Cytotoxic Effect of Geranylphenol Derivatives in the Human Breast Cancer Cell Line MDA-MB-231 and Gastric Cancer Cell Line MKN74

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Abstract:

Background: Previous studies have demonstrated that geranyl metabolites, found mainly in marine organisms, exhibit interesting antimicrobial and antifungal activities. In addition, linear geranyl derivatives have been synthesized as drugs and reported cytotoxic and anti-cancer activities. Aims: Considering the biological testing and the structural features of these compounds, we evaluated seven linear geranylphenol derivatives on the human breast cancer cell line MDA-MB-231, and the human gastric cancer cell line MKN74. Results: We found that compounds 2, 5 and 7 were cytotoxic for both cancer cell lines. From them, the most potent was compound 2, with an IC₅₀=7.5 µM for MKN74 cells, and compound 7, with an IC₅₀=14.73 µM for MDA-MB-231 cells. By nuclear staining and immunocytochemistry, we detected that the three compounds induced cell death, and by Western blot analysis, we observed a remarkable decrease in the expression of cyclin D1 and the retinoblastoma protein, key regulators of the cell cycle. Conclusion: Considering that compounds 2 and 7 were the most potent compounds inducing cell death, and were able to decrease the expression of retinoblastoma protein and cyclin D1, proteins usually altered in different types of cancers, they appear as promising therapeutic agents against cancer.

Keywords: cytotoxicity; geranylphenol; cyclin D1; retinoblastoma protein; MDA-MB-231; MKN74

Introduction

Linear 2-geranylbenzoquinone and 2-geranylhydroquinone (Figure 1A) are a sub-class of secondary metabolites whose terpene portion may have a length of up to 8 isoprene units. They are found mainly in marine organisms, such as brown algae, sponges, corals, gorgonians and ascidians. 2-geranylhydroquinone and 3-demethylubiquinone Q₂ (Figure 1A) have been synthesized as drugs and they have been reported to have some value as radioprotective and anti-cancer agents. Other synthetic geranylphenols, such as 2-geranyl-5-methyl resorcinol and 4-geranyl-5-methyl resorcinol (Figure 1A), showed antimicrobial and antifungal activities. Recently, our research group has developed the synthesis, structural determination and

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antifungal activity of a series of linear geranylphenols derivatives of 2-geranylhydroquinones, such 2-geranylphloroglucinol (Figure 1A) 20-26.

Cytotoxic activity was found for 2-geranylbenzoquinone and 2-geranylhydroquinone, and some synthetic geranylphenol-geranylmetoxy derivatives were also described as cytotoxic27-31. Another interesting study reported the anticancer activity and probable mechanism of action of 3-demethylubiquinone Q2 32. These authors previously evaluated the cancer preventive activity and structure-activity relationships (SARs) of natural 3-demethylubiquinone Q2, and its geranylmetoxyphenol/quinones synthetic analogues. They found that all geranylmet oxyquinones tested were able to inhibit the transformation of JB6 C141 cells, and in addition, they were able to induce apoptosis and the activation of AP-1 and NF-κB, as well as the inhibition of p53. Additionally, compounds containing isoprene units (geranyl chains) and a methoxyl group to a polyprenyl substitution, containing isoprene units (geranyl chains) and a methoxyl group to a polyprenyl substitution, presented the most promising effects. These effects depended on the length of the side chains (geranyl or farnesyl) and on the positions of the methoxyl groups in the quinone nucleus of the molecule 33. Taking into consideration the biological evaluations and the structural features of these compounds, we reported the synthesis, antiproliferative effect and apoptotic activity of a series of linear geranylnaphenol derivatives from phloroglucinol and orcinol. These compounds contain in their structures, hydroxyl, acetates and methoxyls groups as substituents in the arene nucleus, and mono or disubstitution with geranyl chains 34.

In this report, considering the interesting antiproliferative effect and apoptotic activity of mono and digeranylphenoxymethylenophenols, we present the cytotoxic effect of linear geranylmetoxyphenol derivatives (compounds 1-7, Figure 1B), on the human breast cancer cell line MDA-MB-231, a highly aggressive, invasive and poorly differentiated adenocarcinoma, and the gastric cancer cell line MKN74, a moderately-differentiated tubular adenocarcinoma. Our results showed that the biological activity depend on: first, the number of geranyl chains on the aromatic exhibited different cytotoxic effects depending on the cell type; and second, the increasing order in biological activity of the oxygenated function in the aromatic ring could be established as OH > OAc >> OMe. The nuclear staining results of both cancer cell lines showed that the compounds were inducing cell death. In addition, active caspase-3 immunostaining of MDA-MB-231 cancer cells, showed that the compounds were inducing apoptosis. We also observed a decrease in the expression of cyclin D1 and the retinoblastoma protein, key proteins involved in the regulation of the cell cycle that are usually altered in different types of cancers 35-38. The results obtained with two particular compounds are promising, but require further in vivo studies to determine their therapeutic effectiveness against cancer.

