Abstracts
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Conference venue

Historical building Faculty of Economy located at Via Partenope, 36 on the opposite side of Castel dell’Ovo.

http://www.phytochemicalsociety.org/naples
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11.50 12.10 O8 – Mario Zoratti (Italy) “Mitochondria-targeted Polyphenol Derivatives”

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12.30-12.50 O10 – Tania Pannellini (Italy) “Dietary Supplementation of Tomato Prevents Prostate Cancer in Tramp Mice”

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15.40-16.00 O11 – Pinarosa Avato (Italy) “Cytotoxic Effect of Saponins from Medicago on Tumor Cellular Lines”

16.00-16.20 O12 – Wieslaw Szeja (Poland) “Sugar Moiety Structure as a Principal Determinant of Antitumor Glycosides Biological Activity. Genistein Case Study”

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17.40-18.00 O13 – Daniela Smejkalova (Italy) “Metabolic Profile of Uterine Leiomyomas Using High Resolution Magic-angle Spinning 1H NMR Spectroscopy”

18.00-18.20 O14 – Yee V. Tang (Malaysia) “Photosensitisers from Malaysian Seaweeds for Photodynamic Therapy of Cancers”

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09.40-10.00 O15 – Yu-Dong Zhou (U.S.A.) “Pharmacological and Preclinical Evaluation of Manassantins: Potent Inhibitors of Hypoxia-induced HIF-1 Activation”

10.00-10.20 O16 – Salvatore Passarella (Italy) “Mitocondria as a Target of Drugs in Cancer and Diseases”

10.20-10.40 O17 – Maria V. D’Auria (Italy) “Homophymines, a New Family of Marine Cyclodepsipeptides”

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11.50 12.10 O18 – Antonio Randazzo (Italy) “Interaction of Distamycin A and its Derivatives with DNA Quadruplex Structures”
12.10-12.30 O19 – Dagmar Blaesius (Germany) “On The Pathways of Cell Death Induction by Myrtucommulone from Myrtus communis”
12.30-12.50 O20 – Antonio Evidente (Italy) “Anticancer Evaluation of Structurally Diverse Amaryllidaceae Alkaloids and their Synthetic Derivatives”
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17.40-18.00 O23 – Marialuisa Menna (Italy) “Antitumor Potential of Natural Products from Mediterranean Ascidians”
18.00-18.20 O24 – Tatjana Mijatovic (Belgium) “Sodium Pump Antagonist UNBS1450: a Novel Means to Combat Apoptosis and Multi-drug Resistant Cancer Cells”
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Plenary Lectures

International PSE Symposium on
Natural Products in Cancer Therapy

23-26 September 2008
Naples, Italy

Gordon M. Cragg*
NIH Special Volunteer, Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, US National Cancer Institute
*gmcragg@verizon.net

Over 60% of the current anticancer drugs have their origin in one way or another from natural sources. Nature continues to be the most prolific source of biologically active and diverse chemotypes, and it is becoming increasingly evident that associated microbes may often be the source of biologically active compounds originally isolated from host macroorganisms. While relatively few of the actual isolated compounds advance to become clinically effective drugs in their own right, these unique molecules may serve as models for the preparation of more efficacious analogues using chemical methodology such as total or combinatorial (parallel) synthesis, or manipulation of biosynthetic pathways. In addition, conjugation of toxic natural molecules to monoclonal antibodies or polymeric carriers specifically targeted to epitopes on tumors of interest can lead to the development of efficacious targeted therapies. The essential role played by natural products in the discovery and development of effective anticancer agents, and the importance of multidisciplinary collaboration in the generation and optimization of novel molecular leads from natural product sources will be reviewed.
Synthetic and Computational Approaches towards the Development of New HDAC Inhibitors Useful in Cancer Therapy

Raffaele Riccio*, Maria Giovanna Chini, Stefania Terracciano, Giuseppe Bifulco, Ines Bruno
Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte don Melillo, 84084 Fisciano (SA), Italy
* riccio@unisa.it

The epigenetic modulators have emerged as new attractive therapeutics as a consequence of their ability to induce post-translational modifications of nucleosome proteins and hence to influence transcriptional events. In this context, HDAC have been recently highlighted as promising targets for the treatment of several disorders included cancer, as they are responsible, together with HAT, of the acetylation level of histone proteins, which is highly involved in the modulation of chromatin functions. In more details, the hyperacetylation status of histones, promoted by HDAC inhibition, is related to a more open chromatin structure, which determines the activation of transcriptional process. In this regard the great potential of HDAC inhibitors as anticancer drugs, has to be related to the transcription and expression of genes which are proved to be silent in cancer pathology.

Based on these premise, in the recent years we focused our interest on the cyclopeptidic class of HDAC inhibitors, and in particular we selected for our investigations, FR235222, whose total synthesis has been successfully realized. With the aim of gaining more information on the nature of interaction between this structural class of inhibitors and the biological target, we synthesized unnatural variants of the lead and we proved their putative affinity for the enzyme, performing molecular modeling studies on HDPL, the bacterial homologue of human HDAC.

Data obtained allowed us to propose a model of interactions between cyclopeptide-type compounds and the active site of the enzyme. Additionally, the computational approach clearly indicated that an efficient HDAC inhibitor should contain three structural features: an hydrophobic region, involved in the molecular recognition process, a Zn$^{2+}$ chelating element, essential for the crucial inhibition process, and a five to seven-atoms spacer, linking the hydrophobic region to the coordinating function.
Signal Transduction Network as a Roadmap in Searching for Natural Products with Cancer Chemopreventive and Cytoprotective Activities

Young-Joon Surh
National Research Laboratory of Molecular Carcinogenesis & Chemoprevention, College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

Chemoprevention is an attempt to use either naturally occurring or synthetic substances to intervene in or halt the progress of carcinogenesis, before the malignancy manifests. Numerous phytochemicals derived from dietary and medicinal plants have been reported to inhibit, retard, or reverse a specific stage of the carcinogenic process. A wide array of molecules and events are involved in relaying intracellular signals to maintain cellular homeostasis. Cancer arises when fine-tuning of the sophisticated cellular growth signaling network is deregulated or disrupted. Since the cellular signaling network often goes awry in carcinogenesis, it is fairly rational to target intracellular signaling cascades for achieving chemoprevention. Targeted modulation or restoration of the intracellular signaling network by use of phytochemicals thus offers a unique strategy for preventing abnormal cell proliferation and other malfunctions. Research directed toward elucidating underlying molecular mechanisms of chemoprevention or chemoprotection with edible phytochemicals has recognized components of signal transduction networks as potential targets. These include transcription factors and their upstream protein kinases, such as the family of proline-directed serine/threonine kinases named mitogen-activated protein (MAP) kinases, protein kinase C, phosphatidylinositol-3-kinase, protein kinase B/Akt, glycogen synthase kinase, etc.

A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Components of the cell signaling network, especially those converge on the ubiquitous eukaryotic redox-sensitive transcription factors, particularly nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) have been implicated in pathogenesis of many inflammation-associated disorders. Modulation of cellular signaling involved in chronic inflammatory response hence provides a pragmatic strategy in molecular target-based chemoprevention and cytoprotection.

Induction of phase-2 detoxifying or antioxidant enzymes represents an important cellular defence in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. Many antioxidants derived from dietary and medicinal plants have been found to activate this particular redox-sensitive transcription factor, thereby potentiating cellular antioxidant or detoxification capacity. It is noteworthy that there is a good correlation between anti-inflammatory activity of selected chemopreventive/cytoprotective agents and their ability to induce antioxidant gene expression. The current research in our laboratory concerns evaluation of chemopreventive effects of some edible antioxidative and anti-inflammatory phytochemicals and elucidation of their underlying molecular mechanisms. For this purpose, we have attempted to unravel common events mediated by transcription factors, such as NF-kappa B, AP-1 and Nrf2, and upstream kinases involved in the cellular signaling network as prime targets for selected edible phytochemicals.

Supported by the NRL Grant from the Ministry of Science and Technology, Republic of Korea.

Anticancer Activity of the Bioactive Compound Inositol Pentakisphosphate

Marco Falasca
Inositide Signalling Group, Centre for Diabetes and Metabolic Medicine, Institute of Cell and Molecular Science, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK

Bioactive compounds are extra nutritional constituents found in small quantities in foods. We have recently shown that a bioactive compound, inositol pentakisphosphate (IP$_5$), a naturally occurring substance that is present in most legumes, wheat bran and nuts, inhibits cell growth of ovarian, lung and breast cancer cells (1-3). We demonstrate that IP$_5$ specifically blocks the activation of the critical phosphoinositide 3-kinase downstream effector Akt, a serine/threonine kinase which plays a key role in different intracellular processes such as cell survival and proliferation (3). Due to its role in cancer development and progression the PI3K/Akt pathway is an attractive target for therapeutic intervention. Interestingly, IP$_5$ possesses anti-tumour activity in mice to the same extent than cytotoxic drug cisplatin (4). Furthermore, IP$_5$ enhances the effect of cytotoxic drugs in ovarian and lung cancer cells respectively. These results support a role for IP$_5$ as an anti-tumour agent that may sensitise cancer cells to the action of commonly used anticancer drugs. Given that administration of standard chemotherapy agents is usually associated with at least mild toxicity, the possible use of non-toxic, natural compounds to target cancer is attractive. In the present study we have investigated the potential additive or synergistic activities of combinations of IP$_5$ with other bioactive compounds. Data revealed that combination of IP$_5$ with curcumin and taxol resulted in a more than additive effect in different human cancer cell lines.

In addition we demonstrate that specific modifications of the IP$_5$ structure may result in compound with the same solubility and lack of toxicity in vivo but broader range of action and a higher activity compared to parental molecule indicating that represents a promising molecule for further development of novel anticancer drugs. Therefore our study reveals a new pharmacologically active nutrient ("nutraceutical") as a potential chemopreventive agent and a lead compound for possible development of potent small molecule Akt inhibitors.

Chemopreventive Effects of Orange Peel Extract

Randolph Arroo*, Vasilis Androutsopoulos, Kenneth Beresford, Ketan Ruparelia, Somchaiya Surichan, Gerry Potter
Leicester School of Pharmacy, De Montfort University, The Gateway, Leicester, LE1 9BH, United Kingdom
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There are many reasons why vegetables and fruits may protect against cancer. As well as containing vitamins and minerals, which help keep the body healthy and strengthen the immune system; they are also good sources of biologically active compounds, which can help to protect cells in the body from damage that can lead to cancer [1]. Notably, dietary flavonoids and other polyphenols are thought to have an important role as chemopreventive agents.

Most studies on the possible mechanism of the chemopreventive action of dietary compounds have assumed that free hydroxyl groups of flavonoids and other polyphenols are necessary for their biological effects. However, in the human body dietary polyphenols are rapidly conjugated by glucuronosyltransferases and sulfotransferases, two enzymes that are abundantly present in the small intestine and liver, through which all of the oral dose must pass. Thus, most polyphenols that have been studied, e.g. quercetin, kaempferol, diosmetin, curcumin and resveratrol, would not be expected to reach internal organs beyond sites directly along the gastrointestinal tract [2].

When the hydroxyl groups in polyphenols are methylated, the resulting compounds are much less prone to glucuronidation and sulfation. Thus methoxylated compounds are more metabolically stable, increasing their bioavailability. The peel of various Citrus species can contain high concentrations of polymethoxyflavones, whereas the juice mainly contains hydroxylated flavones.

At present, very little is known about the mechanisms by which methoxylated flavones may affect growth and development of tumour cells. Recently, it was shown that tumour specific enzymes can catalyze the O-demethylation of methoxylated flavones, resulting in the formation of flavones with free hydroxyl groups [3]. We propose that demethylation of methoxylated flavones is another example of bioactivation of naturally occurring prodrugs [4].

Recent Developments in the Rapid Analysis of Plants and Tracking their Bioactive Constituents

Teris A. van Beek*, Kishore Tetala, Irina I. Koleva, Suzanne, M.F. Jeurissen, Frank W. Claassen, Elbert van der Klift
Laboratory of Organic Chemistry, Natural Products Chemistry Group, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands
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Natural products chemistry has recently witnessed many new developments like LC/MS and LC/SPE/cryo-NMR, UPLC, UTLC, MS-based preparative HPLC, comprehensive chromatography (GCxGC, LCxLC), new detectors, high throughput screening, various monolithic materials, miniaturisation, automated identification and extraction with subcritical water and ionic liquids. Nevertheless identifying bioactive constituents in complex plant extracts remains a tedious process. The classical approach of bioassay guided fractionation is time-consuming while broad screening of extracts does not provide information on individual compounds and sometimes suffers from false positives or negatives. One way out of this is by coupling chromatography with chemical or biochemical assays.

An example is the development of HPLC on-line assays for antioxidants. By the post-column addition of a relatively stable coloured radical like DPPH* or ABTS**, radical scavengers are detected as negative peaks because in a reaction coil they reduce the model radical to its reduced, non-coloured form [1-2]. When combined with LC/DAD/MS and LC/SPE/NMR, reliable identification of active constituents becomes possible without the necessity of ever isolating them in a classical sense [3]. For finding new leads combining HPLC with biochemical assays is more interesting but also technically more difficult. Most enzymes do not work at the organic modifier concentrations commonly encountered in RP-HPLC and the reaction time is often longer requiring dilution and lengthy coils respectively. This in turn leads to sensitivity problems and peak broadening. Some partial solutions will be shown. For stable analytes high temperature LC offers a solution to the problem of low percentage organic modifier. When enzymes are highly expensive, like those used in the screening for CytP450 inhibitors [4], miniaturisation to chip format may offer a way out.

Microreactors (chips) are not only useful for miniaturising larger assays but also offer completely new prospects in phytochemical analysis. One such application is in the sample clean-up of acids and bases like alkaloids. In a lay-out of three parallel channels of 50 - 100 µm width with the middle one containing organic phase and the two outer ones water of high pH (feed phase) and low pH (trapping phase) this chip functions similar to two classical LLE steps (Tetala et al., unpublished).

Time permitting a few more exciting developments in natural products analysis will be briefly highlighted. Anyhow it is clear that phytochemical analysis is still a vibrant field.

