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Cytotoxicity Studies of Eugenol Amino Alcohols Derivatives †

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Abstract: Eugenol is a phenylpropanoid displaying a wide range of biological activities. In this study, the cytotoxic activity of various β-amino alcohols derivatives from eugenol was evaluated in AGS (gastric cancer) and A549 (lung cancer) cell lines. The results show that some structural modifications resulted in enhanced cytotoxic activity towards cancer cells. In addition, the activation of caspase-3 and hence apoptosis induced by these molecules, was also explored. Considering the obtained results, some structure/activity relationships can be drawn, which may guide future structural improvements for anticancer agents.

Keywords: eugenol; β-amino alcohols; essential oils; cytotoxicity; caspase-3; anticancer

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1. Introduction

Humans have relied on plants for treating a wide range of diseases since pre-historical times [1,2]. Drug discovery and design continues to be inspired by natural products, with 23.5% of 1881 new drugs, approved between 1981 and 2019, being unaltered natural products, botanical drugs, or natural derivatives [3].

Essential oils, natural matrices comprising several secondary metabolites of low molecular weight, gained interest due to their biological activities and application across several industries. Eugenol, the major constituent of clove oil, displays numerous biological properties such as anti-inflammatory, antioxidant, anti-tumorigenic, anti-microbial and cytotoxic activity [4,5]. Eugenol also demonstrated to have an interesting structure to be used as a starting molecule to obtain several synthetic derivatives. For these reasons several studies with eugenol or its synthetic derivatives have been done [6].

Cancer is a multistep process that involves dynamic changes in the genome and allows cells to invade several tissues, increasing cell proliferation and escape from programmed cell death processes, such as apoptosis [7,8]. Apoptosis is a type of cell death characterized by specific morphological and biochemical changes and requires the activation of caspases, a group of cysteine-aspartic acid proteases. It can be triggered by two pathways: the intrinsic/mitochondrial pathway and the extrinsic/death receptor pathway [9–12]. The ability to escape from apoptosis is a hallmark of cancer and, for this reason, new therapies that reactivate apoptotic mechanism are a great promise to counteract cancer [8,9]. Noteworthy, eugenol had already been shown to induce apoptosis in different cancer cell lines [13].

Considering all these facts, cytotoxicity against AGS (human gastric adenocarcinoma) and A549 (human lung adenocarcinoma) cell lines of several β-amino alcohols from eugenol were screened. The most potent molecules were selected and evaluated for their capacity to induce apoptosis.

2. Results and Discussion

2.1. Synthesis of Eugenol β-Amino Alcohols Derivatives **3–9**

Eugenol **1** was reacted with *m*-chloroperbenzoic acid in dichloromethane to give the epoxide **2** [14], which was further reacted with several aromatic and aliphatic amines in ethanol/water as solvent by using a known procedure [15], to yield the corresponding β-amino alcohol derivatives **3–9** [16] (Figure 1).

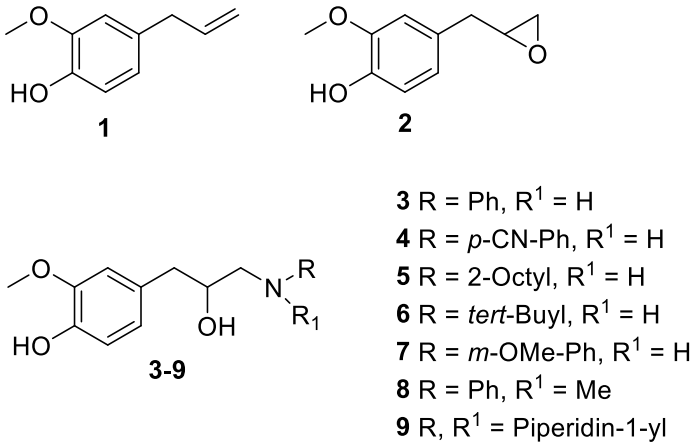

Figure 1. Structures of eugenol **1**, eugenol epoxide **2** and the corresponding β-amino alcohols **3–9**.

2.2. Toxicity of β-Amino Alcohols Eugenol Derivatives **3–9**

The effect of β-amino alcohols **3–9** as well as precursor molecules **1** and **2** were studied in AGS and A549 cell lines at 24 h (100 μM) (Figure 2).

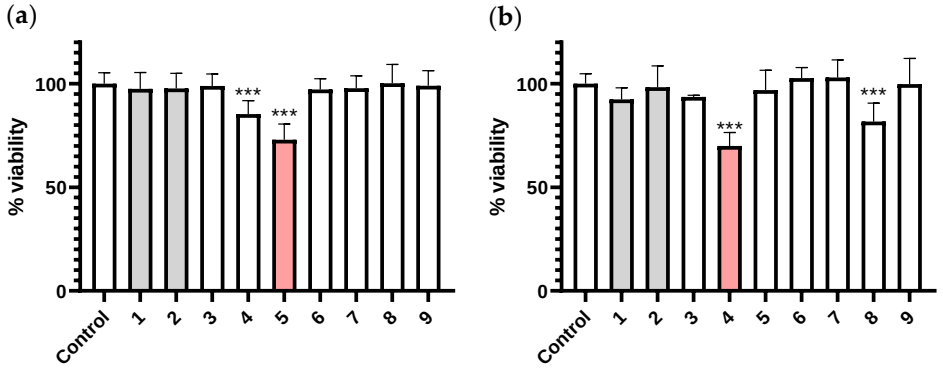

Figure 2. Effect of eugenol and its derivatives (100 μM) in (**a**) AGS and (**b**) A549 cell lines. The results correspond to the mean of each well of three independent experiments performed in triplicate. *** $p < 0.001$.

