Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(6):24-35
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Annonaceous Acetogenins as a new anticancer agent

Wesam S. Qayed, Ahmed S. Aboraia, Hamdy M. Abdel-Rahman and Adel F. Youssef

Department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt

---

ABSTRACT

In spite of the many advances in modern scientific medicine, plant-based traditional medicine still plays an important role in the health care. The acetogenins of Annonaceae are known by their potent cytotoxic activity. In fact, they are promising candidates as a new future generation of antitumoral drugs to fight against the current chemiotherapic resistant tumors. The present review will describe the source and chemistry of Annonaceousacetogenins, their diverse biological activities and their anticancer mechanism of action with highlighting on their toxicity and previously reported total synthesis.

Keywords: Annonaceous acetogenins, Annonacin, antitumor agents, cytotoxic agent, HIF-1 inhibitors, Natural products.

---

1. Natural product as a source of new drugs:

Natural products (NPs) and their synthetic derivatives have been widely used in drug discovery because of their structural diversity and their highly selective and specific biological activities [1-3]. Numerous NPs, semi-synthetic NPs and modified NPs compounds are undergoing clinical testing [4]. Worldwide, between 2005 and 2010, nineteen new NP-based drugs were approved for marketing [5]. Cragg and Newman continuously present updates on NP drugs based on traditional medicine both as pharmaceutical agents and/or as leads for bioactive molecules against serious diseases [1, 2, 6]. It is anticipated that nature will continue to be a major source of new structural leads, and effective drug development will depend on multidisciplinary collaborations. A wide number of studies, including in *vitro*, in *vivo* and enzymatic experiments, have reported effective activity in NP extracts and have isolated compounds from them[7-9].

There are a significant number of NPs drugs in development. It is reported that 100 NPs and NPs-derived compounds and Antibody Drug Conjugates (ADCs) with a NPs-derived cytotoxic component being evaluated in clinical trials or in registration at the end of 2013.Thirty eight of these NPscompounds and thirty three ADCs are being investigated as potential oncology treatments, twenty six as anti-infectives, nineteen for the treatment of cardiovascular and metabolic diseases, eleven for inflammatory and related diseases and six for neurology, **Figure 1[1, 4, 10]**

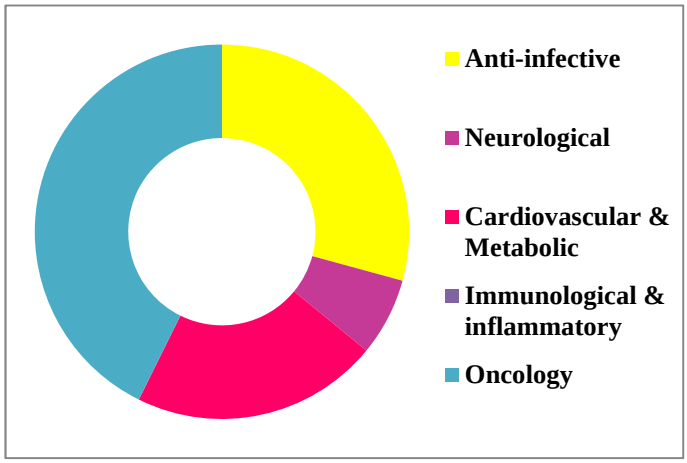


Figure 1.Classificationofthe100NPs-derivedcompoundsinclinicaldevelopmentbytherapeuticarea at the end of 2013.

It is estimated that well over 50% of cancer patients use at least one or more complementary and alternative medicine (CAM) therapies as part of their treatment[11]. Among the alternative medicines, products (e.g., PawPaw Cell-Reg, Graviola Max, Royal Graviola, Graviola Liquid Extract) that contain twig extracts of paw paw (*Asiminatriloba*) or Brazilian paw paw (*Annonamuricata*, graviola or soursop) are reported to have exhibited antitumor efficacy both in animal models and in a limited number of clinical studies. However, the lack of rigorously controlled clinical trials has cast a shadow on the observation that the administration of paw paw capsules rich in acetogenins decreased tumor size, reduced tumor blood flow, suppressed metastasis, and improved survival in cancer patients[12, 13].

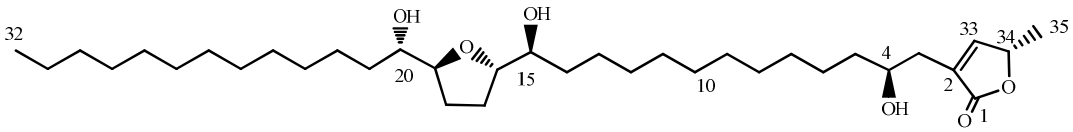
2. Acetogenins

2.1. Annonaceous Plants

The *Annonaceae*, or custard-apple, family consists of many different genera including *Annona* and *Asimina*, each containing several species.*Asiminatriloba* (paw paw), **Figure 2**, is the only temperate species; the rest of the family is tropical or subtropical.TheUnited States Department of agriculture provides a complete list of the 205 Annonaceae genera on the Germplasm Resources Information Network (GRIN, http://www.ars-grin.gov/cgi-bin/npgs/html/exgnlist.pl) including *Annona* and*Asimina*individual species taxonomy.

The fruits of pawpaw have nourished wild animals and mankind in Eastern North America for thousands of years, and Paw pawfestivals are to be found in September throughout the Midwestern United States. Many of the tropical species bear edible fruits and have been naturalized from central and South America to other warm climates in Asia and Africa. The latest articles[14, 15] has summarized the economic potential of the annonaceous fruits and noted that certain parts of several species are poisonous and/or pesticidal.*Soursop* (graviola, guanabana) and *Cherimolia*, **Figure3(a& b)**are two of the best known annonaceous fruits and are sold either fresh or in processed forms; a much amount of the seeds of these commercial species, with their rich concentrations of acetogenins, are discarded during the processing.[16, 17]

Annonacin**(1a)** has been reported to be present in the bark and seeds of *Asiminatriloba* (paw paw, prairie banana, poor man's banana, Ozark banana, Banango, also commonly referred to as its native states ''banana'', e.g. Indiana/Hoosier banana, Kentucky banana, etc.) which grows throughout the Eastern United States and is used as a potential alternative cash crop to tobacco. Furthermore, there is **(1a)**containing commercial supplement made from paw pawtwig extracts, Paw-Paw Cell-Reg TM, that is marketed as beneficial for overall health (www.naturessunshine.com) and as a safe complement to cancer therapy.[18]



(1a) 10-H **(1b) 10- CO** **(1c) 2, 33-dihydro**

Figure 2*: Asiminatriloba* (paw paw) tree and fruit

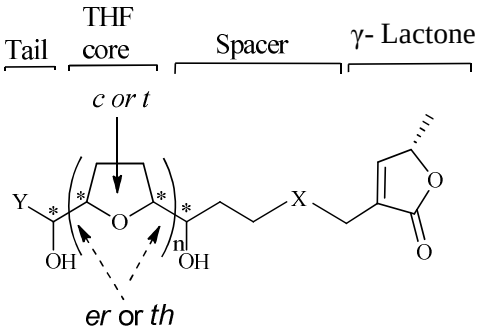
Figure 3a:*Graviola* fruit Figure 3b:*Cherimolia* fruit

