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Chalcones: Synthesis and Their Interaction with Serum Proteins

MEETU and N. RAGHAV*
Department of Chemistry, Kurukshetra University, Kurukshetra-132 119. India
E-mail: noira@rediffmail.com

Two series of chalcones were synthesized by Claisen-Schmidt condensation and their effect on bovine serum albumin and human serum proteins was evaluated. Their structure-effect relationship and comparative interaction with serum proteins is discussed.

Key Words: Synthesis, Chalcones, Serum Proteins.

INTRODUCTION

The term 'chalcones' coined by Kostanecki[1], constitute one of the major classes of natural products having widespread distribution in fruits, vegetables, spices, tea and soya based foodstuffs are also the established precursors of other biologically important class of natural products[2] such as flavones, flavanones, anthocyanins, isoflavanones, *etc*. Various natural or synthetic chalcones have been found to possess diverse biological activities[1,3-11] such as antibacterial, antiviral, anticancer, antiprotozoal, antimalarial, antiulcerogenic, antiinflammatory, anthelmintic, antifungal, *etc*. Variety of chalcones have been found to inhibit nitric oxide synthase[12], cyclooxygenase[12], glutathionetransferase[13], epoxide hydrolase[14], monoamine oxidase[15], prostaglandin dehydrogenase[16], xanthine oxidase[17], *etc*. The diverse biological activities offered by chalcones were the source of inspiration for the work in the present form *i.e.* interactions of chalcones with serum proteins, which are responsible for the transportation of any biologically active compound to the target site, *i.e.*, enzymes, proteins, receptors, nucleic acids, *etc*.

In view thereof series of 1-(5'-chloro-2'-hydroxyphenyl)-3-(4''-substituted phenyl)-prop-2-en-1-one **1(a-d)** and 1-(5'-chloro-2'-methoxyphenyl)-3-(4''-substituted phenyl)-prop-2-en-1-one **2(a-d)** were synthesized and their effect on bovine serum albumin and human serum proteins was observed.

EXPERIMENTAL

Reactions were monitored by thin-layer chromatography. TLC plates were coated with silica gel G (suspended in $CHCl_3$-MeOH) and iodine vapours were used as visualizing agent. Melting points were determined in open capillaries and are thus uncorrected. IR spectra were recorded on a Buck Scientific 500 spectrophotometer. Samples were analyzed using the KBr pellets and frequencies are expressed in cm^{-1}. ^1H NMR spectra were recorded on a 300 MHz Bruker spectrometer using

TMS as internal standard. Chemical shifts are reported on δ scale relative to tetramethylsilane. Yields were determined from isolated products. Digital spectrophotometer 166 (340-990 nm) Systronic make was used for recording absorbance in visible range. Remi - R8C centrifuge were used for centrifugation purposes.

General method for the synthesis of chalcone, 1-(5'-chloro-2'-hydroxyphenyl)-3-phenylprop-2-en-1-one (1a): To a well stirred suspension of powdered NaOH (1.2 g, 0.03 mol) in ethanol (30.0 mL) at 0 ºC were added 5-chloro-2-hydroxy acetophenone (1.70 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol). The reaction mixture which became deep red in colour after 0.5 h was stirred further for 3 h. Thereafter, it was poured over ice and was neutralized with dil. HCl to obtain acrylophenone that was crystallized from ethanol.

Different chalcones **1(b-d)** were prepared by using this procedure starting from 4-substituted aromatic carboxaldehyde, respectively.

Chalcone (1a): Yield 73.88 %, light yellow solid; m.p. 91-92 °C (lit. m.p. 91-93 °C); ν_{max} (cm^{-1}): 1643 (C=O), 1577 (C=C); ^{1}H NMR ($CDCl_3$) δ: 12.72 (1H, -OH), 7.97 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3), 7.89 (1H, d, J_m = 2.7 Hz, H-6'), 7.71 (1H, dd, J_m = 2.7 Hz, J_o = 9.0 Hz, H-4'), 7.59 (1H, dd, $J_{2,3}$ = 15.6 Hz, H-2), 7.49-7.45 (5H, m, 3-phenyl protons), 7.02 (1H, d, J_o = 9.0 Hz, H-3').

