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Synthesis and biological evaluation of xanthen-1,8-dione derivatives

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Abstract: Fourteen previously synthesized 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione derivatives were evaluated *in vitro* for their antimicrobial, antiproliferative and antioxidant activity. Also, in this work 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione (**15**) was synthesized according to the same procedure and structure was confirmed by IR, NMR spectroscopy and mass spectrometry. Compounds were screened against Gram negative bacterium *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The antifungal activity of synthetized xanthene compounds was tested against fungus *Saccharomyces cerevisiae*. The cell lines HeLa, SW620, HEpG2, A549 and 3T3 were targets for antiproliferative effects of synthesized compounds. The results showed that the most potent, as antimicrobial and antiproliferative agent, was compound with two atoms of bromine substituted on aryl ring, and the most potent antioxidant agent was compound 3,3,6,6-tetramethyl-9-(2-methoxy-3-hydroxy-4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione with IC_{50} of 0.045 mM and 70.41% of inhibition DPPH.

INTRODUCTION

Xanthene and their derivatives are important heterocyclic compounds with interesting biological activities, such as antibacterial (Lall, Hussein and Meyer, 2006), anti-inflammatory (Hafez *et al.*, 2008), antiviral (Ram *et al.*, 2000), antioxidant (Veljović *et al.*, 2015), and antitumor (Pinto, Sousa and Nascimento, 2005). Xanthenes were shown to function well as hydrogen donor and suppress the oxidation of linoleic acid in homogeneous solution. Unlike most other phenolic antioxidants, xanthenes react with peroxyl radicals and their antioxidant activities were close to that of α-tocopherol (Nishiyama, *et al.*, 1998). Xanthene derivatives have moderate to excellent activities against number of biological targets. With changing substituent on the xanthene nucleus, the biological targets vary from microbial diseases to viral problems, and variety of cancerous cells. Xanthene derivatives target different biological problems by interacting with enzymes and proteins (Gunjegaonkar *et al.*, 2018). Based on biological activities of xanthenes described in literature, we became interested in the evaluation of the obtained xanthen-1,8-dione derivatives as antioxidant, antimicrobial and antiproliferative agents.

EXPERIMENTAL

General Procedure for Synthesis

A mixture of 4-acetamidobenzaldehyde (1 mmol), 5,5-dimethylcyclohexane-1,3-dione (2 mmol) and DABCO (10 mmol%) in H_2O (20 mL) was refluxed for 30 min (Figure 1). The progress of the reaction was monitored by TLC, using silica gel $60GF_{254}$ plates, and as a mobile

phase dichlormethane:hexane=1:1. After completion of the reaction, the mixture was cooled to room temperature, and the solid was filtered off and washed with distilled water. The crude product (3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione) was purified by recrystallization from 95% ethanol (Paliwal *et al*., 2013).

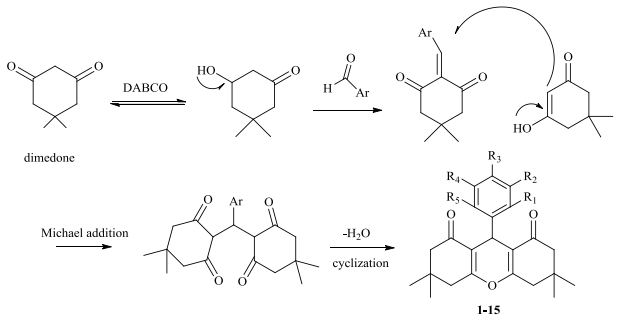

Figure 1. *Synthetic route for xanthen-1,8-dione derivatives.*

In our previous work (Veljović *et al*., 2017) according to described procedure, we synthesized fourteen 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione derivatives, that are structural analogs with different substituents on aryl ring (Table 1).

Table 1. Substituents of synthesized xanthenes aryl ring.

