https://doi.org/10.15255/KUI.2023.013
KUI-56/2023
Original scientific paper
Received March 1, 2023
Accepted March 21, 2023

DNA Binding Affinity Assessment of Xanthene Compounds: *In Vitro* and *In Silico* Approach

E. Veljović,[a] *A. Osmanović*,[a] *M. Salihović*,[a*] *N. Ljubijankić*,[b] *S. Begić*,[b] and *S. Špirtović-Halilović*[a]

[a] University of Sarajevo-Faculty of Pharmacy, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina

[b] University of Sarajevo-Faculty of Science, Zmaja od Bosne 33-35, Sarajevo 71 000, Bosnia and Herzegovina

Abstract

Xanthene derivatives are an important class of heterocyclic compounds with a wide spectrum of pharmacological activities. In our previous investigations, we found the good antiproliferative activity of two xanthene derivatives, with minimal toxicity investigated by *in vitro* tests. In this study, we tested the interaction of compound 1 (powerful potent antiproliferative compound) with calf thymus DNA (CT-DNA) under physiological conditions by spectrophotometric titration. The probable prediction of binding and the type of interaction forces involved in the arrangement between xanthene derivatives and CT-DNA were explored also through molecular docking studies.

The results indicated that compound 1 interacts with CT-DNA by grove binding. The binding constant was found to be $2.5 \cdot 10^4$ M^{-1} indicating the non-covalent binding of compound 1 to CT-DNA. Docking study results proposed possible binding modes, with binding energies of -9.39 and -8.65 kcal mol^{-1} for compounds 1 and 2, respectively, which supported previously obtained *in vitro* results for antiproliferative activity.

In addition to experimental investigation, density functional theory (DFT) calculation with B3LYP/6-31G*, B3LYP/6-31G**, and B3LYP/6-31+G* levels of theories was performed on compounds 1 and 2 to obtain optimised geometry, spectroscopic and electronic properties.

These studies could help in understanding the mechanisms of toxicity, resistance, side effects of xanthene derivatives, and their binding action mechanism to DNA.

Keywords

DNA binding, docking, DFT, xanthene

1 Introduction

The interaction of small molecules with CT-DNA represents one of the ways of testing their biological activities. Given that DNA plays a significant role in biological replication and protein synthesis, many studies have shown that CT-DNA is a favourite target for many small molecules.[1] In general, a double stranded CT-DNA could bind to molecules by direct intercalation, groove binding or electrostatic interactions. The manner of their interaction with CT-DNA is examined by different methods. Spectrophotometric titration as one of them correlates with the way in which these molecules interact with DNA, whereby the insertion of small molecules into adjacent base pairs provides a number of ways in which intercalation and groove binding play a key role.[2,3] This study was undertaken to explore the molecular mechanisms of interaction of xanthene derivatives with CT-DNA.

Xanthene derivatives are biologically active substances displaying broad therapeutic applications, such as anticancer agents,[4] antimicrobial,[5] immunomodulating,[6] antioxidant,[7] antiinflammatory,[8] and other biological activities.[9–11]

To investigate the mechanism of binding to CT-DNA using spectroscopic methods, we chose compound 1, which in our previous tests showed the best antiproliferative activity against cells of cervical carcinoma (HeLa) and adenocarcinomic human alveolar basal epithelial cells (A549). Molecular docking analyses were performed for compound 1 and compound 2, which in our previous investigations showed the best antiproliferative activity against cells of colorectal adenocarcinoma (SW 620) and liver hepatocellular cells (HEpG2).[12] The experimental results of binding to CT-DNA by using spectrophotometric titration were corroborated with the results from molecular docking.

