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Review

Bufadienolides from amphibians: A promising source of anticancer prototypes for radical innovation, apoptosis triggering and Na^+/K^+-ATPase inhibition

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ABSTRACT

Amphibians present pharmacologically active aliphatic, aromatic and heterocyclic molecules in their skin as defense against microorganisms, predators and infections, such as steroids, alkaloids, biogenic amines, guanidine derivatives, proteins and peptides. Based on the discovered bioactive potential of bufadienolides, this work reviewed the contribution of amphibians, especially from members of Bufonidae family, as source of new cytotoxic and antitumor molecules, highlighting the mechanisms responsible for such amazing biological potentialities. Bufonidae species produce bufadienolides related to cholesterol through the mevalonate-independent and acidic bile acid pathways as polyhydroxy steroids with 24 carbons. *In vitro* antitumor studies performed with skin secretions and its isolated components (specially marinobufagin, telocinobufagin, bufalin and cinobufagin) from *Rhinella*, *Bufo* and *Rhaebo* species have shown remarkable biological action on hematological, solid, sensitive and/or resistant human tumor cell lines. Some compounds revealed higher selectivity against neoplastic lines when compared to dividing normal cells and some molecules may biochemically associate with Na^+/K^+-ATPase and there is structural similarity to the digoxin- and ouabain-Na^+/K^+-ATPase complexs, implying a similar mechanism of the Na^+/K^+-ATPase inhibition by cardenolides and bufadienolides. Some bufadienolides also reduce levels of antiapoptotic proteins and DNA synthesis, cause morphological changes (chromatin condensation, nuclear fragmentation, cytoplasm shrinkage, cytoplasmic vacuoles, stickiness reduction and apoptotic bodies), cell cycle arrest in G_2/M or S phases, mitochondrial depolarization, PARP [poly (ADPribose) polymerase] and Bid cleavages, cytochrome *c* release, activation of Bax and caspases (−3, −9, −8 and −10), increased expression of the Fas-Associated protein with Death Domain (FADD), induce topoisomerase II inhibition, DNA fragmentation, cell differentiation, angiogenesis inhibition, multidrug resistance reversion, and also regulate immune responses. Then, bufadienolides isolated from amphibians, some of them at risk of extinction, emerge as a natural class of incredible chemical biodiversity, has moderate selectivity against human tumor cells and weak activity on murine cells, probably due to structural differences between subunits of human and mice Na^+/K^+-ATPases.

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1. Natural products as prototypes with drug-like properties

Between 1981 and 2010, 1073 new chemical entities (New Chemical Entities - NCEs) were approved as drug by Food and Drug Administration (FDA) of the United States and 64% of them are natural, derived or synthesized molecules based on natural compounds. Despite the interest in molecular modeling, in combinatorial chemistry and other chemical synthesis techniques, natural products remains as important source of new therapeutic agents against infections (fungal or bacterial), disease vectors, cancer, dyslipidemia and immunomodulation (Butler, 2004; Balunas and Kingnorn, 2005; Newman and Cragg, 2012; Farias et al., 2013; Santos et al., 2013; Pereira et al., 2015; Simoes et al., 2015; Ferreira et al., 2016).

It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity, molecular flexibility and other molecular properties that make them more favorable as lead structures for drug discovery, help us to differentiate them from libraries of synthetic and combinatorial compounds. Moreover, fraction of natural product structures with two or more 'rule-of-five' violations is quite low (≈10%) and similar to that of trade drugs (Lipinski et al., 1997; Feher and Schmidt, 2003; Ferreira et al., 2011; Newman and Cragg, 2012; Pascolutti and Quinn, 2014). All these advantages partially explain why about 60% of the NCEs introduced as pharmaceutical choices were directly or indirectly based on natural compounds. Definitely, these NCEs mimicking natural-product pharmacophores have already been selected and validated over thousands of years by environmental conditions, and possess interesting drug-like properties commonly found in natural molecules.

