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SPECTROPHOTOMETRIC ANALYSIS OF BOVINE SERUM ALBUMIN IN PRESENCE OF SYNTHESIZED 1-(2'-THIENYL)-3(SUBSTITUTEDPHENYL)--2-PROPEN-1-ONES

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ABSTRACT: Serum albumin an important constituent of blood, involved in transportation of a number of compounds interacts with a vast array of chemically diverse ligands, including drugs by various binding sites. Chalcones, 1,3-diaryl propenones have been found to possess diverse pharmacological activities. In the present work we report binding of bovine serum albumin with some chalcones. Series of 1-(2'- thienyl)-3(substitutedphenyl)-2-propen-1-ones were synthesized by the Claisen-Schmidt condensation and their effect was observed on bovine serum albumin. It was found that the synthesized chalcones interacted with bovine serum albumin irrespective of the nature and position of the substituent.

Key words: Bovine serum albumin, interaction studies, 1-(2'- thienyl)-3(substitutedphenyl)-2-propen-1-ones, chalcones

INTRODUCTION

Serum albumin is an important and most abundant plasma protein in mammals. It is a monomeric protein and comprises about one-half of the blood serum protein. In addition to maintenance of osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues, this also serves as a transport protein for several endogenous compounds. It acts as a carrier protein for several hydrophobic steroids, fatty acids, thyroid hormones and is also capable of binding a number of therapeutic agents. Binding of drug to serum albumin can affect its half life. Binding property of drugs to serum albumin has become one of the most important factors in determining their pharmacokinetics (Kragh-Hansen et al, 2002).

Chalcones, a class of important secondary metabolites of natural products have been found to be very versatile molecules to synthetic chemists due to their ease of synthesis and are also important synthetic intermediates for different types of heterocyclic compounds (Dhar, 1981, Kidwai and Misra, 1999). A number of chalcones synthesized by different research groups have been reported to possess vast array of biological activities such as antimicrobial (Mokle, et al., 2004), antiviral (Onyilagna, et al., 1997), antiprotozoal and antileishmanial (Dimmock, et al.,1999, Neilson et al., 1995), antimalarial (Liu, et al. 2001), antitubercular (Kumar et al, 2007), anticancer (Beutler et al.,1993, Francesco et al., 2007, Ramanathan et al., 1992, Satoni et al., 1993, Wattenberg el. al., 1994, Yit et Al., 1994), antiulcerative (Mukarami, et al., 1991), anti-inflammatory (Hsieh, et al., 2000, Nowakowska, et al., 2007,), analgesic (Viana, et al., 2003), antiplatelet (Zhao, et al., 2005), antioxidant(Miranda et al., 2000), antihyperglycaemic (Satyanarayana et al., 2004), and immunomodulatory (Barford et al., 2002) etc.. Chalcones have also been found to inhibit release of chemical mediators (Ko et al., 2003) and leukotriene B_4 (Deshpande et al., 1999).

A number of enzymes are also reported to be inhibited by chalcones such as nitric oxide synthase, cyclooxygenase (Ahmed et al., 2006), glutathione-5-transferases (GST) (Miyamoto et al., 1987), isolated from mouse liver epoxide hydrolases (Mullin et al., 1987), Mitochondrial monoamine oxidase (Tanaka et al., 1987), isolated from rat liver cyclic adenosine monophosphate phosphodiesterase (Nikaido et al., 1984), xanthine oxidase (Niu et al., 2011) tyrosinase (Khatib et al., 2005), aldolase reductase (Severi et al., 1998), etc. We, in the present work report the interaction of serum albumin with chalcones, since this protein is involved in the transportation of a number of compounds including drugs. Human serum albumin and bovine serum albumin share about 80% primary sequence identity with each other (Peters Jr., 1985). Therefore, the present study performed with BSA will give an idea about the interaction of chalcones with HSA. 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 (Meetu and Raghav, 2009). 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 (Raghav and Malik, 2011a and 2011b).

MATERIALS AND METHODS

Thin layer chromatography was used to study reaction progress and to establish the purity of products. It 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 thus uncorrected. 96-well 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 purposes.

Synthesis of Chalcones- The 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones were synthesized from 2-acetylthiophene (0.01 mole), substituted aryl aldehydes (0.01 moles) in presence of potassium hydroxide (0.03 mole) by the method used earlier in our laboratory (Meetu and Raghav, 2009, Raghav and Malik, 2011b). The progress of reaction and the purity of the products were confirmed through TLC. The structures were confirmed by their IR and 1HNMR spectra.

