Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1505-1529 | ISSN:2251-6727

Exploring the Binding Stability and Sub Domains of Bovine Serum Albumin (BSA) In the Presence of Phenolic Derivatives of Benzoic Acids and Cinnamic Acids Through Molecular Docking Approach

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(Received: 11 June 2024 Revised: 16 July 2024 Accepted: 10 August 2024)

KEYWORDS

Hydroxy derivatives of benzoic acids and cinnamic acids; Bovine Serum Albumin; molecular docking; binding energy; Hydrogen-bonding; hydrophobic interactions

ABSTRACT:

Variation in binding energies and stability of complex between Bovine Serum Albumin (BSA) with hydroxyl derivatives of benzoic acid (DBA) and cinnamic acid (DCA) derived from *Psidium guajava L.* were studied by employing molecular docking (Mol.Doc) techniques. The binding energies of DCA-BSA show more favorable interactions than that of DBA-BSA complex. Among the DCAs, Caffeic acid (CA) and Coumaric acid (CoA) which possess phenolic –OH has larger stability compared to Sinapic acid (SA) and Ferrulic acid (FA) wherein the -OH is replaced by -OCH_3 in these acids. On the contrary, in the case of DBAs, the acid containing -OCH_3 (Vanillic acid (VA)) has a better binding efficiency with BSA compared to acids containing -OH although all the DBAs possess lesser energetics compared to DCAs. The variation in their binding energies are attributed to the binding site, sub domains and the nature of the bimolecular interactions between the BSA and guest (DBA and DCA) molecules. FA and SA prefer to dock in the Sudlow binding site II whereas for CA and CoA, the energetically stable site is warfarin site (Site I). Interestingly, for Syringic acid (SyA) and Pyrogallic acid (PyA) the energetically most favored binding sub domain is IB which is the non-Sudlow binding site (III). However, Gentistic acid (GeA) and Procatechiuc acid (PrA) prefers both drug binding sites. Docking studies provides an excellent approach in determining the various forces governing the stability of ligand-protein complexes.

Introduction

It has been well established and reported in literature regarding the ethnobotanical significance of Guava plant [**1-4**] and the application towards medicinal aspects from the bark, seeds, fruits, flowers as well as leaves of it. The guava plant *(Psidium guajava L.)*, is a small tree belonging to the myrtle family of *Myrtaceae* and play a significant role on the treatment of type II diabetes and hypertension. Interestingly, all the parts of the plant are used in some form of medicine **[5-6]** for treatment towards respiratory problem, coughs, flu diarrhoea, dysentery, cholera, heart burn, constipation, stomach ache and digestive problems. A considerable role on the medicinal effects are further attributed to the leaves and edible fruits which are of more prominence and are of major interest in the field of pharmacology. Since the extracts from fruits **[7,8]** and leaves vary in proportion, the protein content is less than 1% but the fibre content (2% to 6%) has been considered to be very much significant. However, the fruit pulp and peel fractions exhibit very high contents of dietary fibre around 50 % and extractable polyphenols ranging from 2.5 to 8% (2.62–7.79%)

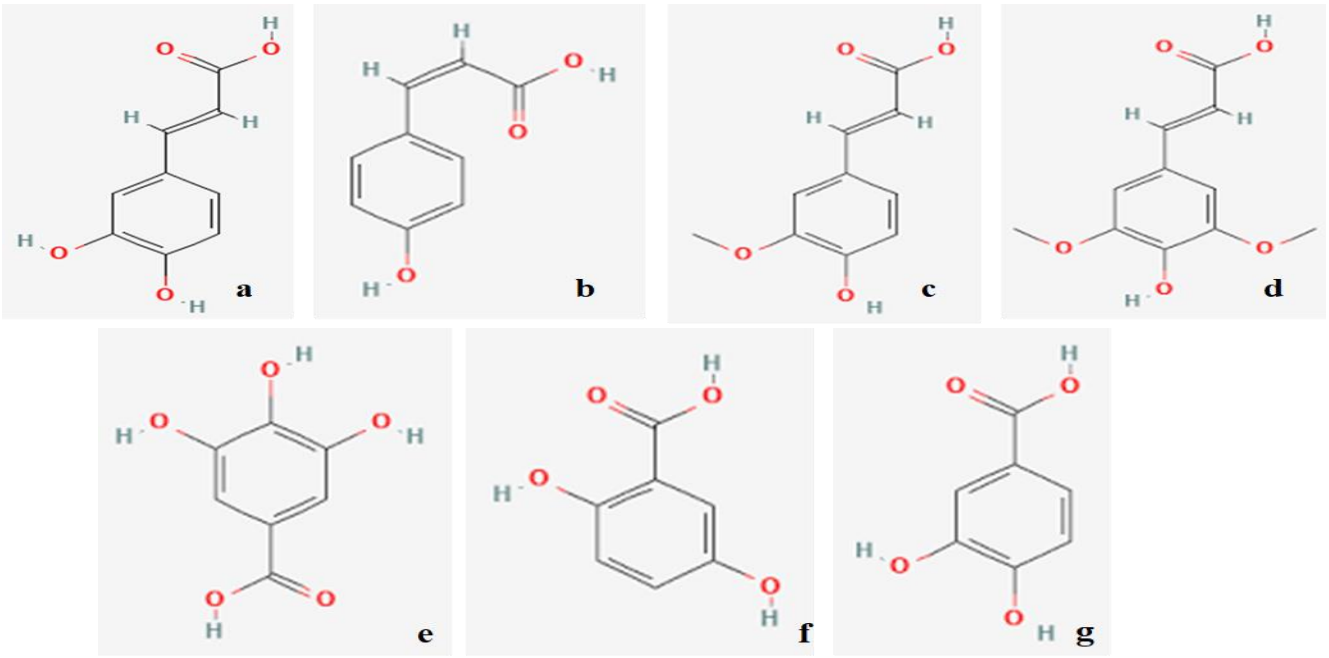
Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(5), 1505-1529 | ISSN:2251-6727

Polyphenols are usually strong reducing agents referred as antioxidants that exhibit a similar role to that of vitamin C, vitamin E and carotenoids. Polyphenols primarily protect the various tissues of our body against oxidative stress and associated pathologies such as cancers, coronary heart disease and inflammation. Literature studies and reports reveals that a longer period of consumption of diets supplemented with polyphenols protects and prevents against certain cancers, cardiovascular diseases, type II diabetes, osteoporosis, pancreatitis, gastrointestinal problems, lung damage, and neurodegenerative diseases**[9-12]**.The range of phenolic acids isolated from guava are predominantly derivatives of benzoic acid (DBA) and cinnamic acid (DCA) and a detailed analysis was carried out on antidiabetic studies **[13]** . The dominant explanation for these benefits are that both DBA and DCA act as a biochemical scavenger such that polyphenolic compounds negate free radicals by forming stabilized chemical complexes, thus preventing further reactions which usually results in cancer and tumours. Among the functional groups, phenolics have been in constant study for research purposes and have attracted the attention of many chemists and biologists due to their diverse biological activities consisting of high pharmacological value **[14-17].**

Our group has involved in various photophysical and theoretical studies on ligand interaction with proteins in the presence of competing ligands, wherein molecular docking (Mol.Doc) approach has been widely employed in establishing the nature of interaction governing the host-guest complex. In such studies, the protein which is large biomolecule acts as the host and the ligands (drugs/fluorophores) as the guest **[18-26].** Our present investigation focuses on the binding studies of these phenolic acids **[27-29]** with most widely studied large globular protein namely Bovine Serum Albumin (BSA) through Mol.Doc approach. The following phenolic acids Caffeic acid (CA), Coumaric acid (CoA), Ferullic acid (FA) and Sinapic acid (SA) have been classified as hydroxy derivatives of cinnamic acid, whereas Gallic acid (GA), Gentisic acid (GeA) Protocatechuic acid (PrA), Pyrogallic acid (PyA), Syringic acid (SyA) and Vanillic acid (VA) belongs to the hydroxy derivatives of benzoic acid. The structures of all the DBAs and DCAs are shown in **figure 1** with representation of the hydrogen-bonding (HB) donor and acceptor sites**.** All the DBAs and DCAs act as the guest molecule and are employed in binding studies with BSA.



