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Review

Identification of Important Compounds Isolated from Natural Sources that Have Activity Against Multidrug-resistant Cancer Cell Lines: Effects on Proliferation, Apoptotic Mechanism and the Efflux Pump Responsible for Multi-resistance Phenotype

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Abstract. *The focus of this mini-review is to identify non-toxic compounds isolated from natural sources (plants) that exhibit specific activity against efflux pumps of specific multidrug-resistant (MDR) cancer cell lines, inhibit proliferation of the MDR cancer cell lines and inhibit the activity of overexpressed efflux pumps of the MDR cancer cell line.*

Therapy of cancer, if not completely effective, results in the overexpression of genes that code for efflux pumps that extrude the noxious agent (anticancer drug) before it reaches its intended target of the cancer cell (1). The overexpressed efflux pump renders the cancer cell immune to not only the initial drug used in therapy but to a wide range of unrelated noxious compounds (1). The arising of this multidrug-resistant (MDR) phenotype makes therapy highly problematic and the end result is that the patient succumbs to the disease (1). Therefore, it is the consensus of many that therapy of MDR cancer may succeed if the responsible efflux pump is inhibited from its activity, therefore, affording the increased concentration of anticancer drug to a level consistent with its toxic targeted action needed to kill the cancer cell. To this extent, over 100,000 synthetic compounds for activity against the known efflux pumps of cancer cell types have been screened by the National Cancer Institute (USA) and, to date, not a single inhibitor has found its way toward successful therapy of MDR cancer. In fact, because normal counter type cells have a fully functional efflux pump, albeit at a much lower level of activity, clinical trials with selected efflux pump inhibitors have produced high toxicity and even death.

During the past two decades, traditional medicine has become a source for the identification of plants that harbor compounds that may have important potential for the therapy of cancer. With respect to the laboratory of one of the authors of this mini-review (JM), literally, hundreds of plants have been studied for their activity against specific targets of the cancer cell and it is the focus of this mini-review to identify selected compounds that have no toxicity at the level of their *in vitro* study and which have activity against proliferation or induction of the apoptotic mechanism or the efflux pump responsible for the phenotype of the MDR cancer cell.

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Targets of MDR Cancer Cells

Proliferation. Proliferation is affected by many drugs and, although the search for non-toxic compounds that inhibit proliferation was initiated more than 60 years ago, remains the focus of most studies; however, the inhibition of proliferation by compounds isolated from natural sources will not be discussed here at the level of its mechanism. Rather, suffice to say that our observations for anti-proliferative activity as presented in the text remain, for the most part, not completely understood.

Apoptosis. Programmed cell death or apoptosis maintains homeostasis; furthermore, it has a major impact on the evolution of organs. However, defect in apoptosis can cause cancer and the suppression of apoptosis during carcinogenesis may play a crucial role in the development of some cancer types (2). Apoptosis is characterized by different morphological changes, such as cell membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentation (3, 4). In order to promote phagocytosis of apoptotic cells by macrophages, apoptotic cells present specific membrane morphologies to activate this process. One of these changes is the translocation of phosphatydilserine from the inside of the cell to the outer surface, followed by membrane blebbing and small vesicles called apoptotic bodies (5). In the early stages of apoptosis, caspases are activated in order to initiate and accomplish the cleavage of essential cellular components. Caspases can be activated by either of the two known apoptotic signaling pathways, *i.e.* intrinsic (mitochondria-mediated) and extrinsic (death receptor-mediated) pathways. In addition, there is a third pathway, described as intrinsic endoplasmic reticulum (ER) pathway, which involves the ER and is based on the oxidative stress response (6). These pathways and their components are targets for agents that are designed to kill cancer cells and, because many compounds isolated from botanical sources have effects on cancer cells that display qualitative and quantitative properties that differ significantly from their normal counterpart cells, we have investigated the potential effect of compounds isolated from natural sources to induce apoptosis in cancer cells and distinguish these effects from effects of the agents on overexpressed efflux pumps that contribute to the multidrug resistance of some cancers.

Efflux Pumps

The major mechanism responsible for the MDR phenotype of MDR cancer cells is the overexpression of ATP-dependent transporters that belong to the ATP-binding cassette (ABC) family. These ATP-dependent transporters extrude noxious agents from the cancer cell before they reach their intended targets. These transporters are termed efflux pumps (EPs) and three major types have been identified for specific cancer cell lines: the ABCB, which has been the most studied of the three EPs, also known as ABCB1 or MDR1 or P-glycoprotein (Pgp1), the ABCC, also known as ABCC1 or MRP1 or ABCC2 or MRP2 or, infrequently, specified as ABCC3–6 and ABCC10–11, as well as the ABCG also known as ABCG2 or MXR or BCRP. The overexpression of these ABC EPs and the resistance of MDR cancer cells to anticancer agents are of obvious importance for chemotherapy of MDR cancer.

Pgp1 (ABCB1/MDR1) was isolated from Chinese hamster cell lines in the laboratory of Victor Ling in 1976 and its amino acid sequence determined by the same investigating laboratory in 1989 (7). From these and other studies involving the human Pgp1, the structure of Pgp1, as it may appear on the plasma membrane of an MDR cancer cell, is presented by Figure 1.

Briefly, Pgp1 is a 170-kDa transmembrane protein that consists of two halves; the N-terminal half of the molecule contains 6 transmembrane domains that is followed by a large cytoplasmic domain with an ATP-binding site and, then, a second section with 6 transmembrane domains and an ATP-binding site that has amino acid similarity with the first half of the polypeptide. The substrate binding sites are formed by the transmembrane domains. The binding of the substrate takes place simultaneously with the binding of ATP and its hydrolysis. Hydrolysis of ATP alters the conformation of the Pgp1 and the bound substrate is released to the surface of the cell. The release of the substrate results in the restoration of Pgp1 conformation that is now ready to accept another noxious molecule for binding and another ATP for hydrolysis.