Density functional theory (DFT) is a very important and frequently used tool in studies on biological systems. A varied range of calculations using DFT helps to develop a close relationship between theoretical and experimental data by giving clues related to molecular geometry, electric and spectroscopic properties. These techniques have become much reliable in predicting properties of molecules with high accuracy.[13]

2 Experimental

2.1 Synthesis of xanthen-3-one derivatives

In our previous work[12], we synthesised and confirmed structure 4′-trifluoromethyl-2,6,7-trihydroxy-xanthen-3-one and 9-(2′-chloro-6′-fluorophenyl)-2,6,7-trihydroxy-xanthen-3-one derivatives from 1,2,4-triacetoxybenzene and benzaldehyde under acidic alcoholic conditions. After a

* Corresponding author: Professor Mirsada Salihović
Email: mirsada.salihovic@ffsa.unsa.ba

two-fold Friedel-Crafts alkylation, intermediate A was obtained. For accomplishing the transformation, a single trihydroxy benzene moiety of (A) had to be oxidised using potassium peroxodisulphate to the corresponding p-benzoquinone. To avoid decomposition of potassium peroxodisulphate, the reaction of oxidation occurred at 80 °C. Benzoquinone intermediate (B) subsequently underwent a cyclocondensation reaction to the xanthenone fragment. To remove potassium peroxodisulphate after completed oxidation, refluxed suspension was poured onto ice water and filtered. The residue was dried under vacuum at 60 °C. The synthetic pathway was adapted from.[14,15]

2.2 DNA Binding Studies

The electronic absorption spectra were recorded on a Perkin Elmer lambda 35 spectrophotometer, and carried out in phosphate buffer (pH 7.42) at room temperature using a DMSO solution of the compound 1.[16] Calf-thymus DNA (CT-DNA) (Sigma, Germany) was dissolved in phosphate buffer and the its purity was checked from the ratio of the absorbance values at 260 and 280 nm. The experimental results showed that the ratio was 1.8, which provided a good estimation of the purity of the DNA. The CT-DNA concentration was determined from its absorbance intensity at 260 nm using a molar extinction coefficient value 6 600 $\mathrm{l\,mol^{-1}\,cm^{-1}}$.[17] Spectrophotometric titration experiments were performed by keeping the compound 1 concentration constant while varying the CT-DNA concentration.

2.3 Molecular docking analysis

The molecular docking study was performed in YASARA Structure 19.12.14 software,[18,19] using AutoDock 4.2 protocol.[20] The crystal structure of target molecule, 6-bp DNA in complex with ellipticine (PDB ID: 1Z3F), was downloaded from RCSB Protein Data Bank (https://www.rcsb.org/). The structure of the target was prepared by removing ligand, water molecules, adding polar hydrogen atoms, and optimising in the AMBER03 force field.[21] The search area box was set around the space that was previously occupied by the ellipticine, as the cuboid shape. The 3D structures of the xanthene molecules were prepared and geometries optimised by the DFT, B3LYP/6-31+G* level of theory, using Spartan 14 software program.[22] The Lamarckian genetic algorithm was employed with the following parameters: 150 docking runs per molecule, with a maximum of 15,000,000 energy evaluations and 27,000 generations for each run, with a grid point spacing of 0.375 Å, providing this way the lowest energy docked poses.

2.4 Density function theory (DFT) study

Quantum chemical computations were carried out in Spartan 14,[22] with full geometry optimisations in order to investigate the theoretical-experimental consistency. Geometry optimisation was carried out at B3LYP/6-31G*, B3LYP/6-31G**, and B3LYP/6-31+G* levels of theory. By computing global chemical reactivity indices, models can be used to clarify the reactivity of compounds 1 and 2. The DFT-calculated chemical reactivity descriptors were as follows:

- Total energy (E);
- Chemical hardness (η), which may be determined using Eq. (1), is related to the stability and reactivity of a chemical system;

$$\eta = (E_{\mathrm{LUMO}} - E_{\mathrm{HOMO}})/2 \quad (1)$$

- Electronic chemical potential (μ), which can be derived using Eq. (2), is the negative of a molecule's electronegativity;

$$\mu = (E_{\mathrm{LUMO}} + E_{\mathrm{HOMO}})/2 \quad (2)$$

- The electronic chemical potential and chemical hardness are used to calculate the global electrophilicity index (ω), as given in Eq. (3).[23]