Solid tumors develop hypoxic regions, which correlate with advanced stages of cancer and treatment resistance. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that promotes tumor cell adaptation and survival under hypoxic conditions. HIF-1 is currently recognized as an important molecular target for anti-cancer drug discovery. A T47D breast tumor cell-based reporter assay was developed and used to evaluate samples for HIF-1 inhibitory activity. Extracts from our University of Mississippi repository and the NCI Open Repository of marine invertebrates and algae were evaluated using reporter gene-based assays for the ability to inhibit HIF-1 activation in tumor cells.

The first marine natural product found to inhibit hypoxia-induced HIF-1 activation was laurenditerpenol, a diterpene from the marine red alga Laurencia intricata. Bioassay-guided isolation has since yielded an array of HIF-1 inhibitors from sponges and other marine organisms. The macrolide latrunculin A from a Red Sea sponge Negombata magnifica and the phenolic pyrrol alkaloid 7-hydroxyneolamellarin A, recently isolated from the sponge Dendrilla nigra, act as moderately potent inhibitors of hypoxia-induced HIF-1 activation in T47D cells (IC50 values 6.7 and 1.9 μM, respectively). Mycothiazole, an antitumor compound from the sponge Cacospongia mycofijiensis, was the most potent marine natural product inhibitor of hypoxia-induced HIF-1 activation (IC50 1 nM). These natural products inhibit the hypoxic induction of HIF-1 target genes and the pathophysiological processes associated with HIF-1 activation (i.e. expression of the angiogenic factor VEGF, induction of tumor angiogenesis, and tumor cell chemotaxis). These compounds vary widely in potency, selectivity, and function through distinctly different mechanisms that include suppression of mitochondria-associated HIF-1 signaling and disruption of nuclear HIF-1α protein translocation.
New Insights into Antitumor Polyketide Biosynthesis in Marine Sponges

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Marine sponges belong to the most important sources of natural products with anticancer properties. However, drug development is often hampered by the limited amounts that are available from the natural sources. Most pharmacologically relevant sponges contain large numbers of symbiotic bacteria that belong to diverse phyla, and it has often been proposed that these consortia are the true source of many of the isolated compounds. Since cultivation of these bacteria is often difficult, if not impossible, we use a culture-independent, metagenomic approach to study their chemistry. Via construction and screening of large fosmid libraries, biosynthetic genes are isolated from the total sponge DNA, which then provides information into the nature of the producer and into how the compound is assembled in the cell. In addition to this information, the availability of the genes provides the opportunity to generate sustainable production systems by heterologus expression in culturable bacteria. The talk will focus on methods that we have developed in recent years to efficiently study the often enormously complex sponge metagenomes. This has allowed us to contribute to a better understanding of antitumor polyketide biosynthesis in a range of marine sponges and revealed new and unexpected insights about natural product enzymology and the evolution of chemical defenses in symbionts.
Plant Products and Synthetic Derivatives as Specific Modulators of Multidrug ABC Transporters Responsible for Resistance to Anticancer Chemotherapeutics


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Three main types of ABC transporters are involved in cancer cell multidrug resistance: P-glycoprotein/ABCB1, BCRP/ABCG2 and MRP1/ABCC1. P-glycoprotein was firstly discovered and shown to be of clinical relevance in various types of cancer, especially leukemia. Many efforts have been spent to discover efficient inhibitors, and several third-generation compounds are undergoing advanced clinical trials, like LY335979 (Zosuquidar), R101933 (Laniquidar), VX-710 (Biricodar), GF120918 (Elacridar) or XR-9576 (Tariquidar). Interestingly, natural compounds, such as jatrophane and lathyrane diterpenes from Euphorbia helioscopia, were found to specifically inhibit P-glycoprotein [1].

In contrast, very few inhibitors are known for BCRP/ABCG2 [2,3], a stem-cell marker, identified in many types of tumors. Fumitremorgin C and its synthetic analogue Ko143 have the particularity to alter both ATP hydrolysis and drug transport activities. We have found that hydrophobic derivatives of both flavones [4], like tectochrysin and 6-prenylchrysin, and rotenoids [5] were potent and specific inhibitors. In addition, acridone analogues structurally mimicking the P-glycoprotein-inhibitor GF120918, were similarly efficient [6] with ABCG2 but quite specific. Such a selectivity of inhibitors for either multidrug transporter contrasts with the high overlapping patterns towards transported substrates, suggesting that these inhibitors are non-competitive, and therefore bind outside the drug-binding site(s). The inhibitors with low intrinsic cytotoxicity are suited for sensitization to drugs of cancer cell proliferation, and constitute potential future candidates usable as adjuvants to chemotherapeutics such as irinotecan (CPT-11), a prodrug of the BCRP-substrate SN-38.

Since MRP1/ABCC1 has the peculiar ability to transport negatively-charged substrates, as either glutathione-, glucuronide- or sulfate-conjugates, competitive inhibitors would have a limited accessibility to cytosolic drug-binding site(s) due to low membrane diffusion. Interestingly, MRP1 is also able to efflux hydrophobic substances such as vincristine by co-transport with intracellular glutathione. Probably by mimicking vincristine but without being transported, various hydrophobic compounds such as S-verapamil [7] and higher-affinity iodinated derivatives [8], as well as some flavonoids were able to strongly stimulate MRP1-mediated glutathione efflux, producing a fast and extensive depletion of intracellular glutathione, inducing MRP1-expressing cells into apoptosis [7,8]. Such compounds open the possibility of a new strategy to selectively eliminate multidrug-resistant cell lines overexpressing MRP1 in the absence of any chemotherapeutic.

The activity of these BCRP and MRP1 specific modulators, as selected and optimized in vitro, is currently being checked in vivo by using nude/SCID mice xenografted with human tumors for their ability to inhibit the growth of tumor cells.

Antitumoral Activity of Naturally Occurring 1-4 Phenanthrenequinones. Mechanisms of Action

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For about two millennia, a causal relationship between inflammation and cancer has been suspected and this association has been supported by more recent epidemiological data that led to the estimate that approximately 20% of cancer deaths are linked to chronic infections and persistent inflammation. One of the most important molecular mediators in inflammation is NF-κB (nuclear factor kappa B) transcription factor that is also involved in tumour promoting processes. The activation of NF-κB in chronic inflammation is especially relevant as far as gastrointestinal carcinogenesis is concerned, and especially in colitis associated cancer (CAC), which represents up to 5% of all colonic cancer. Therefore, it has been suggested that many of the proteins involved in the NF-κB activation pathway, and hence responsible for inflammation and cancer, can be molecular targets for many drugs.

In our screening program to study phytoextracts and natural products with anti-inflammatory and anticancer activities we have identified a variety of Cannabis sativa L. (variety CARMA) with a very low content of the psychoactive cannabinoid Δ9-THC. The biological activity of an acetone extract from this plant was investigated in vitro and in vivo, and we found that this extract is a potent inhibitor of the NF-κB pathway and prevents the release of proinflammatory cytokines and the development of inflammatory bowel disease and CAC in mice. The phytochemical analysis of this extract revealed the presence of cannabinoids such as cannabidiol, cannabigerol and cannabinomere; stilbenoids such as canniprene, cannabispironol and cannabispirane; prenylated flavones such as canflavin A and B; and the 1,4-phenanthrenequinone denbinobin. A detailed analysis of the bioactivities of those compounds indicated that denbinobin is the principal responsible for the anti-NF-κB activity of the phytoextract.

Phenanthrenequinones of non-terpenoide origin occur relatively rarely in the plant kingdom and denbinobin was isolated for the first time from the orchid Dendrobium nobile. Interestingly, denbinobin has been shown to have anti-tumoral activities against leukaemia and colon cancer cell lines. Through kinase over-expression and biochemical and transcriptional assays we found that denbinobin inhibits the NF-κB pathway by targeting the TAB/TAK1 in T cells. Moreover, denbinobin induces reactive oxygen species and sustained activation of the MAPKs (JNK, p38 and ERK) that contribute to the disruption of the mitochondrial permeability transition, activation of effector caspases and cell death by apoptosis and necrosis. Chemistry and SAR analysis led us to identify that different pharmacophores are responsible for distinct biological activities.

We also found that denbinobin inhibits the Wnt pathway in colon cancer cell lines by targeting the PI3K axis. Thus, denbinobin and perhaps other 1,4-phenanthrenequinones represent a group of valuable lead compounds for the development of novel anticancer drugs that inhibit tumorigenesis and inflammation promoted cancer.
In malignant cells, altered expression of cyclin-dependent kinases (CDKs) and their modulators, including overexpression of cyclins and loss of expression of CDK inhibitors, results in deregulated CDK activity, providing a selective growth advantage. In contrast to CDKs governing the transitions between cell cycle phases, transcriptional CDKs, including cyclin H-CDK7, and cyclin T-CDK9 (pTEFb), promote initiation and elongation of nascent RNA transcripts by phosphorylating the carboxy-terminal domain (CTD) of RNA polymerase II. Because of their critical role in cell cycle progression and cellular transcription, as well as the association of their activities with apoptotic pathways, the CDKs comprise an attractive set of targets for novel anticancer drug development. Our research focused on the primary mechanism of action of plant hormones cytokinins (N6-substituted adenine derivatives) in cell division cycle has showed that natural plant cytokinins are also rather non-specific inhibitors of various protein kinases. Surprisingly, among aromatic cytokinin derivatives, we have discovered a compound, 2-(2-hydroxyethylamino)-6-benzylamino-9-methylpurine, named “olomoucine” (OC), which specifically inhibits some CDKs at micromolar concentration. One of the inhibited kinases, the p34cdc2/cyclin B kinase, assumed to be a key mitotic factor, which is highly conserved and strongly implicated in cell cycle transitions in all eukaryotic cells. The total lack of the inhibitory effect of olomoucine on major kinases, such as cAMP- and cGMP-dependent kinases, protein kinase C, and others, suggests that OC might be a useful tool for cell cycle regulation studies. The design and inhibitory activity of OC was further improved by modifications at positions 2, 6, and 9, i.e., the positions that control binding to CDK1. This led to discovery of novel specific CDK inhibitors named roscovitine, olomoucine II and purvalanol A (Figure 1), which display an enhanced inhibitory activity toward CDK1, a higher selectivity toward some CDKs, an increased antimitotic activity at the G1/S and G2/M points of the cell cycle, and stronger and more selective antitumour effects. The compounds are also effective in vivo and one of them is already in clinical trials in USA and Europe (roscovitine ⇒ Seliciclib®, Cyclacel Pharmaceuticals Ltd, U.K.). Seliciclib® is currently in Phase IIb clinical trials as a single therapy in multiple myeloma as well as two other B-cell hematological malignancies: B-cell Chronic Lymphocytic Leukemia and Mantle Cell Lymphoma. An additional Phase IIb clinical trial is in progress investigating the effects of Seliciclib® in patients with Non-Small Cell Lung Cancer in combination with gemcitabine and cisplatin. Recently, we have developed also first CDK9 inhibitors and discovered whole range of new structural motifs for development of CDK inhibitors which are derived from anticytokinin structures.
Marine Compounds as Promising Anti-cancer Agents

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Over the past twenty years, numerous drug discovery programmes based on a continuously growing knowledge about the signal-transduction network that drives neoplastic transformation, and using rationally designed cancer therapeutics that target specific molecular events, have been launched. Most importantly, anti-cancer drug development has shifted dramatically from conventional cytotoxic drugs that affect DNA synthesis in both cancerous and healthy cells to drugs that modulate the activity of proteins which are specifically associated with cancer. For several years, the National Cancer Institute (NCI) in the United States of America has included marine natural products in its screening for novel anticancer drugs. To date, numerous terrestrial natural products and several marine natural products, such as bryostatin 1 isolated from the bryozoan Bugula neritina, dolastatin isolated from the sea hare Dolabella auricularia, halichondrin B isolated from the various sponge species, including Halichondria okadai and Axinella sp., and aplidine (dehydrodidemnin B) isolated from the tunicate Aplidium albicans have entered clinical trials as anti-tumour agents, and several natural anticancer compounds are currently being used in the clinic.
Synthesis of Antiproliferative Anion Transporters Inspired by Nature

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The ion-coupled processes occurring in the plasma membrane regulate most of the physiologically relevant cell processes \cite{1}, however, in contrast with the inspiring specificity of natural ion channel \cite{2} and despite the advances in the ion-transport field, only a restricted number of synthetic ionophores demonstrated efficient anion transport and selectivity \cite{3}.

The quest for the design and synthesis of artificial anion conductors, is justified by the surprising scarcity of anion-transporting secondary metabolites \cite{4}, by the concurrent unfortunate high number of diseases due to insufficient chloride transport \cite{5} and by their interesting antiproliferative activities \cite{4a}. In this context, newly conceived transporters may represent interesting leads for the treatment of channelopathies and cancer \cite{3}.

Calix[4]arene represents a versatile scaffold for the design of synthetic ionophore and, considering the strong correlation between $\text{H}^+$/Cl\textsuperscript{-} symport transport rates and in vitro cytotoxic activity demonstrated for prodigiosin analogues \cite{4a}, we synthesised and evaluated the cytotoxic potential of calix[4]arene 1, against the J774.A1 (murine monocyte/macrophage) cancer cell line.

In this communication we demonstrate that cationic calix[4]arenes mediate HX efflux, induce block of the chloride transport in the presence of appropriate interfering anions, and show a moderate antiproliferative activity against murine monocyte/macrophage J774.A1 cancer cells.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{calixarene.png}
\caption{Structure of calix[4]arene 1.}
\end{figure}

\begin{thebibliography}{9}
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\end{thebibliography}
Taxol demonstrated clinical efficacy, but the mechanisms by which it kills cancer cells remain unknown. We showed that taxol, beside targeting tubulin, is able to directly interact also with Bcl-2, thereby facilitating the initiation of apoptosis and explaining the Bcl-2 down-regulation in resistant cells. Molecular modeling predicted the binding site for taxol in the disordered loop domain of Bcl-2 and revealed an extraordinary similarity between the taxol binding sites in Bcl-2 and β-tubulin, leading us to speculate that taxol is a peptidomimetic compound. We tested the hypothesis that taxol mimics Nur77, which, like taxol, changes the function of Bcl-2. This premise was confirmed by Nur77 interacting with both Bcl-2 and β-tubulin, and by the rational design of a peptide sequence mimicking the Nur77 structural region which includes “the taxol message”. The peptide reproduced the taxol-like effects of tubulin polymerization and opening of the permeability transition pore channel in mitochondria.

