SYNTHESIS AND CHARACTERIZATION OF N-BENZYL-2-(2-NITRO-1H-IMIDAZOL-1-YL)ACETAMIDE DERIVATIVE CYCLOPALLADATED COMPOUND AS A LYSOSOMAL CYSTEINE PROTEASES INHIBITOR

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Introduction and objectives: Human lysosomal cathepsin family present proteolytic activity in different physiological or pathological processes. They are involved in the tumor progression such as cell proliferation, apoptosis, tumor angiogenesis, tissue invasion and metastasis. Therefore cathepsins are potential targets in development of drugs for cancer treatment. Studies have shown that the class of cyclopalladated compounds are potential antitumor agents. Our goal was to synthesize, identify, evaluate and characterize a new palladacycle derived from N-benzyl-2-(2-nitro-1H-imidazol-1-yl)acetamide (benznidazole) as potential inhibitors of human cathepsins B, K, L, S and V.

Materials and methods: Cyclopalladated compound [Pd(C₂,N-N-benzyl-2-nitro-imidazol acetamide)Cl]₂ was synthesized by reaction of Li₂PdCl₄ with N-benzyl-2-(2-nitro-1H-imidazol-1-yl)acetamide as cyclometallation agent, using methanol as solvent. The compound was characterized by elemental analysis (% C, H, N), melting point (°C), NMR and FTIR by KBr pellets technique. Recombinant human cathepsins B, K, L, S and V were expressed in Pichia pastoris. The inhibition assays were carried out in 100 mM sodium acetate buffer, 5 mM EDTA, pH 5.5, 2.5 mM DTT for cathepsins B, K, L and V, and 100 mM sodium phosphate buffer, 1 mM EDTA, pH 6.5, 2.5 mM DTT for cathepsin S, on HITACHI F-2500 spectrofluorometer using the fluorogenic substrate Z-FR-MCA (λₜₐₓ = 360 nm, λₜₐₘ = 480 nm). Results: Synthesis of [Pd(C₂,N-N-benzyl-2-nitro-imidazoacetamide)Cl]₂. Yield: 60%. Elemental analysis, found % (calculated): C, 40.42(35.93); H, 3.7(2.76); N, 15.42(13.97). NMR 1H (δ, ppm, C₃D₅O): 7.47-7.62 (d,d, 2H-imidazole); 7.22–7.33(m, H-aromatic rings); 4.44(d, 2H-CH₂); 5.38(s, 2H-CH₂). FTIR (cm⁻¹, KBr): 3300(ν N-H); 1661(ν C=O); 1536 e 1336(ν NO₂); 743(ν Pd-C). M.P.: ~230 °C. M.W.: 801.8. The IC₅₀ values were 6.73µM, 15.59µM, 17.43µM, 55.90µM and 2.47µM for cathepsins B, K, L, S and V, respectively. Conclusion: The new palladacycle [Pd(C₂,N-N-benzyl-2-nitro-imidazol acetamide)Cl]₂ was able to inhibit all cathepsins, being more efficient against cathepsins V and B. Acknowledgements: CAPES, FAPESP, CNPq, FAEP and Prof. Dr. Antonio Carlos Fávero Caires (Centro Interdisciplinar de Investigação Bioquímica, UMC, SP, Brazil) in memorian†. Key Words: cathepsin, palladacycle, benznidazole