

## ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF SOME PHENYL SUBSTITUTED CHALCONES

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Received: 28 Dec 2012, Revised and Accepted: 02 Feb 2013

### ABSTRACT

Objective: Synthesis of some phenyl substituted chalcones and spectrophotometric analysis of bovine serum albumin in presence of synthesized chalcones.

Methods: 1-Biphenyl-3-(substitutedphenyl)-2-propen-1-ones were synthesized by the reaction of substituted benzaldehydes with 4-phenylacetophenone and in the presence of a base. The structures were confirmed by their IR and <sup>1</sup>HNMR spectra. After establishing the structures of 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones, their effect were observed on BSA in solution.

Results: Out of synthesized chalcones, 1-biphenyl-4-(nitrophenyl)-2-propen-1-one is most reactive chalcone as it decreased the availability of BSA in solution to maximum extent.

Conclusion: 1-Biphenyl-3-(substitutedphenyl)-2-propen-1-ones interact with the bovine serum albumin which is responsible for the transportation of a number of compounds.

**Keywords:** Bovine serum albumin, Interaction studies, Chalcones.

### INTRODUCTION

The most abundant plasma protein, formed principally in the liver and constitutes up to two thirds of the 6 to 8 per cent protein concentration in the plasma. Albumin is responsible for much of the colloidal osmotic pressure of the blood, and thus is a very important factor in regulating the exchange of water between the plasma and the interstitial compartment. A decrease in the serum albumin is an indicative of diseased conditions related to liver disease, malnutrition, malfunction of the kidney etc.

Chalcones and their derivatives have been reported to exhibit a wide variety of pharmacological effects including antimalarial[1-4], antiplatelet<sup>5</sup>, antiviral[6-8], antibacterial[9-12], antitubercular [13,14], antifungal[15], anticancer[16,17], antitumor[18], antileishmanial[19], analgesic[20,21], antiulcerative[22], antihyperglycemic[23], antioxidant[24], antiinvasive[25], antiinflammatory[26], and a number of chalcone derivatives have also been identified for their role in inhibition of several important enzymes in cellular systems, such as epoxide hydrolase[27], protein tyrosine kinase[28], xanthine oxidase[29], alkaline phosphatase[30] and quinone reductase[31].

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[32], 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one[33], 1-(2'-furyl)-3-(substitutedphenyl)-prop-2-en-1-one[34], 1-(2'-thienyl)-3-(substitutedphenyl)-prop-2-en-1-one[35], 1-(4-hydroxyphenyl)-3-(substitutedphenyl)-2-propen-1-ones and 1-(4-nitrophenyl)-3-(substitutedphenyl)-2-propen-1-ones[36] with bovine serum albumin, we here report the interaction of bovine serum albumin with 1-biphenyl-3-(substitutedphenyl)-2-propen-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[37], it is also suggested that the present study performed with BSA can give an insight about the interaction of chalcones with human serum albumin.

### 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<sub>3</sub>-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 Spectrofuuge (model 16M) was used for centrifugation purpose.

### Synthesis of Chalcones

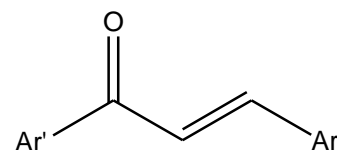
A series of chalcones 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones was synthesized by the grinding of substituted aldehyde (0.01 mole) with 4-phenylacetophenone (0.01 mole) in presence of potassium hydroxide (0.03 mole) respectively with a mortar and pestle. The progress of reaction and the purity of the products were confirmed through TLC. The structures were confirmed by their IR and <sup>1</sup>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[38]. The results are presented in figure 1.

### MATERIALS AND METHODS

A series 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 4-phenylacetophenone. Their physical parameters such as melting points, R<sub>f</sub> values, % yields are reported in Table 1. The given R<sub>f</sub> values were determined with the help of TLC in benzene. The IR and <sup>1</sup>HNMR data of different chalcones is presented in tables 2 and 3 respectively.



In Table 3, <sup>1</sup>HNMR (CDCl<sub>3</sub>) 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.

