**Spectral Analysis Reviews, 2015, 3, 1-8**
Published Online January 2015 in SciRes. http://www.scirp.org/journal/sar
http://dx.doi.org/10.4236/sar.2015.31001

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

**Beáta Veliká[1#], Vladimíra Tomečková[1#], Krisztina Fodor[2], Ivan Kron[1], Pál Perjési[2†]**

[1]Department of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine UPJŠ, Trieda SNP 1, Košice, Slovakia
[2]Institute of Pharmaceutical Chemistry, University of Pécs, Pécs, Hungary
Email: †pal.perjesi@aok.pte.hu

Received 8 January 2015; accepted 23 January 2015; published 30 January 2015



## 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 kinet 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.

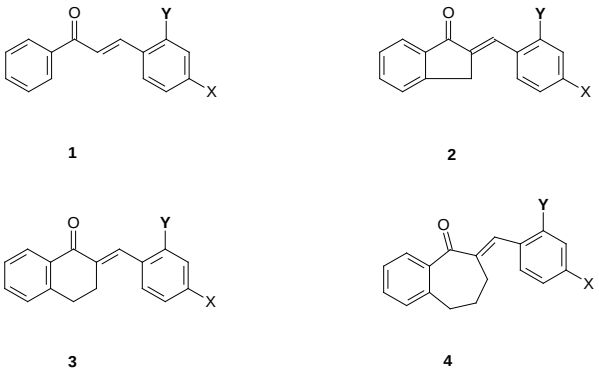
**How to cite this paper:** Veliká, B., *et al*. (2015) (*E*)-2-Benzylidenecycloalkanones XII. Kinetic Measurement of Bovine and Human Serum Albumine Interaction with Selected Chalcones and Their Cyclic Chalcone Analogues by UV Spectrophotometry. *Spectral Analysis Reviews*, **3**, 1-8. http://dx.doi.org/10.4236/sar.2015.31001

## 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 ($K_2HPO_4$), ethylenediamine tetraacetic acid (EDTA), magnesium chloride ($MgCl_2$), potassium chloride (KCl), potassium dihydrogen phosphate ($KH_2PO4$), sodium succinate ($NaOOCCH_2CH_2COONa$) 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), $MgCl_2$ (6 mM), TRIS HCl (4 mM), KCl (0.08 M), $K_2HPO_4$ (0.3 M) and $KH_2PO_4$ (0.3 M) was prepared using bidistilled water. UV-VIS spectra of the solutions ($2.5 \times 10^{-5}$ 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




a.) X=$OCH_3$, Y=H, b.) X=Y=$(OCH_3)_2$, c.) X=$N(CH_3)_2$, Y=H

**Figure 1.** Structure of 4-dimethylaminochalcone (**1c**), (*E*)-2-(2',4'-dimethoxybenzylidene)-1-indanone (**2b**), (*E*)-2-(4'-X-benzylidene)-1-tetralones (**3**) and (*E*)-2-(4'-X-benzylidene)-1-benzosuberones (**4a**, **4c**).

freshly prepared DMSO solutions ($2.5 \times 10^{-3}$ M) have been diluted with the respiration medium containing 1 mM sodium succinate to give a final concentration of 25 nmol/ml ($25 \times 10^{-6}$ 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 1.** UV-VIS absorption maxima and absorbances (A) of compounds **1c**, **4a**, **4c** and **2b** in the presence of BSA (c = 10 μg/ml).

| Compound | Without protein | | With BSA (c = 10 μg/ml) | | With BSA after 1 hour (c = 10 μg/ml) | |
|---|---|---|---|---|---|---|
| | λ (nm) | A | λ (nm) | A | λ (nm) | A |
| **1c** | 270 | 0.383 | 270 | 0.306 | 268 | 0.215 |
| | 427 | 0.551 | 429 | 0.529 | 429 | 0.391 |
| **4a** | 340 | 0.461 | 336 | 0.389 | 335 | 0.159 |
| **4c** | 271 | 0.485 | 268 | 0.376 | 272 | 0.232 |
| | 416 | 0.511 | 403 | 0.450 | 409 | 0.233 |
| **2b** | 260 | 0.279 | 261 | 0.303 | 233 | 0.605 |
| | 382 | 0.460 | 382 | 0.490 | 393 | 0.139 |