Thus, the presence of two intracellular receptor for taxol is explained by taxol functionally mimicking the endogenous proapoptotic signal mediated by Nur77. This discovery could help in development of novel anticancer agents that are able to engage the taxol receptors as well as in identifying the clinical subsets responsive to taxol-based therapy.
Alternative Production of Thapsigargin
A Prostate Cancer Drug

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The moss Physcomitrella patens will be transformed into an efficient producer of thapsigargin or a precursor to be used for simple chemical synthesis. Thapsigargin is currently being developed into a drug for prostate cancer therapy. Prostate cancer is the 2nd most common class of cancer in males in the Western world and accounts for 40,000 deaths per year in the US with a survival rate of 45 % after five years. We will transform Physcomitrella to produce thapsigargin because in the future the collection of wild plants for isolation of the compound will not meet the market demand. The genes for the biosynthesis of thapsigargin will be obtained from Thapsia garganica, this species produce thapsigargin in the majority of the plant tissue and store it in the fruit capsule and roots. Plant material has been obtained through our collaboration partners, Prof. Avato, University of Bari. Large parts of the expressed genome sequence of T. garganica will be obtained as part of this project through pyrosequencing.

In the proposed biosynthesis we currently work on several approaches to obtain the genes downstream. The first gene of the pathway has been obtained and has also been found in normal carrot. The enzyme is currently undergoing characterisation. The subsequent steps in the pathways involve several P450’s and acyltransferases, and from the obtained sequence we aim designing microarrays to gain knowledge of genes co-regulated with the isolated terpene cyclase. The second step in the pathway has been described in Chicory and the acylation in the third step is important to protect carbon 8 to direct the lactone formation towards carbon 6.
Anticancer Activity of a Novel Structural Class of Spiroketalts Isolated from Australian Rainforest Plants

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Many drugs in current use owe their origin to the structural diversity and bioactivity of natural compounds. During a systematic search for new bioactive structures from Australia’s tropical rainforests, we have discovered a number of compounds with promising anticancer activity.

One such group comprises a series of long chain aliphatic cyclic ketals, the lead compound of which is designated EBC23. This novel structure, which is synthetically tractable, selectively inhibits the growth of human tumor cell lines in culture and shows activity against several tumor models in mice.

The pattern of cell line inhibition of EBC23 has so far been different from that of known anticancer compounds. Cell cycle arrest occurs partly in G2/M, with no significant level of apoptosis. Results of current work on efficacy of this compound and progress towards understanding its mode of action will be presented.
Resveratrol and EGCG Differently Regulate p66Shc

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Particular interest has been recently pointed on several plant antioxidants such as resveratrol and epigallocatechin-3-gallate (EGCG), because of their wide-ranging biological activity and remarkable clinical potential. Resveratrol and EGCG exert antioxidant, metabolism-regulating and pro-apoptotic/anti-cancer effects on a wide variety of model systems. Human skin is chronically exposed to both endogenous and environmental pro-oxidant agents, leading to the harmful generation of reactive oxygen species (ROS). Many studies have documented their role in skin aging, inflammatory disorders, and carcinogenesis. Both resveratrol and EGCG have been suggested to protect skin from UV induced photo damaging and photo aging. In parallel also the biological significance of p66Shc, a member of the SHC family of intracellular adaptor proteins, is getting further attention. p66shc is a redox enzyme that generates reactive oxygen species in mitochondria by oxidation of cytochrome c and consequently induces apoptosis [1]. There is a striking intersection among many of the activities of resveratrol and EGCG with those of p66Shc.

Therefore, we investigated whether treatment of cultured human keratinocytes with resveratrol and EGCG would result in the activation of p66Shc.

Although both antioxidants were able to induce ERK activation only resveratrol, but not EGCG, induced Ser36 phosphorylation of p66Shc. This is the first evidence linking resveratrol and p66Shc and may provide insight into the mechanisms underlying the effect of resveratrol on cell proliferation and function. Furthermore, the different effect of resveratrol and EGCG provides additional support to the concept that plant antioxidants drive different pathways according to their different structures that are not necessarily or uniquely linked to their antioxidant, free radical scavenging activity.

Beneficial Effects of Apple Polyphenol Extract in the Gastrointestinal Tract

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Within the gastrointestinal tract there is massive production of reactive oxygen species which may contribute to a number of pathological conditions, including nonsteroidal anti-inflammatory drugs (NSAIDs)-induced gastric damage or cancer.

Fresh fruit and vegetables have been reported to exert multiple biological effects on the mucosa of the gastrointestinal tract due to their antioxidant content. In the past few years we have sought to investigate the anti-oxidant properties of polyphenols obtained from “Annurca” apple. Also, we have studied whether an apple polyphenol extract (APE) was able to prevent NSAID injury to gastric epithelial cells in vitro and to gastric mucosa in vivo. Finally, we explored the hypothesis that APE might exert anti-carcinogenic effects in colon cancer cell lines. Our studies demonstrated that APE significantly prevented reactive oxygen species-induced damage to gastric epithelial cells in tissue culture. The protective effect was associated with an increase in the intracellular anti-oxidant potential and with a decrease in the lipid peroxidation induced by the oxidative stress. In vivo, pre-treatment with APE prevented indomethacin- or aspirin-induced injury to the rat gastric mucosa without affecting gastric secretion, thus suggesting a potential role of APE in the prevention of gastric injury in patients on long term therapy with NSAIDs.

Colorectal cancer is the 4th most common cancer and the 3rd most common cause of cancer-related death in western countries. However, the Mediterranean area is characterized by a lower incidence of cancers, including colon cancer. This has been attributed to the beneficial effects of Mediterranean diet, rich in fruit, vegetables, olive oil, and red wine and dietary polyphenols. We studied the effects of APE in a number of colon cancer cell lines and found that APE was able to decrease cell proliferation and to induce apoptosis. This anti-carcinogenic effect was associated with an increase in the protein expression of p53 (i.e. tumor suppressor gene). DNA methylation and consequent silencing of selected tumor suppressor genes plays an important role in colon cancer carcinogenesis and might represent a target for novel strategies to prevent cancer. We found that APE treatment strongly reduced DNA methylation in the promoter region of a number of tumor suppressor genes and induced restoration of their normal expression. These effects were qualitatively comparable with those obtained with 5-aza-2dc which is used in the treatment of a number of malignancies with limited clinical responses and a wide spectrum of side effects. The demethylating activity of APE was due to its inhibition of DNA methyl transferases which are responsible for DNA methylation and inactivation of tumor suppressor genes.

Our studies lend further support to the concept that food-derived natural products may exert beneficial effects in the gastrointestinal tract. In particular, APE might be regarded as a safe and effective agent in the prevention of NSAIS-induced gastric injury. Also, due to its anti-carcinogenic effects in vitro and because its lack of toxicity, we may envision the use of APE, in combination with other anti-tumor compounds, as a chemopreventive or therapeutic agent against colorectal cancer.
Antiproliferative Activity of Zinc-fortified Fruit Beverages Subjected to *in vitro* Gastrointestinal Digestion in Human Colon Cancer Caco-2 Cells

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Dietary modification through polyphenolic-rich foods could be a good strategy for preventing colorectal cancer incidence due to direct exposure of intestinal epithelia to these dietary ingredients [1]. Fruit beverages (Fb) posses many bioactive components such as ascorbic acid, tocopherols, carotenoids and polyphenols that exert their protective properties acting additively and synergistically. The polyphenolic profile of four fruit beverages fortified with zinc (Zn) (1.6 mg/100 ml Fb) with/without iron (Fe) (3 mg/100 ml Fb) and with/without skimmed milk (M) (11% v/v) were analysed by RP-HPLC-DAD before and after being subjected to *in vitro* gastrointestinal digestion. Prior to digestion FbZn and FbZnFe showed the highest phenolic content (p<0.05) followed by FbZnM and FbZnFeM. On the other hand, after digestion a dramatic decrease in phenolics was observed in all samples (up to 30% respect original fruit beverages) due to the mild alkaline conditions achieved during the *in vitro* digestion to which phenolics are sensitive. FbZnFeM showed again the lowest phenolic content (p<0.05).

In addition, Caco-2 cells were incubated during 24 h with bioaccessible fractions of fruit beverages (7.5% in culture medium) obtained after the *in vitro* digestion, in order to test the their antiproliferative effects. Cell proliferation and viability were measured using Trypan blue test. Viability was always above 90% and FbZnM was the only sample that significantly (p<0.05) inhibited cell proliferation (35% respect blank of digestion). To discard citotoxicity, MTT test was applied to the four fortified fruit beverages, showing no difference (p>0.05) in mitochondrial enzyme activities respect untreated control cells. Thus, for subsequent assays only FbZnM digests were used. Cell cycle distribution was analyzed using flow cytometry. After the 24 h period, FbZnM significantly (p<0.05) arrested cell cycle in the S-phase. Apoptosis, in turn, was not observed when determined microscopically by means Hoechst dye. These results indicate that *in vitro* inhibition of cancer cell proliferation by FbZnM did not involve apoptosis but was likely due to cell cycle arrest.

Compounds capable of accumulating in mitochondria are needed to act on a variety of processes of pathophysiological relevance involving these organelles. Mitochondria are the main source of radical species (ROS) in the cell. Mitochondrial ROS are involved in processes ranging from aging to ischemia/reperfusion damage, and have recently been shown to be a major determinant of the metastatic potential of cancers [1]. Polyphenols – many reported to have anti-cancer properties – act either as anti-oxidants or pro-oxidants, depending on circumstances. In either case, they may produce anti-tumoral effects by opposing metastasis (if anti-oxidant) or inducing death of cancer cells via a "redox catastrophe" (if pro-oxidant). A prerequisite is, however, that they reach a sufficient concentration at the intended site of action.

We have used quercetin (3,3',4',5,7-pentahydroxyflavone) and resveratrol (3,4',5-trihydroxy stilbene) as models to produce proof-of-principle “mitochondriotropic” polyphenol derivatives. For this purpose, the polyphenols have been linked to a triphenylphosphonium moiety, a membrane-permeant permanent cationic group which drives accumulation in regions held at negative electrical potential, such as the mitochondrial matrix (and the cytoplasm). Thus, sufficiently high concentrations may be locally achieved to make a significant impact on cellular processes. The compounds accumulate into isolated and in situ mitochondria as expected, and they are only slowly metabolized by human colon cancer cells (HCT116) and by blood. They inhibit the mitochondrial ATPase, induce the permeability transition and otherwise affect isolated mitochondria like the parent compounds, but – if the mitochondria are energized and they are accumulated – at lower concentrations. In the µM range they are cytotoxic for fast-growing (tumoral or embryonal) cultured cells, but not for slower-growing ones. They thus offer promise as new chemotherapeutic agents.

Quantitative Determination of Rumenic Acid, a Putative Anti-cancer Compound from Butter, by 1H-NMR

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Rumenic acid is the major conjugated linoleic acid (CLA) isomer encountered in milk-products and ruminant fats, accounting for about 90% of the total CLA in dairy products. Its chemical name is cis-9, trans-11-octadecadienoic acid. For this CLA many beneficial effects are claimed, among which anti-cancer and antioxidant activities [1].

The quantification of fatty acids in butter is generally performed by GC-MS analysis after hydrolysis of the triglycerides, a rather laborious method. Here we present a method permitting direct quantification by 1H-NMR. In the 1H-NMR spectrum rumenic acid displays specific signals at 6.28, 5.94 and 5.65 ppm, which are well separated from other signals of butter components. The ratio of the integrals of these signals and those of the glycerol signals yields a direct measure of the quantity of this fatty acid. A series of 14 samples of butter from Brazil was investigated and the rumenic acid content was found to vary between 0.50 and 1.08 %. In two margarine samples no rumenic acid was found.

An additional advantage of this method is that a fingerprint profile is obtained from the buttersample revealing further details of its composition, such as the content of diglycerides and linoleic acid. Also the amount of unsaturated fatty acids can be easily estimated.

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Dietary Supplementation of Tomato Prevents Prostate Cancer in Tramp Mice

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Prostate cancer (PCa) is the most common non-cutaneous malignant neoplasm in men in Western countries and epidemiologic studies indicate that the consumption of tomatoes, particularly processed tomato products, is associated with a 30% to 40% reduction of PCa risk.

The transgenic adenocarcinoma of the mouse prostate (TRAMP) is a model for PCa in which progressive form of the disease occurs in a manner similar to the human disease. This feature makes TRAMP mice a suitable preclinical model for studying chemoprevention of PCa. They have been used to elucidate the antitumorigenic effects of several classes of chemopreventive regimens, including phytochemicals such as green tea polyphenols, phytoestrogens, grape seed extract and flavonolignans.

In this study the effects of a diet enriched with freeze-dried whole tomatoes (12,5 g/100g) on survival, tumorigenesis and progression of PCa as well as on the antioxidant and inflammatory status of TRAMP mice were investigated.

Kaplan-Meier survival curves indicated that tomato-treatment resulted in a significant increase of the overall survival ($P<0.01$). In particular, 12 out of 18 of the animals in the toma-to-treated group were alive up to 47 weeks of age while only 2 out of 18 survived in the control group. Parallel experiments showed that, tomato treatment markedly delayed the progression from prostatic intraepithelial neoplasia to adenocarcinoma and to poorly differentiated, androgen independent tumor. The concentration of serum all-trans lycopene in tomato treated mice decreased after the week 25th. On the contrary an increase of serum antioxidant activity along the treatment time was observed. Accordingly, serum markers of angiogenesis and inflammosis as well as pro-inflammatory cytokines were significantly reduced by the tomato treatment.

In conclusion, tomato dietary supplementation was shown to reduce the incidence of poorly differentiated prostate tumour (the lethal phenotype in the TRAMP model) and than to increase the overall survival of TRAMP mice. To the best of our knowledge no other dietary intervention and/or phytochemical administration was demonstrated to be such effective, as tomato in this study, to modulate so many markers correlated to cancer progression other than the reduction of tumour dimension.
Cytotoxic Effect of Saponins from Medicago on Tumor Cellular Lines

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Saponins are a large group of plant metabolites with a broad spectrum of biological and pharmacological properties such as fungicidal, nematicidal, molluscicidal, antibacterial, antiviral and antitumor activities. The genus Medicago represents a rich source of saponins consisting of a complex mixture of triterpene glycosides with medicagenic acid, hederagenin, zanhic acid, bayogenin and soyasapogenols A and B as the main aglycones. Although some of their biological effects are known [1-3], cytotoxic and tumor-promoter inhibitory properties of those saponins have not been investigated in great details.