$$\omega = (\mu^2/2\eta) \quad (3)$$

3 Results and discussion

3.1 DNA Binding Studies

Spectroscopic titration is a universal method used to investigate the binding mode of DNA with small molecules. The absorption spectra of compound 1 were conducted in the absence and presence of CT-DNA in the range of 200–600 nm. The titration was carried out by gradual addition of CT-DNA ($0.00 - 42.9 \cdot 10^{-6}$ mol $\mathrm{l^{-1}}$) to the compound 1 of fixed concentration ($5.77 \cdot 10^{-5}$ mol $\mathrm{l^{-1}}$) (Fig. 1). The binding constant K_b was calculated using Wolfe–Shimmer Eq. (4) through a plot of [CT-DNA]/ ($\varepsilon_a - \varepsilon_f$) *versus* [CT-DNA], where ε_a, ε_f, and ε_b are apparent extinction coefficients corresponding to A_{obs}/[compound 1], free compound 1, and completely bound form, respectively, and [CT-DNA] is the concentration of DNA in base pairs.[24]

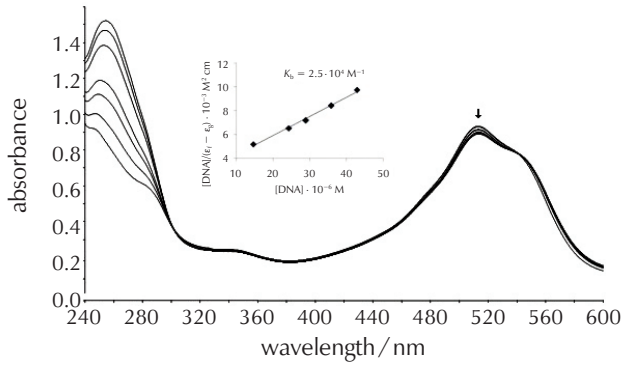


Fig. 1 – UV/vis absorption spectra of $5.77 \cdot 10^{-5}$ mol $\mathrm{l^{-1}}$ compound 1 with increasing concentration of CT DNA ($0.00 - 42.9 \cdot 10^{-6}$ mol $\mathrm{l^{-1}}$) in phosphate buffer (pH 7.42)

Slika 1 – UV/vis apsorpcijski spektri $5{,}77 \cdot 10^{-5}$ mol $\mathrm{l^{-1}}$ spoja 1 s rastućom koncentracijom CT DNA ($0{,}00 - 42{,}9 \cdot 10^{-6}$ mol $\mathrm{l^{-1}}$) u fosfatnom puferu (pH 7,42)

$$\frac{[\mathrm{DNA}]}{[\varepsilon_a - \varepsilon_b]} = \frac{[\mathrm{DNA}]}{[\varepsilon_b - \varepsilon_f]} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)} \quad (4)$$

The value of the binding constant (K_b) is given by the ratio of slope to the intercept, and was calculated on the basis of decrease of absorptions at 513 nm. A "hypochromic effect" was observed for increasing concentration of CT-DNA, while no noticeable change in the absorption band position of the compound 1 was observed. The binding constant was found to be $2.5 \cdot 10^4$ M^{-1} and indicated non-covalent binding of compound 1 to CT-DNA. As there were no bathochromic or hypsochromic shifts of absorption bands, it could be concluded that compound 1 displayed groove binding interactions to CT-DNA.[25]

3.2 DNA Results of docking study

Docking studies were carried out to investigate xanthene DNA binding affinity *in silico*. Results are presented in Table 1. Binding energy corresponds to the stability of the formed complex. The lower the energy, the more stable is the ligand-target complex, and the better affinity of the small molecule toward the target. Dissociation constant indicates the concentration at which half of the target molecules are occupied by the ligand. Again, the lower the constant, the less compound is needed to achieve the wanted effect. Comparing the docking results for compounds 1 and 2, lower binding energy for compound 1 indicates better affinity toward building complex with the DNA. Compound 2 binding energy is close in value, with difference of only 0.74 kcal mol^{-1}. However, having analysing the values of dissociation constant, apparently compound 2 requires 3.5 times the amount of compound 1 to achieve the same effect.

