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Spectrophotometric Analysis Of Bovine Serum Albumin In Presence Of 3-Phenyl-1-(Pyridin-2-yl)Prop-2-en-1-ones

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Abstract: We have synthesized a series of 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones by the Claisen-Schmidt condensation and after establishing the structures of 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones, their effect were observed on Bovine Serum Albumin in solution. We have found that the synthesized 3-phenyl-1-(pyridin-2-yl)prop-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, chalcones of 2-acetylpyridine.

Introduction

Serum albumin as one of the most abundant carrier proteins plays an important role in the transport and disposition of endogenous and exogenous compounds present in blood. Distribution and metabolism of many biologically active compounds such as metabolites, drugs and other organic compounds in the body are correlated with their affinities towards serum albumin, and the binding ability of drugs albumin in blood stream may have a significant impact on free concentration and metabolism of drugs[1,2]. Strong binding can decrease the concentrations of free drugs in plasma, whereas weak binding can lead to a short lifetime or poor distribution. Consequently, the investigation of the binding between drugs and serum albumin is of fundamental importance in pharmacology and pharmacodynamics. Therefore, the binding of drugs to serum albumin in vitro, considered as a model in protein chemistry to study the binding behavior of proteins, has been an interesting research field in chemistry, life sciences and clinical medicine and has been studied for many years[3-6]. In this work, bovine serum albumin (BSA) was selected as our protein model because of its low cost, ready availability, unusual ligand-binding properties, and the results of all the studies are consistent with the fact that bovine and human serum albumins are homologous proteins[7-9].

Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants). However, their occurence is not restricted to flowers but include all parts of the plant[10]. Dietary flavonoids, commonly present in edible plants, are known to have beneficial effects, such as antioxidative effects, tumor cell growth inhibitory activity, and apoptosis induction in cancer cell lines. Therefore dietary flavonoids have attracted attention as chemopreventive agents[11]. Chalcones are the immediate precursors in the biosynthesis of flavonoids. Chalcones are abundantly present in nature starting from ferns to higher plants[12]. flavonoids and chalcones regulate expression of VEGF, matrix metalloproteinases (MMPs), EGFR and inhibit NFkappaB, PI3-K/Akt, ERK1/2 signalling pathways, thereby causing strong antiangiogenic effects[13]. Chalcones and their derivatives have been reported to exhibit a wide variety of pharmacological effects including antimalarial[14-17], antiplatelet[18], antiviral[19-21], antibacterial[22-25], antitubercular[26,27], antifungal[28], antitumor[29], antileishmaninal[30], analgesic[31,32], antiulcerative[33], antihyperglycemic[34], antioxidant[35], antiinvasive[36], cytotoxic[37]. A large noumber of synthetic

chalcones have been reported to have a wide range of biological properties. In an effort to develop a potent anti-inflammatory and cancer chemopreventive agents, a series of chalcones was synthesized[38]. These compounds were tested for their inhibitory effects on the activation of mast cells, neutrophils, macrophages, and microbial cells. It is conceivable that mast cells, neutrophiles, and macrophages are important players in inflammatory disorders[38].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[39], protein tyrosine kinase[40], xanthine oxidase[41], alkaline phosphatase[42] and quinone reductase[43].

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[44], 1-phenyl-3-(substituted phenyl)-prop-2-en-1-one[45], 1-(2'-furyl)-3-(substitutedphenyl)-prop-2-en-1-one[46], 1-(2'-thienyl)-3-(substitutedphenyl)-prop-2-en-1-one[47], 1-(4-hydroxyphenyl)-3-(substitutedphenyl)-2-propen-1-ones and 1-(4-nitrophenyl)-3-(substitutedphenyl)-2-propen-1-ones[48],1-biphenyl-3-(substitutedphenyl)-2-propen-1-ones[49], bischalcones[50] with bovine serum albumin, we here report the interaction of bovine serum albumin with 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones. It is reported that there is about 80% primary sequence identity between bovine serum albumin and human serum albumin[51], it can be concluded that the present study performed with BSA can give an insight about the interaction of chalcones with human serum albumin.

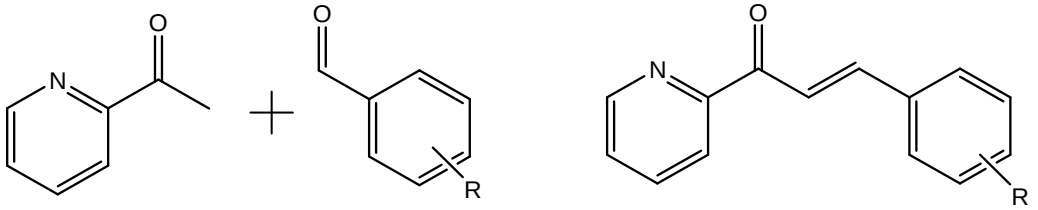
Experimental:

A series 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and 2-acetylpyridine. 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 $CHCI_3$-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 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones was synthesized by the grinding of substituted benzaldehyde (0.01 mole) with 2-acetylpyridine (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.