**Table 2.** UV-VIS absorption maxima and absorbances (A) of compounds **1c**, **4a**, **4c** and **2b** in the presence of HSA (c = 10 μg/ml).

| Compound | Without protein | | With HSA (c = 10 μg/ml) | | With HSA after 1 hour (c = 10 μg/ml) | |
|---|---|---|---|---|---|---|
| | λ (nm) | A | λ (nm) | A | λ (nm) | A |
| **1c** | 270 | 0.383 | 268 | 0.252 | 270 | 0.222 |
| | 427 | 0.551 | 427 | 0.522 | 429 | 0.368 |
| **4a** | 340 | 0.461 | 335 | 0.432 | 335 | 0.379 |
| **4c** | 271 | 0.485 | 267 | 0.399 | 271 | 0.289 |
| | 416 | 0.511 | 399 | 0.479 | 405 | 0.294 |
| **2b** | 260 | 0.279 | 260 | 0.333 | 233 | 0.594 |
| | 382 | 0.460 | 383 | 0.516 | 394 | 0.127 |

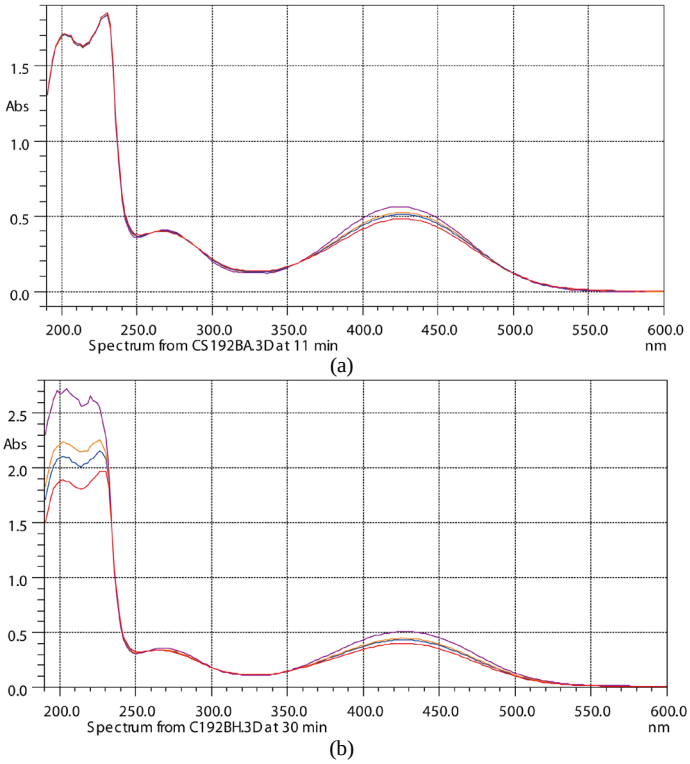



**Figure 2.** Spectrophotometric measurement of **1c** with BSA (a) and HSA (b) at the 0, 10, 30 and 60 minutes timepoints. (purple—0 minute, orange—10 minute, blue—30 minute, red—60 minute).