Compound	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
2	NO_2	H	H	H	H
3	H	H	NO_2	H	H
4	H	F	H	H	H
5	H	H	F	H	H
6	H	H	CF_3	H	H
7	H	Cl	H	H	H
8	H	H	Cl	H	H
9	H	H	Br	H	H
10	H	Br	H	Br	H
11	OH	H	H	Br	H
12	H	OCH_3	OCH_3	H	H
13	H	OCH_3	OH	NO_2	H
14	H	OCH_3	OH	OCH_3	H

Instrumentation

All melting points (m.p.) are uncorrected and expressed in °C. Melting points of the compounds were determined using BŰCHI Melting Point B-545. IR spectra were recorded by Shimadzu IR Prestige 21 ID using KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, in $CDCl_3$ at 25°C using NMR spectrometer Bruker AV600, with tetramethylsilane (TMS) as internal reference. Elemental analyses of synthesized compounds were recorded by Vario EL III C, H, N, S/O Elemental Analyzer, Elementar Analysensysteme GmbH, Hanau-Germany. ESI-MS measurements were performed on a high performance liquid chromatography–mass spectrometry (HPLC-MS) triple quadrupole 6420 instrument equipped with an autosampler (Agilent Technologies, Palo Alto, CA, USA). The desolvation gas temperature was 300°C with flow rate of 6.0 L min^{-1}. The fragmentor voltage was 135 V and capillary voltage was 4.0 kV. Mobile phase was 0.1% formic acid in 50% methanol and a flow rate of mobile phase was 0.2 mL min^{-1}.

Antimicrobial activity

The antimicrobial activity of synthesized compounds was assessed against bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 15442 and fungi strain *Saccharomyces cerevisiae* ATCC 9763. Stock solutions were prepared by dissolving compounds in 99.5% dimethyl sulfoxide (DMSO) to obtain a 1 mg mL^{-1}. Overnight cultures of bacteria were kept for 18 h at 37°C, while fungi strain was incubated for 48 h at 25°C. As nutrition bases for diffusion method Müller-Hinton and Sabouraud microbiological growth medium were used. The antibacterial activity of synthesized compounds, that showed good activity in diffusion method, was studied by employing a dilution method, using Casein soya bean digest broth (Triptic soya bujon). The inoculum was prepared as described previously. The synthesized compounds were dissolved in DMSO and diluted with a culture broth to a concentration of 0.5–0.00024 mg mL^{-1}. Chloramphenicol and fluconazole were used as referent compounds in concentration of 500 μg mL^{-1}.

Antiproliferative activity evaluation

The synthesized compounds were also evaluated for in vitro antiproliferative activity against HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), hepatocellular carcinoma (HEpG2), lung carcinoma cells (A549), and mouse embryo fibroblast cell line (3T3). For investigation 96-well microtiter plates was used and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 72 h. To all of the culture media, 100 μg mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, 2 mM L-glutamine and 10% fetal bovine serum were added. In order to evaluate cell proliferation, the cells were treated with compounds at concentrations of 0.01 μM to 100 μM for 72 h. DMSO, which was used as solvent, was also tested for antiproliferative activity in the working concentration. In the present work, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) was used to assess the antiproliferative activity of the synthesized compounds, while absorbance was measured at 570 nm. The results were presented as a cell percentage growth (PG) using the formulas proposed by NIH and described previously (Gazivoda *et al.*, 2008). The cell viability was determined with Trypan blue solution based on the quantification of the color intensity in each culture well by using an automatic cell counter (Countess, Invitrogen, USA), while morphology was determined under light microscope (Axio Vision-Zeiss, Germany). The IC_{50} curves were plotted and the IC_{50} and LC_{50} values were calculated by using linear regression analysis.

Evaluation of antioxidant activity by using DPPH method

The antioxidant activity of the synthesized xanthene derivatives was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The

reducing abilities of the synthesized compounds were determined by their interaction with the free stable radical 1,1-diphenyl-2-picrylhydrazine (DPPH) at 0.2 mM concentrations for 30 min, by measuring absorbance at 517 nm. The antioxidant activity of tested compounds was determined by measuring the percentage of DPPH neutralization by them and compared to the standard antioxidant Trolox. The EC_{50} (the concentration of antioxidant which eliminate 50% of DPPH radicals) was determined for each compound using calibrated curves. The inhibition percentage (%) of radical scavenging activity was calculated using the formula:

$$\%\ inhibition = \frac{Ac(0)-AA(t)}{Ac(0)} \times 100 \qquad (1)$$

where Ac(0) is the absorbance of control at t=0 and AA(t) is the absorbance of antioxidant at t=30 min. All measurements were done in triplicate (Burda and Oleszek, 2001).

