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

S. Garg and N. Raghav*

Department of Chemistry, Kurukshetra University, Kurukshetra-136119 (India)

Abstract: We have synthesized a series of 1-(4-fluorophenyl)-3-phenylprop-2-en-1-ones by the Claisen-Schmidt condensation and after establishing the structures of 1-(4-fluorophenyl)-3-phenylprop-2-en-1-ones, their effect were observed on Bovine Serum Albumin in solution. We have found that the synthesized 1-(4-fluorophenyl)-3-phenylprop-2-en-1-ones interacted with bovine serum albumin irrespective of the nature and position of the substituent with a little difference.
Key words: Bovine serum albumin, interaction studies, 1-(4-fluorophenyl)-3-phenylprop-2-en-1-ones.

Introduction

Albumin is the major protein in serum and is present typically at around 50 mg/ml, where it makes up around 60% of the total protein. Approximately 60% of total body albumin is in the extravascular space, including within the interstitial space of tissues, which infers an important role in the physiological well-being of cells[1] (Ellmerer et al. 2000). However, its physiological actions and the molecular mechanisms involved are not well understood[2, 3] (Quinlan et al. 2005; Ahn et al. 2008). Despite this the main functions of albumin have been summarized to include (1) maintenance of blood oncotic pressure and pH (2) binding and transport of physiologically important ligands, including lipids, metal ions, amino acids and other factors, and (3) antioxidant functions, but mainly from the perspective of its role in the circulation. Clearly these basic functions of the albumin molecule also apply to the interaction between albumin and cells in animal tissues. It is known that the interaction between drugs and serum albumin plays an important role in the distribution and metabolism of drugs. The studies on the interaction can provide information on the therapeutic effectiveness of drugs and other information, such as the information of storage, and transportation of drugs. Generally we used BSA as a model protein, because BSA is a small protein with a single polypeptide chain containing 585 amino acid residues, which is cross-linked by 17 difusulfide bonds. BSA is made up of three linearly arranged structurally distinct, homologus domains (I–III), which were divided into nine loops (L_1–L_9) and each domain contains two sub-domains (A and B). The specific hydrophobic cavities in the sites existing on BSA are sites I and II, which are located in IIA and IIIA sub domains[4]. BSA has two tryptophan residues that possess intrinsic fluorescence[5].

The chalcones, or 1,3-diaryl-2-propene-1-ones, constitute a relatively simple but pharmacologically important class of organic compounds with reported biological activity as antifungal agents[6], antimicrobial (e.g., bacteria and protozoa) agents[7], anti-inflammatory agents[7] and potential cancer therapeutics[8]. Variants bearing methoxy and hydroxy ring substituents have in some instances been observed to display enhanced efficacy[9], possibly because of improved water solubility, improved binding ability to *in vivo* substrate(s) via hydrogen bond formation, or both. The ease with which a diverse array of chalcone derivatives can be synthesized and their usefulness for the further synthesis of other, biologically important heterocyclic compounds continue to motivate research involving their preparation and the evaluation of their properties.

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[10], 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one[11], 1-(2'-furyl)-3-(substitutedphenyl)-prop-2-en-1-one[12], 1-(2'-thienyl)-3-(substitutedphenyl)-prop-2-en-1-one[13], 1-(4-hydroxyphenyl)-3-(substitutedphenyl)-2-propen-1-ones and 1-(4-nitrophenyl)-3-(substitutedphenyl)-2-propen-1-ones[14], 1-biphenyl-3-(substituted phenyl) -2-propen-1-ones[15], bischalcones[16], 1-(4-methylphenyl)-3-phenylprop-2-en-1-ones[17] with bovine serum albumin, we here report the interaction of bovine serum albumin with 1-(4-fluorophenyl)-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[18], it is also suggested that the present study performed with BSA can give an insight about the interaction of chalcones with human serum albumin.

Experimental:

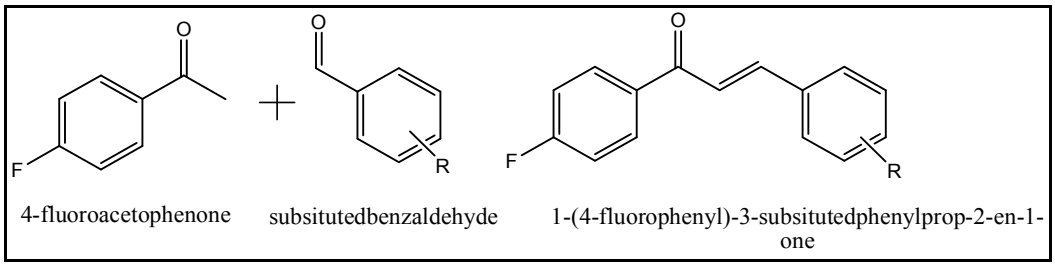
A series 1-(4-fluorophenyl)-3-phenylprop-2-en-1-ones was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 4-fluoroacetophenone. Their IR and ^{1}HNMR data are reported in Table1 & 2.

