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Original scientific paper

Alkylamino and aralkylamino derivatives of avarone and its mimetic as selective agents against non-small cell lung cancer cells, their antibacterial and antifungal potential

MARKO JEREMIĆ[1#], JELENA DINIĆ[2], MILICA PEŠIĆ[2], MARIJA STEPANOVIĆ[2], IRENA NOVAKOVIĆ[3#], DEJAN ŠEGAN[4] and DUŠAN SLADIĆ[4#*]

[1]Innovation Center of the Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, 11000 Belgrade, Serbia, [2]Institute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia, [3]Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Center for Chemistry, Njegoševa 12, 11000 Belgrade, Serbia and [4]Faculty of Chemistry, University of Belgrade, Studentski trg 12–16, 11000 Belgrade, Serbia

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Abstract: In this paper, the synthesis of fourteen alkylamino and arylamino derivatives of sesquiterpene quinone avarone and its model compound *tert*-butylquinone is described. Branched, cyclic, allylic and benzylic alkylamino/arylamino groups were introduced into the quinone moiety. For all the obtained derivatives, their biological activity and redox properties were studied. The cytotoxic activity of the synthesized derivatives towards multidrug resistant (MDR) human non-small cell lung carcinoma NCI-H460/R cells, their sensitive counterpart NCI-H460 and human normal keratinocytes (HaCaT) was investigated. The antimicrobial activity towards Gram-positive and Gram-negative bacteria, and fungal cultures was determined. Some of the synthesized derivatives showed selectivity for cancer cells, including MDR cells. Regarding their cell death induction potential, the most promising compounds were allylamino derivatives, preferentially triggering apoptosis, with high selectivity for cancer cells, including MDR cells. Several compounds showed promising antimicrobial activity, comparable to those of commercial antibiotic and antimycotic agents.

Keywords: quinones; anticancer activity; multidrug resistant; apoptosis; antimicrobial activity; cyclic voltammetry.

INTRODUCTION

Cancer is one of the leading causes of death worldwide, with over 8 million deaths in 2015.[1] Therefore, a great amount of resources and effort have been

* Corresponding author. E-mail: dsladic@chem.bg.ac.rs
# Serbian Chemical Society member.
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invested in developing novel strategies and drugs for combating this disease. Tackling this serious issue includes various forms of therapies, such as radiotherapy, chemotherapy, immunotherapy, surgery as well as prevention of cancer by reducing the risk of carcinogenesis.[2] Chemotherapy involves the application of drugs or cocktails of drugs in order to eliminate developed cancer cells. The limitations of chemotherapy are low efficacy, lack of selectivity, severe toxicity, metastasis and development of drug resistance.[3] The phenomenon of multi-drug resistance (MDR) is a particular problem, caused by the fast proliferation and metabolism of cancer cells together with a proneness to mutations, which enables cancer cells to overcome the toxicity of the applied drugs, resulting in resistant forms of cancer.[4]

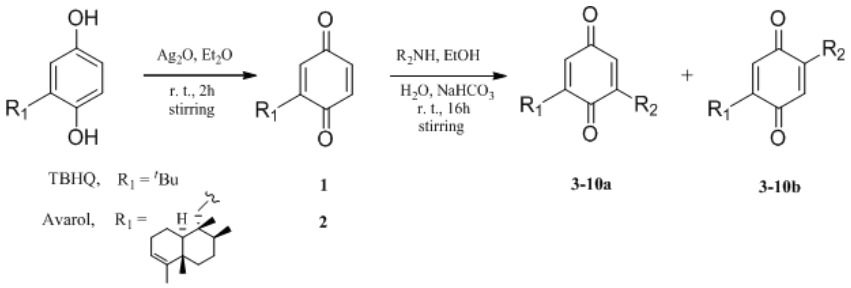
Quinones are a class of organic compounds that have a versatile array of activities, some of which are herbicidal,[5] antimalarial,[6] antiviral,[7] antipsoriatic,[8] antileishmanial[9] and cytotoxic activity.[10–12] Biological activity of quinones originates from dual mode of action. It is a combination of 1,4-Michael addition of cellular nucleophiles to conjugate enone system and the generation of reactive oxygen species (ROS) in redox cycling reactions of quinone and its hydroquinone pair *via* a semiquinone anion radical.[13] Quinone/hydroquinone core-containing compounds are very abundant in nature, being involved in crucially important processes, such as photosynthesis,[14] the mitochondrial electron transport chain[15] and protection from oxidizing species.[16] Natural products of marine origin are of particular interest for their diversity of structures and a myriad of activities, together with the vast possibilities for their modifications.[17–19]

