J Pharm Biopharm Res, 2020, 2(1): 118-125
DOI: 10.25082/JPBR.2020.01.003

RESEARCH ARTICLE

(*E*)-2-Benzylidenecyclanones: Part XVI.† Study on the interaction of some (*E*)-2-benzylidenebenzosuberone derivatives with serum albumin by UV-Vis method, inhibitory effect on topoisomerase

Zsuzsanna Rozmer[1*] Pál Perjési[1]

Abstract: Interaction of some cyclic chalcone analogs, (*E*)-2-(4-X-benzylidene)-1-benzosuberone derivatives with bovine serum albumin (BSA) and human serum albumin (HSA) has been investigated using UV-Vis spectroscopic methods. Recording the UV-Vis spectra of compounds in the presence of BSA or HSA indicated interaction of the molecules with the hydrophobic binding site(s) of the proteins. Investigated analogs have shown remarkable topo I and topo II inhibitory activity compared to camptothecin and etoposide, respectively, at 40 μM concentration. The observed interactions between the cyclic chalcone analogs and the cellular macromolecules might play a role in the previously detected cytotoxicity against several tumor cell lines.

Keywords: cyclic chalcone analogs, enones, bovine serum albumin, human serum albumin, UV-Vis spectroscopy, DNA topoisomerases I and II

1 Introduction

The chemistry and biological activities of chalcones (1,3-diphenyl-2-propenones) (**A**) (Figure 1) have been of interest for a long time. Chalcones are intermediary compounds of the biosynthetic pathway of natural flavonoids[1]. The wide range of biological activities of both naturally occurring and synthetic analogs, among others cytotoxic, antitumor, anti-inflammatory, and chemopreventive properties, are well documented in the literature[1–7]. To study the structure-activity relationship of chalcones, cyclic analogs were synthesized, and their molecular structure, chemical reactivity, and biological activity were investigated[8–12].

Several cyclic chalcone analogs with various substituents at different positions have been synthesized, and their *in vitro* antineoplastic activity (IC_{80} values) has been investigated against murine and human cancer cell lines[8,13]. It was found that the cytotoxic activity of the compounds was mainly influenced by the ring size and the nature and position of substituents. Among the compounds investigated (*E*)-2-(4'-methoxybenzylidene)-1-bensosuberone (**B1**) and (*E*)-2-(4'-dimethylaminobenzylidene)-1-bensosuberone (**B3**) (Figure 1) showed the most significant tumor toxicity[8]. Previously we have investigated the effect of compound **B1** and its methyl-substituted analog (**B2**) (Figure 1) on the cell cycle of Jurkat cells by flow cytometry[14,15]. It was found that equitoxic doses of these compounds induced apoptosis, but they had different effects on cell cycle progression. Compound **B1** caused an immediate G_1 lift and G_2/M arrest, which was followed by cell death (apoptosis and/or necrosis) accompanied by formation of hyperdiploid cells. Such a remarkable effect of **B2** on the G_1 and G_2 checkpoints could not be observed[14]. While investigating the mechanism of cytotoxicity of the compounds, the effect on the mitochondrial outer membrane of some (*E*)-2-arylmethylene-1-benzosuberone analogs was tested by fluorescence spectroscopy[16,17]. The effect of some analogs on isolated rat liver mitochondrial functions were also investigated. It was demonstrated that compound **B1** increased the mitochondrial oxygen consumption and enhanced the activity of the ATPase enzyme. Alteration of mitochondrial functions may play a role in the cytotoxic effect[19].

Interaction of the compounds (**B1-B4**) with ctDNA exhibited relative high intrinsic binding constants in the range of 3.1×10^4–1.0×10^5 M^{-1}, which indicates the existence of weak, non-covalent interaction. The ex-

Received: July 12, 2020 **Accepted:** August 9, 2020 **Published:** August 14, 2020

† Part XV. MOJ Biorganic and Organic Chemistry, 2018, **2**(1): 00046.

* **Correspondence to**: Zsuzsanna Rozmer, Department of Pharmaceutical Chemistry, University of Pécs, Rókus Str. 2, Pécs 7624, Hungary; Email: rozmer.zsuzsanna@pte.hu

[1] Department of Pharmaceutical Chemistry, University of Pécs, Rókus Str. 2, Pécs 7624, Hungary

Citation: Rozmer Z and Perjési P. (*E*)-2-Benzylidenecyclanones: Part XVI.† Study on the interaction of some (*E*)-2-benzyldenebenzosuberone derivatives with serum albumin by UV-Vis method, inhibitory effect on topoisomerase. *J Pharm Biopharm Res*, 2020, **2**(1): 118-125.

perimental analysis reveals primarily groove binding of chalcones investigated with ctDNA[20]. Furthermore, the DNA cleavage activity of the compounds (**B1-B4**) was investigated by agarose gel electrophoresis. Each compound showed slight DNA cleavage activity with pBR322[20]. It was concluded that biological activity, including the cytotoxic effect of the cyclic chalcone derivatives, might be partially a consequence of non-covalent interaction between the compounds and cellular macromolecules[8,13,18].

