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Review Article

BUFADIENOLIDES AND THEIR MEDICINAL UTILITY: A REVIEW

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ABSTRACT

Bufadienolides are a type of cardiac glycoside originally isolated from the traditional Chinese drug Chan'Su which increases the contractile force of the heart by inhibiting the enzyme Na^+/K^+–ATPase. They also show toxic activities to livestock. They are widely used in traditional remedies for the treatment of several ailments, such as infections, rheumatism, inflammation, disorders associated with the central nervous system, as antineoplastic and anticancer component. Structural changes in functionality could significantly alter their cytotoxic activities.

The novel oxy-functionalized derivatives of bufalin obtained could provide new platforms for combinatorial synthesis and other further investigations for the development of new bufadienolides antitumor drugs. In this review, naturally occurring bufadienolides which are isolated from both plant and animal sources are reviewed and compiled with respect to their structural changes and medicinal utility.

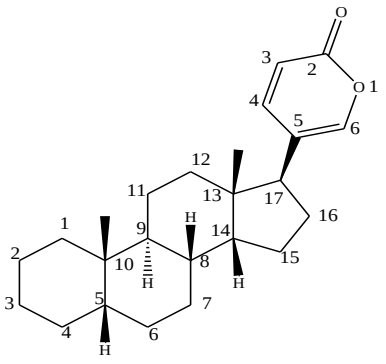
Keywords: Bufadienolides, Cell growth inhibitory activity, Antitumor drugs, Cardenolides, Bufalin.

INTRODUCTION

Bufadienolides are C-24 steroids; its characteristic structural feature is a doubly unsaturated six membered lactone ring having a 2-pyrone group attached at the C-17β position of the perhydrophenanthrene nucleus. On account of this chromophoric ring, they possess a characteristic UV absorption. Many possess a 5b-hydroxyl (A/B-cis ring junction), a trans-B/C ring junction, a 14b-hydroxyl (C/D-cis ring junction) and an aldehydic group at C-19 (e.g. hellebrigenin). Furthermore, these compounds are characterized by the trans-junction of rings B and C and usually the cis-junction of rings C and D [1-3].

C-24 derivatives are collectively known as bufadienolides, including many in the form of bufadienolide glycosides (bufadienolides that contain structural groups derived from sugars). These are a type of cardiac glycoside, the other being the cardenolide glycosides. Both bufadienolides and their glycosides are toxic; specifically, they are heart-arresting. Bufadienolides are important cardiac glycosides which increase the contractile force of the heart by inhibiting the enzyme Na^+/K^+–ATPase. They also show toxic activities to livestock. Some bufadienolides have antineoplastic and cell growth inhibitory properties.

The term "Bufodienolides" derives from the toad genus *Bufo* that contains bufadienolide glycosides, the suffix -*adien*- that refers to the two double bonds in the lactone ring, and the ending -*olide* that denotes the lactone structure. Consequently, related structures with only one double bond are called *bufenolides*, and the saturated equivalent is *bufanolide*. Molecular formula of bufadienolides is $C_{24}H_{34}O_2$.



Bufadienolide

Bufadienolides are in use from more than 1000 years ago. Physician of antiquity and traditional oriental medicine had been known to use medicines prepared from toad in the treatment of cardiac dysfunction. Bufadienolides are a new type of natural steroids with potent antitumor activities, originally isolated from the traditional Chinese drug Chan'Su [2-4]. They have been reported to exhibit significant inhibitory activities against human myeloid leukemia cells (K562, U937, ML1, HL60), human hepatoma cells (SMMC7221), and prostate cancer cells (LNCaP, DU105, PC3). The activities are mediated by induction of cell apoptosis and cell differentiation, and the regulations of a variety of genes and proteins are involved in the process [5-10].

Occurrence

Bufadienolides from plant sources

The bufadienolide class of compounds constitutes the core skeleton of structurally unique 2-pyranone natural products [11-13] in which a steroid moiety is attached at position five of the lactone ring, e.g. bufalin (**1**). This class of compounds is widely used in traditional remedies for the treatment of several ailments, such as infections, rheumatism, inflammation and disorders associated with the central nervous system [14, 15]. On the contrary, bufadienolide glycosides represent a vital cause of mortality among cattle due to cardiac poisoning [16, 18]. The plants belonging to the Crassulaceae and Hyacinthaceae families are rich sources of bufadienolides, which show conservity in the lactone scaffold and diversity in the steroid ring skeleton. Other plant families such as Iridaceae, Melianthaceae, Ranunculaceae and Santalaceae are also sources of the bufadienolide class of compounds. Several of the bufadienolides isolated from species of the *Kalanchoe* (syn. *Bryophyllum*), *Tylecodon* and *Cotyledon* of the plant family Crassulaceae cause acute and sub-acute intoxication affecting the central nervous system and muscular system and producing cardiac poisoning in small animals [19]. In traditional medicine, Kalanchoe species have been used to treat ailments such as infections, inflammation and have immunosuppressive effect as well [20]. List of various bufadienolides obtained from plants is given in Table no. 1.