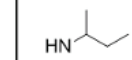
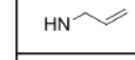
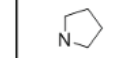
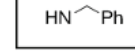
As a continuation of previous work on alkylamino, aralkylamino and amino acid derivatives of avarone and its mimetic *tert*-butylbenzoquinone (TBQ),[20,21] the synthesis and investigation of the cytotoxic activity of a series of alkylamino and aralkylamino derivatives of avarone and *tert*-butylbenzoquinone on three cell lines, *i.e.*, non-small cell lung cancer cells, both the sensitive NCI-H460 and multi-drug resistant NCI-H460/R, and healthy human keratinocytes HaCaT are reported herein. Since in a previous work, benzylamino derivatives of *tert*-butylquinone showed good selectivity for tumour cells, including MDR cells, benzylamino derivatives of avarone were synthesized in the present study. Other amines selected for derivatization of both quinones were allylamines due to similar electronic effects of the allyl and benzyl group, and amines leading to sterically more compressed derivatives, *i.e.*, *sec*-butylamine and pyrrolidine. Additionally, their antibacterial and antifungal activities were determined. Cyclic voltammetry was used to study the redox behaviour of newly synthesized compounds in the system quinone–semiquinone–hydroquinone, in order to gain information on the structure−activity relationship.

RESULTS AND DISCUSSION

Chemistry

As a continuation of previous work, fourteen aminoquinones **3**–**10a** and **3**–**10b** were synthesized (preparation of compounds **6a** and **6b** was presented in a previous paper[20]). Treatment of the parent quinones *tert*-butylquinone (**1**) and avarone (**2**) with various amines produced the corresponding two regioisomers of aminoquinones *via* 1,4-Michael addition reactions (Scheme 1).



R_2	R_1	
	^{t}Bu	
	**3a,3b**	**7a,7b**
	**4a,4b**	**8a,8b**
	**5a,5b**	**9a,9b**
	**6a,6b**	**10a,10b**

Scheme 1. Synthesis of the quinone derivatives.

As expected, with *tert*-butylquinone, the 2,6-disubstituted quinone products were dominant over the 2,5-disubstituted products. On the other hand, with avarone, only in the reaction with pyrrolidine was the 2,6-disubstituted product dominant while in the reaction with allylamine, the 2,5-disubstituted product was the main product and with the other two nucleophiles, similar amounts of two products were obtained. Racemic *sec*-butylamine was used in the reactions. The NMR spectra of the products obtained in its reaction with avarone indicate the presence of only one diastereoisomer. Unfortunately, the configuration of the asymmetric carbon could not be determined, since all attempts to crystallize the products were unsuccessful.

Half-wave potentials were recorded at a glassy carbon disk (3 mm diameter) in DMSO towards a silver wire immersed in the electrolyte solution containing 0.01 M silver ions as the reference electrode, and ferrocene as the reference compound. The results are given in Table I and shown in Fig. S-1 of the Supplementary material to this paper.

TABLE I. Voltammetric half-peak potentials and standard redox potentials of the synthesized compounds (V *vs*. silver/silver chloride electrode)

Compound	E_{c1}	E_{a1}	E_{c2}	E_{a2}	$E^0{}_1/F_c$
3a	–1.210	–1.126	–1.890	–1.741	–1.170
3b	–1.218	–1.154	–1.868	–1.744	–1.186
4a	–1.180	–1.121	–1.812	–1.712	–1.149
4b	–1.210	–1.152	–1.848	–1.733	–1.181
5a	–1.276	–1.214	–1.876	–1.694	–1.244
5b	–1.276	–1.214	–1.865	–1.749	–1.243
6a[20]	–1.128	–1.053	–1.800	–1.619	–1.159
6b[20]	–1.141	–1.071	–1.803	–1.593	–1.174
7a	–1.193	–1.127	–1.881	–1.774	–1.158
7b	–1.201	–1.133	–1.859	–1.713	–1.168
8a	–1.170	–1.113	–1.830	–1.741	–1.142
8b	–1.177	–1.117	–1.799	–1.689	–1.150
9a	–1.267	–1.163	–1.883	–1.710	–1.217
9b	–1.259	–1.177	–1.861	–1.704	–1.218
10a	–1.115	–1.043	–1.865	–1.733	–1.147
10b	–1.123	–1.054	–1.840	–1.685	–1.157

