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Structural Features And Biological Activities Of Bufadienolides.

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ABSTRACT

Bufadienolides represent a class of compounds belonging to cardiac glycosides. Bufadienolides are present in various plants and also in animals. In addition, bufadienolides were found in human plasma, eye lens, and placenta. However, it is not clear how they got there.The most abundant source of already described and also of new bufadienolides is a venom from different types and kinds of toads. The mechanism of action of cardiac glycosides is mediated through the inhibition of membrane-bound Na^+-K^+-ATPase pump leading to an increase in intracellular sodium and calcium concentrations.Bufadienolides are structurally steroids and they are defined as a group by a substituent at the carbon number 17 of the steroidal skeleton. Bufadienolides were discovered to be present as a major bioactive constituent in some well-known folk, especially Chinese, medicines, i.e. in Chan'Su and KY preparations. These are (and were) used as remedies for ulcer problems, sore throat, toothache, sinusitis, and cancer for hundreds of years in China and in most eastern Asian countries. However, its use has not been recognized in modern medicine, despite the fact that current scientific investigation has shown that bufadienolides can be therapeutically beneficial in some specific conditions. Some animal studies indicatetheir anti-cancer, anti-bacterial, antiprotozoal and anti-viral activity together with their more less obvious benefits in some cardiovascular conditions. Also, it has been shown that some bufadienolides have insecticidal, analgesic and surface anesthetic activities. At present, many applications of bufadienolides are only potential due to the insufficient information on them, especially in regards to applications to humans. The goal of this literature review is topresentavailable data on bufadienolides, their structure, biological activity and possible interactions with known drugs, their adverse effectsand possibilities for their future medical applications.

Keywords: bufadienolides, cardiac glycosides, cardiotonic glycosides, structural requirements, mechanism of action

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INTRODUCTION

Bufadienolides are steroid substances containing a characteristic unsaturated six-membered lactone ring (2- pyrone substituent) with two double bonds attached at the 17β position of the cyclopentanoperhydrophenanthrene skeleton [1] (Fig. 1). Bufanolides as aglycones form various bufadienolide glycosides acting as cardiac glycosides. All of their aglycones are based on 24 carbon skeleton.

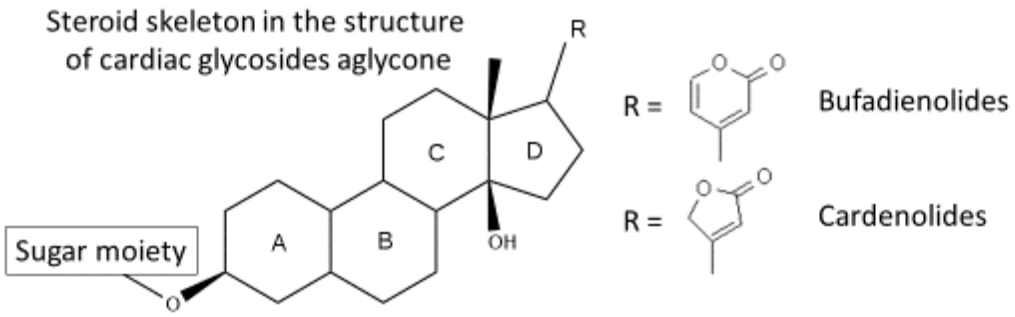

Fig 1: Chemical structure of cardiac glycosides – bufadienolides and cardenolides.

The other important group of cardiac glycosides is formed by cardenolides differing from bufadienolides by a substituent at position 17 of the steroid structure.Bufadienolides contain a "dienolide," a six-membered lactone ring with two double bonds, while cardenolides contain an "enolide," a five-membered lactone ring with a single double bond in their structure. Both bufadienolides and cardenolides are known to affect the cardiac output of the heart. However, some other scientific reports provide information about their other biological and possible therapeutic activities. So far, more attention was directed towardscardenolides (and they are more commonly used medicinally) primarily because of their greater presencein plants traditionally used in western medicine [2].

Cardenolides are known to be present in several plant families. When detected in animals (i.e. butterflies), they are from plants consumed. On the other hand, bufadienolides are found in many animals and plants sources. As even the name of the group suggests,the toad venom is the most important and famous animal source of them.Bufadienolides were discovered also in other animal sources, i.e. in*Photinus* (fireflies) and *Rhabdophis* (snakes). The most abundantly, they are present in six plant families - *Crassulaceae*, *Hyacinthaceae* (*Liliaceae*), *Iridaceae*, *Melianthaceae*, *Ranunculaceae* and *Santalaceae* [1]. Very often, the name of the source for an individual bufadienolide is reflected in the name of isolated bufadienolide. Bufalin, cinobufagin, telocinobufagin, hellebrigenin, resibufagin are some of many examples of this 'tradition'.Bufadienolides as cardiac glycosides possess other than cardiac biological activities, including anti-cancer [3-6], anti-thrombin [7], anti-inflammatory [8,9], antinociceptive [9],antiviral [10], anti-leishmanial and anti-trypanosomal [11] and other activities.

