Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *87* (11) 1245–1258 (2022)
JSCS–5591
Original scientific paper
Published 30 September 2022

Synthesis and biological activity of alkylthio and arylthio derivatives of *tert*-butylquinone

JELENA ĐORĐEVIĆ[1,2], STOIMIR KOLAREVIĆ[3], JOVANA JOVANOVIĆ MARIĆ[3], MARIANA OALĐE PAVLOVIĆ[4], DUŠAN SLADIĆ[5], IRENA NOVAKOVIĆ[6]*# and BRANKA VUKOVIĆ-GAČIĆ[2]

[1]University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia, [2]University of Belgrade, Centre for Genotoxicology and Ecogenotoxicology, Faculty of Biology, Belgrade, Serbia, [3]University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of the Republic of Serbia, Belgrade, Serbia, [4]University of Belgrade, Chair of Plant Morphology and Systematics, Faculty of Biology, Belgrade, Serbia, [5]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia and [6]University of Belgrade, Institute for Chemistry, Technology and Metallurgy, Department for Chemistry, Belgrade, Serbia

(Received 4 March, revised 13 May, accepted 16 May 2022)

Abstract: Biological activity of 2-*tert*-butyl-1,4-benzoquinone (TBQ) and its derivatives, 2-*tert*-butyl-5-(2-propylthio)-1,4-benzoquinone, 2-*tert*-butyl-5--(propylthio)-1,4-benzoquinone, 2-*tert*-butyl-5,6-(ethylenedithio)-1,4-benzoquinone, 2-*tert*-butyl-5-(phenylthio)-1,4-benzoquinone and 2-*tert*-butyl-6-(phenylthio)-1,4-benzoquinone, were tested for their antioxidant, antibacterial, toxic, cytotoxic and genotoxic potential. Using the DPPH test, all derivatives showed good antioxidant activity, better than ascorbic acid, and the 2-tert--butyl-5-(propylthio)-1,4-benzoquinone derivative showed the strongest effect. Better antibacterial potential was observed against Gram-positive bacteria in the broth microdilution method in which the 2-*tert*-butyl-5-(phenylthio)-1,4--benzoquinone derivative showed the strongest activity (*MIC* = 15.6 μM). The results of toxicity tests, using the Brine shrimp test, indicated that the derivatives lose their toxic potential compared to TBQ, except for 2-*tert*-butyl-6--(phenylthio)-1,4-benzoquinone, which showed a 3 times stronger effect. Cytotoxicity was assessed by the MTT assay in 24 and 72 h treatments in MRC-5, HS 294T and A549 cell lines in threefold decreasing gradient (11, 33 and 100 μM). Modifications potentiate the cytotoxic effect, and the strongest effect was observed with the 2-*tert*-butyl-5,6-(ethylendithio)-1,4-benzoquinone derivative. In addition, the genotoxic potential was examined in the MRC-5 cell line using the comet assay. All tested derivatives of TBQ showed a genotoxic effect at all

* Corresponding author. E-mail: irenan@chem.bg.ac.rs
# Serbian Chemical Society member.
https://doi.org/10.2298/JSC220304044D

applied subtoxic concentrations. In general, the chemical modifications of TBQ enhanced its biological activity.

Keywords: TBQ; toxicity; MTT assay; antibacterial activity; antioxidant activity; comet test.

INTRODUCTION

Cancer is a disease of altered signaling and metabolism, which causes uncontrolled division and survival of the transformed cells.[1] According to the World Health Organization, in 2018 alone, there were 18.1 million new cancer patients and 9.6 million cancer deaths.[2] The process of developing this disease is not sufficiently elucidated and modern treatments, although much more effective, are not effective enough to reduce the mortality rate to an acceptable level. Biologically active compounds originating from a variety of natural sources, plants, animals, and microorganisms, have great potential for use in the treatment of malignancies.[3] According to the work of Amaral *et al.*,[4] from 1980 to 2019, a total of 174 new compounds with indications for cancer treatment were commercialized, with 53 % of these drugs being natural products, compounds based on them, or compounds that mimic their action. Since the biodiversity of marine organisms is approximately half of the total biodiversity on Earth, marine organisms are a good basis for the creation of modern chemotherapeutics and have been involved in various clinical trials.[5] However, although they show good biological activities, natural products originating from marine organisms have certain limiting factors in the form of availability and an adequate amount of the active compound obtained because of their low yield. This problem could be solved by creating synthetic compounds modeled from natural products. Many biologically active compounds isolated from marine organisms are hydroquinones and quinones, such as avarol/avarone redox pair isolated from the Mediterranean sponge *Dysidea avara*. Avarol and avaron have shown great and diverse biological activity, such as antimicrobial, antiviral, antioxidant and antitumor.[6–10] In previous studies, the biological activity of avarol/avaron and their methoxy and methylamino derivatives,[11] alkylamino and aralkylamino derivatives,[12] amino acid derivatives[13] and alkyl(aryl)thio derivatives[10] were examined. The yield of biologically active compounds originating from marine organisms is generally good, as is the case with avarol/avaron, but the problem is in the availability of marine organisms and, hence, the solution could be to design specific synthetic compounds that are similar in structure and their derivatives in order to enhance activity and reduce potential side effects. Bearing this in mind, in previous research a relatively crude model was used, based on *tert*-butylquinone – TBQ.[14,15] The results of previous studies showed that modifications increase the cytotoxic and antibacterial activity of TBQ and the alkylamino and aralkylamino derivatives of TBQ show a more selective effect over avarone derivatives.[14,15] In