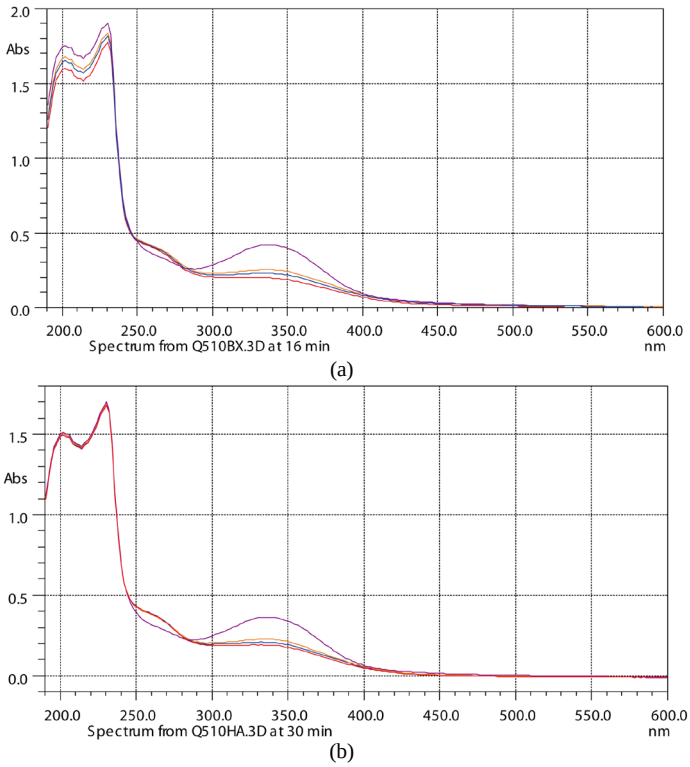
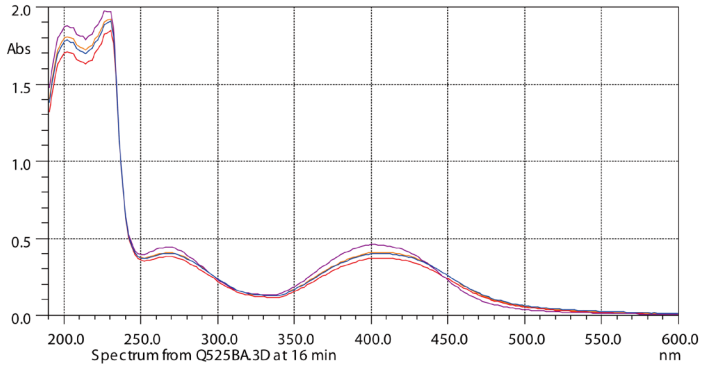
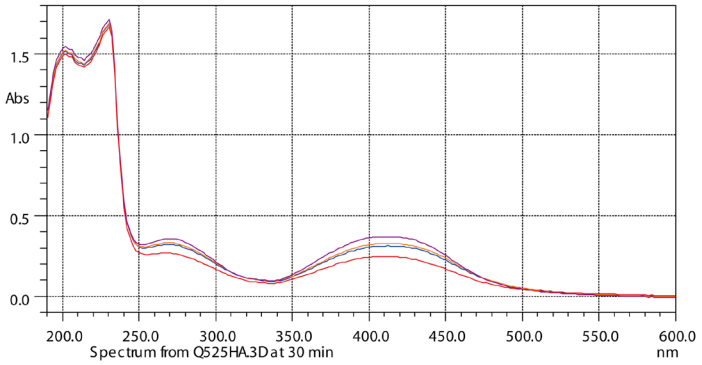



**Figure 3.** Spectrophotometric measurement of **4a** with BSA (a) and HSA (b) at the 0, 10, 30 and 60 minute timepoints. (purple—0 minute, orange—10 minute, blue—30 minute, red—60 minute).