STRUCTURAL REQUIREMENTS FOR BUFADIENOLIDES' BIOLOGICAL ACTIVITY

Aglycones of all bufadienolides contain 24 carbon skeleton. Additionally, all bufadienolides contain basic unsaturated six-membered lactone ring attached at the 17β position of the cyclopentanoperhydrophenanthrene skeleton with A/B rings junction *cis*, B/C rings junction *trans* and C/D rings junction *cis*. The *cis* junction between A/B and C/D rings is an essential requirement for biological activity [1,12,13].

3β-Hydroxyl group presence is essential for glycoside formation. The presence of hydroxyl groups at carbon 5, 11 and 16 is possible as it is possible for carbons number 1, 6, 7, 12 and 15.

It was recorded that introduction of hydroxyls at positions 6, 11 and 15 reduces the cytotoxic activity of bufadienolides. A slight reduction of cytotoxic activity also occurs when a modification at positions 1, 5, 7, and 12 occurs. A hydroxyl group at carbon 14 is not the essential requirement for inotropic activity, however, its removal results in decreased potency [1,12].

Various sugars are present in a glycon part of bufadienolides, such as glucose, rhamnose, cymarose and other. The formation of biologically active glycoside increases absorption and distribution. Replacement of the hydroxyl at the position 3 decreases biological activity as glycoside formation at this site is prevented.

GENERAL CHEMICAL IDENTIFICATION OF BUFADIENOLIDES (& OTHER CARDIAC GLYCOSIDES)

There are many classical non-specific chemical tests for individual structural elements of bufadienolides. These tests are in every pharmacognosy textbook and are not being specifically referenced. Some of them are:

Presence of a lactone ring (Raymond's test, Legal's test, Baljet test):

These tests are all based on the interaction of used chemicals with double bonds of a lactone ringAll of these tests are performed in basic conditions to destabilize a lactone ring.

Raymond's test

A few ml of 50% ethanol and 0.1 ml of 1 % solution of m-dinitrobenzene in ethanol are added to a bufadienolide. Violet color appears after the addition of 2-3 drops of 20% sodium hydroxide due to the presence of double bonds.

Legal's test

A few ml of pyridine and 2drops of nitroprusside and a drop of 20% sodium hydroxide solution are added to the solution of bufadienolide. A deep red color is produced.

Baljet's test:

This test is based on an addition of sodium picrate to a bufadienolide. Addition of sodium hydroxide This results in a yellow to orange color of the solution tested.

Presence of deoxy sugars in glycon part of bufadienolides:

There are several suitable tests. The following example is just illustrative.

Xanthydrol test:

The crude is heated with 0.1 to 5% solution of Xanthydrol in glacial acetic acid containing 1% hydrochloric acid. A red color is produced due to the presence of 2-deoxysugar.

INSTRUMENTAL METHODS IN IDENTIFICATION OF BUFADIENOLIDES

Modern separation methods paired usually with a mass spectrometer are essential in the modern analysis of natural (and synthetic) bufadienolides. Currently, there is an abundance of scientific reports on the application of these methods.

INSTRUMENTAL METHODS IN IDENTIFICATION OF BUFADIENOLIDES

Modern separation methods paired usually with a mass spectrometer are essential in the modern analysis of natural (and synthetic) bufadienolides. Currently, there is an abundance of scientific reports on the application of these methods. Interesting examples of mass spectrometry (MS) application for the study of bufadienolides are references shown [12,13]. MS used after powerful separation techniques of liquid chromatography (LC) in the form of, high-performance LC (HPLC) [14-16] or even of ultra-high pressure LC (UHPLC or UPLC) [15,17,18] not only provides the information regarding bufadienolide identification but is also used for isolating individual compounds.