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JCHR (2024) 14(5), 1505-1529 | ISSN:2251-6727

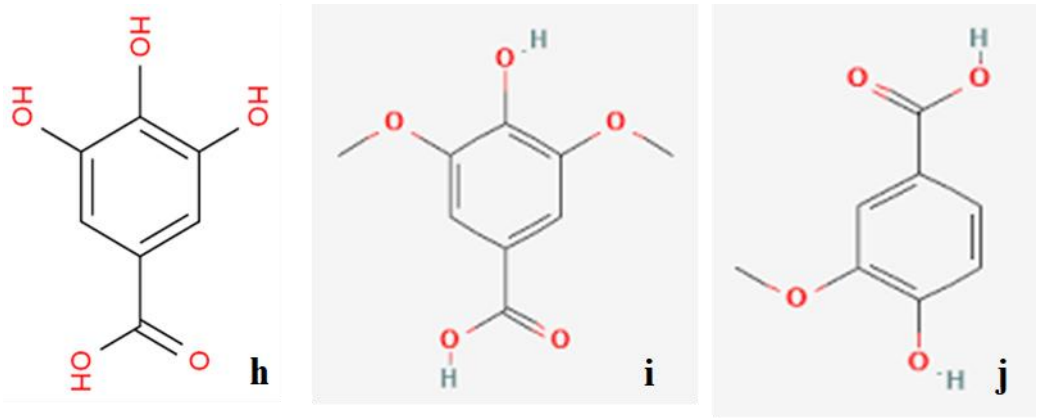

Figure 1: Structure of Phenolic acids of Cinnamic acid and Benzoic acids. (a-d) represents Cinnamic acid derivatives: Caffeic acid (CA), Coumaric acid (CoA), Ferullic acid (FA) and Sinapic acid (SA). (e-j) represents Benzoic acid derivatives: Gallic acid (GA), Gentistic acid (GeA), Protocatechuic acid (PrA), Pyrogallic acid (PyA), Syringic acid (SyA) and Vanillic acid (VA).

Reports portray that the interaction of protein with phenolic compounds can influence the protein hydrophobicity, structure and size thereby affecting the protein interfacial properties **[33-34].** The protein-phenolic interactions through hydrophobic attractive forces results in the formation of aggregates. As a result, the absorption rate of the proteins decreases and this is responsible for the lateral interactions on the interface **[35].** Similarly, protein-phenol interactions are governed by the chemical structure of the phenols, such as degree of polymerization, conformational flexibility, and hydrophobicity **[36, 37].** These properties can lead to non-covalent and/or covalent interactions with the proteins. The non-covalent interactions occur via HB comprising of both conventional hydrogen-bonding (cHB) and non-conventional hydrogen-bonding (NcHB), van der Waals forces, electrostatic and hydrophobic interactions [**31**]. Xu et al. [**38**] reports reveal that 50% of phenols in rapeseed interacted non-covalently with rapeseed proteins which is based on their pH conditions and 40% exist as unbound phenols in the aqueous solution, and the remaining 10% of the phenols interact covalently with the proteins **[39]**. Covalent interactions between phenols and proteins usually occur upon oxidation of the phenols. Hydroxyl groups on phenyl rings can be converted into quinones by enzymes or auto-oxidation, and the latter is accelerated at alkaline pH or in the presence of oxidizing agents **[40-42].**

The interaction of the anticancer plant alkaloid sanguinarine with BSA reveals that the formation of stable complex with the plant alkaloids. Both variants of sanguinarine bind to site I (subdomain IIA) on BSA, as shown by competitive binding using the binding site markers warfarin (site I) and ibuprofen (site II).**[43]** The binding interaction between alpinetin-BSA in the competitive experiments of site markers reveals that alpinetin is located at the Sudlow binding site I (subdomain II A) of BSA [**44**]. Similarly, the interaction of Naringenin, a flavonoid found in grape fruit with BSA was studied with site marker competitive displacement experiments exhibits that naringenin binds to site I (subdomain IIA) of BSA with a high affinity**.[45]** The studies pertaining to BSA with several drugs extracted from plant kingdom has been explored through theoretical methods wherein the binding sites and sub domains are ascertained. Herein, we explore the role of functional groups of DBAs and DCAs responsible for the formation of complex with BSA. Further, we also elucidate the stability of this complex is attributed to the phenolic –OH or the –OH moiety of the carboxylic acid involved in binding with amino acids. Further, the nature of the amino acids (polar or non-polar) that are involved upon complex formation resulting in stability of the complex and the binding domains are identified through docking studies.

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JCHR (2024) 14(5), 1505-1529 | ISSN:2251-6727

Docking methodology and structural aspects

All the phenolic acid structures of benzoic acid and cinnamic acid retrieval were systematically carried out based on Lipinski rule of five **[46]**. This methodology was applied for all the DCAs and DBAs identically without any modification as carried out in our earlier studies involving ligand interaction with proteins **[18-26]**. All these structures were optimised by employing chem sketch software and were finally saved in PDB format using open Babel converter.

The complete docking properties of the ligands based on their molecular formula, molecular weight and properties regarding SMILES notations are provided in **table 1**. The docking procedures were carried out as reported by incorporating the principles of docking methodologies **[47-52]**. Ten different conformers were generated through the software auto dock 4.2 and the structure were arranged based on the corresponding energetics (Kcal mol^{-1}) with the formulation of several possible energy parameters leading to the ligand-BSA binding complex. Based on the docking efficiency and the conformers generated were arranged in the order of stability which is based on several factors regarding the complete energetics that comprises of BE, torsional energy, intermolecular energy, energy related to bimolecular interactions. The results and discussion have been segmented into ligand-DCAs interaction properties in comparison with ligand-DBAs. A comprehensive and detailed comparison on the outcome of the docking studies has been explained in detail in the discussion part.

Results and Discussion

All the DBAs and DCAs structures retrieval carried out systematically obeys the Lipinski rule of five [**46].** This methodology has been employed in several studies of our work involving BSA and HSA interaction with various fluorophores and drugs that can acts as an efficient ligand **[20-26].** The pdb structure of BSA used was the same as in our earlier studies **[21]** and is provided in **supporting information figure S1.** The complete docking properties along with the compound molecular formula, molecular weight and SMILES notations of all the ligands are provided in **table1**. By employing SMILES notation properties, the docking procedures were carried out and docking efficiency (Binding energy between host guest complexes) were ascertained. A detailed comparison on the outcome of the docking studies has been explained in depth based on the energetics, molecular interactions, binding sites and domains of the ligands with protein. Ten different conformers of each ligand with BSA were generated through the software auto dock 4.2 and the resulting conformers were arranged based on the corresponding energetics (Kcal mol^{-1}) with the formulation of several possible energy parameters leading to the ligand- BSA binding complex.

Table 1: SMILES notation of ligands

Ligand	Type	Mol.wt g/mol	Molecular Formula	XLog P3	HB Donor Count	HB Acceptor Count	Rotatable Bond Count	Topological Polar Surface Area (TSA) in Å²	Heavy atom Count
Sinapic Acid	DCA	224.21	$C_{11}H_{12}O_5$	1.5	2	5	4	76	16
Syringic Acid	DBA	198.17	$C_9H_{10}O_5$	1	2	5	3	76	14
Ferullic Acid	DCA	194.18	$C_{10}H_{10}O_4$	1.5	2	4	3	76	14
Caffeic Acid	DCA	180.16	$C_9H_8O_4$	1.2	3	4	2	78	13
Pyrogallic acid	DBA	170.12	$C_7H_6O_5$	1.1	4	5	1	98	12
Gallic Acid	DBA	170.12	$C_7H_6O_5$	0.7	4	5	1	98	12
Vannilic Acid	DBA	168.15	$C_8H_8O_4$	1.4	2	4	2	68	12
Coumaric Acid	DCA	164.16	$C_9H_8O_3$	1.5	2	3	2	57.5	12
Protocatechuic Acid	DBA	154.12	$C_7H_6O_4$	1.1	3	4	1	77.8	11
Gentistic Acid	DBA	154.12	$C_7H_6O_4$	1.6	3	4	1	77.8	11

