Chemico-Biological Interactions 247 (2016) 22–29

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Antiproliferative effect and apoptotic activity of linear geranylphenol derivatives from phloroglucinol and orcinol

Lautaro Taborga [a, *, 1], Luis Espinoza [a], Alejandra Moller [b], Héctor Carrasco [c], Mauricio Cuellar [d], Joan Villena [b, **, 1]

[a] Departamento de Química, Universidad Técnica Federico Santa María, Av. España 1680, Valparaíso, 2390123, Chile
[b] Centro Investigaciones Biomédicas (CIB), Facultad de Medicina, Universidad de Valparaíso, Hontaneda 2653, Valparaíso, 2341386, Chile
[c] Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Llano Subercaseaux 2801, San Miguel, Santiago, 8900000, Chile
[d] Facultad de Farmacia, Universidad de Valparaíso, Av. Gran Bretaña 1093, Playa Ancha, Valparaíso, 2360102, Chile

ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
23 December 2015
Accepted 21 January 2016
Available online 28 January 2016

Keywords:
Cytotoxic activity
Cancer cell lines
Apoptosis
Mitochondrial membrane permeability
Caspase-3 activity
Linear geranylphenols

ABSTRACT

Sixteen synthetic linear derivatives geranylphenols, were obtained from phloroglucinol and orcinol, and cytotoxic activity was evaluated *in vitro* against cancer cell lines (HT-29, PC-3, MDA-MB231, DU-145) and one non-tumor cell line, human dermal fibroblast (HDF). IC50 values were determined at concentrations of 0–100 μM of each compound for 72 h. Compounds **12**, **13**, **17**, **21**, **22** and **25**, showed cytotoxic activity. To elucidate whether these compounds reduce cell viability by inducing apoptosis, cell lines MCF-7, PC-3 and DHF were treated with each active compound **12**, **13**, **17**, **21**, **22** and **25** and were examined after Hoechst 33342 staining. The compounds **12**, **13** and **17** induced apoptosis in various cancer cell lines, as shown by nuclear condensation and/or fragmentation. In addition, it was found that compounds **12** and **13**, induced changes in mitochondrial membrane permeability in those cancer cell lines. Such induction was associated with the depletion of mitochondrial membrane potential. These activities led to the cleavage of caspases inducing the cell death process.

1. Introduction

Prenylated and polyprenylated 1,4-benzoquinones and hydroquinones such as ubiquinones, plastoquinones, and tocopherols are widespread in plants and animals, in which they play important roles in electron transport, photosynthesis, and as antioxidants [1,2]. Prenyl benzoquinones have been also isolated from brown algae of the order Fucales [3–6], sponges [7–10], alcyonaceans [11], gorgonaceans [12], and ascidians belonging to the genus *Aplidium* [13–18]. These substances present a terpenoid portion ranging from one to nine isoprene units.

It has been extensively documented that multiple metabolites, obtained from species belonging to the plant kingdom, have special biological properties suitable for controlling several types of animal and plant pathogen. For instance, linear geranylquinones or geranylhydroquinones, present in higher plants and in marine urochordates [19], exhibit cytotoxic activity and inhibit larval growth and development. Some particular compounds such as 2-geranylbenzoquinone (**1**), isolated from *Ascindian Synoicum castellatum* [20], 2-geranylhydroquinone (**2**) isolated from the *Cordia alliodora* tree [21], *Phacelia crenulata* [22–24], *Aplidium antillense* [25] and the tunicate *Amaroucium multiplicatum* [26], have been related to biological activities including toxicity, cytotoxicity, antimicrobial, anti-cancer protective and antioxidant effects, among others [23,26–30]. Additionally, linear geranylmethoxyphenol/acetates (compounds **3**–**5**, see Fig. 1), isolated from *Phaceliaixodes* [25], are cytotoxic, allergenic and insecticidal, and topical application of 100 μg of geranylbenzoquinone on pupae of *Tenebrio* caused severe abnormalities and death [31]. However, considering the activity against phytophagous insects and pathogens reported for compounds **3**–**5** (Fig. 1) [31] and assuming the presence of this property in other geranylphenol analogs, we have recently reported the synthesis, structure determination and their effect on mycelial