2.2. Structures and nomenclature ofacetogenins:

Natural compounds, known as Annonaceousacetogenins (ACGs), are considered the active ingredients in paw paw products. Found only in Annonaceae species, the ACGs are derivatives of fatty acids which are combined with an alkanolmoiety at the 2-position of2,4-disubstituted γ-lactone[19]. Usually, one, two or three THF ringsoccur in the middle of the long hydrocarbon chain, whichoften contains a number of oxygenated moieties (hydroxyl, acetoxy, carbonyl, epoxide) and/or a double bond, **Figure 4**.All of the acetogenins have multiple stereocenters, andtheir relative and absolute configuration can be determined**.** [20]

The ACGs was classified according to the characteristic number of their oxygen-containing groups appeared on a long chain fatty acids moiety. Linear ACGs, epoxy ACGs, mono-tetrahydrofuran ACGs and bis-tetrahydrofuran ACGs can be found.[21]

The ACGscan be also classified according to the number and arrangement of the tetrahydrofuran rings within the moleculeinto subgroups asmono-THF, adjacent bis-THF, nonadjacent bis-THF,non-THF or tetrahydropyran (THP), nonadjacent THFand THP, and adjacent tris-THF ring compounds, according to the number and arrangement of the THF rings. Thechemical structures of the ACGs areshown in **Figure 5**. Mono-THF compounds are the most popular components of ACGs. Some of them have two flanking hydroxyls, **Figure 5**, **A**, while others have only one.Adjacent bis-THF ring compounds are the second largestgroup. These ACGs are characterized by carrying variable number of hydroxylgroups or carbons, placement of the bis-THF rings andhydroxyl groups on the chain, the stereochemistryaround the bis-THF rings, the type of terminal γ-lactone and/or the existence of some specific functionalgroups, **Figure 5, B**. Nonadjacent bis-THF acetogeninscontain two nonadjacent THF rings which are usually separated by a four-carbon chain; one THF ring is flankedby two hydroxyl groups while the other THF ring has onlyone adjacent hydroxyl group which is positioned betweenthe two THF rings, **Figure 5, B**. Mucocin**(2)** is a typical nonadjacent THF and THP acetogenin, and goniocin**(3)** is an example of adjacent tris-THF acetogenin**.** They contain a methylated α-β-unsaturated γ-lactone at one end, but do not have THF or THPrings. Diepomuricanin**(4)**is an example of a non-THF/non-THP acetogenin, **Figure 5,C.**[22]



n=1-3
X and Y= Hydrocarbon chains having oxygenated moieties and/or double bonds
c = cis,t = trans,er = erthro,th = threro

Figure 4 .Representative structure of the ACGs

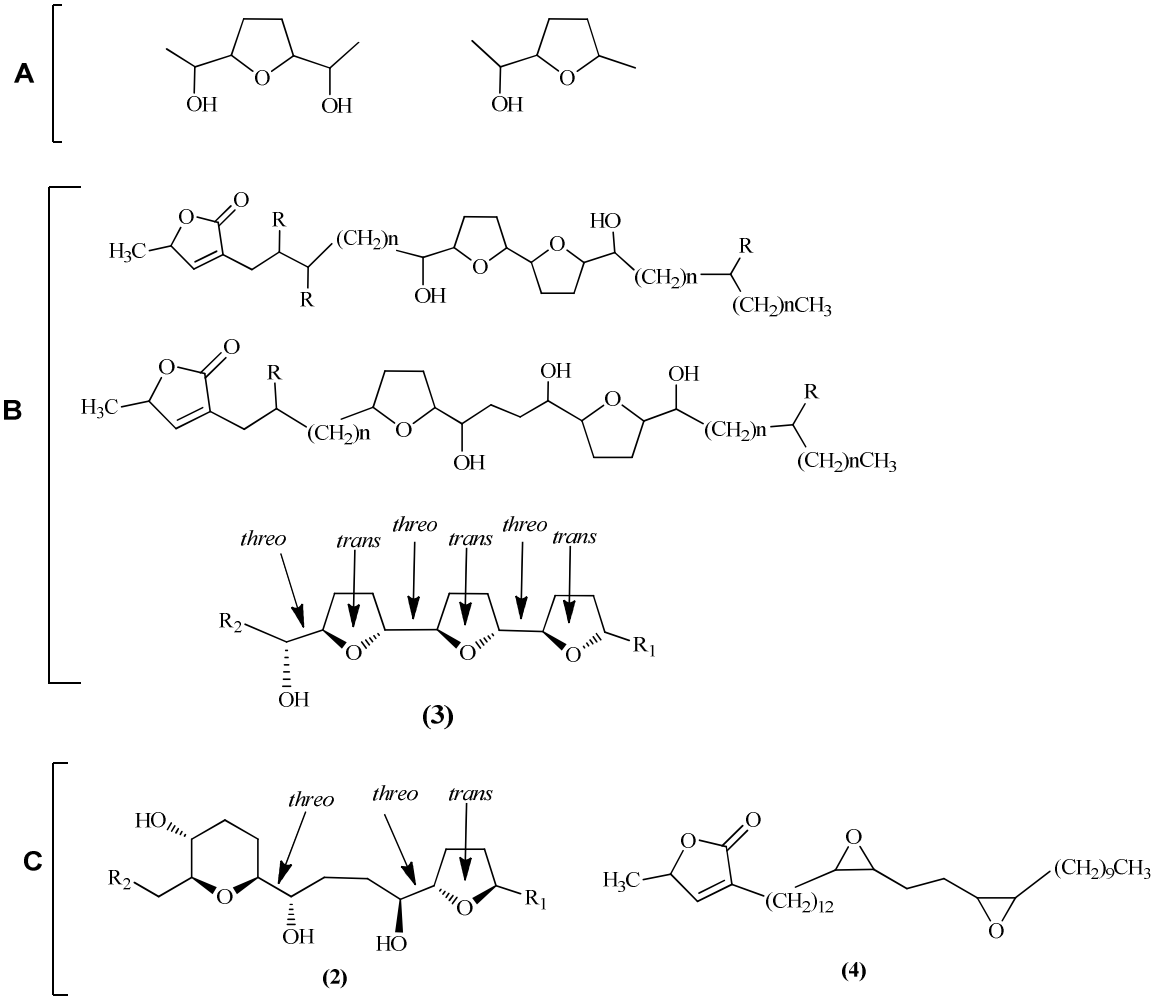
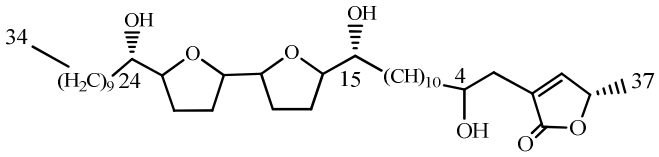


Figure 5. Chemical structure of ACGs

2.3. Acetogenins biological activity

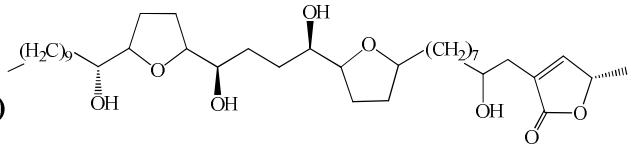
Quantitative evaluation of the published data on cytotoxic activity of these compounds has led to information on the structure-activity relationships. Generally, the data indicate that the most potent cytotoxic compounds possess an adjacent bis-THF ring subunit; the nonadjacent bis-THF ring acetogenins show less cytotoxicity. Mono-THF acetogenins are, in turn, more potent than the non-ring compounds. Acetogenins with different configurations usually show different selectivities or potencies in bioassays. Thus, the importance of determining their relative and absolute configuration is obvious. It should also be pointed out that chlorination decreases bioactivity but indicates some cytotoxic selectivity (**Table 1 shows** a brief list of bioactive ACGs)[23]