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DCAs-BSA Interaction studies

The energetics resulting from various molecular interaction parameters of the ten stable conformers of CA, CoA, FA) and SA were analysed and a comparative stability of the conformers were arranged based on their BE. The stable conformer for any acid taken for docking with BSA is conformer1 which is represented as BSACA1, BSACoA1 BSAFA1 and BSASA1 for CA, CoA, FA and SA respectively. The stability of the host-guest complex is of the order CA = CoA > SA > FA based on the docking score, which refers to the negative ΔG value. The total energetics resulting from various parameters of all the DCAs are provided in **supporting information tables ST1 to ST4**. Interestingly, the conformers generated vary in their BE which is attributed to the different inhibitory constant values. The decrease in the BE of the conformer is due to the difference in inhibitory constant parameter so that different conformer stability varies based on the BE and the molecular interactions existing between the acid derivatives and amino acid moieties in BSA. The extent of decrease in the BE of the conformers of all the DCAs with BSA is provided in **figure 2** which reflects the stability of the individual conformers to one another. Similarly, the number of molecular interactions existing in each conformer of DCAs with BSA is shown in **figure 3** and the molecular interactions resulting from hydrogen-bonding alone is shown in **figure 4.** Interestingly, the conformers stability are predominantly due to cHB and NcHB compared to several hydrophobic interactions. The contribution from several hydrophobic interactions (pi-alkyl, pi-pi, pi-sigma/cation/anion) along with HB and weaker forces /unfavourable interactions existing between the various conformers of DCAs with BSA is provided in **tables 2-5** and the stable sub domains and binding sites of DCAs with the protein are given in **tables 6-9.** The variation in the BE of the conformers are presumably attributed to several factors such as the nature of the amino acids involved in bonding, number of molecular interactions, position and site of binding domains of the ligand with the protein molecule.

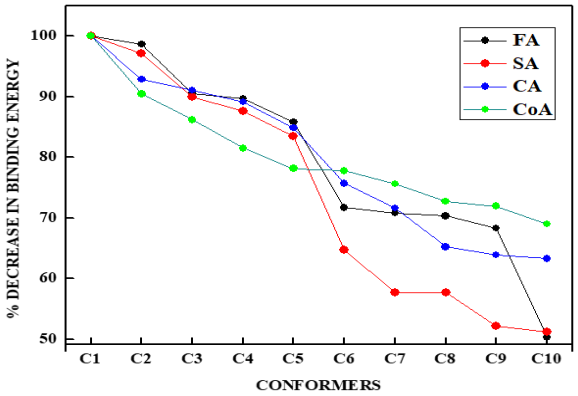

Figure 2: Extent of decrease in the % of BE of the various conformers with respect to Conformer 1 of each DCAs with BSA.

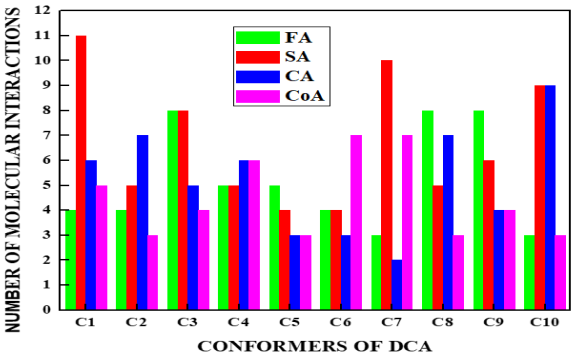

Figure 3: Representation of number of molecular interactions of each conformer of DCAs with BSA. C1 to C10 represents Conformer 1 to Conformer 10 respectively of FA (Green), SA(Red) , CA(Blue) and CoA (Magenta).

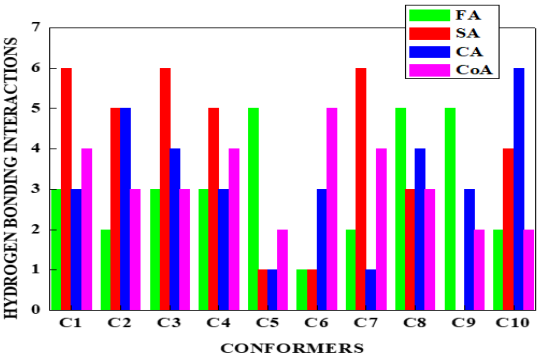

Figure 4: Representation of number of interactions due to hydrogen-bonding alone of each conformer of DCAs with BSA. C1 to C10 represents Conformer 1 to Conformer 10 respectively of FA (Green), SA(Red) , CA(Blue) and CoA (Magenta).

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JCHR (2024) 14(5), 1505-1529 | ISSN:2251-6727

Table2 : Representation of overall interactions existing between CA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi- Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSACA1	**3**	**-**	**2**	**-**	**1**	**-**	**-**	**6**
BSACA2	**5**	**-**	**2**	**-**	**-**	**-**	**-**	**7**
BSACA3	**4**	**-**	**1**	**-**	**-**	**-**	**-**	**5**
BSACA4	**3**	**-**	**1**	**-**	**-**	**-**	**2**	**6**
BSACA5	**1**	**-**	**2**	**-**	**-**	**-**	**-**	**3**
BSACA6	**3**	**-**	**-**	**-**	**-**	**-**	**-**	**3**
BSACA7	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**2**
BSACA8	**4**	**-**	**3**	**-**	**-**	**-**	**-**	**7**
BSACA9	**3**	**-**	**1**	**-**	**-**	**-**	**-**	**4**
BSACA10	**6**	**-**	**2**	**1**	**-**	**-**	**-**	**9**

Table 3: Representation of overall interactions existing between CoA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSACoA1	**4**	**-**	**1**	**-**	**-**	**-**	**-**	**5**
BSACoA2	**2**	**1**	**-**	**-**	**-**	**-**	**-**	**3**
BSACoA3	**3**	**-**	**1**	**-**	**-**	**-**	**-**	**4**
BSACoA4	**4**	**-**	**2**	**-**	**-**	**-**	**-**	**6**
BSACoA5	**2**	**-**	**-**	**-**	**1**	**-**	**-**	**3**
BSACoA6	**5**	**-**	**2**	**-**	**-**	**-**	**-**	**7**
BSACoA7	**4**	**-**	**2**	**-**	**1**	**-**	**-**	**7**
BSACoA8	**3**	**-**	**1**	**-**	**-**	**-**	**-**	**3**
BSACoA9	**2**	**-**	**2**	**-**	**-**	**-**	**-**	**4**
BSACoA10	**2**	**-**	**-**	**-**	**1**	**-**	**-**	**3**

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Table 4: Representation of overall interactions existing between FA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAFA1	**3**	**-**	**-**	**-**	**1**	**-**	**-**	**4**
BSAFA2	**2**	**-**	**1**	**-**	**-**	**-**	**1**	**4**
BSAFA3	**2**	**1**	**2**	**-**	**-**	**-**	**3**	**8**
BSAFA4	**2**	**1**	**2**	**-**	**-**	**-**	**-**	**5**
BSAFA5	**4**	**1**	**-**	**-**	**-**	**-**	**-**	**5**
BSAFA6	**1**	**-**	**1**	**-**	**1**	**-**	**1**	**4**
BSAFA7	**2**	**-**	**1**	**-**	**-**	**-**	**-**	**3**
BSAFA8	**4**	**1**	**3**	**-**	**-**	**-**	**-**	**8**
BSAFA9	**5**	**-**	**2**	**-**	**-**	**-**	**1**	**8**
BSAFA10	**2**	**-**	**1**	**-**	**-**	**-**	**-**	**3**

