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Chemoenzymatic synthesis of new derivatives of glycyrrhetinic acid with antiviral activity. Molecular docking study

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ABSTRACT

We present an efficient approach to the synthesis of a series of glycyrrhetinic acid derivatives. Six derivatives, five of them new compounds, were obtained through chemoenzymatic reactions in very good to excellent yield. In order to find the optimal reaction conditions, the influence of various parameters such as enzyme source, nucleophile:substrate ratio, enzyme:substrate ratio, solvent and temperature was studied. The excellent results obtained by lipase catalysis made the procedure very efficient considering their advantages such as mild reaction conditions and low environmental impact. Moreover, in order to explain the reactivity of glycyrrhetinic acid and the acetylated derivative to different nucleophiles in the enzymatic reactions, molecular docking studies were carried out. In addition, one of the synthesized compounds exhibited remarkable antiviral activity against TK + and TK- strains of Herpes simplex virus type 1 (HSV-1), sensitive and resistant to acyclovir (ACV) treatment.

1. Introduction

The pentacyclic triterpenoid glycyrrhetinic acid (GA) is the major bio-active constituent isolated from the roots of *Glycyrrhiza glabra*, which is a sweet-tasting material and has greater sweetening power than sugar, making it a widely used as additive in the food industry [1–3]. GA has been used as a lead compound to search more potent derivatives with different pharmacological properties. Glycyrrhetinic acid and some derivatives have been shown to exhibit anti-inflammatory [4,5] and anti-viral [6,7] activities. In addition, it has been reported derivatives of GA as inhibitors of cholinesterases [8] and 11β-HSD1 and 11β-HSD2 [9–11]. Despite these reports, few potential applications of GA derivatives for the development of new pharmacological agents have been investigated.

The application of enzymes as biocatalysts in the synthesis and transformation of different substrates with engaging properties has widely expanded in the last years [12–14]. Enzymes catalyze diverse type of reactions in a highly regio, chemo, and enantioselective way using a wide ranging of compounds. Therefore, biocatalysts constitutes a good alternative to obtaining of compounds through a Green Chemistry approach carrying out chemical transformations more easly, particularly in substrates with several functional groups [15–19]. In our laboratory we have studied the application of lipases in diverse reactions like esterifications, transesterifications and aminolysis of multiple substrates, obtaining a wide diversity of new compounds, which show interesting biological activities [20,21]. Recently, we have reported the synthesis of numerous compounds with potential applications as antiparasitic [22,23], antitumoral [24] and antiviral agents [25].

Continuing with our project on enzymatic transformations, we report here the results from the application of lipases in the preparation of a series of glycyrrhetinic acid derivatives.

In addition, all synthesized compounds were biologically evaluated as potential antiviral agents against TK+ and TK− strains of Herpes simplex virus type 1 (HSV-1), sensitive and resistant to acyclovir (ACV) treatment, respectively.

HSV-1 is a member of the Herpesviridae family and infects a high proportion of human population, causing a range of diseases from mild uncomplicated mucocutaneous to more serious infections, such as keratitis and encephalitis. HSV-1 is the causal agent of cold sore and encephalitis [26]. The current gold standard treatment for HSV-1 infections is ACV, a guanosine analogue antiviral drug. With the emergence of Herpesvirus strains resistant to nucleoside analogues, including ACV, there is an urgent need for new and more effective treatments for HSV infections [27]. In this work,

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we evaluated the antiviral capacity of five glycyrrhetinic acid (GA) derivatives against HSV-1.

2. Results and discussions

Many glycyrrhetinic acid derivatives were chemically synthesized and displayed a wide spectrum of applications in different areas. Some of them, with modifications in C-3, C-11 and C-29 positions, were used in the treatment of metabolic diseases [28]. Other derivatives with modifications at C-3 and C-30, showed a selective inhibition of the enzyme BChE [8]. Some chemically prepared alkanolamides, amides and esters derived from glycyrrhetinic acid had a high anti-inflammatory activity [11].

In this section we are going to describe the results obtained by applying lipases as catalysts for obtaining derivatives of glycyrrhetinic acid (GA, **1**).

Our initial experiments to obtain the ester derivatives were performed using **1** as substrate and ethanol as nucleophile. We studied the esterification catalyzed by lipases from several sources: from *Candida antarctica* B (CAL B), from *Candida rugosa* (CRL), Lipozyme from *Rhizomucor miehei* (LIP), *Rhizopus oryzae* lipase (ROL), Lipozyme from *Thermomyces lanuginosus* (TLL) and from *Carica papaya* (CPL), which is the remaining solid fraction of papaya latex after wash off of proteases, in a variety of solvents of different polarity (acetonitrile, acetone, diisopropyl ether, dioxane, hexane and toluene). Unfortunately, none of the enzymes tested catalyzed the esterification reaction of **1** with ethanol.

Then, in order to obtain amides derivatives, we tried the reaction of **1** with *n*-butylamine as model nucleophile. Several different conditions (diverse lipases and solvents) were applied but the same unsatisfactory results were observed.

Additionally, taking into account the previous successful results in the enzymatic esterification reaction of acetylated bile acids [21,22], we examined the possibility of acetylate the OH group of **1** using ethyl acetate as acylating agents and different lipases as biocatalysts at several conditions but no reaction was observed. The reaction with activated acylating agents such as vinyl or isopropenyl acetate or other acylating agents like ethyl caproate or diethyl succinate conducted to the same unsuccessful results.