Bufadienolides from animal sources

The animal sources of bufadienolides include *Bufo* (toad), *Photinus* (fireflies) and *Rhabdophis* (snake), in which an abundance of bufadienolides has been found in some species of toad. Bufadienolides are the major bioactive constituents of the traditional Chinese drug Ch'an Su, and these are major products of the skin secretions of local toads such as *Bufo gargarizans* Cantor or *Bufo melanostrictus* Schneider. Several bufadienolides have been isolated from the bodies of toads of the genus *Bufo*. Five new cancer cell growth inhibitory bufadienolides, 3β-formyloxyresibufogenin, 19-oxobufalin, 19-oxodesacetylcinobufagin, 6α-hydroxycinobufagin and 1β-hydroxybufalin, have been isolated from the Ch'an Su drug, which is used traditionally to treat heart failure and cancer [38].

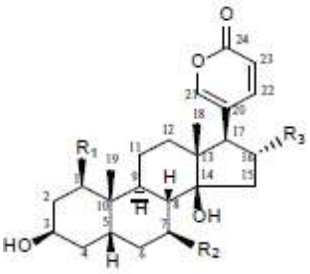
Bufadienolides bearing epoxide substitution in the steroid nucleus, particularly at the C-14 and C-15 positions, are common, but bufadienolides bearing epoxide at the C-20 and C-21 positions are rare. Recently, five new 20,21-epoxybufenolides, 20*S*,21-epoxyresibufogenin, 20*R*,21-epoxyresibufogenin, 3-*O*-formyl-20*S*,21-epoxyresi bufogenin, 3-*O*-formyl-20*R*,21-epoxyresibufogenin and 3-oxo20*S*,21-epoxyresibufogenin with the rarely encountered 17β-2-pyranone ring epoxide have been isolated from toad venom[38]. Some of the bufadienolide class of phytotoxins such as poaefusarin and sporofusarin has been isolated from *Fusarium poae* and *Fusarium sporotrichiella*, respectively. The phytotoxic symptoms of these natural products include the death of branches of peas, beans, and tomatoes. In mammals, these phytotoxins caused temporary inflammation of skin and haemorrhagic or leukocytosic reactions [38]. Various bufadienolides obtained from animals are given in Table no. 2.