Table 5 : Representation of overall interactions existing between SA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSASA1	**3**	**3**	**2**	**-**	**-**	**-**	**3**	**11**
BSASA2	**3**	**2**	**-**	**-**	**-**	**-**	**-**	**5**
BSASA3	**4**	**2**	**1**	**-**	**-**	**-**	**1**	**8**
BSASA4	**3**	**2**	**-**	**-**	**-**	**-**	**-**	**5**
BSASA5	**1**	**-**	**-**	**-**	**-**	**-**	**3**	**4**
BSASA6	**1**	**-**	**1**	**-**	**-**	**-**	**2**	**4**
BSASA7	**4**	**2**	**1**	**-**	**-**	**1**	**2**	**10**
BSASA8	**2**	**1**	**1**	**-**	**-**	**-**	**1**	**5**
BSASA9	**-**	**-**	**1**	**2**	**-**	**-**	**3**	**6**
BSASA10	**4**	**-**	**2**	**-**	**-**	**-**	**3**	**9**

Table 6: Confinement of conformers of CA in various domains of BSA

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Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in BSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in BSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in BSA
BSACA1	**IIIA**	**II**	**IIA,IIB**	**I**	-	-
BSACA2	**IIIA**	**II**	**IIIA**	**II**	-	-
BSACA3	**IIA,IIB**	**I**	**IIB**	**I**	-	-
BSACA4	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSACA5	**IIA**	**I**	**IIA**	**I**	-	-
BSACA6	**IIIA,IIIB**	**II**	**-**	-	-	-
BSACA7	**IIA**	**I**	**IIB**	**I**	-	-
BSACA8	**IIIA**	**II**	**IIIA**	**II**	-	-
BSACA9	**IB**	**III**	**IB**	**III**	-	-
BSACA10	**IIIA**	**II**	**IIIA**	**II**	-	-

Table 7: Confinement of conformers of Coumaric acid in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in HSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in HAS
BSACoA1	**IIA IIB**	**I**	**IIIA**	**II**	-	-
BSACoA2	**IIB**	**1**	**IIIA**	**II**	-	-
BSACoA3	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSACoA4	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSACoA5	**IIA**	**I**	**IIA**	**I**	-	-
BSACoA6	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSACoA7	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSACoA8	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSACoA9	**IIIA**	**II**	**IIIA**	**II**	-	-
BSACoA10	**IIA IIB**	**I**	**IIA**	**1**	-	-

Table 8: Confinement of conformers of FA in various domains of BSA

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Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in BSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in BSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in BSA
BSAFA1	**IIA**	**I**	**IIA**	**I**	**-**	**-**
BSAFA2	**IIIA,IIIB**	**II**	**IIIB**	**II**	**IIIB**	**II**
BSAFA3	**IIA,IIB**	**I**	**IIA**	**I**	**IIA,IIB**	**I**
BSAFA4	**IIIA,IIIB**	**II**	**IIIA,IIB**	**II**	-	-
BSAFA5	**IIIA**	**II**	**IIIA**	**II**	-	-
BSAFA6	**IIIB**	**II**	**IIIB**	**II**	**IIIA**	**II**
BSAFA7	**IIIB**	**II**	**IIIA**	**II**	-	-
BSAFA8	**IIIA,IIIB**	**II**	**IIIA,IIIB**	**II**	-	-
BSAFA9	**IIIA,IIIB**	**II**	**IIIB**	**II**	**IIIB**	**II**
BSAFA10	**IIIB**	**II**	**IIIA**	**II**	-	-

Table 9 : Confinement of conformers of SA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in BSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in BSA
BSASA1	**IIIA,IIIB**	**II**	**IIIA,IIIB**	**II**	**IIIA,IIIB**	**II**
BSASA2	**IIA,IIIA**	**I,II**	**-**	-	-	-
BSASA3	**IIA,IIB,IIIA**	**I,II**	**IIIA**	**II**	**IIIA**	**II**
BSASA4	**IIIA**	**II**	**-**	-	-	-
BSASA5	**IIIB**	**II**	**-**	**-**	**IIIA,IIIB**	**II**
BSASA6	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSASA7	**IIA,IIIA**	**I,II**	**IIIA**	**II**	**IIB,IIIA**	**I,II**
BSASA8	**IIIB**	**II**	**IIIA**	**II**	**IIIA**	**II**
BSASA9	**-**	**-**	**IIIA,IIIB**	**II**	**IIIA,IIIB**	**II**
BSASA10	**IIIA,IIB**	**II**	**IIIA,IIIB**	**II**	**IIIA,IIIB**	**II**

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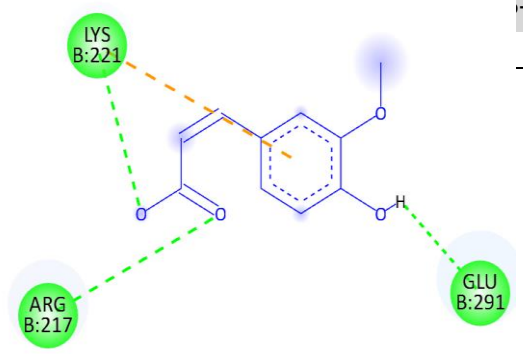
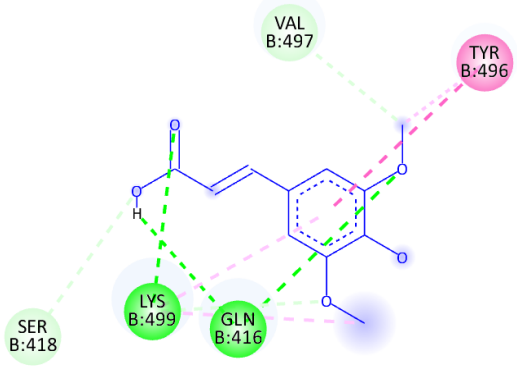
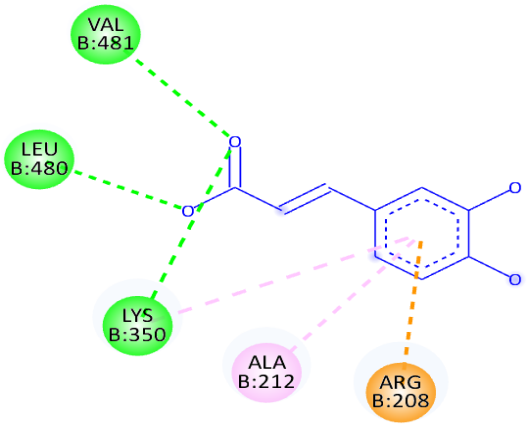


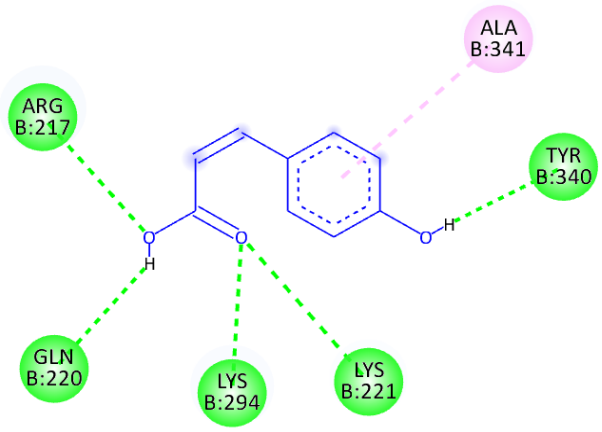
BSAFA1



BSASA1



BSACA1



BSACoA1

Figure 5: The 2D diagram of **BSAFA1, BSASA1, BSACA1, BSACoA1,** visualized using Biovia Discovery Studio visualizer. Green colour dotted line indicates hydrogen bonds with electronegative elements like N and O atoms, light green colour dotted line indicates carbon-hydrogen bonds; light purple colour indicates pi-alkyl interactions, violet colour dotted line indicates – pi-sigma interaction. Light green colour amino acids without interactions represent van der Waals interactions and red colour interaction represents unfavourable interaction. The blue halo surrounding the interacting residues represents the solvent accessible surface that is proportional to its diameter.

The variation in the nature of amino acids involved in cHB, NcHB and hydrophobic interactions of each DCA varies when it forms complex with BSA **(Figure 5).** Apart from conformer1 of each DCAs namely BSAFA1, BSASA1, BSACA1 and BSACoA1, all other unique conformers of all the DCAs with BSA are provided in **supporting information figures S1-S4** which clearly illustrates the amino acids that are involved belong to different sub domains corresponding to different binding sites. **Figures S1, S2, S3** and **S4** represents the unique conformers of FA-BSA, SA-BSA, CA-BSA and CoA-BSA complexes respectively wherein the role of polar and non-polar amino acids that are involved in binding provides information of the sequence site of ligand attachment with the protein molecule.