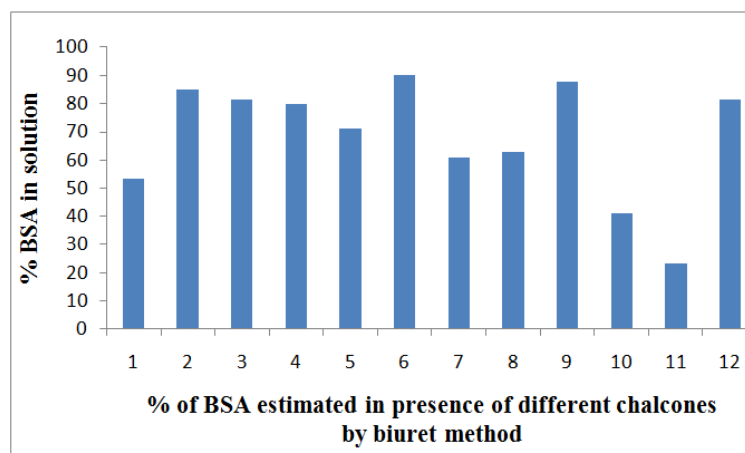


Fig. 1: The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

Table 1: Physical Parameters and Elemental Analysis of Synthesized Chalcones

CompNo	Ar', Ar-	Mol. Formula	Mol. Wt	M.P°C	R <sub>f</sub> value	% yield
1	C <sub>6</sub> H <sub>5</sub> , C <sub>6</sub> H <sub>5</sub>	C <sub>15</sub> H <sub>12</sub> O	208	135-136	0.34	91.12
2	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>5</sub>	C <sub>21</sub> H <sub>16</sub> O	284	90-91	0.740	92.19
3	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> OCl	318.5	110-112	0.851	88.34
4	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -Cl-C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> OCl	318.5	190-193	0.340	81.55
5	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> OCl	318.5	123-125	0.674	89.15
6	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>22</sub> H <sub>18</sub> O <sub>2</sub>	314	168-170	0.901	78.18
7	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>22</sub> H <sub>18</sub> O <sub>2</sub>	314	91-93	0.621	82.18
8	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>22</sub> H <sub>18</sub> O <sub>2</sub>	314	180-182	0.761	73.13
9	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> NO <sub>3</sub>	329	160	0.742	80.79
10	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> NO <sub>3</sub>	329	243-245	0.881	94.40
11	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>21</sub> H <sub>15</sub> NO <sub>3</sub>	329	185-187	0.41	74.89
12	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	C <sub>23</sub> H <sub>21</sub> NO	327	189-190	0.619	77.91

Table 2: IR Data [ν max (cm<sup>-1</sup>)] of Chalcones (Ar'-CO-CH=CH-Ar)

Comp No	Ar', Ar-	[C=O]	[C=C]	[CH]	[O-N-Osym]	[O-N-Oasym]
1	C <sub>6</sub> H <sub>5</sub> , C <sub>6</sub> H <sub>5</sub>	1657	1595	3015	-	-
2	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>5</sub>	1652	1597	2095	-	-
3	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	1652	1598	3115	-	-
4	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -Cl-C <sub>6</sub> H <sub>4</sub>	1653	1598	3089	-	-
5	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub>	1652	1598	3020	-	-
6	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	1652	1599	3035	-	-
7	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>m</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	1656	1599	3125	-	-
8	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	1652	1599	3189	-	-
9	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	1658	1595	3073	1335	1528
10	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	1651	1595	2995	1342	1528
11	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	1650	1597	3091	1343	1529
12	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -N(CH <sub>3</sub> ) <sub>2</sub> -H <sub>4</sub>	1649	1591	3089	-	-

Table 3: <sup>1</sup>HNMR (δ ppm) Data obtained for Chalcones (Ar'-CO-CH=CH-Ar)