Asimicin (*th/t/th/t/th*) **(5)**
Bullatacin (*th/t/th/t/er*) **(6)**
Rilobacin (*th/t/er/c/th*) **(7)**

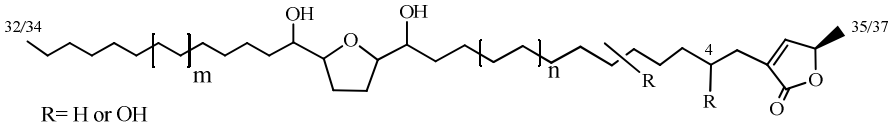


Bullatalicin (8a)

2,35-Dihydrobullatalicin (8b)



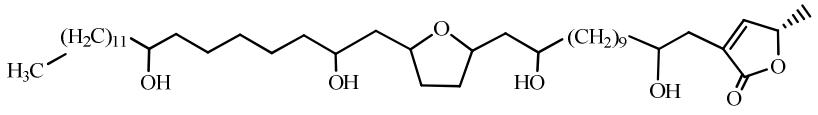
(9-11)

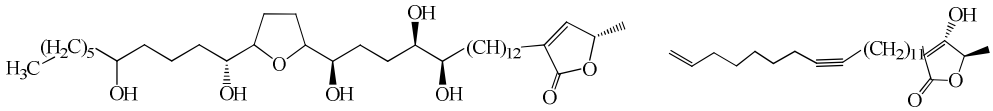


R= H or OH

Comp. No	Compound	OH-positions	n	m	THF-relative configuration	Molecular formula
(9)	Asitrocin	4,12, 15, 20	1	1	*th/t/er*	$C_{35}H_{64}O_7$
(10)	Asitrilobin-C	4, 15, 17, 22	2	1	*th/t/th*	$C_{37}H_{68}O_7$
(11)	Asitrilobin-D	10, 17, 19, 24	3	0	*th/t/th*	$C_{37}H_{68}O_7$

(12)





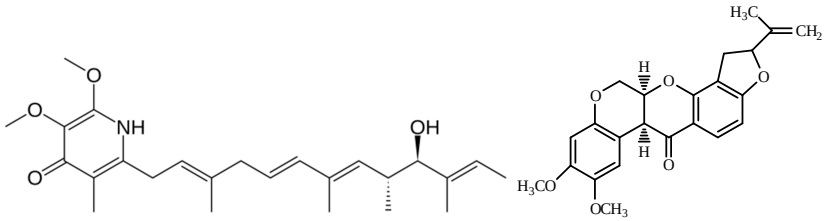
(13) **(14)**

Table 1: Examples of biologically active ACGs as complex I inhibitors

Comp. No.	ACGS name (MW)	Species	Mol. Formula	THF Profile	THF-relative configuration	Ref.
(1a)	Annonacin (596)	*As. triloba* *An. muricata* *An. densicoma*	$C_{35}H_{64}O_7$	Mono	*th/t/th*	[18]
(4)	Diepomuricanin (546)	*An. muricata*	$C_{37}H_{62}O_4$	No THFs, rather bis-epoxy rings		[24]
(5)	Asimicin (622)	*As. triloba* *An. comifolia*	$C_{37}H_{66}O_7$	Adjacent bis-	*th/t/th/t/th*	[25-27]
(6)	Bullatacin (a. k. a. rollin (622)	*As. triloba,* *An. bullata,* *An. comifolia,* *R. mucosa*	$C_{37}H_{66}O_7$	Adjacent bis-	*th/t/th/t/er*	[25, 27]
(7)	Trilobacin (622)	*As. triloa*	$C_{37}H_{66}O_7$	Adjacent bis-	*th/t/er/c/th*	[28]
(8)	Bullatalicin (a. k. a. cherimolin-1) (638)	*As. triloba,* *An. bullata,* *An. comifolia,*	$C_{37}H_{66}O_8$	Non-adjacent bis-	*t/th-th/t/er*	[24]
(9)	Asitrocin (596)	*As. triolba*	$C_{35}H_{64}O_7$	Mono	*er//t/th*	[29]
(10, 11)	Asitrilobin-C and D (642)	*As. triloba*	$C_{37}H_{68}O_7$	Mono	*th/t/th*	[30]
(12)	Gigantetrocin-A (596**)**	*G. giganteus*	$C_{35}H_{64}O_7$	Mono	*th/t-th*	[31]
(13)	Squadiolin A (640)	*An. squamosal*	$C_{35}H_{68}O_8$	Mono	*er/t/th*	[32]
(14)	Butyrolactone-1 (390)	*P. macrocarpa*	$C_{25}H_{42}O_3$	Linear ACG with no THFs	--	[33]

THF stereochemistry refers to the stereochemistry of the bonds between the hydroxyl groups and the THF ring(s) and between the THF rings.
Abbreviations: As. = Asimina,An. = Annona, R. = Rollinia, G. = Goniothalamus, P. = Phaleria;th=threo, t = trans, er =erythro, c = cis.

In particular, the inhibitory effect of acetogenins on mitochondrial NADH-ubiquinone oxidoreductase (complex I) is worthy of note for the following reasons: (a) the diverse biological activities are thought to be attributable to this effect; (b) some of the compounds, such as Bullatacin**(6)** are the most potent inhibitors of the enzyme identified to date [34, 35][IC_{50}(1.2 nM (± 0.1)] and (c) it is quite difficult to visualize structural similarities between the acetogenins and ordinary complex I inhibitors such as piericidin A and rotenone, although the acetogenins act at the terminal electron transfer step of complex I similarly to the ordinary complex I inhibitors.[36]

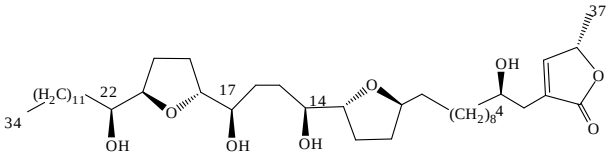


Piericidin A Rotenone

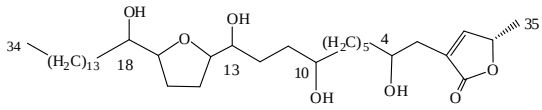
As a result, the powerful cytotoxicity of these compounds indicated a myriad of potentially useful applications. Commercial development of these compounds uses natural mixtures of active components, incorporated into pesticide, topical and dietary supplement products. Successful applications and commercial products include a shampoo, highly effective in treating infestations of head lice, fleas, and ticks; a series of pesticidal sprays, which protects host plants against a diversity of pests; and an ointment for treatment of oral herpes (HSV-1) and other skin afflictions[27]. The extract (in capsule form) enhances a mixture of natural anthelmintics. In addition, an encapsulated extract has been effectively used by certain cancer patients as a botanical supplement product.[2]