Typical quinone electrochemical behaviour was observed for all the synthesized compounds, *i.e.*, two waves that could be attributed to the reduction of quinone to the semiquinone radical and, subsequently, to the hydroquinone dianion (Fig. S-1). First reduction wave was fully reversible, indicating a diffusion controlled process. However, second reduction process was not reversible, having higher peak separation potentials. The derivatization did not perturb the nature of these two redox processes, but rather shifted the peak potentials. As expected, all the derivatives had more negative reduction potential than the parent quinone. It could be assumed that amino substituents with electron-donating ability destabilize the intermediate semiquinone relative to the corresponding quinone. This effect leads to a negative shift in the peak potential. Since the inductive effect of the alkyl part of the alkylamino substituent influences the electron-donating ability of the substituent, the pyrrolidino derivatives have the most negative reduction potentials, while benzylamino and allylamino derivatives have the least negative one. As previously described,[20] the position of the substituent plays a particular role in shifting the peak potential. It was observed that the 4′-derivatives generally had a peak potential more negative by about 10 to 20 mV than the corresponding 3′-derivatives. The probable reason is that the arrangement is more

favourable when the electron-donating amino and alkyl substituents in semiquinone anion radical are both in the meta position to the negative oxygen.

Anticancer activity

Derivatives of TBQ were compared with corresponding avarone derivatives regarding their growth inhibition activity against human cancer and normal cell lines. The cell growth inhibition activity was studied in non-small cell lung carcinoma cells – NCI-H460/R, their multidrug resistant (MDR) counterpart – NCI--H460/R and human keratinocytes – HaCaT cells. The differences in response between cancer and normal cells, evaluated by the MTT assay after 72 h treatment, are presented in Table II and Fig. S-2 of the Supplementary material.

TABLE II. Growth inhibition activity (IC_{50} in μM) of TBQ, avarone and their derivatives in human non-small cell lung carcinoma cell lines (NCI-H460 – sensitive and NCI-H460/R – MDR variant) and human normal keratinocytes (HaCaT); the IC_{50} values were calculated from a minimum of three independent experiments (average ± standard deviation)

Compound	Cell line		
	NCI-H460	NCI-H460/R	HaCaT
CDDP[a]	5.2±0.4	1.7±0.1	0.7±0.1
1[20a]	>100	72±8	95±4
2[20a,b]	84±9	24±4	37±8
3a[b,c]	10.5±0.4	16.3±1.3	24.4±1.5
3b[b,c]	36.6±1.8	96.3±3.9	72.5±1.5
4a	9.6±0.1	12.6±0.4	8.5±0.4
4b	18.3±0.6	21.8±0.2	23.9±0.7
5a[c]	40.9±0.4	65.9±2.6	36.8±4
5b[c]	44.4±4.0	85.7±2.6	26.8±2.4
6a[20b]	14±2	10±2	21±1
6b[20b]	19±4	15±2	28±2
7a	3.5±0.04	3.8±0.1	4±0.1
7b	37.5±1.1	44.4±0.9	40.6±2
8a	2.8±0.03	3.0±0.1	3.5±0.1
8b	18±1.3	24.2±0.7	19.2±0.4
9a	28.2±3.7	20.4±1.2	19.3±0.8
9b[b]	8.9±1.5	7.1±0.2	15.1±0.3
10a[c]	54.4±2.2	26.3±2.6	12±1
10b	>100	>100	42.2±3.0

[a]Selectivity towards MDR cells (higher efficacy in NCI-H460/R compared to NCI-H460 *i.e.*, IC_{50} of sensitive cancer cells ≥ 1.5 fold than IC_{50} of corresponding MDR cells); [b]selectivity towards cancer cells (IC_{50} of either sensitive or MDR cancer cells ≤ 1.5 fold than IC_{50} of normal cells. The dose response curves are shown in Fig. S-2); [c]resistance (lower efficacy in NCI-H460/R compared to NCI-H460 *i.e.*, IC_{50} of sensitive cancer cells ≤ 1.5 fold than IC_{50} of the corresponding MDR cells)

