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Antiproliferative and genotoxic potential of xanthen-3-one derivatives

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Twelve previously synthesized, biologically active 2,6,7-trihydroxyxanthen-3-one derivatives were evaluated *in vitro* for antiproliferative activity. Compounds were screened against HeLa, SW620, HepG2 and A549 tumor cell lines. Compound with the trifluormethyl group on C-4′ position of the phenyl ring showed the best inhibitory activity towards HeLa and A549 tumor cells with IC_{50} of 0.7 and 4.1 μmol L^{-1}, resp. Compound with chlorine and fluorine substituents on aryl ring showed the best antiproliferative activity against SW620 with IC_{50} of 4.1 μmol L^{-1} and against HepG2 tumor cell line with IC_{50} of 4.2 μmol L^{-1}. Analyses of cytotoxic and genotoxic potential of the trifluormethyl derivative were performed with cytokinesis-block micronucleus cytome assay in human lymphocyte culture and revealed no genotoxic and cytotoxic effects. The most potent compounds were subjected to molecular docking simulations in order to analyse bindings to molecular targets and, at the same time, further support the results of experimental cytotoxic tests. Docking studies showed sites of importance in forming hydrogen bonds of the most potent compounds with targets of interest.

Keywords: antiproliferative activity, genotoxic potential, docking study, xanthen-3-one derivatives, cytokinesis-block micronucleus cytome assay

Xanthene derivatives are biologically active compounds with broad therapeutic applications that naturally occure. Some of the derivatives possess anticancer (1), antimicrobial (2), immunomodulating (3), antioxidant (4), anti-inflammatory (5) and other positive biological activities (6–8). One of the initial phases in the testing of the medicinal compounds' potential is the examination of genotoxicity (mutagenicity) (9–11). In the last decade, *in vitro* tests on peripheral blood lymphocytes cultures have been developed to understand the genotoxicity. Measurement of micronuclei (MNi) in human peripheral blood lymphocytes (PBLs) is one of the best-established cytogenetic methods for measuring of chromosomal

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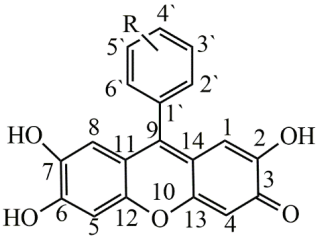
DNA damage in humans. The most reliable method for measuring MNi in PBLs is the cytokinesis-block micronucleus (CBMN) assay (12, 13). Nowadays, cytokinesis-block micronucleus cytome (CBMN-cyt) assay includes the analysis of nuclear buds, nucleoplasmic bridges, proliferative status, apoptotic and necrotic cells in addition to micronuclei, thus, it is recognised as a comprehensive system for measuring DNA damage, cytostasis, and cytotoxicity (14). All of the above mentioned is an incentive for the synthesis of new derivatives and research of their desired biological activity (like antiproliferative), as well as genotoxicity as an unwanted effect.

In our previous work (15, 16), we had prepared twelve 9-aryl substituted 2,6,7-trihydroxyxanthen-3-one derivatives using a reliable one-pot synthesis followed by structure elucidating measurements, and had performed antiplatelate and antimicrobial evaluation. Antiproliferative studies of similar xanthenes were reported (17–19), however, none of these particular derivatives have been evaluated so far. Presented study aimed to evaluate the antiproliferative activity of synthesized 9-aryl substituted xanthen-3-ones against HeLa, SW620, HepG2 and A549 tumor cell lines. The most potent compounds were subjected to molecular docking simulations and genotoxic effects on peripheral blood lymphocytes cultures.

EXPERIMENTAL

In our previous work, we had synthesized and confirmed the structure of twelve xanthen-3-one derivatives **1–12** from 1,2,4-triacetoxybenzene and different aromatic aldehydes under acidic alcoholic conditions (15, 20, 21). Structures of the tested compounds are shown in Fig. 1.



1-12

1: 2'-hydroxy-5'-bromo	**7:** 4'-ethoxy
2: 2'-hydroxy-3'-methoxy	**8:** 4'-dimethylamino
3: 3',4'-dihydroxy	**9:** 4'-trifluoromethyl
4: 3',4'-dimethoxy	**10:** 4'-acetamido
5: 3',5'-dimethoxy-4'-hydroxy	**11:** 3'-bromo
6: 3'-methoxy-5'-nitro-4'-hydroxy	**12:** 2'-chloro-6'-fluoro

Fig. 1. Structures of the tested xanthen-3-one derivatives.

