A novel mechanism for the control of clinical cancer: Inhibition of the production of adenosine triphosphate (ATP) with a standardized extract of paw paw *(Asimincz triloba,* Annonaceae)

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Summary
The North American paw paw tree *[Asiniina triloba (L.)* Dunal, Annonaceae] contains complex mixtures of over 50 Annonaceous acetogenins. These are derivatives of long chain (C-32 or C34) fatty acids which are powerful inhibitors of mitochondrial (the PSST protein of complex I) and cytoplasmic (the plasma membrane NADH oxidase) production of adenosine triphosphate (ATP). A standardized extract of paw paw reduced tumor markers, reduced tumor sizes, and increased longevities among 94 cancer patients while causing minimal side effects. Ten case studies are presented. Inhibition of cellular energy (ATP), using the paw paw supplement, thus offers a novel, safe, and effective mechanism for the control of various types of clinical cancer.

1. Introduction

The paw paw tree, *Asiniina triloba (L.)* Dunal (Annonaceae), is native to the eastern U.S. Its edible fruits are popular in song, and a fluid extract of its seeds was sold by Eli Lilly and Company, at the end of the 1800's, as an emetic. Thus, it has a history of safe human use and consumption. Bioactivity-directed fractionation has resulted in the isolation of over fifty bioactive components of paw paw. The major active compounds are called the annonaceous acetogenins; these are long chain fatty acid derivatives that emanate from an a, (3-unsaturated y lactone ring; they typically contain from 0 to 3 tetrahydrofuran (or tetrahydropyran) rings in the chain. Several related tropical and subtropical species in the Annonaceae (e.g., species in the annonaceous genera *Annona, Asimina, Goniothalanaus, Rollinia, Uvaria, Disepalum,* and *Xylopia)* have yielded an additional 350 compounds in this class. Five comprehensive reviews on the annonaceous acetogenins describe the chemistry and biology of the large number of compounds now known in this class [1-5]. Three additional reviews also focus on this work [68].

The numerous acetogenins in paw paw are described in a series of research papers [9-29]. The chemical structures [9,11,12,16], with their absolute stereochemistries defined [30,31], for bullatacin, asimicin, and trilobacin, represent the most potent, major, bioactive structural types of acetogenins that are found in the paw paw concentrate.

These annonaceous acetogenins are potently cytotoxic, and mechanistically, are the most powerful inhibitors known of complex I in the

electron transport system in mitochondria [29, 3237]. Schuler et aL [38] localized the specific binding site of acetogenins in complex I to the 23kDa PSST subunit. Structure-activity relationships for the acetogenins have been evaluated in cancer cell cultures, brine shrimp, mosquito larvae, isolated mitochondria, and mitochondrial fragments [20,29,3C,37,39,40], and the positioning of the acetogenins in biological membranes has been predicted using nuclear magnetic resonance spectroscopy [41,42]. The acetogenins also inhibit the NADH oxidase found in the plasma membranes of tumor cells [43]. Their immediate effect is depletion of ATP levels. This suggests that they should be excellent candidates for development as new antitumor agents, and they are especially effective against multi-drug resistant (MDR) tumors in which the resistance is due to ATP-dependent efflux pumps [39, 4447]. The acetogenins circumvent MDR by decreasing the efflux pump (P-170 glycoprotein) function and increasing cellular drug accumulation [48]. Successful *in vivo* studies, against murine leukemia, myeloma, and human ovarian carcinoma in athymic mice, attest to the antitumor effectiveness of several of the acetogenins and demonstrate potencies in animals as high as 300 times that of taxol with concurrent weight gain rather than the drastic weight loss caused by taxol [4,35].

In spite of the success of these *in vivo* studies, the inhibition of ATP production has been dogmatically deemed as too general a mechanism for systemic cancer chemotherapy. It has been regarded that all cells require ATP, and, thus, ATP inhibitors would be simultaneously cytotoxic to essential tissues as well as cancer cells. However, the animals in the *in vivo* studies cited above would have succumbed if this were true. Studies with cell Cultures of normal versus cancerous cells showed early on that the acetogenins are relatively less toxic to normal cells [45]; indeed, certain acetogenins are often selectively cytotoxic toward one or only a few cancer cell lines, e.g., squamotacin for PC-3 prostate cells [25] and the 9-keto compounds for PACA-2 pancreatic cells [49]. Over 30 have been repeatedly evaluated and show selectivities in the panel of 60 human tumor cell lines at the test facility of the National Cancer Institute, Frederick, MD. Furthermore, the endogenous molecular biology of cancer cells is now understood to involve autocrine and paracrine secretion of insulin and insulin-like growth factors (IGF-I & II) which subserve enhanced energy production and growth stimulation, respectively, in these cells [50]. Breast cancer cells have an average of seven times more insulin receptors [51] and ten times more IGF receptors [52] than normal breast and other tissue cells within the host. Thus, these cancer cells can take up glucose seventeen times faster than normal cells, and, it must be presumed that, they can also utilize glucose seventeen times faster than normal cells. Mitochondrial inhibitors, such as the annonaceous acetogenins, thus, should show a selection for cancer cells, and they do.

