Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

SPECTROPHOTOMETRIC ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF SOME HYDROXY- AND NITRO- SUBSTITUTED CHALCONES

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Received: 19 July 2012, Revised and Accepted: 30 Aug 2012

ABSTRACT

Chalcones, 1, 3-diaryl propenones, possessing variety of pharmacological activities have been found to interact with bovine serum albumin. Serum albumin is an important constituent of blood. It is involved in transportation of a number of compounds. It interacts with a vast array of chemically diverse ligands, including drugs by various binding sites. In the present work we report binding of bovine serum albumin with some hydroxyl- and nitro- substituted chalcones. Two series of chalcones, 1 - (4 - hydroxyphenyl) - 3- (substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen -1- ones; were synthesized by the Claisen-Schmidt condensation and their effect was observed on bovine serum albumin. We have found that the synthesized chalcones interacted with bovine serum albumin irrespective of the nature and position of the substituent.

Keywords: Bovine serum albumin, Interaction studies, 1 - (4 - hydroxyphenyl) - 3 -(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones, Chalcones.

INTRODUCTION

Serum albumin, a constituent of blood is one of the most important, most abundant plasma proteins, synthesized by the liver and associated with the binding and transport of several small molecules such as fatty acids, dyes, metals, amino acids, drugs, as well as several pharmaceutical compounds. It accounts for ~ 60 % of the total globular protein in blood plasma[1-5]. Bovine serum albumin (BSA) is a very interesting biophysical and biochemical system. Its primary structure consist of 585 amino acid residues and the secondary structure formed of 67 % of alpha helix and 17 disulfide bridges, is responsible for its remarkable stability[6]. The interaction of drug with globular proteins, especially BSA can affect its half life. Binding property of drugs to serum albumin has become one of the most important factors in determining their pharmacokinetics[7].

Chalcones, 1, 3-diarylprop-1-enones, constitute the backbone of numerous natural products belonging to the flavonoid family[8-9]. The derivatives of chalcone have been reported to exhibit a wide variety of pharmacological effects including antimalarial[10-13], antiviral[14-16], antibacterial[17-20], antitubercular[21,22], antifungal[23], anticancer[24,25], antitumor[26], antileishmaninal[27], antiinflammatory[28], analgesic[29,30], antiulcerative[31], antihyperglycemic[32], antioxidant[33], antiinvasive[34], antiplatelet[35] and a number of chalcone derivatives have also been reported for the inhibition of several important enzymes in cellular systems, including xanthine oxidase[36], epoxide hydrolase[37], protein tyrosine kinase[38] and quinone reductase[39]. Effect of chalcones has been observed on alkaline phosphatase[40] and the report concluded that chalcones can be safely used in various pathological conditions without affecting the activity of alkaline phophatase.

We have reported the interaction of some chalcones with BSA. In continuation of our previous work we here report the interaction of bovine serum albumin with 1 - (4 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen - 1 - ones. Since this protein is involved in the transportation of a number of compounds including drugs and reported about 80% primary sequence identity with Human serum albumin[41] suggests that the present study performed with BSA can give an insight about the interaction of chalcones with Human serum albumin. Effect of 1-(5'-chloro-2'- hydroxyphenyl)-3-(4"-substituted phenyl)-prop-2-en-1-one and their methoxy derivatives have already been reported to be similar towards BSA and Human serum proteins[42]. A similar type of interaction between 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one and 1- (2'-furyl)-3-(substituted phenyl)-prop-2-en-1-one with bovine serum albumin is also reported [43-45].

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.

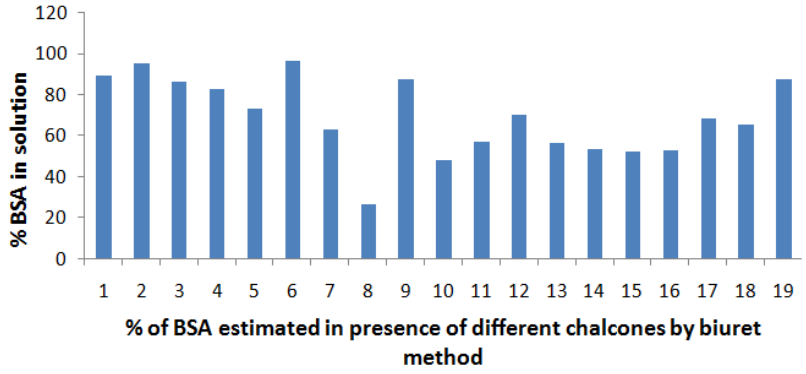

Fig. 1: The results presented are calculated as % of BSA left in solution after Interaction with chalcones with respect to control where no chalcone was added but an equal amount of solvent was added