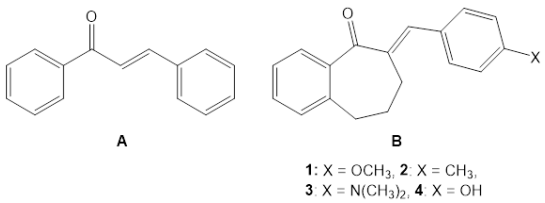

Figure 1. Structure of chalcone (**A**) and the investigated (*E*)-2-(4'-X-benzylidene)-1-benzosuberon analogs (**B1-B4**)

Serum albumins are multifunctional proteins with high ligand binding ability, which act as transporters for a diverse range of drugs and metabolites. Human serum albumin (HSA) is the dominating plasma protein in man. It is a 66 KDa monomer containing three homologous helical domains (I-III), and each is divided into A and B subdomains[21]. Bovine serum albumin (BSA) is usually employed as a model protein due to its low cost and ready availability. Moreover, bovine and human serum albumins are homologous proteins; they display approximately 76% sequence homology[22]. The 3D structure of BSA is believed to be similar to that of HSA. BSA has two tryptophan residues (Trp 134 and Trp 212), located in sub-domains IA and IIA, respectively[23]. HSA, however, has only one tryptophan residue (Trp 214) located in the physiologically important subdomain IIA[24]. Analysis of fluorescence properties of these residues is a useful tool of investigation of binding characteristics of small molecules to the proteins nearby these chromophores[23,24]. The affinity between drugs and serum albumin can change the overall distribution, metabolism, and efficacy of drugs since serum albumins often increase the apparent solubility of hydrophobic drugs in plasma and modulate their delivery to cells[25,26]. Thus, the investigation of drugs binding to serum albumin is of important significance.

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology. There are two types of topoisomerases in humans, namely topoisomerase type I (topo I) and type II (topo II). Topo I makes single-strand brakes of the double helix, while topo II makes double-strand breaks and passes double-stranded DNA through the nick to allow relaxation of over-coiled DNA[27]. Topoisomerases are highly conserved enzymes; they function in DNA replication, chromosome condensation, and segregation. Topoisomerases are one of the most promising targets for the development of anticancer agents because they are highly overexpressed in proliferating cancer cells[28–31].

Earlier, interaction with DNA of the cyclic chalcone analogs **B1-B4**[20] and analogs of **B3** with different ring sizes[32] was investigated. Furthermore, the interaction of BSA and HSA with the 4-dimethylamino-substituted **B3** and its analogs with varying ring sizes was reported[32,33]. As a continuation of our previous works[20,34], here we report the results of UV-Vis studies on the interaction of selected (*E*)-2-(4'-X-benzylidene)-1-benzosuberones (**B1-B4**) (Figure 1) with BSA and HSA, and topoisomerase inhibitory activity of the compounds.

2 Materials and methods

2.1 Materials

Cyclic chalcone analogs **B1-B4** (Figure 1) were synthesized and purified as described before[8,9]. Their purity was checked by thin-layer chromatography and high-pressure liquid chromatography[35]. Their structures were established by X-ray[8,13], MS[10], and ^{1}H NMR spectroscopy[9,12]. All chemicals used were of the analytical grade and if otherwise not specified, purchased from Sigma-Aldrich Hungary (Budapest, Hungary).

2.2 Albumin binding studies

UV-Vis measurements were performed on Jasco V-670 (Japan) spectrophotometer using 1 cm path length quartz cuvettes at ambient temperature.

For BSA/HSA binding studies, stock solution of BSA/HSA (50 μM) was prepared by dissolving BSA/HSA in sodium phosphate buffer (0.1 M, pH 7.4). Variation in the absorption spectrum of BSA/HSA (10 μM) has been studied as a function of chalcone concentration from 0 to 20 μM. Compounds **B1-B4** were dissolved in DMSO immediately before used. The freshly prepared solutions were diluted with 0.1 M sodium phosphate solution (pH 7.4) to the final concentrations (0-20 μM). The concentration of DMSO in the mixtures was 1% v/v. Measurements were performed in the presence of BSA/HSA (10 μM) after 2 min equilibration at room temperature in the dark. Alteration of absorbance in time was followed for 30 minutes in a solution containing chalcone (20 μM) and BSA/HSA (10 μM), prepared as described before.

2.3 In vitro DNA topoisomerase I-mediated relaxation assay

A DNA topo I inhibition assay was performed as previously described by Fukuda et al. with minor modifica-

tions[36,37]. Briefly, compounds **B1-B4** and camptothecin were dissolved in DMSO as 4 mM stock solutions. The activity of DNA topo I was determined by assessing the relaxation of supercoiled DNA pBR322. To this end, a mixture of 100 ng of plasmid pBR322 DNA and 1 unit of recombinant human topo I (TopoGEN INC., USA) was incubated with or without the compounds (40 μM) at 37 °C for 30 min in relaxation buffer (10 mM Tris HCl (pH 7.9), 150 mM NaCl, 0,1% BSA, 1 mM spermidine and 5% glycerol). The reaction was terminated by adding 5 μL of a stop-solution (5% sarcosyl, 0.0025% bromophenol blue, and 25% glycerol) to a final volume of 20 μL. The samples were then electrophoresed on a 1% agarose gel at 50V for 1h in Tris-acetate-EDTA (TAE) running buffer. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by UV transillumination.

