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Synthesis and evaluation of sesquiterpene lactone inhibitors of phospholipase A_2 from *Bothrops jararacussu*

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ABSTRACT

Several sesquiterpene lactone were synthesized and their inhibitive activities on phospholipase A_2 (PLA_2) from *Bothrops jararacussu* venom were evaluated. Compounds Lac01 and Lac02 were efficient against PLA_2 edema-inducing, enzymatic and myotoxic activities and it reduces around 85% of myotoxicity and around 70% of edema-inducing activity. Lac05–Lac08 presented lower efficiency in inhibiting the biological activities studied and reduce the myotoxic and edema-inducing activities around only 15%. The enzymatic activity was significantly reduced. The values of inhibition constants (K_I) for Lac01 and Lac02 were approximately 740 μM, and for compounds Lac05–Lac08 the inhibition constants were approximately 7.622–9.240 μM. The enzymatic kinetic studies show that the sesquiterpene lactones inhibit PLA_2 in a non-competitive manner. Some aspects of the structure–activity relationships (topologic, molecular and electronic parameters) were obtained using *ab initio* quantum calculations and analyzed by chemometric methods (HCA and PCA). The quantum chemistry calculations show that compounds with a higher capacity of inhibiting PLA_2 (Lac01–Lac04) present lower values of highest occupied molecular orbital (HOMO) energy and molecular volume (VOL) and bigger values of hydrophobicity (LogP). These results indicate some topologic aspects of the binding site of sesquiterpene lactone derivatives and PLA_2.

1. Introduction

PLA_2 are enzymes that hydrolyze glycerophospholipid membranes (PL) in the *sn*-2 position, releasing, among other fatty acids, arachidonic acid (AA). AA is involved in the inflammatory process, producing the pro-inflammatory prostaglandins (PGs) and leukotrienes (LTs). The excessive production of PGs and LTs is associated with many physiopathological processes such as asthma, cerebral illnesses, cancers, cardiovascular disorders, and inflammation (Funk, 2001). The inhibition of PLA_2 can prevent the excessive production of PGs and LTs, since the formation of AA is avoided (Yedgar et al., 2000; Balsinde et al., 2002). Venoms from different snake specimens are utilized as a PLA_2 source, due to the abundance of these materials. Thus, these enzymes are utilized as a tool for several pharmacological studies (Jabeen et al., 2005; Yedgar et al., 2006; Romero et al., 2010).

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Lactones are esters formed from the cyclisation reaction between a hydroxyl group and another acid in the same molecule. Lactones with 5 or 6 carbons are more stable due to their low tension energy in the ring. Some studies have demonstrated the capacity of different lactones to inhibit phospholipase A_2. The bromoenol lactone can inhibit calcium-independent PLA_2 (Balsinde and Dennis, 1996; Dentan et al., 1996; Jenkins et al., 2002; Da Silva et al., 2006; Song et al., 2006; Da Silva et al., 2007). In addition, wedelolactone and its derivatives from the class of coumestans, are capable of inhibiting the toxic action of both venom and PLA_2, isolated from *Bothrops jararacussu* and *Crotalus durissus terrificus* (Melo and ownby, 1999; Diogo et al., 2009; Melo et al., 2010).

In this study, we synthesized eight sesquiterpene lactone compounds and evaluated their ability to inhibit some of the toxic effects of both whole venom, and PLA_2 isolated from the venom of *B. jararacussu*. To analyze the toxic effects induced by this venom and provoked by PLA_2, edema-inducing, enzymatic and myotoxic activities of these substances were determined. After these experimental analyses, all lactones compounds were submitted to *ab initio* quantum calculations (DFT – Density Functional Theory – UB3LYP/6-31G*) and the values of their physical–chemistry properties were analyzed by chemometric methods, in order to recognize patterns that correlate the lactone structures with their biological activities. The results obtained may aid in the development of new selective inhibitors for phospholipases A_2 and, consequently, the treatment of poisoning by snake bites.

2. Material and methods

2.1. Chemicals

All reagents, including Lac01 (α-santonin), were purchased from Aldrich or Sigma Co (USA). *B. jararacussu* venom was purchased from a private serpentarium in Formiga, MG, Brazil.