2.3.1. Anticancer effects

Most of the acetogenins evaluated *in vitro* exhibitedsimilar IC_{50}values (10^{-2} to 10^{-7}µg/ml). However, it was observed that some acetogenins appear to be selectivelycytotoxic for certain cancer types. For example, cytotoxicity results for Asimicin**(5)** and Bullatacin**(6)** showed potentselectivity against A2780 human ovarian tumor cells.Other results demonstrating selectivity for certain cancercell lines are summarized in **Table 2**[37]. The ACGs have also been shown to inhibit the growthof multidrug-resistant (MDR) tumor cells. The resistanceof MCF-7/Adr human breast cancer cells and KBv200human nasopharyngeal carcinoma cells to doxorubicinand vincristine, respectively, was about 100-told compared to their parental sensitive cell lines MCF-7 and KB.Whereas the acetogenins exhibited potent cytotoxicity toboth cell lines [38]. Bullatacin**(6)**was also found to be potent against MDR human mammary adenocarcinoma cells[39]. MDR tumor cells were resistant to apoptosis induced by natural anticancer drugs, however, the acetogenins induced apoptosis of both sensitive and MDR cells. It is concluded that adjuvant acetogenins therapy with anticancer drugs may be effective in treating MDRtumors.[40, 41]

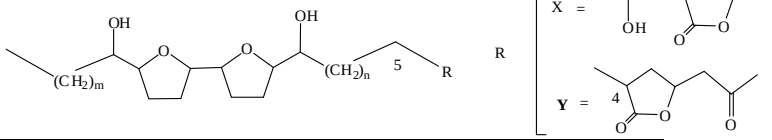


(15)

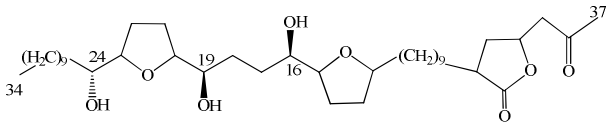


(16)

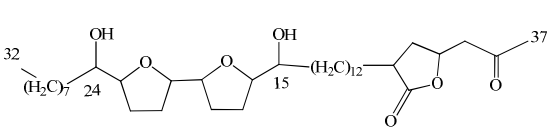
(17-22)



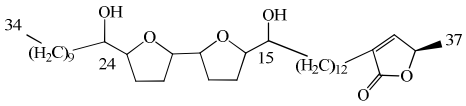
Comp No.	Compound	n	m	OH-positions	THF-relative configuration	R
(17)	Asimin	9	9	10, 15, 24	*(th/t/th/t/th)*	X
(18)	Asiminocin	9	9	15, 24, 30	*(th/t/th/t/th)*	X
(19)	Astribin	9	9	15, 24, 28	*(th/t/er/c//th)*	X
(20)	Bullanin	9	9	15, 24, 30	*(th/t/th/t/er)*	X
(21)	Bullatacinone	9	9	15, 24	*(th/t/th/t/er)*	Y
(22)	Rollincin	7	10	15, 24	*(th/t/th/t/er)*	Y



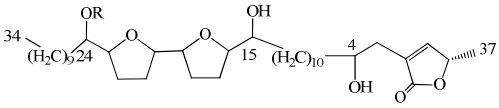
(23)



(24)



(25) (*th/t/th/t/er)*



(26b) R= H
(26a) (*th/t/th/t/er*) R= $COCH_3$

Table 2. Selectivity of ACGs for particular cancer cell types[37, 38, 42]

Comp.No.	Compound	Cell line	ED_{50} (µg/mL)
(1)	cis-annonacin	HT-29	$1x10^{-8}$
(5)	Asimicin	9PS HT-29	$<10^{-12}$ $3.3x10^{-11}$
(6)	Bullatacin	MCF-7 9PS A-549	$<10^{-12}$ $1x10^{-15}$ $1.3x10^{-13}$
(15)	Gigantecin	MCF-7	$4.1x10^{-9}$
(16)	Longicin	PaCa-2	$1.25x10^{-9}$
(17)	Asimin	A-549 MCF-7 HT-29	$8x10^{-9}$ $9.5x10^{-9}$ $<10^{-12}$
(18)	Asiminocin	A-549 MCF-7	$3.1x10^{-12}$ $2.9x10^{-12}$
(19)	Astribin	A-549	$2.25x10^{-10}$
(20)	Bullanin	A-549 MCF-7 HT-29	3.11x10-^{14} $3.22x10^{-14}$ $4.77x10^{-14}$
(21)	Bullatacinone	9KB HT-29	$<10^{-12}$ $5x10^{-12}$
(22)	Rollanicin	9PS	$2.9x10^{-8}$
	Adriamycin *	A-549 PaCa-2 MCF-7 PC-3 HT-29	$1.78x10^{-3}$ $2.42x10^{-3}$ $1.7x10^{-1}$ $1.89x10^{-2}$ $4.28x10^{-3}$

** Reference compound. A-549: human lung carcinoma. HT-29: human colon carcinoma, 9KB: human nasopharyngeal carcinoma, MCF-7: human breast cancer, PaCa-2: human pancreatic carcinoma, 9PS: murine lymphocytic leukemia,* PC-3*: human prostate cancer cell.*

ACGs have also been evaluated for *in vivo* antitumor activity. Increased life spans were observed in the*in vivo* studies using acetogenins against 3PS and L1210 leukemia in mice. In addition, when xenografts of A2780 human ovarian tumor cells were established in athymic nude mice, acetogenin treatment significantly inhibited tumor growth, **Table 3.** The *in vivo* antitumor efficacy of acetogenins suggests that rather evaluation is warranted to define optimal routes and schedules of administration and to verify *in vitro*selectivties.[43]

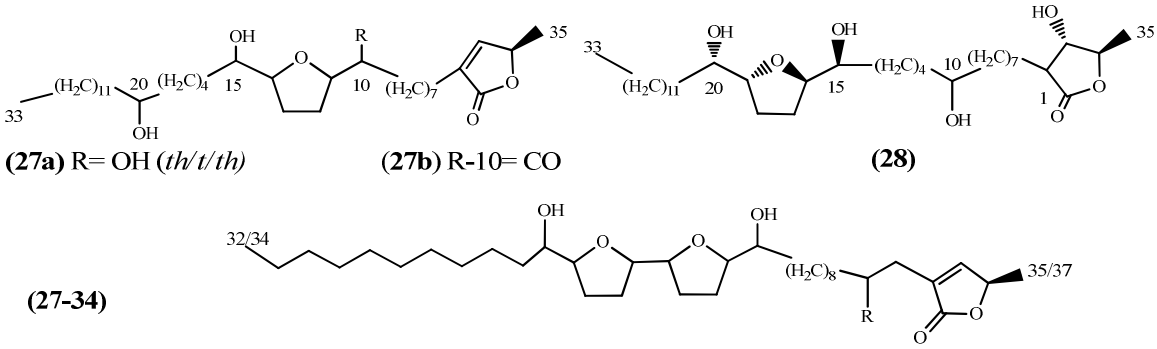
Table 3.Antitumor efficacy of acetogenins in mice.[43, 44]

Comp. No	Compound	Tumor cell line	(daily dose, mg/kg)	Antitumor activity
(1a)	Annonacin	3PS	0.95	124 *
(5)	Asimicin	3PS L1210	0.025 0.2	124* 131*
(6)	Bullatacin	L1210 A2780	0.05 0.1	138 * 68 **
(21)	Bullatacinone	L1210 A2780	0.4 0.13	144 * 52 **
(8)	Bullatalicin	A2780	1.0	75 **
(23)	Bullatalicinone	L1210	0.63	113 *
(24)	Rolliniastatin	3PS	0.25	128*
(25)	Squamocin	L1210	0.63	113 *
(26a)	Uvaricin	3PS	1.4	157 *