(a)




(b)

**Figure 4.** Spectrophotometric measurement of **4c** 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].

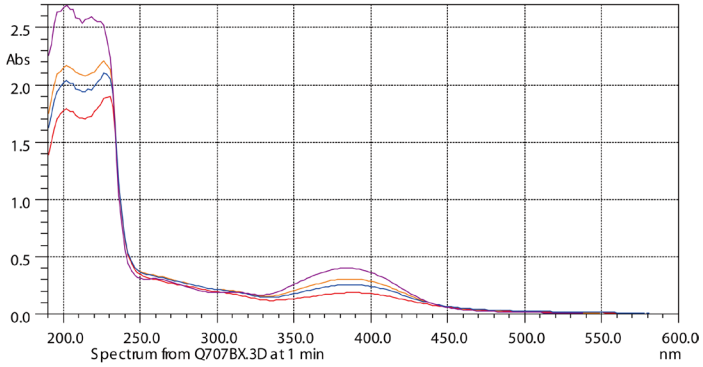
We determined the kinetics of interactions of the tested compounds (**1c, 2b, 4a, 4c**) with the two proteins (BSA, HSA) by measuring the absorbance of the compounds at the 0, 10, 30, 60 minute timepoints. As it is shown, interaction of the compounds with BSA and HSA resulted in a time-dependent hypochromic 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:

$$v = \Delta c/\Delta t \quad (1)$$

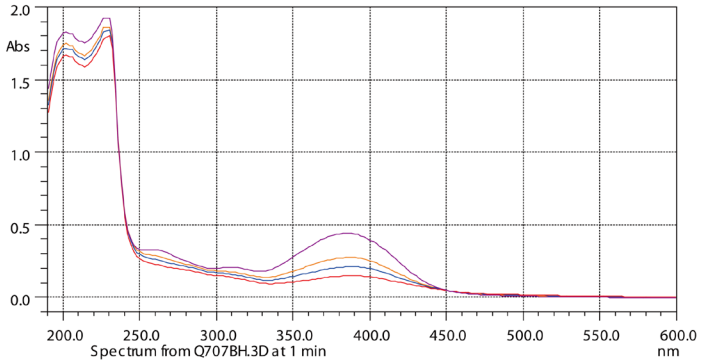
It was found that the compounds interact with the proteins with a slightly different rate. There is negligible difference between the rates of interaction of the studied compounds with BSA and HSA. Compound **4a** (with 4'-methoxy substituent) showed the highest rate of interaction with both proteins (**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-




(a)




(b)

**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).

| Compounds | Reaction with BSA (c = 10 μg/ml) | | | | Reaction with HAS (c = 10 μg/ml) | | | |
|---|---|---|---|---|---|---|---|---|
| | λ (nm) | A | | v (μmol/min) | λ (nm) | A | | v (μmol/min) |
| | | 0 min | 10 min | | | 0 min | 10 min | |
| **1c** | 427 | 0.565 | 0.528 | $0.164 \times 10^{-6}$ | 427 | 0.513 | 0.450 | $0.307 \times 10^{-6}$ |
| **4a** | 340 | 0.422 | 0.256 | $0.983 \times 10^{-6}$ | 340 | 0.366 | 0.229 | $0.936 \times 10^{-6}$ |
| **4c** | 416 | 0.441 | 0.404 | $0.209 \times 10^{-6}$ | 416 | 0.370 | 0.329 | $0.277 \times 10^{-6}$ |
| **2b** | 382 | 0.403 | 0.308 | $0.589 \times 10^{-6}$ | 382 | 0.444 | 0.276 | $0.932 \times 10^{-6}$ |

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

## Acknowledgements

The authors express their sincere thanks to the Austrian grant (ASO) SK-06/07-14/2007 and the Faculty of Mediccine Research Fund (University of Pécs) AOK-KA-213/20.

## References

[1] Huber, I., Zupkó, I., Kovács, I.J., Minorics, R., Gulyás-Fekete, G., Maász, G. and Perjési, P. (2015) Synthesis and Antiproliferative Activity of Cyclic Arylidene Ketones: Direct Comparison of Monobenzylidene and Dibenzylidene Derivatives. *Monatshefte für Chemie*.