Table 1 – Results of docking studies for compounds 1 and 2
Tablica 1 – Rezultati docking studije za spojeve 1 i 2

Compound	Binding energy / kcal mol^{-1}	Dissociation constant / μM	H-bond interactions
1	−9.39	0.13	2-OH with O from phosphate group; 6-OH and 7-OH with O from the sugar
2	−8.65	0.46	2-OH with O from phosphate group; 6-OH with O from the sugar

It is interesting to notice that both compounds occupied the binding pocket in the same manner, owing to its xanthene planar scaffold, with aryl substituent oriented in the same direction (Figs. 2 and 3). Numerous π-π stacking interactions were observed between xanthene ring and guanine and cytosine residues (not shown in Figs. 2 and 3 due to clarity purposes). Two hydrogen bonds were also identical for both compounds, C_2−OH group with O from phosphate, and C_6−OH with O from the sugar. Moreover, compound 1 formed an additional bond between C_7−OH and the sugar's oxygen, which further explained the lower binding energy of 1 and better affinity toward DNA.

Similar findings were observed in other studies,[26,27] where planar part of the molecules (benzimidazole and quinoline) was found to form π-π stacking interactions with adenine and guanine (benzimidazole), and guanine and cytosine (quinoline) residues. In addition, H-bonds with oxygen atom of the residue via nitrogen (NH) atom of the ligand were observed.

The results of the molecular docking study support the *in vitro* results for xanthene compounds where 1 was the most potent antiproliferative agent, and 2 was the second best among 12 tested xanthene compounds.[12]

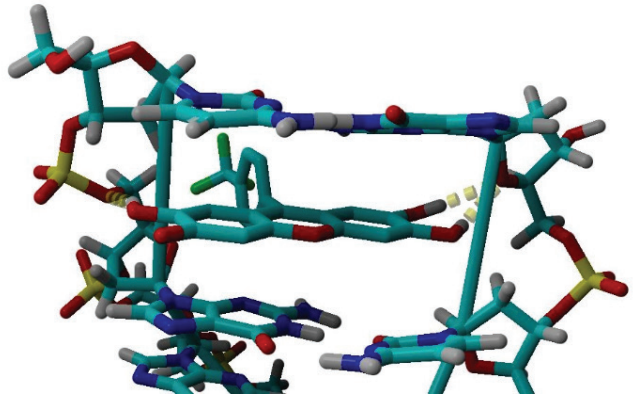


Fig. 2 – Binding of compound 1 to DNA (pdb ID: 1Z3F)
Slika 2 – Vezanje spoja 1 na DNA (pdb ID: 1Z3F)

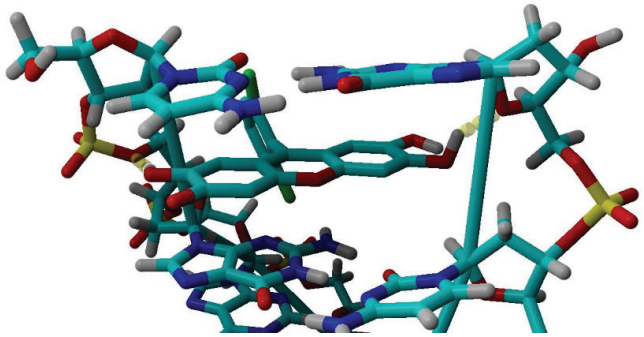


Fig. 3 – Binding of compound 2 to DNA (pdb ID: 1Z3F)
Slika 3 – Vezanje spoja 2 na DNA (pdb ID: 1Z3F)

3.3 DNA results of DFT study

Table 2 shows the calculated global physicochemical properties. The chemical hardness (η) of a compound determines how stable or reactive it is. As a result, compound 2 is harder and less reactive than compound 1.

The electronic chemical potential (μ) is defined as a molecule's negative electronegativity, which is represented as

Table 1 – Global chemical reactivity indices of compounds 1 and 2
Tablica 1 – Globalni indeksi kemijske reaktivnosti spojeva 1 i 2