**Increase of life span (%). ** Tumor growth inhibitor (%) in athymic nude mice. 3PS and L1210: murine leukemia cell lines. A2780: human ovarian carcinoma cell line.*

Although acetogenins are extremely potent against tumor cells *in vitro*, the effect on growth of noncancerous cells such as rat Gl epithelial cells is minimal. **Table 4** shows that acetogenins display a much higher activity against KB human nasopharyngeal carcinoma cells as compared to Vero normal monkey epithelioid renal cells. *In vivo* studies have also reported little weight loss with acetogenin treatment, indicating favorable drug tolerance among the test animals. In addition, a standardized acetogenin mixture from *Asiminatriloba*, was found to be non-mutagenic in the Ames test, suggesting that acetogenins would not be carcinogenic and therefore may have an added advantage over many current antitumor drugs. Furthermore, it has been shown that the NADH oxireductase activity of rat liver plasma membrane is largely unaffected by Bullatacin**(6)** whereas that of HeLa (human cervical carcinoma origin) and HL-60 (human promyelocytic leukemia origin) plasma membranes is strongly inhibited, **Table 5**. These different responses of NADH oxireductase activity to Bullatacin**(6)** explain, in part, the ability of certain members of the *Annonaceae* family to selectively kill cancer cells while leaving normal cells unharmed.[37]

Table 4: Effect of acetogenins on the growth of tumor and normal cells *in vitro*[37, 45]



Comp. No	Compound	OH-positions	THF- relative configuration	Molecular Formula	ED_{50} (µg/mL)	
					KB cells	VERO cells
(27a)	Corossolin		*(th/t/th)*	$C_{35}H_{64}O_6$	$0.3x10^{-2}$	10^{-1}
(28)	Jetein		*(th/t/er)*	$C_{35}H_{66}O_7$	10^{-5}	10^{-2}
(29)	Molvizarin	4, 13, 22	*th/t/th/t/er*	$C_{35}H_{62}O_7$	10^{-3}-10^{-5}	10^{-2}-10^{-3}
(25)	Squamocin	15, 24, 28	*th/t/th/t/er*	$C_{37}H_{66}O_7$	$4x10^{-4}$	$1x10^{-2}$
(30)	Motrillin	15, 24, 29	*th/t/th/t/er*	$C_{37}H_{66}O_7$	10^{-3}-10^{-5}	10^{-2}-10^{-3}
(31)	Spinencin	15, 24, 28, 29	*th/t/th/c/er-th*	$C_{37}H_{66}O_8$	$1x10^{-5}$	$6x10^{-3}$
(32)	Carolin-A	15, 24, 28	*th/t/th/c/er*	$C_{37}H_{66}O_7$	$1x10^{-7}$	$2x10^{-3}$
(33)	Carolin-B	15, 24, 29	*th/t/th/c/er*	$C_{37}H_{66}O_7$	$5x10^{-8}$	$4x10^{-3}$
(34)	Carolin-C	13, 22, 26	*th/t/th/c/er*	$C_{35}H_{62}O_7$	$2x10^{-4}$	$5x10^{-2}$

KB: human nasopharyngeal carcinoma cell line. VERO: normal monkey epitheloid renal cell line.

Table 5.Summary of inhibition of NADH oxireductase activity by acetogenins[35]

Comp. No	Compound	ED_{50}(nM)	
		Rat liver	HeLa cells
(1a)	Annonacin	>10.000	1000
(5)	Asimicin	>10.000	5
(6)	Bullatacin	>10.000	5-10
(21)	Bullatacinone	>10.000	100-1000

Tamoxifen resistance is common in estrogen receptor-(ER)-positivebreast cancers. α-ER-positive MCF-7 cells, annonacin**(1a)** (half-effective dose ED_{50} = 0.31 µM) and 4-hydroxytamoxifen (ED_{50} = 1.13µ M) decreased cell survival whereas annonacin**(1a)** (0.5-1 µM) increased cell death at 48 h. Annonacin**(1a)** induced growth arrest and apoptosis in ER-related pathways in MCF-7 cells. Annonacin**(1a)** and 4-hydroxytamoxifen were additives in inhibiting cell survival and ER transcriptional activity. Moreover, annonacin**(1a)** attenuated MCF-7 xenograft tumor growth while inhibiting ER, cyclin D1and Bcl-2 protein expressions in nude mice.[46]

2.3.2. Potential mechanism of the anticancer action of ACGs

It is reported recently that the inhibitory effects exerted by paw paw crude extract and purified acetogenins on the important anticancer molecular target hypoxia-inducible factor-1 (HIF-1). The transcription factor HIF-1 activates the expression of more than 100 target genes that promote cellular adaptation and survival under hypoxic conditions. It is a heterodimer of the oxygen-regulated HIF-1α and the constitutively expressed HIF-1/ARNT subunits. The classical oxygen-dependent post-translational modification of HIF-1α protein includes the prolyl hydroxylation that tags HIF-1α for pVHL-mediated ubiquitination and subsequent proteasome degradation, and the asparaginyl hydroxylation that inactivates HIF-1 as shown in **Figure 6**.[11]