MECHANISM OF ACTIONOF BUFADIENOLIDES

When a bufadienolide is ingested bya human, 50% of the compound absorbs passively at the intestine and subsequently bounds to Na^+-K^+-ATPase pump subunits in the kidneys, skeletal muscle and mainly in the heart [19]. The powerful ionotropic effect of bufadienolides on the cardiac muscle is due to the inhibition of this membrane-bound Na^+-K^+-ATPase pump as it controls multiple essential cellular functions. It maintains the electrical membrane potential necessary for excitability, nerve transmission, and muscle contraction. Some other cellular functions also depend on the sodium-potassium gradients. Na^+-K^+-ATPase pumpconsists of two polypeptides: α catalytic subunit and the glycosylated β glycoprotein subunit, which regulates the activity of the pump [19]. The α subunit contains the binding sites for Na^+ and cardiotonic steroids on the extracellular segments and the binding sites for K^+ and ATP on the intracellular loops [1]. The pump is acting through pumping sodium ion outside the cell membrane while the potassium ion is pumped inside of the cell.Both ion currents go against existing concentration gradients in an energy-dependent process [20]. Inhibition of Na^+-K^+-ATPase pump by cardiac glycosides results in an increase of sodium concentrations inside the cell.Consequently, this results in reversing the action of calcium and sodium exchanger. After that, an increased intracellular calcium concentration leads to a more intensive binding of calcium ions to troponin, actin and myosin [19]. As a result of high Ca^+ and Na^+ concentrations, contractility of the heart muscle increases, the heart requires less oxygen and less energy for its activity. Thus, this mechanism benefits patients suffering from heart failure.

ENDOGENOUS BUFADIENOLIDES

Endogenous cardiotonic steroids, also called digitalis-like factors, play an important role in health, especially in regulation of renal sodium transport and arterial pressure. Recent discoveries including specific endogenous cardenolides (glycoside ouabain, Fig. 2A) and bufadienolide (aglycone marinobufagenin, Fig. 2B) in humans implicate these substances in the regulation of cell growth, differentiation, apoptosis, and carbohydrate metabolism [1]. Moreover, these substances control various central nervous functions and behavior [21,22]. It is noteworthy to mention that amphibians, which migrate from a dry to an aquatic environment, are known to produce cardiotonic steroids of bufadienolide nature. The highest levels of bufadienolides were detected in the skin of these species. These bufadienolides are responsible for the regulation of water and electrolyte homeostasis in the amphibian's body. In addition, some researchers detected bufalin-immunoreactive material in human bile and plasma [23]. A substance called marinobufagenin (Fig. 2C) with a vasoconstrictor activity, sodium pump-inhibitory activity and positive inotropic effects was isolated from mammalian plasma and urine-based NMR data [24].Other bufadienolides that have also been found in human plasma are bufalin and telocinobufagin (Fig. 2C &2D) [25].Telocinobufagin is the reduced form of marinobufagenin. On the other hand, some studies performed in normotensive rats have shown that plasma marinobufagenin increases in response to acute plasma volume expansion after chronic administration of a high NaCl diet. Also, in patients with pre-eclampsia, essential hypertension, primary aldosteronism and end-stage renal disease, the levels of marinobufagenin are increased [26]. Moreover, it has been observed that immunoneutralization of marinobufageninwith the administration of a specific antibody in vivo reduces blood pressure and renal sodium excretion in NaCl-loaded Dahl salt-sensitive (Dahl-S) rats. This is the most important phenomenon that helped researchers to predict the existence of an endogenous vasoconstrictor and natriuretic substance [27]. It has been observed, in both acute and chronic NaCl loading in Dahl-S rats that a transient increase in circulating endogenous ouabain precedes a sustained increase in circulating marinobufagenin. This indicates that endogenous ouabainacts as a neurohormone that triggers marinobufagenin production. Secretion of marinobufagenin results in peripheral vasoconstriction, natriuresis and an increase in cardiac contractility [19]. On the other hand, it is important to mention that the biosynthesis of endogenous cardiotonic steroids, including bufadienolides, is poorly understood. It is known that both classes of cardiac glycosides can be synthesized in the adrenal cortex and that cholesterol serves as a precursor for the synthesis of endogenous bufadienolides and cardenolides despite the fact that the biosynthetic pathways for these two groups differ [19].

EXAMPLES OF SELECTED BUFADIENOLIDES ORIGINATING FROM DIFFERENT SOURCES

Formulas of bufadienolides mentioned are from PubChem, US National Library of Medicine, National Institutes of Health, USA [28].