In the present work we describe the cytotoxicity of saponins from M. arabica (tops and roots), M. arborea (tops), M. sativa (tops and roots) and pure soyasapogenol I from M. sativa seeds on HeLa and MCF-7 (cisplatin-resistant) tumor cell lines. Bioactivity of related pro-sapogenins from M. arborea and M. sativa (tops) has also been evaluated. Saponins were tested by in vitro assays (MTT) at doses in the range of 0.01-200 \( \mu \text{g/mL} \). Medicago sapogenin-mediated potentiation of cisplatin activity was also investigated. Cisplatin alone was used in the bioassays as the reference anticancer drug.

Saponins from M. arabica were the most active with a toxicity comparable to that of cisplatin at 100 and 200 \( \mu \text{g/mL} \), especially against HeLa cell lines (~ 80%). Saponin toxicity was in general increased in combination with cisplatin (1 and 10 \( \mu \text{M} \)), even the effect was not highly significant as compared to the administration of cisplatin alone. However, interesting results were obtained with the cisplatin-resistant MCF-7 cell line; a saponin-mediated potentiation of the cytotoxic activity of cisplatin against these tumor cells was in fact evident down to 40-45 % cell survival when using soyasapogenol I and pro-sapogenins from M. sativa combined with cisplatin 10 \( \mu \text{g/mL} \), instead of cisplatin alone (60%).

Saponins are known to increase the permeability of the plasma cell membranes. The observed potentiation of the cytotoxic effect of cisplatin in combination with some of the tested compounds suggests that saponins from Medicago may as well influence the cell uptake of the antineoplastic drug. These results could lead to new possible strategies to circumvent cisplatin resistance of some cancers, such as breast carcinoma.

Sugar Moiety Structure as a Principal Determinant of Antitumor Glycosides Biological Activity. Genistein Case Study

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Among natural products which found therapeutic applications, glycosides constitute a considerable proportion, but the role of glycon/aglycon parts are seldom clearly defined in terms of molecular pharmacology [1-2]. Our own experience in the field of anthracycline antibiotics provides a good example of antitumor activity tune up by introduction of relatively simple structural changes to sugar moiety of the prototype metabolites, used as drugs in clinical oncology since 1970 [3-4].

The case of genistein, soy isoflavone presently engaged in numerous clinical trials as a prospective antitumor drug, is distinctly different. Although numerous glycosides of genistein are known as secondary metabolites of higher plants, studies of biological activity are sharply focused on the aglycon, supported by general belief that glycosidic bond like the one present in widespread genistin cannot withstand typical biodistribution along the pathway of an orally administered xenobiotic.

A number of synthetic glycosides of genistein have been obtained in our laboratories, using various chemical glycosidation methods, in order to study their biological activity in comparison with underivatized aglycon. The results clearly demonstrated that glycone split off is not necessarily favorite biotransformation of such derivatives, even for compounds which are considered to posses a weak and easily biodegradable glycosidic bond [5-6]. Moreover, some synthetic glycosides exhibited distinctly different mechanism of antiproliferative action that the one observed for the aglycon. Effects of unsaturated genistein pyranosides on cycloskeleton and cell cycle in selected tumor cell lines will be discussed in some detail.

Metabolic Profile of Uterine Leiomyomas Using High Resolution Magic-angle Spinning 1H NMR Spectroscopy

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Uterine leiomyomas are common solid pelvic tumors occurring in 20% of reproducing aged women [1]. Although these leiomyomas rarely undergo malignant degeneration, they may cause excessive uterine bleeding, pelvic pain, urinary disturbances or anemia and can lead to infertility and abortion. In spite of the fact that biochemical alteration of tumor tissues are expected to be detectable before any visible morphological changes, in case of uterine leiomyomas only the histopathological and morphological changes are well recognized, while the metabolic pathways and chemical analysis remain unknown [1].

Among available methods, high-resolution magic-angle spinning (HRMAS) 1H NMR spectroscopy is considered to be an ideal technique for investigation of metabolic composition of intact tissues [2]. HRMAS technically represents a hybrid method between classical solution- and solid-state NMR. Similarly to solid-state NMR, HRMAS uses magic angle spinning in order to reduce anisotropic interactions in solid material, while at the same time has a resolution comparable to that of conventional solution-state NMR. In addition, unlike solution state NMR that requires separated extracts of water- and fat-soluble tissue fractions, HRMAS is capable to simultaneously observe both soluble lipid and aqueous metabolites.

The metabolic composition of healthy uterus tissue (myometrium) and uterine leiomyomas was studied by 1H HRMAS NMR spectroscopy using 1D and 2D NMR pulse sequences. Three different 1D NMR spectral editing methods were applied: (i) conventional solvent presaturation to obtain an overall 1H spectrum, (ii) Carr-Purcell-Meiboom-Gill spin-echo to selectively observe small metabolites, and (iii) diffusion to selectively edit signals from large molecules. Almost 30 metabolites were detected and assigned combing information observed from 1D and 2D NMR (COSY, TOCSY, HSQC) spectra. Similar metabolites were detected in both myometrium and leiomyomas, however, subtle changes in metabolic profiles were noted due to their different contribution. These differences were analyzed by principal component analysis (PCA) that was able to differentiate myometrium from leiomyomas due to the smaller amount of myoinositol, larger amount of taurine and alternation in phospholipids signal (larger amount of =CH₂−CH₂=).

Photosensitisers from Malaysian Seaweeds for Photodynamic Therapy of Cancers

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Photodynamic therapy (PDT) has emerged as one of the treatments for various cancers in many European and Northern America countries. It involves the selective killing of tumors upon activation of a non- or low-toxic photosensitiser by light in the presence of oxygen. The formation of highly reactive oxygen species upon activation of the photosensitiser causes cellular damage, shuts down the vasculature and activates the immune response to eradicate the tumor [1]. The selective accumulation of drug in tumor tissues as compared to the surrounding tissues and targeted delivery of light at the site improve the efficacy and accuracy of the treatment as compared to conventional chemotherapy.

Most of the photosensitisers currently under investigations or already approved for clinical use are synthetically-derived compounds based on a cyclic tetrapyrrole core. Nature has hitherto been an excellent source for unique structures that have pharmacological applications. In the field of PDT, hypericin from Hypericum perforatum and hypocrellin from Hypocrella bambusae sacc. are some examples of non-tetrapyrrolic photosensitisers found in nature, suggesting that other equally interesting structures with potential as photosensitisers may also exist in nature.

In the search of novel photosensitisers, we embarked on a program to systematically screen the biodiversity in Malaysia for unique structures that may be developed into clinically useful agents. This paper outlines our efforts in studying the marine natural resources which has so far offered unique classes of compounds that are not commonly seen in the terrestrial organisms [2]. Fourteen species of seaweeds were collected from Cape Rachado, Port Dickson and 20 µg/ml of their methanolic extracts were evaluated in an MTT-based assay for photo-cytotoxicity against promyelocytic leukemia cell-line, with or without exposure to 9.6 J/cm\textsuperscript{2} of light. Six of the seaweed species show varying degrees of photocytotoxicity (7.25-33.41% cell survival). A brown (Turbinaria conoides) and a green algae (Cladophora parentiramea) were selected for further fractionation to isolate the active photosensitisers. Details of the collection, bioassay, compound isolation and characterization are described in this paper.

Pharmacological and Preclinical Evaluation of Manassantins: Potent Inhibitors of Hypoxia-induced HIF-1 Activation


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A natural product chemistry-based approach was employed to discover small molecule inhibitors of hypoxia-inducible factor-1 (HIF-1), an important tumor-selective molecular target for anticancer drug discovery. Using a human breast tumor T47D cell-based reporter assay, libraries of plant extracts were evaluated for low molecular weight natural products that inhibit hypoxia-induced HIF-1 activation. Bioassay-guided fractionation of the lipid extract of the aquatic/wetland plant Saururus cernuus L. (Saururaceae) resulted in the isolation of a series of manassantin-type dineolignans and saucerneol-type sesquineolignans as highly potent inhibitors of HIF-1 activation (IC₅₀ values range from 0.0021 to 0.85 μM). These lignans inhibit HIF-1 activation by blocking hypoxia-induced nuclear HIF-1α protein accumulation.

Manassantins were shown to inhibit hypoxic induction of HIF-1 target genes (CDKN1A, GLUT-1, and VEGF), block the hypoxic induction of the angiogenic factor secreted VEGF protein, suppress tumor angiogenesis in vitro, and inhibit tumor cell chemotaxis.

Recent in vivo studies with manassantin B in genetically engineered mouse tumor models support the hypothesis that HIF-1 inhibitors will suppress the growth of tumors with activated HIF-1, and will not significantly affect tumors with the hif1a gene deleted (non-functional HIF-1). Three murine astrocytoma tumor models were used for this study: 1) wild type hif1a and vhl genes (wt); 2) hif1a gene deleted (HIFko); and 3) vhl gene deleted (VHLko). Each was implanted subcutaneously into nude mice. As predicted, manassantin B treatment suppressed the growth of VHLko tumors that have constitutively activated HIF-1, and did not inhibit the HIFko tumors that do not have a functional HIF-1. The decrease in tumor growth correlates with apoptosis induced by manassantin B treatment. This finding supports further development of HIF-1 inhibitors as adjunct agents for the treatment of cancer.

manassantin B
Mitochondria as a Target of Drugs in Cancer and Diseases

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The essential hallmarks of cancer are linked with an altered cancer cell-intrinsic metabolism, either as a consequence or as a cause. Mitochondria have major roles in bioenergetics and vital signalling of the mammalian cell. Consequently, these organelles have been implicated in the process of carcinogenesis, which includes alterations of cellular metabolism and cell death pathways. In this regard, recently it become evident that mitochondria serve as integrators of upstream effector mechanisms in apoptosis. Thus, strategies aimed at directly triggering this event by either blocking the activity of antiapoptotic factors or by interfering with vital mitochondrial functions may help to overcome resistance to standard cancer therapy. On the other hand mitochondria have a role in a variety of diseases and consequently can be target of different drugs in therapy.

Mitochondria are "suspicious of incoming materials", in fact metabolite traffic across the mitochondrial membrane is mediated by many carriers, which due to their localization are special target for externally added compounds.

Among the mitochondrial carriers the adenine nucleotide translocator (ANT) play a major role as it export to cytoplasm the ATP synthesised in the oxidative phosphorylation.

It will be shown that
1. ANT is strongly impaired as a result of cancer photodynamic therapy
2. ANT is impaired during apoptosis of cerebellar granule cells
3. ANT impairment is responsible for the ATP syndrome which occurs in AIDS therapy by AZT.
Homophymines, a New Family of Marine Cyclodepsipeptides

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Sponges in the order Lithistida have been a prolific source of new peptides with unusual structures and remarkable biological activity [1]. Within them is a growing family of cyclic depsipeptides, all displaying potent antiviral activity. The fractionation of the polar extracts of the New Caledonian sponge Homophymia sp. has led to the isolation of a new family of cyclodepsipeptides, named homophymins. The distinguishable feature of these metabolites is the presence of previously unknown amino acid residues. In the present communication the structural characterization, including the stereochemistry of the non-proteinogenic residues, and the biological activity of the new natural compounds will be discussed.

Interaction of Distamycin A and its Derivatives with DNA Quadruplex Structures

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The ends of the chromosomes in all eukaryotic species have specialized non-coding DNA sequences that, together with associated proteins, are known as telomeres. Telomere protects the ends of the chromosome from damage and recombination and its shortening has been implicated in cellular senescence. Telomeric DNA consists of tandem repeats of simple short sequences, rich in guanine residues. In the presence of metal ions such as K+ or Na+, telomeric DNA can form structures of potential biological significance, the G-quadruplexes [1]. Telomerase, the enzyme which elongates the G-rich strand of telomeric DNA, is active in about 85% of tumors, leading the cancer cells to infinite lifetime. The inhibition of telomerase has become an attractive strategy for the anticancer therapy and, because telomerase requires a single-stranded telomeric primer, the formation of G-quadruplex complexes by telomeric DNA inhibits the telomerase activity. Furthermore, small molecules that stabilize G-quadruplex structures have been found to be effective telomerase inhibitors and, then, the use of drugs to target G-quadruplexes is emerging as a promising way to interfere with telomere replication in the tumors cells and to act as anticancer agents [2].

In this frame, the interactions between distamycin A and its two carbamoyl derivatives (compound 1 and 2) and DNA quadruplexes have been studied by 1H NMR spectroscopy and isothermal titration calorimetry (ITC). In particular, the binding to the target [d(TGGGGT)]4 and d[AG3(T2AG3)3] quadruplexes from the Tetrahymena and human telomeres, respectively, will be reported. The study has been conducted also using the recently developed Differential-Frequency Saturation Transfer Difference (DF-STD) method for assessing the ligand-DNA binding mode and using modified quadruplexes. The interactions were examined using two different buffer solutions containing either K+ or Na+ at a fixed ionic strength, to evaluate any influence of the ions present in solution on the binding behaviour. Experiments reveal that distamycin A and compound 1 bind the investigated quadruplexes in both solution conditions; conversely, compound 2 appears to have a poor affinity in any case. Moreover, these studies indicate that the presence of different cations in solution affects the stoichiometry and thermodynamics of the interactions. The three-dimensional structure of the 4:1 distamycin A / [d(TGGGGT)]4 complex has also been determined by an in-depth NMR study followed by dynamics and mechanics calculations.

On the Pathways of Cell Death Induction by Myrtucommulone from *Myrtus communis*

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Myrtucommulone (MC) is a nonprenylated acylphloroglucinol present in the leaves of the Mediterranean plant *Myrtus communis* (myrtle) [1].

We previously showed that MC potently induces cell death in various cancer cell lines (EC\(_{50}\) = 3-8 µM) with characteristics of apoptosis, but not so in normal non-transformed peripheral blood mononuclear cells (PBMC) [2].