The growth inhibition abilities of parent compounds TBQ and avarone, as well as CDDP, an FDA-approved drug for non-small cell lung carcinoma treatment,[22] were investigated in our previous study.[20] CDDP showed stronger effect

in MDR cancer cells in comparison with their sensitive counterparts, but it was not selective against cancer cells due to the pronounced activity obtained in normal human keratinocytes. TBQ was largely ineffective in all cell lines while avarone showed high selectivity towards MDR cells. The derivatives had generally a higher activity than the parent compounds. Avarone 2,6-disubstituted *sec*-butylamino and allylamino derivatives (**7a** and **8a**, respectively) exhibited the highest activity in all tested cell lines with IC_{50} values below 5 μM and, importantly, their activity was not affected by the presence of MDR phenotype. Corresponding 2,5-disubstituted derivatives **7b** and **8b** showed the same pattern of activity (without selectivity against cancer cells) but their efficacy was significantly diminished as their IC_{50} values were around one order of magnitude higher than those obtained by **7a** and **8a**. Similarly, 2,6-disubstituted allylamino derivative **4a** with IC_{50} values near 10 μM was more efficient than **4b** but without selectivity to cancer cells. Benzylamino avarone derivative **10b** was the least active compound in all cell lines, while the IC_{50} values for corresponding **10a** and two pyrrolidino *tert*-butylquinone derivatives **5a** and **5b** were above 25 μM in cancer cells. These four derivatives were more active in normal cells. Compounds **3a** and **9b** exhibited both high cytotoxicity and selectivity towards cancer cell lines and most notably MDR phenotype did not reduce their activity. Although direct correlation between redox properties and cytotoxicity of derivatives with different substituents could not be established, it should be noticed that out of two regioisomers, the isomer with a less negative cathodic potential of the first wave, *i.e.*, the isomer that is more prone to the formation of semiquinone radicals, is more active.

In the *tert*-butylquinone series, the 6-derivatives were always more active to tumour cells than the 5-derivatives. The introduction of the bulky *sec*-butylamino group into the 6-position, *i.e.*, closer to the *tert*-butyl group, did not have much influence on the activity to tumour cells, but it decreased the toxicity to normal cells, and hence, contrary to the 6-(butylamino) derivative, the corresponding *sec*-butylamino substituted quinone showed a pronounced selectivity to tumour cell lines. This did not apply for a more remote 5 substituent. The introduction of allylamino group led to products with higher cytotoxicity than similar derivatives with linear saturated alkylamino substituents, but the selectivity for tumour cells displayed by 6-(ethylamino) derivative was lost.[20] 5-(Allylamino) and 5-(benzylamino) products were the only 5-substituted derivatives that showed a relatively high cytotoxicity, but only the benzylamino derivative showed selectivity for tumour cell lines. The pyrrolidino derivatives were only moderately active, without selectivity for tumour cells. If the whole set of compounds is considered, for a strong cytotoxic activity against tumour cells, an unsaturated or bulky substituent should be present in position 6. For achieving selectivity, the set of sub-

stituents was narrowed to bulky groups, and even more to aralkyl groups (benzylamino, phenethylamino) if selectivity to MDR cells is considered.

As for avarone derivatives, the 3′-derivatives (2,6-disubstitution) were always much more active than the 4′-derivatives (2,5-disubstitution), with the exception of the pyrrolidino derivatives when the inverse was true. Within this series, the introduction of branching or unsaturation into alkylamino substituent increased the cytotoxicity regardless of the position of the substituent. 3′-(*sec*-Butylamino) and 3′-(allylamino) derivatives showed IC_{50} values in the range 3–4 μM. Unfortunately, the selectivity for MDR cells, shown by 3′-(butylamino)-avarone,[20] was lost. The benzylamino derivatives were less active to tumour cells than the phenethylamino ones. The 4′-pyrrolidino derivative (the more active isomer) showed strong activity to the tumour cell lines, with an IC_{50} value of 7.1 μM against the multi-drug resistant cells, and selectivity for tumour cells. The 3′-derivative showed moderate activity, without selectivity. Summarizing these results, the necessary prerequisite for an avarone derivative to have a strong activity against tumour cells is a substituent with a relatively low number of carbon atoms at the position 3′ or pyrrolidine at position 4′. Of these substituents, only unbranched saturated butylamino derivative and heterocyclic pyrrolidino derivative showed the desired selectivity.