Cell culturing for the antiproliferative evaluation

HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), 3T3 (mouse embryo fibroblast cell line), HEpG2 (liver hepatocellular cells) and A549 (adenocarcinomic human alveolar basal epithelial cells) cell lines were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM, Lonza, Austria) supplemented with 10 % fetal bovine serum (FBS), 2 mmol L^{-1} L-glutamine, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin in a humidified atmosphere with 5 % CO_2 at 37 °C. Trypan blue solution was used to determine cell viability using an automatic cell counter (Countess, Invitrogen, USA). Morphology was determined under a light microscope (Axio Vision-Zeiss, Germany).

Proliferation assays

To a panel of adherent cell lines, tested compounds were added in five, 10-fold dilutions (0.01 to 100 μmol L^{-1}) and incubated for 72 h. The solvent dimethylsulfoxide was also tested for eventual inhibitory activity. After 72 h of incubation, the cell growth rate was evaluated by performing the MTT assay.

Each test point was performed in quadruplicate in three individual experiments. Experimentally determined absorbance values (at 570 nm) were calculated into a cell percentage growth (PG) using the formulas proposed by NIH and described previously (22). The IC_{50} and LC_{50} values for each compound were calculated from dose-response curves using linear regression analysis.

Docking study

Lamarckian Genetic Algorithm of the AutoDock 4.0 program was used to perform the flexible-ligand docking studies (23). Drug target's X-ray crystal structures obtained from the protein data bank (www.pdb.org) were applied in docking studies. Drug targets relevant in these studies were vascular endothelial growth factor 2 (VEGFR2, PDB: 1VR2), transforming growth factor-α (TGF-α, PDB: 4TGF), transforming growth factor β (TGF-β, PDB: 2TGI), and thymidylate synthase (PDB: 2TSC).

Prior to actual docking run, AutoGrid 4.0 was introduced to precalculated grid maps of interaction energies of various atom types. In all dockings, a grid map with 126*126*126 points, a grid spacing of 1.000 Å was used. In an AutoGrid procedure, the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The energy of interaction of this single atom with the protein is assigned to the grid point. Autodock 4.0 uses these interaction maps to generate an ensemble of low energy conformations. It uses a scoring function based on the AMBER force field and estimates the free energy of binding of a ligand to its target. For all dockings, 10 independent runs with step sizes of 0.2 Å for translations and 5 Å for orientations and torsions, an initial population of random individuals with a population size of 150 individuals, a maximum number of 250000 energy evaluations and 27000 maximum generations have been done.

Binding modes between ligands and target macromolecule were analyzed, visualized and edited by using Autodock tools program (ADT, Version 1.5.4) and PyMol-1.1 software (24).

Cytokinesis-block micronucleus cytome assay

For testing of genotoxic potential in human lymphocyte culture, compound with the best antiproliferative activity was dissolved in DMSO and added to the culture medium to the final concentrations of 0.05, 0.5 and 5.0 μmol L^{-1}. Concentrations were determined according to the results of MTT assay in tumour cell lines.

Healthy, non-smoking blood donor signed informed consent forms and donated 8 mL of peripheral blood samples for this study. Ethical Committee of the Institute for Genetic Engineering and Biotechnology has approved blood collection from a healthy volunteer for *in vitro* genotoxicological analysis of compounds tested in this study (Approval No: 15-1/16 from June 15th, 2016).

Analysis of the cytotoxic and genotoxic potential was performed applying cytokinesis-block micronucleus cytome assay (CBMN-cyt assay) in human lymphocyte culture according to OECD Guideline for the Testing of Chemicals (2014).

Lymphocyte cultures were set up by adding 400 μL of whole blood in 5 mL of PB-MAX Karyotyping Medium (Life Technologies, Grand Island, NY, USA). Cultivation lasted 72 h at 37 °C. The tested compound was added to the cultures 24 h after cultures initiation in the final concentrations of 0.05, 0.5 and 5.0 μmol L^{-1}. The same volume of DMSO was added to negative controls. Mitomycin C was used as a toxic agent in the positive control (0.25 μg mL^{-1}). Cytokinesis blocking agent – cytochalasin B (Sigma-Aldrich Co., USA) was added at the beginning of the 45th hour of cultivation in the final concentration of 4.5 μg mL^{-1}. For each tested concentration and controls, duplicated cultures were used. After 72 h of cultivation, cultures were briefly treated with 0.56 % KCl, fixed three times in absolute ethanol/glacial acetic acid 3:1 fixative and dropped on the coded microscope slides. Slides were stained in 5 % Giemsa for 7 minutes.

