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Synthesis and biological activity of amino acid derivatives of avarone and its model compound

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ABSTRACT

A series of eighteen derivatives of marine sesquiterpene quinone avarone and its model system *tert*-butylquinone with amino acids has been synthesized by nucleophilic addition of amino acids to the quinones. In vitro cytotoxic activity toward human cancer cell lines (HeLa, A549, Fem-X, K562, MDA-MB-453) and normal MRC-5 cell line was determined. Several compounds showed very strong inhibitory activity with IC_{50} values less than 10 μM. Avarone derivatives were more active than the corresponding *tert*-butylquinone derivatives. The results of the cytofluorimetric analysis of cell cycle of HeLa cells showed that apoptosis might be one of possible mechanism of action of these compounds in cancer cells. In order to examine the influence of caspases on cell death, the apoptotic mechanisms induced by the tested compounds were determined using specific caspases 3, 8 and 9 inhibitors. For all compounds antibacterial activities against six strains of Gram-positive and four strains of Gram-negative bacteria were determined, as well as antifungal activity against three fungal species.

1. Introduction

Marine organisms represent a rich source of metabolites with a high pharmacological potential and exceptionally different structures.[1–4] Many natural products with a decalin-type system and a quinoid moiety are important compounds with powerful and diverse biological properties.[5–7]

Sponges of the order Dictyoceratida are a rich source of bioactive secondary metabolites, sesquiterpene quinones and hydroquinones such as avarol, avarone, illimaquinone, nakijiquinone and bolinaquinone. These compounds have attracted considerable interest due to their remarkable biological activities—antiproliferative, cytotoxic, antiviral and antimicrobial properties. All the above features of marine products provide the possibility for the further development of new agents which can be successfully identified as new drugs and/or factors which participate in clarifying intracellular events.

The hydroquinone avarol and its quinone derivative, avarone, were isolated from the marine sponge *Dysidea avara*.[8,9] This redox couple has a large range of pharmacological properties including antitumor,[10–12] antiinflammatory,[13–15] antibacterial,[16,17] antiviral,[18,19] antioxidant,[20,21] antipsoriatic[22,23] and antibiofouling[24] activities. Also, the compounds induced DNA damage[5,25–27] as a result of reactive oxygen radicals generation, as well as enzyme inhibitory[28–30] activities incurred as a consequence of modification of biomolecules by nucleophilic addition.

During the search for new metabolites from marine organisms, nakijiquinones have been isolated. Nakijiquinones have identical sesquiterpene skeleton as avarone, including the position of the double bond, and additional hydroxyl and either amino acid or amine substituents at the quinone moiety. Kobayashi et al. have isolated a great number of these bioactive metabolites from the Okinawan marine sponge extracts (family Spongiidae). Nakijiquinones A and B (which possess glycine and valine residue attached to the quinone ring, respectively, as shown in Fig. 1) were the first isolated sesquiterpenoid quinones with a normal amino acid residue from natural origin.[31] It should be emphasized that the presence of aminoquinone compounds is not very rare in natural sources.[32–34] Nakijiquinone A and B show a noticeable cytotoxicity against L 1210 murine leukemia cells and KB human epidermoid carcinoma cells, as well as antifungal activity against fungi *Candida albicans* and *Aspergillus niger*.[31] Nakijiquinones C and D (Fig. 1), containing serine and threonine residue, respectively, were isolated from the same sponge family and display

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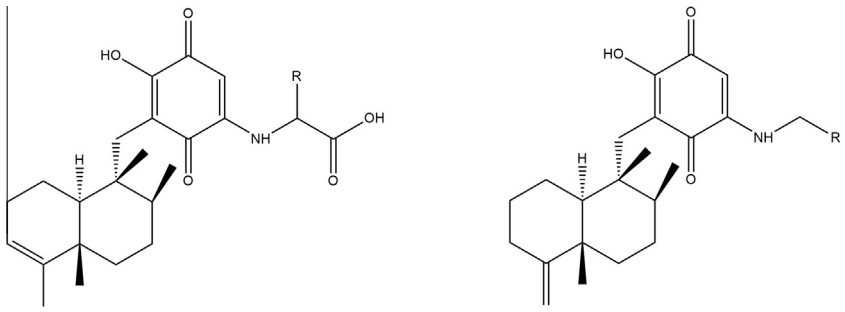

Nakijiquinone

Smenospongine

A R = H **B** R = $CH(CH_3)_2$ **C** R = CH_2OH **D** R = $CH(OH)CH_3$

B R = COOH **C** R = CH_2COOH

Figure 1. Sesquiterpene quinones with an amino acid in side chain (nakijiquinones A–D; smenospongines B and C).

