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SYNTHESIS AND CYTOTOXIC ACTIVITY OF GERANYLMETHOXYHYDROQUINONE DERIVATIVES

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ABSTRACT

The new synthetic geranyl-2,4-methoxyhydroquinone **1** and the known geranyl-4,5-methoxyhydroquinone **2** were prepared by Electrophilic Aromatic Substitution (*EAS*) reactions between geraniol and 1,3,5-trimethoxyphenol using $BF_3{\cdot}Et_2O$ as a catalyst. Furthermore, the new geranylmethoxyhydroquinones derivatives (**3-6**) were obtained by chemical transformations of **1** and **2**. The compounds have been evaluated for their cytotoxic activities against PC-3 human prostate cancer cell line, MCF-7 and MDA-MB-231 human breast cancer cells lines and dermal human fibroblasts DHF. IC_{50} values for compounds **1** and **5** ranged in the 80 μM level.

Keywords: Geranylmethoxyhydroquinone, synthesis, cytotoxic activity

INTRODUCTION

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. On the other hand, different studies of the structure activity relation (SAR) in a series of nonmethoxylated 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[19, 20]. Additionally, these authors informed that all tested quinones (3-Demethylubiquinone Q2 and its synthetic derivatives) have inhibited of JB6 Cl41 cell transformation and p53 activity and induction of apoptosis, AP-1 and NF-kB activity.

Due to the importance that showed by these compounds in the mentioned biological activities, in this work we describe the synthesis and cytotoxic activity of geranylmethoxyhidroquinones derivatives (**1-6**). The new synthetic geranyl-2,4-dimethoxyhydroquinone **1** and known geranyl-4,5-dimethoxyhydroquinone **2** were synthesized using the strategy of Electrophilic Aromatic Substitution (*EAS*) reactions, according to the reported protocol[21-23]. The geranylmethoxyhidroquinones derivatives (**3-6**) are synthetic analogs of **1** and **2**, while the known compound **5** is analogous to the marine natural product verapliquinone A. All the compounds were evaluated *in vitro* against various human cancer cells lines in order to analyse the influence of the molecular structure on the cytotoxic activity.

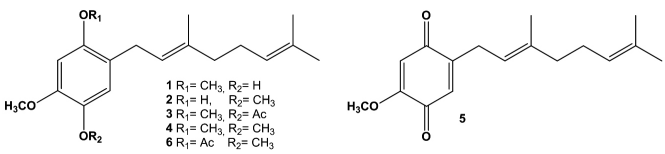
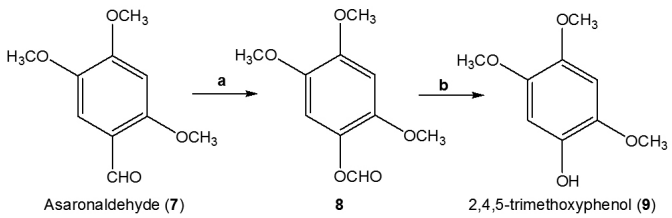


Figure 1. Structure of synthesized compounds.

RESULTS AND DISCUSSION

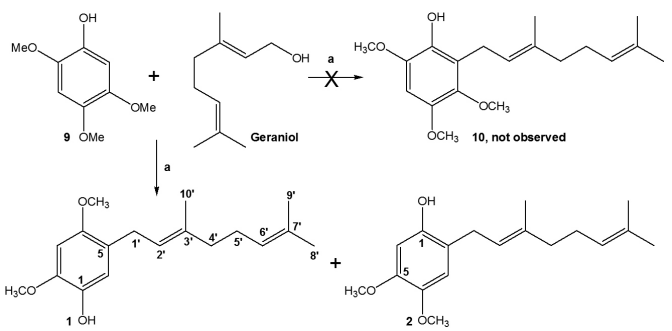
Chemistry

Our first objective was the preparation of compound **(9)** from commercially available asaronaldehyde **(7)**. The first step involves a Baeyer Villiger oxidation reaction and subsequent saponification of formate **8**[24], according to the synthetic route shown in scheme 1.



Scheme 1. Synthesis of 2,4,5-trimethoxyphenol **9** (**a**) MCPBA/CH_2Cl_2, r.t. 1.87 g, 86%; (**b**) CH_3OH/Et_3N, r.t. 2.45 g, 78%.