1-(5'-Chloro-2'-hydroxyphenyl)-3-(4"-methoxyphenyl)prop-2-en-1-one (1b): Yield 71.06 %, yellow solid, m.p. 102-103 °C (lit. m.p. 104 °C); ν_{max} (cm^{-1}): 3444 (OH), 1638 (C=O), 1565 (C=C); ^{1}H NMR ($CDCl_3$) δ : 1287 (1H, s, -OH), 7.94 (1H, d, $J_{3,2}$ = 15.3 Hz, H-3), 7.87 (1H, s, H-6'), 7.66 (2H, d, J_o = 8.1 Hz, H-3", H-5'), 7.48-7.43 (2H, d, $J_{2,3}$ = 15.3 Hz, H-2, H-4'), 6.98 (3H, d, J_o = 8.1 Hz, H-3", H-2', H-6"), 3.88 (3H, s, -OCH_3).

1-(5'-Chloro-2'-hydroxyphenyl)-3-(4"-chlorophenyl)prop-2-en-1-one (1c): Yield (73.38 %), yellow solid, m.p. 180-181 °C (lit. m.p. 181-182 °C), ν_{max} (cm^{-1}): 1644 (C=O), 1563 (C=C), ^{1}H NMR ($CDCl_3$) δ: 12.58 (1H, s, -OH), 7.82 (1H, d, $J_{3,2}$ = 15.3 Hz, H-3), 7.78 (1H, d, J_m = 2.4 Hz, H-6'), 7.55 (2H, d, J_o = 8.4 Hz, H-3", H-5'), 7.47 (1H, d, $J_{2,3}$ = 15.3 Hz, H-2), 7.39 (1H, dd, J_m = 2.4 Hz, J_o = 8.7 Hz, H-4'), 7.36 (2H, d, J_o = 8.4 Hz, H-2", H-6"), 6.93 (1H, d, J_o = 8.7 Hz, H-3').

1-(5'-Chloro-2'-hydroxyphenyl)-3-(4"-nitrophenyl)prop-2-en-1-one (1d): Yield (72.81 %), dark yellow solid, m.p. 213-215 °C, ν_{max} (cm^{-1}): 1641 (C=O), 1575 (C=C),1510 (asymmetric NO_2), 1336.5 (symmetric NO_2); ^{1}H NMR ($CDCl_3$) δ: 12.50 (1H, s, -OH), 8.33 (2H, d, J_o = 7.8 Hz, H-3", H-5"), 7.96 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3'), 7.86 (3H, d, J = 7.8 Hz, H-6, H-2", H-6"), 7.68 (1H, d, $J_{2,3}$ = 15.6 Hz, H-2), 7.51 (1H, d, J_o = 9.0 Hz, H-4'), 7.06 (1H, d, J_o = 9.0 Hz, H-3').

Synthesis of 1-(5'-chloro-2'-methoxyphenyl)-3-(4"-methoxy-phenyl)prop-2-en-1-one (2a): A suspension of chalcone, **1a** (1.034 g, 0.004 mol), MeI (0.6 g, 0.004 mol) and anhydrous K_2CO_3 (1.2 g) in dry acetone (50 mL) was refluxed for 6-7 h. The reaction mixture was cooled, poured into ice cold water and solid thus obtained was filtered and crystallized from alcohol, **2a**. Other chalcones **1(b-d)** were methylated under similar conditions to yield chalcones **2(b-d)**, respectively.

Chalcone (2a): Yield (64.28 %), yellow solid, m.p. 130-135 °C; ν_{max} (cm^{-1}): 1669 (C=O), 1574 (C=C); ^{1}H NMR ($CDCl_3$) δ: 7.97 (1H, d, $J_{3,2}$ = 15.3 Hz, H-3), 1H, d, J_m = 2.1 Hz, H-6'), 7.52 (1H, dd, J_m = 2.1 Hz, J_o = 8.7 Hz, H-4'), 7.48 (1H, d_2, $J_{2,3}$ = 15.3 Hz, H-2), 7.45-7.40 (5H, m, 3-phenyl protons), 6.97 (1H, d, J_o = 8.7 Hz, H-3'), 3.85 (3H, s, -OCH_3).