[2] Rozmer, Z. and Perjési, P. (2014) Naturally Occuring Chalcones and Their Biological Activities. *Phytochemistry Reviews*. http://dx.doi.org/10.1007/s11101-014-9387-8

[3] Go, M.L., Wu, X. and Liu, X.L. (2005) Chalcones: An Update on Cytotoxic and Chemoprotective Properties. *Current Medicinal Chemistry*, **12**, 483-499. http://dx.doi.org/10.2174/0929867053363153

[4] Dimmock, J.R., Kandepu, N.M., Nazarali, A.J., Kowalchuk, T.P., Motaganahalli, N., Quail, J.W., Mykytiuk, P., Audette, G.F., Prasad, L., Perjési, P., Allen, T.M., Santos, C.L., Szydlowski, J., De Chercq, E. and Balzarini, J. (1999) Conformational and Quantitative Structure-Activity Relationship Study of Cytotoxic 2-arylidenebenzocycloalkanones. *Journal of Medicinal Chemistry*, **42**, 1358-1366. http://dx.doi.org/10.1021/jm9806695

[5] Dimmock, J.R., Zello, G.A., Oloo, E.O., Quail, J.W., Kraatz, H.-B., Perjési, P., Aradi, F., Takács-Novák, K., Allen, T.M., Santos, C.L., Balzarini, J., DeClerq, E. and Stables, J.P. (2002) Correlations between Cytotoxicity and Topography of Some 2-arylidenebenzocycloalkanones Determined by X-Ray Crystallography. *Journal of Medicinal Chemistry*, **45**, 3103-3111. http://dx.doi.org/10.1021/jm010559p

[6] Perjési, P., Das, U., De Clercq, E., Balzarini, J., Kawase, M., Sakagami, H., Stables, J.P., Loránd, T., Rozmer, Z. and Dimmock, J.R. (2008) 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*, **43**, 839-845. http://dx.doi.org/10.1016/j.ejmech.2007.06.017

[7] Fodor, K., Tomeckova, V., Kőszegi, T., Kron, I. and Perjési, P. (2011) (E)-2-Benzylidenebenzocyclanones: 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—Chemical Monthly*, **142**, 463-468. http://dx.doi.org/10.1007/s00706-011-0463-0

[8] Tomečková, V., Perjési, P., Guzy, J., Kušnír, J., Chovanova, Z., Chavkova, Z. and Marekova, M. (2004) Comparison of Effect of Selected Synthetic Chalcone Analogues on Mitochondrial Outer Membrane Determined by Fluorescence Spectroscopy. *Journal of Biochemical and Biophysical Methods*, **6**, 135-141. http://dx.doi.org/10.1016/j.jbbm.2004.04.010

[9] Tomeckova, V., Poskrobova, M., Stefanisinova, M. and Perjési, P. (2009) Some Fluorescence Properties of Dimethylaminochalcone and Its Novel Cyclic Analogues. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **74**, 1242-1246. http://dx.doi.org/10.1016/j.saa.2009.09.048

[10] Pengfei, W. and Shikang, W. (1994) A Study on the Spectroscopy and Photophysical Behaviour of Chalcone Derivatives. *Journal of Photochemistry and Photobiology A*: *Chemistry*, **77**, 127-131.

[11] Wang, P.F. and Wu, S.K. (1995) Spectroscopy and Photophysics of Bridged Enone Derivatives: Effect of Molecular Structure and Solvent. *Journal of Photochemistry and Photobiology A*: *Chemistry*, **86**, 109-113. http://dx.doi.org/10.1016/1010-6030(94)03921-G

[12] Wang, H., Mei, M., Xie, H.Z., Fang, Y., Zhang, X.H. and Wu, S.K. (2003) A Study on the Fluorescence Quenching of Modified $\beta$-Cyclodextrin by Transition Metal Ions in Different Solvents. *ARKIVOC*, **2**, 173-181.