2.4 In vitro DNA topoisomerase II-mediated inhibition assay

The DNA topo II inhibitory activity of the compounds investigated were measured as follows[37,38]. A mixture of supercoiled pBR322 plasmid DNA and 1 unit of human DNA topoisomerase IIα (TopoGEN INC., USA) was incubated with or without the compounds **B1-4** or etoposide as reference (40 μM) for 30 min at 37 °C in assay buffer (10 mM Tris HCl (pH 7.9), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP and 15 μg/mL BSA). The reaction was terminated by the addition of 3 μL of 7 mM EDTA to a final volume of 20 μL. The samples were then electrophoresed on a 1% agarose gel at 50V for 1h in TAE running buffer. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by UV transillumination.

3 Results and Discussion

UV-Vis absorption measurements are applicable to explore the structural change of protein and to identify the formation of a complex between serum albumin and small molecules[23,26]. The UV absorption spectra of chalcones usually have one or two main bands. Band I (320-400 nm) is possible from the conjugated system of B-ring and the carbonyl group in the molecule and Band II (220-270 nm) is attributed to the absorption for the conjugated system of A-ring and the carbonyl group[39].

The absorption spectra of the cyclic chalcone analogs **B1-B4**, the free BSA/HSA, and the mixture of the chalcones and BSA/HSA were recorded and presented in Figure 2 and Figure 3, respectively. The absorption maxima of complexes of the compounds **B1**, **B2,** and **B3** with BSA and HSA exhibited hypsochromic shift (blue shift) relative to the absorption maxima of the respective free chalcones. However, in the case of **B4,** the opposite phenomenon, a weak red shift could be observed compared to the absorption maxima of the free compound (Table 1). The observed hypsochromic shifts are in accord with the interaction of the molecules with the hydrophobic binding site(s) of the two proteins[32]. On the other hand, the observed bathochromic shift of **B4** can be rationalized by deprotonation of the phenolic hydroxyl group, resulting in extension of the conjugation[21]. Moreover, it was suggested that conjugation of the molecule could be impaired during the binding[21].

Table 1. UV-Vis absorption maxima of chalcone derivatives **B1-B4** (20 μM) without and in the presence of BSA/HSA (10 μM)

Compound	free compound λ_{max}(nm)	with BSA λ_{max}(nm)	with HSA λ_{max}(nm)
B1	342	338	334
B2	322	320	314
B3	414	411	404
B4	339	342	341

Table 2. Time dependence of UV-Vis absorbances of chalcone derivatives **B1-B4** (20 μM) in sodium phosphate buffer (0.1 M, pH 7.4) without and in the presence of BSA (10 μM) at the λ_{max} values of chalcone-BSA complexes

	Absorbance			
	B1 (334 nm)	**B2** (314 nm)	**B3** (404 nm)	**B4** (341 nm)
Without protein	0.485	0.384	0.369	0.314
With BSA				
0 min	0.401	0.354	0.374	0.289
1 min	0.395	0.352	0.372	0.297
2 min	0.394	0.350	0.371	0.298
5 min	0.393	0.345	0.370	0.300
10 min	0.390	0.337	0.369	0.301
15 min	0.386	0.330	0.363	0.302
20 min	0.383	0.326	0.356	0.305
25 min	0.381	0.323	0.350	0.309
30 min	0.375	0.318	0.345	0.313

Table 3. Time dependence of UV-Vis absorbances of chalcone derivatives **B1-B4** (20 μM) in sodium phosphate buffer (0.1 M, pH 7.4) without and in the presence of HSA (10 μM) at the λ_{max} values of chalcone-HSA complexes

	Absorbance			
	B1 (338 nm)	**B2** (320 nm)	**B3** (411 nm)	**B4** (342 nm)
Without protein	0.287	0.282	0.342	0.214
With HSA				
0 min	0.356	0.372	0.409	0.212
1 min	0.357	0.371	0.409	0.214
2 min	0.354	0.372	0.408	0.214
5 min	0.352	0.369	0.413	0.214
10 min	0.344	0.366	0.411	0.213
15 min	0.335	0.364	0.410	0.214
20 min	0.324	0.359	0.401	0.215
25 min	0.315	0.350	0.386	0.214
30 min	0.300	0.344	0.369	0.215

Using BSA, a hypochromic effect was observed for each compound (except **B3**) on mixing them with the protein (Figure 2). On the contrary, a comparison of the