Based on the energetics and analysis of the B.E values, we found that the acids having –OH functional group (CA and CoA) in the phenyl ring alone results in a lager stability in terms of BE upon complex formation with BSA. Similarly, when both the –OH moieties are substituted by $–OCH_3$ moieties, SA the BE is found to be lesser than CA and CoA. However, the acid containing presence of both –OH and $–OCH_3$ moieties in the phenyl ring (FA) exhibits a lesser

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binding affinity with BSA compared to all other DCAs. The above observation was based on the docking score of the most stable conformer which is the first conformer. However, the contributions leading to the stability of the conformers are further determined by the nature of the amino acids and number of amino acids involved in HB as well as hydrophobic interactions. An illustration on the HB and hydrophobic interactions existing in DCAs-BSA complex with the amino acids are provided in **table 10** and the complete illustration of the total molecular interactions comprising weaker forces and unfavourable interactions are provided in **supporting information tables ST5 to ST8**

Table 10: Amino acids involved in HB and hydrophobic interactions

DCA	Total Conformers	Conformers with cHB/NcHB interactions	Amino acids involved in cHB/NcHB interactions	Conformers with Hydrophobic interactions	Amino acids involved in Hydrophobic interactions	Molecular interaction Influence on the complex
CA	**10**	**10**	ARG GLN GLU LEU, LYS, PHE SER and TYR	**10**	ALA, ARG, LEU, LYS, TYR and VAL	Polar amino acids governs the binding stability
CoA	**10**	**10**	GLU, LEU, LYS, PRO, SER, THR, TYR and VAL	**10**	LEU and LYS	Polar amino acids governs the binding stability
FA	**10**	**10**	ARG, GLU, LEU LYS, PRO, THR, TYR and VAL	**9**	ALA, LEU, LYS, TYR and VAL	Both Polar and non –polar amino acids governs the binding stability
SA	**10**	**10**	ARG, ASP, CYS, GLN, GLU, LYS, SER, and TYR	**8**	ALA, LEU, LYS, MET, TYR and VAL	Polar amino acids involved in HB and non-polar involved in hydrophobic interactions

On the basis of molecular interactions as provided in **Supporting information tables ST5-ST8** and **tables 6-10** it is evident that FA and CoA behave as a site specific and site selective ligand wherein, it prefers to dock only in subdomains IIIA and IIIB respectively of Sudlow Site II, known as ibuprofen binding site. However, SA prefers to reside in both Sudlow binding sites. Among the DCAs CA is the only acid derivative which tend to reside in all the binding sites of BSA. Interestingly, some of the DCAs does not prefer the non-Sudlow binding site, which is generally not preferred by drugs and fluorophores, when docked with proteins. Based on the above studies we further ascertain that

i) HB interactions primarily govern the stability of DCA-BSA complex and those acids that containing –OH group alone possess a larger docking score and enhanced binding affinity.
ii) When the –OH groups are substituted by $–OCH_3$, the contributions from hydrophobic interactions competes along with HB interactions resulting in destabilization of the conformers and this is evident in the case of SA interaction with BSA.
iii) SA has more number of molecular interactions with BSA than any other DCAs involved in docking with BSA resulting in lesser docking score compared to that of CA and CoA.
iv) The binding site I which are governed by hydrophobic interactions is not the preferred binding site for any of the DCAs. This clearly portrays that the combined influences of HB, hydrophobic and electrostatic interactions govern the overall stability of the conformers.
v) Comparatatively lesser number of pi-sigma, pi-cation, and pi-anion interactions was visualized in all the conformers generated, however all the conformers exhibit HB interaction eventually also possesses pi-alkyl interactions.
vi) Aspargine (ASN), Histidine (HIS). Glycine (GLY) and Tryptophan (TRP) are the amino acids that are not involved in any form of molecular interaction with DCAs, whereas, LEU, LYS, VAL, ARG,

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SER, GLN, TYR are the amino acids involved in HB as well as hydrophobic interactions.

vii) In general, polar amino acids form HB interactions and are also involved in hydrophobic interactions with DCAs rather than non-polar amino acids such that the governing stability is presumably influenced by polar amino acids only in the case of CA and CoA,

viii) Both polar and non-polar amino contribute to the stability of SA-BSA and FA-BSA complex.

DBAs-BSA Interaction studies

As carried out for cinnamic acid derivatives, the energetics resulting from various molecular interaction parameters belonging to the conformers of GA, GeA, PrA, PyA, SyA and VA were explored and arranged primarily based on their BE. The first conformer for any acid was considered as the stable conformer as carried out in the case of DCA. The stability of the DBAs-BSA complex is of the order VA = GeA > GA > PrA > SyA > PyA based on the first conformer. The total energetics resulting from various intermolecular energies of all the DBAs-BSA are provided in **supporting information tables ST9 to ST14**. The extent of decrease in the BE of all the conformers with respect to that of stable conformer of DBAs with BSA is provided in **figure 5** which reflects the stability of the individual conformers with one another and also provides the comparison between the stability of each conformer. Similarly, the number of molecular interactions existing in each conformer of DBAs with BSA is shown in **figure 6** and the molecular interactions due to hydrogen-bonding contribution alone, and also ascertaining the nature of the governing forces influencing the host-guest complex is shown in **figure 7.** As observed in the case of DCA-BSA complex, the conformer stability in the case of various DBAs are also attributed to cHB and NcHB. The contribution of number of hydrophobic interactions with that of number of HB interactions are lesser when compared to that of DCAs-BSA complex as provided in **tables 11-16.** Similarly, the preferred sub domains and the binding sites of the individual conformers of all the ligands of DBAs with BSA are given in **tables 17-22** respectively.

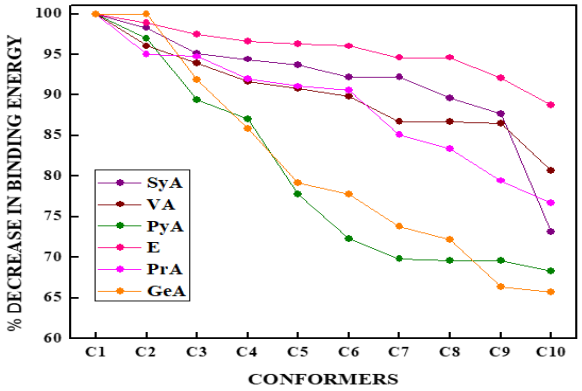

Figure 5: Extent of decrease in the % of BE of the various conformers with respect to Conformer 1 of each DBAs with BSA.

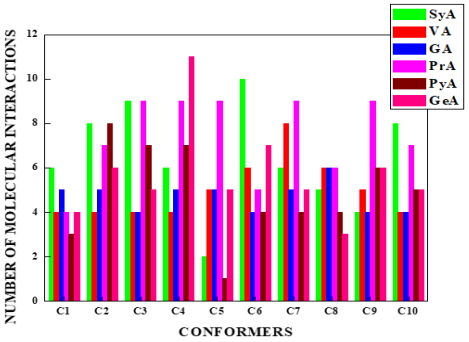

Figure 6: Representation of number of molecular interactions of each conformer of DBAs with BSA. C1 to C10 represents Conformer 1 to Conformer 10 respectively of SyA (Green), VA(Red) , GA(Blue) and PrA (Magenta), PyA (Brown) and GeA (Pink).

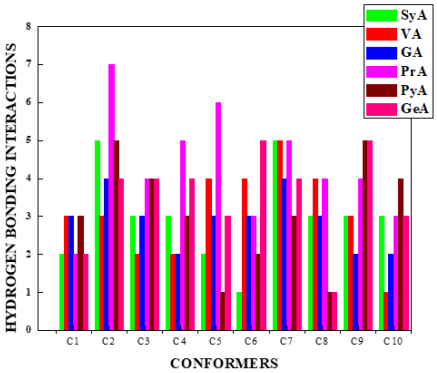

Figure 7: Representation of number of interactions due to hydrogen-bonding alone of each conformer of DBAs

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with BSA. C1 to C10 represents Conformer 1 to Conformer 10 respectively of SyA (Green), VA(Red) , GA(Blue) and PrA (Magenta), PyA (Brown) and GeA (Pink).