Table 1: Bufadienolides from plant sources

S. No.	Plant	Family	Bufadienolides	References
1.	*Kalanchoe daigremontiana*	Crassulaceae	Daigremontianin, Bersaldegenin-1,3,5-orthoacetate, 3-*O*-acetyldaigredorigenin, 1-*O*-acetylbersaldegenin, 3-*O*-acetyl bersaldegenin	21-23
2.	*Kalanchoe lanceolata*	Crassulaceae	Hellebrigenin, Lanceotoxin A, Lanceotoxin B	24
3.	*K. tomentosa and K. tubiflorum*	Crassulaceae	Kalanchoside, Bryotoxin A, Bryotoxin B, Bryotoxin C (bryophyllin A)	25,26
4.	*K. pinnata*	Crassulaceae	Bryophyllin A, Bryophyllin C	27
5.	Kalanchoe gracilis	Crassulaceae	kalanchosides A-C	28
6.	Tylecodon ventricosus	Crassulaceae	Tyledoside D	29
7.	*Tylecodon grandiflorus*	Crassulaceae	Tyledoside A, Tyledoside B, Tyledoside C, Tyledoside D, Tyledoside F, Tyledoside G	30
8.	*Cotyledon orbiculata*	Crassulaceae	Orbicuside A, Orbicuside B, Orbicuside C	31
9.	*Urginea maritima*	Hyacinthaceae/ Liliaceae	11α-acetylgamabufotalin-3-O-(4-O-β-D-glucosyl)-α-L-rhamnoside, proscillaridin A, 11α-hydroxyscilliglaucoside, Scillirosid, gammabufotalin, scillirosidin, glucoscillaren A, Scilliphaeoside, glucoscilliphaeoside,12-epi-scilliphaeoside, gammabufotalin-3-o-alpha-L-rhamnoside, 16β-o-acetyl-γbufotalin-3-o-α-L-rhamnoside, scilliglaucoside, scillicyanoside, 5α-4,5-dihydroproscillaridin A, 5α-4,5-dihydro glucoscillaridin A, 19-oxo-5α-4,5-dihydro proscillaridin A.	32,33
10.	*Urginea hesperia*	Hyacinthaceae/ Liliaceae	scillarenin, scilliphaeosidin, scillarenin-3-O-α-L-rhamnoside, scilliphaeosidin-3-O-α-L-rhamnoside, gamabufotalin-3-O-α-L-rhamnoside, 11α-hydroxyscilli glaucosidin-3-O-α-L-rhamnoside, scillarenin-3-O-α-L-2',3'-diacetyl rhamnosido-4'-β-D-glucoside, scillarenin-3-O-α-L-rhamnosido-4'-β-D-glucosido-3''-β-D-glucoside, scillarenin-3-O-α-L-rhamnosido-4'-β-D-glucosido-4''-β-D-glucoside, scillarenin-3-O-α-L-2',3'-diacetylrhamnosido-4'-β-D-glucosido-3''-β-D-glucoside, scillarenin-3-O-α-L-2',3'-diacetylrhamnosido-4'-β-D-glucosido-4''-β-D-glucoside, scilliphaeosidin-3-O-α-L-rhamnosido-4'-β-D-glucosido-3''-β-D-glucoside, scilliphaeosidin-3-O-α-L-rhamnosido-4'-β-D-glucosido-4''-β-D-glucoside.	34
11.	*Drimia robusta*	Hyacinthaceae	12β-hydroxyscillirosidin, 6β-acetoxy-3β,8β, 12β,14β-tetrahydroxybufa-4,20,22-trienolide (12β-hydroxyscillirosidin), 14β-hydroxybufa-4,20,22-trienolide-3β-*O*-{α-l-rhamnopyranosyl-[(1→4)-β-d-gluco pyranosyl]-(1→3)-α-l-rhamnopyranoside} (urginin)	32
12.	*Urginea altissima*	Hyacinthaceae	Urginin	32
13.	*Urginea sanguinea*	Hyacinthaceae	Phloroglucinol, 1β-D-glucopyranoside (phlorin), scillaren A, 5α-4,5-dihydro scillaren A, scillirosidin, desacetyl scillirosidin, 12β-hydroxy-scillirosidin, 12β-hydroxy-desacetyl-scillirosidin, 12β-hydroxy-scilliroside, 5α-4,5-dihydro-12β-hydroxy-scillirosidin, 12β-hydroxy-scilli rosidin-3-one, 12β-hydroxy-scilli-rubrosidin-3-one,7β,15α-dihydroxy-yamogenin.	35,36
14.	*Urginea lydenburgensis*	Hyacinthaceae	16β-acetoxy-3β,14β-dihydroxy-19-formyl -bufa-4,20,22-trienolide (scillicyanosidin), 4β,8β,11α,14β-tetrahydroxybufa-5,20,22-trienolide-12one, 2α,3β-o-4,6-dideoxy-L-glucose (Lydenburgenin).	37
15.	*Mimosa pudica*	Leguminoseae	Hellebrigenin-3-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-galactopyranoside	38
16.	*Millettia ovalifolia*	Leguminoseae	4,5-dehydro-14-β-hydroxyscilladienolide-3-O-β-D-glucopyranoside	39
17.	*Helleborus torquatus*	Ranunculaceae	Hellebortin A, Hellebortin B, Hellebortin C	38

Table 2: Bufadienolides from animal sources

S. No.	Animal	Bufadienolides	References
1.	Toad venom	20*S*,21-epoxyresibufogenin, 20*R*,21-epoxyresibufogenin, 20*R*,21-epoxyresibufogenin, 3-*O*-formyl-20*R*,21-epoxy resibufogenin, 3-oxo-20*S*,21-epoxyresibufogenin	40
2.	*Fusarium poae*	Poaefusarin	41
3.	*Fusarium sporotrichiella*	Sporofusarin	41
4.	*Bufo marinus*	11α-hydroxy hellebrigenin, marinoic acid, 11α,19-dihydroxy-telocinobufagin (marinosin), 11α–hydroxytelo cinobufagin, 11α,19-dihydroxymarinobufagin, 11α-hydroxymarinobufagin, 19-hydroxytelocinobufagin.	42
5.	Bufo viridis toad	Bacagin	43
6.	toad Bufo rubescens	Marinobufagin, telocinobufagin	44
7.	Bufo bufo gargarizans	Bufogargarizins A & B	25



1 bufalin
$R_1=R_2=R_3=H$

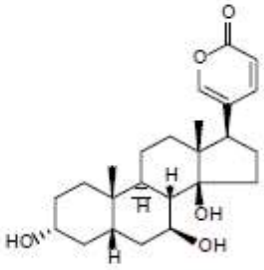
3 7β-hydroxyl bufalin
$R_1=R_3=H\ R_2=OH$