this work, TBQ and its alkylthio derivatives 2-*tert*-butyl-5-(isopropylthio)-1.4--benzoquinone, 2-*tert*-butyl-5-(propylthio)-1.4-benzoquinone, 2-*tert*-butyl-5,6--(ethylenedithio)-1.4-benzoquinone) and arylthio derivatives (2-*tert*-butyl-5--(phenylthio)-1.4-benzoquinone and 2-*tert*-butyl-6-(phenylthio)-1.4-benzoquinone) were examined for their antioxidant, antibacterial, toxic, cytotoxic and genotoxic potentials. According to the work of Božić *et al.*,[10] alkylthio and arylthio derivatives were selected to cover the range of redox potentials between those of alkoxy and alkylamino derivatives on the one end, and the chloro derivatives on the other. The alkylthio group is also found in many compounds and mixtures of natural origin, such as allicin (a thiosulfinate compound originating from *Allium sativum* L.) that induces apoptosis in tumor cells.[16,17] Allicin reduces cell viability and cell proliferation in healthy and cancers cells.[18] By selecting this group, the aim was to combine the action of two strong functional groups of natural origin - quinones and alkylthio sulfides.

EXPERIMENTAL

Synthesis of derivatives

Alkylthio-derivatives of 2-*tert*-butyl-1,4-benzoquinone were synthesized by nucleophilic addition of thiols to quinones.[19] Synthesis of TBQ and derivatives commenced with commercially available *tert*-butylhydroquinone which were oxidized using silver oxide to the corresponding quinone. Conditions described in the paper by Božić *et al.*,[10] for the synthesis of alkylthio and aralkylthio derivatives of avarone were the same for the synthesis of TBQ and its derivatives. Briefly, the optimum reaction conditions for the synthesis of alkylthio derivatives were: equimolar amount of reactants, slightly alkaline medium, nitrogen atmosphere, temperatures of 60 °C, and a 30-min reaction time. Phenylthio derivatives were synthesized under neutral conditions in the air atmosphere while the other conditions were the same. The names and chemical structures of the derivatives are shown in Fig. 1.

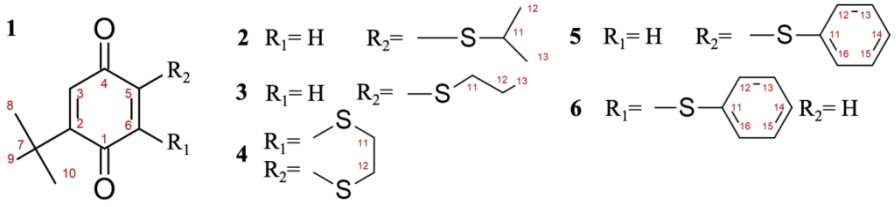

Fig. 1. Chemical structure of *tert*-butylquinone-TBQ and its derivatives: TBQ (**1**) 2-*tert*-butyl--1.4-benzoquinone, **2**) 2-*tert*-butyl-5-(isopropylthio)-1.4-benzoquinone, **3**) 2-*tert*-butyl-5-(propylthio)-1,4-benzoquinone, **4**) 2-*tert*-butyl-5,6-(ethylenedithio)-1.4-benzoquinone, **5**) 2-*tert*--butyl-5-(phenylthio)-1.4-benzoquinone and **6**) 2-*tert*-butyl-6-(phenylthio)-1.4-benzoquinone.

Details on synthesis and spectral data are given in Supplementary material to this paper.

Biological activities testing

Assessment of antioxidant potential by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test. The antioxidant effects of TBQ and its derivatives were determined using the DPPH assay, which measures the radical scavenging potential of selected compounds. The test was per-

formed according to the protocol developed by Blois (1958).[20] A total of 180 μL of freshly prepared methanolic DPPH solution (concentration of 40 μg mL^{-1}) was added to each well of the microtiter plate, followed by 20 μL of TBQ and its derivatives in DMSO at the appropriate concentration. Seven concentrations were tested for each test compound in a threefold gradient with the highest concentration of 1 mM. The test was performed in triplicate. Samples were incubated for 30 min in the dark at room temperature, after which the absorbance was measured at 517 nm using a microplate reader (Multiskan Sky Thermo Scientific, Finland).

Evaluation of antibacterial activity by the broth microdilution method. The antibacterial activity of TBQ and its derivatives against four strains of Gram-positive (*Enterococcus faecalis* ATCC 29212, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and three strains of Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 15442) bacteria was tested using the double microdilution method according to Sarker *et al*.[21] The highest concentration tested for TBQ and its derivatives were 1 mM. *E. faecalis* and *L. innocua* were grown in brain heart infusion broth (BHI), while the other bacteria were grown in Mueller Hinton broth (MHB). After double dilution of the derivatives in the appropriate broth, 10 μL of bacterial suspension (10^4 cells per well), and resazurin at a final concentration of 67.5 μg mL^{-1} were added per well. After thermostating for 24 h at 37 °C, the *MIC* value was determined as the lowest concentration that led to a change in the color of resazurin from blue to pink. Rifampicin and streptomycin with the highest concentrations of 30 and 43 μM, respectively, were used as positive controls. As the solvent control 5 % DMSO was tested. The antibacterial effect was categorized as strong (0–100 μM), moderate (100–500 μM) and weak (500–1000 μM).[22]

Assessment of toxic potential by the Brine shrimp test with Artemia salina (ARC test). A commercially available mixture (Artemia eggs, Dajana, Czech Republic) of lyophilized *A. salina* eggs were put in saltwater solution (3.3 % of sea salt). The test was performed according to Vanhaecke and Persoone[23] in triplicate over a 24-hour incubation period. TBQ and its derivatives were tested in six concentrations in a triple or double gradient (depending on the test compound) with a maximum tested concentration of 500 μM. The experiment had a negative control (saltwater only), positive control ($K_2Cr_2O_7$, concentration range 50–10 μg mL^{-1}), and solvent control (5 % DMSO).