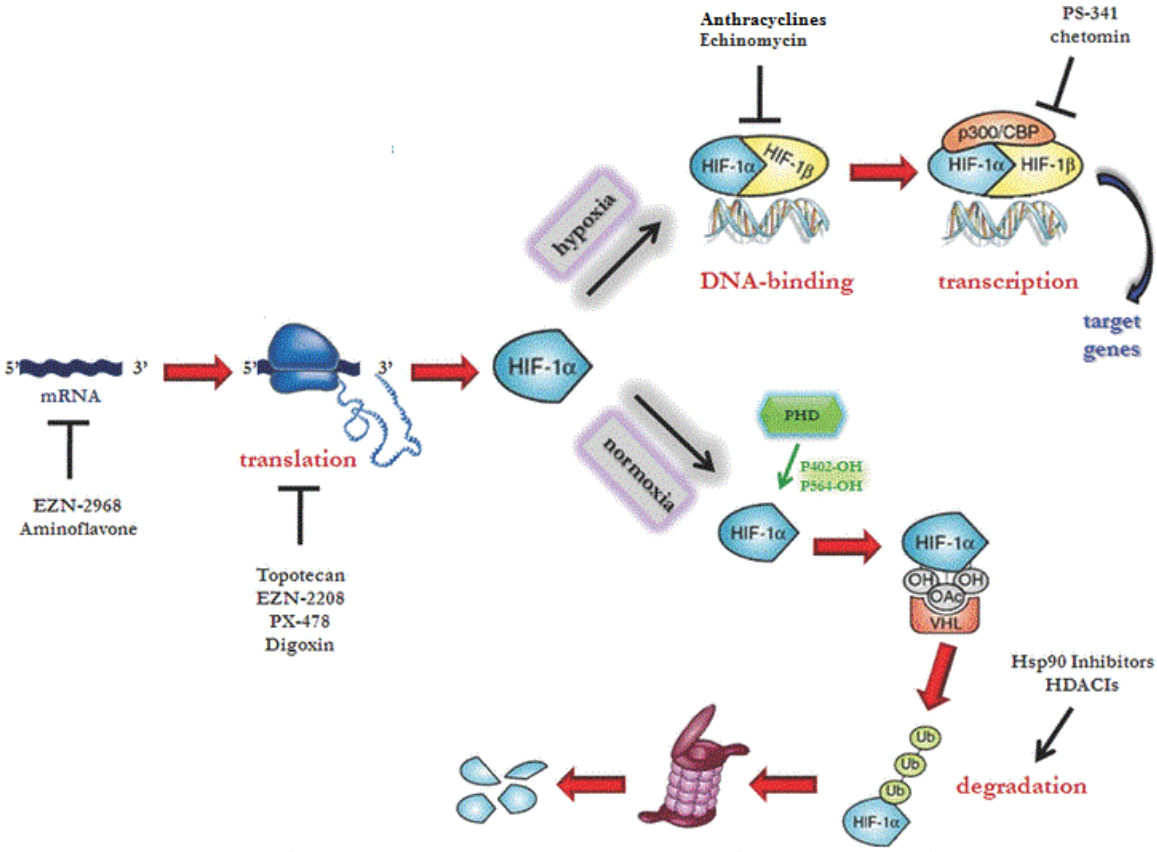


Figure 6.Pathways and inhibitors of HIF-1α synthesis, degradation, and regulation of HIF-1 activity. The figure has been adapted, with some modifications, from Ref [47]

Solid tumors are often subjected to hypoxia and need to adapt to the hypoxic environment to sustain their rapid growth, **Figure 6**. As a transcription factor, HIF is the master regulator of the cellular hypoxia response. HIF is a heterodimer composed of a hypoxia-regulated subunit (HIF-α) and a constitutively expressed subunit HIF-1β. Among three HIF-α isoforms, HIF-1α is the most critical regulator of hypoxia responses in solid tumors, and its activity is indispensable for tumors to adapt to hypoxia conditions and recover from damages caused by hypoxic insult. While HIF-1β is constitutively expressed in the nucleus, HIF-1α remains at a low level through proteasome-dependent mechanisms under normoxia. Upon hypoxia, HIF-1α is quickly stabilized and translocated to the nucleus, where it forms a heterodimer with HIF-1β and subsequently binds to the hypoxia responsive element (HRE) (59-RCGTG-39), resulting in transactivation of more than 200 genes required for the cell to adapt to hypoxic conditions. Since many of the HIF-1 target genes can promote cell survival under hypoxic conditions, it is not surprising that HIF-1α is often overexpressed in various cancers, including breast, lung, pancreatic and renal cancer. Therefore, inhibition of HIF-1α activity represents an attractive strategy for cancer treatment. Indeed, small molecules targeting HIF-1α transcription, translation, and stabilization have been developed, and some of them (e.g., PX- 478, Topetican and BAY87-2243) have entered clinical trials for treating cancer patients.[48]

Both the HIF-1 inhibitory and cytotoxic/cytostatic activities of acetogenins and crude extract appear to be cell line-dependent. Tumor cell populations that rely solely on mitochondria to generate ATP are most sensitive to inhibitors of the electron transport chain. In contrast, aggressive and malignant tumor cells often utilize aerobic glycolysis to fuel cellular metabolism and function. Thus, they are less sensitive to antitumor agents that affect mitochondria. Although neither purified acetogenins nor paw paw crude extracts have been approved by the FDA for cancer treatment, dietary supplements that contain these substances are consumed by cancer patients as alternative medicines.[47, 49]

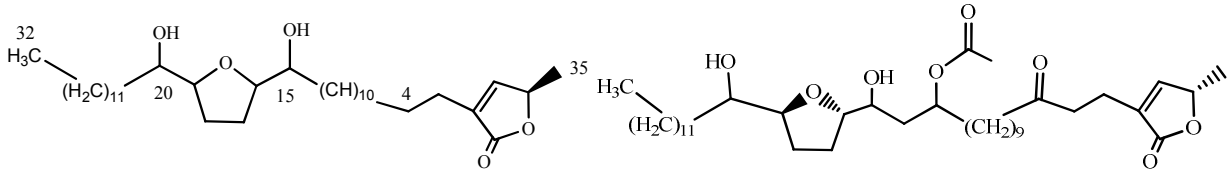
The toxicity of AGEs in both cases probably arises from the strong inhibitory ability of mitochondrial electron transport with specific action at complex I. DegliEsposti *et al.* [50] first used mammalian mitochondria to study the action of AGEs toward the NADH-ubiquinone oxoidoreductase (complex I) and reported that Bullatacin**(6)**inhibited the proton pumping function of complex I with similar efficiency under steady-state and nonsteady-state conditions, as compared to the action of piericidin and rotenone. Because of the ability to inhibit the mitochondrial complex I, the main gate of the energy production in the cells, AGEs have been regarded as candidates for future generations of antitumour drugs with different mechanisms. [51]

Besides blocking the NADH ubiquinone oxidoreductase (complex I) in the electron transport system, AGEs are also powerful inhibitors of the NADH oxidases peculiar to the plasma membranes of cancer cells. Both mechanisms of action result in the inhibition of ATP production and may account for the observation that AGEs are more effective at killing multiple-drug resistant (MDR) tumors than their nonresistant counterparts since the MDR pumps on the cell membranes require ATP to function.

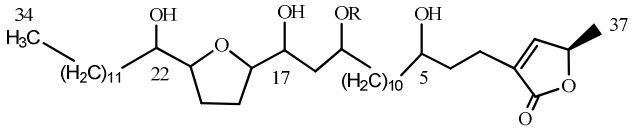
In addition, Oberlies *et al.*[52] observed that AGEs could selectively inhibit the cell growth of cancerous cells by in vitro cell inhibition assays against three murine (P388, PO3, and M17/Adr) and two human (H8 and H125) cancer cell lines. Interestingly, the work of Oberlies *et al.* proposed that this class of natural products showed a certain biological activity against some drug-resistant cancers. Currently, multidrug-resistant cancers are hard to cure because the cancer cells have developed a mechanism to overcome the anticancer agents. Based on the biochemical differences between MDR and parental cancer cells, such as the ATP-dependent P-glycoprotein-mediated pumps (P□gp) and the higher demand for ATP in the MDR cancer cells.[53]Oberlies *et al.* used Bullatacin**(6)** to test two cell lines, MDR human mammary adenocarcinoma (MCF-7/Adr) cells and the parental, nonresistant wild type (MCF-7/wt) cells. Therefore, ATP depletion could be another mode of action of AGEs that offers a special advantage in the chemotherapeutic treatment of MDR tumors. Shimada *et al*. also proposed a model for explaining the action of AGEs. They suspected that the lactone ring alone could directly interact with the binding to complex I, and the THF rings with flanking OH groups function just as hydrophilic anchors at the membrane surface that allow lateral diffusion (or random distribution) of the lactone ring in the membrane interior.[11, 18]