[13] Fayed, T.A. and Awad, M.K. (2004) Dual Emission of Chalcone-Analogue Dyes Emitting in the Red Region. *Chemical Physics*, **303**, 317-326. http://dx.doi.org/10.1016/j.chemphys.2004.06.023

[14] Perjési, P., Nusser, T., Tarczay, Gy. and Sohár, P. (1999) E-2-Benzylidenebenzocycloalkanones. Stereostructure and NMR Spectroscopic Investigation. *Journal of Molecular Structure*, **479**, 13-19. http://dx.doi.org/10.1016/S0022-2860(98)00805-9

[15] Perjési, P., Perjessy, A., Kolehmainen, E., Ősz, E., Samalikova, M., Linnanto, J. and Virtanen, E. (2004) E-2-Benzylidenebenzocyclanones III. Studies on Transmission of Substituent Effects on IR Carbonyl Stretching Frequencies and $^{13}$C NMR Chemical Shifts of E-2-(X-benzylide)-1-Indanones. Comparison of the IR Data of E-2-(X-benzylide)-1-Indanones, -Tetralones and -Benzosuberones. *Journal of Molecular Structure*, **697**, 41-47. http://dx.doi.org/10.1016/j.molstruc.2004.02.006

[16] Perjési, P., Linnanto, J., Kolehmainen, E., Ősz, E. and Virtanen, E. (2005) E-2-Benzylidenebenzocycloalkanones IV. Studies on Transmission of Substituent Effects on $^{13}$C NMR Chemical Shifts of E-2-(X-benzylidene)-1-tetralones, and -benzosuberones. Comparison with the $^{13}$C NMR Data of Chalcones and E-2-(X-benzylidene)-1-Indanones. *Journal of Molecular Structure*, **740**, 81-89. http://dx.doi.org/10.1016/j.molstruc.2004.10.013

[17] Wolfbeis, O.S., Begum, M. and Geiger, H. (1984) Fluorescence Properties of Hydroxyl- and Methoxyflavones and the Effect of Shift Reagents. *Zeitschrift für Naturforschung*, **39**, 231-237.

[18] Asiri, A.M. (2003) Synthesis and Absorption Spectral Properties of Bis-Methine Dyes Exemplified by 2,5-Bis-Arylidene-1-Dicyanomethylene-Cyclopentanes. *Bulletin of the Korean Chemical Society*, **24**, 426-430. http://dx.doi.org/10.5012/bkcs.2003.24.4.426

[19] Zhang, C.H., Chen, Z.B. and Jiang, Y.B. (2004) Intramolecular Charge Transfer Dual Fluorescence of p-dimethylaminobenzoates. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy*, **60**, 2729-2732.

http://dx.doi.org/10.1016/j.saa.2004.01.011

[20] Reichardt, C. (1994) Solvatochromic Dyes as Solvent Polarity Indicators. *Chemical Reviews*, **94**, 2319-2358. http://dx.doi.org/10.1021/cr00032a005

[21] Wang, H., Borguet, E. and Eisenthal, K.B. (1997) Polarity of Liquid Interfaces by Second Harmonic Generation Spectroscopy. *Journal of Physical Chemistry A*, **101**, 713-718. http://dx.doi.org/10.1021/jp962074w

[22] Marder, S.R., Cheng, L.T., Tiemann, B.G., Friedli, A.C., BlanchardDesce, M., Perry, J.W. and Skindhoj, J. (1994) Large First Hyperpolarizabilities in Push-Pull Polyenes by Tuning of the Bond Length Alternation and Aromaticity. *Science*, **263**, 511-514. http://dx.doi.org/10.1126/science.263.5146.511

[23] Domagalska, B.W., Wilk, K.A. and Zielinski, R. (2006) Spectroscopic and Electrochemical Properties of New Amphiphilic Donor-Acceptor Conjugated Polyenes. *Journal of Photochemistry and Photobiology A*: *Chemistry*, **184**, 193-203. http://dx.doi.org/10.1016/j.jphotochem.2006.04.015

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

**Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.**