Synthesis of Chalcones

Two series of chalcones 1 - (4 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones; were synthesized by the grinding of substituted aldehyde (0.01 mole) with 4-hydroxyacetophenone (0.01 mole) and 4-nitroacetophenone 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 ^{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 [46]. The results are presented in figure1.

EXPERIMENTAL

Two series 1 - (4 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen - 1 - ones; were synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 4-hydroxyacetophenone and 4-nitroacetophenone respectively. Their physical parameters such as melting points, R_f values, % yields are reported in Table 1. The given R_f values were determined with the help of TLC in benzene. The IR and 1HNMR data of different chalcones is presented in tables 2 and 3 respectively.

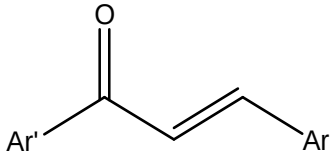

Table 1: Physical Parameters and Elemental Analysis of Synthesized Chalcones

Comp No	Ar', Ar-	Mol.Formula	Mol. Wt	M.P°C	R_f value	% yield
1	C_6H_5, C_6H_5	$C_{15}H_{12}O$	208	135-136	0.34	94.42
2	*p*-NO_2- C_6H_4, C_6H_5	$C_{15}H_{11}NO_3$	253	100-101	0.41	82.09
3	*p*-NO_2-C_6H_4,*o*-Cl-C_6H_4	$C_{15}H_{10}NO_3Cl$	287.5	140-142	0.761	78.34
4	*p*-NO_2- C_6H_4,*m*-Cl-C_6H_4	$C_{15}H_{10}NO_3Cl$	287.5	90-93	0.40	87.65
5	*p*-NO_2- C_6H_4,*p*-Cl-C_6H_4	$C_{15}H_{10}NO_3Cl$	287.5	153-155	0.65	80.95
6	*p*-NO_2-C_6H_4,*o*-OCH_3-C_6H_4	$C_{16}H_{13}NO_4$	283	148-150	0.892	74.78
7	*p*-NO_2-C_6H_4,*m*-OCH_3-C_6H_4	$C_{16}H_{13}NO_4$	283	95-96	0.674	85.08
8	*p*-NO_2- C_6H_4,*p*-OCH_3-C_6H_4	$C_{16}H_{13}NO_4$	283	160-162	0.851	77.53
9	*p*-NO_2- C_6H_4,*o*-NO_2-C_6H_4	$C_{15}H_{10}N_2O_5$	298	150	0.881	70.89
10	*p*-NO_2- C_6H_4,*m*-NO_2-C_6H_4	$C_{15}H_{10}N_2O_5$	298	213-215	0.742	88.00
11	*p*-NO_2- C_6H_4,*p*-NO_2-C_6H_4	$C_{15}H_{10}N_2O_5$	298	155-157	0.740	94.09
12	*p*-OH-C_6H_4, C_6H_5	$C_{15}H_{12}O_2$	224	160-163	0.55	87.67
13	*p*-OH-C_6H_4,*o*-Cl-C_6H_4	$C_{15}H_{11}O_2Cl$	258.5	174-176	0.625	75.97
14	*p*-OH-C_6H_4,*m*-Cl-C_6H_4	$C_{15}H_{11}O_2Cl$	258.5	111-113	0.83	89.78
15	*p*-OH-C_6H_4,*p*-Cl-C_6H_4	$C_{15}H_{11}O_2Cl$	258.5	130-133	0.56	91.21
16	*p*-OH-C_6H_4,*o*-OCH_3-C_6H_4	$C_{16}H_{14}O_3$	256	110-112	0.74	93.07
17	*p*-OH-C_6H_4,*p*-OCH_3-C_6H_4	$C_{16}H_{14}O_3$	256	120-122	0.521	90.14
18	*p*-OH-C_6H_4,*m*-NO_2-C_6H_4	$C_{15}H_{11}NO_4$	269	240-242	0.359	82.66
19	*p*-OH-C_6H_4,*p*-NO_2-C_6H_4	$C_{15}H_{11}NO_4$	269	156-158	0.815	9.23