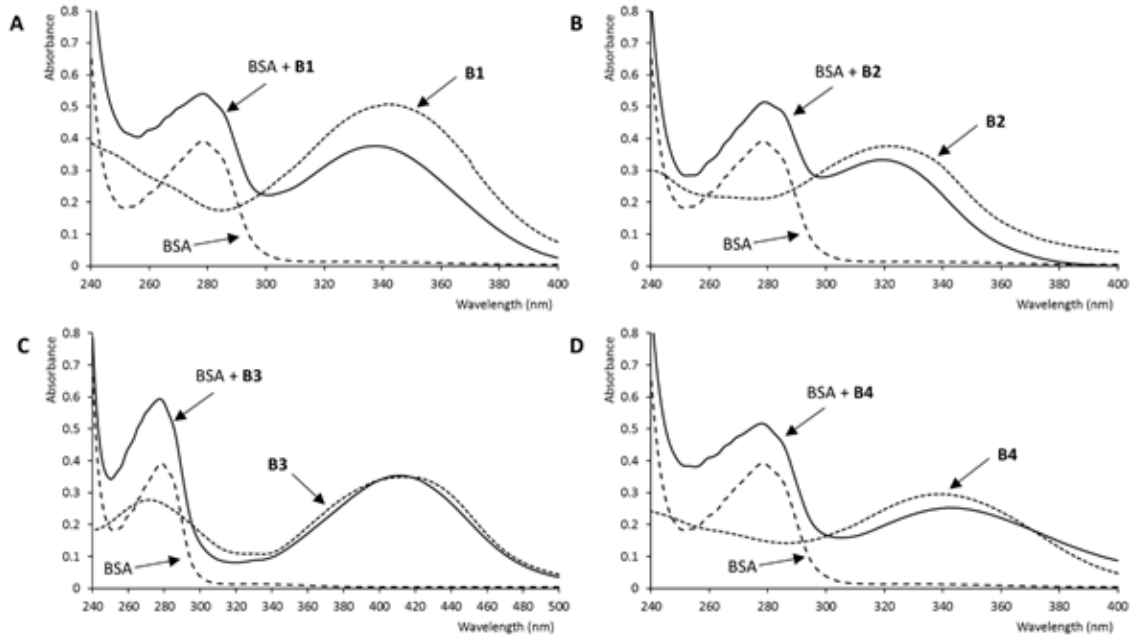

Figure 2. UV-Vis absorption spectra of BSA (10 μM), compound **B1** (**A**), **B2** (**B**), **B3** (**C**) and **B4** (**D**) (20 μM) and the mixture of BSA and chalcone compounds (10 μM; 20 μM) in sodium phosphate buffer (0.1 M, pH 7.4).

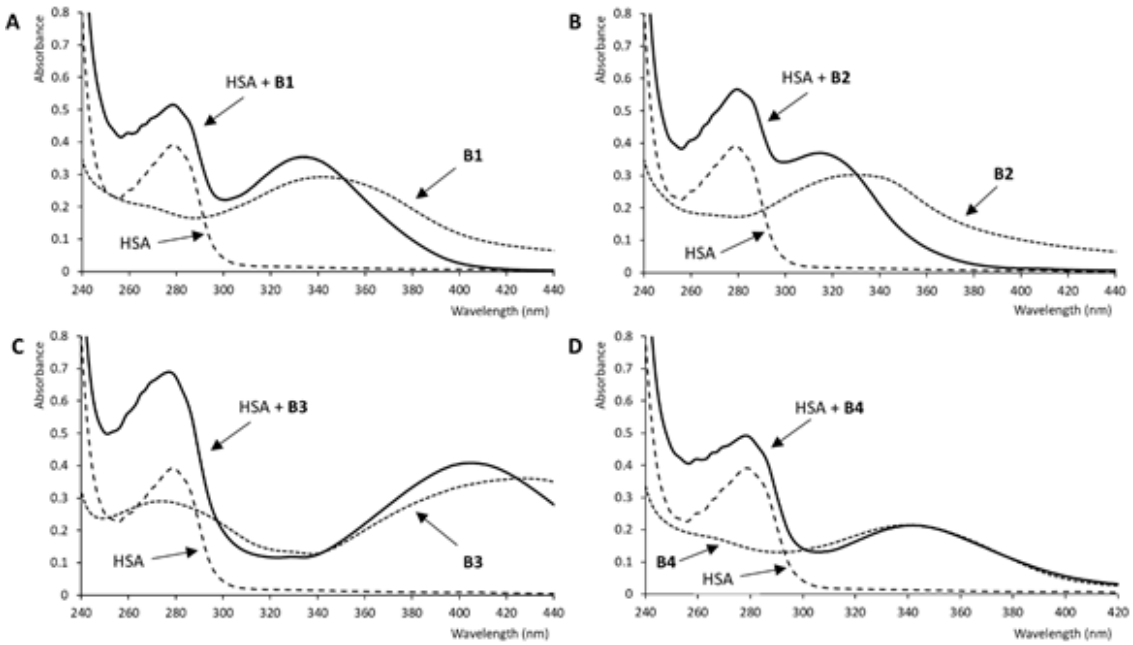

Figure 3. UV-Vis absorption spectra of HSA (10 μM), compound **B1** (**A**), **B2** (**B**), **B3** (**C**) and **B4** (**D**) (20 μM) and the mixture of HSA and chalcone compounds (10 μM; 20 μM) in sodium phosphate buffer (0.1 M, pH 7.4).