**Material and methods**

**Chemical synthesis**

Compounds 1-7 (Figure 1B) were synthesized through the electrophilic aromatic substitution reaction by direct geranylation of activated phenols, which proceeds by the slow addition of geraniol to a solution of phenol derivatives in dioxane and in the presence of BF3·Et2O, which acts as catalyst. Compounds 1 and 2 were reported in reference 23, compounds 3, 4 and 6 were reported in reference 26, and compounds 5 and 7, in reference 23.

**Cell culture**

The human breast cancer cell line MDA-MB-231, and the human gastric cancer cell line MKN74, were obtained from American Type Culture Collection, and were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing high glucose (17.5 mM) or RPMI-1640 medium with 25 mM HEPES, respectively. Each medium was supplemented with 10% heat inactivated fetal bovine serum and 1% antibiotic-antimycotic solution (Hyclone stock solution: EP/fungizone, 10,000 U/mL penicillin G, 10,000μg/mL streptomycin, and 25 μg/mL amphotericin B.), and incubated at 37°C in 5% humidified CO2 atmosphere.

**Cytotoxicity assay**

MDA-MB-231 cells were plated at a density of 1 x 10⁴ cells/well in 96-well microtiter plates. For MKN74 cells, the density was 5 x 10³ cells/well. The following day, the media was replaced with new complete media containing the tested compounds (Figure 1B): compound 1: 4,6-bis((E)-3,3,7-dimethylocta-2,6-dien-1-yl)-5-methylbenzene-1,3-diol; compound 2: 2,4-bis((E)-3,7-dimethylocta-2,6-dien-1-yl)-5-methylbenzene-1,3-diol; compound 3: 3,5-bis((E)-3,7-dimethylocta-2,6-dien-1-yl)-2,4,6-trimethoxyphenol; compound 4: 2,6-bis (E)-3,7-dimethylocta-2,6-dien-1-yl)-
3,4,5-trimethoxyphenol; compound 5: (E)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-methoxybenzene-1,4-diol; compound 6: (E)-4-(3,7-dimethylocta-2,6-dien-1-yl)-2,3-dimethoxyphenol; compound 7: (E)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-methoxy-1,4-phenylene diacetate; and 2-geranylhydroquinone (2-GHQ, Figure 1A), as the positive control. Compounds and control stock solutions were prepared at 40 mM in dimethylsulfoxide (DMSO). Different amounts of the compounds and the positive control were diluted in the respective culture media to achieve final concentrations between 0.1 - 90 µM, and then, they were applied to the wells. DMSO was used as a negative control, with final concentrations equivalent to the corresponding compound (the highest concentration never exceeded 0.2% DMSO). The cells were then incubated at 37°C for 72 h. The uptake was stopped by washing the cells at room temperature (RT) in 1x Hank’s Balanced Salt Solution (HBSS, without calcium, magnesium, phenol red; Hyclone). Cytotoxicity was measured by the sulforhodamine B (SRB) dye assay, as previously described, with the following modifications: cells were fixed in 10% acetic acid during 10 min. at RT (same incubation temperature in subsequent steps); the solution was discarded and replaced with 1% acetic acid. Afterwards, the 1% acetic acid solution was removed and 100 µl of SRB solution (0.1% w/v in 1% acetic acid) was added and incubated for 30 min. protected from light and with gently shaking. Unbound SRB was removed by washing 4x with 1% acetic acid. Plates were air-dried for 5 min. and bound stain was solubilized with 10 mM Tris base (100 µL) and shaken for 2 min. Binding was detected by measuring optical densities at 492 nm and 620 nm (to discount background), on an automated plate reader (Synergy H1 Hibrid Reader, BioTek). Experiments were performed in quadruplicate and repeated 3 times. The cytotoxic IC50 (concentration of the compound that is lethal to 50% of the cancer cell lines), was determined by Graph Pad Prism (version 7.0), and was calculated based on mortality obtained at each concentration of the samples.