Amongst environmental resources, toxin-producing animals are part of the traditional medicine in several countries around the world. Amphibians, especially from Bufonidae family, present pharmacologically active aliphatic, aromatic and heterocyclic molecules in their skin with a diversified range of steroids, alkaloids, biogenic amines, guanidine derivatives, proteins and peptides as defense against microorganisms, predators and infections (Clarke, 1997; Daly, 1995; Duellman and Trueb, 1996; Prates and Bloch-Júnior, 2000; Daly et al., 2004; Costa-Neto, 2005; Gutierrez, 2016). These animal venoms have different biological activities. Briefly, reports have described trypanocidal, leishmanicide, antibacterial, antifungal (Riera et al., 2003; Cunha-Flho et al., 2005; Tempone et al., 2008), antiproliferative (Cunha-Filho et al., 2010; Gao et al., 2011; Moreno Y Banuls et al., 2013a; Ferreira et al., 2013; Sciani et al., 2013), insecticide (Supratman et al., 2000), antiviral (Wang et al., 2011) and cardiotonic (Imai et al., 1965; Mijatovic et al., 2012) actions. In this context, cardenolides (ouabain and digoxin, for example) are the most widely studied cardiotonic steroids and since PubMed database provides a total of about 39,739 cardenolides references. Thus, based on the large and recently discovered pharmacological potential of bufadienolides, this work reviewed, in a systematic and critical way, the contribution of amphibians, especially from members of Bufonidae family, as source of new cytotoxic and antitumor molecules, highlighting the mechanisms responsible for such amazing biological potentialities.

2. Bufonidae family: classification, distribution, components of glandular secretions and environmental factors

Amphibians have emerged in late Devonian period with the change from aquatic to terrestrial environment due to the progressive behavioral and morphofunctional adaptations (Toledo and Jared, 1995).

From the quantitative point of view, there is an amazing global diversity of amphibian species. Brazil (789 species) and Colombia (642) have the largest number of species. China (335) has the largest number of species in the Old World. The Democratic Republic of the Congo (215) has the largest number from continental Africa and 239 species are recorded from Madagascar. Australia and Papua New Guinea have 225 and 289 species, respectively. In North America, Mexico has the largest number of species (357) but there are 291 species in the United States (Wake and Vredenburg, 2008).

Amphibians (Class Amphibia, Subclass Lissamphibia) include toads and frogs (Order Anura, ≈ 5600 currently recognized species), salamanders (Order Caudata, ≈ 570 species), and caecilians (Order Gymnophiona, ≈ 175 species), with up to 6345 species (Vitt and Caldwell, 2014). Anura order is the major Amphibia class group (Fig. 1). Most of them live in the Tropics, and about 600 species are found in the Amazonian basin. They are well adapted and one of the most abundant vertebrates present in a wide multiplicity of habitats. Other amphibians (salamanders and caecilians) are represented by a few species in Amazonian region, and they are also found in Mexico, Costa Rica, Asia, Africa and Europe (Gower and Wilkinson, 2005; Haseyama and Carvalho, 2011; Jia and Gao, 2016).

Bufonidae family has about 51 genus and 596 species distributed around the world, except Australia, Oceanic regions, Sahara desert, Madagascar, Artic and Antarctic (Fig. 1). Studies molecular and phylogenetic relationships have recently reexamined this family and taxon nomenclatures were changed. For example, Chaparro et al. (2007) suggested that most of South American *Bufo* species were placed in genus *Rhinella* (Frost et al., 2006; Pramuk, 2006; Chaparro et al., 2007; Frost, 2015).

In Brazil, Bufonidae family is represented by seven genera, and *Rhinella* is the most representative with nearly 40 species. They are commonly known by its generic name "sapo-cururu" (*kuru'ru* from Tupi, meaning big toad). One of the most analyzed species in Amazonian region is *Rhinella marina* (Fig. 2A), former *Bufo marinus* (Easteal, 1963). Other specimens found in the Amazon basin are