Table 12 : Representation of overall interactions existing between GeA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAGeA1	**2**	**-**	**2**	**-**	**-**	**-**		**4**
BSAGeA2	**2**	**1**	**2**	**-**	**-**	**-**	**1**	**6**
BSAGeA3	**4**	**-**	**1**	**-**	**-**	**-**	**-**	**5**
BSAGeA4	**7**	**1**	**2**	**-**	**1**	**-**	**-**	**11**
BSAGeA5	**3**	**-**	**2**	**-**	**-**	**-**	**-**	**5**
BSAGeA6	**5**	**-**	**2**	**-**	**-**	**-**	**-**	**7**
BSAGeA7	**4**	**-**	**1**	**-**	**-**	**-**	**-**	**5**
BSAGeA8	**1**	**-**	**2**	**-**	**-**	**-**	**-**	**3**
BSAGeA9	**5**	**-**	**-**	**-**	**1**	**-**	**-**	**6**
BSAGeA10	**3**	**-**	**1**	**-**	**-**	**-**	**1**	**5**

Table 13: Representation of overall interactions existing between PrA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAPrA1	**3**	**-**	**-**	**-**	**-**	**-**	**-**	**3**
BSAPrA2	**5**	**-**	**2**	**-**	**1**	**-**	**-**	**8**
BSAPrA3	**4**	**-**	**-**	**1**	**1**	**1**	**-**	**7**
BSAPrA4	**3**	**-**	**3**	**-**	**1**	**-**	**-**	**7**
BSAPrA5	**1**	**-**	**-**	**-**	**-**	**-**	**-**	**1**
BSAPrA6	**1**	**1**	**-**	**-**	**-**	**-**	**2**	**4**
BSAPrA7	**3**	**-**	**-**		**1**	**-**	**-**	**4**
BSAPrA8	**1**	**-**	**1**	**-**	**-**	**-**	**-**	**4**
BSAPrA9	**5**	**-**	**1**	**-**	**-**	**-**	**-**	**6**

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BSAPrA10	**4**	**-**	**-**	**-**	**1**	**-**	**-**	**5**

Table 14 : Representation of overall interactions existing between PyA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAPyA1	**2**	**-**	**1**	**-**	**-**	**-**	**1**	**4**
BSAPyA2	**5**	**-**	**1**	**-**	**1**	**-**	**-**	**7**
BSAPyA3	**4**	**-**	**2**	**2**	**-**	**-**	**1**	**9**
BSAPyA4	**5**	**2**	**-**	**-**	**1**	**-**	**1**	**9**
BSAPyA5	**4**	**1**	**1**	**-**	**2**	**-**	**1**	**9**
BSAPyA6	**2**	**1**	**1**	**-**	**-**	**1**	**-**	**5**
BSAPyA7	**4**	**1**	**1**	**1**	**1**	**-**	**1**	**9**
BSAPyA8	**4**	**-**	**1**	**-**	**1**	**-**	**-**	**6**
BSAPyA9	**4**	**-**	**2**	**-**	**2**	**-**	**1**	**9**
BSAPyA10	**2**	**1**	**3**	**-**	**1**	**-**	**-**	**7**

Table 15 : Representation of overall interactions existing between SyA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSASyA1	**2**	**-**	**1**	**-**	**-**	**-**	**3**	**6**
BSASyA2	**2**	**3**	**-**	**-**	**1**	**-**	**2**	**8**
BSASyA3	**2**	**1**	**2**	**2**	**-**	**-**	**2**	**9**
BSASyA4	**1**	**2**	**1**	**-**	**-**	**-**	**2**	**6**
BSASyA5	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**2**
BSASyA6	**1**	**-**	**4**	**-**	**-**	**-**	**5**	**10**
BSASyA7	**5**	**-**	**-**	**-**	**-**	**-**	**1**	**6**
BSASyA8	**3**	**-**	**1**	**-**	**-**	**-**	**1**	**5**
BSASyA9	**3**	**-**	**-**	**-**	**1**	**-**	**-**	**4**

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BSASyA10	**3**	**-**	**1**	**-**	**-**	**-**	**4**	**8**

Table 16: Representation of overall interactions existing between VA and BSA

CONFORMER	(cHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAVA1	**1**	**2**	**-**	**-**	**-**	**-**	**1**	**4**
BSAVA2	**3**	**-**	**-**	**-**	**1**	**-**	**-**	**4**
BSAVA3	**2**	**-**	**1**	**-**	**-**	**-**	**1**	**4**
BSAVA4	**2**	**-**	**1**	**-**	**-**	**-**	**1**	**4**
BSAVA5	**1**	**1**	**1**	**-**	**-**	**-**	**2**	**5**
BSAVA6	**3**	**1**	**1**	**-**	**1**	**-**	**-**	**6**
BSAVA7	**3**	**2**	**1**	**-**	**1**	**-**	**1**	**8**
BSAVA8	**2**	**2**	**-**	**-**	**1**	**-**	**1**	**6**
BSAVA9	**2**	**1**	**1**	**-**	**-**	**-**	**1**	**5**
BSAVA10	**1**	**-**	**1**	**-**	**-**	**-**	**2**	**4**

Table 17: Representation of overall interactions existing between GA and BSA

CONFORMER	(CHB)	(NcHB)	Pi-Alkyl	Pi-Sigma	Pi-Cation	Pi-Anion	Other Interactions (weak/ unfavourable)	Total Number of molecular Interactions
BSAGA1	**2**	**-**	**1**	**-**	**-**	**-**	**1**	**4**
BSA GA2	**4**	**-**	**2**	**2**	**1**	**-**	**1**	**10**
BSAGA3	**3**	**1**	**1**	**-**	**-**	**-**	**2**	**7**
BSAGA4	**5**	**-**	**2**	**2**	**1**	**-**	**-**	**10**
BSAGA5	**5**	**-**	**2**	**2**	**1**	**-**	**-**	**10**
BSAGA6	**3**	**1**	**1**	**-**	**-**	**-**	**1**	**6**
BSAGA7	**3**	**1**	**1**	**-**	**-**	**-**	**2**	**7**
BSAGA8	**4**	**-**	**2**	**2**	**1**	**-**	**-**	**9**
BSAGA9	**5**	**-**	**2**	**2**	**-**	**-**	**-**	**9**

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BSAGA10	**3**	**-**	**1**	**-**	**-**	**-**	**1**	**5**

Table 18: Confinement of conformers of GeA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in HSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in HSA
BSAGeA1	**IIA IIB**	**I**	**IIIA**	**II**	-	-
BSAGeA2	**IIB**	**1**	**IIIA**	**II**	-	-
BSAGeA3	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSAGeA4	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSAGeA5	**IIA**	**I**	**IIA**	**I**	-	-
BSAGeA6	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSAGeA7	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSAGeA8	**IIIA IIIB**	**II**	**IIIB**	**II**	-	-
BSAGeA9	**IIIA**	**II**	**IIIA**	**II**	-	-
BSAGeA10	**IIA IIB**	**I**	**IIA**	**1**	-	-

Table 19: Confinement of conformers of PrA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in HSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in HAS
BSAPrA1	**IIIA IIIB**	**II**	-	-	-	-
BSAPrA2	**IIIA**	**II**	**IIIA**	**II**	-	-
BSAPrA3	**IB IIA IIB IIIA**	**I II III**	**IIA IIIA**	**I II**	-	-
BSAPrA4	**IIIA**	**II**	**IIIA**	**II**	-	-
BSAPrA5	**IIIA**	**II**	-	-	-	-
BSAPrA6	**IIIA IIIB**	**II**	-	-	**IIIA IIIB**	**II**
BSAPrA7	**IIA IIB**	**I**	**IIA**	**I**	-	-
BSAPrA8	**IIIB**	**II**	**IIIB**	**II**	-	-

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BSAPrA9	**IIIA**	**II**	**IIIB**	**II**	-	-
BSAPrA10	**IIA IIB**	**I**	**IIA**	**I**	-	-