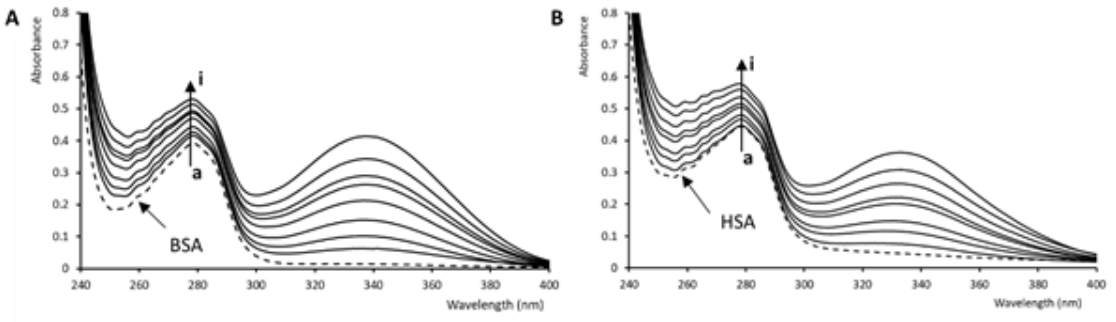

Figure 4. Effect of compound **B1** to UV-Vis absorption of BSA (A) and HSA (B) (10 μM) Concentration of compound **B1** was 0-20 μM for curves **a-i,** respectively, with an increment of 2.5 μM (pH 7.4).

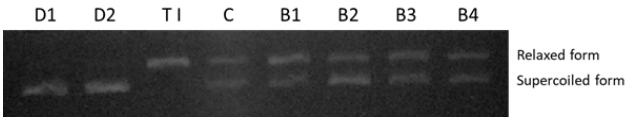

Figure 5. Topoisomerase I inhibitory activity of compounds **B1-4**. Lane D1: pBR322 only; Lane D2: pBR322 + DMSO (as control); Lane TI: pBR322 + topo I; Lane C: pBR322 + topo I + camptothecin; Lane B1-4: pBR322 + topo I + compounds **B1-4**. The compounds were examined in a final concentration of 40 μM.

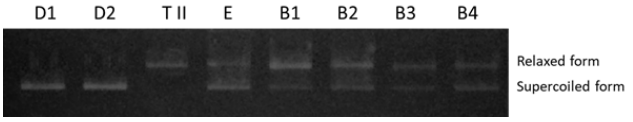

Figure 6. Topoisomerase II inhibitory activity of compounds **B1-4**. Lane D1: pBR322 only; Lane D2: pBR322 + DMSO (as control); Lane TII: pBR322 + topo IIα; Lane E: pBR322 + topo IIα + etoposide; Lane B1-4: pBR322 + topo IIα + compounds **B1-4**. The compounds were examined in a final concentration of 40 μM.

absorbance of the chalcones before and after combining with HSA, initially, a hyperchromic effect could be detected for each compound, except **B4**. In both series of experiments, the initial absorbance gradually decreased (Table 2 and Table 3). Change in intensities of absorbance can be explained by the different molecular environment of the bound chalcone molecules. Reduced planarity results in a decrease in intensity of Band 1 of flavonoids[40]. Based on these observations, it is reasonable to assume that the observed intensity changes are the consequences of the conformational changes caused by interactions of the molecules with the proteins. According to our previous results with cyclic chalcones with related structures, the kinetics of development of the binding equilibrium in the case of the two proteins are different[33]. The binding properties of HSA and BSA have been frequently discussed on the assumption of their structural similarities because the three-dimensional structure was determined only for HSA[41]. However, the present results also indicate that attention should be paid to the small differences in their structures in discussing the correlation between structure and binding properties[42–44].

The UV-Vis absorption spectra of BSA/HSA titrated by individual chalcone solutions were monitored to explore the structural changes of the proteins caused by interaction with the compounds. The absorption spectra of BSA/HSA in the absence and presence of chalcone analog **B1**, as a representative of the investigated compounds, are given in Figure 4. The absorption peak of BSA observed at 278 nm is mainly attributed to the aromatic rings in tryptophan residues (Trp 134 and Trp 212), located in sub-domains IA and IIA, respectively[23]. Similarly, in the case of HSA, the aromatic rings in tryptophan (Trp 214), tyrosine (Tyr 411), and phenylalanine residues result in the absorption peak at 278 nm[25]. The absorption intensity of BSA and HSA increased at 278 nm with the increasing concentration of each compound, indicating that compounds could interact with albumin, and the peptide strands of BSA/HSA were extended[25]. However, the shift of the absorption peak at 278 nm could not be observed. The lack of red shift in the absorption maximum of tryptophan residues indicates no significant changes in its microenvironment[45]. The results suggest a non-covalent interaction between the compounds and albumin, which occurs *via* the π-π stacking between aromatic rings of the chalcones and the Trp, Tyr, and Phe residues located in the binding cavity of BSA/HSA[25,26,46].

Topoisomerase relaxation inhibitory activities were evaluated using recombinant topo I and human topo IIα (TopoGEN) by observation of relaxation of supercoiled pBR322 plasmid DNA[37]. Camptothecin and etoposide well-known topo I and topo II inhibitors, respectively, were used as positive controls, Inhibitory activities were evaluated at 40 μM. The results are shown in Figure 5 and Figure 6.

