Chemical constituents and antiproliferative activity of *Rhinella marina* "Sapo Cururu" from Southern Amazon

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Introdução

The skin secretions and venom of amphibians are rich sources of bioactive compounds, such as peptides, alkaloids, bufadienolides, biogenic amines and proteins. These molecules play a crucial role in the physiological functions of these animals, especially for predation and protection against microorganisms.[1,2] Previous studies of our group with *R. marina* venom resulted in the identification by LC-MS of the four bufadienolides: telocinobufagin (**1**), marinobufagin (**2**), bufalin, and resibufogenin.[3] As part of our ongoing research on bioactive compounds from Brazilian poison frogs, the objectives of the present study were investigated the chemical composition and the antiproliferative effect of the compound **2** from venom of *R. marina* found in Southern Amazon.

Resultados e Discussão

Toad venom was collected from the secretion of *R. marina* in Mato Grosso State, Brazil. The animals were identified by one of the authors (D. J. Rodrigues – IBAMA, SISBIO: number 30034-1). Voucher specimen (*R. marina* – ABAM-H 1262) was deposited in the Acervo Biológico da Amazônia Meridional (Sinop, Mato Grosso, Brazil). The MeOH extract (1.1 g) from venom was chromatographed on Sephadex LH-20 column, using MeOH as eluent. The fractions 31 (178.4 mg) and 75 (14.0 mg) yielding the compounds **3** and **2**+**4** (34:66), respectively. Fraction 66 (173.1 mg) was subjected to SiO_2 column, using hexane/AcOEt as eluent. The subfractions 91, 112, 113 and 128 yielding the compound **2** (23.1 mg) and the subfraction 140 the compound **1** (25.7 mg). Compound **2** was evaluated in a variety of tumor cell lines using the colorimetric MTT assay (Table 1).[4] The compounds (Figure 1) were identified by spectrometric methods (mass, NMR ^{1}H and ^{13}C). The bufadienolides **1** and **2** were reported previously in *R. marina* venom and were identified as telocinobufagin and marinobufagin, respectively.[1,3] The substances **3** and **4** were identified as an alkaloid and a steroid, named dehydrobufotenine and cholesterol, respectively, previously reported in *Bufo marinus*.[5,6] The compound **2** revealed higher cytotoxic activity when compared to doxorubicin (0.3 μg mL^{-1}), with IC_{50} values of 0.07 μg mL^{-1} (HL-60 and HCT-116), 0.12 μg mL^{-1} (OVCAR-8) and 0.18 μg mL^{-1} (SF-295) (Table 1).

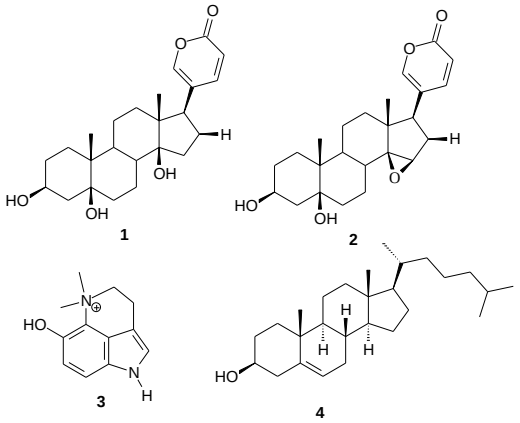

Figure 1. Chemical constituents isolated from *R. marina*

Table 1. Cytotoxic potential (IC_{50} in μg mL^{-1}) of **2** on human tumor cell lines after 72 h of exposure.

Compound/Cell	HL-60	SF-295	HCT-116	OVCAR-8	HEP-2	NCIH292
2	0.07 0.06-0.08	0.18 0.16-0.21	0.07 0.05-0.08	0.12 0.09-0.13	2.9 2.0-4.1	1.1 0.7-1.9

Positive control: doxorubicin (0.3 μg mL^{-1})

Conclusões

The chemical study of the *R. marina* venom resulted in the isolation of four compounds, two bufadienolies, an alkaloid and a steroid. Compound **2** showed potent cytotoxicity against tumor cell lines.

Agradecimentos

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