Slides were analyzed at 400× magnification on the Olympus BX51 microscope (Tokyo, Japan) by two experienced scorers, according to established criteria (14, 25, 26). Genotoxic potential of substance was determined by analyzing frequencies of micronuclei, nucleoplasmic bridges, and nuclear buds in 2000 binuclear (BN) cells per each replication (4000 per treatment).

Frequencies of mononuclear, binuclear, trinuclear, and quadrinuclear cells, as well as apoptotic and necrotic cells, were scored in the total number of at least 500 counted cells per replicate (1000 per treatment). Cytostatic and cytotoxic effects of the tested compound were examined by the calculation of the nuclear division index (NDI) and nuclear division cytotoxicity index (NDCI), according to recommended equations (25).

Statistic analysis

Significance of differences between means of all observed genotoxicity parameters in treated and control cultures was analyzed using the Fisher exact test and Monte-Carlo permutation test. For the testing of differences between values of cytotoxicity indexes, two-way ANOVA was used. Statistical analyses were conducted in the PAST 3.18. (27). Influence of concentrations on frequencies of the observed genotoxicity and cytotoxicity parameters was tested by linear regression analysis using MedCalc 10.4.0.0. software.

RESULTS AND DISSCUSION

Antiproliferative activity

MTT assay was used in order to determine IC_{50} values (values at which 50 % of cells are inhibited) of the tested compounds.

Different substituents play a very important role in the activity of this series of compounds. The addition of hydroxy or methoxy group to aryl ring decreases the antiproliferative activity against tested tumor cells. In support of that, compounds **5** and **6** with methoxy and hydroxy groups on the aryl ring showed weak antiproliferative activity (> 100 μmol L^{-1}). Activity is improved with the addition of halogen substituents such as chlorine, fluorine and bromine. Compound **12** with chlorine and fluorine substituents at the aryl ring (9-(2′-chloro-6′-fluorophenyl)-2,6,7-trihydroxyxanthen-3-one) showed the best antiproliferative activity against SW620 and HepG2 (4.2 and 4.1 μmol L^{-1}, respectively) tumor cell lines, whereas compound **9** with the trifluoromethyl group on C-4′ position on the phenyl ring showed the best inhibitory activity towards HeLa and A549 tumor cell lines (0.7 and 4.1 μmol L^{-1}, respectively).

Docking study

Docking studies were performed for the most potent synthesized compounds for individual tumor cell lines. In docking studies for HeLa tumor cell lines, vascular endothelial growth factor 2 (VEGFR2, PDB: 1VR2), an important factor in tumor cell angiogenesis, was

Table I. IC_{50} *values of compounds* ***1*–*12*** *in tested cell lines*

Compd.	IC_{50} (μmol L^{-1})				
	SW620	HEpG2	3T3	HeLa	A549
1	29.1	27.2	3.9	5.3	30.9
2	49.8	35.1	6.3	8.9	52.8
3	56.8	42.7	8.9	27.5	74.1
4	21.7	16.9	0.8	4.9	32.9
5	> 100	> 100	< 0.01	> 100	> 100
6	> 100	> 100	< 0.01	36.6	> 100
7	6.8	13.1	< 0.01	2.9	37.1
8	8.8	5.8	< 0.01	5.0	39.7
9	6.1	4.8	1.7	0.7	4.1
10	91.9	59.6	> 100	40.8	> 100
11	6.6	5.1	3.4	1.5	4.3
12	4.2	4.1	1.9	4.6	6.5

SW620, A549, HepG2 and HeLa are carcinoma cell lines and 3T3 is healthy control mouse embryo fibroblast cell line.

used as a drug target. Tyrosine kinase in VEGFR2 is on the surface of tumor cells and is responsible for transfosforilation and activation of antitumor drugs (28, 29).

For the most prominent xanthen-3-one derivative **9**, docking studies have shown that it binds to the target by three hydrogen bonds: proton from OH groups in the C-2 position with oxygen from Arg 1080; proton from OH group at position C-7 with oxygen from Ile 1025; oxygen from the OH group in the C-6 position with nitrogen from Ile 1025 (Fig. 2). The binding energy of compound **9** with the target was –4.61 kcal mol^{-1}.