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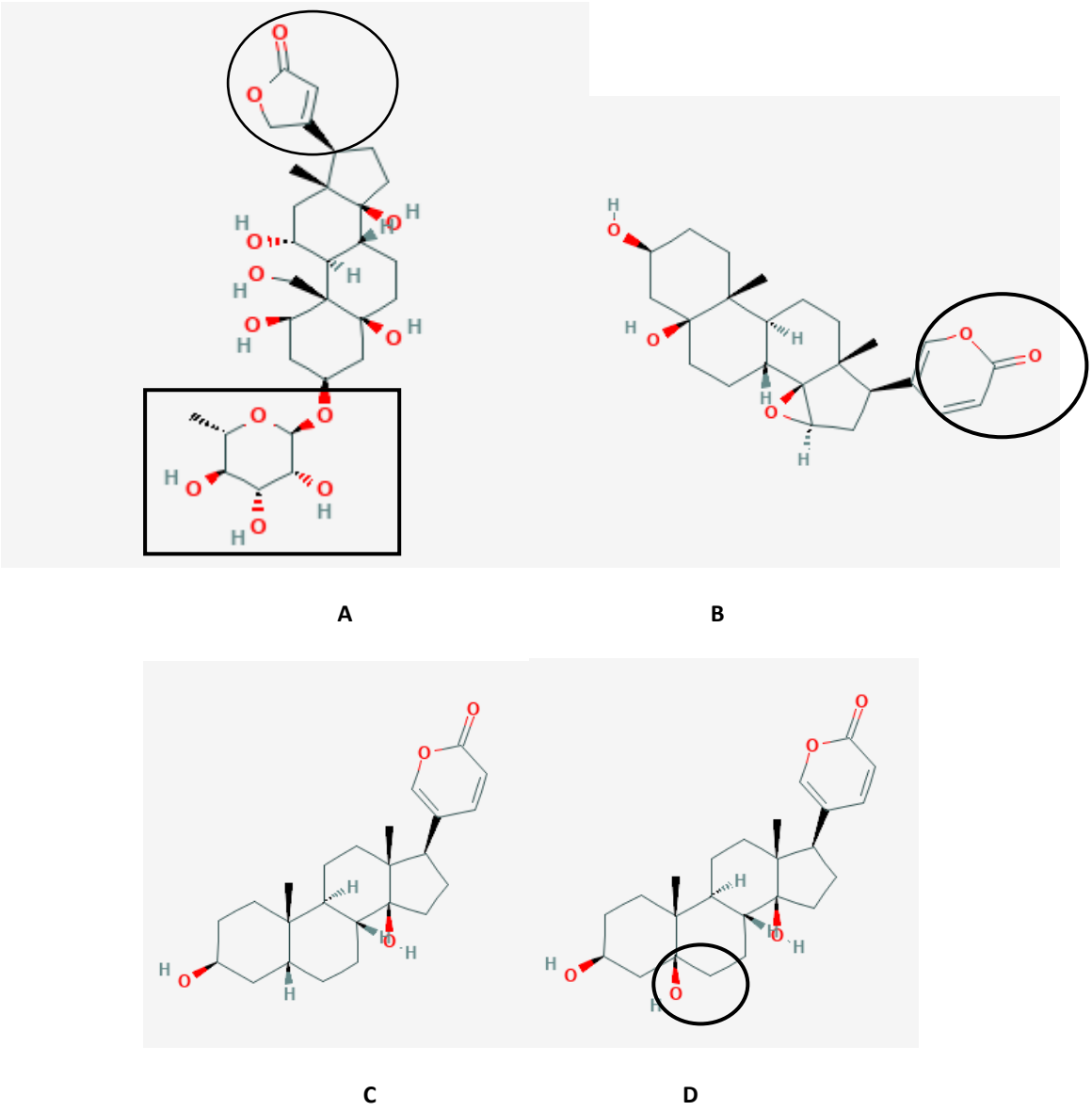

Fig 2: Chemical structure of ouabain (A), marinobufagenin (B), bufalin (C) and telocinobufagin (D)

Bufalin

Bufalin (Fig. 2C) is one of the cytotoxic bufadienolides. Hydroxylation of the structure of bufalin at different position altersthe cytotoxic activity of the parent molecule. 1β-hydroxylbufalin and 12β-hydroxylbufalin are more cytotoxic in comparison to bufalin. Bufalin has a summary chemical formula of $C_{24}H_{32}O_6$ of the molecular weight 416.514 g/mol. IUPAC name of bufalin is 5-[(3S,5R,8R,9S,10S,11S,13R,14S,17R)-3,11,14-trihydroxy-10,13-dimethyl-12-oxo-2,3,4,5,6,7,8,9,11,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl]pyran-2-one[4,10].

