From the very beginning to a promising application: synthesis, characterization, *in vitro* metabolism and *in vivo* preliminary activity of $[Ru_3O(CH_3COO)_6(thiq)_3]PF_6$

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Introduction. Trinuclear ruthenium complexes are known by their interesting chemical and electrochemical properties[1], but the use of these complexes in biological media is relatively new[2]. On the other hand, the antiparasitic properties of quinolines are explored since the beginning of 20th century[3]. *In vitro* metabolism employing microsomes is an important tool to evaluate if a drug candidate is metabolized by the CYP450 enzymes, and these results are necessary to determine if the substance is able to continue to the next phases of drug development[4]. After *in vitro* studies succeed, the *in vivo* experiments are essential to verify the possibility of applying the drug candidate in clinical trials, for example. In view of that, we present in this work the synthesis, characterization, *in vitro* metabolism for both rat and human liver microsomes, and previous *in vivo* studies looking for a treatment to Chagas Disease. ***Results and discussion.*** The title complex was obtained by stirring a methanolic solution of $[Ru_3O(CH_3COO)_6(CH_3OH)_3](CH_3COO)$ with six times excess of 5,6,7,8-tetrahydroisoquinoline, the counterion was changed to PF_6^- by adding NH_4PF_6 to the medium, and the complex was purified by column chromatography using neutral aluminum oxide as stationary phase and a 9:1 mixture of acetonitrile and dichloromethane as mobile phase. Anal. Calcd for $C_{39}H_{51}O_{13}F_6N_3PRu_3$ (found): %C, 38.45 (37.58), %H, 4.22 (4.06), %N, 3.45 (3.42). ESI-Q-TOF MS [**1**-PF_6]$^+$ (found): m/z 1075.0 (1074.9). Absorption spectrum showed four bands (ε, M cm^{-1}): 240 nm (*4.33*, *IL*), 283 nm (*4.31*, *IL*), 340 nm (sh, *CLCT*), 693 nm (*3.82*, *IC*). Infrared spectrum presented the typical profile of this class: ν(C-H) 2936m, 2862m; ν(C=C) 1556br, 1494m, $\nu_{as}(COO^-)$ 1616m, $\nu_s(COO^-)$ 1428s. ^{1}H-NMR δ(CH_3) 4.35 ppm (18H, s), δ(α') -0.16 ppm (3H, s), δ(α) 0.12 (3H, s), δ(β) 5.89 ppm (3H, s), δ(γ) 1.39 ppm (6H, q), δ(δ) 8.07 ppm (6H, t), δ(ε) 1.46 ppm (6H, t), δ(ζ) 1.74 ppm (6H, d). Cyclic voltammetry exhibited the typical behavior to totally delocalized systems: $[Ru_3O]^n$, n= -1/0 (E/2 -1.33 V), 0/+1 (E/2 0.09 V), +1/+2 (E/2 1.24 V). *In vitro* metabolism of $[Ru_3O(CH_3COO)_6(thiq)_3]PF_6$ was performed employing 0.5 mg/mL of microsomal protein concentration during 1 h at 37 ºC for both mammals models. In the rat model, 73% of the complex was metabolized and in human model, 52% was metabolized, which showed that CYP450 enzymes is involved in complex metabolism. The trypanocidal activity *in vivo* was performed in a murine model of acute phase of Chagas disease (Animal Ethics Committee No 15.1.713.60.7). The complex was effective in reducing the parasitemia of mice treated with a concentration 4 times lower than the concentration normally used to benznidazole treatment (clinically used at a concentration of 10 mg kg^{-1} Day^{-1}). In addition, complex was effective in reducing inflammation of the heart of mice infected. ***Final remarks.*** The complex was successfully obtained and by the results presented, it is possible to affirm that the proposed structure is correct. *In vitro* metabolism assays showed that the compound is metabolized by CYP450 enzymes in both rat and human models. Furthermore, the complex proved to be a potent trypanocidal agent.

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