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Research article

SPECTROPHOTOMETRIC ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF 1-(4-METHYLPHENYL)-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-methylphenyl)-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 and produce a great effect on their presence.
Key words: Bovine serum albumin, interaction studies, chalcones of *p*-methylacetophenone.

INTRODUCTION

Albumin is one of the most extensively utilized proteins in the medical industry and in biological research. It acts as a powerful antioxidant in cell culture. It binds, sequesters and stabilizes a variety of molecular species which are often unstable. This acidic, soluble protein has both high-affinity and secondary binding sites, optimizing the roles that fatty acids, metals, disulfides, and other molecules play in cellular metabolism.

Chalcones (1,3-diphenyl-2-propen-1-ones) have been a subject of great interest for chemists and biochemists all over due to their ease of synthesis, vast and interesting pharmacological activities. These are one of the major classes of natural products with widespread distribution in spices, tea, beer, fruits and vegetables. Chalcones also act as intermediate compounds for various heterocyclic compounds (Dhar, 1981).

Chalcones are abundantly present in nature starting from ferns to higher plants (Mark, et al., 1991). Chalcones and their derivatives have been reported to exhibit a wide variety of pharmacological effects including antimalarial (Narender et al., 2011, Dominguez, et al., 2001, Kenyon, et al., 1995, Liu, et al., 2001), antiplatelet (Zhuo, et al., 2005), antiviral (Cheenpracha et al., 2006, Wu, et al., 2003, Wood, et al., 1999), immunomodulatory (Barford, et al., 2002), antiangeogenic (Boumendjel, et al., 2009), antiparasitic (Nielsen, et al., 1995). These compounds have been found to be inhibitors of chemical mediators release(Ko, et al., 2003), leukotriene B4(Deshpande, et al., 1999), tyrosinase kinase (Khatib, et al., 2005), aldose reductase (Severi, et al., 1998), epoxide hydrolase (Morisseau, et al., 1998), xanthine oxidase (Khobragade, et al., 2008) and quinone reductase (Miranda, et al., 2000). The effect of chalcones has also been studied on acid (Raghav, et al., 2010) and alkaline phosphatase (Raghav, et al., 2012). The molecules which interfere with the metabolic system of the host will lead to alteration in metabolic processes and will certainly be having some side effects.

We have reported the interaction of some 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(Raghav, et al., 2009), 1-phenyl-3-(substitutedphenyl)-prop-2-en-1-one (Raghav, et al., 2011a), 1-(2'-furyl)-3-substitutedphenyl)-prop-2-en-1-one (Raghav, et al., 2011b),1- (2'-thienyl) -3- (substitutedphenyl)- prop-2-en-1-one (Raghav, et al., 2011c), 1-(4-hydroxyphenyl)-3- (substitutedphenyl) -2- propen-1-ones and 1- (4-nitrophenyl) -3-(substitutedphenyl) -2-propen-1-ones(Raghav, et al., 2012a), 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones(Raghav, et al., 2012b), bischalcones (Raghav, et al., 2013) with bovine serum albumin, we here report the interaction of bovine serum albumin with 1-(4-methylphenyl)-3-(substitutedphenyl)-2-propen-1-ones. It is reported that there is about 80% primary sequence identity between bovine serum albumin and human serum albumin (Peters, 1985), it is also suggested that the present study performed with BSA can give an insight about the interaction of chalcones with human serum albumin.

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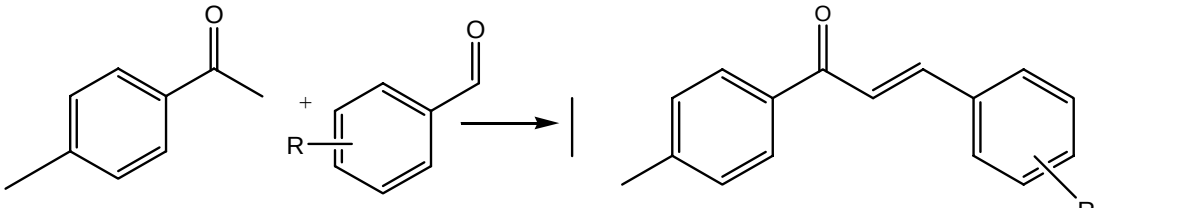
MATERIALS AND METHODS

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-methylphenyl)-3-phenylprop-2-en-1-one was synthesized by the grinding of substituted benzaldehyde (0.01 mole) with 4-methylacetophenone (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-methylacetophenone subsitutedbenzaldehyde 1-(4-methylphenyl)-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 (Gornall, 1949). The results are presented in figure1.