* Corresponding author.
** Corresponding author.
E-mail addresses: lautaro.taborga@usm.cl (L. Taborga), luis.espinozac@usm.cl (L. Espinoza), alejandra.moller@uv.cl (A. Moller), hector.carrasco@uautonoma.cl (H. Carrasco), mauricio.cuellar@uv.cl (M. Cuellar), juan.villena@uv.cl (J. Villena).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cbi.2016.01.016
0009-2797/

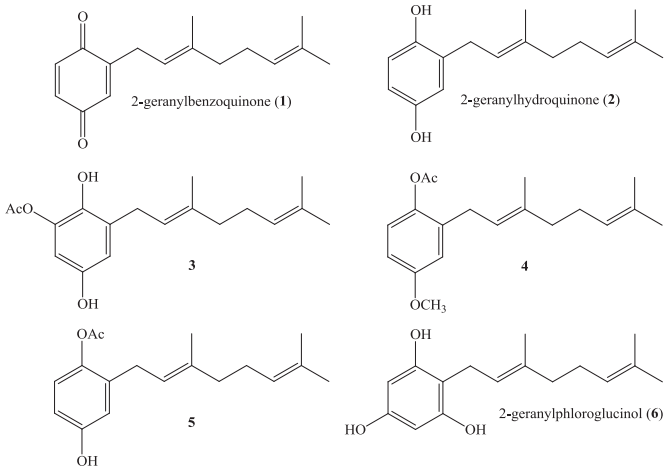

Fig. 1. Structure of some active natural and synthetic linear geranylphenols.

growth of plant pathogen *Botrytis cinerea* of eight linear geranylphenols molecules, wherein the compounds **2** and 2-geranylphloroglucinol (**6**) were the more active ones, showing an inhibitory effect on the mycelial growth that depends on the applied concentration [32].

On the other hand, different studies of the structure activity relation (SAR) in a series of non methoxylated and methoxylated prenylated quinones with side chains containing from one to eight isoprene units reported that the optimum length of the side-chain is two isoprene units and in the para-position relative to the methoxy-group [28,33]. Additionally, these authors informed that all tested quinones (compounds **7**–**10**, Fig. 2) have inhibited JB6 Cl41 cell transformation and p53 activity inducing apoptosis and also the activities of AP-1 and NF-κB. Additionally, in previous studies we have reported the synthesis and cytotoxic activity of 2-geranylbenzoquinone (**1**) and 2-geranylhydroquinone (**2**) and some geranylmethoxyphenol/acetate analogs (Fig. 1) [34,35].

Therefore, in this research we report the cytotoxic activity of a series of linear geranylphenol, acetylated and methoxylated derivatives of phloroglucinol (compounds **6**, **11**–**14**, see Fig. 3) and orcinol (compounds **15**–**25**, see Fig. 4). These compounds were obtained by traditional methodology [33–37] and by a modification of a previously reported synthetic method [32]. Compounds **6** and **11**–**25** were evaluated *in vitro* against various human cancer cells lines in order to analyze the cytotoxic activity.

2. Results and discussion

2.1. Chemical

2-geranylphloroglucinol (**6**) and derivatives **11**–**13** were obtained by a previously reported synthetic method [32]. While the trimethoxylated derivative **14** was obtained by methylation reaction of **6**, (86.8% yield), in dimethyl sulfate/K_2CO_3 system under reflux conditions in acetone. The structure of **14** was mainly established by NMR, where the signals at $\delta_H = 3.92$ ppm (s, 3H, OCH_3) and $\delta_H = 3.79$ ppm (s, 6H, $2 \times OCH_3$) confirmed the presence of three methoxyl groups. Additionally, in the ^{13}C NMR spectrum, the signal at $\delta_C = 55.3$ ppm was assigned to $2 \times OCH_3$ and $\delta_C = 54.9$ ppm was assigned to the third methoxy group.

