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Research article

SPECTROPHOTOMETRIC ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF SOME BISCHALCONES

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ABSTRACT: We have synthesized a series of bischalcones by the Claisen-Schmidt condensation and their effect was observed on bovine serum albumin. We have found that the synthesized bischalcones 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, bischalcones.

INTRODUCTION

Chalcones belong to a class of α,β-unsaturated aromatic ketones which occur abundantly in nature, and have drawn much attention because of their benefits to human application(Dhar, 1981). The chemistry of chalcone has been recognized as a significant field of study. The phenomenal growth of publications in this area is undoubtedly a reflection of the interest in this field throughout the world. An interesting feature of chalcones is that they serve as starting materials for the synthesis of various heterocyclic compounds such as pyrimidines, pyrazolines, flavones, flavonols, flavanones, aurones and benzoylcoumarones as well as certain compounds like deoxybenzoins and hydantions which are of some therapeutic importance.

The pharmaceutical importance of bischalcone derivatives lies in the fact that they can be effectively utilized as ameliorative(Sarojini, *et al.*, 2011), NO production inhibitors and cytotoxic agents(Reddy, *et al*, 2012), Antimicrobial(Asiri, et al., 2011, Husain, et al., 2012) anticancer(Modzelewska, et al, 2006), antimalarial(Ram, *et al.*, 2000, Srivastava, *et al*., 2008), radioprotective and antiviral activities(Raj, *et al.,* 2012a), *in- vivo* peritoneal antiangiogenesis and *in - vitro* antiproliferative agents (Raj, *et al.*, 2012b) etc.

Serum albumin is involved in the transportation of a number of compounds including drugs. It is reported that there is about 80% primary sequence identity between bovine serum albumin and human serum albumin (Peters, 1985). We have reported the interaction of some chalcones with BSA. It is also suggested here that the present study performed with BSA can give an insight about the interaction of bischalcones with human serum albumin. 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., 2012) with bovine serum albumin, we here report the interaction of bovine serum albumin with bischalcones.

MATERIALS AND METHODS

Melting points were determined by electrochemical capillary Melting points apparatus and are uncorrected. IR spectra (KBr, cm-1) were recorded on a Horizon 300 MHz spectrometer. ^{1}H NMR spectra was recorded on Brucker 300 MHz NMR spectrometer (chemical shifts in δ ppm) using TMS as an internal standard. The purity of the compounds was ascertained by thin layer chromatography on aluminium plates percoated with silica gel G (Merck) in various solvent systems using iodine vapors as detecting agent or by irradiation with ultraviolet lights (254 nm). ELISA plate reader was used for measuring absorbance in the visible range. Refrigerated ultracentrifuge Remi C-24BL was used for centrifugation purpose under cold conditions.

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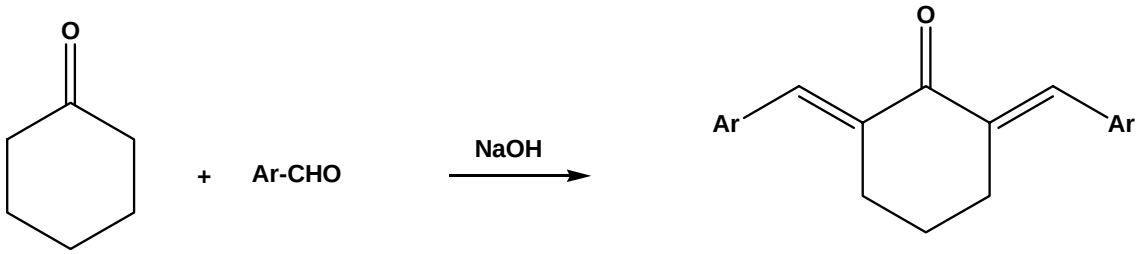
General Procedure for the synthesis of bischalcones: Mixture of cyclohexanone (0.01 mol) and different aromatic aldehydes (0.02mol) in methanol (25ml) was cooled for 10-15 ^{o}C in ice bath. To the cooled solution 40% sodium hydroxide (5ml) was added drop wise with continuous stirring for 30 minutes using magnetic stirrer and then left overnight. The reaction mixture was poured into crushed ice and acidified carefully using dilute hydrochloric acid. The solid obtained was filtered, washed with ice-cold water, dried and recrystallised from ethanol to give compounds.