The inhibition of Pgp1 can occur *via* three pathways: (i) Direct binding of the inhibitor by the substrate binding site of the Pgp1 domain. Binding may be competitive, meaning that, as the concentration of the inhibitor is increased, the greater the probability that noxious agent remains in the cytoplasm of the cell, or, it may be non-competitive, which means that the affinity of the inhibitor for the substrate binding site is significantly greater than that of the noxious substrate. Irreversible binding of the inhibitor, although possible, is not useful since the same irreversible binding would take place with Pgp1 proteins of normal cells, thus compromising the life of the patient. (ii) Inhibition of the ATP binding domain denying the conformational changes needed for translocation of the noxious substrate. (iii) Inhibition of ATP synthesis from glycolysis within the MDR cancer cell. Although this latter path may also affect normal counterpart cells, because most cancer cells have impaired mitochondria, ATP sources are primarily glycolytic, normal cells may not be as severely affected as the MDR cancer cell.

Efflux Pump Models Used for the Investigations Described in this Mini-review

A cell line used for determination of effects of compounds from natural sources on efflux is a murine lymphoma cell that has been transfected with the human *MDR1* gene that codes for Pgp1 (9). It is important to note that the parental mouse lymphoma cell line has its own efflux pump. However, the sensitivity of the method used for determining the effects of a compound on the retention of an anticancer drug (efflux assay employing the fluorescent substrate rhodamine 123 and its assay *via* flow cytometry) is below

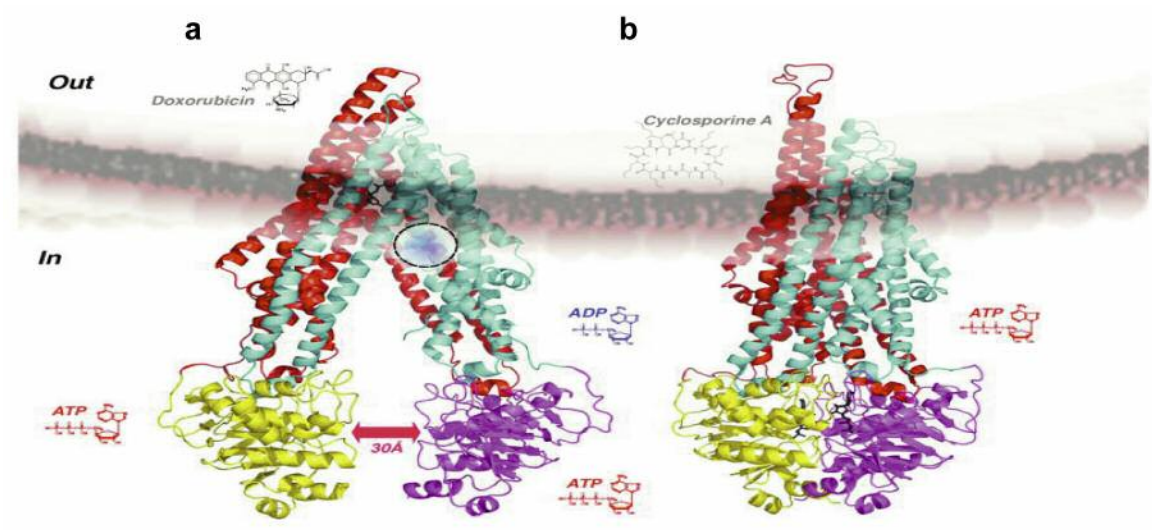

Figure 1. *Theoretical function of Pgp and its location on the plasma membrane. (a) Ribbon-like structure of P-glycoprotein in a unit plasma membrane. ATP (red) and ADP (blue) molecules present in the cytoplasm and two P-gp substrate/inhibitor molecules (black) are in the extracellular space. N-terminal transmembrane domain and nucleotide-binding domain are colored red and yellow, respectively, and the C-terminal transmembrane domain and nucleotide-binding domain are violet and magenta, respectively. The bound QZ59-SSS inhibitor in the transmembrane (TM) region is shown as a stick model in black. The dashed black circle area is proposed to be one of the portals for entry of substrates or modulators directly from the membrane. (b) Model of human P-gp based on the structure of S. aureus SAV1866 bound to ADP and open to the extramembrane space (PDB: 2HYD). Two bound ADP molecules are shown in black. The color code follows that in (a) (8).*

the threshold of detection. Hence, the effects noted are directly related to those on the efflux of rhodamine 123 by the presence of the human *MDR1* gene that has been transfected into the mouse lymphoma cell line.

An additional method that is fully automated has been developed by our laboratory for the measurement of efflux of the fluorescent substrate ethidium bromide. This method, completely described by Spengler *et al*. (10) provides real-time data on influx/efflux parameters under defined conditions of temperature, time, concentrations and physiological constituents of the media, such as pH, ionic strength, *etc*. Examples of data obtained from flow cytometry and ethidium bromide methods are presented in Figure 2.

Activity of Non-toxic Compounds Isolated from Natural Sources (Plants) on MDR Cancer Properties

Traditional medicine is now widely considered for the identification of plants that are known to have medicinal qualities. Because, for almost all cases, the remedial activity of the untreated plant is moderate to nominal for specific pathologies due to the limitations imposed by the largess of plant that must be ingested, the need for isolation of the responsible compound and subsequent medicinal chemistry approaches must be taken. With respect to our two plus decades' attention to plant sources for compounds that may have significant potential for therapy of MDR, the following sections identify the plant source, the identity of the compound isolated and the property of the MDR cancer cell affected (proliferation, apoptotic mechanism, efflux pumps).