Assessment of the cytotoxic potential using the MTT assay on different cell lines. The cytotoxic effect was assessed by the MTT assay on three cell lines: MRC-5 (healthy human fetal lung cell line; ECACC No. 84101801), HS 294T (cancer cell line – human melanocytes; ATCC HTB-140) and A549 (human lung cancer cell line; ATCC CCL-185) according to the protocol described by Kolarević *et al*.[11] Cells in monolayer were treated with TBQ and its derivatives at concentrations of 11, 33 and 100 μM while 5 % DMSO was used as the solvent control. The following passages were used for cell lines MRC-5, A549, HS 294 T: passage 25 and 26; passage 7, 10, 12 and 19; passage 14, respectively. The MTT assay was performed in 3 individual experiments during 24- and 72-hour incubation.

Assessment of the genotoxic potential in MRC-5 cell line by the Comet assay. As described in Kolarević *et al*.,[11] cells were grown 24 h until monolayer formation, washed with 1 x PBS and treated with non-toxic concentrations of the test compound (TBQ: 1.3, 3.7 and 11 μM; derivative **4**: 0.41, 1.23 and 3.7 μM, derivatives **2**, **3**, **5**, **6**: 3.7, 11 and 33 μM). Etoposide (33 μM) and 5 % DMSO were used as the positive and solvent control, respectively. The experiments were performed in triplicate in mini gel format as described in Azqueta *et al*.[24] with some modifications. Aliquots of cell suspensions obtained after treatment (30 μL) were mixed

with 70 μL of 1 % low melting point agarose. For each sample, 15 μL of the mixture was placed in duplicate on slides pre-coated with 1 % normal melting point agarose. Each microscope slide (prepared individually for each substance) contained duplicates of negative and positive controls and three concentrations of tested substances. Lysis, denaturation, electrophoresis, and neutralization were performed as described in Gačić *et al.*[25] A total of 150 nuclei were analyzed for each compound tested, controls were made separately for each microscopic plate examined. Acridine Orange (2 μg mL^{-1}) was used for staining and the analysis was performed with a fluorescence microscope (Leica, DMLS, Austria, under magnification 400×, excitation filter 450–490 nm, barrier filter 510 nm) by Comet IV computer software (Perceptive Instruments, UK). DNA damage was monitored *via* the tail intensity parameter (*T.I.*). Statistical analysis was performed by IBM SPSS Software. The results did not show a normal statistical distribution using the Kolmogorov–Smirnov test. Non-parametric tests were further performed: Kruskal–Wallis one-way ANOVA followed by Wilcoxon signed ranks test for pairwise comparison of treated groups with negative and positive controls with the significance level at $p < 0.05$.

RESULTS AND DISCUSSION

Assessment of the antioxidant potential of TBQ and its derivatives by the DPPH test

Based on the results obtained by DPPH (Table I), it could be concluded that the strongest antioxidant effect was observed for derivative **3,** which was also stronger than TBQ. On the other hand, **6** exhibits the lowest antioxidant activity. Additionally, derivatives **2**, **4** and **5** show activity similar to the parent compound TBQ. It should be noted that derivative **3** with a propyl group on the quinone moiety has a higher activity compared to derivative **2** with a voluminous isopropyl substituent. In addition to the size of the substituents, it is obvious that the activity is also influenced by their position on the quinone nucleus, since a striking difference in activity occurs with two regioisomers **5** and **6**, the 5-phenylthio isomer **5** being more active than 6-phenylthio regioisomer **6**. It should be noted that all tested compounds have higher activity than ascorbic acid, which is a control compound.

TABLE I. DPPH test results (IC_{50} / μM) of *tert*-butylquinone and its derivatives, AsAc – ascorbic acid as control

Compound						
TBQ	**2**	**3**	**4**	**5**	**6**	AsAc
77.27±5.07	80.75±4.10	70.48±3.04	81.98±1.96	80.61±2.14	108.03±1.08	177.81±2.68

The scavenging activity of TBQ and its alkylthio and arylthio derivatives was evaluated using the DPPH assay. DPPH is a relatively stable free radical. This assay is based on the color change of the DPPH solution (from purple to yellow) as the radical receives an electron or a hydrogen atom from the antioxidant. It is a simple and widely used method to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors.[26] The obtained

results show that all the tested compounds have good antioxidant activity, better than that of ascorbic acid. The antioxidant activity of thio derivatives is not surprising. Namely, by the introduction of alkylthio and arylthio groups, the molecules get a sulfur atom that can lose an electron and thus increase the antioxidant activity of the compounds. Furthermore, alkylthio derivatives have a slightly stronger activity than that of the arylthio derivatives. This may be due to electron delocalization in the phenylthio group, which reduces the electron availability for sulfur atoms.

Evaluation of antibacterial activity

The microdilution method on different bacterial strains showed antibacterial effects of different strengths depending on the tested compound. Ethylenedithio **4** and phenylthio derivatives **5** and **6** show a strong antibacterial effect against *S. aureus*, and TBQ and the **2**, **4** and **5** derivatives against *B. subtilis* (Table II). Other derivatives showed a moderate to weak effect on the tested Gram-positive bacterial strains, while no antibacterial effect was observed against Gram-negative bacteria. It could be concluded that both TBQ and all the tested compounds show selectivity towards Gram-positive bacterial strains. Against *E. faecalis* and *S. aureus*, derivatives **4**, **5** and **6** show a stronger antibacterial activity than the parent compound (TBQ) which justifies the synthesis and introduction of S-substituents to the quinone moiety.