5 1β-hydroxyl bufalin
$R_1=OH\ R_2=R_3=H$

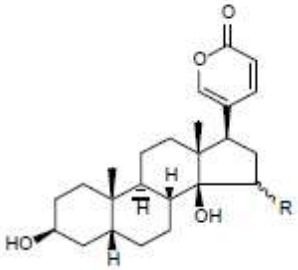
11 1β,7β-dihydroxyl bufalin
$R_1=R_2=OH\ R_3=H$

12 16α-hydroxyl bufalin
$R_1=R_2=H\ R_3=OH$

13 7β,16α-dihydroxyl bufalin
$R_1=H\ R_2=R_3=OH$

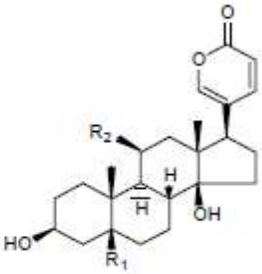


4 3-*epi*-7β-hydroxyl bufalin



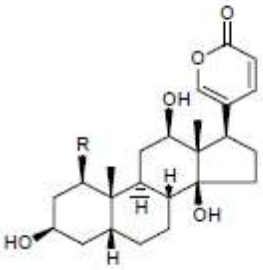
6 15α-hydroxyl bufalin
R=α-OH

7 15β-hydroxyl bufalin
R=β-OH



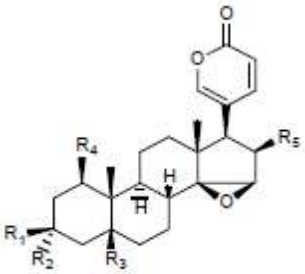
8 telocinobufagin
$R_1=OH\ R_2=H$

9 11β-hydroxyl bufalin
$R_1=H\ R_2=OH$



10 12β-hydroxyl bufalin
R=H

14 1β,12β-dihydroxyl bufalin
R=OH



2 cinobufagin
$R_1=OH\ R_2=R_3=R_4=OH\ R_5=OAc$

15 desacetylcinobufotalin
$R_1=R_3=R_5=OH\ R_2=R_4=H$

16 3-*epi*-desacetylcinobufagin
$R_1=R_3=R_4=H\ R_2=R_5=OH$

17 1β-hydroxyl desacetylcinobufagin
$R_1=R_4=R_5=OH\ R_2=R_3=H$

18 3-*epi*-desacetylcinobufotalin
$R_1=R_4=H\ R_2=R_3=R_5=OH$

19 cinobufagin 3-O-β-D-glucoside
$R_1=O\text{-glc}\ R_2=R_3=R_4=H\ R_5=OAc$

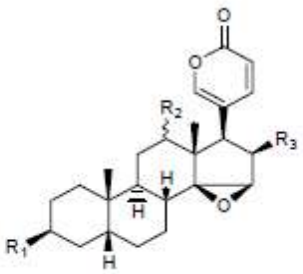
20 3-*oxo*-desacetylcinobufagin 16-O-β-D-glucoside
$R_1+R_2=O\ R_3=R_4=H\ R_5=O\text{-glc}$

21 desacetylcinobufagin 16-O-β-D-glucoside
$R_1=OH\ R_2=R_3=R_4=H\ R_5=O\text{-glc}$

22 3-*epi*-desacetylcinobufagin 16-O-β-D-glucoside
$R_1=R_3=R_4=H\ R_2=OH\ R_5=O\text{-glc}$

23 cinobufotalin
$R_1=R_3=OH\ R_2=R_4=H\ R_5=OAc$

24 desacetylcinobufagin
$R_1=R_5=OH\ R_2=R_3=R_4=H$



25 12β-hydroxyl cinobufagin
$R_1=OH\ R_2=\beta\text{-OH}\ R_3=OAc$

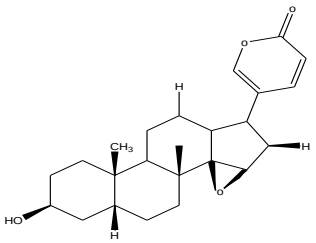
26 12β-hydroxyl desacetylcinobufagin
$R_1=R_3=OH\ R_2=\beta\text{-OH}$