RESULTS AND DISCUSSION

According to described procedure in this work, we synthesized 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione (Figure 2). This compound was previously synthesized (Kaya, Demir and Bekci, 2013), but by different synthetic route. Its structure was confirmed by IR, NMR spectroscopy and mass spectrometry. Introducing acetamido group in structure of similar compounds increased antimicrobial activity (Sotirovaa *et al*., 2014; Shah, 2017); therefore, we also evaluated this compound for antimicrobial, antiproliferative, and antioxidative activity along with 14 previously synthesized similar compounds (Figure 2).

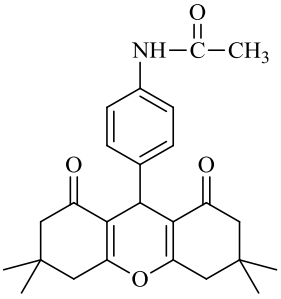

Figure 2. *Structure of 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthen-1,8(2H)-dione (**15**).*

*3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione (**15**):*
mp 170-171°C. IR (KBr) n 3000, 1700, 1620, 1604, 1400, 1300, 1150 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$) d 1.09 (s, 6H, H-14 and H-16), 1.21 (s, 6H, H-15 and H-17), 2.12 (s, 3H, H-8', $NHCOC\underline{H}_3$), 2.20-2.52 (m, 8H, H-2, H-4, H-5 and H-7), 5.48 (s, 1H, H-9), 7.01 (d, $J_{2',3'}$ 9.2 Hz, 2H, H-2'and H-6'), 7.40 (d, 2H, H-3' and H-5'), 7.56 (s, 1H, $N\underline{H}COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) d 24.45 (C-8', $NHCO\underline{C}H_3$), 27.31 (C-14, C-16), 29.55 (C-15, C-17), 31.34 (C-3, C-6), 32.30 (C-9), 46.37 (C-4, C-7), 47.00 (C-2, C-5), 115.50 (C-10, C-12), 119.56 (C-2', C-6'), 127.27 (C-3', C-5'), 133.77 (C-4'), 135.79 (C-1'), 168.29 (C-7', $NH\underline{C}OCH_3$), 189.35 (C-11, C-13), 190.48 (C-1, C-8). MS (m/z) 426.3 $(M+Na)^+$. Anal. Calcd. for $C_{25}H_{29}O_4N$: C 73.68, H 7.17. Found: C 73.72, H 7.09.

Antimicrobial activity evaluation
Microbiological activity was determined by diffusion and dilution method on three strains of bacteria and one strain of fungi. The tests were carried out on Gram negative bacterium *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The antifungal activity of synthetized xanthene compounds was tested against fungus *Saccharomyces cerevisiae*. Chloramphenicol (for antibacterial testing) and fluconazole (for the examination of antifungal activity) were used as standards.
The results of antimicrobial activity of synthesized compounds by diffusion method are shown in Table 2 and Table 3.

Table 2: Results of antimicrobial activity by diffusion method.

Compound	Zone of inhibition (mm)			
	S. cerevisiae	*P. aeruginosa*	*S. aureus*	*B. subtilis*
1	14.5	12	14	15
2	16.5	14	12.5	15
3	17	16	18.5	17.5
4	18.5	16.5	17.5	20.5
5	16	14	**10**	**14**
6	**13.5**	14.5	18.5	14
7	16.5	12	12	19
8	17	12.5	**10**	**14**
9	18.5	15.5	14	18
10	19.5	18.5	20.5	21.5
11	-	12	21.5	14
12	14	14	15	18.5
13	19.5	17.5	20	19.5
14	15.5	13.5	16	18
15	14	**11**	**10**	**14**
Fluconazole	21	-	-	-
Chloramphenicol		20	24	22.5