The next objective consisted in the coupling reaction between geraniol with 2,4,5-trimethoxyphenol **(9)**. For this purpose the strategy of Electrophilic Aromatic Substitution (EAS), was used[21-23]. Nevertheless, in this reaction was not possible to obtain the wished compound **10** (scheme 2) and unexpectedly two compounds were obtained: 2,4-dimethoxy-5-((*E*)-3',7'-dimethylocta-2',6'-dienyl)phenol **(1)** (15% yield) and 4,5-dimethoxy-2-((*E*)-3',7'-dimethylocta-2',6'-dienyl)phenol **(2)** (13% yield). The mass difference corresponds to geraniol not reacted, geraniol decomposition and not reacted 2,4,5-trimethoxyphenol.



Scheme 2. Synthesis of geranylmethoxyhydroquinone derivatives. (**a**) dioxane/$BF_3{\cdot}Et_2O$, N_2, r.t.: **1**, 79.1 mg 15% and **2** 65.3 mg 13% yield.

The formation of compounds **1** and **2** was not possible to explain it by the EAS strategy, probably the coupling reaction ocurred from a different pathway, competing with the EAS. In the presence of BF_3, the compound **9** afforded the allyl aryl ethers **9a** wich presumably undergoes Claisen rearrangement[27-28] giving the dienones **9b** y **9c**. The dienones recover the aromaticity through the formation and later loss of a formaldehyde molecule. The mechanism which was proposed to explain the formation of compounds **1** and **2** is showed in scheme 3.

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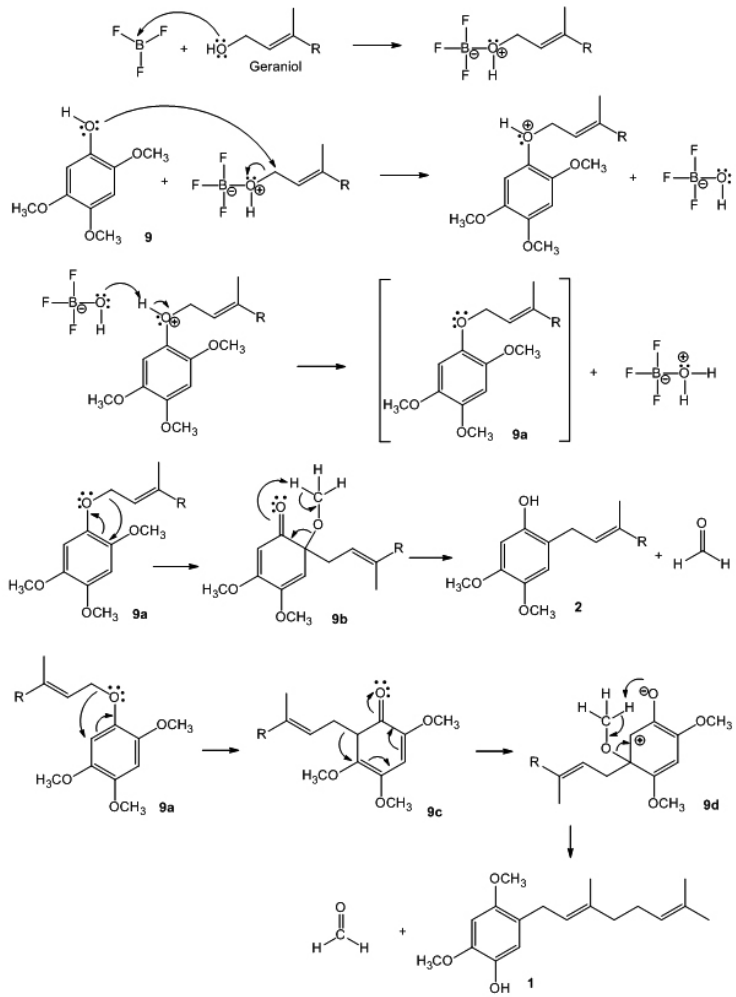


Scheme 3. Proposed reaction mechanism for the formation of compounds **1** and **2**.