Statistical analysis

Cell survival was determined, after normalization, by the following equation: % Viability = (optical density of treated cells/ optical density of control) x 100%. Data are expressed as the mean ± SD. In all cases, p values <0.05 were considered statistically significant. Cytotoxicity data was analyzed using one-way analysis of variance (one-way ANOVA), and differences between groups were calculated by Tukey’s multiple comparisons test.

Nuclear staining

MDA-MB-231 cells were plated at a density of 1.5 x 10^4 cells/well, and MKN74 cells at a density of 2 x 10^5 cells/well, in 6-well plates. The following day, the media was replaced with new complete media containing compounds 2, 5 and 7, at their respective IC50 concentration. DMSO was used as a negative control and 80 mM isopropyl-beta-D-1-tiogalactopyranoside (IPTG), as a positive control. After 48 h of incubation at 37°C, supernatant was saved and adherent cells were trypsinized, mixed with the supernatant, centrifuged at 800 rpm for 5 min, and resuspended in 100 µl 1x HBSS. Afterwards, Hoechst 33342 was added at 2 µg/mL and cells were incubated for 7 min at 37°C, followed by 0.5 µg/µl propidium iodide (PI) incubation during 15 min. at RT and protected from light. Samples were placed on microscope slides and visualized under fluorescent microscope (SCOPE.A, Zeiss; 365 nm and 590 nm).

Immunofluorescence

MDA-MB-231 cells were plated at a density of 5 x 10^4 cells/well on round coverslips placed in 12-well plates. The following day, the media was replaced with new complete media containing the compounds at the following concentrations: compound 2: 24 µM, compound 5: 27 µM, and compound 7: 20 µM. DMSO was used as a negative control and 80 mM IPTG, as a positive control. Cells were incubated at 37°C for 24 h, washed 2x at RT with PBS, 5 min. each. Coverslips were taken out and cells where fixed and permeabilized in cold methanol for 20 min. at -20°C. Afterwards, cells where blocked with PBS/1% albumin (PBS-A), during 30 min. at RT, washed 3x (5 min. each), with PBS/0.5% Tween (PBS-T). Cells where then incubated overnight with human/mouse monoclonal cleaved caspase-3 primary antibody in PBS-A (1:500; MAB835 R&D Systems), at 4°C in a humidity chamber. The following day, cells were washed as before, and incubated with secondary antibody directly coupled to Alexa’s dye (1:400; Life Technologies A11070), in PBS-A for 1 h at RT and protected from light in the humidity chamber. Finally, 3x washes were performed as previously described, and slides were mounted with ProLong Gold Antifade with DAPI (Invitrogen). Slides were left at RT and protected from light for 24 h, sealed with...
nail polish and visualized by fluorescent microscopy (SCOPE.A, Zeiss).

**Protein extraction, Western blot analysis and antibodies**

MDA-MB-231 cells were plated at a density of 6 x 10⁴ cells/well in 12-well plates, and MKN74 cells at a density of 7 x 10⁴ cells/well in 6-well plates. The following day, the media was replaced with new complete media containing compounds 2, 5 and 7, at three different concentrations. DMSO was used as a negative control and 80 mM IPTG, as a positive control. After 24 h of incubation at 37°C, the cells were lysed in 1x RIPA buffer, and proceeded according to manufacturer instructions (Cell Signaling). Protein concentrations were determined with the micro-BCA Protein Assay kit (Pierce), as previously described. Following SDS-PAGE (either 10% for cyclin D1 detection or 8% for Rb detection), the proteins were transferred to polyvinylidene difluoride (PVDF) membranes for 1.5 h at 300 mAs. Afterwards, membranes were blocked with either 0.2% casein in 1x TBS/0.1% Tween-20 (TBS-T) or 5% BSA/TBS-T (depending on the antibodies requirements), at RT for 1.5 h, followed by overnight incubation at 4°C with the corresponding primary antibodies. Then, membranes were washed 3x with 1x TBS-T at RT and incubated with the respective HRP-conjugated secondary antibodies at RT for 1.5 h in TBS-T. The membranes were washed 3x with 1x TBS-T at RT, and visualization was by enhanced chemiluminescence (Pierce).

The following antibodies were used: anti-cyclin D1 monoclonal antibody (mAb) (1:200; Sta. Cruz Biotechnologies), recognizes full-length human cyclin D1 protein; anti-retinoblastoma (Rb) mAb (1:1,000; Cell Signaling), recognizes human total Rb protein; anti-phosphorylated-Rb (P-Rb) mAb (1:1,500; Cell Signaling), recognizes human Rb protein phosphorylated only at Ser780; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mAb (1:4,000; Sigma Aldrich), was used as loading control. Secondary antibodies were from Sta. Cruz Biotechnologies (1:10,000).