The resulting depletion of ATP and related nucleotides (all of which are precursors of DNA and RNA) has been demonstrated (Table *I) in vitro* in human leukemic cells (Fotopoulos, personal Communication); and the result is an upset

of cell timing with subsequent apoptosis (programmed cell death) as demonstrated by DNA laddering in malignant B-cells (Geahlen, personal communication). Subsequent studies have confirmed that acetogenin-induced cell death, indeed, involves apoptosis, and these studies are helping to define the molecular consequences such as caspase-3 activation and decreases in cyclic adenosine monophosphate and cyclic guanosine monophosphate levels [53,54]. The depletion of the nucleotide pool not only blocks replication of chromosomal, mitochondrial, and ribosomal nucleic acids in cancer cells but, as well, should block the replication of viral particles in viral-infected host cells and account for the previously observed antimalarial and antimicrobial effects [2].

Previous clinical studies of the acetogenins in cancer patients have been thwarted not so much by a lack of interest in these compounds and their novel mechanism of action but by their unavailability in pure form. Several lengthy synthetic procedures have been reported (see the review by Alali et al., [1]), but these would be expensive on a commercial scale. A few suppliers of botanical supplements are selling products containing powdered leaves and twigs of *Annona muricata. A. muricata* has been cultivated for its fruit throughout the tropics and is named graviola, guanabana, and Brazilian paw paw; it is probably the most abundant annonaceous plant in the world. Unfortunately, it contains only the less potent monotetrahydrofuran acetogenins [55], and the producers of the graviola supplements have made no effort to maximize acetogenin levels or standardize the product to assure consistent potency. The utility of biologically standardized crude extracts as botanical supplements, containing the natural complexes of the acetogenins rather than the single acetogenins as drugs in the alleviation of clinical cancer, has not been previously reported.

Table I.
Depletion of nucleotide pools by bullatacin in human CEM leukemia cells (Fotopoulos, personal communication).

Percent of Control [1,2]				
Bullatacin 100 ng/ml	UTP[3] %	CTP[4] %	ATP[5] %	*GTP*[6] %
2 Days	74	75	98	125
3 Days	24	36	46	69

1. Nucleotide pools determined initially in units of pmol/106 units
2. Average of two experiments
3. UTP = Uridine 5' – ti phosphate
4. CTP = Cytidine 5' - tri-phosphate
5 ATP = Adenosine 5' - tri-phosphate
6. GTP = Guanosine 5' - tti-phosphate

2. Methods

2.1 Plant Materials

The bioactive acetogenins of paw paw have been isolated and identified individually using bioassay-guided fractionation directed by the brine shrimp lethality test (BST) [6, 56]. Using this bioassay, the acetogenins have been found to be concentrated in the twigs, which, thus, serve as a convenient, renewable source of biomass [57]. The BST and high performance liquid chromatography/tandem mass spectrometry (LC/MS/MS) [58] have demonstrated that the concentrations of the acetogenins in the twigs are maximal in the months of May-June; thus, collections of the biomass are best made at that time of year [59].

In May-June, 2000, some 18,000 pounds of paw paw twigs were collected and acquired through American Botanicals, Eolia, MO. The twigs (<1/2 inch diameter) were collected from wild stands in the states of Indiana, Kentucky, and Missouri. Bundles of the twigs were dried in a forced-air drier at <50°C and reduced in a chipper/shredder through an 1/4 inch sieve. Samples of the various lots were extracted with dichloromethane or methanol, and 20 mg portions of the residues were subjected to the BST. Only those lots that showed BST LC_{50} values of <0.8 ppm were acceptable. In some cases subpotent lots were combined with superpotent lots to furnish a blend that was acceptable.

2.2 Extraction

Extractions of the plant material were made at Botanicals International Extracts, Inc. Boonton, NJ. A typical extraction run began with ca. 1500 pounds of the dried, shredded twigs that had passed the BST criteria. The material was placed into a large percolator.

Extraction was initiated with hot (city) water at one gallon/pound of twigs. After soaking for 8 hours the water was drained and discarded, and the water extraction was repeated three times. The damp mass was then extracted with 95% ethanol, four times, in a similar manner. The ethanolic extracts were combined and concentrated, *in vacuo,* at <40°C, to a syrup. Upon sitting under refrigeration, a water layer formed and was removed and discarded leaving a thick, syrupy, crude extract. The crude extract was standardized for 0% moisture and an ideal LC_{50} value of 0.5 ppm in the BST. Generally, the extract will contain from 10-40% moisture, and the BST LC_{50} value will range from 0.2-0.8 ppm. Adjustments were subsequently made in the amounts of extract per capsule to accommodate the standard values from lot to lot. The mixture of acetogenins was monitored chemically by using LC/MS/MS on a Hewlett Packard (Palo Alto, CA) model no. 1100 hplc and a Finnegan Corporation (San Jose, CA) model LCQ Duo [58,59] to be

assured of the presence of the major bioactive acetogenins (asimicin, bullatacin, trilobacin, and bullatalicin) as marker compounds.