TABLE II. Antibacterial activity (*MIC* / μM) of *tert*-butylquinone and its derivatives, AB – antibiotics rifampicin ([a]) or streptomycin ([b]) as positive controls

Compound	Bacterium						
	E. faecalis	*L. innocua*	*S. aureus*	*B. subtilis*	*S. typhimurium*	*P. aeruginosa*	*E. coli*
TBQ	1000	250	125	62.5	>1000	>1000	>1000
2	>1000	250	125	62.5	>1000	>1000	>1000
3	>1000	1000	500	250	>1000	>1000	>1000
4	125	250	62.5	62.5	>1000	>1000	>1000
5	500	250	15.6	62.5	>1000	>1000	>1000
6	250	125	31.2	125	>1000	>1000	>1000
AB	3.79[a]	1.90[a]	10.75[b]	10.75[b]	21.50[b]	42.99[b]	42.99[b]

TBQ and its derivatives were assigned values (1, 2, 4, 8, 16, 32, 64, 128) depending on the obtained minimum inhibitory concentration (*MIC*: 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81 μM). The assigned values obtained for the derivatives are compared with the assigned values for TBQ and shown on the radial diagram in Fig. 2, to show how many times stronger the derivatives are relative to the starting compound TBQ. The derivatives **4**, **5** and **6** showed better antibacterial activity against bacterial strains: *E. faecalis* and *S. aureus*, while towards *L. innocua*, derivative **6** showed better activity than TBQ. The highest

activity, 8 times stronger than TBQ, was shown by derivatives **4** (against *E. faecalis*) and **5** (against *S. aureus*).

The introduction of a substituent on the quinone moiety of TBQ, generally leads to an improvement of the antibacterial activity for most synthesized derivatives. Selectivity of both TBQ and all its derivatives to Gram-positive bacteria was observed.

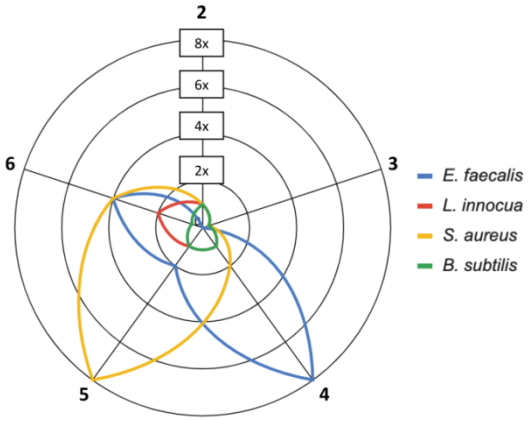

Fig. 2. Antimicrobial activity of derivatives relative to TBQ against bacterial strains: *E. faecalis*, *L. innocua*, *S. aureus* and *B. subtilis*.

From a structure–activity relationship (SAR) analysis, it could be noticed that the derivatives with a more voluminous substituent show stronger antibacterial activity. In accordance with this data, the isopropylthio derivative showed better activity than the propylthio derivative, while bulky groups ethylenedithio and phenylthio also exhibited an increased activity. On the other hand, two phenylthio regioisomers (**5** and **6**) showed different activities, but with no clear preference. Derivative **5** showed promising antibacterial properties because of an activity against *S. aureus* similar to that of streptomycin. Alkylthio and arylthio derivatives of avaron were less or equally active than avarone on *S. aureus* and *E. coli* bacterial strains.[10] On the same bacterial strains, alkylamino, aralkylamino and amino acid derivatives of TBQ mostly showed stronger activity compared to TBQ.[12–14]

Assessment of toxic potential by the Brine shrimp test

Based on the obtained results (Table III), it could be concluded that all the tested derivatives, except **6,** showed lower toxicity than TBQ. Toxicity of TBQ and derivatives **3**, **4** and **6** were higher than the toxicity of potassium dichromate ($K_2Cr_2O_7$), which was used as a positive control (**6** > TBQ > **3** > **4**).

The lowest activity was observed for derivative **5** which is interesting since it contains the same substituent as derivative **6** but at a different position.

Since *A. salina* shrimps live in symbiosis with some types of bacteria and that the toxicity of the compounds may be related to the lysis of the cell wall of bacteria present in the digestive tract of *A. salina* adults,[27] false-positive toxicity results are avoided by using nauplii in which the digestive tract is not yet developed. The fact that the most toxic TBQ derivatives are not those which are the most active against bacterial cells corroborates with this conclusion. The most toxic compound is derivative **6** that is almost 10 times more toxic than its regioisomer **5**. Since quinone toxicity is associated with either ROS formation or alkylation of cellular nucleophiles, a possible explanation for this activity difference is the fact that in derivative **6,** the most active position for nucleophilic attack is available. The toxicity of other derivatives could be explained by ROS formation since nucleophilic addition is either unlikely (derivatives **2**, **3**, **5**) or impossible (derivative **4**).