Here, we attempted to elucidate the respective underlying mechanisms of cell death induction in cancer cell lines. We found that MC has two different modes of action: (I) inhibition of proliferation, and (II), induction of apoptosis via the intrinsic pathway involving mitochondria. MC potently (IC\(_{50}\) approx. 3 µM) decreases the phosphorylation of mitogen-activated protein kinases (MAPK), Akt and the PKC substrates MARCKs that are all signalling molecules governing cell proliferation. On the other hand, MC (3 µM) efficiently reduced the mitochondrial membrane potential (ΔΨ\(_m\)) in leukemic cells but not in PBMC which are hardly susceptible to MC. This loss of ΔΨ\(_m\) might be the trigger for cyt c release and subsequent formation of the apoptosome that leads to activation of caspase-9. Intriguingly, MC failed to induce apoptosis in a leukemic T cell line lacking caspase-9, whereas cell lines deficient in distinct elements within the extrinsic apoptosis pathway (FAS, FADD, caspase-8) were still susceptible to MC. In summary, MC exerts antiproliferative actions apparently by blocking various signalling kinases, and causes apoptotic cell death via the intrinsic pathway involving mitochondria seemingly initialized by a loss of the ΔΨ\(_m\) and recruitment of caspase-9.

Anticancer Evaluation of Structurally Diverse Amaryllidaceae Alkaloids and their Synthetic Derivatives

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Lately, plants of the Amaryllidaceae family have been under intense scrutiny for the presence of the specific metabolites responsible for the medicinal properties associated with them. The study began in 1877 with the isolation of alkaloid lycorine from \textit{Narcissus pseudonarcissus} and since then more than 100 alkaloids, exhibiting diverse biological activities, have been isolated from the Amaryllidaceae plants.

Based on the present scientific evidence, it is likely that isocarbostyril constituents of the Amaryllidaceae, such as narciclasine, pancratistatin and their congeners, are the most important metabolites responsible for the therapeutic benefits of these plant species in the folk medical treatment of cancer. Notably, \textit{Narcissus poeticus} L. used by the ancient Greek physicians, as was eluded before, is now known to contain some 0.12 g of narciclasine per kg of fresh bulbs.

Continuing along this intriguing path, the focus of the present research work is the chemistry and biology of these compounds as specifically relevant to their potential use in medicine [1].

\begin{center}
\begin{tabular}{c c}
\textbf{Lycorine} & \textbf{Narciclasine} \\
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In particular, the anticancer evaluation of lycorine, narciclasine as well as of other Amaryllidaceae alkaloids and their synthetic derivatives will be presented in this communication. The structure-activity relationships among several groups of Amaryllidaceae alkaloids will be discussed.

Jatrophane Diterpenes from *Euphorbia* spp. as Modulators of Multidrug Resistance in Cancer Therapy

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A phytochemical investigation of the aerial parts of *Euphorbia* spp., considered the most common elements of the Mediterranean landscape, lead to the isolation of a large number of bioactive plant metabolites, belonging to the diterpenes family. Above all, over sixty jatrophane [1,3-6], modified jatrophane [2], lathyrane [6], pepluane [7,8], and paralian diterpenoids [8], fifty of them reported for the first time, were extracted, purified and characterized from *E. dendroides*, *E. characias*, *E. peplus*, *E. amygdaloides*, *E. helioscopia*, and *E. paralias*. These compounds showed interesting pharmacological activities. In particular, jatrophanes, modified jatrophanes, and lathyranes exhibit a potent inhibitory activity against P-glycoprotein, a membrane protein that confers upon cells the ability to resist lethal doses of certain cytotoxic drugs by pumping them out of the cells, thus resulting in a reduced cytotoxic effect. Among the others, our chemical survey led to the isolation of the most powerful inhibitors of daunomycin efflux activity isolated to date, named Euphodendroidin D [1] and Pepluanin A [3]. Their efficiency was found to be at least two-fold higher than conventional modulator ciclosporin A, taken as a reference. In addition, the isolation of a high number of structurally-related analogues allowed to perform Structure Activity Relationship studies without any chemical modification which gave information on the key pharmacophoric elements of these new class of promising drugs. Those belonging to the rare classes of pepluane and paralian were showed to be promising anti-inflammatory agents in vivo [7,8], due to the reduction in the production of nitric oxide, prostaglandin E₂ and TNF-α by inhibiting the expression of inducible nitric oxide synthase, cyclooxygenase-2 and TNF-α mRNA respectively, through the down-regulation of NF-κB binding activity.


**Pergularia tomentosa**: a Rich Source of Antiproliferative Cardenolide Glycosides Targeting Na⁺/K⁺-ATP-ase Pump

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Several reports suggest the sodium pump as an interesting oncology target since its subunit expression is markedly altered in cancer. The sodium pump consists of two subunits in equimolar ratios: the catalytic α subunit which is a multi-pass trans-membrane protein containing the binding sites for Na⁺, K⁺ and ATP and the β regulatory subunit, a trans-membrane protein, required for the biogenesis and activity of the enzyme complex.

Na⁺/K⁺-ATP-ase α subunits seem to be up-regulated in some malignant cells [1].

Cardiotonic steroids (CSs) represent a group of compounds able to bind to the extracellular surface of the Na⁺/K⁺-ATP-ase α subunit. This fact supports the possibility of their potential development as anti-cancer agent targeting over expressed Na⁺/K⁺-ATP-ase α subunits, as shown in a number of studies that have emphasized their potential use in oncology. Several CSs demonstrate marked anti-proliferative effects against human cancer cells line in vitro [1].

Unusual cardenolide glycosides, characterized by the presence of a sugar moiety double-linked to the steroid aglycone, have been isolated from the roots of *P. tomentosa* (Asclepiadaceae), an Egyptian wild perennial shrub [2]. On the basis of the above reports, these compounds were tested against six different human cell lines: non-small cell lung cancer (NSCLC) A549, glioblastoma U373, refractory prostate cancer PC-3, pancreatic cancer cell Bx-PC3, colon cancer LoVo and breast cancer MCF-7. All the compounds displayed high anti-proliferative activity (IC50 in nM range). Moreover, these compounds were tested against three murine cancer cell lines (BI16F10, SCVII, MXT), resulting much less active. Since the α-1 subunit of the sodium pump is mutated in rodents and is about 1000 times less sensitive to cardiotonic steroids than the corresponding subunit in the human cells, it clearly appeared that compounds isolated from *P. tomentosa* exerted their anti-tumor effects by targeting mainly the α-1 subunit of the sodium pump. Thus, the ability of cardenolide glycosides to inhibit the activity of the porcine cerebral cortex extracted Na⁺/K⁺-ATP-ase (containing α-1, -2 and 3 isoforms) was tested, and the compounds showed quite potent IC50 in the range 280-680 nM which is the same range displayed by classic cardenolides like digoxin (IC50 ~367nM).


O22
Approximately one third of today’s best selling drugs are either natural products or have been developed based on lead structures provided by nature. However, it is surprising that up to now almost all medicinally used natural products or derivatives thereof were obtained from terrestrial organisms rather than from those inhabiting the sea, considering that the oceans cover more than 70% of the earth’s surface. With regard to drug discovery and development, natural products marine sources started to attract interest from pharmaceutical companies and research institutions only 40 years ago, with the advent of high performance liquid chromatography (HPLC), and the new NMR and Mass techniques. Since then, marine sources have provided well over 18,000 different natural products many of them being structurally unique and absent in terrestrial organisms. Incidence of biological activity in marine derived compounds is high, especially with regard to cytotoxicity where marine-derived extracts surpass those of terrestrial origin. It is no surprise therefore that marine natural products have their stronghold in the area of anti-cancer chemotherapy as indicated by the list of compounds currently under clinical investigation. Ascidians, invertebrates belonging to the subphylum Urochordata (Tunicata), are renowned for their great chemical diversity and, during the last 25 years, they have been shown to produce an array of cytotoxic molecules. Among the first six marine-derived compounds that have reached clinical trials as antitumor agents, three are derived from ascidians [1], as evidence of the high potential of these organisms as a new source of antitumor compounds. In the course of our research activities on marine ascidians from the Mediterranean Sea, we discovered a number of new molecules with different structural features but all endowed with antiproliferative or cytotoxic activity [2-6]. Reported in this communication are some of our recent results on the chemistry and bioactivity of these natural products, which strongly support the hypothesis that ascidians natural products could play a highly significant role in anticancer drug discovery and development process.

Sodium Pump Antagonist UNBS1450: a Novel Means to Combat Apoptosis and Multi-drug Resistant Cancer Cells

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Cardiotonic steroids (including cardenolides and bufadienolides) are characterised by their abundance in nature, potential for chemical modification and use in cardiology for heart failure management. Cardiotonic steroids are the natural ligands of sodium pump (Na+,K+-ATPase) α subunits. α1 subunits over-expressed in a large number of solid cancers potentially represent a new target for anti-cancer therapy. Modification of 2”-oxovorusscharin (a novel cardenolide we extracted from Calotropis procera) has led to the identification of UNBS1450 characterized by markedly more potent anti-tumor activity than classic cardenolides. Structurally UNBS1450 differs significantly from classic cardenolides and it displays a markedly higher binding affinity for the alpha subunits of the sodium pump.

UNBS1450’s marked anti-cancer activity is characterized by both anti-proliferative and anti-migratory features, resulting from its ability to disorganize the actin cytoskeleton, leading to autophagy-related cell death rather than apoptosis. UNBS1450 also proved effective against apoptosis and multi-drug resistant cancer cells. Even limited exposure (1-2h) of cancer cells to UNBS1450 induced irreversible growth arrest. By binding to the sodium pump, UNBS1450 affects downstream signaling pathways, provoking nucleolar targeting and irreversible c-Myc down-regulation. In vivo in aggressive and metastatic orthotopic NSCLC, refractory prostate cancer and glioma models, UNBS1450 was more potent than tested reference compounds. Safety pharmacology studies in rats indicated no compound-related effects on the CNS or on respiratory function. Cardiovascular evaluations in dogs demonstrated a shortening of ventricular repolarisation which was not considered adverse, although digoxin-like arrhythmia at i.v. doses ≥0.07mg/kg was evident; with pro-arrhythmic potency/toxicity similar to digoxin. UNBS1450 post i.v. was rapidly cleared from rat and dog plasma with a half-life between 0.09-0.76h. Total clearance was high in both species; approaching or exceeding hepatic blood flow.

The structural uniqueness of UNBS1450 taken with its novel mechanism of action, support its clinical development as an anti-cancer agent targeting over-expressed sodium pump α1 subunits and using c-Myc expression as a biomarker.
O25

Prenyloxyphenylpropanoids as a Novel Class of Colon Cancer Chemopreventive Agents

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Oxyprenylated natural products (isopentenyloxy-, geranyloxy- and the less spread far-nesyloxy- compounds and their biosynthetic derivatives) represent a family of secondary metabolites that have been considered for years just as biosynthetic intermediates of C-prenylated derivatives. Only in the last decade these natural products have been recognized as interesting and valuable biologically active phytochemicals [1]. Many of the isolated oxyprenylated natural products were shown in the last decade to exert in vitro and in vivo remarkable anti cancer and anti-inflammatory effects.

Among prenyloxyphenylpropanoids, geranylated coumarins and cinnamic acid derivatives, such auraptene 1, collinin 2 and 4’-geranyloxyferulic acid 3 has been discovered as valuable chemopreventive agents of several types of cancers, in particular those affecting gastrointestinal apparatus. We elaborated a high yield and “eco-friendly” synthetic scheme of compounds 1-3, starting from cheap and non toxic reagents and substrates, that allowed us to handle quantities of all compounds sufficient to perform in vivo studies as colon cancer chemopreventive agents.

Colon cancer was induced in animals by administration of azoxymethane and sodium destrane sulphate along a period of 3 weeks, followed by administration in the basal diet of compound under investigation for 17 weeks at different concentrations [2].

All natural geranyloxy natural products listed above showed a good chemopreventive effect for both benign (adenoma) and malign form (adenocarcinoma) of colon cancer. Incidence, multiplicity of tumors, as well as proliferation and inflammatory indices (COX-2 and iNOS expression) decreased in all cases especially in animals treated with the highest concentration level of oxygeranylated compound.

\begin{tikzpicture}
\begin{scope}[scale=0.8]
\node at (0,0) [anchor=west] {1 \(R = \text{H}\)};
\node at (1,0) [anchor=west] {2 \(R = \text{OCH}_3\)};
\node at (2,0) [anchor=west] {3 \(\text{OCH}_3\)};
\end{scope}
\end{tikzpicture}


Phytotherapy of Malignant and Benign Tumors with Mixtures of Extracts of Angiosperms from Arid Zones of North of Mexico

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Introduction. Diverse angiosperms from the arid and semiarid regions from northern Mexico, are profusely applied in ethnotherapy for the treatment of digestive disorders: mainly gastric ulcers and not specified neoplasms \cite{1}. If natural compounds are employed individually with direct action against cancerous cells, require of excessive and insecure doses. Lower doses permits less adverse reactions and its combinations, have the possibility of additive and synergistic effect \cite{2}.

Objective: Our Group applied combinations of watery extracts of several angiosperms with antineoplastic activity: roots of Cucurbitaceae family. Shafts of Cactaceae family. Leaves and shafts of the Meliaceae family, and leaves and shafts of the Zygophyllaceas family; in patients with malignant and benign tumors. The phytochemical content is actually investigated (alkaloids, steroids, etc.) \cite{3}.

Methodology: Phytotherapy as causal treatment was used in 5 patients with malignant (3) and benign (2) tumors. Case No.1. Feminine, 42 years old; with papillary thyroid cancer with metastases to the right lymphs glands of neck. Phytotherapy: November 11, 2005. Evolution: December 4, 2007. Without evidence of metastases, February 18, 2008. Asymptomatic.


Conclusions: The combination of watery extracts of several angiosperms of the mexican deserts showed clinically, antineoplastic activity.


Investigation of Cytotoxic, Genotoxic and Proliferative Effects of Rhein, an Anthraquinone Compound Isolated from Cassia Species, on Caco-2 Human Adenocarcinoma Cells

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Among laxatives, anthranoids are the oldest used drugs in clinical practice and as self-medications [1]. In recent years the use of anthraquinone laxatives, in particular senna, has been associated with damage to the intestinal epithelial layer and an increased risk of developing colorectal cancer [2], although there are conflicting data [3]. In the present study we evaluated the cytotoxicity of rhein, the active metabolite of senna, on human colon adenocarcinoma cells (Caco-2) and its effect on cell proliferation. Cytotoxicity studies were performed using MTT and TEER assays whereas \textsuperscript{3}H-thymidine incorporation and western blot analysis were used to evaluate the effect of rhein on cell proliferation. Moreover, for geno-protection studies, comet assay and oxidative biomarkers measurement (malondialdehyde and reactive oxygen species) were used. Rhein (0.1-10 μg/ml) had no significant cytotoxic effect on proliferating and differentiated Caco-2 cells. Rhein (0.1 and 1 μg/ml) significantly reduced cell proliferation as well as MAP kinase activation; by contrast, at the high concentration (10 μg/ml) rhein significantly increased cell proliferation and ERK phosphorylation. Moreover, rhein (0.1-10 μg/ml) (i) did not adversely affect the integrity of tight junctions and hence epithelial barrier function, (ii) did not induce DNA damage rather it was able to reduce H\textsubscript{2}O\textsubscript{2}-induced DNA damage and (iii) significantly inhibited the increase in malondialdehyde and ROS levels induced by H\textsubscript{2}O\textsubscript{2}/Fe\textsuperscript{2+}. In conclusion our data show that rhein is devoid of cytotoxic and genotoxic effects in colon adenocarcinoma cells. Moreover, at concentrations present in the colon after a human therapeutic dosage of senna, rhein inhibits cell proliferation via a mechanism which seems to involve directly the MAP kinase pathway. Finally, rhein prevents the DNA damage probably via an anti-oxidant mechanism.