The compounds displayed comparable topoisomerase I inhibitory activity to camptothecin at the test concentrations (Figure 5). The compounds have shown a topo II inhibitory effect as well; however, they were less effective than etoposide, which was used as a positive control. Although there were no clear correlations between the topoisomerase inhibitory activities and the structures of

the investigated derivatives, the observed results suggest that the shape of the cyclic chalcone system and the nature of the functional groups are an important factor for interaction between the DNA-protein complex and chalcone analogs during DNA unwinding process[47]. The biological role of topo enzymes is consistent with their expression pattern across the cell cycle, whereby topo IIα primarily expressed in late S and G_2/M phase cells[48]. The observed topo inhibitory effect might play an important role in the previously detected G_2/M arrest caused by compound **B1**[14].

4 Conclusions

In this work, the interaction of four cyclic chalcone analogs, substituted (*E*)-2-benzylidene-1-bensosuberone derivatives (**B1**-**B4**), with BSA and HSA, was studied by UV-Vis spectroscopy. The binding reaction with the cellular macromolecules was spontaneous, and in general, non-covalent, hydrophobic interaction played a significant role in the interactions. The observed differences in the spectral features suggest the importance of a spatial arrangement of the electron-rich moieties of the compounds. Among the investigated compounds, chalcone **B1** displayed the most pronounced cytotoxic effect against different tumor cell lines. Structure-activity relationship analyses of these toxicity studies also indicated the importance, in addition to the ring size, the presence of the electron-rich aromatic substituent[8, 13]. The interaction with cellular macromolecules, like DNA and proteins, might be one of the contributing vectors of the observed cytotoxicity of the compounds. Furthermore, the investigated compounds showed topoisomerase inhibitory activity, which might be an attribute of their previously reported biological effects. The obtained results provide additional knowledge on the pharmacological effect of the cyclic chalcone analogs.

Acknowledgments

The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary. This study was supported by the EFOP Operational Program (Grant No. EFOP-3.6.1-16-2016-00004).

References

[1] Rozmer Zs and Perjési P. Naturally occuring chalcones and their biological activities. Phytochemistry Reviews, 2016, **15**: 87-120. https://doi.org/10.1007/s11101-014-9387-8

[2] Dimmock JR, Elias DW, Beazely MA, *et al*. Bioactivities of chalcones. Current Medicinal Chemistry, 1999, **6**: 1125-1149.

[3] Ni L, Meng CQ and Sikorski JA. Recent advances in therapeutic compounds. Expert Opinion on Therapeutic Patents, 2004, **14**(12): 1669-1691. https://doi.org/10.1517/13543776.14.12.1669

[4] Go ML, Wu X and Liu XL. Chalcones: an update on cytotoxic and chemoprotective properties. Current Medicinal Chemistry, 2005, **12**(4): 483-499. https://doi.org/10.2174/0929867053363153

[5] Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. European Journal of Medicinal Chemistry, 2007, **42**(2): 125-137. https://doi.org/10.1016/j.ejmech.2006.09.019

[6] Mahapatra DK, Bharti SK and Asati V. Anti-cancer chalcones: Structural and molecular target perspectives. European Journal of Medicinal Chemistry, 2015, **98**: 69-114. https://doi.org/10.1016/j.ejmech.2015.05.004

[7] Mahapatra DK, Bharti SK and Asati V. Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. European Journal of Medicinal Chemistry, 2015, **101**: 496-524. https://doi.org/10.1016/j.ejmech.2015.06.052

[8] Dimmock JR, Kandepu NM, Nazarali AJ, *et al*. Conformational and quantitative structure-activity relationship study of cytotoxic 2-arylidenebenzocyclanones. Journal of Medicinal Chemistry, 1999, **42**(8): 1358-1366. https://doi.org/10.1021/jm9806695

[9] Perjési P, Nusser T, Tarczay Gy, *et al*. E-2-benzylidenebenzocycloalkanones. Stereostructure and NMR spectroscopic investigation. Journal of Molecular Structure, 1999, **479**: 13-19. https://doi.org/10.1016/S0022-2860(98)00805-9

[10] Tarczay Gy, Vékey K, Ludányi K, *et al*. E-2-benzylidenebenzocyclanones. II. IR and mass spectrometric investigations. Journal of Molecular Structure, 2000, **520**: 97-102. https://doi.org/10.1016/S0022-2860(99)00330-0

[11] Perjési P, Perjéssy A, Kolehmainen E, *et al*. E-2-benzylidenebenzocycloalkanones III. Studies on transmission of substituent effect on IR carbonyl stretching frequencies and 13C NMR chemical shifts of E-2-(X-benzylidene)-1-indanones. Comparison with the IR date of E-2-(X-benzylidene)-1-indanones, -tetralones, and -benzosuberones. Journal of Molecular Structure, 2004, **697**: 41-47. https://doi.org/10.1016/j.molstruc.2004.02.006

[12] Perjési P, Linnato J, Kolehmainen E, *et al*. E-2-Benzylidenebenzocycloalkanones. IV. Studies on transmission of substituent effects on 13C NMR chemical shifts of E-2-(X-benzylidene)-1-tetralones, and -benzosuberones. Comparison with the 13C NMR data of chalcones and E-2-(X-benzylidene)-1-indanones. Journal of Molecular Structure, 2005, **740**: 81-89. https://doi.org/10.1016/j.molstruc.2004.10.013