Compound **6** with CF_3 group on *para* position on aryl ring, was most potent compound against *S. cerevisiae* in diffusion method. The new synthesized compound **15** with acetamido group in *para* position of aryl ring, showed the best activity against Gram negative bacterium *P. aeruginosa* , while compounds with substituents on *para* position of aryl ring (compounds **5**, **8** and **15**) were most potent against Gram positive bacteria *S. aureus* and *B. subtilis.*
In dilution method, compound **10** with bromine as substituent, was most potent. Biological comparative effects, as function of the nature of the substituents, reveals that the insertion of bromine atoms in the 3 and 5-position of the aryl ring of the xanthene derivatives, is an important factor on the antimicrobial activity.
Already published studies have shown that the introduction of bromine as a substituent, contributes to the antimicrobial activity (Shridhar *et al.*, 2009). Thus, the compound with bromine (compound **10**), has shown a good activity against *S. cerevisiae* and *S. aureus* with MIC 0.313 mg mL^{-1}, against *B. subtilis* with MIC of 0.626 mg mL^{-1} while the activity against *P. aeruginosa*,

with MIC 1.25 mg mL^{-1}, was significantly weaker. The results of antimicrobial activity by dilution method of synthesized compounds are presented in Table 3.

Table 3: Results of antimicrobial activity by dilution method.

Compound	MIC, mg/mL			
	S. cerevisiae	*P. aeruginosa*	*S. aureus*	*B. subtilis*
1	1.25	2.50	1.25	2.50
2	1.25	2.50	1.25	2.50
3	1.25	1.25	1.25	1.25
4	2.50	1.25	1.25	1.25
5	1.25	1.25	1.25	2.50
6	1.25	1.25	1.25	2.50
7	1.25	1.25	1.25	1.25
8	1.25	1.25	1.25	1.25
9	1.25	1.25	1.25	1.25
10	**0.313**	1.25	**0.313**	**0.625**
11	/	2.50	0.313	2.50
12	0.625	1.25	1.25	2.50
13	0.625	1.25	1.25	1.25
14	1.25	2.5	2.5	1.25
15	1.25	2.50	5.5	2.5
Fluconazole	0.313	-	-	-
Chloramphenicol	-	0.313	0.01	0.01

Antioxidant activity

The antioxidant activity was performed using DPPH radical scavenging method where Trolox was used as a positive control for comparison. The results of antioxidant activity of the compounds **1-15** are shown in Table 4. The results of the radical scavenging was expressed in terms of half-inhibition concentration (IC_{50}) which denotes the concentration required to scavenge 50% of DPPH radicals.

Table 4: Results of antioxidant activity of synthesized compounds using DPPH method.

Compound	Absorbance (t=30 min)	% Inhibition	IC_{50}, mM
1	0.456	66.76	0.09
2	0.478	65.16	0.1
3	0.558	59.33	0.14
4	0.504	63.27	0.12
5	0.544	60.35	0.131
6	0.631	54.01	0.195
7	0.574	58.16	0.165
8	0.580	57.73	0.18
9	0.603	56.05	0.17
10	0.667	51.38	0.193
11	0.621	54.74	0.19
12	0.431	68.59	0.16
13	0.406	**70.41**	**0.045**
14	0.485	64.65	0.13
15	0.575	58.10	0.16
Trolox	0.212	84.54	0.018

Compound **13,** with IC_{50} of 0.045 mM and 70.41% of inhibition DPPH, seemed to be most active. It is assumed to be due to the presence of nitro substituent group in the molecule, as the presence of nitro group in any organic molecule in general, confers significant biological activity like in metronidazole (Al-Masoudi and Abbas, 2016), and nitrazepam (Gilli *et al.*, 1977). Also, in literature, it is described that introduction of nitro group as substituent, significantly increases antioxidative activity, and compounds with halogen derivatives, and methoxy substituents also showed promising antioxidant activity (Karmaker *et al.*, 2018), which correlate well with results of our antioxidant study, while compound **13** possesses methoxy group at position C3 and nitro group at C5 on aryl ring of xanthene derivative.

Antiproliferative activity

The synthesized compounds **1-15** were tested for cytotoxicity in four human cancer cell lines, which contained human HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), hepatocellular carcinoma (HEpG2), lung carcinoma cells (A549), and mouse embryo fibroblast cell line (3T3). The IC_{50} values of the xanthene derivatives for antiproliferative activity are listed in Table 5.

Table 5: Antiproliferative activity for xanthene derivatives.