2.3 Capsule Preparation

The standardized crude extract was adsorbed onto microcrystalline cellulose (Endurance MCC VE-090, FMC, Princeton, NJ) and dried at <40°C. Up to 2% silicon dioxide (Syloid 244, W.R. Grace, Baltimore, MD) and vegetable magnesium stearate were added, as needed, to improve flow and to aid encapsulation. Additional Endurance was added, as needed, to adjust the standard extract concentration to 12.5 mg/size O capsule.

2.4 Safety and Toxicology

In previous work, using a modified guinea pig maximization test, a paw paw extract was found to be only a weak skin sensitizer, and asimicin was found to be only a weak skin irritant; neither produced the vesication or ulceration typical of urushiol (poison ivy) components [60]. Ames test results (Sitek Research Laboratories, unpublished results) on a paw paw extract were negative in 9 out of 10 tests and only slightly positive (2.5% above background reversions) on one histidine mutant of *Salrrionella typhimuriurrz* after enzyme activation of the extract. These results have been confirmed in a publication in which two paw paw acetogenins were found to be antimicrobial but not mutagenic [61].

In previous feeding experiments [56], mice tolerated the paw paw extract mixed in their diet at 1% (a no choice diet). The mice ate this for four days without lethal effects. However, at 5% and above in their diets, they succumbed after three days, showing lethargy (as is typical from ATP deprivation), with their internal organs appearing normal. In other unpublished results (Asta Laboratories), bullatacin was emetic in pigs; this result demonstrated that the acetogenins very likely explain the former use of Eli Lilly's fluid extract of paw paw seeds as an emetic preparation. Thus, emesis is a definite safety factor should someone ingest excessive amounts of this supplement either intentionally or unintentionally.

Toxicology of ascending oral dosing of paw paw capsules (at 25 mg of the standardized extract each) in beagle dogs at White Eagle Toxicology Laboratories, Doylestown, PA, directed by A. D'Ver, DVM (unpublished results), failed to reach a lethal dose. There was a gradual increase in signs of emesis and loose stools as the dose of paw paw extract increased. There were no effects on alertness, appetite, or weight. These doses ranged from 50 mg four times a day (QID) to 800 mg QID. Five to seven resting days were permitted between doses. At the maximum dose of 800 mg QID per dog, there were no severe effects

(other than emesis and loose stools) in the dogs. Thus, any potential systemic toxic effects are conveniently thwarted by emesis as a safety valve.

2.4 Patient Case Studies

Volunteer subjects were recruited by physicians and other health care providers whose patients agreed to participate. Only subjects diagnosed with clinical cancer were included, and many were those whose cancer at stage four was deemed as "terminal." Those who concurrently were undergoing chemotherapy or radiation were included, along with those who had not had long-term success with chemotherapy/radiation and those who had refused these options due to their known devastating, effects on the immune system and general well-being. Patients suffering from a variety of tumor types were included in order to ascertain tumor types that might be most responsive.

A total of 94 participants were enrolled. Each subject signed an informed consent and medical records release statement. Subjects were monitored by their health care provider for any adverse as well as positive effects. An in-house Institutional Review Board, comprised of outside professionals, reviewed the protocols and found no concern regarding the safety of the subjects. The health care providers were requested to discuss any adverse events with patients and to contact the investigators to report any adverse event within 24 hours. If the provider were unable to be contacted, the subject could call an after hours number printed on the informed consent form. The study coordinator compiled the signed consent forms from the participants and recorded adverse events, compliance, positive results, dates of treatment and marker determinations, and other concerns the participant or health care provider may have had.

Preliminary studies in three volunteers with terminal ovarian cancers determined that a regimen of one capsule containing 12.5 mg of the standardized extract, taken four times a day with food, was tolerable and avoided nausea and vomiting; within one week the CA125 levels of one patient were reduced by 66%. Determination of the usual blood parameters, for possible deleterious effects on liver and bone marrow in the early patients, revealed no untoward effects. The study was continued over a period of 1 1/2 years with new patients being added as the results kept looking more and more promising.