Apoptosis and necrosis are the two main forms of cell death. Apoptosis is regarded as a programmed process, with minimal level of ATP required for the assembly of a apoptosome complex and activation of caspases, while necrosis is often referred to as the complete decay of cell metabolism.[23] They can be distinguished from one another by various morphological and biochemical characteristics, although there is no clear-cut distinction between these two (hence, other forms of cell death, *i.e.*, necroptosis, aponecrosis, *etc.*).[24] The most obvious characteristics of apoptosis are cell shrinkage, DNA fragmentation, condensation of chromatin, formation of apoptotic bodies, disruption of mitochondrial redox processes and generation of ATP. Cells undergoing apoptosis do not release their constituents into the surrounding tissue because the integrity of the cell membrane is preserved. In addition, macrophages quickly phagocytose apoptotic bodies and the surrounding cells do not produce anti-inflammatory cytokines.[25] Therefore, no inflammatory reaction occurs during the apoptotic process. Necrosis, on the other hand, is usually followed by inflammation, because one of the main characteristics of necrosis is the loss of membrane integrity and the release of the cytoplasmic content into the surrounding area, leading to inflammation.[26] Inflammation, for its part, can damage cells and even cause cancer.[27] Bearing this in mind, apoptosis is clearly the preferred type of cell death, considering the removal of either healthy, aging or tumour cells. Besides inflammation, the other characteristics of necrosis are cell swelling and complete abolition of ATP pro-

duction. However, many types of cancer employ anti-apoptotic mechanisms for their survival, so necrosis could be useful in promoting the death of tumour cells.

The cell death inducing capacity of 25 μM TBQ and the avarone derivatives was analyzed after 72 h treatment (Table III, Fig. S-3 of the Supplementary material). Both pyrrolidino *tert*-butylquinone derivatives (**5a** and **5b**) showed significant activity towards sensitive cancer cells, triggering both apoptosis and necrosis, which was not observed in normal cells. The allylamino derivatives **4a** and **4b** induced apoptotic type of cell death more prominently in both cancer cell lines than in HaCaT cells. The *sec*-butylamino derivatives **3a** and **3b** also induced apoptosis, most effectively in NCI-H460 cells. Cells treated with the avarone pyrrolidino derivatives **9a** and **9b** underwent necrosis as the predominant cell death type. Consistent with the cell growth analysis, the inverted efficacy pattern of **9a** and **9b** as well as the selectivity of **9b** towards cancer cells was confirmed by cell death induction. Compounds **8a** and **8b** (allylamino derivatives of avarone) also predominantly induced necrosis in cancer cells with **8a** being the significantly more potent derivative. *sec*-Butylamino derivative **7a** was considerably more active against MDR cancer cells in triggering necrotic cell death compared to **7b**. A similar but reduced effect was also observed in NCI-H460 and HaCaT cells. Importantly, according to the cell death analysis, **7a** and **8a** were shown to be selective against cancer cells.

TABLE III. Cell death induction by 25 μM TBQ, avarone and their derivatives in human non-small cell lung carcinoma cell lines (NCI-H460 – sensitive and NCI-H460/R – MDR variant) and human normal keratinocytes (HaCaT)). CDDP is included as a positive control. The values represent percentages of viable, apoptotic and necrotic cells

Compound	Viable cells	Early apoptosis	Late apoptosis	Necrosis
	AV–PI–	AV+PI–	AV+PI+	AV–PI+
		NCI-H460		
Control	95.5	2	1.1	1.3
CDDP	66	1.6	5.2	27.2
1[20]	95.2	0.5	0.5	3.8
2[20]	74	3.4	16.3	6.3
3a	43.8	21.9	28.2	6.1
3b	85.6	3.5	4.9	6
4a	5.1	24.9	61.4	8.6
4b	4.9	23.1	63.9	8.1
5a	67.4	4.7	11.4	16.5
5b	51	5.3	18.1	25.6
7a	53.9	6.6	5.7	33.8
7b	81.4	0.7	3.9	13.9
8a	28.6	12.5	18	40.9
8b	72.1	3.5	7	17.4
9a	83.6	2	3.5	10.9
9b	31	3.4	10	55.6