In the ^{1}H-NMR spectrum of geranyl-2,4-dimethoxyphenol **1** the signals at δ 3.82 ppm (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3) of the two methoxyl groups, δ 3.25 ppm (d, J = 7.0, 2H, H-1') of the point of coupling, 5.49 ppm (s, 1H, OH) of the phenol and two aromatic hydrogens at δ 6.68 ppm (s, 1H, H-6), 6.54 (s, 1H, H-3) were mainly observed. In the ^{13}C-NMR spectrum the presence of the methoxyl groups at δ 56.8 ppm, 56.1 ppm, the signals of two aromatic carbons at δ 99.3 ppm (C-3) and 112.8 ppm (C-6) also were confirmed. These data also were corroborated by 2D HMBC ^{3}J heteronuclear correlations between H-1' and the carbon signals at δ 151.8 (C-4), 112.8 (C-6), 136.0 (C-3') ppm and ^{2}J with δ 122.8 (C-2'), 120.9 (C-5) ppm (figure 2a). The *E*-geometry of the trisubstituted C2'-C3' double bond was established from gs-sel-^{1}H ^{1}D-NOESY experiments (figure 2b), where H-1' showed a long range interaction with the methyl group in position 10' (1.70 ppm). In addition, the configuration of the C2'-C3' double bond was compared with the chemical shifts of C-10' reported for similar compounds[19].

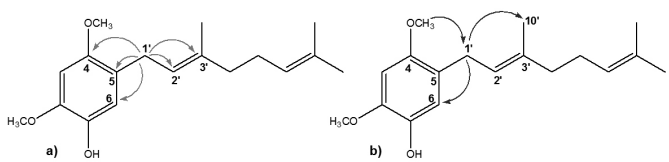


Figure 2. Structure of compound **1**. (**a**) HMBC correlations; (**b**) NOE correlations.

Nevertheless the ^{1}H-NMR spectrum of **2** was compared with the reported spectral data[20]. This compound showed the existence of an hydroxyl group at δ 4.93 ppm and the presence of two aromatic hydrogens at δ 6.62 ppm (s, 1H, H-5); 6.45 ppm (s, 1H, H-6). In addition, the point of coupling was confirmed by the presence of the signal at δ 3.30 (d, J = 7.0 Hz, 2H, H-1'). While in the ^{13}C-NMR spectrum the signals at δ 113.7 (C-3), 101.2 (C-6) of two aromatic carbons and 29.6 (C-1') corroborate the molecular structure.

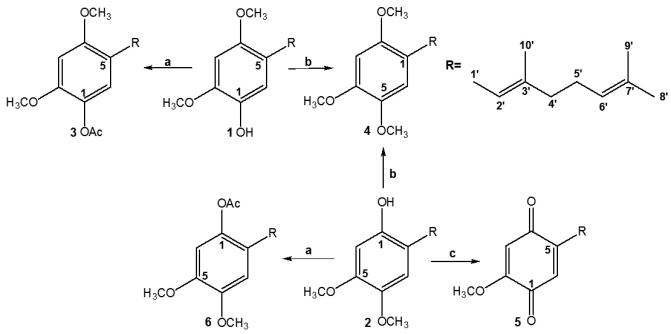
For the preparation of 2,4-dimethoxy-5-((*E*)-3',7'-dimethylocta-2',6'-dienyl)phenyl acetate derivative **3**, the geranyl-2,4-dimethoxyphenol **1** was acetylated with acetic anhydride and dimethylaminopyridine (DMAP) as catalyst, then the compound **3** was obtained with a 55% of yield (scheme 4). The molecular structure was corroborated for the signals at δ 2.31 ppm, 20.7 ppm, 169.2 ppm of the acetyl group, in addition for comparison of their spectral data with geranyl-2,4-dimethoxyphenol **1**.

The preparation of 2,4,5-trimethoxy-1-((*E*)-3',7'-dimethylocta-2',6'-dienyl)benzene derivative **4**, was carried out by reaction of methylation with $(CH_3)_2SO_4$ in slightly basic conditions of the compound **1** or **2** with a 40 % yield (scheme 4). ^{1}H-NMR spectrum showed three singlets at δ 3.87 ppm 3.82 ppm and 3.80 ppm, the signals corresponding to the methoxyl groups. These signals also were observed in the ^{13}C-NMR spectrum at δ 56.3 ppm, 56.7 ppm and 56.6 ppm.

