(E)-2-Benzylidene cycloalkanones XII.*
Kinetic Measurement of Bovine and Human Serum Albumine Interaction with Selected Chalcones and Their Cyclic Chalcone Analogues by UV Spectrophotometry

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Abstract
UV-VIS spectroscopic investigations of interaction of bovine and human serum albumin with selected chalcones (1) and their cyclic chalcone analogues: (E)-2-(4’-X-benzylidene-1-tetralones (3), benzosuberones (4) with dimethylamino and methoxy substituents and (E)-2-(2’,4’-dimethoxybenzylidene)-1-indanone (2) were performed in polar respiration medium. Absorption maxima of the tested compounds were investigated in the presence of bovine and human serum albumin at the 0, 10, 30 and 60 minute timepoints of the interaction. The absorbance of all studied compounds in the presence of proteins decreased after one hour of the reaction. Molecule 4a showed the strongest and fastest kinetic initial interaction with both albumins.

Keywords
Chalcones, Cyclic Chalcone Analogues, UV Spectra, Kinetic Measurements, Binding Constant Bovine Serum Albumin, Human Serum Albumin

*For part XI of this series, see ref [1].
#These authors contributed equally.
†Corresponding author.

1. Introduction

Chalcones (1) are intermediary compounds of the biosynthetic pathway of a very large and widespread group of plant constituents known collectively as flavonoids [2]. Among the naturally occurring chalcones and their synthetic analogues, several compounds displayed antineoplastic activity [2] [3]. Recently we have investigated in vitro antineoplastic activity of several synthetic chalcones and chalcone analogues [4]-[6]. (E)-2-(4’-methoxybenzylidene)-1-benzosuberone (4a) and (E)-2-(4’-dimethylaminobenzylidene)-1-benzosuberone (4c) (Figure 1) had the greatest tumor cytotoxicity of all studied molecules. Structural characterization of selected (E)-2-arylmethylene-1-tetralones (3) and (E)-2-arylmethylene-1-benzosuberones (4) with 4’-methyl and methoxy substituents and their interactions with proteins, and mitochondria were investigated by absorption and fluorescence spectroscopy [7]-[9]. Based on the literature, chalcones and cyclic chalcone analogues seem to be promising molecular tools to investigate changes of molecular surroundings both in solution and in biological systems [10]-[13]. Earlier results indicated that the ring size of the cyclic chalcone analogues has a remarkable impact on their spectroscopic properties [14]-[17]. In order to gain a better understanding, the dynamic interaction of the compounds with proteins, UV-VIS kinetic investigations of selected 4-X-chalcones (1c) and cyclic chalcone analogues: (E)-2-(4’-X-benzylidene)-1-indanones (2b) and (E)-2-(4’-X-benzylidene)-1-benzosuberones (4a, 4c) have been performed (Figure 1). The compounds represent open chain (1) and cyclic (2-4) structures with substituents of different electron donating capacity on their benzylidene moiety [18]-[21]. Earlier results suggest that the chalcone derivatives exert their biological activities through noncovalent interactions with cellular macromolecules [4]-[6]. As a continuation of our previous works, UV-VIS studies of kinetic interaction of the above compounds with bovine (BSA) and human serum albumin (HSA) have been performed.

2. Material and Methods

Compounds 1c, 2b, 4a and 4c were synthesized as described before [4] [5]. Their purity was checked by thin layer chromatography and gas chromatography methods. Dimethyl sulfoxide (DMSO), dipotassium hydrogen phosphate (K2HPO4), ethylenediamine tetraacetic acid (EDTA), magnesium chloride (MgCl2), potassium chloride (KCl), potassium dihydrogen phosphate (KH2PO4), sodium succinate (NaOOCCH2CH2COONa) and Tris HCl were obtained from Serva (Heidelberg, Germany). All solutions were prepared immediately before use. Lyophilized powder of bovine serum albumin (BSA) and human serum albumin (HSA) — purchased from Sigma—Aldrich, Germany) — were dissolved in bidistilled water to give the final concentrations of 2 mg/ml and 10 mg/ml. The respiration medium (pH 7.4) containing EDTA (0.78 mM), MgCl2 (6 mM), TRIS HCl (4 mM), KCl (0.08 M), K2HPO4 (0.3 M) and KH2PO4 (0.3 M) was prepared using bidistilled water. UV-VIS spectra of the solutions (2.5 × 10−5 M) and interactions with BSA and HSA were run/studied on a Shimadzu MultiSpec-1501 UV-VIS spectrophotometer using 1 cm path length quartz cuvettes at ambient temperature. Compounds 1c, 2b, 4a and 4c have been dissolved in dimethyl sulfoxide (DMSO) immediately before use and kept in the dark. The
freshly prepared DMSO solutions \((2.5 \times 10^{-3} \text{ M})\) have been diluted with the respiration medium containing 1 mM sodium succinate to give a final concentration of 25 nmol/ml \((25 \times 10^{-6} \text{ M})\) of the investigated compounds. Concentration of DMSO in the mixtures was 1% v/v. Kinetic measurements have been performed in the presence of 10 μg/ml BSA and HAS over a 60 minute incubation period at room temperature in the dark.

3. Results and Discussion

Comparison of UV spectra of (methoxy and dimethylamino substituted) cyclic chalcone analogues in the polar respiration media showed a decrease of absorption maxima in the order of \(2a > 4a > 4c\) (Table 1, Table 2, Figures 2-5) indicating the strongest conjugation of the rigid, planar compound \(2a\). It is worth mentioning that similar decreasing order of effectiveness of transmission of substituent effects of some \(para\)-substituted 1-4 derivatives could be observed by SSP (single substituent parameter) analysis of their IR carbonyl wave numbers [15]. Both methods indicated the strongest conjugation of the most planar structure of compounds 2.