Here, Ar = *p*-F-C_6H_4, *p*-Cl-C_6H_4, *p*-Br-C_6H_4, p-CH_3-C_6H_4, *p*-OCH_3-C_6H_4, p-NO_2-C_6H_4, C_6H_5-CH=CH, *p*-$N(CH_3)_2$-C_6H_4, H

Synthesis of Bischalcones

Scheme- 1

Reaction of bischalcones with Bovine Serum Albumin- To 10 ml solution of 0.1mM BSA, 1ml solution of 50 mM bischalcone solution was added drop wise with constant stirring. After interaction between bischalcone and BSA, some albumin gets precipitated. The remaining protein in solution was estimated by biuret method (Gornall, et al., 1949). The results are presented in figure1.

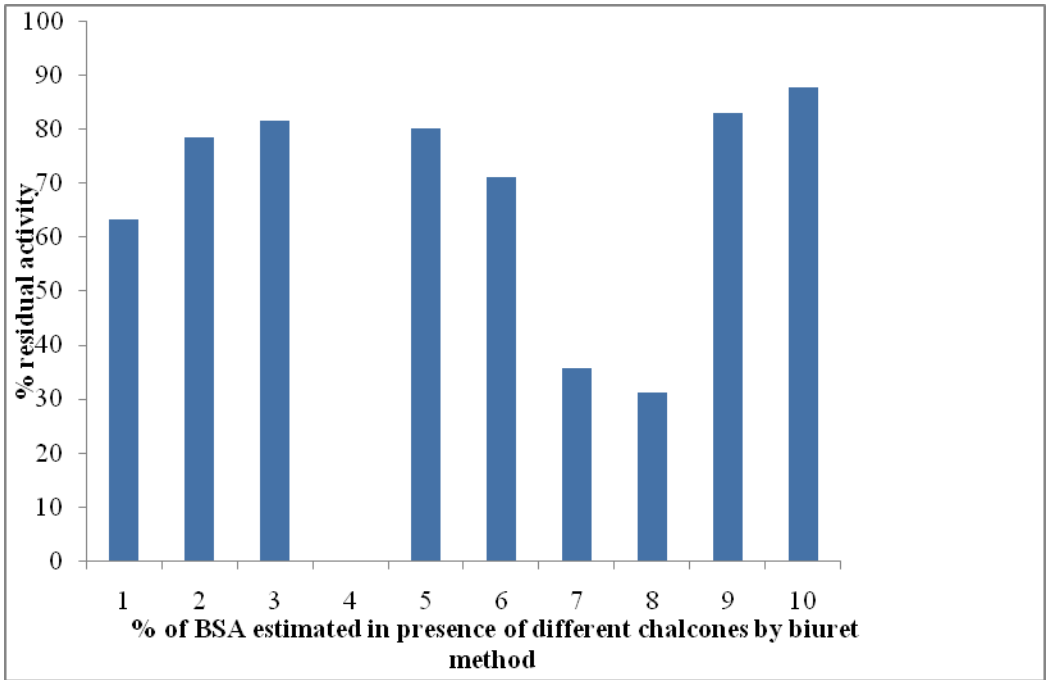


Fig.1 The results presented are calculated as % of BSA left in solution after Interaction with chalcone with respect to control where no chalcone was added but an equal amount of solvent was added

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EXPERIMENTAL

A series of bischalcones was synthesized in good yields by Claisen Schmidt reaction between substituted benzaldehydes and cyclohexanone. The physical parameters such as melting points, % yields, IR and ^{1}HNMR data of different bischalcones are reported in Table no.1.