The 2-methoxy-5-((*E*)-3',7'-dimethylocta-2',6'-dienyl)-1,4-benzoquinone compound **5**, was prepared by oxidation reaction of geranyl-4,5-dimethoxyphenol **2** with nitrate of cerium and ammonium (CAN), and was obtained with a 15 % yield (scheme 4). The molecular structure was confirmed for the presence of the signals at δ 6.46 ppm (t, 1H, H-6) and 5.92 ppm (s, 1H, H-3) of the quinonic hydrogens in the ^{1}H-NMR spectrum and the existence in the ^{13}C-NMR spectrum of two carbonyl carbons at δ 182.4 ppm (C-1), 187.6 ppm

(C-4), in addition to comparison with the spectral data reported for the compound[20].

Finally the 4,5-dimethoxy-2-((*E*)-3',7'-dimethylocta-2',6'-dienyl)phenyl acetate derivative **6**, was prepared using the same protocol of synthesis for the derivative **3** with a 70 % yield (scheme 4). ^{1}H-NMR and ^{13}C-NMR spectra showed the signals corresponding to the acetyl groups at δ 2.29 ppm, 20.8 ppm and 169.8 ppm.



Scheme 4. Synthesis of geranylmethoxyhydroquinone derivatives. (**a**) Ac_2O/CH_2Cl_2/DMAP, r.t. for **3**, 30 mg 55%; for **6**, 30 mg 70% yield (**b**) $(CH_3)_2SO_4/K_2CO_3$, acetone, r.t. for **4** from **1**, 37 mg, 40% whereas for compound **4** from **2** the same yield was obtained (**c**) CAN/acetonitrile/water, r.t. 20 mg 15% yield.

Antiproliferative activity

The cytotoxicity of the compounds was evaluated *in vitro* against three different cancer cell lines: PC-3 human prostate cancer, MCF-7 and MDA-MB-231 human breast cancer and one non-tumoral cell line, dermal human fibroblasts (DHF) by using sulforhodamine dye assay. This conventional colorimetric assay was set up to estimate the IC_{50} values which represents the concentration of a drug that is required for 50% inhibition *in vitro* after 72 h of treatment with the test compounds. Five serial dilutions (from 12.5 to 100 μM) for each sample were evaluated in triplicate. The results obtained from these assays are shown in Table 1. The compounds **2-4** and **6** did not affect the viability of the cells lines studied, however the compounds **1** and **5** showed IC_{50} values with inhibitory activity, ranged in the 80 μM level for breast cancer cell line MCF-7. Moreover the compound **5** showed IC_{50} values with inhibitory activity, ranged in the 80 μM level for breast cancer cell line MDA-MB-231, these values may be due to the presence of a quinone moiety. In the case of the compound **1** the cytotoxicity could be influenced by the position of the hydroxyl group in the ring, *orto* to the methoxyl group. Furthermore, it was demonstrated by the results obtained for the acetylated derivative **3** and the *meta* position of the hydroxyl group in the compound **2**, which did not display cytotoxic activity. Moreover, compound **1** and **5** showed some selectivity for the cancer cells versus fibroblast cells because the IC_{50} for human fibroblast were on 100 μM, which could provide an approach to obtain compounds with potential less toxicity in normal human cells. The increased citotoxicity induced by compounds **1** and **5** on human breast cancer cell lines was due probably to the presence of a quinone moiety and *orto* position of the hydroxyl group on the structure of these compounds versus the compounds **2**, **3**, **4** and **6**.

EXPERIMENTAL

General

Unless otherwise stated, all chemical reagents purchased (Merck or Aldrich) were of the highest commercially available purity and were used without previous purification. IR spectra were recorded as thin films in a Nicolet Impact 420 spectrometer and frequencies are reported in cm^{-1}. Low resolution mass spectra were recorded on a Shimadzu QP-2000 spectrometer at 70eV ionising voltage and are given as m/z (% rel. int.). ^{1}H, ^{13}C (DEPT 135), sel. 1D ^{1}H NOESY, 2D HSQC and 2D HMBC spectra were recorded in $CDCl_3$ solutions and are referenced to the residual peaks of $CHCl_3$ at δ 7.26 ppm and δ 77.0 ppm for ^{1}H and ^{13}C, respectively, on a Bruker Avance 400 Digital NMR spectrometer, operating at 400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C. Chemical shifts are reported in δ ppm and coupling constants (*J*) are given in Hz. Silica gel (Merck 200-300 mesh) was used for C.C. and silica gel plates HF-254 for TLC. TLC spots were detected by heating after spraying with 25% H_2SO_4 in H_2O.