Telocinobufagin

Telocinobufagin (Fig. 2D) has a molecular weight of 402.531 g/mol and its chemical formula is $C_{24}H_{34}O$. The IUPAC name for telocinobufagin is5-[(3S,5S,8R,9S,10R,13R,14S,17R)-3,5,14-trihydroxy-10,13-dimethyl-2,3,4,6,7,8,9,11,12,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl] pyran-2-one and it has 37 synonyms [11,12]. It was isolated from skin secrets of Brazilian toad *Buforubescens*[24]. It exhibits anti-microbial activity against *Staphylococcus aureus* and *Escherichia coli*. Telocinobufagin is related to marinobufagin but it has a hydroxyl group at the carbon 14 of its steroidal system whereas the marinobufagin

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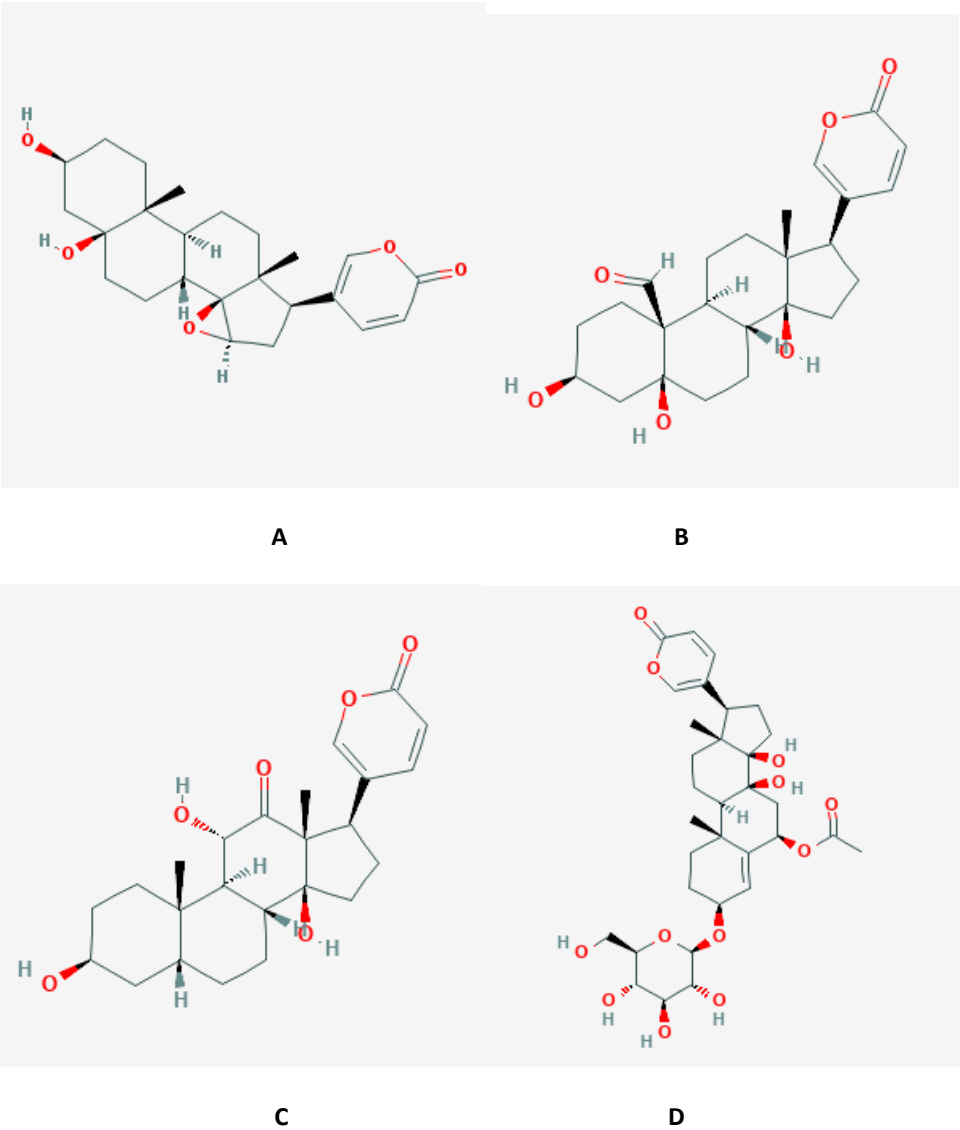
contains an epoxy groupon carbons 14 and 15 (Fig. 3A). It was hypothesized that telocinobufagin may be a natural precursor of marinobufagin (Fig. 3A) [24].