Materials and Methods

The reaction progress and purity of products were monitored by thin layer chromatography. Thin layer chromatography was performed with silica-gel G (suspended in $CHCl_3$-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-fluorophenyl)-3-phenylprop-2-en-1-ones was synthesized by the grinding of substituted benzaldehyde (0.01 mole) with 4-fluoroacetophenone (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.



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[19]. The results are presented in figure1.

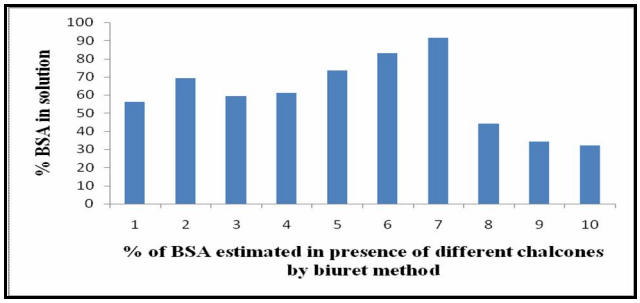


Figure 1. The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

Table 1: IR Data [υ max (cm^{-1})] of Chalcones (FC_6H_4-CO-CH=CH-C_6H_4R)

Comp No	R	[C=O]	[C=C]	[CH]	[O-N-O sym]	[O-N-O asym]
1	H	1656	1607	3059	-	-
2	*o*-Cl	1670	1605	3059	-	-
3	*m*-Cl	1672	1604	3076	-	-
4	*p*-Cl	1679	1608	3063	-	-
5	*o*-OCH_3	1681	1598	3066	-	-
6	*m*-OCH_3	1680	1598	3063	-	-
7	*p*-OCH_3	1667	1598	3064	-	-
8	*o*-NO_2	1659	1598	3016	1345	1518
9	*m*-NO_2	1656	1605	2925	1345	1518
10	*p*-NO_2	1655	1605	3076	1353	1531

Table 2: ^{1}HNMR (δ ppm) Data obtained for Chalcones (FC_6H_4-CO-CH=CH-C_6H_4R)

Comp No	R	H-2	H-3	J2-3 (Hz) -	Ar-H	3H,-OCH3
1	H	7.450 (d)	7.882 (d)	15.7	7.129-8.526(m)	-
2	*o*-Cl	7.445 (d)	7.687 (d)	15.2	7.191-8.313(m)	-
3	*m*-Cl	6.668(d)	7.197(d)	15.2	7.176-8.426(m)	-
4	*p*-Cl	7.426(d)	7.421(d)	15.7	7.196-8.426(m)	
5	*o*-OCH_3	7.202 (d)	7.751 (d)	15.8	7.191-8.323(m)	3.862 (s)
6	*m*-OCH_3	7.635(d)	7.727 (d)	15.8	7.179-8.341(m)	3.862 (s)
7	*p*-OCH_3	7.876 d)	8.221 (d)	15.8	7.116-8.356(m)	3.862 (s)
8	*o*-NO_2	7.587(d)	7.950 (d)	15.8	7.329-8.516(m)	-
9	*m*-NO_2	6.985 (d)	7.820 (d)	15.8	7.299-8.643(m)	-
10	*p*-NO_2	7.353 (d)	8.261 (d)	15.8	7.156-8.456(m)	-

In Table 2, ^{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 (FC_6H_4-CO-CH=CH-C_6H_4R)

Comp No	R-	% of BSA left in solution after Interaction with chalcones
1.	H	56.2
2.	*o*-Cl	69.29
3.	*m*-Cl	59.2
4.	*p*-Cl	61.29
5.	*o*-OCH_3	73.5
6.	*m*-OCH_3	83.2
7.	*p*-OCH_3	91.4
8.	*o*-NO_2	44.2
9.	*m*-NO_2	34.1
10.	*p*-NO_2	32.3

Results and Discussion

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-fluorophenyl)-3-phenylprop-2-en-1-ones by the reaction of substituted benzaldehydes with 4-fluoroacetophenone and in the presence of a base.

The synthesis of different chalcones was established by their spectral data. In the IR spectra of chalcones (1-11) as mentioned in table 1, the peak at 1656 – 1680 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-fluorophenyl)-3-phenylprop-2-en-1-ones, their effect were observed on BSA in solution.

We have earlier reported spetrophotometric analysis of BSA in presence of different series of chalcones[10-17]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1-(4-fluorophenyl)-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[18]. 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-fluorophenyl)-3-(4-nitrophenyl)-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.

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