37 (**b**) and 45 °C (**c**)

In all, it can be seen that a marked hypochromic effect was observed with BSA-ligand interactions relative to the neat BSA in the higher energy band. The changes observed in the absorbance could be due to hydrophobic interactions between the aromatic rings of the amino acids and the ligands. These changes signify a modification in the BSA conformation which could result in the backbone unfolding and increase the hydrophobicity of the microenvironment of the aromatic amino acid residues [26]. It could also be due to hydrogen bonding, electrostatic forces or van der Waal's interactions.

3.2 Binding studies

Essentially, there are two types of binding involved in ligand-biomolecular interactions: the reversible non-covalent and the irreversible or covalent binding. In non-covalent interactions, the collaborating atoms do not share electrons and are weaker than covalent connections. They are of great importance in biochemical processes, where they determine the structure, dynamics, and function of biomolecules [27, 28]. They are, nevertheless, distinct, appealing and being reversible, can be built and broken without consuming much energy. They also have an impact on pharmacokinetic properties of molecules such solubility, partitioning, distribution, and permeability, which are critical to medication development and functionality [29]. Their interactions could be within the amino acids of the protein or inter-molecularly between the protein and the ligand in a protein–ligand complex [30]. Intermolecular forces, such as ionic bonds, hydrogen bonds and Van der Waals forces, are responsible for non-covalent ligand-binding interactions and these associations with ligands are almost always reversible. On the other hand, covalent bonds are created when a reactive functional group of the ligand, such as hydroxyl, epoxy, or carbonyl, interacts with a nucleophilic cysteine, serine, threonine, or, in rare cases, lysine. This process creates covalent adducts [31]. This bond forms an irreversible bond within the target protein's half-life and is long enough to produce a ligand–protein complex that is not susceptible to classical equilibrium [32]. It has been demonstrated that substances that are genotoxic can bind covalently to nuclear proteins (as against genotoxicity as a result of covalent bond formation with DNA) [33].

The binding constants of ligands SB, CP and SP were determined at the three temperature levels using nonlinear regression based on the quadratic equation [34] stated below:

$$\frac{Ao - A}{Ao} = ([P] + [L] + Kf) - \frac{\sqrt{([P] + [L] + Kf)^2} - 4[P][L]}{2[P]} \quad (1)$$

where Ao and A are the absorbance of free and bound BSA respectively, $\frac{Ao-A}{Ao}$ is the fraction of bound ligand, [P] and [L] are the concentrations of the protein and ligand respectively while K_f is the binding constant, these parameters are determined mathematically using nonlinear regression in the Eq. (1). The binding curve when fraction of bound ligand is plotted against its concentration the graphs are in Figs. 4, 5, 6, while the binding constants, $K_{f.}$ obtained are depicted in Table 1.

3.3 Thermodynamic parameters and nature of binding forces

The estimation of thermodynamic parameters is critical to gain further insight into how BSA binds to dietary ingredients. The overall free energy change (ΔG) was calculated using Eq. (2)

$$\Delta G = -RT\,ln\,K \quad (2)$$

where T is the temperature in Kelvin and R is the universal gas constant. The modified Van't Hoff Eq. (3) and (4) was used to calculate the thermodynamic enthalpy (ΔH) and entropy (ΔS) change of BSA and ligand complexation. Enthalpy change is derived from the slope using Eq. (4) and entropy change from the intercept when the natural logarithm of the binding constants $K_{f.}$ is plotted against the reciprocal of the temperatures (T).

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

The thermodynamic parameters for the food additives were derived as shown in Table 1.

For the binding interactions of the ligands with BSA except for BSA-CP where a more stable complex was formed with temperature, the binding constants decreased slightly with increasing temperature which depicts that this is

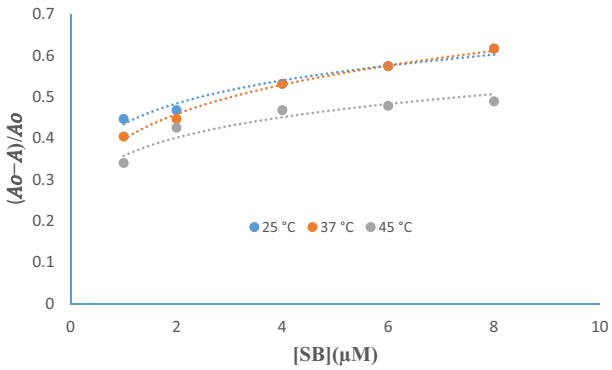

Fig. 4 Non-linear regression plots for the formation of BSA-SB complex at 25, 37 and 45 °C