Following the same procedure with other substituted chalcones **1(b-d)** methylated chalcones **2(b-d)** were prepared. Their spectral parameters and other characteristics are given below:

1-(5'-Chloro-2'-methoxyphenyl)-3-(4"-methoxyphenyl)prop-2-en-1-one (2b): Yield 73.55 %, creamy solid, m.p. 76-77 °C; ν_{max} (cm^{-1}): 1652 (C=O), 1566 (C=C); ^{1}H NMR ($CDCl_3$) δ: 7.63 (2H, d, J_o = 8.0, H-3", H-5"), 7.58 (1H, d, $J_{3,2}$ = 16.0, H-3), 7.49 (1H, d, J_m = 2.8 Hz, H-6'), 7.44 (1H, dd, J_m = 2.8 Hz, H-8.8 Hz, H-4'), 7.20 (1H, d, $J_{2,3}$ = 16.0, H-2), 7.01 (1H, d, J_o = 8.8 Hz, H-3'), 6.92 (2H, d, J_o = 8.0 Hz, H-2", H-6"), 3.90 (3H, s, -OCH_3), 3.85 (3H, s, -OCH_3).

1-(5'-Chloro-2'-methoxyphenyl)-3-(4"-chlorophenyl)prop-2-en-1-one (2c): Yield 69.22 %, creamy solid, m.p. 85-86 °C; ν_{max} (cm^{-1}): 1663 (C=O), 1565 (C=C); ^{1}H NMR ($CDCl_3$) δ: 7.95 (1H, d, $J_{3,2}$ = 16.0 Hz, H-3), 7.82 (1H, d, J_m = 2.8 Hz, H-6'), 7.69 (2H, d, J_o = 8.4 Hz, H-3", H-5"), 7.49 (1H, d, $J_{2,3}$ = 16.0 Hz, H-2), 7.48 (1H, dd, J_m = 2.8 Hz, J_o = 8.8 Hz, H-4'), 7.39 (2H, d, J_o = 8.4 Hz, H-2", H-6"), 7.02 (1H, d, J_o = 8.8 Hz, H-3'), 3.92 (3H, s, -OCH_3).

1-(5'-Chloro-2'-methoxyphenyl)-3-(4"-nitrophenyl)-prop-2-en-1-one (2d): Yield 70.92 %, deep orange solid, m.p. 108-110 °C; ν_{max} (cm^{-1}): 1652 (C=O), 1516 (asym, NO_2), 1343 (sym., NO_2); ^{1}H NMR ($CDCl_3$) δ: 8.42 (2H, d, J_o = 8.4 Hz, H-3", H-5"), 8.02 (1H, d, $J_{3,2}$ = 15.6 Hz, H-3), 7.99 (2H, d, J_o = 8.4 Hz, H-2", H-6"), 7.79 (1H, d, J_m = 2.8 Hz, H-6'), 7.70 (1H, d, $J_{2,3}$ = 15.6 Hz, H-2), 7.60 (1H, dd, J_m = 2.8 Hz, J = 8.8 Hz, H-4'), 6.92 (1H, d, J_o = 8.8 Hz, H-3').

Interaction of chalcones with serum proteins

Interaction of chalcones 1(a-d) and 2(a-d) with bovine serum albumin (BSA): 0.1 M stock solutions of chalcones **1(a-d)** and **2(a-d)** were prepared in MeOH/DMSO, separately. 0.5 mL of BSA solution (5 mg/mL) was interacted with varying concentrations of chalcones containing a total of 100 µL solvent. After 0.5 h the reaction mixture was warmed to 50 ºC. The complexed protein got precipitated and protein was estimated by usual Biuret method in the supernatant. 200 µL of supernatant was used for protein estimation and the results are presented in Table-1.