	Compound 1			**Compound 2**		
	B3LYP/6-31G*	B3LYP/6-31G**	B3LYP/6-31+G*	B3LYP/6-31G*	B3LYP/6-31G**	B3LYP/6-31+G*
E/au	−1444.39	−1444.41	−1444,45	−1665.82	−1665.84	−1665.88
E_{HOMO}/eV	−5.52	−5.52	−5.95	−4.52	−4.52	−4.99
E_{LUMO}/eV	−2.48	−2.48	−2.90	−2.74	−2.74	−3.19
Dipole moment/debye	7.32	7.32	7.67	8.8	8.79	9.35
μ/eV	−4.00	−4.00	−4.43	3.63	3.63	4.09
η/eV	1.52	1.52	1.53	0.89	0.89	0.90
ω/eV	5.26	5.26	6.41	7.40	7.40	9.28
conformers	8	8	8	16	16	16
tautomer	1	1	1	1	1	1

the tendency of electrons to escape from an equilibrium system.[23] The molecule is less stable or reactive the higher the electrical chemical potential. Compound 1 is thus more reactive than compound 2.

Results of both chemical hardness and electronic chemical potential support results obtained by molecular docking study, where compound 1, as harder and more reactive than 2, is more efficient in binding to DNA. This is expressed as 3.5 lower dissociation constant value of 1 compared to 2 (0.13 μM for 1, compared to 0.46 μM for 2).

Electrophilicity (ω) measures a species' propensity or capacity to accept electrons.[23] Compound 1 is a stronger nucleophile because of its lower ω value, but compound 2 is a stronger electrophile because of its higher ω value.

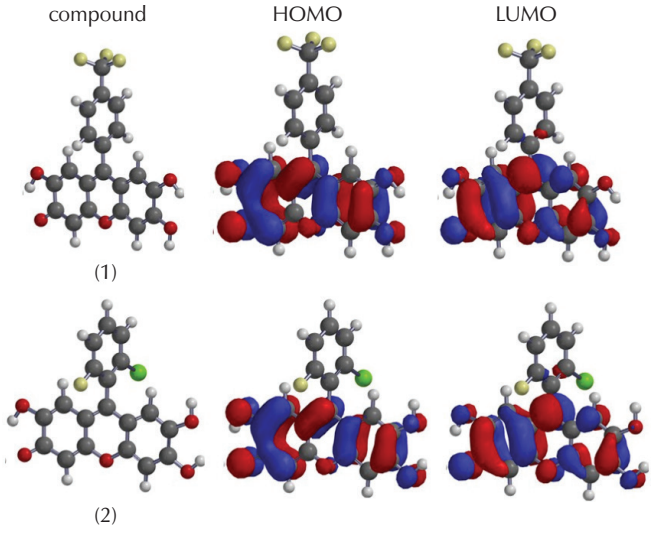
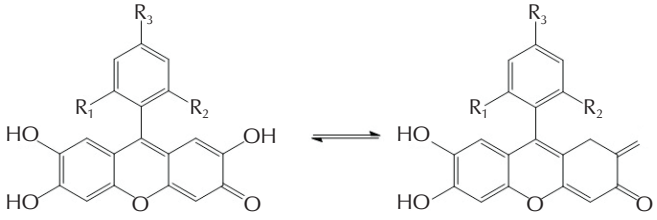


Fig. 4 – Schematic representation of HOMO and LUMO molecular orbital of compounds 1 and 2 at the B3LYP/6-31G** level
Slika 4 – Shematski prikaz HOMO i LUMO molekularne orbitale spojeva 1 i 2 na razini B3LYP/6-31G**

The positive charge and the distance between the charges combine to form the electric dipole moment. Table 2 also shows the value of the dipole moment of molecules. A large dipole moment indicates a large charge separation. In chemistry, the electrical dipole moment is useful for explaining many intermolecular interactions because the most interesting ones are usually dipoles.

Fig. 4 shows diagrams of the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals of compounds 1 and 2.

The results show a pronounced tautomer in the structure between the OH and the C=O group for compounds 1 and 2, as illustrated in Fig. 5. Because of the stable ring, the enol form is preferred.



Compound 1: R_1=H; R_2=H; R_3=CF_3
Compound 2: R_1=F; R_2=Cl; R_3=H

Fig. 5 – Tautomerisation of compounds 1 and 2
Slika 5 – Tautomerizacija spojeva 1 i 2

4 Conclusion

The present study delivers important information about binding mechanisms of xanthen-3-on derivatives with CT-DNA using spectrophotometric and molecular docking methods. Results of DNA binding studies using spectroscopic titration indicate non-covalent binding of compound 1 to CT-DNA, which was confirmed by the results of docking studies. In addition, the results of DFT studies

showed xanthene compounds of minor reactivity, which enables further testing of these compounds as potential antitumor drugs.