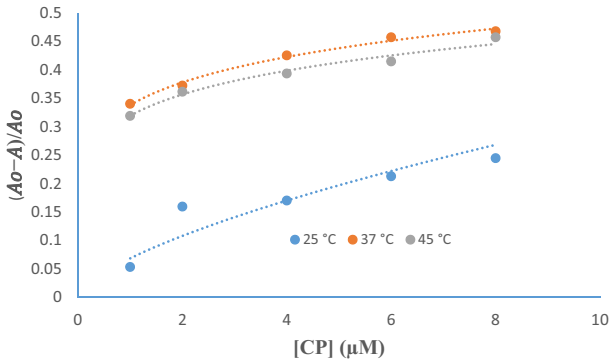

Fig. 5 Non-linear regression plots for the formation of BSA-CP complex at 25, 37 and 45 °C

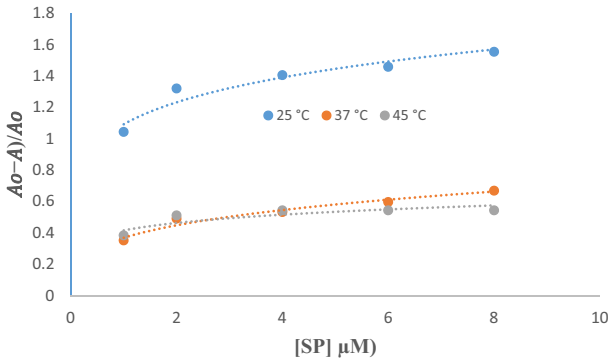

Fig. 6 Non-linear regression plots for the formation of BSA-SP complex at 25, 37 and 45 °C

Table 1 Thermodynamic energy fluctuations and binding connected to BSA's interaction with ligands

Binding	25 °C		37 °C		45 °C		ΔH (KJ mol^{-1})	ΔS (J K^{-1})
	ln K_f	ΔG (KJ mol^{-1})	*ln* K_f	ΔG (KJ mol^{-1})	*ln* K_f	ΔG (KJ mol^{-1})		
BSA-SB	12.72	− 31.52	12.72	− 32.79	12.70	− 33.59	0.64	− 103.66
BSA-CP	12.66	− 31.36	12.70	− 32.73	12.69	− 33.57	− 1.71	− 110.99
BSA-SP	12.91	− 31.98	12.72	− 32.80	12.72	− 33.63	− 7.88	− 80.74

an exothermic reaction process. The negative free energy changes obtained with regards to the formation of the complexes for all the binding interactions reveal that the binding is spontaneous. The consideration of enthalpy and entropy change to the free energy change plays a contributory role in the determination of major type of interaction responsible for binding of the ligand to the biomolecule. Based on previous research [35, 36], it was reported that if the value of ΔH and ΔS are negative, van der Waal's interactions and hydrogen bonds are mainly responsible for the binding reaction. If the value of ΔH and ΔS are positive, hydrophobic interactions are most prominent while

electrostatic forces are more important when ΔH value is negative and ΔS value is positive. The minute positive value of ΔH and the negative ΔS value associated with the interaction of BSA with SB indicate that hydrogen bonding and hydrophobic interactions are probable in the formation and stabilization of the ligand–protein complex. This is not unlikely as sodium benzoate when in solution dissociate to form ions and charge distribution can occur between the ions and the ionic sites of the side chain of amino acids that are constituents of the BSA. The resultant effect of this could impact pharmacokinetic and pharmacodynamic activities when SB is employed as a pharmaceutical excipient or as food additives. Hydrophobic interaction between the aromatic residue of SB can also occur with the hydrophobic pockets of BSA and this is also depicted in the molecular docking studies (Fig. 7). However, the negative values of ΔH and ΔS obtained with BSA-CP and BSA-SP suggest that the protein-complex formation is driven by, van der Waal's interactions and hydrogen bonding. Thus, the binding stabilizing the complex formation is likely between carboxylic functional group of the structure of the propionates and the amino functions of the albumin. Yu and coworkers [37] have also investigated the binding interaction of BSA with SB using spectroscopic and molecular docking studies but this research as far as we are aware is the first investigation of BSA interaction with CP and SP.