Interaction of chalcones 1(a-d) and 2(a-d) with human serum proteins: Similarly, 0.5 mL of 6 times diluted human serum protein samples were interacted with varying concentration of chalcones **1(a-d)** and **2(a-d)** and were processed for protein estimation as mentioned in preceding section and the results are presented in Table 2.

TABLE-1
EFFECT OF VARYING CONCENTRATION OF CHALCONES 2.1-2.8 ON BOVINE SERUM ALBUMIN

Effective concentration of chalcone (mM)	Optical Density (545 nm) in presence of							
	1a	1b	1c	1d	2a	2b	2c	2d
0.00	0.109 (100.00)	0.108 (100.00)	0.109 (100.00)	0.109 (100.00)	0.109 (100.00)	0.108 (100.00)	0.110 (100.00)	0.109 (100.00)
0.16	0.094 (86.25)	0.098 (90.74)	0.90 (82.56)	0.087 (79.81)	0.094 (86.25)	0.0963 (88.07)	0.092 (83.63)	0.091 (83.48)
0.83	0.090 (82.51)	0.093 (86.11)	0.086 (78.89)	0.078 (71.55)	0.087 (79.82)	0.89 (82.41)	0.086 (78.18)	0.083 (76.14)
1.66	0.079 (72.47)	0.083 (76.85)	0.069 (63.30)	0.065 (59.63)	0.080 (73.39)	0.081 (75.00)	0.078 (70.09)	0.076 (69.72)
8.33	0.071 (65.14)	0.073 (67.92)	0.063 (57.80)	0.060 (55.04)	0.069 (63.30)	0.070 (64.81)	0.066 (60.0)	0.064 (58.71)
16.66	0.064 (58.71)	0.069 (63.89)	0.055 (50.46)	0.045 (41.28)	0.060 (55.04)	0.061 (56.48)	0.058 (52.07)	0.054 (49.54)

The results presented are mean of two different experiments. The values given in parentheses are % residual activity with respect to control containing an equivalent amount of solvent.

TABLE-2
EFFECT OF VARYING CONCENTRATION OF CHALCONES 2.1-2.8 ON HUMAN SERUM PROTEINS

Effective concentration of chalcone (mM)	Optical Density (545 nm) in presence of							
	1a	1b	1c	1d	2a	2b	2c	2d
0.00	0.200 (100.00)	0.210 (100.00)	0.210 (100.00)	0.208 (100.00)	0.208 (100.00)	0.208 (100.00)	0.209 (100.00)	0.210 (100.00)
0.16	0.173 (82.77)	0.180 (85.71)	0.168 (80.00)	0.164 (78.09)	0.173 (83.17)	0.175 (84.13)	0.169 (80.86)	0.165 (78.57)
0.83	0.166 (79.42)	0.173 (82.38)	0.155 (73.81)	0.153 (72.86)	0.157 (75.48)	0.160 (76.92)	0.153 (73.20)	0.151 (71.90)
1.66	0.156 (74.64)	0.162 (77.14)	0.149 (70.95)	.0.142 (67.62)	0.147 (70.67)	0.150 (72.11)	0.144 (68.89)	0.143 (68.09)
8.33	0.134 (64.11)	0.139 (66.19)	0.127 (60.47)	0.118 (56.19)	0.138 (66.34)	0.140 (67.31)	0.134 (64.11)	0.131 (62.38)
16.66	0.111 (52.85)	0.116 (55.25)	0.109 (51.90)	0.104 (49.52)	0.122 (58.65)	0.123 (59.13)	0.121 (57.89)	0.118 (56.19)

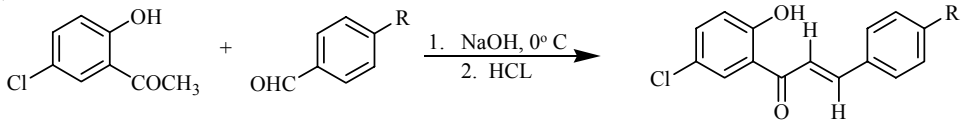
The result presented is mean of two different experiments. The values in parentheses are % residual activity with respect to control having nil concentration of chalcones but equivalent amount of solvent.