Terpenoids: Diterpenes and Triterpenes

Diterpenes and Triterpenes are classes of chemical compounds composed of two and three terpene units, respectively (Figure 3 – example of a triterpene) with the molecular formula C20H32 and C30H48, respectively and are made by animals, plants and fungi.

This class of compounds contains members with biological activities against two of the most important diseases: multidrug-resistant tuberculosis (11-13) and multidrug-resistant cancer (14, 15). Because the triterpene structure is central to all steroids, ecdysteroids produced by plants and insects have been studied for their anticancer properties as well (16-18). Derivatives of anticancer triterpenes have also received attention (19). The anticancer properties of specific diterpenes and triterpenes are inhibition of the replication of cancer cells by affecting the S phase of DNA synthesis (20) supposedly by inhibiting DNA polymerase activity (21), activating the apoptotic pathway (20-23) and inhibiting the activity of the efflux

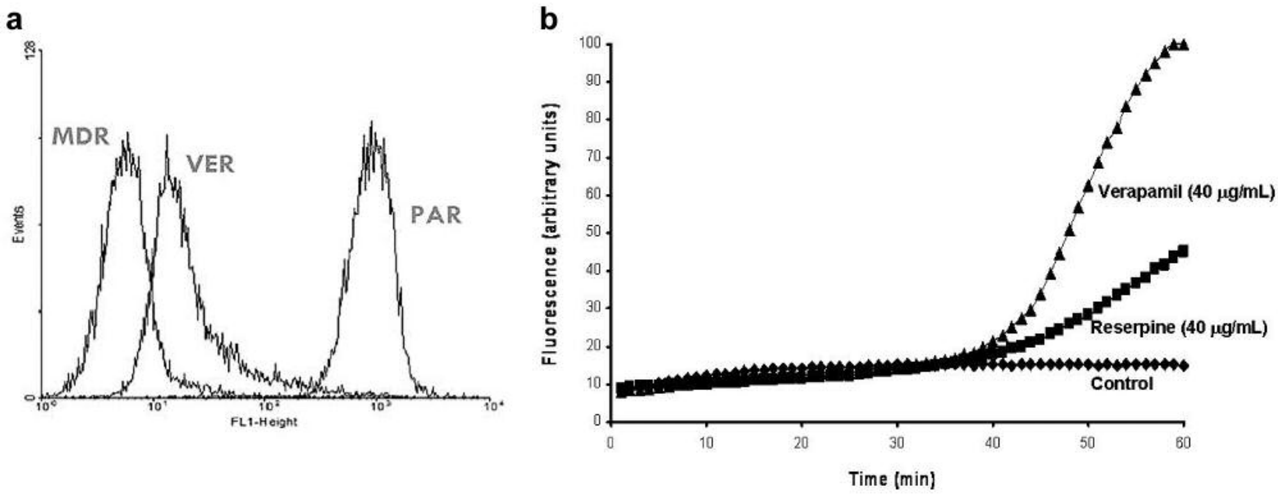

Figure 2. *Examples of data obtained from flow cytometry and ethidium bromide methods. (a) Flow cytometry: The accumulation of rhodamine 123 by parental mouse lymphoma cells (PAR) is at the extreme right of the figure; that of the mouse lymphoma cell transfected with the human MDR 1 gene is at the extreme left of the figure. The addition of the efflux pump inhibitor verapamil to the MDR cell line shifts the accumulation of rhodamine 123 towards that of the PAR. This shift is interpreted to show that the fluorescent rhodamine 123 is now retained and approximates that of the PAR. (b) Example of efflux data obtained from the ethidium bromide method: Mouse lymphoma cells transfected with the human MDR 1 gene are incubated with the efflux pump substrate ethidium bromide with and without the efflux pump inhibitors verapamil or reserpine. Note that these agents increase the retention of ethidium bromide above that than the control lacking these inhibitory agents.*

pump Pgp1 of cancer cell lines (14-23). The inhibition of DNA synthesis by direct inhibition of DNA polymerase and induction of apoptosis are important anticancer targets being affected by some diterpenes and triterpenes. However, it is the effect of this class of compounds on the activity of Pgp1 (the main efflux pump of most multidrug-resistant cancer cells responsible for the multidrug-resistant phenotype) that deserves major attention given that, by inhibiting the pump, the cancer cell becomes susceptible to cytotoxic drugs to which it was initially resistant. Non-cancer cells have the same efflux pump that is overexpressed by multidrug-resistant cancer cells. Consequently, the efflux pump activities of terpenoids would be expected to produce toxicity against normal cells. However, the concentrations that are proven to be effective *in vitro* for the inhibition of the efflux pump of cancer cells, whenever studied, have expressed little or no cytotoxic activity against normal cells. Nevertheless, because the efflux pump of normal cells will similarly be affected by the terpenoid compound, one would expect that the adjuvant use of the terpenoid would promote an even greater negative effect of the anticancer drug against normal cells. Perhaps, this negative effect can be acceptable if the activity of the anticancer agent is effective against multidrug-resistant cancer, which would make therapy of multidrug-resistant cancer less problematic or even successful.