Toxicity of ACGs

Graviola (*A. muricata*), fruta-de-conde (*A. squamosa*), and the paw paw (*A. triloba*) may cause atypical parkinsonism, due to the presence of annonacin**(1a)** and other neurotoxins.[54][17]*A. muricata* that contains high content of annonacin**(1a)** is enough to cause neurodegeneration in rats can also be attained in humans by regular consumption within one year. Graviola and fruta-de-conde were suspected of causing atypical parkinsonism and progressive supranuclear palsy (PSP) on the Caribbean island of Guadeloupe, some areas of London, and the Pacific islands of New Caledonia and Guam [55]. This was based on the abnormally high incidence and relatively high consumption of fruits and tea made from the leaves. At first it was blamed on the presence of neurotoxic benzyltetrahydroisoquinolines. Subsequent authors identified annonacin**(1a)** and other ACGs as a more likely cause. Like benzyltetrahydroisoquinolines, annonacin**(1a)** may cause tau pathologies, including atypical parkinsonism due to their inhibition of mitochondrial complex I. The concentration that caused 50% inhibition (IC_{50}) of the mitochondrial complex I was lowest for squamocin**(25)** (1.4 nM), compared to 54.8, 63.8, 100.3, 11.8, 2.1, 1.6, and 1.7 nM for annonacin**(1a)**, annonacinone**(4b),**isoannonacin**(4c)**, solamin**(41)**, sootepensin B **(42)**, tonkinesin A **(43a)**, and tonkinin C **(43b)** , respectively. The concentrations needed to induce cell death in 50% of the cultured neurons (EC_{50} death) were 134, 246, 121.0, 23.8, 1.7, 3.2, and 3.9 nm for annonacin**(1a),**annonacinone**(1b)**, isoannonacin**(1c)**, solamin**(41)**, sootepensin B **(42),**tonkinesinA**(43a)** and tonkinin C **(43b)**, respectively.



(41) **(42)**



(43a) R= H **(43b) R= $COCH_3$**

About the basic role of tau in development of neurodegenerative diseases, one can refer to the articles of Potts *et al*[56] and other references [57, 58].

These ACGs also decreased ATP levels, with an EC50 of 134 nM for annonacin**(1a)** and 2.9 nM for squamocin**(25)**, and values ranging from 4.0 to 246 nM for the others.These ACGs also induced neuronal cell death with EC50 values of 60.8 nM for annonacin**(1a)**, rolliniastatin**(24)** and 1.1 nM forsquamocin**(25)**, and values rangingfrom 1.7 to 189.7 nM for the others. These ACGs also redistributed thetau protein in cultured neurons. The concentration needed to redistribute tauin 5% of the neurons (EC5) was (0.6 nM) for squamocin**(25)**and rolliniastatin**(24)**,44.1 nM for annonacin**(1a)**, and values ranging from 1.0 to 134.9 nM for the others.[59, 60]

Recently, other workers found that the mechanism underlying the cytotoxicityof ACGs is modulated by the chelation of THF moieties of ACGs withCa^{+2} to form hydrophobic complexes. As shown in **Figure 7**a representative example of annonacin (1a) - Ca^{+2}chelate.The ability of ACGs to bind Ca^{+2} correlated with their levels of cytotoxicity. Also, incubation of cells with bis-THFACGs also increased the mitochondrial Ca^{+2}concentration anddecreased the mitochondrial membrane potential, which may be the maincause of cytotoxicity.[17]

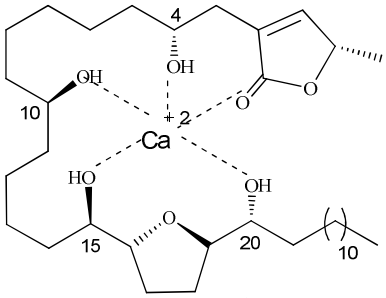


Figure 7.Annonacin- Ca^{+2} complex[61]

REFERENCES

[1] G. M. Cragg, D. J. Newman, *Biochimica Et Biophysica Acta*,**2013**, 1830, 3670.
[2] D. J. Newman, G. M. Cragg, *Journal Of Natural Products*,**2012**, 75, 311.
[3] A. A. G. Lagunin, Rajesh K.; Gawande, Dinesh Y.; Pahwa, Priynka; Gloriozova, Tatyana A.; Dmitriev, Alexander V.; Ivanov, Sergey M.; Rudik, Anastassia V.; Konova, Varvara I.; Pogodin, Pavel V.; Et Al, *Natural Product Reports* **2014**, 31, 1585.
[4] S. Chrubasik, In *Plant Bioactives And Drug Discovery*, John Wiley & Sons, Inc., **2012**. 395.
[5] V. E. Cornelio, M. M. Pedroso, A. S. Afonso, J. B. Fernandes, M. F. G. F. Da Silva, R. C. Faria, P. C. Vieira, *Analytica Chimica Acta*,**2015**, 862, 86.
[6] B. David, J.-L. Wolfender, D. Dias, *Phytochem Rev*,**2014**, 1.
[7] M. M. Cowan, *Clinical Microbiology Reviews*,**1999**, 12, 564.
[8] P. C. Perumal, S. Sundaram, P. Prabhakaran, V. Pratibha, A. Palanirajan, T. Starlin, R. Subban, V. K. Gopalakrishnan, *Pharmacognosy Research*,**2015**, 7, 121.
[9] R. V. C. G. R.P. Severino, E.F. Marques, D. Brömme, M.F.D.G.F. Da Silva, J.B.Fernandes, A.D. Andricopulo, P.C. Vieira, *Bioorg. Med. Chem.*,**2011**, 19, 1477.
[10] M. S. Butler, A. A. B. Robertson, M. A. Cooper, *Natural Product Reports*,**2014**, 31, 1612.
[11] V. Coothankandaswamy, Y. Liu, S.-C. Mao, J. B. Morgan, F. Mahdi, M. B. Jekabsons, D. G. Nagle, Y.-D. Zhou, *Journal Of Natural Products*,**2010**, 73, 956.
[12] A. Bermejo, Figadère, B., Zafra-Polo, M.C., Barrachina, I., Estornell, E., And Cortes, D. , *Nat. Prod. Rep.* ,**2005**, 22, 269.
[13] E. K. Spiridon, G. B. Maria, In *Plants That Fight Cancer*, CRC Press, **2004**. 15.
[14] M. S. Costa, J. F. S. Cossolin, M. J. B. Pereira, A. E. G. Sant'Ana, M. D. Lima, J. C. Zanuncio, J. E. Serrão, *Toxins*,**2014**, 6, 1169.
[15] L. D. Ribeiro, O. Zanardi, J. Vendramim, P. Yamamoto, *Exp Appl Acarol*,**2014**, 64, 87.
[16] J. L. Mclaughlin, *Journal Of Natural Products*,**2008**, 71, 1311.
[17] R. E. Smith, K. Tran, K. M. Richards, In *Studies In Natural Products Chemistry*, Ed. By R. Atta Ur, Elsevier, **2014**, Vol. Volume 41. 95.
[18] S.-S. F. Yuan, H.-L. Chang, H.-W. Chen, Y.-T. Yeh, Y.-H. Kao, K.-H. Lin, Y.-C. Wu, J.-H. Su, *Life Sciences*,**2003**, 72, 2853.
[19] Y. Liu, Q. Xiao, Y. Liu, Z. Li, Y. Qiu, G.-B. Zhou, Z.-J. Yao, S. Jiang, *European Journal Of Medicinal Chemistry*,**2014**, 78, 248.
[20] I. B. Spurr, R. C. Brown, *Molecules*,**2010**, 15, 460.
[21] N. Phonkerd, *KKU Sci. J.*,**2014**, 42, 700.
[22] C.-C. Liaw, T.-Y. Wu, F.-R. Chang, Y.-C. Wu, *Planta Medica*,**2010**, 76, 1390.
[23] D. B. Neha Pandey, *International Journal Of Research In Pharmaceutical And Biomedical Sciences* **2011**, 2, 1404.
[24] M. Takada, K. Kuwabara, H. Nakato, A. Tanaka, H. Iwamura, H. Miyoshi, *Biochimica Et Biophysica Acta (BBA) - Bioenergetics*,**2000**, 1460, 302.
[25] A. Gupta, D. R. Shah, N. R. Seth, S. Pandey, J. S. Yadav, *Systematic Reviews In Pharmacy*,**2011**, 2, 104.
[26] Z. Chen, S. C. Sinha, *Tetrahedron Letters*,**2009**, 50, 6398.
[27] K. W. Pomper, J. D. Lowe, S. B. Crabtree, W. Keller, *Journal Of Agricultural And Food Chemistry*,**2009**, 57, 8339.

