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Synthesis, characterization and cytotoxic evaluation of inclusion complexes between Riparin A and β-cyclodextrin

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ABSTRACT

This study performed a physicochemical characterization of the inclusion complex generated between Riparin A and β-cyclodextrin (Rip A/β-CD) and compared the cytotoxic potential of the incorporated Rip A upon *Artemia salina* larvae. Samples were analyzed by phase solubility diagram, dissolution profile, differential scanning calorimetry, X-ray diffraction, infrared spectroscopy, proton nuclear magnetic resonance, scanning electron microscopy and artemicidal action. Riparin A/β-cyclodextrin complexes presented increased water solubility, A_L type solubility diagram and Kst constant of 373 L/mol. Thermal analysis demonstrated reduction of the melt peak of complexed Rip A at 116.2 °C. Infrared spectroscopy confirmed generation of inclusion complexes, ^{1}H NMR pointed out the interaction with H-3 of β-CD cavities, alterations in the crystalline natures of Rip A when incorporated within β-CD were observed and inclusion complexes presented higher cytotoxic on *A. salina* nauplii, with CL_{50} value of 117.2 (84.9–161.8) μg/mL. So, Rip A was incorporated into β-CDs with high efficiency and water solubility of Rip A was improved. Such solubility was corroborated by cytotoxic evaluation and these outcomes support the improvement of biological properties for complexes between Riparin A/β-cyclodextrin.

1. Introduction

Riparins are natural isolated for the first time from *Aniba riparia* (Ness) Mez fruits. Thenceforth, new potentially active molecules derived from them revealed as promising options for novel drugs. Riparin A (Rip-A) – N-(2-phenylethyl)benzamide – a synthetic alkamide, is the fundamental structural of all riparins (Fig. 1) [1,2].

Recently, we have shown biological *in vitro* properties attributed to the Riparin A, such as antioxidant activity against TBARS, cytotoxic action on HCT-116 colon carcinoma cells and leishmanicidal potential upon *Leishmania amazonensis* promatigostas. Additionally, it exhibits anti-inflammatory properties in carrageenan-induced paw edemas and reduces levels of inflammatory mediators as myeloperoxidase, tumor necrosis factor-α and interleukin-1β [3–5].

Cyclodextrins (CDs) have been used to develop new drugs with new therapeutic systems and produce optimized pharmaceutical formulations since they often increase pharmacological activity and improve physical and chemical properties of drugs. They are macrocyclic oligosaccharides generated by the enzyme glycosyltransferase (CGTase) action on the starch. CDs work as pharmaceutical excipients of natural sources constituted by six (α-CD), seven (β-CD) or eight monomers (γ-CD) of α-D-glucopyranose connected by α-1,4 bonds. However, there are semisynthetic CDs

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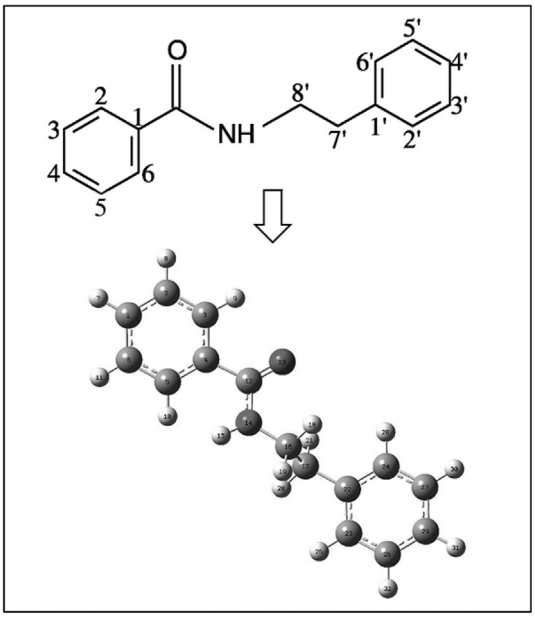

Fig. 1. Molecular structure of Riparin A.

based on substituted derivatives of natural cyclodextrins, such as hydroxypropyl betacyclodextrin [6–8].

Cyclodextrins have a variable concave molecular aspect, hydrophilic external surface with hydroxyl groups and a central lipophilic structure. Thus, they are capable of harboring hydrophobic molecules, improve their solubility, as well as increase their bioavailability. Such inclusion complexes have low oral toxicity even in high doses and superior drug inclusion capacity [9–11]. So, the present study performed a physicochemical characterization of the inclusion complex generated between Riparin A and β-cyclodextrin (Rip A/β-CD) and compared the cytotoxic potential of the incorporated Rip A upon *Artemia salina* larvae.