Chemistry

*General procedure for the Electrophilic Aromatic Substitution (EAS), synthesis of compounds **1**, **2***

$BF_3.Et_2O$ (0.08 g, 0.6 mmol) was gradually added at room temperature to a solution of 2,4,5-trimethoxyphenol (0.3278 g, 1.8 mmol) and geraniol (0.3 g, 1.8 mmol) in freshly distilled 1,4-dioxane (20 mL). The mixture was stirred at room temperature under a nitrogen atmosphere for 24 h, when the completion of the reaction was verified by TLC. The mixture was poured onto crushed ice (about 30 g) and the organic layer was extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with 5% $NaHCO_3$ (30 mL), then with water (2 × 20 mL) and dried over anhydrous Na_2SO_4, filtered and evaporated. The crude residue was redissolved in CH_2Cl_2 (5 mL) and chromatographed on silica gel with petroleum ether/EtOAc mixtures of increasing polarity (19.8:0.2→16.0:4.0 for **1** and 19.8:0.2→13.0:7.0 for **2**).

2,4-dimethoxy-5-((E)-3',7'-dimethylocta-2',6'-dienyl)phenol **1**: colorless viscous oil, 79.1 mg (15%). **^{1}H-NMR**: 6.68 (s, 1H, H-6); 6.54 (s, 1H, H-3); 5.49 (s, 1H, OH); 5.28 (t, *J*= 7.0 Hz, 1H, H-2'); 5.11 (t, *J*= 7.0 Hz, 1H, H-6'); 3.82 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.25 (d, *J*= 7.0, 2H, H-1'); 2.08 (m, 4H, H-5' and H-4'); 1.70 (s, 3H, H-10'); 1.67 (s, 3H, H-8'); 1.60 (s, 3H, H-9'). **^{13}C-NMR**: 144.1 (C-1), 140.1 (C-2), 99.3 (C-3), 151.8 (C-4), 120.9 (C-5), 112.8 (C-6), 56.8 (OCH_3), 56.1 (OCH_3), 27.7 (C-1'), 122.8 (C-2'), 136.0 (C-3'), 39.8 (C-4'), 26.8 (C-5'), 124.3 (C-6'), 131.4 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'). **IR** (cm^{-1}): 2924, 1602, 1512, 1194, 1042. **MS** (m/z, %): M$^+$ 290 (< 1%), 288 (13), 287 (28), 246 (43), 245 (9), 219 (12), 203 (15), 178 (39), 177 (70), 175 (9), 163 (17), 161 (31), 149 (10) 124 (16), 123 (100), 122 (21), 69 (36).

4,5-dimethoxy-2-((E)-3',7'-dimethylocta-2',6'-dienyl)phenol **2**: colorless viscous oil, 65.3 mg (13%). **^{1}H-NMR**: 6.62 (s, 1H, H-5); 6.45 (s, 1H, H-6); 5.30 (t, *J*= 7.0 Hz, 1H, H-2'); 5.07 (t, *J*= 7.0 Hz, 1H, H-6'); 4.93 (s, 1H, OH); 3.82 (s, 6H, OCH_3); 3.30 (d, *J*= 7.0, 2H, H-1'); 2.09 (m, 4H, H-5' and H-4'); 1.78 (s, 3H, H-10'); 1.68 (s, 3H, H-8'); 1.60 (s, 3H, H-9'). **^{13}C-NMR**: 148.4 (C-1), 117.3 (C-2), 113.7 (C-3), 142.8 (C-4), 148.3 (C-5), 101.2 (C-6), 55.9 (OCH_3), 56.6 (OCH_3), 29.6 (C-1'), 121.9 (C-2'), 138.6 (C-3'), 39.6 (C-4'), 26.4 (C-5'), 123.7 (C-6'), 132.0 (C-7'), 25.6 (C-8'), 17.7 (C-9'), 16.2 (C-10'). **IR** (cm^{-1}): 2924, 1619, 1512, 1451, 1195. **MS** (m/z, %): M$^+$ 290 (40), 221 (4), 207 (4), 205 (15), 190 (5), 175 (6), 168 (12), 167 (100), 166 (8), 69 (9).