Compound	Tumor cells IC_{50}, μg mL^{-1}				
	SW620	**HEpG2**	**3T3**	**HeLa**	**A549**
1	>100	>100	86.1	>100	>100
2	>100	>100	0.02	>100	>100
3	>100	>100	11.9	>100	>100
4	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100
6	>100	>100	25.5	>100	>100
7	>100	>100	>100	>100	>100
8	>100	>100	<0.01	>100	>100
9	>100	>100	<0.01	>100	>100
10	**85.6**	>100	<0.01	**62.0**	>100
11	>100	>100	<0.01	62.2	>100
12	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100
15	>100	>100	0.05	>100	>100

The antiproliferative screening results show that synthesized xanthen-1,8-dione derivatives show weak antiproliferative activity against tested tumor cell lines. The results revealed that compound **10** with two bromine atoms substituted on aryl ring of xanthene, exhibited the highest activity against HepG2 cell line with IC_{50} 62.0 μg $mL^{-1}$and IC_{50} 85.6 μg mL^{-1} against SW620 tumor cell lines, while other synthesized xanthen-1,8-dione derivatives had IC_{50} more than 100 μg mL^{-1} against all tested tumor cells.

CONCLUSIONS

A series of 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione derivatives has been *in vitro* biological evaluated as potential antimicrobial, antiproliferative and antioxidant agents. The results showed that compound **10**, having two atoms of bromine substituted on aryl ring at positions C-3 and C-5 was the most potent as antimicrobial and antiproliferative agent. The results revealed that the most potent antioxidant agent was compound **13** (3,3,6,6-tetramethyl-9-(2-methoxy-3-hydroxy-4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione) with

IC_{50} of 0.045 mM and 70.41 % of inhibition DPPH. Further experiments aimed at defining the target and the mechanisms of antimicrobial and antiproliferative activity showed by these molecules, are in progress and the results will be reported in a forthcoming paper.

REFERENCE

Al-Masoudi, N. A., Abbas, Z. A. (2016). Synthesis and biological activity of new metronidazole derivatives. *Monatshefte für Chemie-Chemical Monthly*, *147(2)*, 383-390.

Burda, S., Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of agricultural and food chemistry*, *49(6)*, 2774-2779.

Gazivoda, T., Raić-Malić, S., Krištafor, S., Makuc, D., Plavec, J., Bratulić, S., Kraljević Pavelić, S., Pavelić, K., Naesens, L., Andrei, G., Snoeck, R., Balzarini, J., Mintas, M. (2008). Synthesis, cytostatic and anti-HIV evaluations of the new unsaturated acyclic C-5 pyrimidine nucleoside analogues. *Bioorganic & medicinal chemistry*, *16(10)*, 5624-5634.

Gilli, G., Bertolasi, V., Sacerdoti, M., Borea, P. A. (1977). 7-Nitro1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (nitrazepam). *Acta Crystallographica Section B*, *33(8)*, 2664-2667.

Gunjegaonkar, M. B., Fegade, S. A., Kolhe, R. C. (2018). Review on medicinal importance of xanthene derivatives. *International Journal of Research in Pharmacy and Chemistry*, *8(2)*, 319-328.

Hafez, H. N., Hegab, M. I., Ahmed-Farag, I. S., El-Gazzar, A. B. A. (2008). A facile regioselective synthesis of novel spiro-thioxanthene and spiro-xanthene-9′, 2-[1, 3, 4] thiadiazole derivatives as potential analgesic and anti-inflammatory agents. *Bioorganic & medicinal chemistry letters*, *18(16)*, 4538-4543.

Karmaker, N., Lira, D. N., Das, B. K., Kumar, U., Rouf, A. S. S. (2018). Synthesis and Antioxidant Activity of Some Novel Benzimidazole Derivatives. *Dhaka University Journal of Pharmaceutical Sciences*, *16(2)*, 245-249.

Kaya, M., Demir, E., Bekci, H. (2013). Synthesis, characterization and antimicrobial activity of novel xanthene sulfonamide and carboxamide derivatives. *Journal of enzyme inhibition and medicinal chemistry*, *28(5)*, 885-893.