Table 20: Confinement of conformers of PyA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in HSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in HAS
BSAPyA1	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSAPyA2	**IB**	**III**	**IIIA**	**II**	**IB**	**III**
BSAPyA3	**IIIA**	**II**	**IB IIIA**	**II III**	**IB**	**III**
BSAPyA4	**IB**	**III**	**IIIA**	**II**	**IIIA**	**II**
BSAPyA5	**IB IIIA**	**II III**	**IB IIIA**	**II III**	-	-
BSAPyA6	**IB IIIA**	**II III**	**IB**	**III**	-	-
BSAPyA7	**IB IIIA**	**II III**	**IIIA**	**II**	**IIIA**	**II**
BSAPyA8	**IB**	**III**	**IB IIIA**	**II III**	-	-
BSAPyA9	**IB IIIA**	**II III**	**IB IIIA**	**II III**	**IB**	**III**
BSAPyA10	**IB IIIA**	**II III**	**IB IIIA**	**II III**	-	-

Table 21: Confinement of conformers of SyA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in HSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in HSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in HSA
BSASyA1	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSASyA2	**IIA,IIB,IIIA**	**I,II**	**IIA**	**I**	**IIA,IIB,IIIA**	**I,II**
BSASyA3	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSASyA4	**IIIA,IIB**	**II**	**IIIA**	**II**	**IIIA,IIIB**	**II**
BSASyA5	**IIA**	**I**	**-**	**-**	**-**	**-**
BSASyA6	**IIIA**	**II**	**IIA,IIB,IIIA**	**I,II**	**IIA,IIB,IIIA**	**I,II**
BSASyA7	**IIA**	**I**	**-**	**-**	**IIA**	**I**

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BSASyA8	**IIIA,IIIB**	**II**	**IIIB**	**II**	**IIIB**	**II**
BSASyA9	**IIA**	**I**	**IIA**	**I**	-	-
BSASyA10	**IB**	**III**	**IB**	**III**	**IB**	**III**

Table 22: Confinement of conformers of VA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in BSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in BSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in BSA
BSAVA1	**IIIB**	**II**	**IIIB**	**II**	**IIIA**	**II**
BSAVA2	**IIA,IB**	**I,III**	**IIA**	**I**	-	-
BSAVA3	**IIIB**	**II**	**IIIB**	**II**	**IIIA**	**II**
BSAVA4	**IB**	**III**	**IB**	**III**	**IB**	**III**
BSAVA5	**IIIA,IIIB**	**II**	**IIIB**	**II**	**IIIA,IIIB**	**II**
BSAVA6	**IIA**	**I**	**IIA,IIB**	**I**	-	-
BSAVA7	**IIA**	**I**	**IIA,IIB**	**I**	**IIB**	**I**
BSAVA8	**IIA,IIB**	**I**	**IIA**	**I**	**IIIA**	**II**
BSAVA9	**IIIA**	**II**	**IIIA**	**II**	**IIIB**	**II**
BSAVA10	**IIIB**	**II**	**IIIB**	**II**	**IIIA**	**II**

Table 23: Confinement of conformers of GA in various domains of BSA

Conformer	cHB and NcHB interactions confined to subdomains of BSA	cHB and NcHB interactions Sites in BSA	Hydrophobic interactions confined to subdomains of BSA	Hydrophobic interactions sites in BSA	Unfavourable interactions confined to subdomains of BSA	Unfavourable interactions Sites in BSA
BSAGA1	**IB**	**III**	**IB**	**III**	-	-
BSA GA2	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA3	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA4	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA5	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA6	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-

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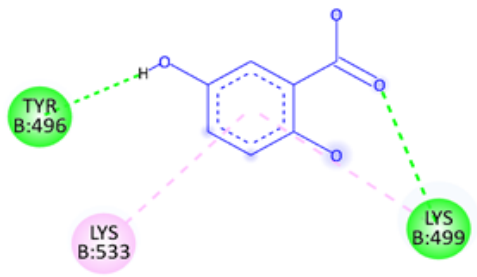
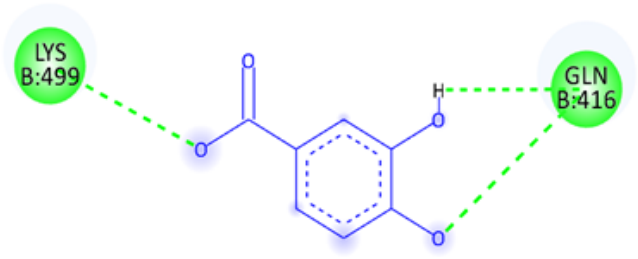
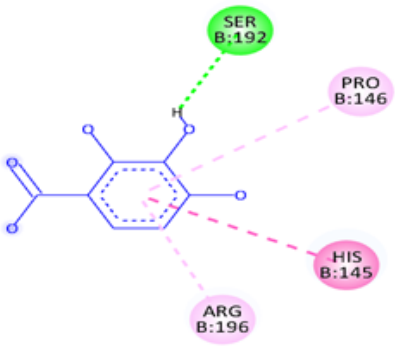
BSAGA7	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA8	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA9	**IB,IIIA**	**III,II**	**IB,IIIA**	**III,II**	-	-
BSAGA10	**IB**	**III**	**IB**	**III**	-	-



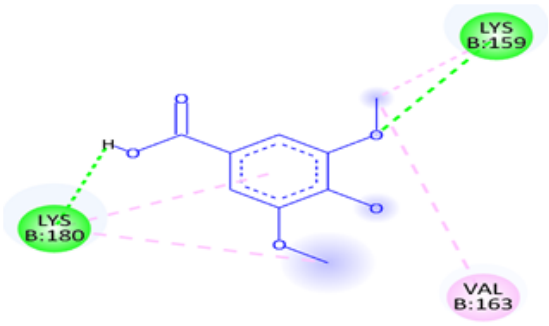
BSAGyA1



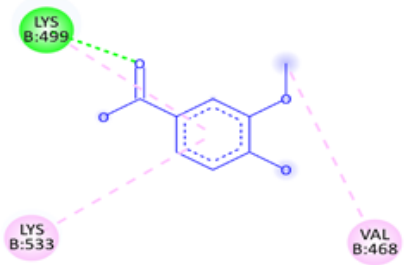
BSAPrA1



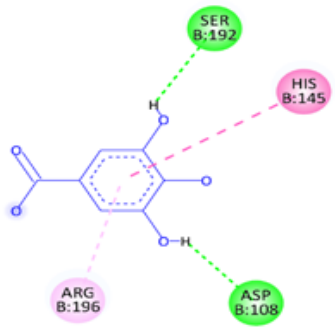
BSAPyA1



BSASyA1



BSAVA1



BSAGA1

Figure 9: The 2D diagram of **BSAGyA1, BSAPrA1, BSAPyA1, BSASyA1, BSAVA1,** visualized using Biovia Discovery Studio visualizer. Green colour dotted line indicates hydrogen bonds with electronegative elements like N and O atoms, light green colour dotted line indicates carbon-hydrogen bonds; light purple

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colour indicates pi-alkyl interactions, violet colour dotted line indicates – pi-sigma interaction. Light green colour amino acids without interactions represent van der Waals interactions and red colour interaction represents unfavourable interaction. The blue halo surrounding the interacting residues represents the solvent accessible surface that is proportional to its diameter.

The variation in the nature of amino acids involved in cHB, NcHB and hydrophobic interactions of each DBA varies when it forms complex with BSA **(Figure 9**) as provided for DCAs **(Figure 5).** Apart from conformer1 of each DBAs namely BSAGA1, BSAGeA1, BSAPrA1 and BSAPyA1, BSASyA1 and BSAVA1 all other unique conformers of all the DBAs with BSA are provided in **supporting information figures S5-S10.** The figures corresponding to unique conformers clearly illustrates the amino acids that are involved belong to different sub domains corresponding to different binding sites. **Figures S5, S6, S7, S8, S9** and **S10** represents the unique conformers of FA-BSA, SA-BSA, CA-BSA and CoA-BSA complexes respectively wherein the role of polar and non-polar amino acids that are involved in binding provides information of the sequence site of ligand attachment with the protein molecule.