Sources of Important Terpenoids with High Potential for Therapy of Multidrug-resistant Cancers

1. Carpobrotus edulis. During Professor Molnar's visit to my home (L. Amaral) in 2001 and one of the many walks along the coastal cliffs of Cascais, Portugal, a particular plant "stole" his attention from our discussion of future research plans and the role of his Cost Action B16 recently funded by the European Commission. As one would have it, the specific plant that drew his attention was prominently displayed at the edge of a cliff some 50 meters above the pounding surf. The precarious position of this plant did not faze Professor Molnar; he approached it and examined it for he had never seen this plant before. He asked me if I knew the species and I responded that, although I did not know its name, the entire coastal area of Portugal is inundated with this plant and that there was no need to take any further risk if he had an interest in the plant. In a much safer area few meters away, the plant was in abundance and Professor Molnar took a few samples that consisted of the yellow flower, fleshy stem and roots supposedly for further study. A few weeks after his return to Szeged, Hungary, he called me and excitedly told me that the methanol extract caused his cell model of multidrug-resistant mouse lymphoma cells transfected with the gene that codes for the human efflux pump protein Pgp1to become fully susceptible to doxyrubicin, a cancer drug to which the hybrid cell line was initially resistant *via* the overexpression of its efflux pump. Knowing that my assistant's father Professor Viveiros was Portugal's premier botanist and Professor Emeritus of the University of Lisbon, I asked his son Dr. Miguel Viveiros if his father could identify the plant and provide as much information as possible. The plant was identified by Professor Viveiros as *C. edulis*, a member of the Aizoaceae family and, hence, a potential source of alkaloids with neuroleptic activity (34). It is about a common, nuisance plant that grows on the coast of Portugal and has its origins in coastal areas of South Africa where it is known as "Hottentot-fig" since its fig-like fruit is edible. The species is represented by two varieties one that produces a yellow flower and the other a purple flower. Cross pollination is common and the hybrid products are indistinguishable from the purple yielding variety. However, the degree of successful frequency of hybrids is dependent upon the salinity of the coastal soil (35). The information provided perked our interest in Portugal since the neuroleptic thioridazine had been shown by Amaral's group to have remarkable *in vitro* (36) and *ex vivo* activity (37). Thus, a joint study was undertaken between the Molnar and Amaral group that would examine the methanol extract for properties against multidrug-resistant cancer and multidrug-resistant *Mycobacterium tuberculosis* and then isolate the active compounds and determine their mode of action(s). Briefly, the results of the cancer component of the study (38) show that the extract is non-toxic at concentrations that inhibit a verapamil-sensitive efflux pump of the L5178 mouse T cell lymphoma cell line, thereby rendering these multidrug-resistant cells susceptible to anticancer drugs. These non-toxic concentrations of the methanol extract also prime THP-1 human monocyte-derived macrophages to kill ingested Staphylococcus aureus, promote the release of lymphokines associated with cellular immune functions and induce the proliferation of THP-1 cells within 1 day of exposure as is the case with phytohaemagglutinin. The potential role of the compound(s) isolated from *Carpobrotus edulis* in cancer biology and related immunological events during development of cancer was intriguing and prompted a study that would isolate the biologically active compounds of the extract and study them in detail with respect to inhibitory properties, such as replication, induction of apoptosis and inhibition of the Pgp1 efflux pump responsible for resistance of multidrug-resistant cancer cell lines. This study involved a bioassay-guided separation protocol, including the testing of the extracts, fractions and pure compounds for their ability to inhibit P-glycoprotein responsible for multidrug resistance of the mouse lymphoma cells containing the human efflux pump gene *MDR1* and led to the isolation of seven compounds from the chloroform and ethyl acetate soluble fractions of the methanolic extract of *Carpobrotus edulis*. The compounds were identified by 1-Dimension, 2-Dimension NMR and MS investigations as triterpenes (beta-amyrin, uvaol and oleanolic acid), monogalactosyldiacylglycerol, catechin, epicatechin and procyanidin B5. The triterpene uvaol was the most effective, non-toxic and promising compound for development as an adjunct to doxyrubicin therapy of multidrug-resistant cancer (39).

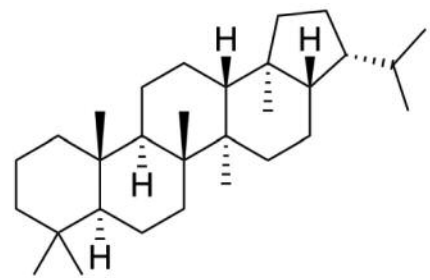

Figure 3. *Hopane: An example of a pentacyclic triterpene.*

2. Euphorbia portlandica. The genus *Euphorbia* contains almost 1,600 species characterized by the presence of white milky, usually toxic, latex. Nevertheless, this group of plants has been the subject of intense phytochemical research during recent years due to the wide-spread use of its members in traditional medicine. The compounds isolated from its extracts include: flavanoids, terpenoids, alkanes, amino acids and alkaloids many of which have shown potential in the therapy of important pathologies. *Euphorbia portlandica*, a species of flowering plant, belongs to this family and is endemic to coasts of Western Europe ranging from Portugal, the northern coasts of Spain and France and as far north as Scotland. The first report of anticancer properties of compounds isolated from a methanol extract of the whole plant of *Euphorbia portlandica* was made in 2004 from the collaboration between Professor Maria-Jose Ferreira of the University of Lisbon and Professor Joseph Molnar (40). The active compounds were identified as diterpenes (5'beta,9'alpha,10'alpha)-7-0-(3alpha-methoxy-8'(12')-drimen-11'-yl)-scopoletin, designated driportlandin (compound 1) and a new abietane quinoid diterpene 16-hydroxy-abieta-8,12-diene-11,14-dione, named portlanquinol (compound 2). Although both compounds 1 and 2 were found to reverse the resistance of mouse lymphoma cells transfected with the *Pgp1* gene, compound 2 was found to be toxic. In 2006, further collaboration between Joseph Molnar and Maria-Jose Ferreira resulted in the isolation and characterization of 12 tetracyclic triterpenes from an acetone extract of *Euphorbia portlandica* (41). The new triterpenes were shown to reverse the resistance of the mouse lymphoma cell line model (42).