**Ethical clearance:** This study was approved by ethics committee of Catholic University of Maule, Talca, Chile.

**Results**

**Chemical synthesis**

All the compounds used in this study were synthesized by direct aromatic electrophilic substitution reaction and reported in references 23, 24 and 26. Their structures are illustrated in Figure 1B.

**Cytotoxicity and cell viability**

The cytotoxic effect of compounds 1-7 were evaluated in the breast cancer cell line MDA-MB-231 and the gastric cancer cell line MKN74, since these cells are widely used to characterize the behavior of the respective human cancers.

As shown in the Table, the more cytotoxic IC₅₀ values determined by the SRB colorimetric assay, were IC₅₀ = 7.5 µM for compound 2 in MKN74 cells, and IC₅₀ = 14.73 µM for compound 7 in MDA-MB-231 cells. Similar IC₅₀ values were obtained for compound 5 in both cancer cell lines, IC₅₀ = 22 µM and IC₅₀ = 19.4 µM for MDA-MB-231 and MKN74 cells, respectively. Compound 1 exhibited a moderately cytotoxic effect only to the MKN74 cell line (Table). The control 2-GHQ showed an IC₅₀ = 13.30 µM for MKN74 cells and an IC₅₀ = 18.30 µM for MDA-MB-231 cells. Compounds 3, 4 and 6 presented elevated IC₅₀ values (Table) and were not further considered. Since compounds 2, 5 and 7 presented considerably cytotoxic effects in both cancer cell lines, we performed additional experiments only with these three compounds. Their respective IC₅₀ graphs are shown in Figure 2 (A-1 to H-1). Data are expressed as the mean ± SE.

The effects of compounds 2, 5, 7 on cell viability are shown on Figure 2 (A-2 to H-2). Clearly, and accordingly to the corresponding IC₅₀ values, MDA-MB-231 cells were killed more efficiently with compounds 7, and with compound 2, in the case of MKN74 cells. In both cancer cell lines, the cytotoxic effects of compounds 2 and 7, respectively, were stronger than the control. Data are expressed as the mean ± SD. In all cases, p values <0.05 were considered statistically significant.

**Morphological cellular alterations and cell death**

In order to determine if the different compounds exerted cellular morphological changes, microscopic examinations were performed. As shown in Figure 3A, MDA-MB-231 cells exposed to compound 7 exhibited a rounded phenotype, which was more evident at 30 µM (at 20 µM the rounded phenotype was also evident; data not shown). Compound 2 also induced a rounding phenotype at 35 µM, and with compound 5 this effect was scarcely observed. For MKN74 cells, the rounding effect was observed with compound 2 predominantly at 20 µM (at 9 µM the
effect was observed; data not shown). Compound 5 also induced the rounding of the cells, but not compound 7 (Figure 3B). As expected, DMSO, used at a concentration equivalent to the highest concentration of the compounds, did not altered the morphology of the two cancer cell lines, and 80 mM IPTG, used as a positive control \(^{43}\), did not induced dramatic morphological cellular changes (Figure 3A-B).

Considering the above results, we looked for nuclear morphological changes that could be induced by compounds 2, 5 and 7 in both cancer cell lines. As shown in Figure 4, the dual nuclear staining with Hoechst 33342 and PI indicated that the three compounds were altering nuclear morphology in both cancer cell lines, in a pattern that resembles the hallmarks of apoptosis \(^{44}\). Compounds 5 and 7 induced nuclear fragmentation and compound 2 nuclear condensation, in both MDA-MB-231 and MKN74 cancer cell lines. Blue nuclear staining corresponded to live cells, and pink nuclear staining to dead cells with apparently apoptotic nuclei.

In order to corroborate if the morphological changes induced by the compounds were due to apoptosis, immunocytochemistry staining was performed with cleaved caspase-3 antibody. As shown in Figure 5, cells exposed to compounds 2, 5 and 7 exhibited cytosolic green fluorescence staining, indicating that these compounds were able to induced apoptosis in MDA-MB-231 cells. As expected, cells without treatment and cells incubated with DMSO, do not induced apoptosis. IPTG was used as positive control. Unfortunately, the immunostaining obtained with MKN74 cells was not specific. Different technical conditions were used, and the background effect did mostly not disappeared (data not shown).