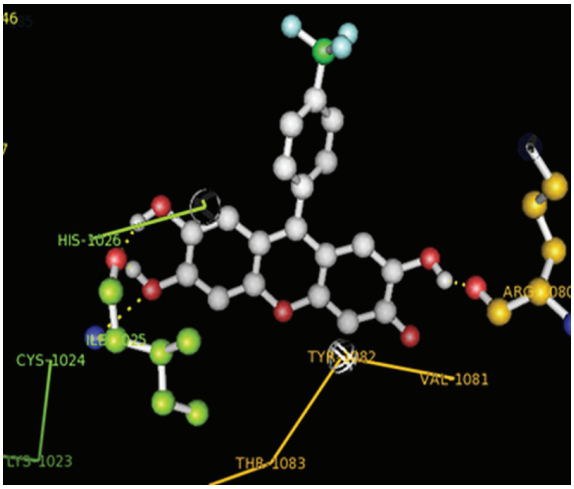


Fig. 2. The binding of compound **9** with vascular endothelial growth factor 2.

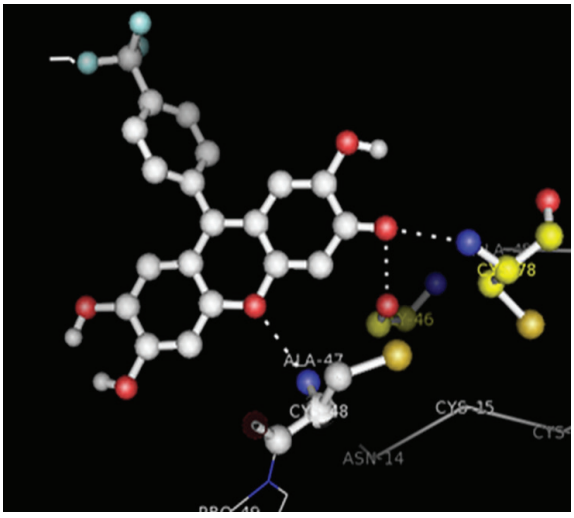


Fig. 3. The binding of compound **9** with transforming growth factor β.

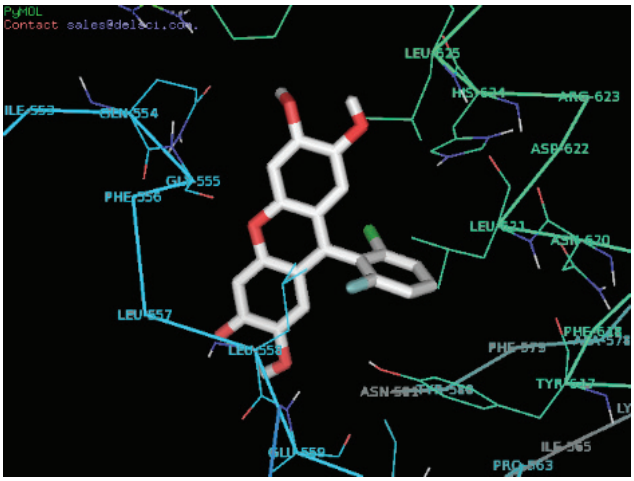


Fig. 4. The binding of compound **12** with thymidylate synthase.

In the docking studies of the antitumor effect for lung carcinoma cells (A549), a transforming growth factor β (TGF-β, PDB: 2TGI) was used as the drug target, which is crucial in the migration processes and metastases of lung carcinoma cells (30, 31). Compound **9**, the most potent xanthen-3-one derivative toward A549 cell line, binds to the target with energy of –4.61 kcal mol^{-1} by constructing three hydrogen bonds, via oxygen in the composition of the xanthene core with Cys 48 and oxygen from a carbonyl group at the C-3, which makes two hydrogen bonds with Cys 78 and Gly 46 (Fig. 3).