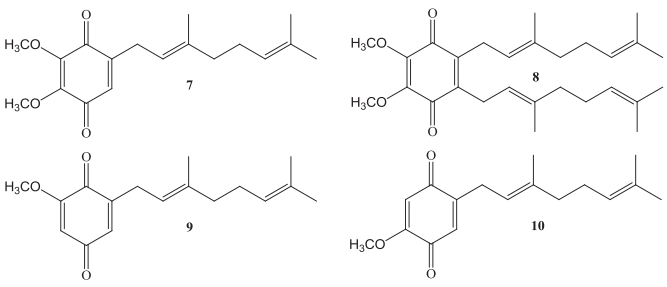

Fig. 2. Structure of some active synthetic linear geranylquinones.

Compounds **15** and **16** were previously synthesized by means of Electrophilic Aromatic Substitutions reactions, catalyzed by mineral acids, such as orcinol reaction with geraniol in aqueous formic acid solutions [38], condensation reaction of geraniol and orcinol in methylene chloride in the presence of *p*-toluenesulfonic acid [39]. However, the yields obtained with these methodologies were low (2–18% yield). Subsequently we report the preparation of compounds **15** and **16** from orcinol and geraniol with 27.6% and 12.6% yields respectively, using Lewis acid (BF_3Et_2O) as catalyst in dioxane [36]. Recently, our research group reported a new synthesis of compounds **15** and **16** with identical yields, by direct geranylation reaction between orcinol and geraniol, using BF_3OEt_2 as catalyst and $AgNO_3$ as secondary catalyst but using acetonitrile instead of dioxane as solvent. Additionally in this reaction the compounds **17** and **18** were obtained with 6.1% and 9.9% yields respectively [40]. Subsequently derivatives **19**–**22** were obtained by standard acetylation reaction (Ac_2O/CH_2Cl_2/DMAP) from phenols **15**–**18** respectively [40].

On the other hand the methoxylated compounds **23**–**25** were obtained by methylation reaction (in dimethyl sulfate/K_2CO_3 system under reflux conditions in acetone) from **15**, **16** and **18**, with 80.1%, 78.7% and 78.1% yield respectively.

The structure of **23**–**25** was mainly established by NMR. For compound **23** in the ^1H NMR spectrum the signals at $\delta_H = 3.81$ ppm (s, 3H, OCH_3) and $\delta_H = 3.80$ ppm (s, 3H, OCH_3) confirmed the presence of two methoxyl groups. Additionally, in the ^{13}C NMR spectrum, the signals at $\delta_C = 55.5$ and 55.1 ppm was assigned to two methoxyl group. For symmetric compound **24**, the signal at $\delta_H = 3.85$ ppm (s, 6H, OCH_3) and the signal at $\delta_C = 55.5$ ppm ($2 \times OCH_3$) in the ^{13}C NMR spectrum, mainly confirm the structure **24**. Similarly compound **25**, showed signals at $\delta_H = 3.82$ ppm (s, 3H, OCH_3) and $\delta_H = 3.72$ ppm (s, 3H, OCH_3) in the ^1H NMR spectrum. While in the ^{13}C NMR spectrum the signals at $\delta_C = 61.5$ and 55.5 ppm was assigned to two methoxyl group respectively.

2.1.1. Biological

2.1.1.1. Cell viability

2.1.1.1.1. In vitro growth inhibition assay. The cytotoxicity of compounds (**6** and **11**–**25**) was evaluated *in vitro* against different cancer cell lines: MDA-MB-231 breast cancer, DU-145 and PC-3 prostate cancer, HT-29 colon cancer and one non-tumor cell line, human dermal fibroblast (HDF). A colorimetric assay was set up to estimate the IC50 values. The IC_{50} obtained from these assays are shown in Table 1.

The highest cytotoxicity values were observed for compounds **12** and **13** in all cell lines tested and were more active than those of the rest of compounds. The cytotoxicity of compounds in human dermal fibroblast (HDF) is similar than in the cancer cell lines under study, even so the cytotoxic activity was not previously described for the compounds **12**, **13**, **17**, **21**, **22**, and **25** in the literature.

Based on the obtained results, the cytotoxic action against studied cell lines would be explained by the structure-activity relationship analysis of the results showed in Table 1. It is clearly seen, that the presence of both geranyl chains on the aromatic ring is critical for apoptotic activity, because monogeranyl compounds have not activity on any of the studied cell lines. In the same way, by