**Effects on the expression of cyclin D1 and Retinoblastoma protein**

In order to determine if compounds 2, 5 and 7 were affecting the expression of key molecules involved in the regulation of the cell cycle, the expression of cyclin D1 and retinoblastoma (Rb) protein were evaluated. IPTG (80 mM) \(^{44}\) and DMSO were used as positive and negative controls, respectively.

As shown in Figure 6A, MDA-MB-231 cells showed a decrease in the expression of Cyclin D1, Rb and phospho-Rb (P-Rb). In the case of cyclin D1, compounds 2 and 7 showed a stronger effect at 35 µM than compound 5, at the same concentration. The same pattern was observed for Rb and P-Rb with compounds 2 and 5. In the case of compound 7, a decrease in the expression of Rb and P-Rb was observed at 25 µM. As expected, DMSO did not affect cyclin D1, Rb and P-Rb expression, and IPTG decreased their expressions.

In the case of MKN74 cancer cell line (Figure 6B), the WB analysis showed a dramatic decrease in the expression of cyclin D1 with 20 µM of compound 2. The same patterns of expression were observed for Rb and P-Rb. For compound 5, higher concentrations (35 µM) were required to induce a significant decrease in the expression of Cyclin D1, Rb and P-Rb. A decrease in the expression of Cyclin D1, Rb and P-Rb were detected at 20 µM of compound 7. Higher concentrations were not used, because the effect was extremely dramatic, even with housekeeping genes. Again, DMSO did not affect cyclin D1, Rb and P-Rb expression, and IPTG decreased the expression of all of them. GAPDH was used as loading control.

**Discussion**

Seven different linear geranylmethoxyphenol derivatives were tested as potential cytotoxic compounds in the human breast and gastric cancer cell lines, MDA-MB-231 and MKN74, respectively. It seems that the bisubstitution with 2 geranyl chains
We observed a dramatic cellular rounding of MDA-
with compound we continued the study of those three compounds.

Structural configuration of the compounds in the indicated that the different cellular characteristics Thus, since the most effective cytotoxic compounds
in both cancer cell lines were compounds 2, 5 and 7, we performed a dual nuclear staining
with Hoechst 33342/PI. The observed nuclear staining indicated nuclear DNA condensation and fragmentation, suggesting that the three compounds were inducing apoptosis in both cancer cell lines. Immunostaining with cleaved caspase-3 antibody clearly demonstrated that the three compounds induced apoptosis in MDA-MB-231 cells. Unfortunately, this antibody did not worked well in MKN74 cells. There was a nonspecific staining of proteins, which even different staining protocols were not able to eliminate.

On the other hand, since compounds 2, 5 and 7 were able to decrease cell viability, we wanted to determine if key molecules involved in the cell cycle were affected. We observed that the treatment with the three compounds led to a reduction in the expression of Cyclin D1, Rb and P-Rb in both cancer cell lines, and compounds 2 and 7 were more effective. The results suggested that these compounds were affecting the cell cycle.

Cyclin D1, together with the cyclin-dependent kinases (CDKs) 4 and 6 (CDK4 and CDK6), are involved in the entry to G1 phase of the cell cycle, promoting cell cycle progression to S phase. Cyclin D1-CDK4/6 adds, during G1, only the first phosphate group to one of the 14 possible different sites in Rb, a tumor suppressor protein that regulates the passage from G1 to S phase of the cell cycle, by acting as the restriction point. Afterwards, many other residues are phosphorylated and Rb becomes inactivated and the cell cycle progress to S phase. It has also been shown that phosphorylated Rb releases most of the E2F transcription factor, in order to allow the entrance to S phase. But, some E2F reminds bound to Rb, regulating transcription of pro-apoptotic genes. In that way, Rb in its hyperphosphorylated stage allows cell proliferation and its loss of expression triggers apoptosis.

In addition, cyclin D1 and CDKs are also associated with apoptosis. It has been shown that the overexpression of cyclin D1 favors the sequestration of the pro-apoptotic protein BAX. Other studies have demonstrated that Rb can be found at the outer mitochondrial membrane, interacting with BAX and promoting apoptosis.

Since cyclin D1 gene is frequently amplified in different types of cancers, and Rb is often mutated or hyperphosphorylated in several cancers, both proteins constitute important targets for cancer.
therapy. Additionally, considering that the ability to evade apoptosis is a hallmark of cancer, the tested compounds appear to be good candidates for the treatment of breast and gastric cancer. More studies should be performed in order to understand the molecular signaling pathways affected by these compounds that lead to cell death. Additionally, more experiments have to be done to determine if these compounds do not affect normal breast and gastric cells.