3. Results and Discussion

Perusal of the records of the participants reveals some exciting observations. Over approximately 18 months, of 20 terminal cancer patients treated by one of us (JWF), 13 have survived and are now in stable condition taking the paw paw capsules every day. Longevities have increased. Levels of

prostate specific antigen (PSA) have been held constant and even decreased. Likewise, levels of breast tumor antigens have been significantly reduced. Tumor sizes, of the primary and secondary tumors, e.g., in breast, colon, prostate cancer and melanomas, and cell counts in lymphomas, have decreased, and some have even disappeared. Adverse effects (the bugaboo of chemotherapy) are practically nonexistent with the regimen, one 12.5 mg capsule taken four times a day (QID) with meals, of supplement servings over 18 or more months. The only side effects reported were nausea and vomiting (3 patients) and itching (1 patient). Many patients reported having "increased energy." Some patients have even tolerated 50 mQ QID over several months with no side effects. However, with one capsule QID most patients experienced positive responses within six to eight weeks. The results, perhaps, are best summarized by the brief presentation of ten case studies.

1. Participant #1 is a 53 year old female. She was diagnosed with breast cancer in 1996 and underwent a lumpectomy and radiation treatments. In January 2000 the cancer returned to the bone. Further radiation treatments were performed to the right hip and spine area concluding in September 2002. Alkaline phosphatase was measured in the blood as a way to monitor the progress of the disease. The normal range is 0-136, and those with cancer in the bone have elevated levels. The blood test taken in September 2002 was at 327. She started taking four paw paw capsules per day in November. By December the levels slightly decreased to 242. In February 2003 the alkaline phosphatase level decreased to 144. Since February the levels have remained stable (between 144 and 150). According to her physician, since the levels are remaining stable it shows that the cancer is contained and not doing further damage to the bone. She reports that she has more energy and stamina when taking the paw paw capsules.

2. Participant #2 is a 63 year old male with a bone tumor in the neck. On July 30, 2002, the bone tumor, which showed on x-ray, was measured as a 7 mm cavity with a 5 mm mass on the neck. He did not undergo any other treatments besides the paw paw capsules. He started taking the paw paw capsules in September 2002. Another x-ray taken on March 13, 2003, showed a significant decrease of the tumor size: the cavity was measured to be 4.5 mm with a 3 mm mass.

3. Participant #13 is a 52 year old female with breast cancer. She has not undergone any conventional treatments since being diagnosed. She has taken the paw paw capsules since October 2002. She reports that the pain in the breast has decreased and the non-cancerous fibrocystic lumps have reduced in size. Her doctor reports she has been doing "remarkably well" considering that she has not had surgery, chemotherapy or radiation. She says that she feels good and also has had some weight gain.

4. Participant #14 is a 59 year old female with breast cancer. She has not had

any surgery, chemotherapy, or radiation treatments. She started taking four capsules of paw paw in November 2002. The blood tests for the breast cancer tumor markers (CA2729) were consistent from 9/12/02 to 12/3/02 with both being 24.6. In March 2003 all the blood tests were within the normal range. The tumor size has also reduced.

5. Participant #15 is a 62 year old female that had breast cancer. She decided to undergo chemotherapy treatments and take the paw paw capsules concurrently. The chemotherapy treatments lasted seven months. The tumor almost completely disappeared as evidenced by magnetic resonance imaging and ultrasound. She decided to undergo surgery as well to remove any traces of the cancer. Removal of 14 subaxillary lymph nodes showed no metastatic cancer. This was followed by radiation. Her most recent screen showed no cancer in the breast. She is in complete remission and believed to be cancer free.

6. Participant #20 is a 70 year old female with stage four breast cancer. Within just six weeks of taking the paw paw capsules she saw a 50% percent reduction in the CA2729 tumor markers which went from 160 to 80. The size of the tumor was also reduced significantly, and she continues in stable condition. Since she had not changed any other treatment protocol, her physician is convinced that the paw paw is responsible for her improvement and stabilization.

7. Participant #42 is a 66 year old male with stage four lung cancer. He previously had undergone two years of chemotherapy, but the lung cancer had become resistant. Within two months of taking the paw paw capsules his CEA tumor markers decreased from 275 to 222. He also had a weight gain of 5 pounds and experienced no side effects to the paw paw capsules. Previous to taking the paw paw he was bedridden or was in a wheelchair. His health improved to the point that he is now able to walk on his own.

8. Participant #51 is a 60 year old male diagnosed with stage four melanoma which had metastasized from his arm to the lungs and lymph nodes. The doctors could not operate to remove the lung mass. The tumor started causing difficulty in breathing and he was expected to expire in February 2003. Since starting the paw paw in November 2002 he had an easier time breathing within a few days, and he has been feeling much better. He has been able to get out of bed and soon progressed to riding a bike, walking uphill, and working on his farm. Interestingly, two fatty tumors on his arm have also decreased considerably in size. He reports that the toe nail fungus he has had for 10 years is clearing up.

9. Participant #64 is a 56 year old male diagnosed with prostate cancer. The cancer was confirmed by biopsy. He started taking 4 paw paw capsules per day in October 2002. His PSA levels dropped to 2.08 on 12/23/02 down from a PSA of 3.85 taken two months previously, and he continued to take the paw paw

capsules until April 2003.