Table 2: IR Data [υ max (cm^{-1})] of Chalcones (Ar'-CO-CH=CH-Ar)

Comp No	Ar', Ar-	[C=O]	[C=C]	[CH]	[O-N-Osym]	[O-N-Oasym]
1	C_6H_5, C_6H_5	1657	1595	3015	-	-
2	*p*-NO_2- C_6H_4, C_6H_5	1652	1597	2095	1340	1528
3	*p*-NO_2-C_6H_4,*o*-Cl-C_6H_4	1652	1598	3115	1345	1525
4	*p*-NO_2- C_6H_4,*m*-Cl-C_6H_4	1652	1598	3089	1340	1522
5	*p*-NO_2- C_6H_4,*p*-Cl-C_6H_4	1652	1598	3020	1342	1528
6	*p*-NO_2-C_6H_4,*o*-OCH_3-C_6H_4	1652	1599	3035	1340	1522
7	*p*-NO_2-C_6H_4,*m*-OCH_3-C_6H_4	1652	1599	3125	1345	1528
8	*p*-NO_2- C_6H_4,*p*-OCH_3-C_6H_4	1652	1599	3189	1345	1522
9	*p*-NO_2- C_6H_4,*o*-NO_2-C_6H_4	1652	1595	3073	1335	1528
10	*p*-NO_2- C_6H_4,*m*-NO_2-C_6H_4	1652	1595	2995	1342	1528
11	*p*-NO_2- C_6H_4,*p*-NO_2-C_6H_4	1652	1595	3075	1340	1522
12	*p*-OH-C_6H_4, C_6H_5	1659	1598	3078	-	-
13	*p*-OH-C_6H_4,*o*-Cl-C_6H_4	1659	1599	3095	-	-
14	*p*-OH-C_6H_4,*m*-Cl-C_6H_4	1659	1599	2985	-	-
15	*p*-OH-C_6H_4,*p*-Cl-C_6H_4	1659	1599	2923	-	-
16	*p*-OH-C_6H_4,*o*-OCH_3-C_6H_4	1659	1603	3155	-	-
17	*p*-OH-C_6H_4,*p*-OCH_3-C_6H_4	1659	1603	2865	-	-
18	*p*-OH-C_6H_4,*m*-NO_2-C_6H_4	1659	1603	2876	1340	1522
19	*p*-OH-C_6H_4,*p*-NO_2-C_6H_4	1659	1603	2812	1335	1528

