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Spectrophotometric Analysis of Bovine Serum Albumin In Presence of 1-(4-Bromophenyl)-3-Phenylprop-2-En-1-Ones

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ABSTRACT

A series of chalcones was synthesized by the Claisen-Schmidt condensation and the structures of 1-(4-bromophenyl)-3-phenylprop-2-en-1-ones were established with the help of IR and NMR study, then their effect was observed on bovine serum albumin. We have found that the synthesized chalcones interacted with bovine serum albumin irrespective of position and nature of substituent. 1-(4-bromophenyl)-3-(3-methoxyphenyl)-prop-2-en-1-one has been found to interact with BSA maximally.

Keywords: Bovine serum albumin, interaction studies, chalcones of *p*-bromoacetophenone.

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INTRODUCTION

Chalcones are compounds from either natural sources or synthetic pathways. The literature describes them as having a great number of biologic activities, being used as a template for the synthesis of more potent and selective analogues to obtain new therapeutic agents[1–6]. Structurally, they are α,β-unsaturated aromatic ketones with an essentially planar core due to the presence of carbon atoms with sp^2 hybridization. However, the substituent's attached to aromatic rings of these compounds are usually responsible for important structural changes that lead to a greater or lesser biological response against specific therapeutic targets[7–9].

Serum albumins are major transport proteins[10,11] found in the blood plasma and are capable of binding to various biologically relevant substances like lysolecithin, bilirubin, bile salts, certain metals, hormones and a variety of therapeutic drugs1[12-14]. As a model protein a large volume of research work around protein BSA is already established in diversified directions. The primary structure of BSA is a sequence of 583 amino acid residues where the secondary. structure contains 67% alpha helix with six turns and 17 disulphide linkages[12,15–17]. The tertiary structure is formed by three homologous domains I→III, each of which is divided into two sub-domains A and B[12,18]. BSA has two tryptophan residues Trp-134 and Trp213 in the IB and IIB sub-domains[19,20] respectively. This protein has two most widely reported principle drug binding sites, site-I and site-II. Site-I is present in the hydrophobic core of sub-domain IIA, whereas site-II is within the IIIA sub-domain[21]. Although the crystal structure of human serum albumin (HSA) is well known, the exact crystal structure of BSA is unknown till date. The pair wise sequence alignment has only one gap over all the residues of the BSA sequence with 75% identity and 87% similarity shared between human and the bovine sequence[22]. Various researchers have studied the structure and properties of serum albumins and their interactions with other proteins and with denaturants such as guanidine hydrochloride, urea and surfactants[23–25] using NMR[26] , dynamic light scattering[27], differential scanning calorimetry[28], circular dichroism.

We have reported the interaction of some series of chalcones with BSA. In continuation of our previous work, with 1-(5'-chloro-2'-hydroxyphenyl)-3-(4"-substituted phenyl)-prop-2-en-1-one and their methoxy derivatives[29], 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one[30], 1-(2'-furyl)-3-(substituted phenyl)-prop-2-en-1-one[31], 1-(2'-thienyl)-3-(substituted phenyl)-prop-2-en-1-one[32], 1-(4-hydroxyphenyl)-3-(substituted phenyl)-2-propen-1-ones and 1-(4-nitrophenyl)-3-(substituted phenyl)-2-propen-1-ones[33], 1-biphenyl-3-(substituted phenyl)-2-propen-1-ones[34], bischalcones[35],1-(4-methylphenyl)-3-phenylprop-2-en-1-ones[36], 3-phenyl-1-(pyridin-2-yl)prop-

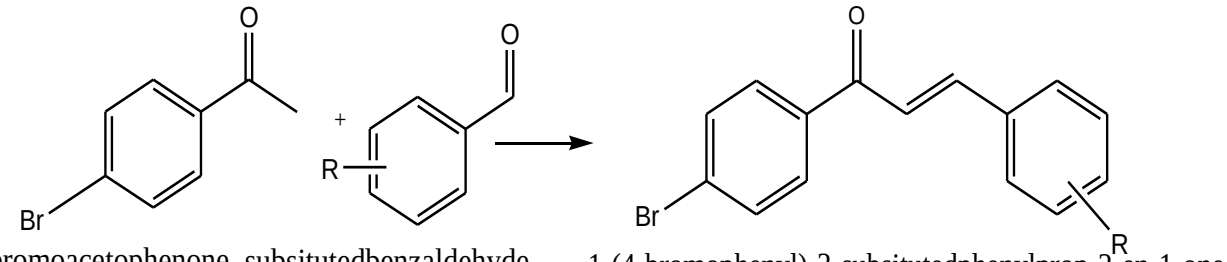
2-en-1-ones[37], 1-(4-(benzylideneamino)phenyl)-3-phenylprop-2-en-1-ones[38] and 1-(naphthalen-3-yl)-3-phenylprop-2-en-1-ones[39] with bovine serum albumin, we here report the interaction of bovine serum albumin with 1-(4-bromophenyl)-3-phenylprop-2-en-1-ones. This protein is involved in the transportation of a number of compounds including drugs. It is also reported that there is about 80% primary sequence identity between bovine serum albumin and human serum albumin[40]. It is also suggested that the present study performed with BSA can give an insight about the interaction of chalcones with human serum albumin and therefore can also help in understanding the mode of transport of molecules like chalcones of immense biological activities having α,β-unsaturated moiety in situ.