A **B**

C **D**

Fig 3: Examples of selected bufadienolides originating from different sources: marinobufagin (A), hellebrigenin or bufotalidin or gellebrigenin (B), arenobufagin (C) and scilliroside (D).

Hellebrigenin (Bufotalidin)

Hellebrigenin, also known as bufotalidin or gellebrigenin (Fig 3B) is an aglycone of glycoside hellebrine. Hellebrigenin was extracted from the plant *Helleborus niger*, some other plants but is also found in various secrets of many toad [11,29,30]. Hellebrigenin is of the chemical formula $C_{24}H_{32}O_6$ and its molecular weight is 416.514 g/mol. The IUPAC name of hellebrigenin is (3S,5S,8R,9S,10S,13R,14S,17R)-3,5,14-trihydroxy-13-methyl-17-(6-oxopyran-3-yl)-2,3,4,6,7,8,9,11,12,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-10-carbaldehyde or 19-oxo-3-beta,5,14-trihydroxy-5-beta-bufa-20,22-dienolide.

Arenobufagin

Arenobufagin (Fig. 3C) was first reported to isolated from the venom of the *Bufo arenarum* toads[31]. Its chemical formula is $C_{24}H_{32}O_6$ and molecular weight is 416.51 g/mol. The IUPAC name of arenobufagin is 5-[(3S,5R,8R,9S,10S,11S,13R,14S,17R)-3,11,14-trihydroxy-10,13-dimethyl-12-oxo-2,3,4,5,6,7,8,9,11,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl]- pyran-2-one.

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BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF BUFADIENOLIDES

Old Egyptians are thought to be the first civilization that used a bufadienolide-containing plant called squill for the treatment of heart diseases. Nowadays, some Asian countries are still using different toad venoms for the preparation of the remedy Chan'Su [11].Chan'Su is a traditional Chinese medicine and it is widely used in China, Japan, Korea, and other Asian countries [21]. This medicine is derived from the skin and parotid venom glands of the toad. The major active components in this Chinese medicine are bufadienolides, such as cinobufagin, resibufogenin, and bufalin [32]. Cinobufagin and bufalin demonstrate digoxin-like immunoreactive activity in serum due to high similarity in structures [20]. Cardioactive bufadienolides exhibit many biological as well as pharmacological activities,including cardiotonic, anesthetic, antineoplastic, antimicrobial and antiviral activity. The therapeutic activity of cardiac glycosides depends mainly on the structure of the aglycone. The type of sugar units attached to the steroidal system at theposition C3 is responsible for modifying potency and duration of action of these compounds [11,33,34].

Surface anesthetic activityof bufadienolides

Ten bufadienolides were investigated to elucidate the relationship between their chemical structure and surface anesthetic activityusing the cornea of guinea pigs [35]. Cocaine hydrochloride served as a control substance in this study.It was determined that all of these drugs were more effective than cocaine, except marinobufagin and resibufogenin. These two compounds did not have any surface anesthetic activity. Bufalin possesses the highest surface anesthetic activity followed by telocinobufagin and other substances:

Bufalin>telocinobufagin>bufotalin>cinobufagin>cinobufotalin>desacetylbufotalin ≥ desacetylcinobufagin ≥ desacetylcinobufotalin [35]. Obtained results highlighted the importance of acetylation for this type of activity as deacetylation leads to diminished surface anesthetic activity [35].

Other studies have also shown that bufotalin, bufalin and cinobufagin possess local anesthetic activity, bufalin being the most potent substance.Its potency and duration of action are higher than with cocaine. Cinobufagin solution was shown to induce numbness of the tonguewhen administered locally. The presence of unsaturated lactone ring with a hydroxyl group in the C3 position of the steroid ring seems to be responsible for the local anesthetic activity [36]. In addition, topical administration of high concentration of Chan'Su also exhibits strong anesthetic effects. This activity is due to disrupting Na^+ and K^+ gradients of the neurons after inhibition of Na^+/K^+-ATPase.This leads to over-excitation of the peripheral nerves followed by paralysis of the neurons responsible for pain signals transmission [21].

Analgesic and anti-inflammatoryactivities of bufadienolides

Bufalin is a representative bufadienolide substance possessing significant analgesic and anti-inflammatory activities as shown inrodents.Bufalin attracts attention as it is a component of Chan'Su preparation. It was demonstrated that this substance decreases carrageenan-induced paw edema at concentrations of 0.3 and 0.6 mg/kg administered intraperitoneally [37]. Additionally, bufalin affects the expression of nitric oxide synthase (iNOS), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), interleukin-1β (IL-1β) and of tumor necrosis factor-α (TNF-α). In the hot-plate test, bufalin increases reaction latencies. Bufalin and Naloxone interaction in these test suggest their interaction with the opioid system[37]. Bufalin was also show to decrease sensitivity to acetylcholine at the motor end-plate by causing prolonged depolarization [38]. Bufalin is not alone among bufadienolides possessing analgesic activity. Other bufadienolides, such as proscillaridinA and scilliroside (Fig. 3D) were also reported to possess analgesic activity [39].