27 3-*oxo*-12β-hydroxyl cinobufagin
$R_1=O\ R_2=\beta\text{-OH}\ R_3=OAc$

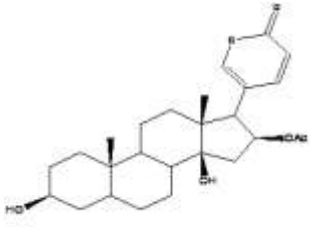
28 3-*oxo*-12β-hydroxyl desacetylcinobufagin
$R_1=O\ R_2=\beta\text{-OH}\ R_3=OH$

29 12-*oxo*-cinobufagin
$R_1=OH\ R_2=O\ R_3=OAc$

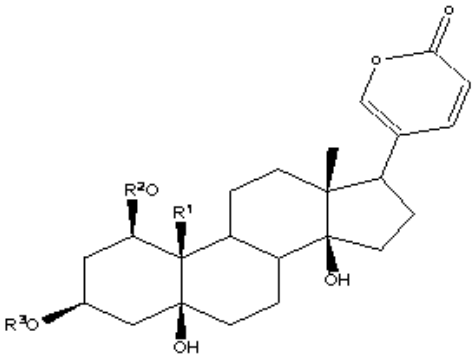
30 3-*oxo*-12α-hydroxyl cinobufagin
$R_1=O\ R_2=\alpha\text{-OH}\ R_3=OAc$



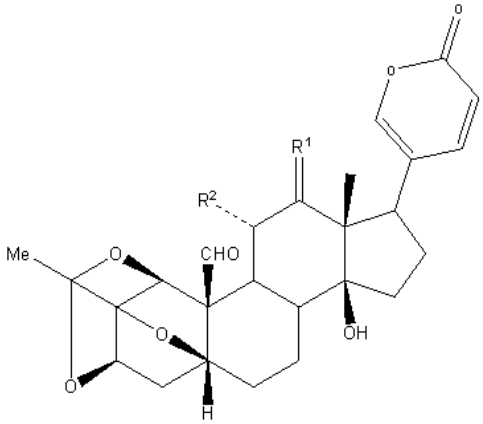
Resibufagenin (31)



Bufotalin (32)



3-O-acetyldaigredorigenin(33)
R^1=Me, R^2=H, R^3=COMe
1-O-acetylbersaldegenin(34)
R^1=CHO, R^2=Ac, R^3=H
3-O-acetylbersaldegenin(35)
R^1=CHO, R^2=H, R^3=COMe



daigremontianin(36)
R^1=O, R^2=OH
bersaldegenin 1,3,5-orthoacetate(37)
R^1=H_2, R^2=H

Structure Activity Relationship

From various studies it was found that subtle changes in functionality of bufadienolides could significantly alter their cytotoxic activities. Biotransformation is an alternative tool in the structural modification of complex natural products due to its great capabilities to catalyze novel reactions and its region and stereo-selectivity [45, 46]. Microorganisms, especially filamentous fungi, are well known as efficient and selective hydroxylation catalysts [47-49].

The biotransformation products (**3**–**14)** obtained is bufalin derivatives hydroxylated at C-1β, C-5, C-7β, C-11β, C-12β, C-15α, C-15β or C-16α positions. All the oxyfunctionalities except 5-hydroxylation are novel for natural bufadienolides, and are obviously difficult to obtain by chemical means [2, 3, 50].

Hydroxylation of bufalin at different sites could remarkably alter the cytotoxic activities. 1β-hydroxylbufalin (**5**) and 12β-hydroxylbufalin (**10**) showed potent cytotoxicities comparable to bufalin. Both compounds are even more active against human gastric cancer BGC-823 cells and human cervical cancer HeLa cells with IC_{50} values of 10^{-8} to 10^{-9} mol/l.

Compounds **3**, **8** and **9** showed a little weaker but still potent cytotoxicities than bufalin. However, hydroxylations at 15α-, 15β- or 16α-positions significantly reduced the activity, and the corresponding compounds **6**, **7**, **12** and **13** exhibited very weak or no cytotoxicities.

The 16-acetoxyl group is essentially important for the activities of cinobufagin derivatives. All deacetylated products (**15**–**18**, **24**, **26** and **28**) have very weak cytotoxicities, and glucosylation of 16-OH does not improve the activity (compounds **20**–**22**). However, the 3-OH glucosylated product (compound **19**), which was obtained as a major biotransformation product by *Catharanthus* cell suspension cultures, is two times more active than cinobufagin against all the four test cancer cell lines.

The 12β-hydroxylation of cinobufagin is a popular reaction by filamentous fungi and slightly reduces the activities. Compound **17** is the only 12α-hydroxylated bufadienolide examined, and is about 10 times more active than its 12β-OH epimer **14**.

Bufalin derivatives are generally more active than corresponding cinobufagin analogues. Thus the 14β, 15β-epoxy ring appears to reduce cytotoxicity.