Lall, N., Hussein, A. A., Meyer, J. J. M. (2006). Antiviral and antituberculous activity of *Helichrysum melanacme* constituents. *Fitoterapia*, *77(3)*, 230-232.

Nishiyama, T., Sakita, K., Fuchigami, T., Fukui, T. (1998). Antioxidant activities of fused heterocyclic compounds, xanthene-2, 7-diols with BHT or catechol skeleton. *Polymer degradation and stability*, *62(3)*, 529-534.

Paliwal, P., Jetti, S. R., Bhatewara, A., Kadre, T., Jain, S. (2013). DABCO catalyzed synthesis of Xanthene derivatives in aqueous media. *ISRN organic chemistry*, doi: 10.1155/2013/526173.

Pinto, M. M. M., Sousa, M. E., Nascimento, M. S. J. (2005). Xanthone derivatives: new insights in biological activities. *Current medicinal chemistry*, *12(21)*, 2517-2538.

Ram, V. J., Saxena, A. S., Srivastava, S., Chandra, S. (2000). Oxygenated chalcones and bischalcones as potential antimalarial agents. *Bioorganic & medicinal chemistry letters*, *10(19)*, 2159-2161.

Shah, M. (2017). Synthesis and Antimicrobial Properties of Chitosan: A Case Study. *EC Microbiology*, *9*, 193-200.

Shridhar, D. M., Mahajan, G. B., Kamat, V. P., Naik, C. G., Parab, R. R., Thakur, N. R., Mishra, P. D. (2009). Antibacterial Activity of 2-(2', 4'-Dibromophenoxy)-4, 6-dibromophenol from *Dysidea granulosa*. *Marine drugs*, *7(3)*, 464-471.

Sotirova, A., Avramova, T., Lazarkevich, I., Lubenetz, V., Karpenko, O., Galabova, D. (2014). Antibacterial potential of novel synthetic derivatives of 1, 4-naphthoquinone and their complexes with biosurfactants. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *5(4)*, 530-541.

Veljović, E., Špirtović-Halilović, S., Muratović, S., Osmanović, A., Badnjević, A., Gurbeta, L., Tatlić, B., Zorlak, Z., Imamović, S., Husić, Đ., Završnik, D. (2017). Artificial Neural Network and Docking Study in Design and Synthesis of Xanthenes as Antimicrobial Agents. In *CMBEBIH 2017* (pp. 617-626). Springer, Singapore.

Veljović, E., Špirtović-Halilović, S., Muratović, S., Valek Žulj, L., Roca, S., Trifunović, S., Osmanović, A., Završnik, D. (2015). 9-Aryl substituted hydroxylated xanthen-3-ones: synthesis, structure and antioxidant potency evaluation. *Croatica Chemica Acta*, *88(2)*, 121-127.

Summary/Sažetak

U našem prethodnom radu sintetizirali smo četrnaest 3,3,6,6-tetrametil-9-aril-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion derivata, koji su evaluirani *in vitro* kao antimikrobni, antiproliferativni i antioksidativni agensi. Također, u ovom radu sintetiziran je i 3,3,6,6-tetrametil-9-(4-acetamidofenil)-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion, po istoj proceduri čija je struktura potvrđena IR, NMR spektroskopijom i masenom spektrometrijom. Spojevi su ispitivani kao antimikrobni agensi prema Gram negativnoj bakteriji *Pseudomonas aeruginosa* i Gram pozitivnim bakterijama *Staphylococcus aureus* i *Bacillus subtilis.* Antifungalna aktivnost sintetiziranih spojeva ispitivana je prema gljivici *Saccharomyces cerevisiae*. Stanične linije HeLa, SW620, HEpG2, A549 i 3T3 bile su meta za ispitivanje antiproliferativnog učinka sintetiziranih spojeva. Rezultati su pokazali da je najbolje antiproliferativno i antimikrobno djelovanje pokazao spoj koji u strukturi na arilnom prstenu ima supstituirana dva atoma broma, dok je najpotentniji antioksidativni agens 3,3,6,6-tetrametil-9-(2-metoksi-3-hidroksi-4-nitrofenil)-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion sa IC_{50} 0.045 mM i 70.41% inhibicije DPPH reagensa.