Synthesis of geranylmethoxyhidroquinones derivatives (**3**–**6**)

2,4-dimethoxy-5-((E)-3',7'-dimethylocta-2',6'-dienyl)phenyl acetate **3**

To geranyl-2,4-dimethoxyphenol **1** (0.0485 g, 0.16 mmol) dissolved in dichloromethane (10 mL), acetic anhydride (0.02 g, 0.02 mL, 0.16 mmol) and dimethylaminopyridine (DMAP, 2 mg, 0.016 mmol) were added. The mixture was stirred at room temperature during 2 hours. The organic phase was washed with water and after it was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product, which gave 30 mg (55 % yield) of compound **3** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→17.8:2.2), colorless viscous oil. **^{1}H-NMR**: 6.80 (s, 1H, H-6); 6.57 (s, 1H, H-3); 5.30 (t, *J*= 7.0 Hz, 1H, H-2'); 5.12 (t, *J*= 7.0 Hz, 1H, H-6'); 3.77 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.30 (d, *J*= 7.0, 2H, H-1'); 2.31 (s, 3H, $CO_2\underline{CH}_3$); 2.09 (m, 4H, H-5' and H-4'); 1.70 (s, 3H, H-10'); 1.67 (s, 3H, H-8'); 1.60 (s, 3H, H-9'). **^{13}C-NMR**: 144.6 (C-1), 137.7 (C-2), 106.1 (C-3), 151.2 (C-4), 128.2 (C-5), 114.4 (C-6), 56.0 (OCH_3), 56.6 (OCH_3), 28.0 (C-1'), 122.0 (C-2'), 136.6 (C-3'), 39.7 (C-4'), 26.7 (C-5'), 124.2 (C-6'), 131.4 (C-7'), 26.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'); 20.7 ($CO_2\underline{CH}_3$); 169.2 (CO_2). **IR** (cm^{-1}): 2929, 1768, 1768, 1510, 1215, 1203, 1040. **MS** (m/z, %): M$^+$ 332 (87), 291 (20), 290 (94), 221 (39), 207 (16), 189 (40), 175 (18), 168 (14), 167 (100), 161 (52), 154 (13), 129 (20), 69 (18).

2,4,5-trimethoxy-1-((E)-3',7'-dimethylocta-2',6'-dienyl)benzene **4**

To **1** or **2** (90 mg, 0.3 mmol) dissolved in acetone (10 mL), potassium of carbonate (0.04 g, 0.3 mmol) and dimethyl sulphate (0.04 g, 0.3 mmol) were added. The mixture was stirred at room temperature during 24 hours. After

filtration, the solution was evaporated to dryness. Dilution with diethyl ether was followed by washing with NaOH solution (5%) and the organic phase was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product, which gave 37 mg (40 % yield) of compound **4** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→18.2:1.8), colorless viscous oil. **^{1}H-NMR**: 6.71 (s, 1H, H-6); 6.52 (s, 1H, H-3); 5.29 (t, *J*= 7.0 Hz, 1H, H-2'); 5.11 (t, *J*= 7.0 Hz, 1H, H-6'); 3.87 (s, 3H, OCH_3); 3.82 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 3.27 (d, *J*= 7.0, 2H, H-1'); 2.08 (m, 4H, H-5' and H-4'); 1.71 (s, 3H, H-10'); 1.67 (s, 3H, H-8'); 1.61 (s, 3H, H-9'). **^{13}C-NMR**: 143.0 (C-1), 147.6 (C-2), 98.2 (C-3), 151.4 (C-4), 121.9 (C-5), 113.9 (C-6), 56.3 (OCH_3), 56.7 (OCH_3), 56.6 (OCH_3), 27.6 (C-1'), 122.7 (C-2'), 136.0 (C-3'), 39.8 (C-4'), 26.8 (C-5'), 124.3 (C-6'), 131.4 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.0 (C-10'). **IR** (cm^{-1}): 2921, 1511, 1459, 1202,1037. **MS** (m/z, %): M^+ 304 (100), 235 (36), 221 (20), 205 (22), 204 (52), 203 (13), 190 (13), 189 (24), 182 (62), 69(11).