Comp No	Ar', Ar-	H-2	H-3	J2-3 (Hz) -	Ar-H	3H,-OCH3
1	C <sub>6</sub> H <sub>5</sub> , C <sub>6</sub> H <sub>5</sub>	7.301 (d)	7.735 (d)	15.6	7.199-8.343(m)	-
2	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>5</sub>	7.587 (d)	8.091 (d)	15.5	7.156-8.456(m)	-
3	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	7.652 (d)	7.980 (d)	15.5	7.129-8.526(m)	-
4	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -Cl-C <sub>6</sub> H <sub>4</sub>	6.965 (d)	7.850 (d)	15.5	7.199-8.343(m)	-
5	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub>	7.357 (d)	8.061 (d)	15.7	7.156-8.456(m)	-
6	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	7.450 (d)	7.882 (d)	15.7	7.129-8.526(m)	3.861
7	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>m</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	7.439 (d)	7.841 (d)	15.8	7.156-8.456(m)	3.824
8	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	7.412 (d)	8.101 (d)	15.8	7.129-8.526(m)	3.932
9	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>o</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	7.548 (d)	8.029 (d)	15.6	7.118-8.299(m)	-
10	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>m</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	7.397 (d)	7.685 (d)	15.3	7.199-8.343(m)	-
11	<i>p</i> -C <sub>6</sub> H <sub>5</sub> - C <sub>6</sub> H <sub>4</sub> , <i>p</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	6.671 (d)	7.546 (d)	15.3	7.156-8.456(m)	-
12	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	7.411(d)	7.881(d)	15.1	7.186-8.416(m)	-

Table 4: Experimental Analysis of Synthesized Chalcones (Ar'-CO-CH=CH-Ar)

Comp No	Ar', Ar-	% of BSA left in solution after interaction with chalcones
1	C <sub>6</sub> H <sub>5</sub> , C <sub>6</sub> H <sub>5</sub>	53.42
2	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , C <sub>6</sub> H <sub>5</sub>	85.5
3	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>o</i> -Cl-C <sub>6</sub> H <sub>4</sub>	81.74
4	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>m</i> -Cl-C <sub>6</sub> H <sub>4</sub>	80.24
5	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -Cl-C <sub>6</sub> H <sub>4</sub>	71.25
6	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>o</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	90.62
7	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>m</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	61.11
8	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub>	63.20
9	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>o</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	87.90
10	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>m</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	41.43
11	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	23.42
12	<i>p</i> -C <sub>6</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>4</sub> , <i>p</i> -N(CH <sub>3</sub> ) <sub>2</sub> -C <sub>6</sub> H <sub>4</sub>	81.90

## 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 the synthesis of one series i.e. 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones by the reaction of substituted benzaldehydes with 4-phenylacetophenone and in the presence of a base.

The synthesis of different chalcones was established by their spectral data. In the IR spectra of chalcones (1-12) as mentioned in table 2, the peak at 1651 – 1659 cm<sup>-1</sup> 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  $\alpha$ ,  $\beta$  – unsaturated carbonyl group in the synthesized compounds. <sup>1</sup>H NMR (CDCl<sub>3</sub>) data of different chalcones is presented in table 3. The synthesis of chalcones is characterized by the presence of two doublets around  $\delta$  7.6 - 6.6 and  $\delta$  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<sub>2,3</sub> is ~ 15.9 - 15.0 Hz. The aryl and other protons were revealed at their respective position. After establishing the structures of 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones, their effect were observed on BSA in solution.

We have earlier reported spectrophotometric analysis of BSA in presence of different series of chalcones [33-37]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones (Figure 1). The chalcones possess  $\alpha$ ,  $\beta$ -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 [39]. 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  $\alpha$ ,  $\beta$ -unsaturated ketone group. We propose that nucleophilic groups of BSA react with  $\alpha$ ,  $\beta$ -unsaturated group in an effective manner. The results suggest that 1-biphenyl-4-(methoxyphenyl)-2-propen-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-biphenyl-3-(substitutedphenyl)-2-propen-1-ones; by Claisen-Schmidt condensation successfully and has been characterized with the help of IR and <sup>1</sup>H NMR spectra. These  $\alpha$ ,  $\beta$ -unsaturated compounds may possess diverse biological activities as reported with this class of compounds. 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.

## ACKNOWLEDGEMENTS

The authors are thankful to Department of Science and Technology, New Delhi, for providing financial assistance.

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