List of abbreviations and symbols
Popis kratica i simbola

DNA – deoxyribonucleic acid
– deoksiribonukleinska kiselina

CT-DNA – circulating tumour DNA
– DNK cirkulirajućeg tumora

DFT – density functional theory
– teorija funkcionale gustoće

DMSO – dimethyl sulfoxide
– dimetil sulfoksid

HOMO – highest occupied molecular orbital
– najviša zauzeta molekularna orbitala

LUMO – lowest unoccupied molecular orbital
– najniža nezauzeta molekularna orbitala

References
Literatura

1. *L. M. Tumir, I. Zonjić, K. Žuna, S. R. Brkanac, M. Jukić, A. Huđek, M. R. Stojković,* Synthesis, DNA/RNA-interaction and biological activity of benzo [k, l] xanthene lignans. Bioorg. Chem. **104** (2020) 104190, doi: https://doi.org/10.1016/j.bioorg.2020.104190.
2. *Y. Fu, Y. Xu, Y. Liu, Y. Wang, J. Chen, X. Wang*, Synthesis, Characterization and Anticancer Efficacy Evaluation of Benzoxanthone Compounds toward Gastric Cancer SGC-7901, Mol. **27** (6) (2022) 1970, doi: https://doi.org/10.3390/molecules27061970.
3. *H. F. Wang, H. Yan, X. Gao, B. Niu, R. Guo, L. Wei, N. Tang,* Cytotoxic activity and DNA-binding properties of isoeuxanthone derivativesm, Chem. Pharm. Bull. **62** (3) (2014) 260–266, doi: https://doi.org/10.1248/cpb.c13-00789.
4. *S. S. Liou, W. L. Sheih, T. H. Cheng, S. J. Won, C. N. Lin*, γ-Pyrone compounds as potential anticancer drugs, J. Pharm. Pharmacol. **45** (9) (1993) 791–794, doi: https://doi.org/10.1111/j.2042-7158.1993.tb05686.x.
5. *M. Kaya, E. Demir, H. Bekci*, Synthesis, characterization and antimicrobial activity of novel xanthene sulfonamide and carboxamide derivatives, J. Enzyme Inhib. Med. Chem. **28** (5) (2013) 885–893, doi: https://doi.org/10.3109/14756366.2012.692087.
6. *B. C. Baguley, L. M. Ching,* Immunomodulatory actions of xanthenone anticancer agents, BioDrugs **8** (2) (1997) 119–127, doi: https://doi.org/10.2165/00063030-199708020-00005.
7. *A. E. Hay, M. C. Aumond, S. Mallet, V. Dumontet, M. Litaudon, D. Rondeau, P. J. Richomme*, Antioxidant Xanthones from *Garcinia vieillardii*, Nat. Prod. **67** (2004) 707–709, doi: https://doi.org/10.1021/np0304971.
8. *Z. Karimi-Jaberi, M. M. Hashemi*, One step synthesis of 14-alkyl-or aryl-14 H-dibenzo [a, j] xanthenes using sodium hydrogen sulfate as catalyst, Monatshefte für Chemie-Chemical Monthly **139** (2008) 605–608, doi: https://doi.org/10.1007/s00706-007-0786-z.
9. *S. Laphookhieo, J. K. Syers, R. Kiattansakul, K. Chantrapromma*, Cytotoxic and antimalarial prenylated xanthones from *Cratoxylum cochinchinense*, Chem. Pharm. Bull. **54**(5) (2006) 745–747, doi: https://doi.org/10.1248/cpb.54.745.
10. *Q. B. Han,H. L. Tian, N. Y. Yang, C. F. Qiao, J. Z. Song, D. C. Chang, H. X. Xu*, Polyprenylated Xanthones from Garcinia lancilimba Showing Apoptotic Effects against HeLa-C3 Cells, Chem. Biodivers. **5** (12) (2008) 2710–2717, doi: https://doi.org/10.1002/cbdv.200890225.