An enzyme thymidylate synthase (PDB: 2TSC) was used as a drug target for docking studies of antitumor effect toward HepG2 cell line, because of its importance in the proliferation and metastases of hepatocellular carcinoma cells (32). The best antitumor activity against hepatocellular carcinoma cells (HepG2) showed compound **12**. Docking studies have shown that xanthen-3- one derivatives bind to thymidylate synthase via a

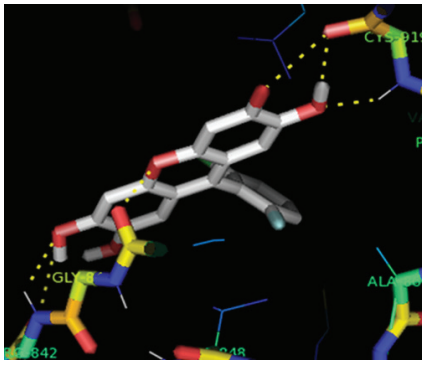


Fig. 5. The binding of compound **12** with transforming growth factor-α.

hydrogen bond between OH group at the C-6 position with the NH group of histidine on the target (His 624). Compound **12** binds to the target with the energy of –6.36 kcal mol^{-1}.

As a drug target in docking studies of synthesized xanthene compounds against colorectal carcinoma cells (SW620), a transforming growth factor-α (TGF-α, PDB: 4TGF) was used. The transforming growth factor is important for the proliferation of the SW620 cells (33). The best antiproliferative activity against the SW620 cell line showed compound **12**, which binds with the target by forming six hydrogen bonds with the target. Oxygens from C-2 and C-3 form three bonds with Cys 919, and O-10 and oxygen from C-6 form three bonds with Gly 841 (Fig. 5). The binding energy of compound **12** with the target was –6.07 kcal mol^{-1}.

The obtained results of the docking studies could be the basis for discovering the mechanism of action for synthesized xanthene compounds, in conjunction with additional *in vitro* tests.

Genotoxicity

Genotoxicity of compound **9** (the most potent antiproliferative compound) was evaluated in cytokinesis blocked human lymphocytes by scoring micronuclei (MNi),

*Table II. Results of cytostatic and cytotoxic activity of compound **9** in cultures of human lymphocytes*

Treatments	Replication	M1	M2	M3	M4	N	Ap	Nec	N	NDI	NDCI
Negative control DMSO	1	329	161	13	34	537	5	0	542	1.54	1.53
	2	397	174	14	25	610	4	2	616	1.45	1.45
	Xav	363	167.5	13.5	29.5	573.5	4,5	1	579	1.50	1.49
0.05 μmol L^{-1}	1	355	168	11	25	559	5	1	565	1.47	1.47
	2	312	160	13	23	508	5	0	513	1.50	1.50
	Xav	333.5	164	12	24	533.5	5	0,5	539	1.49	1.48
0.5 μmol L^{-1}	1	351	137	8	22	518	6	0	524	1.42	1.42
	2	391	135	14	13	553	5	1	559	1.37	1.36
	Xav	371	136	11	17.5	535.5	5.5	0.5	541.5	1.39	1.39
5 μmol L^{-1}	1	353	139	9	15	516	8	1	525	1.39	1.39
	2	352	136	16	27	531	5	3	539	1.47	1.46
	Xav	352.5	137.5	12.5	21	523.5	6.5	2	532	1.43	1.42
Positive control mytC	1	448	79	2	2	531	8	1	540	1.17	1.17
	2	416	113	1	2	532	14	3	549	1.23	1.22
	Xav	432	96	1.5	2	531.5	11	2	544.5	1.20	1.19

M1-M4 – cells with 1–4 nuclei; N – number of total scored cells; Ap – apoptotic cells; Nec – necrotic cells; NDI – nuclear division index; NDCI – nuclear division and cytotoxicity index.

Table III. Means of observed genotoxicity and cytotoxicity parameters in cytokinesis-blocked lymphocytes

Treatments	Xav/1000 BN cells			Xav/500 cells	
	MNi	NBUDs	NPBs	NDI	NDCI
Negative control (DMSO)	6.25[a]	2.5[a]	0	1.50[a]	1.49[a]
0.05 μmol L^{-1}	5.5[a]	2[a]	0	1.49[a]	1.48[a]
0.5 μmol L^{-1}	4.75[a]	0.5[a]	0.25	1.39	1.39
5 μmol L^{-1}	9.75[a]	1.75[a]	0	1.43	1.42
Positive control (mytC)	101.25	8.75	1.5	1.20	1.19

[a] Significantly different against the positive control

nuclear buds (NBUDs) and nucleoplasmic bridges (NPBs) in a total of 4000 binuclear cells (BN) per treatment (2000 per each replicate). Cytostatic and cytotoxic effects, expressed as nuclear division indexes (NDI and NDCI), were calculated after scoring of mono-, bi-, tri- and tetranuclear cells as well as apoptosis and necrosis in at least 500 cells per replication (1000 cells per treatment). Results of the analysis are presented in Table II.