When evaluating the impact of all molecules obtained, it was clear that for both cell lines a few derivatives displayed higher cytotoxicity than eugenol **1** and its epoxide **2**, which are devoid of activity. Among all derivatives, compound **5** was the most potent for AGS cells, leading to 28% reduction in cell viability and for A549 cells compound **4** reduced in 31% cell viability.

Considering these results, we were interested in understanding if a process of programmed cell death, as apoptosis, was taking place.

2.3. Caspase-3 Activity

After the results from the viability assay, the most potent molecule for each cell line, was tested for possible triggering cell death by apoptosis. For this purpose, activation of the effector caspase-3 was studied (Figure 3).

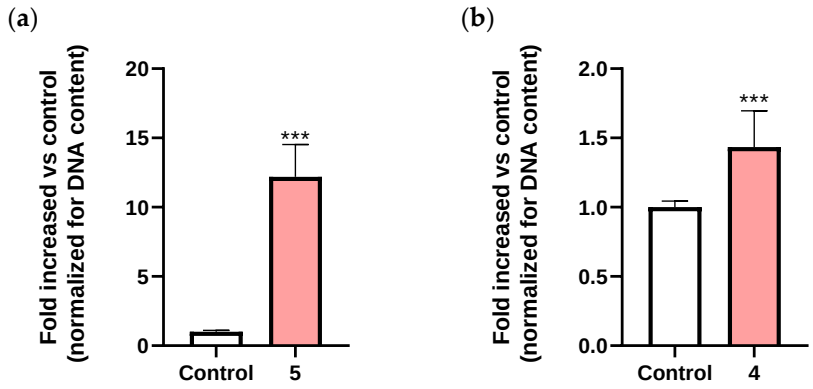

Figure 3. Effect of the compounds **5** and **4** at 100 μM on the activity of caspase-3, on (**a**) AGS cell line and (**b**) A549 cell line. The results correspond to the mean of each well of four independent experiments performed in duplicate. The values were normalized for DNA content. *** $p < 0.001$.

This experiment revealed that for the AGS cell line compound **5** at 100 μM elicited an increase in caspase-3 activity of about 12-fold. Compound **4** was also active, while in much lower magnitude.

In addition, compound **5** have a stronger effect in caspase-3 activation allowing us to conclude that cell viability reduction is caused by activation of apoptosis.

3. Experimental

3.1. Cell Culture and Viability Assessment

AGS (human gastric adenocarcinoma) and A549 (human lung adenocarcinoma) cells were broadly used in pharmacological studies and create a versatile pair, due to the diferent away of the cells to respond to chemoterapy treatmens, being AGS more responsive than A549.

Cells were maintained in DMEM plus GlutaMAX™ with 10% FBS and 1% penicillin/streptomycin, at 37 °C with 5% CO_2 in a humidified incubator. For the assessment of viability, a resazurin-based method was used. AGS and A549 cells were seeded at a density of 15,000 and 10,000 cells/well, respectively, incubated for 24 h, and then exposed to the compounds under study for another 24 h period. After this period, a commercial solution of resazurin was added (1:10) and incubated for 30 min. Finally, the fluorescence was read at 560 nm.

3.2. Caspase Activity

AGS and A549 cells were seeded at the same density decribed for viability assessment and exposed to the compounds under study for the same period of time. After the incubation period, 50 μL of supernatant were removed from each well, followed by the additon of 50 μL of caspase-3 substrate (1:200), the plate was incubated for 45 min. Lastly, fluorexscent was read at 535–620 nm.

3.3. DNA Quantification

Cells were cultured and exposed to the molecules under study, as referred before. Past the incubation period, the culture medium was replaced by 50 μL of ultra-pure water and then the plate was incubated for 30 min in a humidified incubator at 37 °C with 5%

CO_2 and subsequently frozen at −80 °C. DNA quantification was performed by using $Qubit^{TM}$ dsDNA HS/Protein assay kit, the $Qubit^{TM}$ 4 Fluorometer reader was used.

4. Conclusions

The cytotoxic activity of various β-amino alcohols derivatives from eugenol evaluated in AGS and A549 cell lines revealed that compounds **4** and **5** are the most promising at 100 μM. These molecules enhanced the cytotoxic activity in relation to eugenol. Besides that, compound **5** was able to increase caspase-3 activity, which is involved in apoptosis cell death process. Therefore, compound **5** could be used as an anticancer agent and as starting molecule for design of new and more potent anticancer molecules.

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