Additional terpenoids from the genus *Euphorbia* have been isolated, identified, characterized and examined regarding their biological properties by the Molnar-Ferreira collaboration and each has been shown to have distinct properties that inhibited replication, induced apoptosis and inhibited the efflux pump machinery of the mouse lymphoma cell line transfected with the human gene that codes for the Pgp1 transporter (28, 43, 44). These studies indicate that the genus *Euphorbia* is a rich source of terpenoid compounds that have important potential for therapy of multidrug-resistant cancer. Because this genus contains almost 1,600 species, the interested reader has an open field for drug discovery of new anticancer agents whose sources are the species that make up this genus.

3. Momordica balsamina Linn. Momordica is a genus of about 60 species of annual or perennial climbers herbaceous or rarely small shrubs belonging to the family Cucurbitaceae, natives of tropical and subtropical Africa and Asia, as well as Australia. The genus attracted the attention of Professor Maria-Jose Ferreira since one of its members, *Momordica charantia*, was well-known in the traditional medicine widely practiced in China as therapy for hypoglycemia seemingly improving the rate of wound healing. The genus *Momordica* has also provided a rich source of terpenoids with activities against cancer cells. From the aerial parts of *Momordica balsamina*, five new cucurbitane-type triterpenoids (1-5) and two known analogues have been isolated and characterized for cytotoxicity against human breast cancer cells (MCF-7). Their structures were determined by spectroscopic methods, including 2D NMR experiments (COSY, HMQC, HMBC and NOESY). The new compounds presented unusual oxidation patterns in the cucurbitane skeleton at C-29 and C-12 of some of the compounds isolated. Compounds 1-4, 6 and 7 were shown to have high *in vitro* cytotoxicity against a human breast cancer cell line (44). Four of these curcubitane-type triterpenes compounds were studied for their inhibition of the ABC transporter P-glycoprotein coded by the human *ABCB1* gene transfected into mouse lymphoma cells. The evaluation was conducted by flow cytometry using rhodamine 123 and real-time fluorometry assessing accumulation of ethidium bromide on a real-time basis. The most active compound that inhibited efflux of ethidium bromide and rhodamine 123 from the *ABCB1*-transfected mouse lymphoma cell was 7-methoxycucurbita-5,24-diene-3beta,23(R)-diol (44). Interestingly, this compound has a minor effect on the replication of the cell line used by Spengler *et al*. (44) and, hence, we can deduce that it has little toxicity. Although the genus has few species, similar studies should be considered with the remaining unstudied species of this genus.

Conclusion/Summary/Questions

There are some questions that require answers, however, prior to serious consideration of adjuvant use of tripertinoids. A large study that quantifies the ability of each reported active tripertinoid to reverse resistance of members of a panel of multidrug-resistant cancer cells to given anticancer agents (*i.e.* inhibit the main efflux pump responsible for multidrug resistance) is needed. The identified major active compounds would also need to be evaluated for cytotoxic activity against a panel of normal cells. Then, it would be possible to select for clinical trial(s) bioactive tripertinoid compounds as adjuvants for specific therapies of specific multidrug-resistant cancers.

References

1 Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, Hu T, Jiang L and Li J. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. Cancer Lett *370*: 153-164, 2016. http://www.ncbi.nlm.nih.gov/pubmed/?term=1.%09Chen+Z%2C+Shi+T%2C+Zhang+L%2C+Zhu+P%2C+Deng+M%2C+Huang+C%2C+Hu+T%2C+Jiang+L%2C+Li+J.

2 Elmore S: Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol *35*: 495-516, 2007. http://www.ncbi.nlm.nih.gov/pubmed/?term=Apoptosis%3A+A+Review+of+Programmed+Cell+Death+.Toxicol+Pathol.+35%3A+495%E2%80%93516%2C+2007.

3 Tian H, Gao Z, Li H, Zhang B, Wang G, Zhang Q, Pei D and Zheng J: DNA damage response-a double-edged sword in cancer prevention and cancer therapy. Cancer Lett *358*: 8-16, 2015. http://www.ncbi.nlm.nih.gov/pubmed/?term=Tian+H%2C+Gao+Z%2C+Li+H%2C+Zhang+B%2C+Wang+G%2C+Zhang+Q%2C+Pei+D%2C+Zheng+J

4 Su Z, Yang Z, Xu Y, Chen Y and Yu Q: Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol Cancer *14*: 48, 2015. http://www.ncbi.nlm.nih.gov/pubmed/25743109

5 Hassan M, Watari H, AbuAlmaaty A, Ohba Y and Sakuragi N: Apoptosis and molecular targeting therapy in cancer. Biomed Res Int *2014*: 150845, 2014. http://www.ncbi.nlm.nih.gov/pubmed/?term=Hassan+M%2C+Watari+H%2C+AbuAlmaaty+A%2C+Ohba+Y%2C+Sakuragi+N

6 Banerjee S, Uppal T, Strahan R, Dabral P, and Verma SC: The Modulation of Apoptotic Pathways by Gammaherpesviruses. Front Microbiol *7*: 585, 2016, doi: 10.3389/fmicb.2016.00585. eCollection 2016. http://www.ncbi.nlm.nih.gov/pubmed/?term=Banerjee+S%2C+Uppal+T%2C+Strahan+R%2C+Dabral+P%2C+Verma+SC

7 Ng WF, Sarangi F, Zastawny RL, Veinot-Drebot L and Ling V: Identification of members of the P-lycoprotein multigene family. Mol Cell Biol *9*: 1224-1232, 1989. http://www.ncbi.nlm.nih.gov/pubmed/?term=Ng+WF%2C+Sarangi+F%2C+Zastawny+RL%2C+Veinot-Drebot+L%2C+Ling+V

8 Gottesman MM, Ambudkar SV and Xia D: Structure of a multidrug transporter. Nat Biotechnol *27*: 546-547, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19513059