2-methoxy-5-((E)-3',7'-dimethylocta-2',6'-dienyl)-1,4-benzoquinone **5**

To geranyl-4,5-dimethoxyphenol **2** (0.1416 g, 0.5 mmol) dissolved in 20 mL acetonitrile/water (1:1), CAN (0.1725 g, 0.3 mmol) was added. The mixture was stirred at -5 ºC during 20 minutes. Dilution with diethyl ether was followed by washing with water and the organic phase was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product, which gave 20 mg (15 % yield) of compound **5,** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→17.0:3.0), colorless viscous oil. **^{1}H-NMR**: 6.46 (t, 1H, H-6); 5.92 (s, 1H, H-3); 5.15 (t, *J*= 7.0 Hz, 1H, H-2'); 5.07 (t, *J*= 7.0 Hz, 1H, H-6'); 3.81 (s, 3H, OCH_3); 3.13 (d, *J*= 7.0, 2H, H-1'); 2.07 (m, 4H, H-5' and H-4'); 1.69 (s, 3H, H-10'); 1.61 (s, 3H, H-8'); 1.60 (s, 3H, H-9'). **^{13}C-NMR**: 182.4 (C-1), 158.7 (C-2), 107.7 (C-3), 187.6 (C-4), 149.6 (C-5), 130.3 (C-6), 56.2 (OCH_3), 27.3 (C-1'), 117.8 (C-2'), 140.1 (C-3'), 39.6 (C-4'), 26.4 (C-5'), 130.3 (C-6'), 131.9 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.1 (C-10'). **IR** (cm^{-1}): 3018, 2929, 1675, 1651, 1606, 1458. **MS** (m/z, %): M^+ 274 (< 1%), 247 (3), 221 (20), 205 (29), 168 (15), 167 (100), 166 (8), 161 (6), 69 (11).

4,5-dimethoxy-2-((E)-3',7'-dimethylocta-2',6'-dienyl)phenyl acetate **6**

To geranyl-4,5-dimethoxyphenol **2** (0.0367 g, 0.13 mmol) dissolved in dichloromethane (10 mL), acetic anhidric (0.01 g, 0.01 mL, 0.13 mmol) and dimethylaminopyridine (DMAP, 1.5 mg, 0.013 mmol) were added. The mixture was stirred at room temperature during 2 hours. The organic phase was washed with water and after it was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product, which gave 30 mg (70 % Yield) of compound **6** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→17.0:3.0), colorless viscous oil. **^{1}H-NMR**: 6.70 (s, 1H, H-6); 6.56 (s, 1H, H-3); 5.22 (t, *J*= 7.0 Hz, 1H, H-2'); 5.10 (t, *J*= 7.0 Hz, 1H, H-6'); 3.84 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.17 (d, *J*= 7.0, 2H, H-1'); 2.29 (s, 3H, CO_2CH_3); 2.07 (m, 4H, H-5' and H-4'); 1.69 (s, 3H, H-10'); 1.67 (s, 3H, H-8'); 1.59 (s, 3H, H-9'). **^{13}C-NMR**: 146.9 (C-1), 124.8 (C-2), 112.3 (C-3), 141.8 (C-4), 147.5 (C-5), 106.1 (C-6), 56.0 (OCH_3), 56.1 (OCH_3), 28.2 (C-1'), 121.8 (C-2'), 136.8 (C-3'), 39.7 (C-4'), 26.7 (C-5'), 124.1 (C-6'), 131.5 (C-7'), 25.7 (C-8'), 17.7 (C-9'), 16.2 (C-10'), 20.8 (CO_2CH_3), 169.8 (CO_2). **IR** (cm^{-1}): 2922, 1764, 1658, 1620, 1513, 1205, 1174, 1014. **MS** (m/z, %): M^+ 332 (87), 291 (20), 290 (94), 221 (39), 207 (16), 189 (40), 175 (18), 168 (14), 167 (100), 161 (52), 154 (13), 129 (20), 69 (18).

2,4,5-trimethoxyphenyl formate ***8***

To 2,4,5-trimethoxybenzaldehyde **7** (2.02 g, 10 mmol) dissolved in dichloromethane (70 mL), metachloroperoxibenzoic acid (MCPBA, 3.30 g, 19 mmol) and sodium bicarbonate (1.82 g, 21 mmol) were added. The mixture was stirred at room temperature during 2 hours. The organic phase was washed with water (2 × 10 mL) and after it was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product which gave 1.87 g (86 % Yield) of compound **8** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→14,0:6,0), colorless viscous oil **^{1}H-RMN**: 8.28 (s, 1H, CHO), 6.85 (s, 1H, H-6), 6.78 (s, 1H, H-3), 3.91 (s, 3H, OCH_3), 3.85 (s, 6H, OCH_3). **^{13}C-RMN**: 161.3 (CHO), 122.1 (C-6), 101.8 (C-3).