TABLE III. Continued

Compound	Viable cells	Early apoptosis	Late apoptosis	Necrosis
	AV-PI-	AV+PI-	AV+PI+	AV-PI+
		NCI-H460/R		
Control	95.6	1.6	1.9	0.9
CDDP	55.3	3.8	13.3	27.6
1[20]	95.2	0.5	1.2	3.1
2[20]	65.8	2.6	22.7	8.9
3a	68.4	9.2	17.5	4.9
3b	93	2.3	3.2	1.5
4a	7.3	4.1	64.4	24.2
4b	11.8	6.3	63.4	18.5
5a	91.8	2	4.1	2.1
5b	84.8	2	5.5	7.7
7a	38.4	3.8	9.8	48
7b	96.3	1.5	1.4	0.8
8a	21.7	6.6	19.2	52.5
8b	55.1	4.4	7.1	33.4
9a	58.7	5.2	7.7	28.4
9b	26.9	3.9	10.9	58.3
		HaCaT		
Control	93.6	2.9	1	2.4
CDDP	53.5	1.4	9	36.1
1[20]	86.8	1.9	3.8	7.5
2[20]	78.3	2.5	10.1	9.1
3a	64	14.2	9.7	12.1
3b	82	6.8	5.5	5.7
4a	61.5	15.9	8.1	14.5
4b	33.5	18.8	18.9	28.8
5a	85.9	4.8	4.9	4.4
5b	85.3	4.9	4	5.8
7a	72.3	1.1	1	25.6
7b	95.7	0.5	0.6	3.2
8a	73.8	2.4	4.1	19.7
8b	89.3	0.6	0.8	9.3
9a	97.2	0.3	0.3	2.2
9b	83.7	0.9	1.1	14.3

The cell death-inducing activity of compounds **4a** and **4b** significantly stand out from the others in the series. The majority of the cells treated with **4a** or **4b** underwent apoptotic cell death, targeting late apoptosis preferentially. Most importantly, this pair were very selective toward cancer cell lines, both sensitive and resistant ones, particularly the **4a** derivative with over 60 % of viable cells in healthy HaCaT cell lines. This distinction could be attributed to the allyl moiety. The probable reason is the additional generation of ROS *via* allyl radicals, formed by the decomposition of the semiquinone radical generated by the one-

electron reduction of allylamino quinone (the other product would be amino quinone) and/or the formation of cytotoxic acrolein by the oxidative metabolism of allylamino quinones.[28] Considering that allylamino TBQ derivatives preferentially induce (late) apoptosis, and that cancer cells are usually already in a state of oxidative stress,[29] this seems a plausible explanation for the selective action.

Antimicrobial activity

Compounds **3**–**10a** and **3**–**10b** were tested for their antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungal strains, and compared to the commercial antibiotics amikacin and antimycotic nystatin. The results are given in Tables IV and V.

Most of the derivatives showed weak activity in comparison to amikacin, having at least an order of a magnitude higher *MIC*. The most active TBQ derivatives were allylamino derivatives **4a** and **4b**, with activity comparable to amikacin for *E. coli*.

This pair had almost identical *MIC* for all strains except *M. luteus* (ATCC 10240 and 4698) and *S. enterica*. The pair **3a** and **3b** showed generally weaker activity than **4a** and **4b**, with **3a** being only slightly more active against *B. subtilis*, *M. luteus* (ATCC 10240 and 4698) and *E. coli*, and **3b** for *K. rhizophila*. The pair **5a** and **5b** displayed the weakest activity of all the tested TBQ compounds, with some activity only towards *K. rhizophila*, *E. coli* and *M. luteus*, ATCC 4698, (only **5a**). The avarone counterparts showed no activity, as expected from previous results.[20] The probable reason for inactivity is insufficient hydrophilicity. This conclusion is corroborated by the fact that several amino acid derivatives of avarone showed strong antibacterial activity.[21] In general, in order to show a relatively broad activity comparable to amikacin, *tert*-butylquinone derivatives should have a non-branched medium length alkylamino group or an aralkyl group in position 3′. It is interesting that among the avarone amino acid derivatives, the most active were those with aromatic amino acids, implying that there is an aromatic binding site in a putative target. All the tested compounds, except **10a** and **10b**, showed stronger activity toward *C. albicans* than nystatin, with **3a**, **4a**, **4b** and **5a** even having a two orders of a magnitude lower *MIC* (less than 50 μM). Towards *A. brasiliensis*, the activities were generally comparable to that of nystatin, with **4a**, **4b** and **5a** displaying stronger activity. Considering *S. cerevisiae*, **4a**, **5a**, **7b**, **9a** and **9b** possess stronger activity than amikacin. Of all the tested compounds, **4a** showed the strongest antifungal activity, while **10a** and **10b** showed no activity at all. Based on these results and those from a previous paper,[20] it could be concluded that in order to achieve a strong anti-*Candida* activity, a compound should be a 6-substituted 2-*tert*-butyl-1,4-benzoquinone (exception are allylamino derivatives, where both isomers have a similar activity),