Insecticidal activity of bufadienolides

Several bufadienolides were tested for their insecticidal activity.Proscillaridin A, scillaren A, scilliroside, gammabufotalin and scillirosidin obtained from *Urginea maritima* (L.) Baker, family *Asparagaceae*. Obtained results indicated that proscillaridinA, scilliroside and scillirosidin were active against larvae. Scillirosidin was the most lethal aglycone bufadienolide as it caused over 50% mortality in *Tribolium* larvae at a minimum dose of 10 µg/larva. Scillaren A and gammabufotalin were the least toxic among the bufadienolides tested. The LD_{50}value of scilliroside was determined to be 25.5 µg/insect after one and seven days [40].

Antiplasmodial activity of bufadienolides was demonstrated on substances from the toad venoms of *Rhinellamarina* and *Rhaebo guttatus*. Telocinobufagin (Fig. 2D) was found to be part of the original toads' secrets. Whole secrets and telocinobufagin alone demonstrated pronounced lethal effects on chloroquine-resistant *Plasmodium falciparum* [41]. Structure-insecticidalactivity relationship studies shown that both orthoacetate and alpha-pyronemoieties are essential structural features necessary for insecticidalactivity. Additionally, oxygenated substituents in the ring C increases the insecticidalactivity against silkworm instar larvae *(Bombyx mori*) [42].

Bryophyllin A (bryotoxin C) and bryophyllin Cisolated from the leaves of *Kalanchoe pinnata*(Lam.) Pers. (or *Bryophyllum pinnatum* (Lam.)Okenby another name) also demonstrated stronginsecticidalactivity in the model system of instar larvae of the silkworm. Their LD_{50}values were established to be 3 and 5 µg/g of diet [43].

Anti-viral activity

The discovery that cardiac glycosides may possess antiviral, especially anti-HIV, activity brought an interest in their antiviral properties. It was established that that majority of anti-HIV- active cardiac glycosides belong to cardenolides (e.g. digoxin). On the other hand, bufalin represents bufadienolides with anti-HIV properties. Based on these reported data, all cardiac glycosides are of anti-HIV interest with a potential for development of an antiretroviral substance for future clinical use[44].

Cardiac glycosides, specifically bufadienolides, were tested for their activity against some other viruses and many were found to display inhibitory activity *in vitro*.A preparation from the skin of *Bufo bufo gargarizans* Cantor, Bufonidae, is traditionally used in Chinese medicine. When tested for its anti-hepatitis B activity, the preparation huachansu (or cinobufacini), and also its components bufalin and cinobufagin demonstrated activityagainst hepatitis B virological markers. Cinobufacini was more potent than the positive control lamivudine (100 µg/ml) [45]. The activity of bufadienolides against papillomaviruses was also reported [46].

Antimicrobial activity

As skin secrets of amphibians and many plants contain a large number of biologically active bufadienolides, some of them possess antimicrobial activity.*Drimia robusta* Baker, Hyacinthaceae, is a bulbous medicinal plant from South African. It produces a bufadienolide -proscillaridin A that is active against both gram-positive bacteria (*Staphylococcus aureus*or*Enterococcus faecalis*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) [47]. Other bufadienolides exhibiting inhibitory action in*Staphylococcus aureus* and *Escherichia coli* strains are telocinobufagin and marinobufagin isolated from skin secretions of the Brazilian toad *Bufo rubescens* [24,48]. Additionally, whenthe methanolic root extract of *Helleborus bocconei* Ten. subsp. *siculus* and its bufadienolide fraction were evaluated for its antimicrobial activity, it showed antibacterial activity against microorganisms responsible for respiratory infections, such as *Moraxella catarrhalis* and *Streptococcus pneumoniae* [49].