MATERIALS AND METHOD

The reaction progress and purity of products were monitored by thin layer chromatography. Thin layer chromatography was performed with silica-gel G (suspended in $CHCI_3$-EtOH) and plates were viewed under Iodine vapors. Melting points were determined by electrochemical capillary Melting points apparatus and are uncorrected. Elisa plate reader, Systronic make was used for measuring absorbance in the visible range. The Lab-India made Spectrofuge (model 16M) was used for centrifugation purpose.

Synthesis of Chalcones-

A series of chalcones 1-(4-bromophenyl)-3-phenylprop-2-en-1-one was synthesized by the grinding of substituted benzaldehyde (0.01 mole) with 4-bromoacetophenone (0.01 mole) in presence of potassium hydroxide (0.01 mole) respectively with a mortar and pestle. The progress of reaction and the purity of the products were confirmed through TLC. The structures were confirmed by their IR and ^{1}HNMR spectra.



4-bromoacetophenone subsitutedbenzaldehyde 1-(4-bromophenyl)-3-subsitutedphenylprop-2-en-1-one

Reaction of chalcones with Bovine Serum Albumin-

To 10 ml solution of 0.1mM BSA, 1ml solution of 50 mM chalcone solution was added drop wise with constant stirring. After interaction between chalcone and BSA, some albumin gets precipitated. The remaining protein in solution was estimated by biuret method[41]. The results are presented in figure1.

RESULTS AND DISCUSSION

A series 1-(4-bromophenyl)-3-phenylprop-2-en-1-one was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 4-aminoacetophenone. Their IR and ^{1}HNMR data are reported in Table1 & 2.

Table 1: IR Data [υ max (cm^{-1})] of Chalcones (BrC_6H_4-CO-CH=CH-C_6H_4R)

Comp No	R	=O]	[C=C]	[CH]	[O-N-Osym]	[O-N-O asym]
1	*H*	1650	1590	3069	-	-
2	*o*-Cl	1652	1590	3087	-	-
3	*m*-Cl	1655	1592	2967	-	-
4	*p*-Cl	1652	1592	2918	-	-
5	*o*-OMe	1650	1598	3137	-	-
6	*m*-OMe	1652	1605	2869	-	-
7	*p*-OMe	1655	1603	2872	-	-
8	*o*-NO2	1653	1605	2877	1340	1525
9	*m*-NO2	1653	1606	2877	1344	1526
10	*p*-NO2	1650	1601	2812	1335	1529

Table 2: ^{1}HNMR (δ ppm) Data obtained for Chalcones (BrC_6H_4-CO-CH=CH-C_6H_4R)

Comp No	R	H-2	H-3	J2-3 (Hz) -	Ar-H	3H,-OCH3
1	H	6.911 d)	7.821(d)	15.1	7.131-8.123(m)	-
2	*o*-Cl	7.330 (d)	8.345(d)	15.6	7.237-8.435(m)	-
3	*m*-Cl	7.480 (d)	7.671(d)	15.7	7.111-8.523(m)	-
4	*p*-Cl	7.486(d)	7.834 (d)	15.9	7.567-8.346(m)	-
5	*o*-OCH_3	7.413(d)	7.823 (d)	15.3	7.191-8.123(m)	3.821
6	*m*-OCH_3	7.602 (d)	8.178 (d)	15.5	7.671-8.566(m)	3.232
7	*p*-OCH_3	7.643 (d)	8.126(d)	15.5	7.187-8.276(m)	3.211
8	*o*-NO_2	7.754(d)	7.514 (d)	15.1	7.198-8.365(m)	-
9	*m*-NO_2	7.543(d)	7.667 (d)	15.1	7.155-8.321(m)	-
10	*p*-NO_2	6.621 (d)	7.579(d)	15.3	7.144-8.459(m)	-

The biological activities exhibited by chalcones and their potential to be used as synthones for the synthesis of large number of heterocyclic compounds have made our interest in the synthesis of a large number of substituted chalcones. The most widely used method used for the synthesis of chalcones involves Claisen-Schmidt condensation of substituted arylaldehyde with the arylmethyl ketones with the help of mortar and pestle by solvent free synthesis. In the present work we report solvent free synthesis of a series of chalcones i.e. 1-(4-bromophenyl)-3-phenylprop-2-en-1-ones by the reaction of substituted benzaldehydes with 4-bromoacetophenone and in the presence of a base.