Reaction of chalcones with Bovine Serum Albumin- To 10 ml solution of 0.1mM BSA added 1ml solution of 50 mM chalcone solution 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 et al., 1948). The results are presented in figure 1.

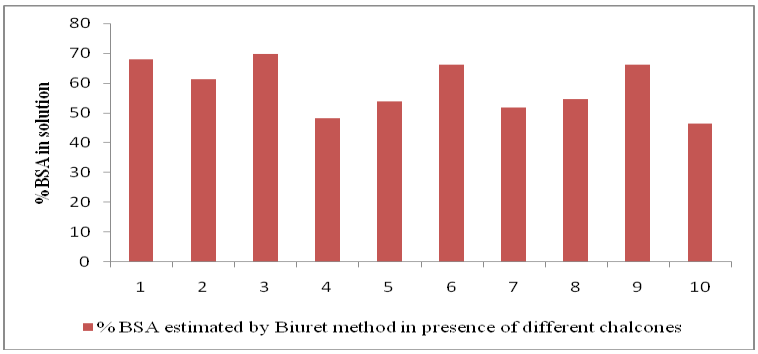


Figure 1. The results presented are calculated as % of BSA left in solution after interaction with chalcones with respect to control where no chalcone was added but an equal amount of solvent was added

RESULTS

1-(2'-thienyl)-3(substitutedphenyl) -2-propen-1-ones were synthesized in good yields by Claisen Schmidt reaction between 2-acetylthiophene and substituted benzaldehydes. Their physical parameters such as melting points, Rf values, % yields are reported in Table 1. The given Rf values are determined in benzene. The IR and 1HNMR data of different chalcones is presented in tables 2 and 3, respectively.

In table 2 the major peaks in IR spectra of chalcones 1-10 are presented. The peak at 1645 – 1651 cm^{-1} represent >C=O streching vibrations. The results show the presence of double bond in conjugation with carbonyl group in the synthesized compounds.

Table 1 : Physical Parameters and Elemental Analysis of Synthesized Chalcones (C_4H_3S-CO-CH:CH-Ar)

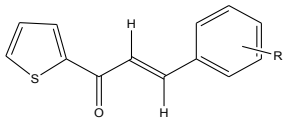
Comp No	Ar-	Mol. Formula	Mol. Wt.	M.Pt.(lit) °C	Rf value	% Yield
1	C_6H_5-	$C_{13}H_{10}OS$	214	90-92 °C	0.611	87.00
2	*o*-Cl-C_6H_4-	$C_{13}H_9ClOS$	248	130-131 °C	0.644	25.30
3	*m*-Cl-C_6H_4-	$C_{13}H_9ClOS$	248	62-65 °C	0.633	74.72
4	*p*-Cl-C_6H_4-	$C_{13}H_9ClOS$	248	118-120 °C	0.644	94.61
5	*o*-OMe-C_6H_4-	$C_{14}H_{12}O_2S$	244	80-82 °C	0.577	96.78
6	*m*-OMe-C_6H_4-	$C_{14}H_{12}O_2S$	244	50-52 °C	0.626	96.17
7	*p*-OMe-C_6H_4-	$C_{14}H_{12}O_2S$	244	144-146 °C	0.604	98.23
8	*o*-NO_2-C_6H_4-	$C_{13}H_9NO_3S$	259	120-122 °C	0.560	51.50
9	*m*-NO_2-C_6H_4-	$C_{13}H_9NO_3S$	259	141-144 °C	0.549	64.93
10	*p*-NO_2-C_6H_4-	$C_{13}H_9NO_3S$	259	200-203 °C	0.549	67.39s

Table 2 : IR Data [$\upsilon_{max}(cm^{-1})$] of Chalcones (C_4H_3S-CO-CH:CH-Ar)

Compound No	Ar-	[C=O]	[C=C]	[CH]	[O-N-O sym]	[O-N-O asym]
1	C_6H_5-	1651	1595	3015	-	-
2	*o*-Cl-C_6H_4-	1651	1597	2995	-	-
3	*m*-Cl-C_6H_4-	1647	1595	3115	-	-
4	*p*-Cl-C_6H_4-	1645	1591	3089	-	-
5	*o*-OMe-C_6H_4-	1651	1605	3020	-	-
6	*m*-OMe-C_6H_4-	1651	1597	3035	-	-
7	*p*-OMe-C_6H_4-	1647	1590	3082	-	-
8	*o*-NO_2-C_6H_4-	1651	1597	3075	1340	1528
9	*m*-NO_2-C_6H_4-	1651	1597	3078	1342	1528
10	*p*-NO_2-C_6H_4-	1651	1595	3060	1345	1525