RESULTS AND DISCUSSION

Synthesis of chalcones 1(a-d) and 2(a-d): The chalcones **1(a-d)** were synthesized by base catalyzed Claisen-Schmidt condensation between 4-substituted benzaldehydes and 5-chloro-2-hydroxycetophenones (**Scheme-I**) in presence of NaOH at 0 ºC[18].

The structures of chalcones **1(a-d)** prepared were confirmed through IR and ^{1}H NMR spectra.

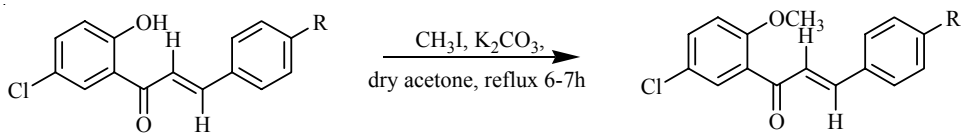
In the IR spectrum of 1-(5'-chloro-2'-hydroxyphenyl)-3-phenylprop-2-ene-1-one (**1a**) the (C=O) group absorption was present at 1643 cm^{-1} suggesting the presence of highly conjugated system in *vicinal* environment. In the 300 MHz, ^{1}H NMR ($CDCl_3$) spectrum, the protons H-3', H-4' and H-6' were revealed at δ 7.02 (1H, d, J_o = 9.0 Hz), 7.71 (1H, dd, J_m = 2.7 Hz, J_o = 9.0 Hz) and 7.89 (1H, d, J_m = 2.7 Hz), respectively. The resonance due to H-2 and H-3 were present as doublets at δ 7.59 and 7.97, respectively. The $J_{2,3}$ of 15.6 Hz indicate the trans stereochemistry across C_2, C_3 double bond. The protons due to 3-phenyl ring were placed as multiplets in the range δ 7.45-7.49. The peak due to 2'-OH proton was observed as singlet at δ 12.72. The structures of chalcones **1(b-d)** were also found to be consistent with their spectral parameters.



1a, R = H; 1b, R = OCH_3
1c, R = Cl; 1d, R = NO_2

Scheme-I: Synthesis of chalcones **1(a-d)**

The series 1-(5'-chloro-2'-methoxyphenyl)-3-(4''-substituted phenyl)prop-2-en-1-one **2(a-d)** was prepared by refluxing chalcones **1(a-d)**, respectively in presence of CH_3I and K_2CO_3 in dry acetone for 6-7 h (**Scheme-II**).



1a, R = H; 1b, R = OCH_3
1c, R = Cl; 1d, R = NO_2

2a, R = H; 2b, R = OCH_3
2c, R = Cl; 2d, R = NO_2

Scheme-II: Synthesis of chalcones **2(a-d)**

The reaction resulted in conversion of 2'-hydroxy group to 2'-methoxy group. The structures of chalcones **2(a-d)** were confirmed by IR and ^{1}H NMR spectra where the broad singlet observed in chalcones **1(a-d)** around δ 12.50-12.40 due to -OH disappeared and a -OCH_3 a singlet was revealed around δ 3.9 to δ 3.8.

Interaction of chalcones 1(a-d) and 2(a-d) with serum proteins: Table-1 presents the amount of bovine serum albumin left in solution after interacting with varying concentrations of chalcones for 0.5 h at room temperature[19]. 1-(5'-Chloro-2'-hydroxyphenyl)-3-phenylprop-2-en-1-one (**1a**), at 0.16 mM concentration resulted