The in vitro cytotoxicities of 30 bufadienolides suggested that 3-OH glucosylation or hydroxylation at C-1β or C-12β positions might be promising reactions to obtain more polar bufadienolides with enhanced cytotoxic activities. The comprehensive preliminary structure–cytotoxic activity relationship of bufadienolides as illustrated in Fig. 1.

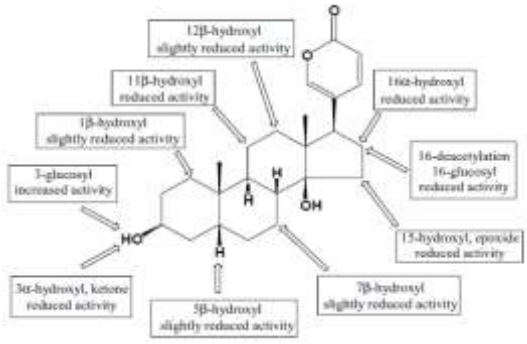


Fig. 1: Effects of structural modifications of bufalin and cinobufagin derivatives on growth inhibition of human cancer cell lines.

The novel oxyfunctionalized derivatives of bufalin obtained in this study could provide new platforms for combinatorial synthesis [51-53] and other further investigations for the development of new bufadienolides antitumor drugs.

Functions

Bufodienolides have the ability to inhibit the adenosine triphosphatase sodium-potassium pump (Na^+-K^+-ATPase), with predilection for its α-1 isoform [54]. This capability enables them to share with other cardiac glycosides the facility to cause an increase in sodium excretion, produce vasoconstriction resulting in hypertension, and act as cardiac inotropes. Bufadienolides have been implicated in instances of volume expansion-mediated hypertension, syndromes in which they are considered capable of causing a vascular leak, interfering with cellular proliferation, and inhibiting cellular maturation [54]. The cytotoxic evaluation showed that all natural bufadienolides and their derivatives exhibited moderate to strong activity against human HL-6, SF-295, MDA-MB-435, and HCT-8 cancer cell strains without hemolysis [54].

Mechanism of action

According to the still most widely accepted mechanism of action for bufadienolides, they act through inhibition of Na^+/K^+-ATPase, thus raising indirectly the intracellular Ca^{2+} concentration. The major ion motive ATPase, in animal cells, is the Na^+, K^+-ATPase or sodium pump. This membrane bound enzyme is responsible for the translocation of Na^+ ions and K^+ ions across the plasma membrane, an active transport mechanism that requires the expenditure of the metabolic energy stored within the ATP molecule [55]. This ubiquitous enzyme controls directly or indirectly many essential cellular functions, such as, cell volume, free calcium concentration and membrane potential. It is, therefore, apparent that alterations in its regulation may play key roles in pathological process. Therapeutic concentrations of bufadienolides produce a moderate enzyme inhibition. When the cell is depolarized, there is a lower amount of enzymes available for the restoration of the Na^+/K^+ balance. The remaining enzymes, non-inhibited, will act faster, because the high Na^+ concentration and the ionic balance must be restored before the following depolarization, although it will take longer than if every enzyme were available. This lag causes a temporary increase of Na^+ concentration reaching higher concentrations than if ATPase activity were not partially inhibited. This temporary increase of $[Na^+]$, modifies $[Ca^{2+}]$ through a Na^+/Ca^{2+} exchanger which allows Na^+ exit from the cell in exchange for Ca^{2+}, or Ca^{2+} exit from the cell in exchange for Na^+ , depending on the prevailing Na^+ and Ca^{2+} electrochemical gradients . This mechanism decreases exchange rate, or even reverses exchanger ion transport, being Ca^{2+} carried into the cell; anyway increasing $[Ca^{2+}]$ and thus increasing contractile force. When the concentration of bufadienolides reaches to toxic levels, enzyme inhibition is too high, thus decreasing Na^+ and K^+ transport to the extent that the restoring of normal levels during diastole is not possible before the next depolarisation. Then, a sustained increase of $[Na^+]$, and thus of $[Ca^{2+}]$, gives rise to toxic effects (i.e. arrhythmia) of these compounds [55].