In summary, our data indicated that two of the tested compounds were more effective in killing cancer cells. Compound 7 was the most cytotoxic for MDA-MB-231 cells and compound 2 for MKN74 cells. Both compounds were able to induce cell death and decrease the expression of cyclin D1 and Rb, probably deregulating the cell cycle. Based on these findings, we suggest that both compounds are promising cancer therapeutic agents.

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Authors’s contribution

Data gathering and idea owner of this study: S. Pilar Zamora-León

Study design: S. Pilar Zamora-León

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Figure 1. Structure of natural and synthetic linear geranylphenols and geranyliqueunones. A: some active natural occurring linear geranyliqueunones and synthetic geranyliqueunols. a: 2-geranylbenzoquinone, b: 2-geranylhydroquinone, c: 3-dimethylubiquinone Q2, d: 2-geranyl-5-methyl resorcinol, e: 4-geranyl-5-methyl resorcinol, f: 2-geranyl-2,3-dimethoxyacetol. B: synthetic linear mono- and digeranylphenols. 1: 4,6-bis(E)-3,7-dimethylocta-2,6-dien-1-yl)-5-methylbenzene-1,3-diol; compound 2: 2,4-bis(E)-3,7-dimethylocta-2,6-dien-1-yl)-5-methylbenzene-1,3-diol; compound 3: 3,5-bis(E)-3,7-dimethylocta-2,6-dien-1-yl)-2,4,6-trimethoxyphenol; compound 4: 2,6-bis(E)-3,7-dimethylocta-2,6-dien-1-yl)-3,4,5-2trimethoxyphenol; compound 5: (E)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-methoxybenzene-1,4-diol; compound 6: (E)-4-(3,7-dimethylocta-2,6-dien-1-yl)-2,3-dimethoxybenzene; compound 7: (E)-2-(3,7-dimethylocta-2,6-dien-1-yl)-5-methoxy-1,4-phenylene diacetate. G: geranyl group (A, B).
Cytotoxic effect of geranylphenol derivatives in breast and gastric human cancer cell lines
Figure 2. *IC50* graphs of compounds 2, 5 and 7 in MDA-MB-231 and MKN74 cell lines and cell viability. A-1 to H-1: *IC50* determinations were performed in MDA-MB-231 cells (A-1 to D-1) or MKN74 (E-1 to F-1) cells, after 72 h of treatment with different concentrations of compound 2 (A, E), compound 5 (B, F), compound 7 (C, G) or 2-GHQ control (D, H). Data are expressed as the mean ± SE. Abs: absorbance. A-2 to H-2: cell viability determination after treatments with compounds 2, 5 and 7 as previously described. A-2 to D-2: percentage of viable MDA-MB-231 cells; E-2 to F-2: percentage of viable MKN74 cells. Data are expressed as the mean ± SD and p values <0.05 were considered statistically significant (* ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001, **** ≤ 0.0001).
Cytotoxic effect of geranylphenol derivatives in breast and gastric human cancer cell lines
Figure 3. Cellular morphological changes induced by treatment with compounds 2, 5 and 7. MDA-MB-231 (A) and MKN74 (B) cells were treated with different concentrations of compounds 2, 5 and 7, 80 mM IPTG, and DMSO. The rectangle within the picture indicates the compound and its concentration (μM).
Cytotoxic effect of geranylphenol derivatives in breast and gastric human cancer cell lines

Figure 4. Composite images of dual Hoechst 33342/PI nuclear staining. MDA-MB-231 (A) and MKN74 (B) cells were treated for 48 h with compound 2 (C2), 5 (C5) and 7 (C7), at their respective IC50 concentrations; 80 mM IPTG, DMSO, and media. First columns: 100x magnification, and second columns: 400x magnification. Blue nuclear staining corresponds to live cells, and pink nuclear staining to dead cells with apparently apoptotic nuclei. Arrows indicate fragmented DNA and arrowheads, condensed DNA. Bar: 50 μm.
Figure 5. Composite image of cleaved caspased-3 staining. MDA-MB-231 cells were treated for 24 h with compound 2 (C2, 24 μM), 5 (C5, 27 μM) and 7 (C7, 20 μM). DMSO was used as a negative control, and IPTG (80 mM), as a positive control. Medium correspond to cells without treatment, and negative control, to incubation without primary antibody. Magnification: 400x, bar: 50 μm.
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