in *ca.* 14 % complexation of BSA. The binding increased to *ca.* 28 % at 1.6 mM concentration of chalcone, **1a** and *ca.* 42 % BSA was precipitated at 16 mM concentration. The similar pattern of protein complexation was observed in case of other chalcones **1(b-d)**. The BSA-chalcone interaction also increased with increase in chalcone concentrations. In this series of 1,3-diarylpropenones prepared **1(a-d)** having different 4"-substitutents, it was found that interaction of bovine serum albumin though was not significantly altered (Fig. 1) but certainly was in the order **1d** > **1c** > **1a** > **1b**, indicating thereby that 4"-electron withdrawing group (-NO_2) had comparatively more effect on protein complexation than 4"-electron donating group (-OCH_3), the effect of other substitutions such as -Cl and -H lying in between. Similar pattern of bovine serum albumin binding was observed in 1-(5'-chloro-2'-methoxy)-3-(4"-substituted phenyl)prop-2-ene-1-one **2(a-d)** and the results are presented in Table-1.

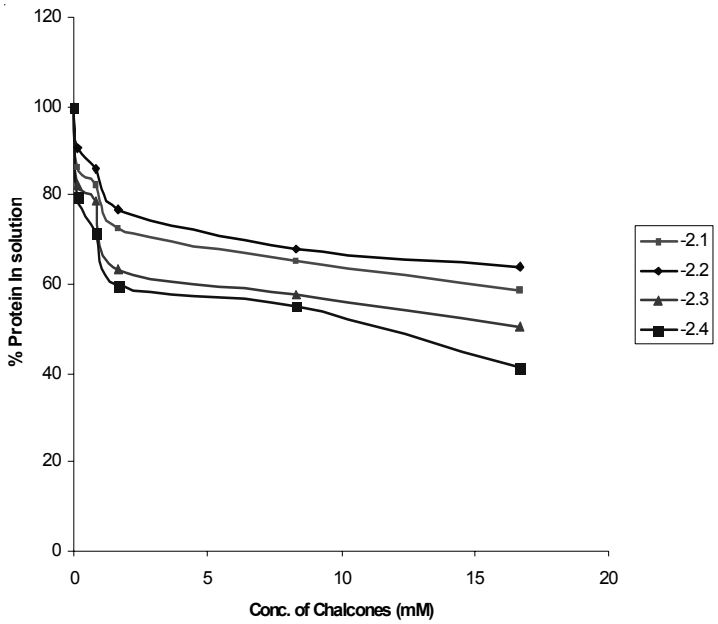


Fig. 1. Effect of 1-(5'-chloro-2'-hydroxyphenyl)-3-(4"-substituted phenyl)prop-2-en-1-ones on bovine serum albumin

Binding of chalcones **1(a-d)** and **2(a-d)** with human serum proteins was also analyzed similarly. Table-2 presents the amount of human serum protein left in solution after interacting with varying concentrations of differently substituted chalcones for 0.5 h at room temperature. The binding pattern was more or less of similar type *i.e.*, the interaction of plasma protein was greater in presence of chalcone **1d** followed by **1c** > **1a** > **1b** in that order. Among the series of chalcones 1-(5'-chloro-2'-methoxy)-3-(4"-substituted phenyl)prop-2-en-1-one **2(a-d)** again, similar type of results were obtained.

The results can be correlated with the cytotoxicity caused by 4-nitrochalcones in a series of chalcones prepared[20] where the electron withdrawing substituents were found to be more mutagenic. Further, the position of -NO_2 substitution was also related to the mutagenicity[21]. The nitrochalcones were found to be less mutagenic when the 4''-position of -NO_2 group was altered.

The binding of bovine serum albumin and human serum protein with different chalcones **1(a-d)** and **2(a-d)** lie with in a variation of *ca*. 8 % indicating thereby that chalcones invariably interacts with serum proteins *i.e.*, with bovine serum albumin and human serum proteins. The synthesized chalcones **1(a-d)** and **2(a-d)** interacted invariably in the similar manner with bovine serum albumin and human serum proteins. And, in a series of 4''-substitued chalcones it was observed that the effect was slightly more pronounced in nitro-substitution followed by chloro-, unsubstituted and methoxy substitution in that order that can be well correlated with the cytotoxicity caused by 4''-nitro chalcones.

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