[13] Dimmock JR, Zello GA, Oloo EO, *et al*. Correlations between cytotoxicity and topography of some 2-arylidenebenzocycloalkanones determined by X-ray crystallography. *Journal of Medicinal Chemistry,* 2002, **45**: 3103-3111. https://doi.org/10.1021/jm010559p

[14] Rozmer Zs, Berki T and Perjési P. Different effects of two cyclic chalcone analogues on cell cycle of Jurkat T cells. Toxicology in Vitro, 2006, **20**: 1354-1362. https://doi.org/10.1016/j.tiv.2014.06.006

[15] Pilatova M, Varinska L, Perjési P, *et al*.In vitro antiproliferative and antiangiogenic effects of synthetic chalcone analogues. *Toxicology in Vitro,* 2010, **24**(5): 1347-1355. https://doi.org/10.1016/j.tiv.2010.04.013

[16] Tomeckova V, Perjési P, Guzy J, *et al*. Comparison of effect of selected synthetic chalcone analogues on mitochondrial outer membrane determined by fluorescence spectroscopy. Journal of Biochemical and Biophysical Methods, 2004, **61**: 135-141. https://doi.org/10.1016/j.jbbm.2004.04.010

[17] Tomeckova V, Stefanisinova M, Velika B, *et al.* investigation of interaction of some chalcones and cyclic chalcone analogues with outer mitochondrial membrane by UV-Vis and fluorescence spectroscopy. Spectral Analysis Review, 2013, **1**: 1-9. https://doi.org/10.4236/sar.2013.11001

[18] Perjési P, Das U, De Clercq ED, *et al*. Design, synthesis and antiproliferative activity of some 3-benzylidene-2,3-dihydro-1-benzopyran-4-ones which display selective toxicity for malignant cells. European Journal of Medicinal Chemistry, 2008, **43**: 839-845. https://doi.org/10.1016/j.ejmech.2007.06.017

[19] Perjési P, Kubalkova J, Chovanova Z, *et al.* Comparison of effects of some cyclic chalcone analogues on selected mitochondrial functions. Pharmazie, 2008, **63**: 899-903. https://doi.org/10.1691/ph.2008.8629

[20] Rozmer Zs, Marton E and Perjési P. (E)-2-Benzylidenecyclanones: part XIV. Study on interaction of some (E)-2-benzylidenebenzosuberone derivatives with calf thymus DNA by TLC and UV-Vis methods, a DNA cleavage study. Medicinal Chemistry Research, 2017, **26**: 2172-2179. https://doi.org/10.1007/s00044-017-1924-8

[21] Jiang YL. Design, synthesis and spectroscopic studies of resveratrol aliphatic ligands of human serum albumin. Bioorganic Medicinal Chemistry, 2008, **16**(12): 6406-6414. https://doi.org/10.1016/j.bmc.2008.05.002

[22] Da Silva JG, Despaigne AAR, Louro SRW, *et al*. Cytotoxic activity, albumin and DNA binding of new copper(II) complexes with chalcone-derived thiosemicarbazones. European Journal of Medicinal Chemistry, 2013, **65**: 415-426. https://doi.org/10.1016/j.ejmech.2013.04.036

[23] Zhang X, Song Q, Cao Z, *et al*.Study on the binding of chloramphenicol with bovine serum albumin by fluorescence and UV-Vis spectroscopy. Spectrochimica Acta A, 2013, **105**: 74-79. https://doi.org/10.1016/j.saa.2012.11.064

[24] Carter DC and Ho JX. Structure of serum albumin. Advances in Protein Chemistry, 1994, **45**: 153-203. https://doi.org/10.1016/s0065-3233(08)60640-3

[25] Yin BT, Yan CY, Peng XM, *et al*.Synthesis and biological evaluation of α-triazolyl chalcones as a new type of potential antimicrobial agents and their interaction with calf thymus DNA and human serum albumin. European Journal of Medicinal Chemistry, 2014, **71**: 148-159. https://doi.org/10.1016/j.ejmech.2013.11.003

[26] Naik KM and Nandibewoor ST. Spectroscopic studies on the interaction between chalcone and bovine serum albumin. Journal of Luminescence, 2013, **143**: 484-491. https://doi.org/10.1016/j.jlumin.2013.05.013

[27] Champoux JJ. DNA topoisomerases: structure, function and mechanism. Annual Review of Biochemistry, 2001, **70**: 369-413. https://doi.org/10.1146/annurev.biochem.70.1.369

[28] Heck MM and Ernshaw WC. Topoisomerase II: a specific marker for cell proliferation. Journal of Cell Biology, 1986, **103**: 2569-2581. https://doi.org/10.1083/jcb.103.6.2569

[29] Larsen AK, Escargueil AE and Skladanowski A. Catalytic topoisomerase II inhibitors in cancer therapy. Pharmacology Therapeutics, 2003, **99**: 167-181. https://doi.org/10.1016/S0163-7258(03)00058-5

