ENZYME CATALISED SYNTHESIS OF NOVEL PERILLYL ALCOHOL ESTERS WITH POTENTIAL CITOTOXIC ACTIVITY

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ABSTRACT

Perillyl alcohol (PA) is a monoterpene widely found in the essential oils of various plant species. In vitro studies have demonstrated the cytotoxicity of this natural product against different cancer cell lines. However, due to its lipophilicity and volatility, its use as a drug is limited. In this context, the esterification of monoterpenes is an alternative to improve their pharmacological properties. Lipase-catalyzed esterification offers a greener approach to traditional chemical synthesis, enabling the production of esterified products under mild conditions. Therefore, we aim to perform the enzymatic synthesis of perillyl esters using different carboxylic acids and evaluate their cytotoxicity against various types of cancer. The enzymatic reactions were conducted using previous optimized conditions, for carboxylic acids with chain lengths of C_3-C_6 and C_8-C_{18}. The first product, synthesized using propanoic acid as the acyl donor, resulted in perillyl propionate with isolated yield of 88% and using octanoic acid the yield was 63%. The product was characterized using ^{1}H NMR, and its structure was confirmed based on literature data. Now, we aim to use different carboxylic acids as acyl donors and begin the cytotoxicity assays.

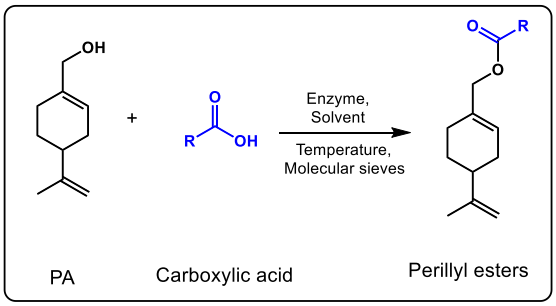
Keywords: Cancer. Esterification. Lipases. Monoterpenes.

1 INTRODUCTION

Perillyl alcohol (PA) is a monoterpene found in essential oils of plants such as peppermint, spearmint, and lavender. It is traditionally used as a fragrance in perfumes and cosmetics. PA has recently shown to be promise in preventing and treating various types of cancer in vitro. However, clinical studies have revealed challenges due to its low water solubility, requiring high dosages that lead to report side effects[1].

Given the PA's potential anticancer activity, novel analogues have been synthesized to improve its physicochemical and pharmacological properties. In this context, esterification of monoterpenes is a promising approach to enhance absorption and pharmacokinetics[2]. Our group has previously developed enzymatic methods using immobilized lipases as catalysts, achieving high yields under mild reaction conditions[3]. We found that the size of the acyl donor directly influences the reaction kinetics.

Based on this perspective, our objective is to perform enzymatic synthesis of novel perillyl alcohol esters (Scheme 1) and assess their cytotoxicity against various cancer cell lines. The most promising compound will undergo further synthesis using a continuous flow reactor, facilitating scale-up for subsequent in vivo studies.



Scheme 1. Enzymatic synthesis of perillyl alcohol using carboxylic acids as acyl donors

2 MATERIAL & METHODS

For the carboxylic acids with carbon chain from C_3 to C_6, the enzymatic esterification was conducted in 4 mL amber vials, using 0.83 mmol of PA, 1.1 mmol of carboxylic acid, 25 mg of the enzyme Novozym 435®, and 3.3 mL of cyclohexane, under orbital shaking at 200 rpm and 30°C for 24 hours. For the carbon chain lengths from C_8 to C_{18}, it was used 0.83 mmol of PA, 1.25 of acyl donor, 16 mg of biocatalyst, while all other remaining reactions parameters were kept the same. The reactions were conducted in duplicate, and the yields were determined using ^{1}H NMR quantitative, with 1,3-benzodioxole as an internal standard. For final product isolation, after 24 hours, the reaction medium was filtered to remove the enzyme and then purified using silica flash and n-hexane/ethyl acetate as eluents.

After the product's purification, the cytotoxicity will be evaluated using the WST-1 colorimetric methodology. Briefly, 90 µL of each cell suspension containing 5×10^4cells/mL will be precultured in 96-well plates. After 24 hours, the perillyl esters, previously weighed and solubilized in cell culture medium, will be added at concentrations ranging from 3.125 to 100 µM. Cellular viability rates will be measured after 24 hours of incubation by the addition of 20 µL of WST-1 to each well, followed by incubation for 1.5 hours. Absorbance at 450 nm and 650 nm will be measured using a multi-well plate reader (Labsystems Multiskan RC, Helsinki, Finland). Their effect on cell viability will also be evaluated. The percentage of viable cells will be calculated in relation to vehicle control cells, using mean values from at least three independent experiments performed in triplicate. The IC50 will be calculated using GraphPad 8 Software.

3 RESULTS & DISCUSSION

Initial results using propanoic and octanoic acid as acyl donors achieved yields of 94% and 63%, respectively (Table 1). After the reaction period, the products were purified by using flash silica chromatography, and their structure were confirmed using ^{1}H NMR. Chemical shifts were compared with those previously reported in literature in order to validate the product's identity.

Acyl donor	Yield (%)
Propanoic acid (C_3)	94[a] (88)[b]
Octanoic acid (C_8)	63[a] (48)[b]

[a]Yield determined using ^{1}H NMR and 1,3-benzodioxole as internal standard; [b]Isolated yield

Based on these previous results, it is possible to observe that the carbon chain size directly affects the product yields. With an increase in carbon chain length, the yield decreased from 94% to 63%. These findings are in accordance with the literature when using lipase as biocatalysts, where yields typically decrease when enhancing the size of the carboxylic acid chain. However, these studies only report conversion to the product and do not include product isolation. It is important to consider that losses can occur during product purification.

4 CONCLUSION

In conclusion, we successfully obtained perillyl propionate and perillyl octanoate esters with good isolated yields and performed product characterization by using ^{1}H NMR technique. We observed that the carbon chain affects directly the esterification yield and best resulted were achieved using propanoic acid as acyl donor. Following this, we will expand the reaction scope to include aliphatic, unsaturated, and (hetero)aromatic acids.

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