TABLE III. Brine shrimp test results (LC_{50} / μM) of *tert*-butylquinone and its derivatives. Control – potassium dichromate ($K_2Cr_2O_7$)

Compound						
TBQ	**2**	**3**	**4**	**5**	**6**	$K_2Cr_2O_7$
58.68± 4.72	159.20± 46.36	69.85± 17.67	85.55± 11.34	183.83± 11.88	19.21± 0.88	92.29± 0.34

Assessment of cytotoxic potential using the MTT assay on different cell lines

The results presented in Table IV confirm that the derivatives show a stronger cytotoxic effect than TBQ and the effect is more pronounced after a longer incubation period (72 h). Exceptions were only observed for derivatives **3**, **4** and **6** on the A549 cell line where the derivatives had a lower cytotoxic effect than TBQ after 72 h. The strongest cytotoxic effect on the MRC-5 cell was exhibited by derivative **4** (20.61 and 11.12 μM for 24 and 72 h incubation periods, respectively), while on HS 294T, the compounds were active only at 72 h treatment, whith derivative **3** showing the strongest effect (53.12 μM). On the A549 cell line, derivative **5** (57.04 and 52.80 μM for 24 and 72 h treatments, respectively) was the most active, while derivative **2** showed a similar effect but only at

TABLE IV. Cytotoxic activity (IC_{50} / μM) of *tert*-butylquinone and its derivatives on different cell lines after 24 and 72 h treatment; nt – not tested

Compound	MRC-5		HS 294T		A549	
	Time, h					
	24	72	24	72	24	72
TBQ	82.93±4.79	54.22±8.02	>100	>100	>100	67.58±0.87
2	64.14±3.66	51.51±5.38	>100	83.77±7.88	>100	52.99±4.01
3	61.15±4.89	35.516±9.79	>100	53.12±9.80	>100	75.72±1.18
4	20.61±12.87	11.12±15.65	>100	94.74±18.07	>100	>100
5	64.32±2.36	55.16±3,68	>100	nt	57.04±6.63	52.80±6.98
6	82.51±2.94	37.11±3.47	>100	68.09±3.66	>100	75.73±1.18

72 h treatment (52.99 μM). In general, the MRC-5 cell line was more sensitive in regard to DNA damage by TBQ and its derivatives when compared to tested cancer lines. All tested compounds showed a low selectivity index (SI < 2), hence, according to Koch *et al.*,[28] they may be considered generally toxic.

In this work, modifications increase the cytotoxic effect of TBQ with a more pronounced effect after a 72-hour incubation period. The tested compounds showed as active (IC_{50} less than 20 μg mL^{-1}) or moderately active (IC_{50} between 20 and 100 μg mL^{-1}) in the inhibition of cell growth.[29] According to Sladic and Gasic,[30] the main mechanism of quinone cytotoxicity may be nucleophilic addition and ROS production, resulting in oxidative stress and cell death. Tumor cells are more susceptible to oxidative stress. This combined mechanism is not possible with derivative **4**, which explains why this derivative is less active on the tumor cell lines than the other derivatives. The stronger cytotoxic effects on the normal MRC-5 cell line compared to tumor cell lines cannot be explained by generation of radicals, and is possibly a consequence of enhanced transport through cell the membrane and/or difference in metabolic transformations in normal and tumor cells. The conflicting results with the previously described antioxidant effect of the derivatives could be explained by the fact that antioxidant supplements, such as vitamins and/or flavonoids, under conditions of increased oxidative stress also exacerbate the pro-oxidant effect.[31] Candidate anticancer drugs should ideally be selective, potent, and relatively non-toxic.[32] However, based on the selectivity index, these compounds show a low selectivity index and are considered generally toxic. The low selectivity index could be a problem when using these derivatives as anticancer drugs. Additional modifications could improve the selectivity and reduce the genotoxicity of the derivatives. All tested compounds, except derivative **4**, showed a lower cytotoxic effect on the MRC-5 cell line at the highest tested concentration (100 μM) compared to avarol and avarone examined in the work of Kolarević *et al*.[11] On the A549 cell line, the cytotoxic effect of the derivatives was lower relative to avarol and lower or similar to avarone. In the work of Božić *et al.*,[10] alkyl(aryl)thio derivatives of avarone were less (or equally) active than avarone, whereas in the present work, the opposite results were obtained. This indicates that alkyl(aryl)thio groups enhance the activity of TBQ but not of avarone. Amino acid derivatives of TBQ showed a lower cytotoxic activity compared to TBQ,[13] while the same avarone derivatives generally proved to be more active. On the other hand, alkylamino and aralkylamino derivatives of TBQ generally had a higher activity than the parent compounds.[12,14]

Assessment of genotoxic potential in MRC-5 cell line by Comet assay

All tested derivatives of TBQ induced DNA damage in a dose-dependent way and show statistically significant differences in comparison with the negat-

ive control (Fig. 3). The highest genotoxic potential was observed for derivative **4**. In the previous work, no statistically significant difference was observed for TBQ compared to the control.[15] The tested TBQ derivatives showed a lower genotoxic potential than the positive control (etoposide).