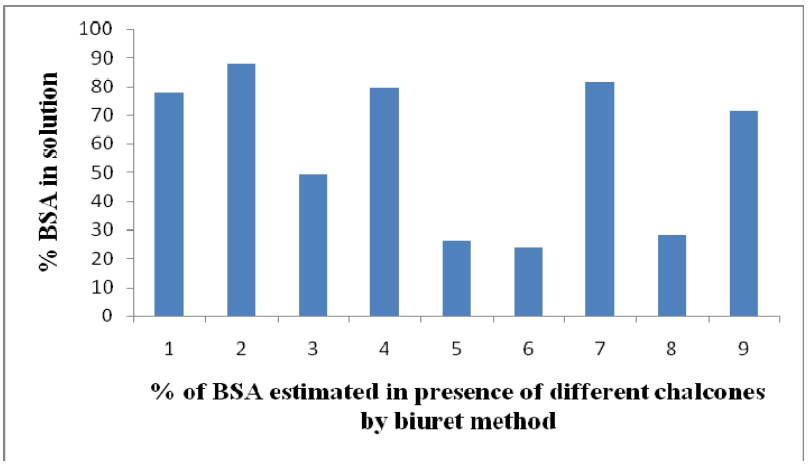


Figure 1. The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

EXPERIMENTAL:

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

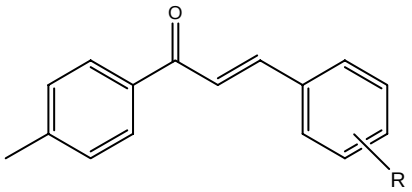


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Table 1: IR Data [υ max (cm^{-1})] of Chalcones (C_7H_7-CO-CH=CH-C_6H_4R)

Comp No	R	[C=O]	[C=C]	[CH]	[O-N-Osym]	[O-N-O asym]
1	*H*	1657	1598	3078	1342	1528
2	*o*-Cl	1657	1599	3095	-	-
3	*m*-Cl	1657	1599	2985	-	-
4	*p*-Cl	1657	1596	2923	-	-
5	*o*-OMe	1657	1596	3155	-	-
6	*m*-OMe	1657	1603	2865	-	-
7	*p*-OMe	1657	1603	2865	-	-
8	*m*-NO2	1657	1603	2876	1340	1526
9	*p*-NO2	1657	1603	2812	1335	1529

Table 2: ^{1}HNMR (δ ppm) Data obtained for Chalcones (C_7H_7-CO-CH=CH-C_6H_4R)

Comp No	R	H-2	H-3	J2-3 (Hz)	Ar-H	3H,-OCH3
1	H	6.965 (d)	7.850 (d)	15.5	7.199-8.343(m)	-
2	*o*-Cl	7.357 (d)	8.061 (d)	15.7	7.156-8.456(m)	-
3	*m*-Cl	7.450 (d)	7.882 (d)	15.7	7.129-8.526(m)	-
4	*p*-Cl	7.450 (d)	7.882 (d)	15.7	7.129-8.526(m)	-
5	*o*-OCH_3	7.439 (d)	7.841 (d)	15.8	7.156-8.456(m)	3.824
6	*m*-OCH_3	7.412 (d)	8.101 (d)	15.8	7.129-8.526(m)	3.932
7	*p*-OCH_3	7.548 (d)	8.029 (d)	15.6	7.118-8.299(m)	3.861
8	*m*-NO_2	7.397 (d)	7.685 (d)	15.3	7.199-8.343(m)	-
9	*p*-NO_2	6.671 (d)	7.546 (d)	15.3	7.156-8.456(m)	-

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 (C_7H_7-CO-CH=CH-C_6H_4R)

Comp No	R-	% of BSA left in solution after interaction with chalcones
1.	H	77.91
2.	*o*-Cl	87.81
3.	*m*-Cl	49.33
4.	*p*-Cl	79.54
5.	*o*-OCH_3	26.28
6.	*m*-OCH_3	23.62
7.	*p*-OCH_3	81.5
8.	*m*-NO_2	28.42
9.	*p*-NO_2	71.41

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RESULTS AND DISCUSSION

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 the synthesis of one series i.e. (4-methylphenyl)-3-phenylprop-2-en-1-one by the reaction of substituted benzaldehydes with 4-methylacetophenone and in the presence of a base. The synthesis of different chalcones was established by their spectral data. In the IR spectra of chalcones (1-9) as mentioned in table 1, the peak at 1651 – 1659 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 7.4 - 6.7 and δ 8.1 - 7.4. 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 ~ 15.7 - 15.0 Hz. The aryl and other protons were revealed at their respective position. After establishing the structures of 1-(4-methylphenyl)-3-phenylprop-2-en-1-one their effect was observed on BSA in solution.

We have earlier reported spetrophotometric analysis of BSA in presence of different series of chalcones (Raghav, et al., 2009, Raghav, et al., 2011a, Raghav, et al., 2011b, Raghav, et al., 2011c, Raghav, et al., 2012a, Raghav, et al., 2012b, Raghav, et al., 2013). In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1-(4-methylphenyl)-3-phenylprop-2-en-1-one (Figure 1). The chalcones possess α, β-unsaturated ketone moiety and are therefore highly reactive. The moiety C2-C3 double bond is most nucleophilic group available and therefore has been used as a tool for the synthesis of large number of heterocycle compound (Dhar, 1981). In proteins also, a number of side chain groups such as thiol, amino, imidazole, alcohol etc. are available. Any of these nucleophilic groups can react with C2-C3 double bond of chalcones. We propose that nucleophilic groups of BSA react with α, β-unsaturated group in an effective manner. The results suggest that 1-(4-methylphenyl)-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.

CONCLUSION

To conclude, we have synthesized a series i.e. 1-(4-methylphenyl)-3-phenylprop-2-en-1-one; by Claisen-Schmidt condensation successfully and has been characterized with the help of IR and ^{1}H NMR spectra. These α, β-unsaturated compounds may possess diverse pharmacological activities. It has been found that these chalcones interact with the bovine serum albumin, a protein mainly responsible for the transportation of a number of compounds.

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