From these results and considering the work previously reported [22], we carried out the chemical acetylation of **1** obtaining 3-acetyl glycyrrhetinic acid (**2**) in excellent yield (98%). Then, we studied the esterification (with ethanol) and amidation (with butylamine) reactions of **2** using several lipases in various solvents at different temperatures, but again no reaction was observed. In summary, under all reaction conditions, neither **1** nor **2** proved to be good substrates for nucleophilic agents such as ethanol or *n*-butylamine.

Lastly, we decided to study the reaction of **1** and **2** with alkanolamines as nucleophiles. We carried out the lipase catalyzed reaction with ethanolamine (**3a**) applying the optimal conditions previously determined for other similar substrates: CAL B as biocatalyst (E/S: 10), hexane as solvent, temperature: 55 °C, a Nu/S: 5. In this case, we observed that while **1** did not reacted, **2** led to the corresponding ethanolamide (**4a**) (Scheme 1). Therefore, considering this result, in order to optimize the reaction conditions, we performed different experiments using **2** as substrate and varying the lipase, solvent, enzyme:substrate ratio (E/S), nucleophile:-substrate ratio (Nu/S) and temperature.

2.1. Amidation of 3-acetyl glycyrrhetinic acid (2)

Six commercial lipases from different sources were evaluated for enzymatic amidation with ethanolamine: from the yeasts *Candida rugosa* (CRL), *Candida antarctica* B (CAL B) and *Candida antarctica* lipase B (CAL B immo plus); from the fungus *Rhizomucor miehei* (LIP), *Rhizopus oryzae* lipase (ROL) and *Thermomyces lanuginosus* (TLL).

Several solvents were also assayed: hexane, diisopropyl ether, acetonitrile and acetone. As it was mentioned, the reactions were carried out at 55 °C using E/S: 10 and Nu/S: 5. It was observed that enzyme activity was variable, giving CAL B the most satisfactory results in acetonitrile; CAL B was also active in DIPE and hexane, but to a lesser extent (Fig. 1). LIP in DIPE and hexane has also showed activity but with a lower performance than CAL B showing conversion 53% at 96 h and 46% at 72 h, respectively, whereas with CRL, ROL, TLIM the enzyme activity were very low. In case of CAL B immo plus, no product was observed under the conditions tested. In the absence of biocatalyst no product was detected.

It is important to note the chemoselective behavior of CAL B since the ethanolamide was the only obtained product; the isomeric aminoester was not detected. Under chemical conditions, alkanolamines are susceptible for acylation both at the amino and hydroxy group. These results are in coincidence with studies which report the same chemoselective behavior of lipase, solely affording the amide [20,24,29–31].

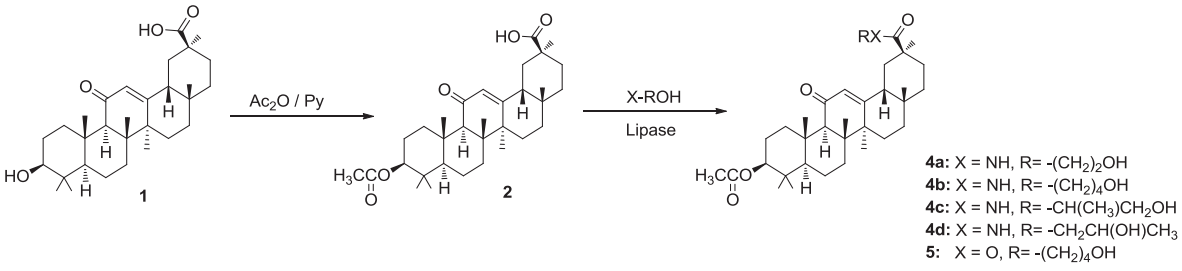
Once determined the appropriate lipase and solvent, the influence of the enzyme:substrate ratio on this reaction was evaluated at 48 h, using a Nu/S ratio of 5, acetonitrile as solvent at 55 °C and variable amounts of CAL B. From the results, it can be concluded that a ratio E/S of 10 is the most acceptable (Fig. 2).

Similarly, the influence of the Nu/S ratio over amidation yield was evaluated at 48 h in acetonitrile using CAL B (E/S = 10) at 55 °C and variable amounts of nucleophile. As it was observed, a ratio Nu/S of 5 is enough to afford the best conversion (Fig. 3).

Finally, we performed the reaction at different temperatures setting the other reaction parameters to their optimal determined values (CAL B, acetonitrile, E/S: 10 and Nu/S: 5). Results showed higher yield with the increase in the temperature. Therefore we selected 55 °C as the best reaction temperature.

Considering the previously mentioned experiments, the determined conditions were applied for enzymatic reactions of **2** with different alkanolamines (**3a-d**) obtaining only the products of amidation (**4a-d**) in very good yields (Table 1).

As it was exhibited, the acetylated glycyrrhetinic acid (**2**) lacked reactivity to both alcohols and amines, whereas amidation reaction



Scheme 1. Chemoenzymatic synthesis of glycyrrhetinic acid derivatives.