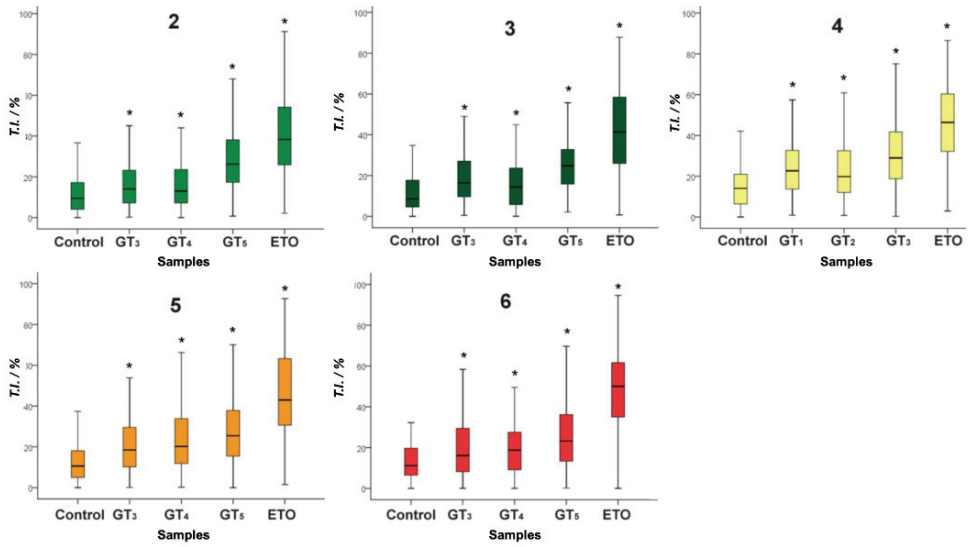

Fig 3. Comet assay results of *tert*-butylquinone derivatives represented by the tail intensity parameter (*T.I.*). Concentrations of test compounds: GT_1–0.41, GT_2–1.3, GT_3–3.7, GT_4–11 and GT_5–33; control-cells without treatment; ETO-etoposide (33 μM); *significant increase of DNA damage in comparison with the negative control (Control).

Various chemical, biological or physical agents damage DNA molecules and thus cause a genotoxic effect. The resulting damage to DNA molecules, if not repaired, can cause mutations and consequent pathological consequences, such as cancer, which is why it is important to examine the possible genotoxic effect of selected TBQ derivatives before their possible use as drugs.

All the tested derivatives at all the tested subtoxic concentrations showed a genotoxic effect, while the opposite effect was observed for TBQ in the work of Đorđević *et al.*[15] Alkylthio and arylthio derivatives showed stronger genotoxic potential than alkylamino and aralkylamino derivatives, where the effect was observed only at the highest tested concentration of 11 μM, except for 2-(benzylamino)-6-(*tert*-butyl)-1,4-benzoquinone, which showed a genotoxic effect at a concentration of 4 μM.[15] The alkylthio and arylthio derivatives of TBQ showed genotoxic potential at three times lower concentrations than 2-(benzylamino)-6--(*tert*-butyl)-1,4-benzoquinone, while derivative **4** was genotoxic even at a concentration that was nine times lower. These results confirm that the selected modifications of the starting compound enhance their genotoxic potential. How-

ever, the genotoxicity of alkylthio and arylthio derivatives was not higher than the genotoxicity of etoposide at the same concentration tested. The stronger genotoxic potential of alkylthio and arylthio derivatives of TBQ compared to TBQ may be related to cytotoxicity and should be further investigated. Avarol and avarone did not exert genotoxic potential in the same concentration range according to the work of Kolarević *et al.*,[11] while 3'-methoxyavarone and 3'--(methylamino) avarone have genotoxic potential. According to Okubo *et al.*,[33] TBQ induced a decrease in the potential of the mitochondrial membrane, disruption of the mitochondrial structure with the formation of cytosolic vacuoles, release of cytochrome *c* from mitochondria, caspase activation, poly(ADP-ribose)polymerase (PARP) cleavage, and a decrease of intracellular GSH and ATP. However, in the same work, neither oligonucleosomal degradation of nuclear DNA nor nuclear fragmentation in DAPI stained cells were detected, which excludes apoptosis as a cell death pathway. The authors suggest, based on research by Fiers *et al.*,[34] that it is possible that there are multiple pathways leading to cell death and different pathways (apoptosis, necrosis, and reactive oxygen damage) could co-exist in the same cell.

CONCLUSIONS

In general, the results indicated that modification of TBQ enhanced its activity, but increased genotoxicity was also observed. The synthesized compounds have good antioxidant activity, better than ascorbic acid, and exhibited antibacterial potential against tested Gram-positive bacteria with the strongest effect on *S. aureus* and *B. subtilis*. The results of the ARC test indicated that the toxicity of the derivatives is diminished compared to TBQ except for 2-*tert*--butyl-6-(phenylthio)-1,4-benzoquinone. The derivatives show a stronger cytotoxic effect than TBQ whereby the effect is more pronounced after a longer incubation period (72 h), with the 2-*tert*-butyl-5,6-(ethylendithio)-1,4-benzoquinone derivative showing the strongest cytotoxic activity. The tested compounds behaved as active or moderately active in the inhibition of cell growth. However, they show a low selectivity index and are considered generally toxic. In the studied concentration range, all tested derivatives possess genotoxic potential while TBQ does not exert genotoxic potential. In further studies, it is necessary to examine the mechanism of action of TBQ derivatives on DNA molecules and to determine whether genotoxicity is a consequence of cytotoxicity or vice versa.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11670, or from the corresponding author on request.

Acknowledgements. The authors are grateful to Luka Gačić who provided improvements to our English. This study was financially supported by the Ministry of Education, Science

and Technological Development of the Republic of Serbia (Grant No. 451-03-9/2021-14/ /200053, 451-03-9/2021-14/200007, 451-03-68/2022-14/200178, 451-03-9/2021-14/200168 and 451-03-68/2022-14/200026).