The statistical analysis revealed no significant differences between frequencies of micronuclei and nuclear buds in the treated and negative control cultures, whereas a significant increase of these parameters was evidenced in the positive control ($p < 0.05$). The frequencies of nucleoplasmic bridges in controls and treatments did not significantly differ. Linear association between logarithmic values of compound **9** concentrations and frequencies of all observed parameters revealed no significant relationship ($p > 0.05$).

Two-way ANOVA for two replicates showed no significance between calculated values of nuclear division indexes in treatments and negative control, while NDI and NDCI in the positive control were significantly lower than that of the negative control and 0.05 μmol L^{-1} treatment (Table III). No linear association between concentration and cytotoxicity indexes was observed.

As used in the food and drug industry, xanthene dyes were extensively studied to analyse their genotoxic potential. Numerous studies confirmed that certain xanthenes (*e.g.* erythrosine) induce DNA damage and should be carefully consumed (34). In the study performed by Eisenträger *et al*. (35) quinoline, 6-methylquinoline and xanthene caused mutagenic effects in the Ames assay with *Salmonella typhimurium* only after the metabolic activation. However, the conducted analysis of compound **9** has not revealed its genotoxic potential in the tested concentrations.

CONCLUSIONS

Majority of tested xanthen-3-on derivatives exerted antiproliferative effects on tumor cell lines. Compound **12**, with chlorine and fluorine substituents at the aryl ring, showed the best antiproliferative activity against SW620 with IC_{50} of 4.1 μmol L^{-1} and against HepG2 tumor cells line with IC_{50} of 4.2 μmol L^{-1}. Towards HeLa and A549 tumor cells, the

best inhibitory activity showed compound **9** with the trifluormethyl group at C-4′ position on the phenyl ring with IC_{50} of 0.7 μmol L^{-1} against HeLa and IC_{50} of 4.1 μmol L^{-1} against A549 tumor cell lines. Docking studies, carried out on vascular endothelial growth factor 2 (VEGFR2), thymidylate synthase, transforming growth factor β (TGF-β) and transforming growth factor-α (TGF-α) as drug targets, showed sites of importance in forming hydrogen bonds with targets for the most potent compounds. The performed analyses revealed no genotoxic and cytotoxic effects of compound **9** (the most potent antiproliferative compound) in concentrations of 0.05, 0.5 and 5 μmol L^{-1}, thus, this compound should be the subject of further research and potential applications.