Pharmacology

From a pharmacological point of view, bufadienolides can act as endogenous steroidal hormones and display a large range of activities related to Na^+/K^+-ATPase enzyme blockage: antiangiogenic, anti-hypertensive, immunosupressor, antiendometriosis, positive inotropic action and a possible association with mood control and ethanol addiction. In a model study, 20S, 21R-epoxy-resibufogenin-3-acetate avoided cancer cachexia by the inhibition of interleucine-6 receptor, with no Na^+/K^+-ATPase inhibition. Bufadienolides and closely related derivatives have been object of several bioassays and the structure-activity relationships studies were related to cardiotonic, antiviral, and Na^+/K^+-ATPase inhibition. Animal, plant and biotransformed bufadienolides are extensively evaluated against a variety of cancer cells, including human leukemia HL-60 and HCT-8 cells. In human breast cancer cellsMDA-MB-231, b-sitosterol promoted apoptosis. Cardiac glycoside digitoxins and their analogs were evaluated against TK-10 renal adenocarcinoma, MCF-7 breast adenocarcinoma, UACC-62 malignant melanoma, and K-562 chronic myelogenous leukemia cell lines. Additionally, some of them showed ability to inhibit the growth of cancer cells at concentrations commonly found in the plasma of patients with cardiac diseases. Digitalis effects on Na^+/K^+ -ATPase from MDA-MB-435s tumor cells suggested that digitoxin and digoxin may have potential therapeutic value for breast cancer treatment. Therefore such steroidal compounds have demonstrated antitumor activities by inducing apoptosis and/or inhibition of cell cycle progression. Bufadienolides also exhibited cytotoxic/anti-proliferative activities against human hematopoietic, pancreas, nasopharnyx, lung, prostate, colon, breast, liver, gastric, melanoma, and renal cancer cells. Furthermore, bufadienolides act as surface anesthetics, antiviral including HIV, anti-proliferative effect, antibacterial, antiparasitic and insecticidal activities.

MEDICINAL IMPORTANCE

Cardiotonic and Kidney diseases

Bufadienolides shows a beneficial effect on congestive heart failure models in rabbits due to its cardiotonic property [40]. The vasodilating effect and positive inotropic action of bufadienolides is due to its beta-adrenergic action [56]. Kyushin (a Japanese medicine containing bufadienolides) significantly inhibits the aconitine-induced and thyroxin induced arrhythmia in guinea pigs. The decrease in heart rate induced by electrical stimulation to the parasympthatic nerve can be restored by Kyushin. Kyushin dose dependently increase the left ventricular pressure and mean aortic pressure and decrease the left ventricular end-diastolic pressure in a dose dependent manner. Bufalin, cinobufagin, and some other bufadienolides like bufotalin, cinobufotalin, gamabufotalin and resibufogenin—all show cardiotonic effect in a concentration dependent manner in guinea pig isolated heart preparations cinobufagin possesses most cardiotonic effect in experimentally induced heart failure due to acute local ischemia[57].The cardiotonic steroids (bufalin, bufotalin, resibufagenin, marinobufagenin) all inhibit Na^+-K^+-ATPase activity [47,58-62].

Immunomodulatory activity

Cinobufagin has been used successfully in high doses in attenuation and treatment of infection and granulocytopenia during combined chemotherapy. In patients with malignant blood disease, after treatment with high dose of cinobufocini, infection was significantly decreased without a significant change in the number of granulocytes before and after the treatment [63]. In experimental animals, bufadienolides significantly increase blood lymphocyte, splenic lymphocyte and macrophages count, strongly suggesting the possible involvement of bufadienolides in first line defense through immunomodulation of lymphoid cell [63-64].

In a study human T-cells were stimulated “in vitro” with mitogens or alloantigens in the presence of bufadienolides. The most active compound totally inhibited T-cell activity at a concentration of 0.75 pmol/105 cells. This effect is 16 384× stronger than that of cortisol and 256× stronger than that of cyclosporin A or tacrolimus. Preactivated T cells were downregulated and, most importantly, suppressed viable T cells could not be restimulated. Lack of the 17β-lactone ring dramatically reduced the activity of bufadienolides. Substitution at C-3 also affected their function: components with a 3-OH group were up to 1000× stronger than those without. The replacement of 14β-OH with an epoxy-group slightly decreased the activity. Because there is evidence that the latter change abolishes the cardiac activity, this finding is relevant for therapeutic applications in which immunosuppression without the risk of cardiotoxicity is attempted. One of the substances analyzed in this study was Proscillaridin A. A similar bufadienolide occurs naturally in mammals. Bufadienolides represent an important bioregulatory link between the cardiovascular, nervous and immune systems.