2,4,5-trimethoxyphenol ***9***

To 2,4,5-trimethoxyphenyl formate **8** (3.68 g, 17 mmol) dissolved in methanol (50 mL) triethylamine (4 mL) were added. The mixture was stirred at room temperature during 30 minutes. Then, the solution was evaporated to dryness. Dilution with EtOAc was followed by washing with HCl solution (5%) (2 × 10 mL) and the organic phase was dried over anhydrous Na_2SO_4. The solution was evaporated to dryness to afford the crude reaction product, which gave 2.45 g (78 % yield) of compound **9** after column chromatography (eluent to hexane/ethyl acetate 19.8:0.2→14,0:6,0), colorless viscous oil **^{1}H-RMN**: 6.58 (s, 1H, H-6), 6.44 (s, 1H, H-3), 3.87 (s, 3H, OCH_3), 3.84 (s, 6H, OCH_3). **^{13}C-RMN**: 104.8 (C-3), 100.1 (C-6).

Cell lines

The experimental cell lines MCF-7, MDA-MB-231 and PC-3 were obtained from American Type Culture Collection (Rockville, MD, USA). MCF-7, MDA-MB-231, PC-3 and DHF cells were grown in DMEM-F12 containing 10% FCS, 100 U/ml penicillin, 100 µg/ml streptomycin and 1 mM glutamine at 37°C under a humidified 5% CO_2.

Cell growth inhibition assay

The colorimetric assay using sulforhodamine B (SRB) following an adaptation of the described method for Skehan[25-26] was used. Cells were seeded in 96-well microtiter plates, at $5x10^3$ cells per well in aliquots of 100 µL of medium, and they were allowed to attach to the plate surface by growing in drug-free medium for 18 h. Afterwards, compounds samples were added in aliquots to achieve a final concentration of 12.5, 25, 50 and 100 µM. Stock solution of compounds was prepared in ethanol and the final concentration of this solvent was kept constant at 1%. Control cultures received 1% ethanol alone. After 72 h exposure, the cytotoxicity was measured by the SRB dye assay. Cells were fixed by adding cold 50% (wt/vol) trichloroacetic acid (TCA, 25 µL) and incubated for 60 min at 4 ºC. Plates were washed with deionized water and dried; SRB solution (0.1% wt/vol in 1% acetic acid, 50 µL) was added to each microtiter well and incubated for 30 min at room temperature. Unbound SRB was removed by washing with 1% acetic acid. Plates were air-dried and bound stain was solubilized with Tris base (100 µL, 10 mM). Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 540 nm. Values shown are the % viability *vs*. Ctrl + SD, n=four independent experiments in triplicate.

Table 1. Cytotoxicity (IC_{50} µM +/- SD) of geranylmethoxyhydroquinone derivatives **1-6.**

Compound	PC-3	MCF-7	MDA-MB-231	DHF
1	> 100	82.2 +/- 6.5	> 100	> 100
2	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	> 100
5	> 100	84.2 +/- 8.9	86.2 +/- 7.1	> 100
6	> 100	> 100	> 100	> 100
Daunorubicin	0.36+/-0.12	0.19 +/- 0.11	0.32 +/- 0.09	1.34 +/- 0.31

CONCLUSIONS

In summary, we have prepared the new synthetic geranyl-2,4-methoxyhydroquinone **1** and known geranyl-4,5-methoxyhydroquinone **2**. These compounds were obtained unexpectedly by Electrophilic Aromatic Substitution (EAS) coupling reactions between geraniol with 2,4,5-trimethoxyphenol. Furthermore, the geranylmethoxyhydroquinone derivatives (**3-6**) were obtained of the chemical transformations of the compounds coupling **1** and **2**. The compound **5** showed cytotoxic activity against cell line MCF-7 and MDA-MB-231 with IC_{50} values of 84.2 and 86.2 µM respectively. The compound **1** showed some selectivity against cell line MCF-7 with an IC_{50} value of 82.2 µM. The increased citotoxicity induced by compounds **1** and **5** on cell lines can be probably due to the presence of a quinone moiety and position of the hydroxyl group on the structure of these compounds versus the compounds **2**, **3**, **4** and **6**.

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