Table 1: Physical Parameters and Elemental Analysis of Synthesized Chalcones

Comp No	Ar-	M.P.^{0}C	IR(cm^{-1})	1H-NMR (300 MHz, CDCl3, δ ppm)	% YIELD
1	*p*-FC_6H_5	148-149	1651(>C=O str), 1489-1612(-C=C- str), 648(-C-F str)	1.78-1.82(s, 2H, >CH_2), 2.90-2.93(s, 4H, >CH_2), 6.94-7.71(m, 8H, arom. H), 7.99(s, 2H, =CH-)	84.76
2	*p*-ClC_6H_5	150-152	1666(>C=O str), 1427-1605(-C=C- str), 774(-C-Cl str)	1.69-1.73(s, 2H, >CH_2), 2.85-2.88(s, 4H, >CH_2), 7.49-7.59(m, 8H, arom. H), 7.55(s, 2H, =CH-)	86.57
3	*p*-BrC_6H_5-	162-164	1659(>C=O str), 1489-1574(-C=C-str), 825(-C-Br str)	1.71-1.73(s, 2H, >CH_2), 2.85-2.92(s, 4H, >CH_2), 7.47-7.65(m,8H, arom. H), 7.56(s, 2H, =CH-)	78.25
4	*p*-$CH_3C_6H_5$-	172-174	1659(>C=O str), 1412-1597(-C=C- str), 2916(-C-H- str)	1.77-1.85(s, 2H, >CH_2), 2.40(s, 3H, -CH_3), 2.92-2.96(s, 4H, >CH_2), 7.22-7.41(m, 8H, arom. H), 7.79(s, 2H, =CH-)	83.00
5	*p*-$OCH_3C_6H_5$-	158-160	1659(>C=O str), 1489-1574(-C=C- stretching), 2932(-C-H- str), 1250(-C-OCH_3 str)	1.74-1.76(s, 2H, >CH_2), 2.46(3H, s, -$COCH_3$), 2.88-2.92(s, 4H, >CH_2), 7.32-7.55(m, 8H, arom. H), 7.65(s, 2H, =CH-)	74.54
6	*p*-$NO_2C_6H_5$-	200-202	1674(>C=O str), 1435-1589(-C=C- str), 1342,1512(-C-NO_2 str)	1.75-1.77(s, 2H, >CH_2), 2.93-2.99(s, 4H, >CH_2), 7.79-7.89(m, 8H, arom. H), 7.68(s, 2H, =CH-)	88.26
7	*p*-C_6H_5CH=CH-	182-184	1651(>C=O str), 1450-1612(-C=C-str)	1.77-1.81(s, 2H, >CH_2), 2.81-2.84(s, 4H, >CH_2), 7.30-7.66(m, 10H, arom. H), 7.09(dd, 1H), 7.21(dd, 1H), 7.30(s, 2H, =CH-)	71.34
8	*p*-$N(CH_3)C_6H_5$-	240-242	1659(>C=O str), 1427-1574(-C=C- str), 2916(-C-H- str)	1.70-1.76(s, 2H, >CH_2), 2.88-2.92(s, 4H, >CH_2), 7.31-7.55(m, 10H, arom. H), 7.63(s, 2H, =CH-)	72.45
9	C_6H_5-	116-118	1659(>C=O str), 1489-1605(-C=C- str)	1.70-1.76(s, 2H, >CH_2), 2.88-2.92(s, 4H, >CH_2), 7.31-7.55(m, 10H, arom. H), 7.63(s, 2H, =CH-)	83.58

In Table 1, ^{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.

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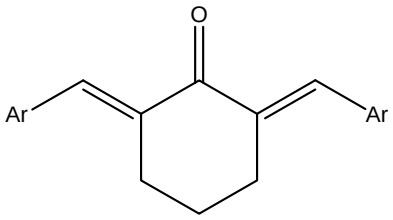
Table 2: Experimental Analysis of Synthesized Bischalcones



Comp No	Ar-	% of BSA left in solution after interaction with bischalcones
1	*p*-FC_6H_5	63.42
2	*p*-ClC_6H_5	78.5
3	*p*-BrC_6H_5-	81.74
4	*p*-$CH_3C_6H_5$-	80.24
5	*p*-$OCH_3C_6H_5$-	71.25
6	*p*-$NO_2C_6H_5$-	35.62
7	*p*- C_6H_5CH=CH-	31.11
8	*p*-$N(CH_3)C_6H_5$-	83.20
9	C_6H_5-	87.90

RESULTS AND DISCUSSION

The synthesis of bischalcones was achieved through base catalysed Claisen-Schmidt reaction (Scheme 1) between cyclohexanone and *p*-substituted arylaldehydes. The synthesis of compounds was confirmed with the help of their physical data, IR and ^{1}H NMR spectra. Bishalcones show a characteristic IR absorption peak at *v*1690-1650 cm-^{1} indicating the presence of a conjugated carbonyl group (>C=O) as well as an olefenic C = C band in the region 1400 – 1605 cm-^{1}. In ^{1}H NMR, the characteristic peak of 2 =CH near δ7.50-7.99 was observed.

The biological activities exhibited by bischalcones 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. In the present work, we report the synthesis of bischalcones by the reaction of substituted benzaldehydes with cyclohexanone in the presence of a base. After establishing the structures of bischalcones, 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., 2012). In the present work, the results are presented on the basis of interaction of serum protein with synthesized bischalcones (Figure 1). We propose that nucleophilic groups of BSA react with α, β-unsaturated group in an effective manner. The results suggest that 2,6-bis(3'-phenylallylidene)cyclohexanone 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 a series of bischalcones 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 bischalcones interact with the bovine serum albumin, a protein mainly responsible for the transportation of a number of compounds.

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