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: ^{1}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_6H_5, C_6H_5	7.301 (d)	7.735 (d)	15.6	7.199-8.343(m)	-
2	*p*-NO_2- C_6H_4, C_6H_5	7.587 (d)	8.091 (d)	15.6	7.156-8.456(m)	-
3	*p*-NO_2-C_6H_4,*o*-Cl-C_6H_4	7.652 (d)	7.980 (d)	15.6	7.129-8.526(m)	-
4	*p*-NO_2- C_6H_4,*m*-Cl-C_6H_4	6.965 (d)	7.850 (d)	15.6	7.199-8.343(m)	-
5	*p*-NO_2- C_6H_4,*p*-Cl-C_6H_4	7.357 (d)	8.061 (d)	15.6	7.156-8.456(m)	-
6	*p*-NO_2-C_6H_4,*o*-OCH_3-C_6H_4	7.450 (d)	7.882 (d)	15.6	7.129-8.526(m)	3.861
7	*p*-NO_2-C_6H_4,*m*-OCH_3-C_6H_4	7.439 (d)	7.841 (d)	15.6	7.156-8.456(m)	3.824
8	*p*-NO_2- C_6H_4,*p*-OCH_3-C_6H_4	7.412 (d)	8.101 (d)	15.6	7.129-8.526(m)	3.932
9	*p*-NO_2- C_6H_4,*o*-NO_2-C_6H_4	7.548 (d)	8.029 (d)	15.6	7.118-8.299(m)	-
10	*p*-NO_2- C_6H_4,*m*-NO_2-C_6H_4	7.397 (d)	7.685 (d)	15.3	7.199-8.343(m)	-
11	*p*-NO_2- C_6H_4,*p*-NO_2-C_6H_4	6.671 (d)	7.546 (d)	15.3	7.156-8.456(m)	-
12	*p*-OH-C_6H_4, C_6H_5	7.805 (d)	8.287 (d	15.3	7.129-8.526(m)	-
13	*p*-OH-C_6H_4,*o*-Cl-C_6H_4	7.706 (d)	7.818 (d)	15.3	7.118-8.299(m)	-
14	*p*-OH-C_6H_4,*m*-Cl-C_6H_4	7.410 (d)	7.837 (d)	15.0	7.156-8.456(m)	-
15	*p*-OH-C_6H_4,*p*-Cl-C_6H_4	7.471 (d)	8.217 (d)	15.9	7.129-8.526(m)	-
16	*p*-OH-C_6H_4,*o*-OCH_3-C_6H_4	7.703 (d)	7.866 (d)	15.9	7.199-8.343(m)	3.866
17	*p*-OH-C_6H_4,*p*-OCH_3-C_6H_4	7.320 (d)	7.873 (d)	15.3	7.118-8.299(m)	3.897
18	*p*-OH-C_6H_4,*m*-NO_2-C_6H_4	6.741 (d)	7.720 (d)	15.3	7.129-8.526(m)	-
19	*p*-OH-C_6H_4,*p*-NO_2-C_6H_4	7.803 (d)	7.687 (d)	15.3	7.156-8.456(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_6H_5, C_6H_5	89.74
2	*p*-NO_2- C_6H_4, C_6H_5	95.5
3	*p*-NO_2-C_6H_4,*o*-Cl-C_6H_4	86.74
4	*p*-NO_2- C_6H_4,*m*-Cl-C_6H_4	82.24
5	*p*-NO_2- C_6H_4,*p*-Cl-C_6H_4	73.25
6	*p*-NO_2-C_6H_4,*o*-OCH_3-C_6H_4	96.62
7	*p*-NO_2-C_6H_4,*m*-OCH_3-C_6H_4	63.10
8	*p*-NO_2- C_6H_4,*p*-OCH_3-C_6H_4	26.90
9	*p*-NO_2- C_6H_4,*o*-NO_2-C_6H_4	88.00
10	*p*-NO_2- C_6H_4,*m*-NO_2-C_6H_4	48.53
11	*p*-NO_2- C_6H_4,*p*-NO_2-C_6H_4	57.52
12	*p*-OH-C_6H_4, C_6H_5	70.56
13	*p*-OH-C_6H_4,*o*-Cl-C_6H_4	56.85
14	*p*-OH-C_6H_4,*m*-Cl-C_6H_4	53.88
15	*p*-OH-C_6H_4,*p*-Cl-C_6H_4	52.58
16	*p*-OH-C_6H_4,*o*-OCH_3-C_6H_4	53.03
17	*p*-OH-C_6H_4,*p*-OCH_3-C_6H_4	68.76
18	*p*-OH-C_6H_4,*m*-NO_2-C_6H_4	65.39
19	*p*-OH-C_6H_4,*p*-NO_2-C_6H_4	87.86

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 two series i.e 1 - (4 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen - 1 - ones; by the reaction of substituted benzaldehydes with 4-hydroxyacetophenone and 4-nitroacetophenone respectively in the presence of a base.

The synthesis of different chalcones was established by their spectral data. In the IR spectra of chalcones (1-19) as mentioned in table 2, 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. ^{1}HNMR ($CDCl_3$) data of different chalcones is presented in table 3.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 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen - 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 [42-45]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 1 - (4 - hydroxyphenyl) - 3-(substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3-(substitutedphenyl) - 2 - propen - 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[47]. 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- nitrophenyl)-3-(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

In summary, we have synthesized two series i.e 1 - (4 - hydroxyphenyl) - 3- (substitutedphenyl) - 2 - propen - 1 - ones and 1 - (4 - nitrophenyl) -3- (substitutedphenyl) - 2 - propen - 1 - ones; by Claisen-Schmidt condensation successfully. These α, β-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.

ACKNOWLEDGEMENT

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

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