2. Material and methods

2.1. Obtention of Riparin A

Riparin A was obtained according to Nunes et al. [4] using the Schoten-Bauman reaction, through the mixture of 0.41 mL acyl chloride and 0.89 mL 2-phenylethylamine with triethylamine (as base), followed by magnetic stirring and purification by column chromatography with yield of 84%. The confirmation of synthesis was characterized using usual spectrometric methods (UV, IR, 1H and 13C NMR) [12].

2.2. Proton nuclear magnetic resonance (^{1}H NMR)

The spectrum of Rip A was obtained by nuclear magnetic resonance spectrometer of ^{1}H operating at 500 MHz (Bruker Avance III) with samples solubilized in deuterated dimethylsulfoxide (DMSO-d_6) at room temperature.

2.3. Physical mixture of Rip A and β-CD

Physical mixture (PM) was obtained by homogenization in porcelain mortar for 30 min with pestle in molar ratio of 1:1. Subsequently, the products were separated in a mesh of 250 μm and stored in a desiccator until analysis [13].

2.4. Preparation of the inclusion complex Rip A/β-CD by spray-drying

Equimolar amounts of Rip A and β-CD (ISP Technologies, São Paulo, Brazil, Batch: B701118) were solubilized at room temperature in ethanol and distilled water, respectively. The solutions were homogenized with magnetic stirrer for 30 min and were injected into the bench spray-drying apparatus (Buchi B-290) with an inlet pressure of 0.9 Bar, inlet temperature of 95 °C and sample flow of 3 mL/min. This technique ensured the obtainment of complex with 62% of yield. Complexes were stored in a desiccator until its analysis [14].

2.5. Characterization of the inclusion complex of Rip A/β-CD

2.5.1. Phase solubility diagram

Solubility studies were performed according to Higuchi and Connors [15]. Riparin A was added in tubes with aqueous solutions of β-CD (0, 2, 4, 6, 8 and 10 mM). The samples were kept in constant agitation for 72 h at temperature of 50 ± 2 °C. Then, they were filtered in 0.22 μm membranes and analyzed by spectrophotometer UV–Vis (Shimadzu® UV – 1800) at 225 nm. It was used a mixture of distilled water with ethanol (1:9) as background medium. Experiments were performed in triplicate for each concentration of β-CD. The stability constant (Kst) was calculated by the following equation, where S_0 is the intrinsic solubility of Rip A [14,16,17]: $Kst = Slope\ [S_0 \times (1-Slope)^{-1}]$.

Subsequently, Rip A, PM and Rip A/β-CD complexes were submitted to the standardization analysis.

2.5.2. Dissolution study

Dissolution investigations were conducted in the dissolutor Ethink Technology Model 299 at 50 rpm and 37 ± 0.5 °C. Samples were added in an equivalent way to 10 mg of Rip A. The reaction media with distilled water (1000 mL) was used to evaluate the variation of aqueous solubility. Analysis was performed in triplicate by UV/VIS spectrophotometry (Shimadzu® UV – 1800) after filtration at 225 nm with intervals of 5, 10, 15, 20, 30, 45 and 60 min. The dissolution profile was determined based on the dissolution efficiency at 60 min [18,19].

2.5.3. Differential scanning calorimetry (DSC)

Samples were put inside closed pots and heated between 50 °C and 400 °C at 10 °C/min in nitrogen atmosphere (DSC-2920 - TA Instruments) to obtain the DSC curves.

2.5.4. X-ray diffraction (XRD)

The diffractograms were obtained by X-ray diffractometer (Minifle x® model, λ = 1.5418 Å) with angle of 2θ ranging from 3° to 120° at 2 °C/min.

2.5.5. Infrared spectroscopy (IR)

The infrared vibration spectra were acquired with solid samples of Rip A, β-CD, PM and Rip-A/β-CD in KBr pellets (Perkin Elmer Frontier 1420) and scans ranging from 400 to 4000/cm. Spectra were smoothed and their baselines were automatically corrected using the spectrophotometer software.

2.5.6. Proton nuclear magnetic resonance (^{1}H NMR)

The spectra of the samples were obtained by hydrogen nuclear magnetic resonance spectrometer at 500 MHz (Bruker Avance III). Samples were solubilized in deuterated dimethylsulfoxide (DMSO-d_6) at room temperature (25 °C).