2.2. Bothrops jararacussu *PLA_2 isolation*

B. jararacussu PLA_2 was isolated employing two chromatographic steps: first gel filtration on Sephadex G-75, followed by cation-exchange chromatography. The column was previously equilibrated with 0.05 M ammonium bicarbonate buffer, pH 8.0. Elution was carried out with a continuous gradient up to a concentration of 0.5 M ammonium bicarbonate. Absorbance of the effluent solution was recorded at a wavelength of 280 nm. PLA_2 homogeneity was assessed by native and SDS-PAGE and reverse-phase HPLC. Fraction II, known as Asp49 BthTX-II, was used in this study. This phospholipase will be denominated in this paper as just PLA_2 (Da Silva et al., 2008a,b).

2.3. Animals

Male Swiss mice, 6–8 weeks old, were matched for body weight (18–22 g). The animals were housed for at least one week before the experiment in laminar-flow cages maintained at a temperature of 22 ± 2 °C and a relative humidity of 50–60%, under a 12:12 h light–dark cycle. The animal experiments were carried out with the approval of the institutional committee of ethics, in accordance with protocols following the recommendations of the Canadian Council on Animal Care. The mice used in this study were kept under specific pathogen-free conditions.

2.4. Synthesis of sesquiterpene lactones

The compounds employed in this study are shown in Fig. 1. Lactones 2, 3, 5, 6, 7, and 8 were prepared by procedures described in the literature (Arantes et al., 2009; De Alvarenga et al., 2009). Lac04 was prepared as described below. To characterization of Lac04: IR spectra were recorded on a Perkin Elmer Paragon 1000 FTIR spectrophotometer, KBr, ν_{max}, cm^{-1}. ^1H and ^{13}C NMR spectra were obtained on a Bruker AVANCE DRX400 spectrometer at 400 and 100 MHz, respectively, and a Varian Mercury spectrometer observing ^1H at 300 MHz and ^{13}C at 75 MHz. All ^1H and ^{13}C spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. Low resolution mass spectra were obtained on a SHIMADZU GC MS-QP5050A instrument by direct injection. The microanalysis was obtained on a PERKIN ELMER *2400* instrument. HRMS data were recorded under conditions of chemical ionization (CI) on a Fisons Autospec– oaTof (resolution = 10,000 FWHM) in CI^+ mode using NH_3 as the ionization gas. All reagents and solvents used were previously purified and dried, as reported in the literature (Perrin et al., 1980).

2.4.1. *(3S)-5a-(1-bromo-1-methylethyl)-3-methyl-3,3a,5,5a,8,9b-hexahydro-4H-furo[2,3-f]chromene-2,7-dione (Lac04)*

To isofotosantonic acid (50 mg, MW 264 g/mol, 0.189 mmol) in dichloromethane (20 mL) was added a solution of bromine (38 mg, 0.238 mmol) in dichloromethane (3 mL) drop wise. The solvent was removed under vacuum to afford a yellow solid. This residue was recrystallized in a mixture of hexane/dichloromethane to give pale white crystals (48 mg, MW 424 g/mol, 60%). Mp = 176–177.3 °C IR ν_{max} 2976, 2935, 2903, 1782, 1734, cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ: 1.25 (d, 3H, $J_{13,11}$ = 6.9, H13), 1.70–1.75 (m, 1H, H6), 1.85 (s, 3H, H15), 1.88–1.94 (m, 1H, H7′), 1.97 (s, 3H, H14), 2.06–2.12 (m, 2H, H8), 2.39–2.50 (m, 1H, H11), 2.75–2.80 (m, 1H, H7), 3.13–3.16 (m, 2H, H2 H2′), 5.03–5.08 (m, 1H, H5), 6.06–6.09 (m, 1H, H3); ^{13}C NMR (75 MHz, $CDCl_3$): 12.7 (C13), 25.5 (C14), 30.2 (C15), 30.8 (C7), 31.0 (C8), 36.6 (C2), 42.1 (C11), 52.7 (C6), 70.4 (C10), 80.8 (C9), 90.0 (C5), 116.2 (C3), 133.5 (C4), 167.7 (C12), 177.9 (C1); MS, m/z (%): 424 – Br_2 [M^+.], 221 (100), 203 (15), 175 (10), 123 (11), 91 (13), 69 (14), 55 (16). (**found**: C, 52.16; H, 5.52. $C_{15}H_{19}BrO_4$ **requires**, C, 52.49; H, 5.58).

2.5. Edema-inducing activity

Male Swiss mice (18–22 g) were used for inducing edema. The edema was induced in the right foot pad by i.d. injection of 50 μL of a solution containing 50 μg of PLA_2, purified from *B. jararacussu* venom dissolved in 1% DMSO (Dimethyl Sulfoxide) in PBS (phosphate-buffered saline –