10. Participant #84 is a 73 year old male with stage four prostate cancer which had spread to other parts (left neck, abdomen, and hip bone) of the body. Within six weeks of taking the paw paw, the CT scan showed *a* 25% reduction in the tumor masses. His PSA levels are remaining constant, and he is in stable condition while continuing on paw paw.

Due to the positive responses from the aforementioned case studies and from many other of the original 94 participants, it is apparent that the paw paw extracts are an effective supplement for the regulation of cancers of various types. It also looks as if the extracts are an effective adjunctive to chemotherapeutic agents. When taken with chemotherapy, in some instances, the patients and physicians noticed an above average amount of tumor shrinkage after the first treatments than would be normally expected with chemotherapy alone. The synergistic benefit may be attributed to the novel action of the acetogenins causing ATP depletion resulting in apoptosis of the cancer cells. Chemotherapy resistant cells with ATP-driven efflux pumps are also thwarted by the ATP-depleting action of the acetogenins [39,44,46,47] resulting in renewed cellular accumulation of the chemotherapeutic agent [48]. Thus, the combination of chemotherapy, which also induces apoptosis, and paw paw extracts had an increased effect.

Yet, paw paw extracts have been effective, by both objective and subjective measurements, even when they are not combined with chemotherapy or radiation. Many participants have enjoyed an extended longevity and also have improved their quality of life with this supplement. Those that were weakened or bed-ridden now have more energy to do those things that they enjoy. Others that were given only a couple of months to live have surpassed the doctors' predictions in a marvelous way. Even more promising are those that have decreased the size of the tumors without the loss of hair, nausea, bone marrow depression, induction of new cancers, or other side effects.

The paw paw extract is unlike drugs because it is a complex mixture of natural compounds rather than a single entity. This represents a new approach in the alleviation of clinical cancer. Paw paw is an herbal supplement product with clinical effectiveness but with a very low toxicity. Paw paw has a unique mode of action focusing on depleting the energy-producing molecule ATP. Rather than poisoning the DNA as with most chemotherapy, paw paw is able to exploit a previously neglected biochemical difference, i.e., the voracious uptake of glucose, in cancer cells versus normal cells. Paw paw is affordable and very cost efficient (less than $2.00/day). Approximately 3000 cancer patients are now taking it every day, and the numbers are growing. It is now apparent that mitochondrial inhibition (ATP depletion) offers a novel mechanism for the control of clinical cancer.

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References

[1] Alali FQ, Liu XW, McLaughlin JL. Annonaceous acetogenins: recent progress. Journal of Natural Products. 1999;62(3):504-540.

[2] Rupprecht JK, Hui YH, McLaughlin JL. Annonaceous acetogenins: a review. Journal of Natural Products. 1990;53(2):237-278.

[3] Fang XP, Rieser MJ, Gu ZM, Zhao GX, McLaughlin JL. Annonaceous acetogenins: an updated review. Phytochemical Analysis. 1993;4(1):27-48 (part 1) and 49-67 (part 2).

[4] Gu ZM, Zhao GX, Oberlies NH, Zeng L, McLaughlin JL. Annonaceous acetogenins: mitochondrial inhibitors with diverse applications. In: Arnason JT, Mata R, Romeo JT, eds. Recent Advances in Phytochemistry. New York, NY: Plenum Press; 1995:249-310.

[5] Zeng L, Ye Q, Oberlies NH, Shi G, Gu ZM, He K, McLaughlin JL. Recent advances in annonaceous acetogenins. Natural Product Reports. 1996;13(4):275-306.

[6] McLaughlin JL. Crown gall tumours in potato discs and brine shrimp lethality: two simple bioassays for higher plant screening and fractionation. In: Hostettmann K, ed. Methods in Plant Biochemistry. London: Academic Press; 1991:1-31.

[7] McLaughlin JL, Chang CJ, Smith DL. Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumor compounds: review of recent progress. In: Kinghorn AD, Balandrin MF, eds. Human Medicinal Agents from Plants, ACS Symposium Series 534. Washington, D.C.: American Chemical Society; 1993:112-137.

[8] McLaughlin JL, Chang CJ. Simple (bench-top) bioassays and the isolation of new chemically diverse antitumor and pesticidal agents from higher plants. In: Romeo JT, ed. Recent Advances in Phytochemistry. New York: Kluwer Academic/Plenum Publishers; 1999:89-132.

[9] Rupprecht JK, Chang CJ, Cassady JM, McLaughlin JL, Mikolajczak KL, Weisleder D. Asimicin, a new cytotoxic and pesticidal acetogenin from the paw paw, *Asiniina triloba* (Annonaceae). Heterocycles. 1986;24(5):1197-1201.

[10] Hui YH, Rupprecht JK, Anderson JE, Liu YM, Smith DL, Chang CJ, McLaughlin JL. Bullatalicin, a novel bioactive acetogenin from *Annona bullata* (Annonaceae). Tetrahedron. 1989;45(22):6941-6948.