In Table 3, 1HNMR ($CDCl_3$) 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 ($CDCl_3$) Data Obtained for Chalcones (C_4H_3S-CO-CH:CH-Ar) δ:



Comp. No	Ar-	H-2	H-3	$J_{2,3}$ (Hz)	Ar-H and thienyl-H	3H, -OCH_3
1	C_6H_5-	7.301 (d)	7.735 (d)	15.6	7.199-8.343(m)	
2	*o*-Cl-C_6H_4-	7.805 (d)	8.091 (d)	15.3	7.295-8.425(m)	
3	*m*-Cl-C_6H_4-	7.706 (d)	7.980 (d)	15.6	7.165-8.407(m)	
4	*p*-Cl-C_6H_4-	7.410 (d)	7.850 (d)	15.6	7.201-7.930(m)	
5	*o*-OMe-C_6H_4-	7.471 (d)	8.061 (d)	15.6	6.872-7.795(m)	3.861 (s)
6	*m*-OMe-C_6H_4-	7.703 (d)	7.882 (d)	15.6	7.023-8.365(m)	3.829 (s)
7	*p*-OMe-C_6H_4-	7.320 (d)	7.841 (d)	15.6	6.960-7.721(m)	3.828 (s)
8	*o*-NO_2-C_6H_4-	6.741 (d)	8.101 (d)	15.7	7.065-8.145(m)	
9	*m*-NO_2-C_6H_4-	7.803 (d)	8.029 (d)	15.7	7.266-8.722(m)	
10	*p*-NO_2-C_6H_4-	6.967 (d)	7.685 (d)	15.6	7.065-8.143(m)	

DISCUSSION

The diverse biological activities possessed by chalcones and their potential to be used as synthetic intermediates for variety of biologically active heterocyclic compounds have generated considerable interest in the synthesis of a large number of substituted chalcones. Claisen-Schmidt condensation of substituted arylaldehyde with the arylmethyl ketones has been the most widely used method for the synthesis of chalcones. In the present work we report the synthesis of 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones from 2-acetylthiophene and substituted benzaldehydes in the presence of a base.

$$C_4H_3SCOCH_3 + R\text{-}C_6H_4\text{-}CHO \xrightarrow{KOH} C_4H_3SCOCH{:}CHC_6H_4\text{-}R$$

R= H-, *o*-Cl-, *m*-Cl-, *p*-Cl-, *o*-OCH_3, *m*-OCH_3, *p*-OCH_3, *o*-NO_2, *m*-NO_2, *p*-NO_2

The synthesis of different chalcones was established by their IR and 1HNMR spectral studies. In the IR spectra of chalcones 1-10 as mentioned in table 2, the peak at 1651 – 1659 cm^{-1} represent >C=O streching vibrations which indicate the presence of α,β – unsaturated carbonyl group in the synthesized compounds. Table 3 represents the 1HNMR ($CDCl_3$) data of different 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones. Synthesis of these chalcones was confirmed by the presence of two doublets around δ 7.6 - 6.6 and δ 8.2 - 7.5 in these spectra, representing C-2 and C-3 protons. The geometry across the double bond has been found out to be trans as established by the value of coupling constant, $J_{2,3}$, which lies in the range of ~ 15.9 - 15.0 Hz. The aryl and other protons were revealed at their respective positions.

Once the structures of the 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones was established; their effect was observed on BSA in solution. The results of the serum protein left in solution after interaction with chalcones is presented in figure 1.

The synthesized 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones possess α,β-unsaturated ketone moiety, which is highly reactive and is susceptible to most nucleophilic group available. Therefore, has been used as synthons for the synthesis of different types of heterocycles (Dhar, 1981, Kidwai and Misra, 1999). A large number of side chain groups such as thiol, amino, imidazole, alcohol etc. are available in proteins for interaction with α,β-unsaturated ketone group. We propose that chalcones interact with the nucleophilic groups of BSA in an effective manner. These interactions may cause a change in the three dimensional structure of albumin under study and therefore causes its precipitation out of solution.

Conclusion

We have successfully synthesized 1-(2'-thienyl)-3(substitutedphenyl)-2-propen-1-ones by Claisen-Schmidt condensation. The synthesized chalcones were characterized by TLC, melting point, IR and 1HNMR spectroscopy. These may possess diverse biological activities as has been shown by in this class of compounds. We have 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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