Anti-neoplastic activity

Bufalin has been shown to have anticancer properties in leukemia as well as melanoma cells. It induces differentiation in human erythroleukemia K562 cells and also produces a strong differentiation-inducing activity in three other human leukemia-derived cell lines HL60, U937, ML1 to monocyte/macrophage like cells [6,67]. Bufalin arrests the growth of ML1 cells preferentially at the G2 phases of the cell cycle [65]. Bufalin induces differentiation of

ML1 cells through the modulation of several protein kinase activities in a distinct way from RA and 1 alpha, 25(OH) 2D3. This effect of bufalin on the cell cycle of leukemia cells is similar to that of topoisomerase inhibitors [8]. Bufalin reduces the level of topoisomerase-2 in human leukemia HL60 cells and also increases the inhibitory effects of anti-cancer drugs like cisplatin and RA on cell growth and enhance the induction of cell death. Na^+-K^+-ATPase inhibition by bufalin initiates the process of K562 cell differentiation [67]. Bufalin or cinobufagin increases the intracellular calcium concentration and apoptosis in prostate cancer cell lines LNCaP, DU145 and PC3 [10]. Bufalin significantly inhibits the cell proliferation and DNA synthesis of cultured ovarian endometriotic cyst stromal cells and induces apoptosis and G0/G1 phase cell cycle arrest of these cells by down-regulation of the cyclin A, Bcl-2 and Bcl-X (L) expression with the simultaneous up-regulation of p21 and Bax expression [68].

Analgesic activity

Amphibian skin secretions are the potential source of many powerful analgesics which also include many of bufogenins and bufotoxins [69]. Bufalin also give analgesic activity. Bufalin exhibited analgesic effects through increase in hepatic blood circulation. Bufadienolides such as proscillaridin A, scilliroside (isolated from bulbs of *Urginea maritima*) also give analgesic activity.

Insecticidal activity

Kalanchoe pinnata exhibited strong insecticidal activities against third instar larvae of silkworm (*Bombyx mori*). Its active compounds are bufadienolide derivatives, bryophyllin A and C (Supratman *et al*, 2000). Another *Kalanchoe* plant that showed insecticidal activity is *Kalanchoe daigremontiana x tubiflora* with daigremontianin, bersaldegenin-1, 3,5 ortoasetat, and methyl daigremonate as its active compounds. *Urginea maritima* bufadienolides (proscillaridin A, scillaren A, scillirosid, gammabufotalin, and scillirosidin) induce anti-insect effects on *Tribolium castaneum* [70].

Antimicrobial, Antileishmanial and Antitrypanosomal activity

Two bufadienolides named telocinobufagin and marinobufagin are active against *Staphylococcus aureus and Escherichia coli* [44]. Bufadienolides also show antileishmanial activity. Telocinobufagin and hellebregenin give antileishmanial activity against *Leishmania (L) chagasi promastigotes* with no activation of nitric oxide production by macrophages. It was found neither cytotoxic against mouse macrophages nor hemolytic. Hellebregenin also give antitrypanosomal activity against *Trypanosoma cruzi trypomastigotes* [71].

Anti-inflammatory Activity

Toad venom is used as an anti inflammatory agent in small doses in China [72]. Bufadienolides are cardioactive steroids responsible for the anti-inflammatory actions of toad venom. Bufadienolides (8 mg/kg) caused arrhythmias, cardiac dysfunction and death in guinea-pigs. Pretreatment with taurine (150, 300 mg/kg) significantly prevented bufadienolide-induced cardiotoxicity and reduced the mortality in vivo. Taurine markedly increased the cumulative doses of bufadienolides and resibufogenin required for lethal arrhythmia in ex vivo isolated guinea-pig heart. Taurine did not compromise the anti-inflammatory activity of the bufadienolides on concanavalin-A stimulated proliferation of guinea-pig splenocytes in vitro. The data indicate that taurine can prevent bufadienolide-induced cardiotoxicity and could be a novel antidote in combination with bufadienolide therapy [72].

Cytotoxicity

The cytotoxic evaluation showed that all natural bufadienolides and their derivatives exhibited moderate to strong activity against human HL-60, SF-295, MDA-MB-435, and HCT-8 cancer cell strains without hemolysis of mouse erythrocytes. The acetylated bufadienolides and the epoxide showed lesser peripheral blood lymphocytes (PBLs) inhibitory activity than their precursors, suggesting that chemical modifications on such compounds can play an important role on the modulation of their cytotoxic profile [73].

CONCLUSION

With regard to naturals, both plants and animals are promising source of bufadienolides: a type of cardiac glycoside widely used traditionally in the treatment of cardiac dysfunction. They also exhibit significant anticancer activities. They also show toxic activity. Future optimization of these compounds through structural alternation may allow the development of pharmacologically acceptable anticancer and antitumor agents with reduced cytotoxicities. In addition to structural modification, investigation on the mechanism of actions of these compounds is likely to be productive area of research. Such information may assist in the optimization of lead compounds activity. Also characterization of the interaction between bufadienolides and their targets with respects to its other medicinal activities could potentially allow the design of new lead compounds.

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