9 Cantrell CL, Franzblau SG and Fischer NH: Antimycobacterial plant terpenoids. Planta Med *67*: 685-694, 2001. http://www.ncbi.nlm.nih.gov/pubmed/11731906

10 Spengler G, Viveiros M, Martins M, Rodrigues L, Martins A, Molnar J, Couto I and Amaral L: Demonstration of the activity of P-glycoprotein by a semi-automated fluorometric method. Anticancer Res *29*: 2173-2177, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19528478

11 Martins M, Ordway D, Kristiansen M, Viveiros M, Leandro C, Molnar J and Amaral L: Inhibition of the *Carpobrotus edulis* methanol extract on the growth of phagocytosed multidrug-resistant *Mycobacterium tuberculosis* and methicillin-resistant Staphylococcus aureus. Fitoterapia *76*: 96-99, 2005. http://www.ncbi.nlm.nih.gov/pubmed/15664469

12 Chudzik M, Korzonek-Szlacheta I and Król W: Triterpenes as potentially cytotoxic compounds. Molecules *20*: 1610-1625, 2015. http://www.ncbi.nlm.nih.gov/pubmed/?term=Chudzik+M%2C+Korzonek-Szlacheta+I%2C+Kr%C3%B3l+W

13 Rodriguez-Rodriguez R: Oleanolic acid and related triterpenoids from olives on vascular function: molecular mechanisms and therapeutic perspectives. Curr Med Chem *22*: 1414-1425, 2015. http://www.ncbi.nlm.nih.gov/pubmed/25515513

14 Martins A, Vasas A, Schelz Z, Viveiros M, Molnár J, Hohmann J and Amaral L: Constituents of *Carpobrotus edulis* inhibit P-glycoprotein of MDR1-transfected mouse lymphoma cells. Anticancer Res *30*: 829-835, 2010. http://www.ncbi.nlm.nih.gov/pubmed/?term=Martins+A%2C+Vasas+A%2C+Schelz+Z%2C+Viveiros+M%2C+Moln%C3%A1r+J%2C+Hohmann+J%2C+Amaral+L.

15 Martins A, Sipos P, Dér K, Csábi J, Miklos W, Berger W, Zalatnai A, Amaral L, Molnár J, Szabó-Révész P and Hunyadi A: Ecdysteroids sensitize MDR and non-MDR Cancer cell lines to doxorubicin, paclitaxel, and vincristine but tend to protect them from cisplatin. Biomed Res Int *2015*: 895360, 2015. http://www.ncbi.nlm.nih.gov/pubmed/?term=Martins+A%2C+Sipos+P%2C+D%C3%A9r+K%2C+Cs%C3%A1bi+J%2C+Miklos+W%2C+Berger+W%2C+Zalatnai+A%2C+Amaral+L%2C+Moln%C3%A1r+J%2C+Szab%C3%B3-R%C3% A9v%C3%A9sz+P%2C+Hunyadi+A

16 Martins A, Csábi J, Balázs A, Kitka D, Amaral L, Molnár J, Simon A, Tóth G and Hunyadi A: Synthesis and structure-activity relationships of novel ecdysteroid dioxolanes as MDR modulators in cancer. Molecules *18*: 15255-15275, 2013. http://www.ncbi.nlm.nih.gov/pubmed/?term=Martins+A%2C+Cs%C3%A1bi+J%2C+Bal%C3%A1zs+A%2C+Kitka+D%2C+Amaral+L%2C+Moln%C3%A1r+J%2C+Simon+A%2C+T%C3%B3th+G%2C+Hunyadi+A.

17 Martins A, Tóth N, Ványolós A, Béni Z, Zupkó I, Molnár J, Báthori M and Hunyadi A: Significant activity of ecdysteroids on the resistance to doxorubicin in mammalian cancer cells expressing the human ABCB1 transporter. J Med Chem *55*: 5034-5043, 2012. http://www.ncbi.nlm.nih.gov/pubmed/?term=Martins+A%2C+T%C3%B3th+N%2C+V%C3%A1nyol%C3%B3s+A%2C+B%C3%A9ni+Z%2C+Zupk%C3%B3+I%2C+Moln%C3%A1r+J%2C+B%C3%A1thori+M%2C+Hunyadi+A

18 Balázs A, Hunyadi A, Csábi J, Jedlinszki N, Martins A, Simon A and Tóth G: 1H and 13C NMR investigation of 20-hydroxyecdysone dioxolane derivatives, a novel group of MDR modulator agents. Magn Reson Chem *51*: 830-836, 2013. http://www.ncbi.nlm.nih.gov/pubmed/?term=Bal%C3%A1zs+A%2C+Hunyadi+A%2C+Cs%C3%A1bi+J%2C+Jedlinszki+N%2C+Martins+A%2C+Simon+A%2C+T%C3%B3th+G

19 Liu H, Liu YQ, Liu YQ, Xu AH, Young CY, Yuan HQ and Lou HX: A novel anticancer agent, retigeric acid B, displays proliferation inhibition, S phase arrest and apoptosis activation in human prostate cancer cells. Chem Biol Interact *188*: 598-606, 2010. http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu+H%2C+Liu+YQ%2C+Liu+YQ%2C+Xu+AH%2C+Young+CY%2C+Yuan+HQ%2C+Lou+HX

20 Murakami C, Ishijima K, Hirota M, Sakaguchi K, Yoshida H and Mizushina Y: Novel anti-inflammatory compounds from Rubus sieboldii, triterpenoids, are inhibitors of mammalian DNA polymerases. Biochim Biophys Acta *1596*: 193-200, 2002. http://www.ncbi.nlm.nih.gov/pubmed/?term=Murakami+C%2C+Ishijima+K%2C+Hirota+M%2C+Sakaguchi+K%2C+Yoshida+H%2C+Mizushina+Y