11. *S. L. Niu, Z. L. Li, F. Ji, G. Y. Liu, N. Zhao, X. Q. Liu, H. M. Hua*, Xanthones from the stem bark of *Garcinia bracteata* with growth inhibitory effects against HL-60 cells, Phytochem. **77** (2012) 280–286, doi: https://doi.org/10.1016/j.phytochem.2012.01.010.
12. *E. Veljović, S. Špirtović-Halilović, S. Muratović, A. Osmanović, S. Haverić, A. Haverić, M. Hadžić, M. Salihović, M. Malenica, A. Šapčanin, D. Završnik*. Antiproliferative and genotoxic potential of xanthen-3-one derivatives, Acta Pharm. **69** (2019) 683–694, doi: https://doi.org/10.2478/acph-2019-0044.
13. *G. S. Suresh Kumar, A. Antony MuthuPrabhu, P. G. Seethalashmi, N. Bhuvanesh, S. Kumaresan*, Self-catalyzed syntheses, structural characterization, DPPH radical scavenging-, cytotoxicity-, and DFT studies of phenoxyaliphatic acids of 1,8-dioxo-octahydroxanthene derivatives, J. Mol. Struct. **1059** (2014) 51–60, doi: https://doi.org/10.1016/j.molstruc.2013.11.016.
14. *E. Veljović, S. Špirtović-Halilović, S. Muratović, L. Valek Žulj, S. Roca, S. Trifunović, D. Završnik*, 9-Aryl substituted hydroxylated xanthen-3-ones: Synthesis, structure and antioxidant potency evaluation, Croat. Chem. Acta **88** (2) (2015) 121–127, doi: https://doi.org/10.5562/cca2595.
15. *P. Schrick, K. Geick, S. R. Waldvogel,* Reliable synthesis of 9-aryl-substituted 2, 6, 7-trihydroxyxanthen-3-ones, Synth. **2008** (14) (2008) 2211–2216, doi: https://doi.org/10.1055/s-2008-1078447.
16. *A. Ilangovan, K. Anandhan, K. M. Prasad, P. Vijayakumar, R. Renganathan, D. A. Ananth, T. Sivasudha*, Synthesis, DNA-binding study, and antioxidant activity of 14-aryl-14 H-dibenzo [a, j] xanthene derivatives, Med. Chem. Res. **24** (2015) 344–355, doi: https://doi.org/10.1007/s00044-014-1124-8.
17. *K. A. Meadows, F. Liu, J. Sou, B. P. Hudson, D. R. McMillin,* Spectroscopic and photophysical studies of the binding interactions between copper phenanthroline complexes and RNA, Inorg. Chem. **32** (13) (1993) 2919–2923, doi: https://doi.org/10.1021/ic00065a020.
18. *E. Krieger, G. Vriend*, New ways to boost molecular dynamics simulations, J. Comput. Chem. **36** (13) (2015) 996–1007, doi: https://doi.org/10.1002/jcc.23899.
19. *E. Krieger, G. Vriend*, YASARA View—molecular graphics for all devices – from smartphones to workstations, Bioinf. **30** (20) (2014) 2981–2982, doi: https://doi.org/10.1093/bioinformatics/btu426.
20. *G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson,* AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. **30** (16) (2009) 2785–2791, doi: https://doi.org/10.1002/jcc.21256.
21. *Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell,* A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations, J. Comput. Chem. **24** (16) (2003) 1999–2012, doi: https://doi.org/10.1002/jcc.10349.
22. *Y. Shao, L. F. Molnar, Y. Jung, J. Kussmann, C. Ochsenfeld, S. T. Brown, A. T. Gilbert, L. V. Slipchenko, S. V. Levchenko, D. P. O'Neill, R. A. DiStasio*, Advances in methods and algorithms in a modern quantum chemistry program package, J. Phys.

Chem. Chem. Phys. **8** (2006) 3172−3191, doi: https://doi.org/10.1039/b517914a.