3.4 Molecular docking

Molecular docking studies were conducted to gain insights into the positions of favourable interactions between the ligands and BSA [38]. The 3-dimensional structure of BSA retrieved from PDB had 3,5-diiodosalicylic acid as co-crystallised ligand. CP, SB, and SP were docked to the binding pocket of 3,5-diiodosalicylic acid, and eight (8) exhaustive poses were obtained for each ligand. From the results in Table 2, the most affinity for BSA was exhibited by SB, whose binding energies were − 5.9 kcal/mol. (RMSD = 0 Å). This is followed by SP with binding affinity of − 4.1 kcal/mol, and CP having binding energy of − 3.7 kcal/mol.

The binding energies (in kcal/mol) obtained for the ligands are justifed by the types and numbers of intermolecular interactions involved. SB was noted to utilise H-bonding, π-alkyl interaction, and van der Waals forces to bind to BSA (Fig. 7). A hydrogen bonding was observed between the carbonyl functional group of SB and the guanidinium ion of ARG 256 residue of BSA. van der Waal's forces were also observed between SB and nine residues within the binding pockect of BSA (TYR149, ARG217, LEU218, LYS221, HIS241, LEU259, ALA260, ILE263, and ILE289). H-bonding and van der Waals interactions are important non-covalent bonds for proteins' molecular recognition by ligands [39]. In addition, the benzene unit formed π-alkyl interactions with the isobutyl group of LEU 237 and methyl group of ALA 290. These interactions corroborate the results obtained from the in vitro studies. CP showed multiple hydrogen bonds between carbonyl functional group and GLY247, LEU249 and LEU250 residues (Fig. 8) and van der Waals ineractions with ALA26, VAL23, PHE70, LEU46, LEU66, ASP248 and HIS67 while SP which had the second highest binding affinity, was posed in such a way that it interacted with ARG256 via H bonding and with TYR149, LEU237, HIS241, LEU259, ALA260, ILE263, SER286, ILE289 and ALA290 via the distance-dependent van der Waals forces (Fig. 9). H-bonds contribute about 10–40 kJ/mol energy to protein–ligand binding [40].

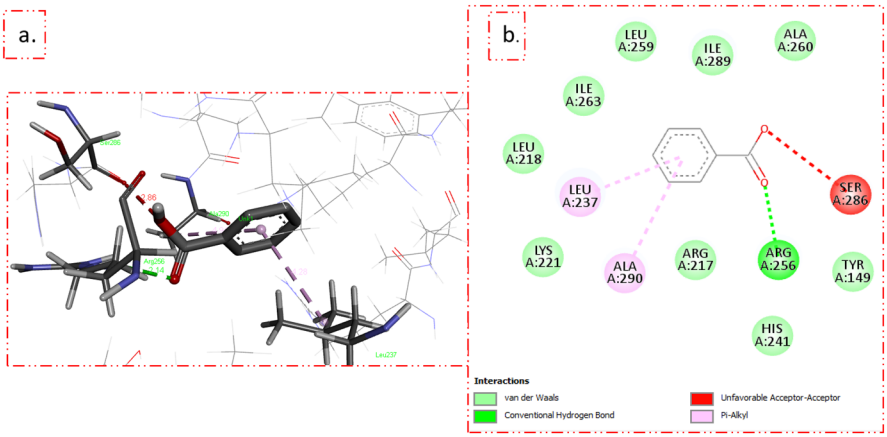

Fig. 7 Visualisation of SB structure interacting with BSA in 3D (**A**) and 2D (**B**) showing H-bond, π-alkyl interaction, and van der Waals interaction

Table 2 Binding affinity scores of the three additives (ligands) when docked on BSA

Rank	Binding affinity (kcal/mol)		
	SB	CP	SP
1	− 5.9	− 3.7	− 4.1
2	− 5.5	− 3.6	− 3.7
3	− 5.5	− 3.6	− 3.6
4	− 5.5	− 3.6	− 3.6
5	− 5.4	− 3.4	− 3.6
6	− 5.4	− 3.3	− 3.4
7	− 5.4	− 3.2	− 3.3
8	− 5.3	− 3.2	− 3.3