2-acetylpyridine subsitutedbenzaldehyde 3-subsitutedphenyl-1-(pyridin-2-yl)prop-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[52]. The results are presented in figure1.

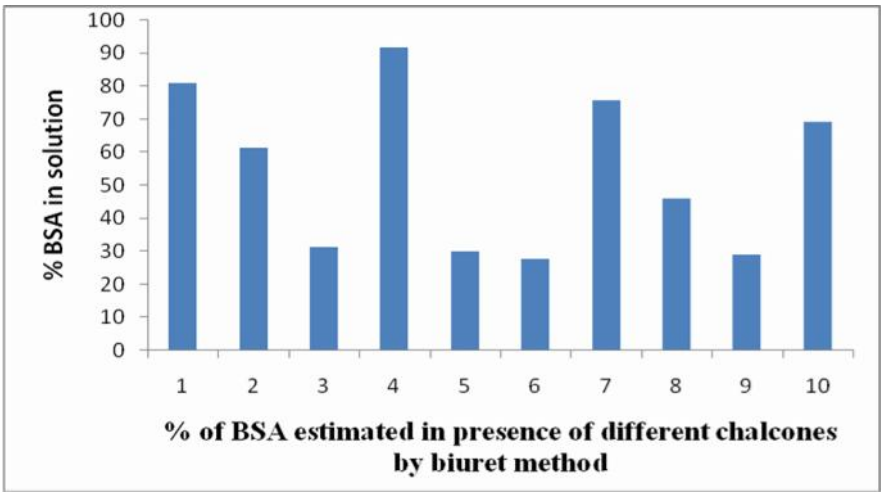


Figure 1. The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

Table 1: IR Data [υ max (cm^{-1})] of Chalcones (NC_5H_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	1666	1605	3055	-	-
2	*o*-Cl	1666	1605	3055	-	-
3	*m*-Cl	1659	1605	3078	-	-
4	*p*-Cl	1659	1605	3063	-	-
5	*o*-OCH_3	1659	1597	3063	-	-
6	*m*-OCH_3	1659	1597	3063	-	-
7	*p*-OCH_3	1659	1597	3063	-	-
8	*o*-NO_2	1659	1597	3009	1342	1512
9	*m*-NO_2	1666	1605	2916	1342	1512
10	*p*-NO_2	1659	1605	3070	1350	1528

Table 2: ^{1}HNMR (δ ppm) Data obtained for Chalcones (NC_5H_4-CO-CH=CH-C_6H_4R)

Comp No	R	H-2	H-3	J2-3 (Hz) -	Ar-H	3H,-OCH3
1	H	7.521 (d)	8.029 (d)	15.6	7.118-8.299(m)	-
2	*o*-Cl	7.447 (d)	7.685 (d)	15.3	7.199-8.343(m)	-
3	*m*-Cl	6.678(d)	7.766 (d)	15.3	7.156-8.456(m)	-
4	*p*-Cl	7.456(d)	7.651(d)	15.1	7.186-8.416(m)	-
5	*o*-OCH_3	7.378 (d)	7.756 (d)	15.6	7.199-8.343(m)	3.867 (s)
6	*m*-OCH_3	7.548 (d)	7.767 (d)	15.6	7.199-8.343(m)	3.867 (s)
7	*p*-OCH_3	7.587 (d)	8.091 (d)	15.5	7.156-8.456(m)	3.867 (s)
8	*o*-NO_2	7.652 (d)	7.980 (d)	15.5	7.129-8.526(m)	-
9	*m*-NO_2	6.965 (d)	7.850 (d)	15.5	7.199-8.343(m)	-
10	*p*-NO_2	7.357 (d)	8.061 (d)	15.7	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 (NC_5H_4-CO-CH=CH-C_6H_4R)

Comp No	R-	% of BSA left in solution after Interaction with chalcones
1.	H	81.10
2.	*o*-Cl	61.29
3.	*m*-Cl	31.53
4.	*p*-Cl	91.72
5.	*o*-OCH_3	30.18
6.	*m*-OCH_3	27.62
7.	*p*-OCH_3	75.8
8.	*o*-NO_2	46.13
9.	*m*-NO_2	29.12
10.	*p*-NO_2	69.24

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 for the synthesis of chalcones involves Claisen-Schmidt condensation of substituted arylaldehyde with the arylmethyl ketones. In the present work we report the solvent free synthesis of one series i.e. 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones by the reaction of substituted benzaldehydes with 2-acetylpyridine and in the presence of a base with the help of mortar and pestle.

The structure of synthesized chalcones was established by their spectral data. In the IR spectra of chalcones (1-10) as mentioned in table 1, the peak at 1651 – 1659 cm^{-1} represent >C=O stretching vibrations due to presence of carbonyl group in conjugation with highly unsaturated system. The synthesis of chalcones is characterized by the presence of two doublets around δ 7.4 - 6.4 and δ 8.6 - 7.9. 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 3-phenyl-1-(pyridin-2-yl)prop-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[44-50]. In the present work, the results are presented on the basis of interaction of serum protein with synthesized 3-phenyl-1-(pyridin-2-yl)prop-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[37]. 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 3-(-3-methoxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-ones 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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