[30] Hande KR. Topoisomerase II inhibitors. Update in Cancer Therapeutics, 2008, **3**: 13-26. https://doi.org/10.1016/j.uct.2008.02.001

[31] Hevener KE, Verstak TA, Lutat KE, *et al.* Recent developments in topoisomerase-targeted cancer chemotherapy. Acta Pharmaceutica Sinica B, 2018, **8**: 844-861. https://doi.org/10.1016/j.apsb.2018.07.008

[32] Stefanisinova M, Tomeckova V, Kozurkova M, *et al.* Study of DNA interactions with cyclic chalcone derivatives by spectroscopic techniques. Spectrochimica Acta A, 2011, **81**: 666-671. https://doi.org/10.1016/j.saa.2011.06.071

[33] Velika B, Tomeckova V, Fodor K, *et al.* (E)-Benzyli- denecycloalkanones XII. Kinetic measurement of bovine and human serum albumin interaction with selected chalcones and their cyclic chalcone analogues by UV spectrophotometry. Spectral Analysis Review, 2015, **3**: 1-8. https://doi.org/10.4236/sar.2015.31001

[34] Fodor K, Tomescova V, Kőszegi T, *et al*. (E)-2-Benzylidenecyclanones: Part VI. Solvent effect on the UV and fluorescence properties of some chalcones and their cyclic analogues. Interaction of 4-dimethylaminochalcones with bovine and human serum albumin: a UV–Vis study. Monatshefte für Chemie, 2011, **142**: 463-468. https://doi.org/10.1007/s00706-011-0463-0

[35] Perjési P. (E)-2-Benzylidenebenzocyclanones: part XIII. Light-induced in solution (E)/(Z) isomerization of some cyclic chalcone analogues. Effect of ring size on lipophilicity of geometric isomers. Monatshefte für Chemie, 2015, **146**: 1275-1281. https://doi.org/10.1007/s00706-015-1463-2

[36] Fukuda M, Nishio K, Kanzawa F, *et al.*Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells. Cancer Research, 1996, **56**: 789-793.

[37] Jun KY, KwonH, Park SE, *et al.* Discovery of dihydroxylated 2,4-diphenyl-6-thiophen-2-yl-pyridine as a non-intercalative DNA-binding topoisomerase II-specific catalytic inhibitor. European Journal of Medicinal Chemistry, 2014, **80**: 428-438. https://doi.org/10.1016/j.ejmech.2014.04.066

[38] Kang DH, Kim JS, Jung MJ, *et al.*New insight for fluoroquinophenoxazine derivatives as possibly new potent topoisomerase I inhibitor. Bioorganic Medicinal Chemistry Letters, 2008, **18**: 1520-1524. https://doi.org/10.1016/j.bmcl.2007.12.053

[39] Ma J, Fan Y, Si Q, *et al.*Interactions of three chalcones with human serum albumin revealed by spectroscopic techniques. Analytical Sciences, 2017, **33**: 493-498. https://doi.org/10.2116/analsci.33.493

[40] Mabry TJ, Markham KR and Thomas MB. The Ultraviolet spectra of flavones and flavonols. In: The systematic identification of flavonoids. Springer, Berlin, Heidelberg, 1970. https://doi.org/10.1007/978-3-642-88458-0_5.

[41] He XM and Carter DC. Atomic structure and chemistry of human serum albumin. Nature, 1992, **358**: 209-215. https://doi.org/10.1038/358209a0

[42] Sudlow G, Birkett DJ and Wade DN. The characterization of two specific drug binding sites on human serum albumin. Molecular Pharmacology, 1975, **11**(6): 824-832.

[43] Moriyama Y and Takeda K. Re-formation of the helical structure of human serum albumin by the addition of small amounts of sodium dodecyl sulfate after the disruption of the structure by urea. A comparison with bovine serum albumin. Lamgmuir, 1999, **15**: 2003-2008. https://doi.org/10.1021/la981442f

[44] Moriyama Y, Sato Y and Takeda K. Reformation of the helical structure of bovine serum albumin by the addition of small amounts of sodium dodecyl sulfate after the disruption of the structure by urea. Journal of Colloid Interface Sciences, 1993, **156**: 420-424. https://doi.org/10.1006/jcis.1993.1132

[45] Trnkova L, Bousova I, Kubicek V, *et al.* Binding of naturally occuring hydroxycinnamic acids to bovine serum albumin. Natural Science, 2010, **2**: 563-670. https://doi.org/10.4236/ns.2010.26071

[46] Chen X, Qian K and Chen Q. Comparison between loureirin A and cochinchinenin C on the interaction with human serum albumin. European Journal of Medicinal Chemistry, 2015, **93**: 492-500. https://doi.org/10.1016/j.ejmech.2015.02.025

[47] Cho HJ, Jung MJ, Woo S, *et al.*New benzoxanthone derivatives as topoisomerase inhibitors and DNA cross-linkers. Bioorganic Medicinal Chemistry, 2010, **18**: 1010-1017. https://doi.org/10.1016/j.bmc.2009.12.069

[48] Woessner RD, Mattern MR, Mirabelli CJ, *et al.* Proliferation- and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. Cell Growth Differentation, 1992, **2**: 209-214.