Antihypertensive activity

Two major pathophysiologic processes involved in the development of high blood pressure. They are expanded extracellular fluid volume and vasoconstriction. It is known the majority of bufadienolides would induce hypertension. Marinobufagenin is one of such bufadienolides. However, structurally-related to marinobufagenin, resibufogenin, that possess an extra hydroxyl at the 5β position,does not cause vasoconstriction with consequent elevated blood pressure. Both resibufogenin and marinobufagenininhibit Na +/ K + -ATPase. However, the reason for the antagonistic behavior of these bufadienolides was not elucidated but the administration of resibufogenin did not affect blood pressure and reduced the blood pressure in experimental rats with volume extension mediated hypertension after marinobufagenin administration [50,51].

Bufadienolides and cancer

Anticancer activity is one of the activities that are very often investigated in bufadienolides as they represent are the major pharmacologic constituents of traditional Chinese medicine Chan'Su, which is frequently used clinically for cancer treatment in China [51].Cardiovascular side-effects in cancer therapy are a limiting factor in developing bufadienolides as chemotherapeutic agents [52]. One of the newer strategies for circumventing this cardiotoxicity is a formation of the fibroblast activation protein α (FAPα) activated tripeptide arenobufagin prodrugs [53] that are a successful example of various bufadienolide prodrugs utilized in the search for cancer agents. The other strategy in discovering new bufadienolide-based anticancer agents is based on studies on less known sources of these molecules and on an identification of new bufadienolides [54]. As more information regarding anticancer activity of various bufadienolides is accumulated, reports on a more specific anticancer mechanism in this group appear: These are, for example finding regarding gamabufotalin and arenobufagin possessing selective cytotoxic activity against tumor cells rather than normal cells [55].A dose-dependent lactate dehydrogenase release, a well-known indicator of necrosis, was observed in cancer cells treated with gamabufotalin. This suggests that gamabufotalin-mediated cell death is predominantly associated with a necrosis-like phenotype. Additionally, treatment with as little as 8 ng/ml of gamabufotalin, efficiently downregulated the percentages of CD4+CD25+Foxp3+ regulator T (Treg) cells in mitogen-activated peripheral blood mononuclear cells [55,56]. Bufadienolides were recently also reported to inhibit cellular proteasome [57], to affect ER2 overexpression in breast cancercells [58] and to intercalate with DNA with consequent G2 cell cycle arrest [59]. Definitely, additional investigations bufadienolides will elucidate other anticancer mechanism or effects.

INTERACTIONS OF BUFADIENOLIDES WITH OTHER DRUGS

Because of the limited data about bufadienolides, there is even less information about their interactions with clinically used drugs. However, some information was obtained on interaction with drugs affecting the cardiovascular system. For example, verapamil was shown tointeract with preparations containing bufadienolides [60] as its co-administration let to anincrease in the uptake of bufadienolides by the heart tissues and worsened the heart block and bradycardia. This information should be taken into consideration as the protective effects of verapamil against bufadienolide intoxication may be decreased or generally compromised [60]. Additionally, it was shown that verapamil reduces serum-binding rates of arenobufagin, consequently increasing bufadienolide concentrations in the blood [61].

Investigations of bufalin revealed that itdoes not damage human normal cervical cells at the low concentration (<20nM) but increases the efficacy of tumor chemotherapy by paclitaxel. It is being suggested that bufalin may be developed as a potential anticancer drug for gynecological cancers [62].

NOTABLE KNOWN ADVERSE EFFECTS OF BUFADIENOLIDES

Bufadienolides have displayed pharmacological and toxic effects on the peripheral and central nervous system. The source of these neurotoxic bufadienolides were plants from *Crassulaceae* family. These caused a unique chronic form of cardiac glycoside poisoning in sheep, a condition known as krimpsiekte. Krimpsiekte is characterized by tremors, paresis and recumbence [63-65]. Moreover, poisoning due to ingestion of the high dose of medication containing toad venom (Chan'Su) may also cause shortness of breath, breathlessness, seizure, coma and cardiac arrhythmia. Cardiac arrhythmias may occur by modifying the functions of cardiomyocytes and Purkinje fibers of the heart. In addition, high concentrations of cinobufagin and resibufogenin - two of the major effective bufadienolides in Chan'Su, induced irreversible over-excitation of mitral cells resulting seizures. [21,66].

CONCLUSION

In summary, bufadienolides are an important class of cardiac glycosides. They are defined as compounds that are structurally similar to cardenolide core but differ bysome specific substituents. The base of their chemical structure is a steroidal aglycone capable of forming glycosideswith various sugar molecules. Bufadienolides are widely used in China and Japan as traditional medicines. However, the scarcity of their sources and limited knowledge of their therapeutic potential together with their side effects and drug

interactions require future investigations before they are included among standard medicines. On the other hand, these investigationswill definitely bring interesting results.

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