TABLE IV. Antibacterial *in vitro* activity (*MIC* / mM) against Gram-positive and Gram-negative bacteria

Compd.	Bacteria									
	Staphylococcus aureus (ATCC 6358)	*Kocuria rhizophila* (ATCC 9341)	*Bacillus subtilis* (ATCC 6633)	*Micrococcus luteus* (ATCC 10240)	*Micrococcus luteus* (ATCC 4698)	*Clostridium sporogenes* (ATCC 19404)	*Escherichia coli* (ATCC 25922)	*Proteus hauseri* (ATCC 13315)	*Salmonella enterica* (ATCC 13076)	*Pseudomonas aeruginosa* (ATCC 9027)
3a	0.667	0.085	0.667	0.085	0.166	0.667	0.021	1.33	0.166	2.656
3b	0.667	0.042	1.33	0.085	0.166	0.667	0.085	1.33	0.166	2.656
4a	0.36	0.045	0.716	0.178	0.178	0.36	0.022	0.36	0.091	0.716
4b	0.36	0.045	0.716	0.36	0.36	0.36	0.022	0.36	0.178	0.716
5a	2.679	0.086	2.679	0.339	0.085	2.679	0.339	2.679	0.673	2.679
5b	2.679	0.167	1.342	0.673	2.679	2.679	0.167	2.679	2.679	2.679
6a[20]	0.009	–	2.323	0.009	4.646	2.323	0.009	–	4.646	2.323
6b[20]	0.072	4.646	4.646	0.018	2.323	2.323	0.036	2.323	4.646	4.646
7a	–	–	–	–	–	–	–	–	–	–
7b	–	–	–	–	–	–	–	–	–	–
8a	–	–	–	–	–	–	–	–	–	–
8b	–	–	–	–	–	–	–	–	–	–
9a	–	–	–	–	–	–	–	–	–	–
9b	–	–	–	–	–	–	–	–	–	–
10a	–	–	–	–	–	–	–	–	–	–
10b	–	–	–	–	–	–	–	–	–	–
Amikacin	0.019	0.003	0.072	0.014	0.003	0.026	0.009	0.012	0.014	0.085

with at least three carbon atoms in the side chain. The avarone derivatives were much less active, but in contrast to antibacterial effects, most of them had some activity, although much lower than the amino acid derivatives.[21] The amino acid derivatives of TBQ had weak antimicrobial activity, probably due to their excessive hydrophilicity.

TABLE V. Antifungal *in vitro* activity (*MIC*/mM)

Compound	Fungi		
	Candida albicans (ATCC 10231)	*Aspergillus brasiliensis* (ATCC 16404)	*Saccharomyces cerevisiae* (ATCC 9763)
3a	0.042	1.33	1.33
3b	0.166	2.656	2.656
4a	0.047	0.36	0.36
4b	0.047	0.716	1.427
5a	0.042	0.339	0.673
5b	0.673	2.679	2.679
***6a**	2.333	2.333	2.333
6b[20]	–	–	–
7a	0.41	1.631	0.817
7b	0.41	1.631	1.631
8a	0.852	1.701	3.401
8b	1.701	1.701	3.401
9a	1.638	1.638	0.82
9b	1.638	1.638	0.411
10a	–	–	–
10b	–	–	–
Nystatin	2.7	1.35	1.35

EXPERIMENTAL

General synthetic procedure

The parent quinones were obtained from hydroquinones according to a previously described procedure.[20] Quinones (300mg; 1.83 mmol **1**; 0.96 mmol **2**) were dissolved in ethanol (50 mL). Amine hydrochloride salts (in large excess, 22×) were prepared as aqueous solutions. The pH of the solution was adjusted to 7–8 by the addition of solid sodium bicarbonate, and the solution was added to quinone. Water and ethanol were added to the reaction mixture to a final ratio water:ethanol of 1:1, and total volume of 300 mL. The reaction mixture was stirred at 60–70 °C for 3 h. Ethanol was removed by vacuum evaporation, and the reaction mixture was extracted two times by dichloromethane, with half the volume of the aqueous phase each time. The organic phase was separated, dried with anhydrous calcium chloride, and the solvent was removed by evaporation under vacuum. The crude products were separated by column or low-bar chromatography and purified by preparative thin-layer chromatography, with the indicated solvents. Numbering scheme for assignment of signals in NMR spectra of all compounds is given in Scheme S-1.