[11] Hui YH, Rupprecht JK, Liu YM, Anderson JE, Smith DL, Chang CJ, McLauahlin JL. Bullatacin and bullatacinone: two highly potent bioactive

acetogenins from *Annona bullata.* Journal of Natural Products. 1989; 52(3):463-477.

[12] Zhao GX, Hui YH, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba.* Journal of Natural Products. 1992;55(3):347-356.

[13] Zhao GX, Rieser MJ, Hui YH, Miesbauer LR, Smith DL, McLaughlin JL. Biologically active acetogenins from the stem bark of *Asiniifia triloba.* Phytochemistry. 1993;33(5):10651073.

[14] Zhao GX, Miesbauer LR, Smith DL, McLaughlin JL. Asimin, asiminacin, and asiminecin: novel highly cytotoxic asimicin isomers from *Asimina triloba.* Journal of Medicinal Chemistry. 1994;37(13):1971-1976.

[15] Zhao GX, Ng JH, Kozlowski JF, Smith DL, McLaughlin JL. Bullatin and bullanin: two novel, highly cytotoxic acetogenins from *Asimina triloba.* Heterocycles. 1994;38(8):1897-1908.

[16] Zhao GX, Gu ZM, Zen- L, Chao JF, Wood KV, Kozlowski JF, McLaughlin JL. The absolute configuration of trilobacin and trilobin, a novel highly potent acetogenin from the stem bark of *Asinzina triloba* (Annonaceae). Tetrahedron. 1995;51(26):7149-7160.

[17] Zhao GX, Chao JF, Zeng L, McLaughlin JL. (2,4-cis)-Asimicinone and *(2,4-trcuzs)*asimicinone: two novel ketolactone acetogenins from *Asimina triloba* (Annonaceae). Natural Toxins. 1996;4(3):128-134.

[18] Zhao GX, Chao F, Zeng L, Rieser MJ, McLaughlin JL. The absolute configuration of adjacent bis-THF acetogenins and asiminocin, a novel highly potent asimicin isomer from *Asimina triloba.* Bioorganic and Medicinal Chemistry. 1996;4(1):25-32.

[19] Ratnayake S, Gu ZM, Miesbauer LR, Smith DL, Wood KV, Evert DR, McLaughlin JL. Parvifloracin and parviflorin: cytotoxic bis-tetrahydrofuran acetogenins with 35 carbons from *Asimina put˙viflora* (Annonaceae). Canadian Journal of Chemistry. 1994;72(3):287-293.

[20] Woo MH, Cho KY, Zhang Y, Zeng L, Gu ZM, McLaughlin JL. Asimilobin and *cis*- and trans-murisolinones, novel bioactive annonaceous acetogenins from the seeds of *AsifTiif2u triloba.* Journal of Natural Products. 1995;58(10):1533-1542.

[21] Woo MH, Zeng L, McLaughlin JL, Asitribin and asimenins A and B, novel bioactive annonaceous acetogenins from the seeds of *Asiniina triloba.* Heterocycles. 1995;41(8):17311742.

[22] Woo MH, Zen- L, Ye Q, Gu ZM, Zhao GX, McLaughlin JL. 16,19-cis-Murisolin and murisolin A, two novel bioactive mono-tetrahydrofuran annonaceous acetogenins from *Asimina triloba* seeds. Bioorganic and Medicinal Chemistry Letters. 1995;5(11):1135-1140.

[23] Woo MH, Kim DH, McLaughlin JL, Asitrilobins A and B: cytotoxic

mono-THF annonaceous acetogenins from the seeds of *Asimina triloba.* Phytochemistry. 1999;50(6):10331040.

[24] Woo MH, Chung SO, Kim DH. Asitrilobins C and D: two new cytotoxic monotetrahydrofuran annonaceous acetogenins from *Asimina triloba* seeds. Bioorganic and Medicinal Chemistry. 2000;8(1):285-290.

[25] Hopp DC, Zeng L, Gu ZM, McLaughlin JL. Squamotacin: an annonaceous acetogenin with cytotoxic selectivity for the human prostate tumor cell line (PC-3). Journal of Natural Products. 1996;59(2):97-99.

[26] Ye Q, He K, Oberlies NH, Zeng L, Shi G, Evert D, McLaughlin JL. Longimicins A-D: novel bioactive acetogenins from *Asisnina longifolia* (Annonaceae) and structure-activity relationships of asimicin type annonaceous acetogenins. Journal of Medicinal Chemistry. 1996; 39(9):1790-1796.

[27] Ye Q, McLaughlin JL, Evert D. Absolute stereochemistries of giganin and loganin, bioactive non-tetrahydrofuran ring annonaceous acetogenins from *Asiniina longifolicr.* Heterocycles. 1996;43(8):1607-1512.