The synthesis of different chalcones was established by their spectral data. In the IR spectra of chalcones (1-10) as mentioned in table 1, the peak at 1650 – 1660 cm-^{1} represent >C=O stretching vibrations which indicate the presence of carbonyl group in conjugation with highly

unsaturated system and the results suggests the presence of α, β – unsaturated carbonyl group in the synthesized compounds. The synthesis of chalcones is characterized by the presence of two doublets around δ 7.6 - 6.6 and δ 8.2 - 7.5. These represents C-2 and C-3 protons and the geometry across the double bond has been found out to be trans as doublets with coupling constant $J_{2,3}$ is ~ 15.9 - 15.0 Hz. The aryl and other protons were revealed at their respective position. After establishing the structures of 1-(4-bromophenyl)-3-phenylprop-2-en-1-ones, their effect were observed on BSA in solution.

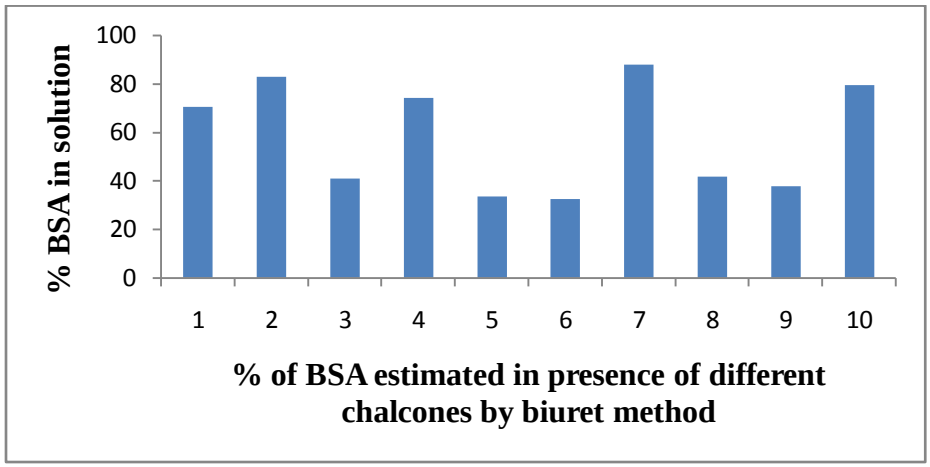


Figure 1. The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

We have earlier reported spectrophotometric analysis of BSA in presence of different series of chalcones[29-39]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1-(4-bromophenyl)-3-phenylprop-2-en-1-ones (Figure 1). The chalcones possess α, β-unsaturated ketone moiety and are therefore highly reactive. The moiety reacts with most nucleophilic group available and therefore has been used as synthons for the synthesis of different types of heterocycles[41]. In proteins also, a number of side chain groups such as thiol, amino, imidazole, alcohol etc. are available. Any of these side chain containing nucleophilic groups can react with α, β-unsaturated ketone group. We propose that nucleophilic groups of BSA react with α, β-unsaturated group in an effective manner. The results suggest that 1-(4-bromophenyl)-3-(3-methoxyphenyl)-prop-2-en-1-one is most reactive chalcone as it decreased the availability of BSA in solution to maximum extent. The resulting interactions may cause a change in the three dimensional structure of albumin under study and finally resulting its precipitation out of solution.

In Table 3, ^{1}HNMR ($CDCl_3$) data of different chalcones are presented. It was observed that C-2 and C-3 protons resonated as doublets with coupling constant ~ 15 Hz. The stereochemistry across C-2, C-3 double bond is Trans. The other protons were revealed at their respective position.

Table 3: Experimental Analysis of Synthesized Chalcones (BrC_6H_4-CO-CH=CH-C_6H_4R)

Comp no	R-	% of BSA left in solution after interaction with chalcones
1	H	70.41
2	*o*-Cl	82.98
3	*m*-Cl	40.87
4	*p*-Cl	74.21
5	*o*-OCH_3	33.56
6	*m*-OCH_3	32.41
7	*p*-OCH_3	87.76
8	*o*-NO_2	41.76
9	*m*-NO_2	37.81
10	*p*-NO_2	79.32

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