ИЗВОД

СИНТЕЗА И БИОЛОШКА АКТИВНОСТ АЛКИЛТИО И АРИЛТИО ДЕРИВАТА *терц*-БУТИЛХИНОНА

ЈЕЛЕНА ЂОРЂЕВИЋ[1, 2], СТОИМИР КОЛАРЕВИЋ[3], ЈОВАНА ЈОВАНОВИЋ МАРИЋ[3], МАРИАНА ОАЉЂЕ ПАВЛОВИЋ[4], ДУШАН СЛАДИЋ[5], ИРЕНА НОВАКОВИЋ[6] и БРАНКА ВУКОВИЋ-ГАЧИЋ[2]

[1]Универзитет у Београду, Институт за мултидисциплинарна истраживања, Београд, [2]Универзитет у Београду, Центар за генотоксикологију и екогенотоксикологију, Биолошки факултет, Београд, [3]Универзитет у Београду, Институт за биолошка истраживања „Синиша Станковић", Институт од националног значаја за Републику Србију, Београд, [4]Универзитет у Београду, Катедра за морфологију и систематику биљака, Биолошки факултет, Београд, [5]Универзитет у Београду, Хемијски факултет, Београд и [6]Универзитет у Београду, Институт за хемију, технологију и металургију, Центар за хемију, Београд

Испитана је биолошка активност 2-*терц*-бутил-1,4-бензохинона (TBQ) и његових деривата: 2-*терц*-бутил-5-(изопропилтио)-1,4-бензохинона, 2-*терц*-бутил-5-(пропилтио)-1,4-бензохинона, 2-*терц*-бутил-5,6-(етиленедитио)-1,4-бензохинона, 2-*терц*-бутил-5-(фенилтио)-1,4-бензохинона и 2-*терц*-бутил-6-(фенилтио)-1,4-бензохинона укључујући њихов антиоксидативни, антибактеријски, токсични, цитотоксични и генотоксични потенцијал. Применом DPPH теста, сви деривати су показали добру антиоксидативну активност, бољу од аскорбинске киселине, а најјаче дејство показао је дериват 2-*терц*-бутил-5-(пропилтио)-1,4-бензохинон. Бољи антимикробни потенцијал је примећен против Грам-позитивних бактерија методом микродилуције у бујону, где је дериват 2-*терц*-бутил-5-(фенилтио)-1,4-бензохинон показао најјачу активност (*MIC* = 15,6 μM). Резултати испитивања токсичности, применом теста на *Artemia salina*, показују да деривати губе токсични потенцијал у односу на TBQ, осим 2-*терц*-бутил-6-(фенилтио)-1,4-бензохинона, који је показао 3 пута јачи ефекат. Цитотоксичност је испитана MTT тестом у третманима од 24 и 72 h на ћелијским линијама MRC-5, HS 294T и A549 у троструко опадајућем градијенту (11, 33 и 100 μM). Модификације појачавају цитотоксични ефекат, а најјачи ефекат је примећен код деривата 2-*терц*-бутил-5,6-(етилендитио)-1,4-бензохинона. Поред тога, генотоксични потенцијал је испитан на ћелијској линији MRC-5 комет тестом. Сви испитивани деривати су показали генотоксични ефекат при свим примењеним субтоксичним концентрацијама. Генерално, хемијске модификације побољшавају биолошку активност 2-*терц*-бутил-1,4-бензохинона.

(Примљено 4. марта, ревидирано 13. маја, прихваћено 16. маја 2022)

REFERENCES

1. A. Upadhyay, *Genes Dis.* **8** (2021) 655 (https://doi.org/10.1016/j.gendis.2020.09.002)
2. IARC, *Press Release* **263** (2018) (https://www.iarc.who.int/wp-content/uploads/2018/09/pr263_E.pdf) (accessed on March 4, 2022)
3. J. Khazir, B. A. Mir, S. A. Mir, D. Cowan, *J. Asian Nat. Prod. Res.* **15** (2013) 764 (https://doi.org/10.1080/10286020.2013.798314)
4. R. G. Amaral, S. A. dos Santos, L. N. Andrade, P. Severino, A. A. Carvalho, *Clin. Oncol* **4** (2019) 1562 (https://www.clinicsinoncology.com/open-access/natural-products-as-treatment-against-cancer-a-historical-and-current-vision-1716.pdf)