21 Fulda S: Modulation of apoptosis by natural products for cancer therapy. Planta Med *76*: 1075-1079, 2010. http://www.ncbi.nlm.nih.gov/pubmed/20486070

22 Mullauer FB, Kessler JH and Medema JP: Betulinic acid, a natural compound with potent anticancer effects. Anticancer Drugs *21*: 215-227, 2010. http://www.ncbi.nlm.nih.gov/pubmed/25893306

23 Valente I, Reis M, Duarte N, Serly J, Molnár J and Ferreira MJ: Jatrophane diterpenes from *Euphorbia* mellifera and their activity as P-glycoprotein modulators on multidrug-resistant mouse lymphoma and human colon adenocarcinoma cells. J Nat Prod *75*: 1915-1921, 2012. http://www.ncbi.nlm.nih.gov/pubmed/?term=Valente+I%2C+Reis+M%2C+Duarte+N%2C+Serly+J%2C+Moln%C3%A1r+J%2C+Ferreira+MJ

24 Csupor-Löffler B, Hajdú Z, Zupkó I, Molnár J, Forgo P, Vasas A, Kele Z and Hohmann J: Antiproliferative constituents of the roots of Conyza canadensis. Planta Med *77*: 1183-1188, 2011. http://www.ncbi.nlm.nih.gov/pubmed/?term=Csupor-L%C3%B6ffler+B%2C+Hajd%C3%BA+Z%2C+Zupk%C3%B3+I%2C+Moln%C3%A1r+J%2C+Forgo+P%2C+Vasas+A%2C+Kele+Z%2CHohmann+J

25 Dehelean CA, Feflea S, Molnár J, Zupko I and Soica C: Betulin as an antitumor agent tested *in vitro* on A431, HeLa and MCF7, and as an angiogenic inhibitor in vivo in the CAM assay. Nat Prod Commun *7*: 981-985, 2012. http://www.ncbi.nlm.nih.gov/pubmed/?term=Dehelean+CA%2C+Feflea+S%2C+Moln%C3%A1r+J%2C+Zupko+I%2C+Soica+C

26 Duarte N, Ramalhete C, Varga A, Molnár J and Ferreira MJ: Multidrug resistance modulation and apoptosis induction of cancer cells by terpenic compounds isolated from *Euphorbia* species. Anticancer Res *29*: 4467-4472, 2009. http://www.ncbi.nlm.nih.gov/pubmed/?term=Duarte+N%2C+Ramalhete+C%2C+Varga+A%2C+Moln%C3%A1r+J%2C+Ferreira+MJ

27 Duarte N, Gyémánt N, Abreu PM, Molnár J and Ferreira MJ: New macrocyclic lathyrane diterpenes, from *Euphorbia* lagascae, as inhibitors of multidrug resistance of tumour cells. Planta Med *72*: 162-168, 2006. http://www.ncbi.nlm.nih.gov/pubmed/?term=Duarte+N%2C+Gy%C3%A9m%C3%A1nt+N%2C+Abreu+PM%2C+Moln%C3%A1r+J%2C+Ferreira+MJ

28 Ramalhete C, Mansoor TA, Mulhovo S, Molnár J and Ferreira MJ: Cucurbitane-type triterpenoids from the African plant *Momordica balsamina*. J Nat Prod *72*: 2009-2013, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19795842

29 Ramalhete C, Molnár J, Mulhovo S, Rosário VE and Ferreira MJ: New potent P-glycoprotein modulators with the cucurbitane scaffold and their synergistic interaction with doxorubicin on resistant cancer cells. Bioorg Med Chem *17*: 6942-6951, 2009. http://www.ncbi.nlm.nih.gov/pubmed/19733087

30 Madureira AM, Gyémant N, Ascenso JR, Abreu PM, Molnar J and Ferreira MJ: Euphoportlandols A and B, tetracylic diterpene polyesters from *Euphorbia portlandica* and their anti-MDR effects in cancer cells. J Nat Prod *69*: 950-953,2006. http://www.ncbi.nlm.nih.gov/pubmed/16792416

31 Molnár J, Gyémánt N, Tanaka M, Hohmann J, Bergmann-Leitner E, Molnár P, Deli J, Didiziapetris R and Ferreira MJ: Inhibition of multidrug resistance of cancer cells by natural diterpenes, triterpenes and carotenoids. Curr Pharm Des *12*: 287-311, 2006. Review. http://www.ncbi.nlm.nih.gov/pubmed/?term=Moln%C3%A1r+J%2C+Gy%C3%A9m%C3%A1nt+N%2C+Tanaka+M%2C+Hohmann+J%2C+Bergmann-Leitner+E%2C+Moln%C3%A1r+P%2C+Deli+J%2C+Didiziapetris+R%2C+Ferreira+MJ.

32 Madureira AM, Spengler G, Molnár A, Varga A, Molnár J, Abreu PM and Ferreira MJ: Effect of cycloartanes on reversal of multidrug resistance and apoptosis induction on mouse lymphoma cells. Anticancer Res *24*: 859-864, 2004. http://www.ncbi.nlm.nih.gov/pubmed/?term=Madureira+AM%2C+Spengler+G%2C+Moln%C3%A1r+A%2C+Varga+A%2C+Moln%C3%A1r+J%2C+Abreu+PM%2C+Ferreira+MJ

33 Smith MT, Crouch NR, Gericke N and Hirst M: Psychoactive constituents of the genus N. E. Br. and other Mesembryanthemaceae: a review. J Ethnopharmacol *50*: 119-130, 1996. http://www.ncbi.nlm.nih.gov/pubmed/?term=Smith+MT%2C+Crouch+NR%2C+Gericke+N%2C+Hirst+M.