[28] T.-I. Sohn, M. J. Kim, D. Kim, *Journal Of The American Chemical Society*,**2010**, 132, 12226.
[29] E.-J. Kim, F. Tian, M.-H. Woo, *Journal Of Natural Products*,**2000**, 63, 1503.
[30] M.-H. Woo, S.-O. Chung, D.-H. Kim, *Bioorganic & Medicinal Chemistry*,**2000**, 8, 285.
[31] F. Q. Alali, W. Kaakeh, G. W. Bennett, J. L. Mclaughlin, *Annonaceous Acetogenins As Natural Pesticides: Potent Toxicity Against Insecticide-Susceptible And -Resistant German Cockroaches (Dictyoptera: Blattellidae)*.Ed., **1998**, Vol. 91, Pp. 641.
[32] C.-C. Liaw, Y.-L. Yang, M. Chen, F.-R. Chang, S.-L. Chen, S.-H. Wu, Y.-C. Wu, *Journal Of Natural Products*,**2008**, 71, 764.
[33] M. Carmen Zafra-Polo, B. Figadère, T. Gallardo, J. Tormo, D. Cortes, *Phytochemistry*,**1998**, 48, 1087.
[34] N. Kojima, M. Abe, Y. Suga, K. Ohtsuki, T. Tanaka, H. Iwasaki, M. Yamashita, H. Miyoshi, *Bioorganic & Medicinal Chemistry Letters*,**2013**, 23, 1217.
[35] H. Miyoshi, In *A Structural Perspective On Respiratory Complex I*, Ed. By L. Sazanov, Springer Netherlands, **2012**, Chap. 4. 81.
[36] Y. J. Miao, X. F. Xu, F. Xu, Y. Chen, J. W. Chen, X. Li, *Natural Product Research*,**2014**, 28, 1929.
[37] Y. Chen, J.-W. Chen, X. Li, *Journal Of Natural Products*,**2011**, 74, 2477.
[38] I. Royo, N. Depedro, E. Estornell, D. Cortes, F. Peláez, J. R. Tormo, *Oncology Research Featuring Preclinical And Clinical Cancer Therapeutics*,**2003**, 13, 521.
[39] Y. Chen, J. W. Chen, J. H. Zhai, Y. Wang, S. L. Wang, X. Li, *Food And Chemical Toxicology : An International Journal Published For The British Industrial Biological Research Association*,**2013**, 58, 394.
[40] N. H. Oberlies, C.-J. Chang, J. L. Mclaughlin, *Journal Of Medicinal Chemistry*,**1997**, 40, 2102.
[41] Azucena Gonza´Lez-Coloma, Ana Guadan˜Oa, C. N. D. I. S. , R. M. N.-D. A. A. D. Cortes, *Z. Naturforsch*,**2002**, 57 C, 1028.
[42] Y. Chen, J.-W. Chen, J.-H. Zhai, Y. Wang, S.-L. Wang, X. Li, *Food And Chemical Toxicology*,**2013**, 58, 394.
[43] Y. Matsui, Takeuchi, T., Kumamoto-Yonezawa, Y., Takemura, M., Sugawara, F., Yoshida, H., & Mizushina, Y. , *Experimental And Therapeutic Medicine*,**2010**, 1, 19.
[44] L. Xiang, C. Xiao-Ling, C. Jian-Wei, S. Dong-Dong, *Chemistry Of Natural Compounds*,**2010**, 46, 101.
[45] N. Kojima, T. Tanaka, *Molecules*,**2009**, 14, 3621.
[46] J. Qu, W. Liu, C. Huang, C. Xu, G. Du, A. Gu, X. Wang, *Toxicology In Vitro*,**2014**, 28, 373.
[47] Y. Xia, H.-K. Choi, K. Lee, *European Journal Of Medicinal Chemistry*,**2012**, 49, 24.
[48] L. Wang, G. Chen, X. Lu, S. Wang, S. Han, Y. Li, G. Ping, X. Jiang, H. Li, J. Yang, C. Wu, *European Journal Of Medicinal Chemistry*,**2015**, 89, 88.
[49] B. Onnis, A. Rapisarda, G. Melillo, *Journal Of Cellular And Molecular Medicine*,**2009**, 13, 2780.
[50] M. Degli Esposti, Ghelli, A., Ratta, M., Cortes, D., Estornell, E, *Biochemical Journal*,**1994**, 301, 161.
[51] J.-F. Shi, P. Wu, Z.-H. Jiang, X.-Y. Wei, *European Journal Of Medicinal Chemistry*,**2014**, 71, 219.
[52] N. H. Oberlies, Croy, V.L., Harrison, M.L., Mclaughlin, J.L., , *Cancer Lett.*,**1997**, 115, 73.
[53] Y. Yang, M. Sun, L. Wang, B. Jiao, *Journal Of Cellular Biochemistry*,**2013**, 114, 967.
[54] R. A. Levine, K. M. Richards, K. Tran, R. Luo, A. L. Thomas, R. E. Smith, *Journal Of Agricultural And Food Chemistry*,**2015**, 63, 1053.
[55] R. A. Levine, K. M. Richards, K. Tran, R. Luo, A. L. Thomas, R. E. Smith, *Journal Of Agricultural And Food Chemistry*,**2015**.
[56] L. F. Potts, F. A. Luzzio, S. C. Smith, M. Hetman, P. Champy, I. Litvan, *Neurotoxicology*,**2012**, 33, 53.
[57] K. Iqbal, F. Liu, C.-X. Gong, A. Alonso, I. Grundke-Iqbal, *Acta Neuropathol*,**2009**, 118, 53.
[58] B. Frost, J. Götz, M. B. Feany, *Trends In Cell Biology*,**2015**, 25, 46.
[59] P. Champy, G. U. Hoglinger, J. Feger, C. Gleye, R. Hocquemiller, A. Laurens, V. Guerineau, O. Laprevote, F. Medja, A. Lombes, P. P. Michel, A. Lannuzel, E. C. Hirsch, M. Ruberg, *Journal Of Neurochemistry*,**2004**, 88, 63.
[60] A. H. Schapira, *Experimental Neurology*,**2010**, 224, 331.
[61] C.-C. Liaw, W.-Y. Liao, C.-S. Chen, S.-C. Jao, Y.-C. Wu, C.-N. Shen, S.-H. Wu, *Angewandte Chemie International Edition*,**2011**, 50, 7885.