5. J. T. Jimenez, M. Sturdíkova, E. Sturdík, *Acta Chim. Slov.* **2** (2009) 63 (http://acs.chtf.stuba.sk/papers/acs_0047.pdf)
6. E. Batke, R. Ogura, P. Vaupel, K. Hummel, F. Kallinowski, M. J. Gasić, H. C. Schröder, W. E. G. Müllerm, *Cell Biochem. Funct.* **6** (1988) 123 (https://doi.org/10.1002/cbf.290060207)
7. M. L. Ferrándiz, M. J. Sanz, G. Bustos, M. Payá, M. J. Alcaraz, S. de Rosa, *Eur. J. Pharmacol.* **253** (1994) 75 (https://doi.org/10.1016/0014-2999(94)90759-5)
8. M. Tsoukatou, J. P. Maréchal, C. Hellio, I. Novaković, S. Tufegdzic, D. Sladić, M. J. Gašić, A. S. Clare, C. Vagias, V. Roussis, *Molecules* **12** (2007) 1022 (https://doi.org/10.3390/12051022)
9. N. Aktaş, B. Gözcelioğlu, Y. Zang, W.-H. Lin, B. Konuklugil, *FABAD J. Pharm. Sci.* **35** (2010) 119 (http://dergi.fabad.org.tr/pdf/volum35/issue3/119-123.pdf)
10. T. Božić, I. Novaković, M. J. Gašić, Z. Juranić, T. Stanojković, S. Tufegdžić, Z. Kljajić, D. Sladić, *Eur. J. Med. Chem.* **45** (2010) 923 (https://doi.org/10.1016/j.ejmech.2009.11.033)
11. S. Kolarević, D. Milovanović, M. Kračun-Kolarević, J. Kostić, K. Sunjog, R. Martinović, J. Đorđević, I. Novaković, D. Sladić, B. Vuković-Gačić, *Drug Chem. Toxicol.* **42** (2019) 130 (https://doi.org/10.1080/01480545.2017.1413108)
12. M. Jeremić, J. Dinić, M. Pešić, M. Stepanovic, I. Novaković, D. Šegan, D. Sladić, *J. Serb. Chem. Soc.* **83** (2018) 1193 (https://doi.org/10.2298/JSC180627062J)
13. J. Vilipić, I. Novaković, T. Stanojković, I. Matić, D. Šegan, Z. Kljajić, D. Sladić, *Bioorg. Med. Chem.* **23** (2015) 6930 (https://doi.org/10.1016/j.bmc.2015.09.044)
14. M. Jeremić, M. Pešić, J. Dinić, J. Banković, I. Novaković, D. Šegan, D. Sladić, *Eur. J. Med. Chem.* **118** (2016) 107 (https://doi.org/10.1016/j.ejmech.2016.04.011)
15. J. Đorđević, S. Kolarević, J. Jovanović, J. Kostić-Vuković, I. Novaković, M. Jeremić, D. Sladić, B. Vuković-Gačić, *Drug Chem. Toxicol.* **43** (2020) 522 (https://doi.org/10.1080/01480545.2018.1514043)
16. X. Li, J. Ni, Y. Tang, X. Wang, H. Tang, H. Li, S. Zhang, X. Shen, *Nat. Prod. Res.* **33** (2019) 2722 (https://doi.org/10.1080/14786419.2018.1465425)
17. M. Sarvizadeh, O. Hasanpour, Z. Naderi Ghale-Noie, S. Mollazadeh, M. Rezaei, H. Pourghadamyari, M. Masoud Khooy, M. Aschner, H. Khan, N. Rezaei, L. Shojaie, H. Mirzaei, *Front. Oncol.* **11** (2021) 650256 (https://doi.org/10.3389/fonc.2021.650256)
18. M. C. H. Gruhlke, C. Nicco, F. Batteux, A. J. Slusarenko, *Antioxidants* **6** (2017) 1 (https://doi.org/10.3390/antiox6010001)
19. I. Novaković, Z. Vujčić, T. T. Božić, N. Božić, N. B. Milosavić, D. Sladić, *J. Serb. Chem. Soc.* **68** (2003) 243 (https://doi.org/10.2298/JSC0305243N)
20. M. S. Blois, *Nature* **181** (1958) 1199 (http://dx.doi.org/10.1038/1811199a0)
21. S. D. Sarker, L. Nahar, Y. Kumarasamy, *Methods* **42** (2007) 321 (https://doi.org/10.1016/j.ymeth.2007.01.006)
22. E. J. Crevelin, S. C. Caixeta, H. J. Dias, M. Groppo, W. R. Cunha, C. H. G. Martins, A. E. M. Crotti, *Evid. Based Complementary Altern. Med. (*2015) 102317 (https://doi.org/10.1155/2015/102317)
23. P. Vanhaecke, G. Persoone, *Ecotoxicol. Test. Mar. Environ.* **2** (1984) 588 (https://www.researchgate.net/publication/36455047)
24. A. Azqueta, K. B. Gutzkow, C. C. Priestley, S. Meier, J. S. Walker, G. Brunborg, A. R. Collins, *Toxicol. in Vitro* **27** (2013) 768 (https://doi.org/10.1016/j.tiv.2012.12.006)

25. Z. Gačić, S. Kolarević, K. Sunjog, M. Kračun-Kolarević, M. Paunović, J. Knežević--Vukčević, B. Vuković-Gačić, *Environ. Pollut.* **191** (2014) 145 (https://doi.org/10.1016/j.envpol.2014.04.024)
26. H. Lai, Y. Lim, *Int. J. Environ. Sci.* **2** (2011) 442 (https://doi.org/10.7763/IJESD.2011.V2.166)
27. S. A. Soto-Rodriguez, A. Roque, M. L. Lizarraga-Partida, A. L. Guerra-Flores, B. Gomez-Gil, *Dis. Aquat. Org.* **53** (2003) 231 (https://doi.org/10.3354/dao053231)
28. A. Koch, P. Tamez, J. Pezzuto, D. Soejarto, *J. Ethnopharmacol.* **101** (2005) 95 (https://doi.org/10.1016/j.jep.2005.03.011)
29. P. Tanamatayarat, P. Limtrakul, S. Chunsakaow, C. Duangrat, *Thai J. Pharm. Sci.* **27** (2003) 167 (https://www.thaiscience.info/Article%20for%20ThaiScience/Article/5/10016408.pdf)
30. D. Sladic, M. J. Gasic, *Molecules* **11** (2006) 1 (https://doi.org/10.3390/11010001)
31. I. Pérez-Torres, V. Guarner-Lans, M. E. Rubio-Ruiz, *Int. J. Mol. Sci.* **18** (2017) 2098 (https://doi.org/10.3390/ijms18102098)
32. C. P. Wu, S. Ohnuma, S. V. Ambudkar, *Curr. Pharm. Biotechnol.* **12** (2011) 609 (https://doi.org/10.2174/138920111795163887)
33. T. Okubo, Y. Yokoyama, K. Kano, I. Kano, *Food Chem. Toxicol.* **41** (2003) 679 (https://doi.org/10.1016/S0278-6915(03)00002-4)
34. W. Fiers, R. Beyaert, W. Declercq, P. Vandenabeele, *Oncogene* **18** (1999) 7719 (https://doi.org/10.1038/sj.onc.1203249).