Table 23: Amino acids involved in HB and hydrophobic interactions

DBA	Total Conformers	Conformers with cHB/NcHB interactions	Amino acids involved in cHB/NcHB interactions	Conformers with Hydrophobic interactions	Amino acids involved in Hydrophobic interactions	Molecular interaction Influence on the complex
GA	**10**	**10**	ARG, ASP, ASN, LEU, SER and TYR	**10**	ARG, ALA and HIS	Polar amino acids governs the binding stability
GeA	**10**	**10**	ARG, ASP, GLN, LEU, LYS, PRO, TYR and VAL	**10**	ARG, LEU, LYS, and VAL	Polar amino acids governs the binding stability
PrA	**10**	**10**	ARG, ASP, GLN, LEU, LYS, PRO, TYR and VAL	**8**	ARG, LEU, LYS, and TRP	Polar amino acids governs the binding stability
PyA	**10**	**10**	ASN, ILE, LEU, SER, THR and TYR	**10**	ALA, ARG, ASP and LYS	Polar amino acids governs the binding stability
SyA	**10**	**10**	ARG, ASP, GLN, GLU, LYS, TYR and VAL	**9**	ALA, LEU, LYS, ILE, PRO, TYR and VAL	Polar amino acids involved in HB and non-polar involved in hydrophobic interactions
VA	**10**	**10**	ARG, GLN, LEU, LYS and VAL	**10**	ALA, ARG,PRO, TYR and VAL	Polar amino acids involved in HB and non-polar involved in hydrophobic interactions

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Based on the energetics and molecular interaction studies, a comparison on the binding nature and stability of DCAs-BSA versus DBAs-BSA are summarised as follows

i) The phenolic acids of cinnmaic acid derivatives (except FA) possess a stronger binding affinity towards BSA than all other phenolic acids of benzoic acid derivatives
ii) HB interaction predominates over hydrophobic interactions in several acids with BSA, but the binding stability of the complex is hindered due to the presence of multiple interactions.
iii) The presence of three –OH groups in the phenyl ring (PyA) results in a lesser binding energy compared to that of acids containing one and two –OH moieties of CA and CoA respectively.
iv) Among all the acids taken in the present study, PyA possesses the lowest binding energy and affinity towards BSA and this is presumably attributed to the docking of the PyA to all the binding sites and domains which includes the less favoured drug binding site (Site III). PyA resides in non-favourable binding site III compared to that of drug favourable binding site I and II when compared to other DBAs and DCAs.
v) Although SA and SyA are structurally similar based on their substitution in the phenyl ring, they differ by one vinylic linkage attached to the carboxylic acid moiety in SA as compared to that of SyA (**Figure 1**). In general a benzoic acid derivative (substituent directly attached to the phenyl ring) (SyA) possesses a lesser negative ΔG **(-4.13 kcal M^{-1})** value than cinnamic acid derivative (SA) **(-4.77 $kcalM^{-1}$)** when it forms a complex with BSA.
vi) An unusual pattern was observed in the case of FA Vs VA where FA which is a cinnamic acid derivative has a lesser - ΔG value **(-4.45 $kcalM^{-1}$)** than VA (benzoic acid derivative) **(-4.81 $kcalM^{-1}$)** even though both FA and VA has a similar position of methoxy and hydroxyl group in the phenyl ring **(Figure 1)**.
vii) The BE for the formation of CA-BSA complex in comparison to CoA-BSA is almost similar to that of GeA-BSA with VA-BSA. This clearly reveals that the presence of OH or COOH functional groups in the phenolic acids predominantly involve HB interactions either through its donor or acceptor site with the amino acids of BSA resulting in the stabilisation of the complex.
viii) The position of –OH group with respect to that of –COOH group influences the binding stability. This was evident in the case of GA and GeA upon complex formation with BSA, wherein GA has –OH groups in 3rd, 4th and 5th position of benzoic acid has a BE of **-4.54 $kcalM^{-1}$** than Pyrogallic acid (PyA) (**-3.55 $kcalM^{-1}$**)) which has –OH groups in 2nd, 3rd and 4th positions.
ix) GeA and PrA behave as site selective and site specific ligand such that it prefers Site II only.
x) SyA and PyA prefer to dock in all the three binding sites of BSA which was not observed in the case of DCAs.
xi) HIS and TRP are the two amino acids that are not involved in any type of molecular interaction with the acid derivatives.
xii) Polar amino acids involve both in HB and hydrophobic interactions resulting in stability of the complex than non-polar amino acids.

Role of OH, OCH_3 and COOH groups substituents in phenyl ring

Studies pertaining to ligand binding studies of phenolic acids interaction with proteins elucidate that both non-covalent and covalent phenol-protein interactions largely influence the protein structure, and in turn alters the naturing/denaturing properties regarding protein functionality. Two mechanisms were postulated based on protein studies with phenolic moieties wherein one mechanism signifies that bound phenols influence the topological surface of the protein hydrophobicity and other mechanism describes that the introduction of hydrophilic groups from the phenol promotes a layer of coating of hydrophobic residues on the protein surface. **[53,54]** Additionally, phenol interaction could alter protein structure, and thereby exposing hydrophobic residues. A decrease of protein surface hydrophobicity was evident in the case of whey proteins upon interaction with phenols **[55,56]**.The alteration of protein structure is another major change in proteins upon phenol binding, which was also confirmed for milk proteins, as non-covalent interactions with phenols resulted in an increase of unordered secondary protein structures. In the earlier studies, the role of –OH groups attached to phenyl ring

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has more influence on the binding interaction with BSA-ligand complexes. In other words, they are stabilizing the protein-ligand complex.

On the contrary, in our present study the presence –OH groups attached to phenyl ring is not found to stabilize the protein-ligand complex, whereas the presence of both –COOH and $–OCH_3$ in addition to –OH group stabilizes the protein-ligand complex. Furthermore, it was also observed that a greater number of –OH groups attached to phenyl ring compared to $–OCH_3$ group destabilize the protein-ligand complex which was clearly visualized in PyA and PrA. Through docking approach a new set of factors influencing the binding pattern with biomolecules is imparted in our studies such that the role of multiple functional groups obtained from the extracts from polyphenols, flavanoids, alkaloids and carotenoids interaction with globular proteins provide an easier and efficient route in determining the stability as well as the binding pattern. An overall view on the interaction parameters regarding energetics has been performed with HSA. (**unpublished work**), wherein the mode of binding pattern and relative stability of phenolic derivatives of various acids with HSA is found to be entirely different from that of BSA even though both these proteins are similar to each other.

Conclusion

Molecular docking, though a simple and efficient and tool in ascertaining the interaction of host-guest complexes, it is more reliable in ascertaining the multiple forces that exist in ligand-protein systems. The energetics and the molecular interactions existing between various phenolic derivatives of benzoic acid and cinnamic acid with BSA provide substantial information regarding the stable binding sites and sub domains. Though, all the derivatives differ by either –OH or $–OCH_3$ group susbstitution, the binding stability of various naturally occurring phenolic derivatives of cinnamic acids are found to be energetically more stable than the benzoic acids. We ascertain that the presence of –COOH and $–OCH_3$ groups attached to phenyl ring stabilizes the protein-ligand complex than -OH groups attached to phenyl ring. The competitive influences through hydrogen-bonding accompanied by several hydrophobic interactions resulting from various amino acids results in large variation in the energetics and stability of the host-guest system that are ascertained accurately which is the most significant factor through in silico studies.

Acknowledgments: The authors from D.G.Vaishnav College thank the Principal, Dr. S. Santhosh Baboo, and the Secretary, Dr. Ashok Kumar Mundra, D.G. Vaishnav College (Autonomous), Chennai, for permitting to avail the laboratory facilities. R.K and M.V thank Mrs. Shoba Gunasekaran, Department of Bio-Technology, D.G. Vaishnav College (Autonomous), for aiding the students in learning the molecular docking techniques. The KSU authors acknowledge the financial support from Researchers Supporting Project number (RSP2023R368), King Saud University, Riyadh, Saudi Arabia.

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