34 Weber E and D'Antonio CM: Germination and growth responses of hybridizing Carpobrotus species (Aizoaceae) from coastal California to soil salinity. Am J Botany *86*: 1257-1263, 1999. http://www.ncbi.nlm.nih.gov/pubmed/?term=Weber+E%2C+D%E2%80%99Antonio+CM

35 Amaral L, Kristiansen JE, Abebe LS and Millett W: Inhibition of the respiration of multidrug-resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: Potential use for initial therapy of freshly diagnosed tuberculosis. J Antimicrob Chemother *38*: 1049-1053, 1996. http://www.ncbi.nlm.nih.gov/pubmed/?term=Amaral+L%2C+Kristiansen+JE%2C+Abebe+LS%2C+Millett+W

36 Ordway D, Viveiros M, Leandro C, Bettencourt R, Almeida J, Martins M, Kristiansen JE, Molnar J and Amaral L: Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother *47*: 917-922, 2003. http://www.ncbi.nlm.nih.gov/pubmed/?term=Ordway+D%2C+Viveiros+M%2C+Leandro+C%2C+Bettencourt+R%2C+Almeida+J%2C+Martins+M%2C+Kristiansen+JE%2C+Molnar+J%2C+Amaral+L.

37 Ordway D, Hohmann J, Viveiros M, Viveiros A, Molnar J, Leandro C, Arroz MJ, Gracio MA and Amaral L: *Carpobrotus edulis* methanol extract inhibits the MDR efflux pumps, enhances killing of phagocytosed *S. aureus* and promotes immune modulation. Phytother Res *17*: 512-519, 2001. http://www.ncbi.nlm.nih.gov/pubmed/?term=Ordway+D%2C+Hohmann+J%2C+Viveiros+M%2C+Viveiros+A%2C+Molnar+J%2C+Leandro+C%2C+Arroz+MJ%2C+Gracio+MA%2C+Amaral+L

38 Martins A, Vasas A, Schelz Z, Viveiros M, Molnár J, Hohmann J and Amaral L: Constituents of *Carpobrotus edulis* inhibit P-glycoprotein of MDR1-transfected mouse lymphoma cells. Anticancer Res *30*: 829-835, 2010. http://www.ncbi.nlm.nih.gov/pubmed/?term=Martins+A%2C+Vasas+A%2C+Schelz+Z%2C+Viveiros+M%2C+Moln%C3%A1r+J%2C+Hohmann+J%2C+Amaral+L

39 Madureira AM, Molnár A, Abreu PM, Molnár J and Ferreira MJ: A new sesquiterpene-coumarin ether and a new abietane diterpene and their effects as inhibitors of P-glycoprotein. Planta Med *70*: 828-833, 2004. http://www.ncbi.nlm.nih.gov/pubmed/15503353

40 Madureira AM, Gyémant N, Ascenso JR, Abreu PM, Molnar J and Ferreira MJ: Euphoportlandols A and B, tetracylic diterpene polyesters from *Euphorbia portlandica* and their anti-MDR effects in cancer cells. J Nat Prod *69*: 950-953, 2006. http://www.ncbi.nlm.nih.gov/pubmed/?term=Madureira+AM%2C+Gy%C3%A9mant+N%2C+Ascenso+JR%2C+Abreu+PM%2C+Molnar+J%2C+Ferreira+MJ.

41 Valente C, Ferreira MJ, Abreu PM, Gyémánt N, Ugocsai K, Hohmann J and Molnár J: Pubescenes, jatrophane diterpenes, from *Euphorbia* pubescens, with multidrug resistance reversing activity on mouse lymphoma cells. Planta Med *70*: 81-84, 2004. http://www.ncbi.nlm.nih.gov/pubmed/?term=Valente+C%2C+Ferreira+MJ%2C+Abreu+PM%2C+Gy%C3%A9m%C3%A1nt+N%2C+Ugocsai+K%2C+Hohmann+J%2C+Moln%C3%A1r+J.

42 Valente I, Reis M, Duarte N, Serly J, Molnár J and Ferreira MJ: Jatrophane diterpenes from *Euphorbia mellifera* and their activity as P-glycoprotein modulators on multidrug-resistant mouse lymphoma and human colon adenocarcinoma cells. J Nat Prod *75*: 1915-1921, 2012. http://www.ncbi.nlm.nih.gov/pubmed/?term=Valente+I%2C+Reis+M%2C+Duarte+N%2C+Serly+J%2C+Moln%C3%A1r+J%2C+Ferreira+MJ

43 Reis MA, Paterna A, Mónico A, Molnar J, Lage H and Ferreira MJ: Diterpenes from *Euphorbia* piscatoria: synergistic interaction of Lathyranes with doxorubicin on resistant cancer cells. Planta Med *80*: 1739-1745, 2014. http://www.ncbi.nlm.nih.gov/pubmed/?term=Reis+MA%2C+Paterna+A%2C+M%C3%B3nico+A%2C+Molnar+J%2C+Lage+H%2C+Ferreira+MJ

44 Spengler G, Ramalhete C, Martins M, Martins A, Serly J, Viveiros M, Molnár J, Duarte N, Mulhovo S, Ferreira MJ and Amaral L: Evaluation of cucurbitane-type triterpenoids from *Momordica balsamina* on P-glycoprotein (ABCB1) by flow cytometry and real-time fluorometry. Anticancer Res *29*: 3989-3993, 2009. http://www.ncbi.nlm.nih.gov/pubmed/?term=Spengler+G%2C+Ramalhete+C%2C+Martins+M%2C+Martins+A%2C+Serly+J%2C+Viveiros+M%2C+Moln%C3%A1r+J%2C+Duarte+N%2C+Mulhovo+S%2C+Ferreira+MJ%2C+Amaral+L

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