REFERENCES

1. S. S. Liou, W. L. Sheih, T. H. Cheng, S. J. Won and C. N. Lin, γ-Pyrone compounds as potential anticancer drugs, *J. Pharm. Parmacol.* **45** (1993) 791–794; https://doi.org/10.1111/j.2042-7158.1993.tb05686.x
2. K. Muharrem, D. Ersin and B. Hatice, Synthesis, characterization and antimicrobial activity of novel xanthene sulfonamide and carboxamide derivatives, *J. Enzyme Inhib. Med. Chem.* **28** (2013) 885–893; https://doi.org/10.3109/14756366.2012.692087
3. B. C. Baguley and L. M. Ching, Immunomodulatory actions of xanthenone anticancer agents, *BioDrugs* **8** (1997) 119–127; https://doi.org/10.2165/00063030-199708020-00005
4. A. E. Hay, M. C. Aumond, S. Mallet, V. Dumontet, M. Litaudon, D. Rondeau and P. Richomme, Antioxidant xanthones from Garcinia vieillardii, *J. Nat. Prod.* **67** (2004) 707–709; https://doi.org/10.1021/np0304971
5. Z. Karimi-Jaberi and M. M. Hashemi, One step synthesis of 14-alkyl-or aryl-14H-dibenzo [a,j] xanthenes using sodium hydrogen sulfate as catalyst, *Monatsh. Chem.* **139** (2008) 605–608; https://doi.org/10.1007/s00706-007-0786-z
6. S. Laphookhieo, J. K. Syers, R. Kiattansakul and K. Chantrapromma, Cytotoxic and antimalarial prenylated xanthones from Cratoxylum cochinchinense, *Chem. Pharm. Bull.* **54** (2006) 745–747; https://doi.org/10.1248/cpb.54.745
7. Q. B. Han, N. Y. Yang, H. L. Tian, C. F. Qiao, J. Z. Song, D. C. Chang, S. L. Chen, K. Q. Luo and H. X. Xu, Xanthones with growth inhibition against HeLa cells from Garcinia xipshuanbannaensis, *Phytochemistry* **69** (2008) 2187–2192; https://doi.org/10.1016/j.phytochem.2008.05.019
8. S. L. Niu, Z. L. Li, F. Ji, G. Y. Liu, N. Zhao, X. Q. Liu, Y. K. Jing and H. M. Hua, Xanthones from the stem bark of Garcinia bracteata with growth inhibitory effects against HL-60 cells, *Phytochemistry* **77** (2012) 280–286; https://doi.org/10.1016/j.phytochem.2012.01.010
9. A. Nersesyan, E. Perrone, P. Roggieri and C. Bolognesi, Genotoxic action of cycloplatam, a new platinum antitumor drug, on mammalian cells *in vivo* and *in vitro*, *Chemotherapy* **49** (2003) 132–137; https://doi.org/10.1159/000070619
10. G. P. Machado, J. I. González Borroto, N. Castañedo, A. Creus and R. Marcos, *In vitro* genotoxicity testing of the furylethylene derivative UC-245 in human cells, Mutagenesis **19** (2004) 75–80; https://doi.org/10.1093/mutage/geg039
11. A. K. Nersesyan, G. S. Melikyan and H. Stopper, The study of genotoxicity of two newly synthesized pyrrolinone derivatives on L5178Y mouse lymphoma and bone marrow cells, *Exp. Oncol.* **25** (2003) 176–179.
12. F. Karaer, Environmental pollution and carcinogenic risk, *J. Environ. Pathol., Toxicol. Oncol.* **15** (1996) 105–113.