[28] He K, Shi G, Zhao GX, Zeng L, Ye Q, Schwedler JT, Wood KV, McLaughlin JL. Three new adjacent bis-tetrahydrofuran acetogenins with four hydroxyl groups from *Asimina trilobu.* Journal of Natural Products. 1996;59(11):1029-1034.

[29] He K, Zhao GX, Shi G, Zen- L, Chao JF, McLaughlin JL. Additional bioactive annonaceous acetogenins from *Asiriaifzcr triloba* (Annonaceae). Bioorganic and Medicinal Chemistry. 1997;5(3):501-506.

[30] Rieser MJ, Hui YH, Rupprecht JK, Kozlowski JF, Wood KV, McLaughin JL, Hoye TR, Hanson PR, Zhuang ZP. Determination of absolute configuration of stereogenic carbinol centers in annonaceous aceto~enins by I H- and ^{1}~~F- NMR analysis of Mosher ester derivatives. Journal of the American Chemical Society. 1992;114(26):10203-10213.

[31] Gu ZM, Zeng L, Fang XP, Colman-Saizarbitoria T, Huo M, McLaughlin JL. Determining absolute configurations of stereocenters in annonaceous acetogenins through formaldehyde acetal derivatives and Mosher ester methodology. Journal of Organic Chemistry. 1994;59(18): 5162-5172.

[32] Londershausen M, Leicht W, Lieb F, Moeschler H, Weiss H. Molecular mode of action of annonins. Pesticide Science. 1991;33(4):427-438.

[33] Lewis MA, Arnason JL, Philogene BJR, Rupprecht 7K, McLaughlin

7L. Inhibition of respiration at site I by asimicin, an insecticidal acetogenin of the paw paw, *Asimina triloba* (Annonaceae). Pesticide Biochemistry and Physiology. 1993;45(1):15-23.

[34] Hollingworth RM, Ahammadsahib KI, Gadelhak G, McLaughlin JL. New inhibitors of complex I of the mitochondrial electron transport chain with activity as pesticides. Biochemical Society Transactions. 1994;22(1):230-233.

[35] Ahammadsahib KI, Hollingworth RM, McGovren JP, Hui YH, McLaughlin JL. Mode of action of bullatacin: a potent antitumor and pesticidal agent. Life Sciences. 1993;53(14):11131120.

[36] Landolt JL, Ahammadsahib KI, Hollingworth RM, Barr R, Crane FL, Buerck NL, McCabe GP, McLaughlin JL. Determination of structure-activity relationships of annonaceous acetogenins by inhibition of oxygen uptake in rat liver mitochondria. Chemico-Biological Interactions. 1995;98(1):1-13.

[37] Alfonso D, Johnson HA, Colman-Saizarbitoria T, Presley CP, McCabe GP, McLaughlin JL. SARs of annonaceous acetogenins in rat liver mitochondria. Natural Toxins. 1996;4(4):181-8 and Erratum 1996; 4(6):295.

[38] Schuler F, Yano T, Di Bernardo S, Yagi T, Yankovskaya V, Singer TP, Casida JE. NADHquinone oxidoreductase: PSST subunit couples electron transfer from iron-sulfur cluster N2 to quinone. Proceedings of the National Academy of Sciences. 1999;96(7):4149-53.

[39] Oberlies NH, Chang CJ, McLaughlin JL. Structure-activity relationships of diverse annonaceous acetogenins against multidrug resistant human mammary adenocarcinoma (MCF7/adr) cells. Journal of Medicinal Chemistry. 1997;40(13):2102-2106.

[40] Miyoshi H, Ohshima M, Shimada H, Akazi T, Iwamura H, McLaughlin JL. Essential structural factors of annonaceous acetogenins as potent inhibitors of mitochondrial complex 1. Biochimica Biophysica Acta. 1998;1365(3):443-452.

[41] Shimada H, Grutzner JB, Kozlowski JF, McLaughlin JL. Membrane conformations and their relation to cytotoxicity of asimicin and its analogues. Biochemistry. 1998;37(3):854-866.

[42] Shimada H, Kozlowski JF, McLaughlin JL The localisations in liposomal membranes of the tetrahydrofuran ring moieties of the annonaceous acetogenins, annonacin and sylvaticin, as determined by 'H NMR spectroscopy. Pharmacological Research. 1998;37(5):357-384.

[43] Moi¨re DJ, de Cabo R, Farley C, Oberlies NH, McLaughlin JL. Mode of

action of bullatacin, a potent antitumor acetogenin: inhibition of NADH oxidase activity of HELA and HL-60, but not liver, plasma membranes. Life Sciences. 1995;56(5):343-348.

[44] Johnson HA, Oberlies NH, Alali FQ, McLaughlin JL. Thwarting resistance: annonaceous acetogenins as new pesticidal and antitumor agents. In: Cutler H, Cutler S eds. Biologically Active Natural Products: Pharmaceuticals ACS Symposium Book. Boca Raton, LA: CRC Press; 1999:173-183.