4 Conclusion

The binding interactions of SB, CP, and SP with BSA were investigated using the UV–visible spectroscopic and molecular docking methods. Spectroscopic results showed a marked hypochromic effect with increased concentration of ligands relative to the BSA alone. Also, the binding constant increased with temperature revealing that a more stable ligand–protein complex is formed at higher temperature. The investigation of thermodynamic parameters showed that electrostatic force is prominent in the binding of SB to BSA and hydrophobic interactions as the major forces driving the interaction of BSA with both CP and SP. Molecular docking results revealed that H bonds, π-alkyl interaction, and van der Waals forces play roles in the binding of SB, CP, and SP to BSA. One important limitation of this study which is hereby acknowledged to ensure a comprehensive understanding of the study's findings is that only one spectroscopic study (UV spectroscopy) was employed. While molecular docking provided complemetary information, a multi-spectroscopic studies could add to the robbustness of the conclusions. Thus, the claim of this study can be verified upon its subject to other spectroscopic methods such as circular dichroism or fluorescence spectroscopy which are beyond the scope of this study.

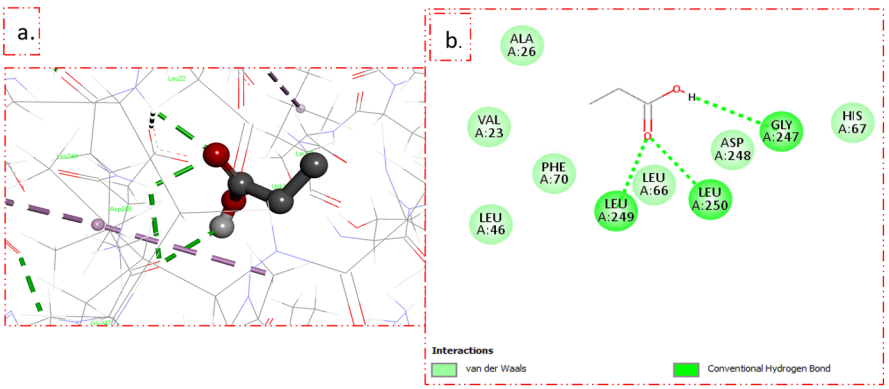

Fig. 8 Visualisation of CP structure interacting with BSA in 3D (**A**) and 2D (**B**) showing H-bond and van der Waals interaction

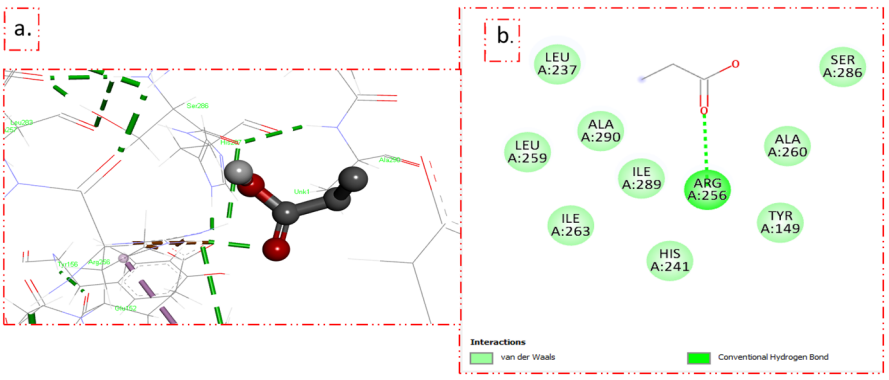

Fig. 9 Visualisation of SP structure interacting with BSA in 3D (**A**) and 2D (**B**) showing H-bond and van der Waals interaction

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Author contributions OMA designed and supervised the study. Material preparation, data collection and analysis were performed by SCA and OOO. Further data interpretation was by YAA. The first draft of the manuscript was written by OOO and YAA and all authors commented on previous versions of the manuscript. Figures 1, 2, 3, 4, 5, 6 and Table 1 were prepared by OOO while Figs. 7, 8, 9 and Table 2 were prepared by YAA. All authors read and approved the final manuscript.

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Data availability Data supporting the findings of this submission can be accessed via this link: https://shorturl.at/FD2mS.

Declarations

Competing interests The authors declare no competing interests.

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