Molecular Mechanism of Plumbagin Action: an Update on its Antitumor Function

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Previous studies show that reduction in BRCA1 mRNA and protein can result in increased proliferation of BG-1 ovarian cancer cells in both in vitro and in vivo conditions, suggesting that BRCA1 may normally be acting as a growth inhibitor in these cells. Also, there are other reports suggesting that wild type BRCA1 protein may repress estrogen receptor (ER) function either directly or indirectly. However, response to antiestrogen drugs in BRCA1 blocked ER positive ovarian cancer cells has not been reported. We have analyzed the effect of about 20 different natural compounds in BRCA1 blocked ER positive BG-1 ovarian cancer cells. Only plumbagin, a plant derived napthaquinone, showed a statistically significant difference in mean viability, i.e., BRCA1 blocked cells were more sensitive than the corresponding control cells against plumbagin. It induced loss of mitochondrial potential, nuclear condensation, DNA fragmentation, and morphological changes observed after 6 h of drug treatment which are suggestive of apoptosis induction in both BRCA1 blocked and control cells. Although we found that all the compounds studied induced apoptosis, the induction was in the order of plumbagin> naphthoquinone, doxorubicin, tamoxifen >juglone>emodin>genistein>cisplatin. The dose of plumbagin needed to kill 50% was 5 $\mu$M in the control cells and 2.68 $\mu$M for the BRCA1 blocked cells indicating that the latter was about two fold more sensitive to plumbagin than the wild type cells. Plumbagin can bind to the active site of ER-$\alpha$ inducing ER-$\alpha$ 46 kDa truncated isoform, which was found abundantly preempted in the cytoplasm compared with a 66-kDa full-length isoform. The truncated isoform is known to inhibit classical ER-$\alpha$ signaling pathways. SiRNA transfected cells for ER-$\alpha$ exhibited lower cytotoxicity upon plumbagin treatment than the control-transfected cells. We have done molecular mechanism of action of plumbagin in BRCA1 blocked ovarian cancer cell line by suppression subtractive hybridization and microarray. This throws light on the fact that plumbagin may have a chemotherapeutic potential as an anticancer agent in BRCA1 mutated ovarian cancer patients. Furthermore, our study in cervical cancer cells using a combination of plumbagin and radiation is effective in altering the expression of the apoptotic genes and in the activation of caspases, and reducing the dose of radiation to have same of better effect than a higher dose of radiation alone. Further studies are going on to establish the effects of plumbagin as an enhancer of radiation effects in experimental cells and animal models.


Poster Session

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Phytochemical Study of the Endemic Algerian Plant *Launaea arborescens*

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The genus *Launaea* belongs to the tribe Lactucea of the Asteraceae family and contains about 40 species, most of which are adapted to dry, saline and sandy habitats. Only few previous investigations of some species of this genus reported the presence of phenolic compounds as the main metabolites such as flavonoids and coumarins [1-3], whereas terpenoids were described [4,5].

*Launaea arborescens* is one of the endemic Algerian plant which is localised in the South-east of the country and used in popular medicine. Only two chemical papers on this species have been so far published [6,7]. As part of a continuing interest in this plant, we undertook a systematic chemical investigation of the liposoluble extract with the objective of characterising the terpenoidic fraction.

The dried and powdered aerial parts of *L arborescens* were extracted by light petroleum ether while the roots were extracted by methanol. The petroleum ether extract of the aerial parts and the ethyl acetate soluble portion of the methanol extract of the roots were submitted to subsequent chromatographic separations and the terpenoids containing fractions were further purified by HPLC to obtain twenty-seven metabolites, five of which were novel compounds.

In this communication, we will present the elucidation of structures of the new metabolites, on the basis of their spectroscopic analysis, including mainly 1D and 2D NMR techniques and by comparison with the literature data.

Finally, preliminary cytotoxic assay for some of these compounds will be presented.

Comparison of the Active Compounds and *in vitro* Anticancer Effects of Three *Hypoxis* Species (African Potato)

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The African potato is used as an African traditional medicine for its nutritional and medicinal properties. *Hypoxis hemerocallidea* topped the list of the 60 most frequently traded plant species in the Eastern Cape, South Africa when a study was done in 2002 [1]. The identification and isolation of the glycoside, hypoxoside [2] in the corms of *Hypoxis hemerocallidea* has shown promising anticancer activities [3]. Most research has been carried out on *H. hemerocallidea* (formerly known as *H. rooperi*), with very little or nothing on other *Hypoxis* spp. TLC and GC were used for the identification and quantification of sterols (especially β-sitosterol)/sterolins in chloroform extracts of *H. hemerocallidea*, *H. stellipilis* and *H. sobolifera* var. *sobolifera*. HPLC was used to identify and quantify hypoxoside content in these *Hypoxis* spp. The cytotoxicity of the chloroform extracts of the three *Hypoxis* spp. and purified hypoxoside/rooperol were tested against HeLa, HT-29 and MCF-7 cancer cell lines. DNA cycle arrest and caspase 3/7 activities were measured as possible mechanisms of action. TLC results showed that *H. sobolifera* contained the most sterols/sterolins compared to the other two *Hypoxis* spp. GC results show that β-sitosterol and campesterol were the main two phytosterols present in the *Hypoxis* extracts. *H. sobolifera* and *H. hemerocallidea* contained the most β-sitosterol and hypoxoside, respectively. Treatment of the three cancer cell lines has shown a difference in the *in vitro* anticancer activity of the chloroform *Hypoxis* extracts. A difference in apoptosis, cell cycle arrest and caspase 3/7 activities may explain possible mechanisms of action. These results show a difference in the sterol/sterolin and hypoxoside contents and anticancer activities between species of the genus *Hypoxis*.

Mitochondriotropic polyphenol-based compounds may have biomedically relevant activities. We are working with derivatives of quercetin and resveratrol bearing a triphenylphosphonium group. Assessment of the pharmacokinetics is a prerequisite for in vivo tests. This in turn requires the development of a suitable analytical method, ensuring complete extraction and stabilization of the polyphenols and their derivatives from whole blood. Analysis of plasma, as reported in several publications, may not be adequate since polyphenols are known to associate with haemoglobin and other blood proteins, and our compounds are expected to be largely sequestered inside mitochondria.

The suitability of different methods has been evaluated measuring (via HPLC-UV analysis) the recovery of polyphenol and internal standard from spiked blood samples. We first developed satisfactory procedures for resveratrol; the two methods ensuring good recovery of resveratrol were then tested with quercetin; only one avoided quercetin oxidation, and was thus tested for the recovery of the mitochondriotropic derivatives, for which it also proved to be suitable. It involves prevention of polyphenol oxidation, protein precipitation, and solubilization/extraction.

The incubation at 37 °C for different times of blood samples spiked with resveratrol, quercetin or their mitochondriotropic derivatives, followed by treatment as mentioned, revealed an important metabolization (mostly methylation) of the parent compounds after 1h, while metabolic processing of the mitochondriotropic derivatives was limited. Directing polyphenols into mitochondria may thus provide not only a way to focus their activity, but also the means to hinder their metabolism by having the compounds accumulate in a subcellular compartment lacking conjugating enzymes.

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Hyperforin against Multiple Myeloma Plasma Cells

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Hyperforin (Hyp) is an active compound contained in the extract of Hypericum perforatum, well known for its antidepressant activity. It has been found to possess several other biological properties, including inhibitory effects on tumor invasion, angiogenesis, and to promote apoptosis of leukemic B cells.

Here we investigated the role of Hyp on survival and migration ability of multiple myeloma (MM) plasma cells (PC). Flow cytometry analysis of six MM cell lines and highly purified malignant PC from eight patients with MM showed that Hyp induced apoptosis, as shown by dose-dependent stimulation of phosphatidylserine externalization and DNA fragmentation, by disruption of the mitochondrial transmembrane potential and cleavage of the caspase substrate PARP-1. Hyp also downregulated CXCR4 expression on malignant PC and restrained their migratory capability towards CXCL12, a CXCR4 specific ligand. Finally, treatment of MM cells with Hyp resulted in a marked inhibition of their capacity to secrete matrix metalloproteinase-9, an essential component in neo-angiogenesis through degradation of the extracellular matrix process. All these effects were dose-dependent and in the µM range measured in the blood of people under Hypericum extract treatment.

Altogether, these properties qualify Hyp as a lead structure for the development of new therapeutic molecules in the treatment of various diseases, including some haematological malignant tumors such as MM.
3-O-methylfunicone, a Metabolite from *Penicillium pinophilum*, Inhibits Proliferation of Human Tumor Cells by Inducing Apoptosis

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3-O-methylfunicone (OMF) is a secondary metabolite produced by the soil fungus *Penicillium pinophilum*. The compound was found to be involved in antagonism against the plant pathogenic fungus *Rhizoctonia solani* [1], and displayed potent cytostatic properties on human tumor cells (HEp-2) [2]. Our previous studies demonstrated the ability of OMF to induce growth arrest and apoptosis in HeLa cells and to inhibit cell motility in MCF7 cells not affecting the normal MCF-10 cell line [3,4]. Furthermore, OMF affected cell growth by inducing apoptosis in a parental melanoma cell line (A375P) and in its metastatic derivative (A375M), displaying low and medium metastatic behavior, respectively [5]. Studies currently in progress show that this compound has also the ability to arrest the growth and induce apoptosis of mesothelioma cells, without affecting the primary cell culture derived from normal mesothelial tissue. The structural similarity to routinely-used anticancer drugs, associated with the antiproliferative and pro-apoptotic properties on different cell lines, make OMF a good candidate in contrasting tumor proliferation and migration, and characterize it as a promising molecule to be included in the strategies for the treatment of cancer.

α-tomatine in Solanum Species: Characterization of its Content and Biosynthetic Pathway

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Tomato plants synthesize the steroidal sapoin α-tomatine, that is constituted of a tetrasaccharide group attached to the aglycone tomatidine. This compound acts in planta as a phytoanticipin against fungi, bacteria and insects. Moreover, it shows interesting pharmaceutical properties including antibacterial, antifungal, antiviral, anticholesterolemic and antitumoral activities. In facts, microculture tetrazolium (MTT) in vitro assay showed that α-tomatine is a strong inhibitor of growth for both human colon and liver cancer cell lines (Lee et al., 2004). Recently, Friedman et al. (2007) reported also the anticarcinogenic effects of α-tomatine in the rainbow trout model.

The main objectives of the present work are: i) the characterization of α-tomatine content in tomato cultivars and wild species; ii) the isolation of genes involved in the metabolic pathway; iii) toxicological studies using α-tomatine and Solanum species extracts have been also performed.

For characterization of content, crude extracts from different organs of five traditional varieties and four wild species of tomato were tested by a bioassay with Trichoderma viridae that showed a dose-dependent dropping in colony diameter related to the concentrations of tomatine in the medium. The growth of Trichoderma viridae was inhibited in a significant way ($P \leq 0.05$) as compared to controls by crude extracts from leaves of San Marzano, Corbarino and Perino Giallo and by crude extracts from immature fruits of San Marzano and Corbarino. All crude extracts from leaves and flowers of wild species significantly inhibited the growth of T. viridae, except those obtained from leaves of Solanum pennelli.

The biosynthetic pathway of α-tomatine is still weakly understood despite the pharmaceutical importance of this secondary metabolite. Since the pathway of steroids is the starting point of tomatine biosynthesis, molecular cloning of the cycloartenol synthase (CAS1), (S)-adenosyl methionyltransferase (SMT1) and cycloeucalenol cycloisomerase (CYC1) genes were pursued in S. lycopersicum by homology-based PCR method. Moreover, to study differential gene expression of CAS1, RT-PCR was performed in different organs and species and results will be discussed.

Toxicological studies shown that all Solanum species analyzed did not exert any toxic effects.


A New Member of the Tambjamine Alkaloids Family from the Nudibranch Tambja ceutae and its Prey Bugula dentata

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Tambjamines are a group of alkaloids that have been isolated from bacteria and marine invertebrates including bryozoans, nudibranchs and ascidians \[1\text{-}4\]. From a structural point of view, the tambjamines belong to the group of 4-methoxypyrrolic natural products including several antitumor compounds such as the tripyrrolic prodigiosin family of alkaloids \(1\) \[5\]. Tambjamine structure in fact comprises a \(2,2'\)-bipyrole ring system containing an enamine moiety at the C5 position of the pyrrole ring, and an adjacent methoxy group at C4. In most of these compounds the enamine nitrogenum is substituted with a two to four carbon saturated alkyl chain.

As most of biological active natural products, tambjamines seem to be implicated in the chemical defense mechanisms of the organisms from which they are obtained \[6,7\]. Besides of the ecological role, a number of these alkaloids has shown to possess a wide spectrum of pharmacological properties including antitumor, antimicrobial and immunosuppressive activities \[2,8\]. In addition, it has been demonstrated that tambjamine E is able to bind DNA and facilitate single-strand DNA cleavage in the presence of Cu(II) and molecular O\(_2\) \[7\].

In the course of our search of new lead compounds among the chemical weapons of shell less mollusks, we have isolated from the never studied nudibranch Tambja ceutae and its prey, the bryozoan Bugula dentata, a new tambjamine, tambjamine K (\(2\)), together with known tambjamine A and the blue pigment 3. Antiproliferative properties of the isolated compounds were tested on human epithelial ovarian cancer (EFO-27) and rat basophil leukemia (RBL-2H3) cell lines.