[45] Oberlies NH, Jones JL, Corbett TH, Fotopoulos SS, McLaughlin JL. Tumor cell growth inhibition of annonaceous acetogenins in an *in vitro* disk diffusion assay. Cancer Letters. 1995; 96(1):55-62.

[46] Oberlies NH, Croy VL, Harrison MH, McLaughlin JL. The Annonaceous acetogenin bullatacin is cytotoxic against multidrug resistant human mammary adenocarcinoma cells.
Cancer Letters. 1997;115(1):173-179.

[47] Oberlies NH, Alali FQ, McLaughlin JL. Annonaceous acetogenins: thwarting ATP dependent resistance. In: Calls I, Ersoz T, Basaran AA, eds. New Trends and Methods in Natural Products Research, Proceedings of the 12'" International Symposium on Plant Originated Crude Drugs, May 20-22, 1998. Ankara, Turkey: The Scientific and Technical Research Council of Turkey; 1999:192-223.

[48] Fu LW, Pan QC, Liang YJ, Huang HB. Circumvention of tumor multidrug resistance by a new annonaceous acetogenin: atemoyacin-B. Zhongguo Yao Li Xue Bao. 1999;20(5):435-439.

[49] Hopp DC, Zeng L, Gu ZM, Kozlowski JF, McLaughlin JL. Novel mono-tetrahydrofuran ring acetogenins, from the bark of *Annona squamosa,* showing cytotoxic selectivities for the human pancreatic carcinoma cell line, PACA-2. Journal of Natural Products. 1997;60(6):581586.

[50] Ayre SG, Garcia y Bellon DP, Garcia Jr. DP. Insulin, chemotherapy, and the mechanisms of malignancy: the design and the demise of cancer. Medical Hypothesis. 2000;55(4):330-334.

[51] Papa V, Pezzino V, Constantino A, Belfiore A, Giuffrida D, Frittitta L, Vannelli GB, Brand R, Goldfine ID, Vigneri R. Elevated insulin receptor content in human breast cancer. Journal of Clinical Investigation. 1990;86(5):1503-1510.

[52] Cullen JK, Yee D, Sly WS, Perdue J, Hampton B, Lippman ME, Rosen N. Insulin-like growth factor receptor expression and function in human breast cancer. Cancer Research. 1990;50(1):48-53.

[53] Chih HW, Chiu HF, Tang KS, Chang FR, Wu YC. Bullatacin, a potent antitumor annonaceous acetogenin, inhibits proliferation of human hepatocarcinoma cell line 2.2.15 by apoptosis induction. Life Sciences. 2001;69(11):1321-1331.

[54] Chiu HF, Chih TT, Hsian YM, Tseng CH, Wu MJ, Wu YC. Bullatacin, a potent antitumor Annonaceous acetogenin, induces apoptosis through a reduction of intracellular cAMP and cGMP levels in human hepatoma 2.2.15 cells. Biochemical Pharmacology. 2003;65(3):319-327.

[55] McLau~hlin JL, Zeng L, Oberlies NH, Alfonso D, Johnson HA, Cummings BA. Annonaceous acetogenins as new natural pesticides: recent progress. In: Hedin P, Hollingwor¯th R, Mujamoto J, Mesler E, Thompson D, eds. Phytochemical Pest Control Agents. Washinaton, D.C.: ACS Symposium; 1997:117-130.

[56] McLau~hlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. Drug Information Journal. 1998; 32(2):513-524.

[57] Ratnayake S, Rupprecht JK, Potter WM, McLaughlin JL. Evaluation of various parts of the paw paw tree, *Asi»iinia triloba* (Annonaceae), as commercial sources of the pesticidal annonaceous acetogenins. Journal of Economic Entomology. 1992;85(6):2353-2356.

[58] Gu ZM, Zhou D, Wu J, Shi G, Zeng L, McLaughlin JL. Screening for Annonaceous acetogenins in bioactive plant extracts by liquid chromatography/mass spectrometry. Journal of Natural Products. 1997;60(3):242-248.

[59] Gu ZM, Johnson HA, Zhou D, Wu J, Gordon J, McLaughlin JL. Quantitative evaluation of annonaceous acetogenins in monthly samples of paw paw *(Asimina triloba)* twigs by liquid chromatography/electrospray ionization/tandem mass spectrometry. Phytochemical Analysis. 1999;10(1):32-38.

[60] Avalos J, Rupprecht JK, McLaughlin JL, Rodriguez E. Guinea pig maximization test of the bark extract of paw paw. Contact Dermatitis. 1993;29(1):33-35.

[61] Guadano A, Gutierrez C, de la Pena E, Cortes D, Gonzales-Coloma A. Insecticidal and mutagenic evaluation of two annonaceous acetogenins. Journal of Natural Products. 2000;63(6): 773-776.