Cyclic voltammetry

Electrochemical behaviour of synthesized aminoquinones was studied as previously described.[20]

Biological activity

Cytotoxic, antibacterial and antifungal activity of synthesized compounds were analyzed according to a previous study.[20]

CONCLUSIONS

Among the 14 newly synthesized compounds, a potential to be antitumor agents was shown for *sec*-butylamino derivatives of *tert*-butylquinone (**3a** and **b**) because of their selectivity for tumour cells, allylamino derivatives of *tert*-butylquinone (**4a** and **b**) because of their selective induction of apoptosis in tumour cells, including MDR cells, 3′-(*sec*-butylamino)avarone (**7a**) and 3′-(allylamino)-avarone (**8a**) because of their higher cytotoxic activity than cisplatin, as well as 4′-pyrrolidinoavarone (**9b**) because of selectivity to tumour cells, including MDR cells. Some derivatives showed promising antimicrobial properties: *sec*-butylamino (**3a**) and allylamino (**4a** and **b**) derivatives of *tert*-butylquinone, because of an activity against *E. coli* similar to that of amikacin, and strong antifungal activity to *C. albicans*, which also applies to the pyrrolidino derivative of *tert*--butylquinone (**5a**).

SUPPLEMENTARY MATERIAL

Additional experimental results, as well as spectroscopic and analytical data, are available electronically on the pages of the journal's website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

АЛКИЛАМИНО И АРАЛКИЛАМИНО ДЕРИВАТИ АВАРОНА И ЊЕГОВОГ МИМЕТИКА КАО СЕЛЕКТИВНИ АГЕНСИ ПРЕМА ЋЕЛИЈАМА НЕСИТНОЋЕЛИЈСКОГ КАРЦИНОМА ПЛУЋА, ЊИХОВ АНТИБАКТЕРИЈСКИ И АНТИФУНГАЛНИ ПОТЕНЦИЈАЛ

МАРКО ЈЕРЕМИЋ[1], ЈЕЛЕНА ДИНИЋ[2], МИЛИЦА ПЕШИЋ[2], МАРИЈА СТЕПАНОВИЋ[2], ИРЕНА НОВАКОВИЋ[3], ДЕЈАН ШЕГАН[4] и ДУШАН СЛАДИЋ[4]

[1]Иновациони центар Хемијског факултета, Универзитет у Београду, Студентски трг 12–16, 11000 Београд, [2]Институт за биолошка истраживања „Синиша Станковић", Универзитет у Београду, Деспота Стефана 142, 11060 Београд, [3]Институт за хемију, технологију и металургију, Универзитет у Београду, Центар за хемију, Његошева 12, 11000 Београд и [4]Хемијски факултет, Универзитет у Београду, Студентски трг 12–16, 11000 Београд

У овом раду, описана је синтеза четрнаест алкиламино и аралкиламино деривата сесквитерпенског хинона аварона и његовог модел-једињења, *терц*-бутилхинона. Алкиламино/аралкиламино групе које су уведене у хинонски остатак биле су разграната, циклична, алилна и бензилна. За све добијене деривате одређена је биолошка активност и редокс особине. Испитана је цитотоксична активност синтетисаних деривата према резистентним ћелијама неситноћелијског карцинома плућа (NCI-H460/R), њиховом осетљивом пандану (NCI-H460) и нормалним хуманим кератиноцитима (HaCaT). Одређена је и антимикробна активност према Грам-позитивним и Грам-негативним бактеријама и културама гљивица. Неки од синтетисаних деривата су показали селективност

према ћелијама канцера, укључујући и резистентне ћелије. Што се тиче потенцијала за индукцију ћелијске смрти, деривати који највише обећавају су алиламино деривати, који преференцијално активирају апоптозу, са великом селективношћу према ћелијама канцера, укључујући и резистентне ћелије. Неколико једињења је показало обећавајућу антимикробну активност, упоредиву са комерцијалним антибиотицима и антимикотицима.

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