The results of this study will be discussed in the present communication.

O-Phosphoryl Derivatives of (E)-Resveratrol: Some Biological Properties

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In the last decade there has been considerable interest in the polyphenol (E)-resveratrol (trans-3,4',5-trihydroxystilbene, 1), a stilbenoid synthesized by a variety of dietary plant species. This compound possesses different and very interesting biological activities, among them differential effects on growth, cell cycle arrest, and induction of apoptosis in human prostate cancer cell lines [1]. Resveratrol is poor water soluble and this property makes somewhat difficult a direct utilization of the free compound in the biological assays where the medium is an aqueous solution.

Recently, we had synthesized three hydrophilic derivatives of (E)-resveratrol employing a chemoenzymatic strategy, namely 4'-O-phosphorylresveratrol (2), 3-O-phosphorylresveratrol (3) and 3,5-di-O-phosphorylresveratrol (4) as sodium salts.

These water-soluble derivatives of resveratrol were subjected to some biological studies in parallel with the lead compound 1.

Firstly, compounds 1-4 were tested in parallel experiments for cell-growth inhibitory activity towards androgen non responsive DU 145 human prostate cancer cells, androgen responsive LNCaP cells and human benign prostatic hyperplasia BPH-1 cells. Normal human fibroblasts were used as non tumor control cells. The assay results showed 3-O-phosphorylresveratrol twice more active than resveratrol against DU 145 prostate cancer cells.

Then, we have explored the interaction of the three phosphoryl derivatives with DNA by means of UV-absorption spectroscopy and Differential Scanning Calorimetry (DSC) investigations. The UV spectra of aqueous solutions of calf thymus DNA at varying concentrations were recorded in parallel experiments, in the presence of fixed amounts of each one of the compounds 1-4. The Benesi-Hildebrand plots of the spectral data gave the best binding affinity for 4'-O-phosphorylresveratrol (2). Moreover, preliminary DSC measurements of thermal denaturation of calf thymus DNA in presence of compound 2 evidenced a prevalent interaction of 4'-O-phosphorylresveratrol with the satellites DNA richest of GC base pairs.

A First Approach to the Synthesis of Anigopreissin A, a Resveratrol-derived Compound with Potential Antiproliferative Activity

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Benzofurans form the core of numerous natural products and have considerable pharmacological potential as antitumoral agents, antidepressants or inhibitory activity of the HIV-protease [1].

Herein we report the first studies towards the total synthesis of the Anigopreissin A, a resveratrol dimer, isolated from roots of \textit{Anigozanthos preissi} and from rhizomes of \textit{Musa Cavendish} [2], possessing a tetrasubstituted benzofuran ring. Because resveratrol analogs as well as polyhydroxylic benzofurans possess antiproliferative properties [3] we decided to synthesize this compound in order to evaluate its biological activities. The two reported retrosynthetic strategy envisioned Pd-catalyzed cross-coupling reactions (Suzuki, Sonogashira, heteroannulation reactions) as key steps to prepare the target compound. The results of the synthesis will be discussed.

Antiproliferation and Antioxidation by Extracts from Seeds of Carob Tree (Ceratonia siliqua L.) on Human Cancer Lines

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Carob tree (Ceratonia siliqua L.) is one of the most useful trees of the Mediterranean basin. The two main constituents of carob fruits are (by weight): pulp (90%) and seed (10%), from which locust bean gum (LBG) is extracted. The germ and a part of the carob seed skin are a waste from the LBG industrial processing, which are known to be rich in tannins. They are, therefore, a cheap source of natural polyphenolic phytochemicals, and their value could be increased if other more valuable uses were found. There is currently an increasing awareness concerning the use of natural antioxidants, since studies have provided evidence for their role in carcinogenesis. However, the high cost of the exploitation of naturally occurring agents constitutes a major limitation towards their use. As a result, efforts have been focused on inexpensive plant sources and on agricultural wastes rich in phenolic compounds, which are probably the most promising class of natural antioxidants.

The aim of this work was the evaluation of the antiproliferative activity of methanolic extracts of germ + seed coat from a female cultivar of carob tree (Mulata), in human cancer cell lines, namely a cervical (HeLa), a prostate (DU-145), a breast (MDA-MB-231) and a colon cell line (HCT-166). Cells were treated with extracts at different concentrations (2.5-20 mg.ml-1), for 24, 48 and 72 h. Citotoxicity was assessed by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay.

It was observed that the extracts significantly inhibited cell proliferation in a dose-dependent manner, and the best periods of incubation were 48 and 72 h. In addition, the extracts were also found to inhibit ROS production. The results suggest that the methanolic extracts from the germ + seed coat of carob tree have antiproliferation and antioxidant activity, which indicates that they have potential for cancer chemoprevention.

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Biomimetic Synthesis of ‘Unnatural’ Lignans with DNA Intercalating Properties

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Lignans and related compounds (neolignans, oxyneolignans and others) are a group of plant metabolites showing a large structural diversity and including many bioactive molecules, among them cytotoxic, antileishmanial, antiangiogenic, cardiovascular and antiviral agents. The most cited example of bioactive lignan is podophyllotoxin, isolated as an anti-cancer drug etoposide. Dimeric lignans and neolignans are biosynthetized by radical coupling of two phenylpropanoid (C\textsubscript{6}C\textsubscript{3}) units. Biomimetic syntheses of lignans can be carried out starting from a natural phenylpropanoid and employing oxidative coupling methods.

In our search of synthetic analogues of bioactive natural products, possibly displaying anticancer properties, we tried to obtain dimeric lignans by oxidative coupling of caffeic acid esters; in particular, we employed as first substrate the caffeic acid phenetyl ester (CAPE); this natural product is a component of propolis and it is reported as an anti-inflammatory, antioxidant and antitumoral agent. Using MnO\textsubscript{2} or Mn(OAc)\textsubscript{3} as oxidative coupling agents, the benzo[k]xanthene lignan (1) was obtained with good yield. A similar reactions was carried out on methyl caffeate, thus obtaining the related lignan 2. These ‘unnatural’ lignans are strictly related to the benzo[k]xanthene lignan yunnaneic acid H, isolated from \textit{Salvia yunnanensis}. Benzoxanthene lignans are rare both among natural products and their synthetic analogues.

Both compounds 1 and 2 were submitted to a DF-STD NMR (Saturation Transfer Difference spectroscopy) study and molecular docking analysis to evaluate a possible DNA binding. Employing a poly(dG-dC)•poly(dG-dC) as model DNA receptor, DF-STD experiment clearly showed that the benzoxanthene lignans 1 and 2 are DNA intercalating agents. An independent docking study corroborated these results and showed that the methyl and phenethyl groups are inserted into the minor groove. The lignans 1 and 2 are currently under investigations as antiproliferative and antiangiogenic agents.
Antiangiogenic Effects of *Hypericum perforatum* L. Essential Oil

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Angiogenesis is also associated as a critical stage in the growth and metastasis of various cancer types. The antiangiogenic approach which corresponds to the inhibition of formation of new blood vessels, is a new advance and target for the treatment of some tumors [1,2]. A wide range of natural products and compounds were proven as angiogenic inhibitors with extension to cancer research [2,3].

In this study we have investigated the essential oil of *Hypericum perforatum* L. (Clusiaceae) obtained by hydrodistillation for its angiogenesis and antiangiogenic properties using the chicken chorio allantoic membrane assay [4]. Gas chromatographic (GC) and gas chromatography-mass spectrometric (GC-MS) analyses of the oil revealed a composition with the main components as α-pinene (33.5±0.2%), β-pinene (12.4±0.2%), 3-methyl nonane (7.9±0.1%), caryophyllene oxide (4.2±0.1%), and 2-methyl decane (4.0±0.1%). Overall, 59 compounds corresponding to 89.9% of the total were identified in the investigated essential oil.

The essential oil at various concentrations (5-50 microgram/pellet) remarkably prevented new blood vessel growth in the in vivo chicken embryo chorioallantoic membrane assay (CAM) compared to the standards suramin and thalidomide. Determination of the active constituents and the antiinflammatory properties of the *H. perforatum* essential oil using a modified CAM assay is still under progress by using bioassay guided fraction techniques.

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Glycopyrannosylglycerols from Red Algae as a New Therapeutic Approach in Cancer Treatment

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Seaweeds constitute an attractive marine resource with a high potential for medical applications. Many biological activities have been already found for a wide range of molecules isolated from seaweeds. However, a class of molecule remains not still well investigated for their biological activities. It concerns the glycopyrannosylglycerols, primo-photosynthesized molecules contained in red algae and composed by floridoside and its analogues. In this study, these natural products have been purified from selected red algae species from Brittany coast in France. ESIMS, 1D and 2D NMR Spectroscopy performed on these low-molecular-weight carbohydrates allowed to elucidate their chemical structure \cite{1}, and \cite{3}. Three molecules of same chemical family, X1, X2 and X3 were extracted and their cytotoxicity was evaluated in daudi cells (human B cells, Burkitt Lymphoma). Apoptosis was quantified by detecting surface exposure of phosphatidylserine using Annexin V-FITC/PI detection kit. No cytotoxicity was revealed after treatment by these compounds. On the other hand, these molecules have shown different immunostimulating properties, especially X3 which increase neutrophil phagocytosis. Furthermore we highlighted a great enhancement of NK cell activation, after exposure to these natural products by an antibody-dependent -cellular-cytotoxicity flow cytometric assay, especially by X3 molecule. This mechanism is now a major axis of therapeutic antibodies-activity potentiation \cite{4}. Moreover, X3-stimulated human cells exhibit an increased capacity to IFN production confirming NK cells activation contrary to X1 or X2-stimulated cells. These cytokine is already known to activate mononuclear phagocytes, one of its most prominent and potent immuno-modulatory effects as it was first demonstrate in vivo by Nathan et al. \cite{5}, and it usually used in the treatment of cancer. So, glycopyrannosylglycerols contained in red algae and more particularly the X3 molecule represent a new potent clinical use in tumor treatment by amplifying the immune response and more effectively kill tumor cells.

\cite{4} Masayasu Hara et al (2008) Interleukin-2 potentiation of cetuximab antitumor activity for epidermal growth factor receptor-overexpressing gastric cancer xenografts through antibody-dependent cellular cytotoxicity Cancer Science.
\cite{5} Carl F. Nathan et al (1985) Administration of recombinant interferon y to cancer patients enhances monocyte secretion of hydrogen peroxide Medical Sciences 82: 8686-8690.
Oncoly’s Anti-cancer, Anti-aging and other Cytoprotective Functions


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Oncolyn is a formulated extract from three edible plants. More than ninety percent of its ingredients are polyphenols, proanthocyanidins, phenolic acid and saponins in synergistic combination with other natural plant ingredients, which together exhibited effective anti-cancer, antioxidant, apoptosis, anti-angiogenesis, anti-inflammatory and cytoprotective function. It neutralized the oxidative damage of H2O2 to human lymphocytes, and ROS damage to endothelial cells. Oncolyn further protected pulmonary macrophages and Wistar rats from inhalation injury by asbestos and silica, as verified by single cell gel electrophoresis and histopathology. Oncolyn is highly inhibitory against various cancer cells in vitro. Cell cycle studies with flow cytometry, and morphological changes observed with electron microscopy demonstrated cancer cells with various stages of apoptosis under the influence of Oncolyn.

Oncolyn inhibited the telomerase [1,2,6] activity of various cancer cell lines correlated with cancer cell apoptosis. Oncolyn extended the life expectancy of Musca domestica and Drosophila melanogaster. Oncolyn further delayed the senescence of mice as verified by brain biochemistry and mice behavior pattern.

In vitro, Oncolyn inhibited HIV replication. It markedly reduced p24 antigen expression [3] after 14 days post-infection. In nude mouse, Oncolyn [5] was found to be very effective against implanted human cancer cells of lung, colon, liver and stomach origin for both prevention and therapy. Expression of FAS genes, a biomarker for apoptosis, was demonstrated by immunohistochemistry for both prevention and therapy groups. Subrenal capsule assay in mice showed Oncolyn caused reduction of implanted tumor fragments from patients with invasive breast carcinoma, rectal adenocarcinoma and squamous cell carcinoma of the lung. Oncolyn reduced the implanted carcinoma by itself, and synergistically with other chemotherapeutic agents such as cytoxan, 5FU and methotrexate, cisplatin and adriamycin. Oncolyn caused objective and subjective improvement of patients with different types of cancers (90%), some in clinical remission, in an in-patient setting, 90 days, in a large teaching hospital.

Median survival of malignant diffuse thoracic mesothelioma is less than 6 months. From the time of definitive pathology diagnosis, approximately 90% of the patients are dead within a period of one year, in spite of combination therapy with surgery, radiation and chemotherapy. In 1999, we applied Oncolyn for one terminal pulmonary mesothelioma patient and achieved a clinical remission in 6 months. The patient is living and well and working full time as of June 2008. A terminal disseminated intraosseous lymphoma patient also achieved clinical remission in 6 months with Oncolyn therapy in July 1999. The lymphoma patient finally passed away from pneumonia in March 2005. Another patient with Embryonal carcinoma of the testis with metastases to the liver and lung achieved a clinical remission in 6 months using Oncolyn, and fathered a healthy child now 8 years old (6/2008) and his wife gave birth to two additional healthy babies.

Concomitantly Oncolyn also caused amelioration of rheumatoid and osteoarthritis and produced a feeling of wellness in patients with various chronic debilitating conditions including COPD, and prevented the reoccurrence of TIA's and caused speedy recovery of stroke patients (Dr. Wang, Tianjin First Central Hospital). Oncolyn further caused remission for canine mammary cancer (Dr. Murley) and amelioration of canine liver adenocarcinoma (Dr. Moscow) [4].

The present poster will report the results of nude mice's response to Oncolyn's prevention therapy vs. various cancer cells implantation, single cell gel electrophoresis assay against injuries caused by asbestos and silica, delaying senescence in mice and Oncolyn's clinical experiences with various malignant solid tumors (prostate, breast, lung, colon and others) with illustrative cytology, surgical pathology, CT, MRI and clinical data [5].

Oncolyn has 5 patents (3 United States, 1 Canadian, 1 EU) one US pending patent and 5 International Trademarks. Supported in part by a grant from Santé International, USA.