13. M. S. Legator and W. W. Au, Application of integrated genetic monitoring: the optimal approach for detecting environmental carcinogens, *Environ. Health Perspect.* **102** (1994) 125–132; https://doi.org/10.1289/ehp.94102s9125
14. M. Fenech, Cytokinesis-block micronucleus cytome assay, *Nat. Protoc.* **2** (2007) 1084; https://doi.org/10.1038/nprot.2007.77
15. L. Applova, E. Veljović, S. Muratović, J. Karličkova, K. Macakova, D. Završnik, L. Saso, K. Durić and P. Mladenka, 9-(4′-Dimethylaminophenyl)-2,6,7-trihydroxy-xanthene-3-one is a Potentially Novel Antiplatelet Drug which Antagonizes the Effect of Thromboxane A2, *Med. Chem.* **14** (2018) 1–10; https://doi.org/10.2174/1573406413666171010102535
16. E. Veljović, S. Špirtović-Halilović, S. Muratović, M. Salihović, I. Novaković, A. Osmanović and D. Završnik, Antimicrobial Activity and Docking Study of Synthesized Xanthen-3-one Derivatives, *Res. J. Pharm. Biol. Chem. Sci.* **9** (2018) 777–783.
17. H. Marona, N. Szkaradek, E. Karczewska, D. Trojanowska, A. Budak, P. Bober, W. Przepiorka, M. Cegla and E. Szneler, Antifungal and Antibacterial Activity of the Newly Synthesized 2-Xanthone Derivatives, *Arch. Pharm.* **342** (2009) 9–18; https://doi.org/10.1002/ardp.200800089
18. S. Gobbi, A. Rampa, A. Bisi, F. Belluti, P. Valenti, A. Caputo, A. Zampiron and M. Carrara, Synthesis and antitumor activity of new derivatives of xanthen-9-one-4-acetic acid, *J. Med. Chem.* **45** (2002) 4931–4939; https://doi.org/10.1021/jm020929p
19. X. Z. Wang, J. H. Yao, G. B. Jiang, J. Wang, H. L. Huang and Y. L. Liu, Synthesis, characterization, cytotoxicity, apoptosis and cell cycle arrest of dibenzoxanthenes derivatives, *Spectrochim. Acta, Part A* **133** (2014) 559–567; https://doi.org/10.1016/j.saa.2014.05.054
20. E. Veljović, S. Špirtović-Halilović, S. Muratović, L. Valek Žulj, S. Roca, S. Trifunović, A. Osmanović and D. Završnik, 9-Aryl Substituted Hydroxylated Xanthen-3-ones: Synthesis, Structure and Antioxidant Potency Evaluation, *Croat. Chem. Acta* **88** (2015) 121–127; https://doi.org/10.5562/cca2595
21. S. Petra, G. Klaus and R. W. Siegfries, Reliable synthesis of 9-aryl-substituted 2,6,7-trihydroxyxanthen-3-ones, *Synthesis* **14** (2008) 2211–2216; https://doi.org/10.1055/s-2008-1078447
22. T. Gazivoda, S. Raić-Malić, S. Krištafor, D. Makuc, J. Plavec, S. Bratulić, S. Kraljević Pavelić, K. Pavelić, L. Naesens, G. Andrei, R. Snoeck, J. Balzarini and M. Mintas, Synthesis, cytostatic and anti-HIV evaluations of the new unsaturated acyclic C-5 pyrimidine nucleoside analogues, *Bioorg. Med. Chem.* **16** (2008) 5624–5634; https://doi.org/10.1016/j.bmc.2008.03.074
23. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility *J. Comput. Chem.* **30** (2009) 2785–2791; https://doi.org/10.1002/jcc.21256
24. M. A. Lill and M. L. Danielson, Computer-aided drug design platform using PyMOL, *J. Comput.-Aided Mol. Des.* **25** (2011) 13–19; https://doi.org/10.1007/s10822-010-9395-8
25. M. Fenech, The *in vitro* micronucleus technique, *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **455** (2000) 81–95; https://doi.org/10.1016/S0027-5107(00)00065-8
26. M. Fenech, W. P. Chang, M. Kirsch-Volders, N. Holland, S. Bonassi and E. Zeiger, HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures, *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **534** (2003) 65–75; https://doi.org/10.1016/S1383-5718(02)00249-8
27. O. Hammer, D. A. T. Harper and P. D. Ryan, PAST-Palaeontological statistics. www. uv. es/~ pardomv/pe/2001_1/past/pastprog/past. pdf, acessado em, 25 (2001) 2009.
28. J. Azizian, M. K. Mohammadi, O. Firuzi, N. Razzaghi-asl and R. Miri, Synthesis, biological activity and docking study of some new isatin Schiff base derivatives, *Med. Chem. Res.* **21** (2012) 3730–3740; https://doi.org/10.1007/s00044-011-9896-6

29. E. Gordian, E. Welsh and T. Muñoz-Antonia, Alvespimycin (17-DMAG) blocks TGFβ-induced EMT and migration in A549 lung cancer cells, *Cancer Res.* **74** (2014) 1916–1930; https://doi.org/10.1158/1538-7445.AM2014-1050
30. P. Xia, F. F. Mou and L. W. Wang, Predictive Role of Computer Simulation in Assessing Signaling Pathways of Crizotinib-treated A549 Lung Cancer Cells, *Asian Pac. J. Cancer Prev.* **13** (2012) 3119–3121; https://doi.org/10.7314/APJCP.2012.13.7.3119
31. A. F. Eweas, Q. M. A. Abdullah and E. S. I. Hassan, Design, synthesis, molecular docking of new thiopyrimidine-5-carbonitrile derivatives and their cytotoxic activity against HepG2 cell line, *J. Appl. Pharm. Sci.* **4** (2014) 102–111; https://doi.org/10.7324/JAPS.2014.41218
32. R. J. Coffey, G. D. Shipley and H. L. Moses, Production of transforming growth factors by human colon cancer lines, *Cancer Res.* **46** (1986) 1164–1169.
33. F. M. Drumond Chequer, V. P. Venâncio, M. L. Bianchi abd L. M. Antunes, Genotoxic and mutagenic effects of erythrosine B, a xanthene food dye, on HepG2 cells, *Food. Chem. Toxicol.* **50** (2012) 3447–3451; https://doi.org/10.1016/j.fct.2012.07.042
34. A. Eisentraeger, C. Brinkmann, H. Hollert, A. Sagner, A. Tiehm and J. Neuwoehner, Heterocyclic compounds: toxic effects using algae, daphnids, and the Salmonella/microsome test taking methodical quantitative aspects into account, *Environ. Toxicol. Chem.* **27** (2008) 1590–1596; https://doi.org/10.1897/07-201.1