It can be seen from the molecular structures that the methoxy and the dimethylamino substituted derivatives have the same donor-acceptor type chromophore [22] [23] where the electron-donating groups (OCH\(_3\) and \(N(CH_3)_2\)) are linked to the electron accepting carbonyl group through a styrene moiety. Substituted benzosuberones (4a and 4c) in the presence of BSA (Table 1) and HSA (Table 2) indicated a slight hypsochromic shift of the Band I maxima indicating change in the molecular environment of the compounds. This observation is in accord with interaction of the molecules with the hydrophobic binding site(s) of the two proteins [7]. Similar studies of the open chain chalcone (1c) did not indicate such interaction suggesting importance of spatial arrangement of the electron-reach moieties of the compounds. It is worth mentioning that the two interacting molecules display the most pronounced biological effects and structure-activity relationship studies also indicated importance of the ring size and the presence of the electron-reach aromatic substituents [4]-[6]. On the contrary,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Without protein</th>
<th>With BSA (c = 10 μg/ml)</th>
<th>With BSA after 1 hour (c = 10 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\lambda) (nm)</td>
<td>(A)</td>
<td>(\lambda) (nm)</td>
</tr>
<tr>
<td>1c</td>
<td>270</td>
<td>0.383</td>
<td>270</td>
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<td>427</td>
<td>0.551</td>
<td>429</td>
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<td>340</td>
<td>0.461</td>
<td>336</td>
</tr>
<tr>
<td>4c</td>
<td>271</td>
<td>0.485</td>
<td>268</td>
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<tr>
<td></td>
<td>416</td>
<td>0.511</td>
<td>403</td>
</tr>
<tr>
<td>2b</td>
<td>260</td>
<td>0.279</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>0.460</td>
<td>382</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Compound</th>
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<th>With HSA after 1 hour (c = 10 μg/ml)</th>
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<td>(A)</td>
<td>(\lambda) (nm)</td>
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<tr>
<td>1c</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>382</td>
<td>0.460</td>
<td>383</td>
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</tbody>
</table>
Figure 2. Spectrophotometric measurement of 1c with BSA (a) and HSA (b) at the 0, 10, 30 and 60 minutes timepoints. (purple—0 minute, orange—10 minute, blue—30 minute, red—60 minute).

Figure 3. Spectrophotometric measurement of 4a with BSA (a) and HSA (b) at the 0, 10, 30 and 60 minute timepoints. (purple—0 minute, orange—10 minute, blue—30 minute, red—60 minute).
interaction of the two proteins with the dimethoxy-substituted indanone (2c) caused a bathochromic shift of the Band I maximum of the compound (Table 1 and Table 2). Such observation indicates opposite change in environmental conditions of the bound chalcone molecules [7].

We determined the kinetics of interactions of the tested compounds (1c, 2b, 4a, 4c) with the two proteins (BSA, HSA) by measuring the absorbance of the compounds at the 0, 10, 30, 60 minute timepoints. As it is shown, interaction of the compounds with BSA and HSA resulted in a time-dependent hypochromic effect on the Band I maxima (Figures 2-4(a) and (b), Table 1, Table 2). The sharpest decrease of absorbance was observed in the first 10 minutes but the maximal decrease of was measured at the 60 minute timepoint. Initial velocity of interactions was calculated based on the difference of the 0 and the 10 minute absorbance and the molar extinction coefficients according to the following formula:

\[ v = \frac{\Delta c}{\Delta t} \]

It was found that the compounds interact with the proteins with a slightly different rate. There is negligible difference between the rates of interaction of the studied compounds with BSA and HSA. Compound 4a (with 4'-methoxy substituent) showed the highest rate of interaction with both proteins (Figure 1, Table 3). Compound 2b (with two methoxy substituents) also displayed remarkably fast kinetics with HSA in comparison with the dimethylamino-substituted 1c and 4c (Figure 1, Table 3).

4. Conclusion

In the present work, interaction of compounds 1c, 2b, 4a and 4c with BSA and HSA was studied by UV-Vis spectroscopy. In the spontaneous binding of 4a and 4c, hydrophobic interaction played a major role in it. The fastest initial rate and the strongest initial interaction with both proteins have been recorded for the seven-membered compound 4a. The obtained results provide useful information about protein binding of the com-
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Figure 5. Spectrophotometric measurement of 2b with BSA (a) and HSA (b) at the 0, 10, 30 and 60 minute timepoints. (purple—0 minute, orange—10 minute, blue—30 minute, red—60 minute).

Table 3. Initial rate of interaction of compounds 1c, 4a, 4c and 2b with BSA (c = 10 μg/ml) and HSA (c = 10 μg/ml).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Reaction with BSA (c = 10 μg/ml)</th>
<th></th>
<th>Reaction with HAS (c = 10 μg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ (nm)</td>
<td>A</td>
<td>v (μmol/min)</td>
<td>λ (nm)</td>
</tr>
<tr>
<td>1c</td>
<td>427</td>
<td>0.565</td>
<td>0.528</td>
<td>0.164 × 10^{-6}</td>
</tr>
<tr>
<td>4a</td>
<td>340</td>
<td>0.422</td>
<td>0.256</td>
<td>0.983 × 10^{-6}</td>
</tr>
<tr>
<td>4c</td>
<td>416</td>
<td>0.441</td>
<td>0.404</td>
<td>0.209 × 10^{-6}</td>
</tr>
<tr>
<td>2b</td>
<td>382</td>
<td>0.403</td>
<td>0.308</td>
<td>0.589 × 10^{-6}</td>
</tr>
</tbody>
</table>

Compounds, which can lead to design new drugs in the future that could be effective in treatment and prevention of cancer and other diseases.

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References


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