similar cytotoxicity.[35] Further research resulted in the isolation of two dimeric sesquiterpenoid quinones, nakijiquinones E and F, which did not show cytotoxicity against murine leukemia P388 and L1210, and KB human epidermoid carcinoma cell.[36] Unlike these, sesquiterpenoid quinones containing a different amine residue derived from amino acids, nakijiquinones G-I, showed a modest cytotoxicity against the forementioned cancer cells and an inhibitory activity against HER2 kinase.[37] Additionally, nakijiquinone H demonstrated a good antibacterial and antifungal activity.[37] Some of the nakijiquinones J–R, with amine residue attached to quinone ring, showed inhibitory activities against EGFR and HER2 tyrosine kinases.[38] Biologically active smenospongines (Fig. 1), isomeric to nakijiquinones, were isolated from the marine sponge *Dactylospongia elegans*[39] and showed a cytotoxic activity against a panel of tumor cell lines.

Considering all of the above, the rationale of this work was to obtain avarone derivatives with amino acids, which could be considered mimics of nakijiquinones. Possible advantage of this approach would be to afford derivatives with better solubility in water, and a better selectivity than avarone, since it is expected that the electronic and steric effects of the amino acid substituent would decrease the reactivity of the quinone moiety with cellular nucleophiles. Different tautomeric forms of the products could have different binding properties with putative targets compared to avarone. Although avarone is the major constituent of the sponge *Dysidea avara*, which has been a subject of cultivation and cell culture projects, availability of large amounts of the compound remains a problem, so it seemed reasonable to investigate whether a simplification of the structure would lead to satisfactory biological activity. Therefore, a very simple model, *tert*-butylquinone was selected as target for modification, with the same amino acids. The model quinone is readily available.

Thus, in this paper, the synthesis and characterization of eighteen new amino acid derivatives of avarone and *tert*-butylquinone are reported. The results of cytotoxic activity investigation of synthesized compounds, against five cancer cell lines and a non-cancerous cell line are also presented. Furthermore, effects of the derivatives on cell cycle analysis of HeLa cells and the caspases activity were analyzed. The antibacterial activities of all the compounds against six strains of Gram-positive and four strains of Gram-negative bacteria were determined, as well as the antifungal activity against three fungal species. The biological activity of all derivatives was also examined by the brine shrimp test, that is, toxicity to *Artemia salina*. Electrochemical parameters of all compounds were also determined in order to better understand structure–activity relationships.

2. Results and discussion

2.1. Chemistry

The preparation of the derivatives is shown in Figure 2. The synthesis started with commercially available *tert*-butylhydroquinone or avarol isolated from *D. avara*. The hydroquinones were oxidized using silver oxide to the corresponding quinones. All of the derivatives were obtained by slowly adding an amino acid dissolved in saturated sodium bicarbonate solution, to the ethanol solution of the appropriate quinone, and stirring at room temperature for several hours. Using all amino acids only products arising from substitution in position 3′ were obtained. The exception was L-proline which afforded only 4′-derivatives. In our previous publication,[40] it was shown that good nucleophiles preferentially add in position 4′ and weaker nucleophiles in position 3′. Proline is much more nucleophilic than the other amino acids.[41]

IR spectra, recorded as KBr disks show that the derivatives are in the form of zwitter-ions, based on the absence of carboxylic carbonyl absorption (except for both glycine derivatives), and the presence of the strong carboxylate ion band at 1600–1580 cm^{-1}. The presence of form III (Fig. 3) in solid state can be evidenced by a strong C–O vibration band at 1150–1100 cm^{-1}, and a strong broad absorption at 3600–2600 cm^{-1} from superimposed O–H and N–H stretching bands. There are two types of carbonyl stretching vibrations, a stronger band at ca. 1630 cm^{-1} from hydrogen bonded carbonyl group, and a weaker band at 1670 cm^{-1} from a non-hydrogen bonded conjugated carbonyl. The tautomeric equilibrium could be inferred from the severe broadening of signals in NMR spectra taken in $CDCl_3$. However, in CD_3OD the signals are sharp and indicate the dominant presence of quinone tautomeric forms. These forms are now favored since there is no need for formation of a tautomer with a hydroxylic group, because the protic solvent plays the role of the hydrogen bond donor.

The position of the substituent on the benzoquinone ring was determined using ^1H NMR and ^{13}C NMR spectroscopy. In the proton spectra, recorded in methanol, of 3′-substituted derivatives signals of quinone protons are doublets with J = 2.2 Hz, while in the spectra of 4′-substituted they are singlets. In ^{13}C NMR spectra the positions of the signals are in accordance with the calculated values.[42]

Cyclic voltammetry was used for examination of electrochemical properties in order to correlate structure and bioactivity. Cyclic voltammetry measurements of avarone and amino acid derivatives in dimethyl sulfoxide gave two waves corresponding to reversible or quasi-reversible one-electron processes (Fig. 4). Such behavior is typical for redox couple quinone/semiquinone anion radical and