23. *C. A. Mebi*, DFT study on structure, electronic properties, and reactivity of *cis*-isomers of $[(NC_5H_4\text{-}S)_2Fe(CO)_2]$, J. Chem. Sci. **123** (2011) 727–731, doi: https://doi.org/10.1007/s12039-011-0131-2.
24. *N. Shahabadi, M. Maghsudi*, Multi-spectroscopic and molecular modeling studies on the interaction of antihypertensive drug; methyldopa with calf thymus DNA, Mol. BioSys. **10** (2) (2014) 338–347, doi: https://doi.org/10.1039/c3mb70340a.
25. *N. Ljubijankić, A. Zahirović, E. Turkušić, E. Kahrović,* DNA binding properties of two ruthenium (III) complexes containing Schiff bases derived from salicylaldehyde: Spectroscopic and electrochemical evidence of CT DNA intercalation, Croat. Chem. Acta **86** (2) (2013) 215–222. doi: https://doi.org/10.5562/cca2216.
26. *S. Murugavel, C. J. Stephen, R. Subashini, D. Anantha Krishnan*, Synthesis, structural elucidation, antioxidant, CT-DNA binding and molecular docking studies of novel chloroquinoline derivatives: Promising antioxidant and anti-diabetic agents, J. Photochem. Photobiol. B. **173** (2017) 216–230. doi: https://doi.org/10.1016/j.jphotobiol.2017.05.043.
27. *S. Singhal, P. Khanna, L. Khanna*, Synthesis, DFT studies, molecular docking, antimicrobial screening and UV fluorescence studies on ct-DNA for novel Schiff bases of 2-(1-aminobenzyl) benzimidazole, Heliyon. **5** (10) (2019) e02596, doi: https://doi.org/10.1016/j.heliyon.2019.e02596.

SAŽETAK

Procjena afiniteta vezanja DNA ksantenskih spojeva: *in vitro* i *in silico* pristup

Elma Veljović,[a] *Amar Osmanović*,[a] *Mirsada Salihović*,[a*] *Nevzeta Ljubijankić*,[b] *Sabina Begić*[b] i *Selma Špirtović-Halilović*[a]

Derivati ksantena važna su klasa heterocikličkih spojeva sa širokim spektrom farmakoloških aktivnosti. U našim prethodnim istraživanjima pronašli smo dobru antiproliferativnu aktivnost dvaju derivata ksantena, s minimalnom toksičnošću ispitanom *in vitro* testovima. U ovoj smo studiji testirali interakciju spoja 1 (snažan antiproliferativni spoj) s DNA telećeg timusa (CT-DNA) u fiziološkim uvjetima spektrofotometrijskom titracijom. Predviđanje vezanja i vrsta interakcijskih sila uključenih u raspored između derivata ksantena i CT-DNA također su istraženi kroz studije molekularnog spajanja.

Rezultati su pokazali da spoj 1 stupa u interakciju s CT-DNA *grove* vezanjem. Nađeno je da konstanta vezanja iznosi $2{,}5 \cdot 10^4\ M^{-1}$ i ukazuje na nekovalentno vezanje spoja 1 na CT-DNA. Rezultati *docking* studije predstavljaju moguće načine vezanja, s energijama vezanja od −9,39 odnosno −8,65 kcal mol^{-1} za spojeve 1 i 2, što je u skladu s prethodno dobivenim *in vitro* rezultatima za antiproliferativno djelovanje.

Uz eksperimentalno istraživanje, izračun teorije funkcionalne gustoće (DFT) s razinama teorija B3LYP/6-31G*, B3LYP/6-31G** i B3LYP/6-31+G* proveden je na spojevima 1 i 2 da bi se dobila optimizirana geometrija, spektroskopska i elektronička svojstva.

Ove studije mogle bi pomoći u razumijevanju mehanizama toksičnosti, otpornosti, nuspojava derivata ksantena i njihovog mehanizma djelovanja na DNA.

Ključne riječi
DNA vezanje, docking, DFT, ksanten

[a] *Univerzitet u Sarajevu, Farmaceutski fakultet, Zmaja od Bosne 8, 71 000 Sarajevo, Bosna i Hercegovina*
[b] *Univerzitet u Sarajevu, Prirodno-matematički fakultet, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosna i Hercegovina*

Izvorni znanstveni rad
Prispjelo 1. ožujka 2023.
Prihvaćeno 21. ožujka 2023.