Cancer-preventive Compounds in Tomato Genotypes

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Many studies suggest that antioxidant compounds like L-ascorbic acid (vitamin C) and phenols, have importance for human health, in particular for their cancer-preventive activity [1]. Thus, an increase in antioxidant in human diet by intake of fruits and vegetables has been suggested worldwide. The fruits of tomato (Solanum lycopersicum), one of the most cultivated plants, are an important source of antioxidants for humans and other mammals. Nevertheless, to date little is known on genetic control mechanisms promoting antioxidant accumulation in tomato plant.

Aims of this study was to investigate accumulation of specific antioxidants in the selected genotypes of three introgression line (IL) population of S. pennelli into S. lycopersicum cv. M82 [2]. Particularly, we focused on IL12-4, IL7-3 and IL10-1 lines which showed to express QTLs for fruit ascorbate accumulation.

Our results showed that ascorbate is formed in the plants of the screened genotypes and the fruits had the major ascorbate amount. IL 12-4 and IL 7-3 lines accumulated higher fruit ascorbate contents compared to M82 parental line. Also total phenols showed to accumulate differentially in genotypes and in specific tissues. The HPLC phenols analysis in the crude organic extracts of different plant organs showed that chlorogenic acid, rutin and ferulic acid were the most representative compounds. No significant differences in rutin and ferulic acid contents were observed in different tomato tissues or lines, while chlorogenic acid was preferentially accumulate in leaves. Instead of, fruits of IL 10-1 showed higher amounts of rutin and chlorogenic acid than M82 variety.

In conclusion, tomato plants synthesize and accumulate a significant amounts of antioxidants. Therefore, as tomato fruit significantly contribute to health benefits, these might be improved by breeding tomato crop for antioxidant accumulation in fruits increasing antioxidant intake in human diet.

Phenolic Glycosides from *Cucumis melo* var. *inodorus* Seeds

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The seeds of *Cucumis melo* L. (Cucurbitaceae) are used in traditional Chinese medicine as antitussive, digestive, febrifuge and vermifuge [1]. Melon seeds extract can be used as an antidiabetic and is beneficial in chronic eczema [2]. More recent studies largely focused on physicochemical properties, fatty acids, tocopherols, sterols and phenolic profiles of seed oil. As our current interest involves the chemistry of biologically active natural products [3,4], we investigated the chemical constituents of the seeds of *C. melo*. This report deals with the phenolic glycosides from methanol extract of melon seeds.

The MeOH extract of powdered seeds of *Cucumis melo* was subjected Kupchan’s [5] partitioning methodology to give three extracts: n-hexane, CHCl3, n-BuOH and the aqueous residue. The n-BuOH extract, after purification by droplet counter current chromatography (DCCC) and reversed phase HPLC gave the new glycoside (1) and the known benzyl O-β-D-glucopyranoside (2). The CHCl3 extract mainly contained multiflorane triterpene esters (3 and 4) identified as new melon constituents.

Their structures were elucidated by extensive NMR experiments including 1H-1H (COSY, TOCSY) and 1H-13C (HSQC and HMBC) spectroscopy and chemical evidence.

(E)-4-hydroxycinnamyl 4-O-(2’-O-β-D-apiofuranosyl)-β-D-glucopyranoside (1)

Antitumoral Ability of Spanish Red Wine

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Wine is a natural product from Vitis vinifera L. fruits that presents qualitative and quantitative differences in its phenolic composition which directly depends on the grape variety from which it is made. Red wine possesses the most antioxidant, neuroprotective and cardioprotector effects, because of its high polyphenols content. Recent studies have shown that the dealcoholized fraction of wines can have important neuroprotective properties which depend on a wide number of factors such as climatic and cultivation process, wine-making technology, conditions of wine storage, etc. [1].

In this study, the antitumoral activity of five monovarietal young Spanish red wines has been studied for the first time: Tempranillo Press (TP), Tempranillo Bud (TB), Merlot Press (MEP), Merlot Bud (MEB) and Cabernet-Sauvignon (CS). Grape-wine was cultivated at one experimental vineyard station of IMIDRA, finca “El Encín”, Alcalá de Henares (Madrid, Spain). Four different concentrations of each dealcoholized wine sample have been tested: 25µg/ml, 2.5µg/ml, 0.25µg/ml and 0.025µg/ml. The preliminary chemical study of the wine samples by high-performance liquid chromatography revealed their richness in phenolic compounds; some differences in the content of total polyphenols, catechins, proanthocyanidins and anthocyanins were found among the different wines.

The in vitro antitumoral activity evaluation is carried on according to the proposed cell lines by the National Cancer Institute for the screening of extracts and potentially antitumoral compounds: MCF-7 (breast adenocarcinoma), UACC-62 (melanoma) and TK-10 (kidney carcinoma). The cellular viability is evaluated by the NCI proposed method: Sulphorhodamine B (SRB) [2]. This technique is applied for the initial screening of the antitumoral activity of the wine fractions with the aim of selecting the most active ones and finally fractionizing and isolating the active components.

The results showed that every assayed wine sample exerts an antitumoral activity towards UACC cell line, as cell viability is significantly decreased. This activity is statistically significant at the highest tested concentration of 25µg/ml. No changes on cell viability were observed for the MCF-7 line with anyone of the studied wines. For the TK10 cell line, an increase in cell viability is obtained with every assayed wine, except for TP. So a lack of antitumoral activity of the studied wine towards this cell line is shown. Taking the above results in account, the most active varieties are TP, CS and MEP. Statistical analyses: Data were analyzed by analysis of variance (ANOVA), statistically significances were considered with \( p \leq 0.05 \).

Antiproliferative, Cytotoxic and Antioxidant Capacity of Methanolic Extracts of *Pinguicula lusitanica*

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*Pinguicula lusitanica* (L.) Lentibulariaceae is a rare insectivorous plant endemic to the coastal area of Western Europe, and Morocco [1]. A methanolic extract of *in vitro* cultured *P. lusitanica* shoots was investigated for its antiproliferative and cytotoxic effects on Hela cells using the crystal violet and WST-1 assays, respectively. In order to investigate the mechanism of cytotoxicity, the distribution of normal cells and apoptotic cells in the various phases of cell cycle was analysed by flow cytometry.

Hela cells were treated with several extract concentrations in the range of 25-1000 µg ml⁻¹ and proliferation and viability was evaluated after 24, 48 and 72 h. Cell viability and cell proliferation were inhibited in a time dependent manner, while little variations were observed over time. After 72 h extract at a concentration of 634 µg ml⁻¹ induced 50% inhibition of cell proliferation. In the cytotoxicity assay, an IC50 of 1 mg ml⁻¹ was obtained after 24 h treatment. Analysis of the flow cytometry results indicated that the extract induced apoptosis, since a great percentage of cells in sub-G1 phase was detected (37.0%) after treatment with extract at 300 µg ml⁻¹, in comparison to control cells (6.42%).

Furthermore, the antioxidant capacity of the extract was determined by the oxygen radical absorbance capacity (ORAC), trolox equivalent antioxidant capacity (TEAC) assay and the Folin-Ciocalteu method which indicated strong antioxidant activity.

Chemical investigation of the extract by HPLC-SPE-NMR indicated that the main secondary metabolites of *P. lusitanica* are iridoid and phenylethanoid glucosides. Bearing in mind the apoptotic potential of the extract, efforts should be made to isolate the main components in order to discriminate the compounds responsible for this activity.

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Antiproliferative and Cytotoxic Effects of Leaf Extracts from *Drosophyllum lusitanicum*

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*Drosophyllum lusitanicum* (L.) Link is an insectivorous plant of the family Drosophyllaceae native to the western Iberian Peninsula and northwest Morocco. Leaves of this species contain flavonoids, phenolic compounds and higher amounts of the naphthoquinone plumbagin [1,2,3]. The antimicrobial and insecticidal activities of extracts from this species were previously demonstrated by our group [4,5].

The aim of this study was to assess the antiproliferative and cytotoxic effects of aqueous, methanolic and hexanic extracts from *D. lusitanicum* on Hela cells. Antiproliferative activity and cytotoxicity were evaluated by the crystal violet and WST-1 assays, respectively. Moreover, the distribution of normal and apoptotic cells in the various phases of cell cycle was analysed by flow cytometry.

All the extracts reduced cell viability and inhibited cell proliferation in a concentration-dependent manner. The most active extract was the hexanic (IC$_{50}$ < 3 µg ml$^{-1}$), followed by the methanolic (IC$_{50}$ < 50 µg ml$^{-1}$). As compared to control, the sub-G1 group significantly increased (P < 0.05) after culturing cells with hexanic extract at 3 µg ml$^{-1}$ (from 6.4 to 17.3%), while in the non-apoptotic population Hela cells accumulated in G2/M phase. These results suggest that *D. lusitanicum* hexanic extract can induce cell cycle arrest in G2/M phase and apoptosis in Hela cells.

The results obtained indicate that *D. lusitanicum* extracts exhibit antiproliferative effects. However further investigation is needed to elucidate the chemical profile of the extracts and to clarify the molecular mechanism mediating anticancer activity.

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Methanolic and Aqueous Extracts from Quercus suber Leaves Induce Apoptosis in HeLa Cells

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Quercus suber L. (Fagaceae), a common tree of the Mediterranean region, is the primary source of industrial cork, a material of stoppers and corkboards used for insulation. The wood, bark, and leaves of Quercus species are rich sources of ellagitannins and condensed tannins. A number of polyphenolic compounds were already isolated and identified from the leaves of Q. suber [1]. Having in mind the biological activities exhibited by polyphenolic compounds, the aim of this work was to investigate the antiproliferative, cytotoxic and apoptotic effects of aqueous and methanolic extracts from leaves of Portuguese Q. suber on Hela cells.

Proliferation and viability were evaluated in cells treated with several concentrations (25-1000 µg ml⁻¹) of extracts during 24, 48 and 72 h. Antiproliferative activity and cytotoxicity were evaluated by the crystal violet and WST-1 assays, respectively. The distribution of normal and apoptotic cells in the various phases of cell cycle was analysed by flow cytometry.

Results indicated that Q. suber leaf extracts inhibit cell proliferation and reduce cell viability in a time- and concentration-dependent manner. After treatment for 72 h with methanolic extract at 133.41 µg ml⁻¹ and aqueous extracts at 97.71 µg ml⁻¹ cell proliferation decrease 50%. In the cytotoxicity assay, IC₅₀ values of methanolic and aqueous extracts were 303.53 and 296.80 µg ml⁻¹, respectively.

The sub-G1 population significantly increased in cells treated with 300 µg ml⁻¹ of methanolic and aqueous extract (control: 6.42%; methanolic: 33.2%, aqueous: 51.67%) indicating apoptotic-associated chromatin degradation.

In conclusion, this study demonstrates that methanol and aqueous extracts of Q. suber inhibited cell proliferation, reduced viability and induced apoptosis in Hela cells.

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A Potentional Antiangiogenic Properties of Brassinosteroids

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Phytohormones brassinosteroids are small-molecule organic compounds occurring in plants. Up to date, more than 70 types of these plant growth regulators were isolated. These substances play an important role in hormone signalling and physiological response in plant organism. They regulate various types of processes, such as growth and development. Naturally occurring in plants (particularly in flowers, pollen, seeds) they cause the increase of number of cells and their elongation. Brassinosteroids enhance the disease resistance against abiotic and biotic stresses in plants. The antiviral effects of natural brassinosteroids and their synthetic derivatives were also reported \cite{1,2}. Brassinosteroids are very intensively studied now, especially their influence on animal and human cells, with the focus on cell viability, proliferation, apoptosis and differentiation. The cytotoxic effect on animal and human cancer cells derived from tumors were estimated recently in vitro \cite{3}.

The cellular and molecular mechanisms of action of these phytohormones in animal and human cells are still unknown. The effects of brassinosteroids were studied on endothelial primary cells HUVEC (Human Umbilical Vein Endothelial Cells) and HMEC (Human Mammary Epithelial Cells). Cells were stimulated with natural brassinosteroids and their synthetic analogs for 16, 24, 48 and 72 hours. Methods used for detection of changes in viability, proliferation, migration and apoptosis were: proliferation assay (staining with Crystal Violet), migration assay, tube formation and flow cytometry.

Both natural brassinosteroids and their synthetic analogs inhibit proliferation and migration of human endothelial cells and cause apoptosis. Especially the inhibition of migration plays a crucial role in development of new blood vessels, formation of new centres of tumor and spreading. These results should help us to understand the effect of brassinosteroids on endothelial as well as on cancer cells.

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Labdane Components from *Juniperus communis* Berries and Cytotoxic Activity

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In a survey of chemical components from useful plants grown in the Molise (Italy), we have identified a number of various types of compounds with labdane skeleton along with flavonoids, in their glycoside form, from the berries of *Juniperus communis* (Cupressaceae).

The predominant flavor and aroma of a modern gin come from dried berries of the *Juniperus communis*. The gin is an alcoholic liquor obtained by distilling grain mash with juniper berries.

New glucosides were isolated from the fresh berries, together with known components. In the present communication, we describe the isolation, structure elucidation, and biological activity of these constituents.

The n-BuOH and CHCl\(_3\) -soluble part were obtained from the MeOH extract by Kupchan’s partition methodology \([1]\). Both extracts were separated by a combination of Sephadex LH-20 column, DCCC fractionation and followed by HPLC separation.

Structural elucidation was obtained by NMR spectroscopy (\(^1\)H and \(^13\)C NMR) and 2D experiments as COSY, HSQC and HMBC and MS and chemical methods.

The chloroform extract was rich of diterpenoids with labdane skeleton; the BuOH extract was rich of interesting glicosides. Labdane-type diterpene was found to exhibit cytotoxic and cytostatic activity cell lines derived from solid tumors \([2]\).


Hellebrin and Hellebrigenin Derivatives from *Helleborus* species Display Marked *in vitro* Anti-tumor Activity through Sodium Pump Targeting

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Several plant genera (particularly the Asclepiadaceae, Apocynaceae, Ranunculaceae and Scrophulariaceae) are recognized to contain cardiotonic steroids (comprising cardenolides & bufadienolides); natural ligands and inhibitors of the sodium pump (Na+/K+\text{-ATPase}). Cardiotonic steroids are also found in animal species and occur mainly in toads (species of the *Bufo* genera). Na+/K+\text{-ATPase} should be considered an important target for the development of anti-cancer drugs as it serves as a versatile signal transducer, it is a key player in cell adhesion and its aberrant expression and activity are implicated in the development and progression of different cancers. By binding to Na+/K+\text{-ATPase}, cardiotonic steroids elicit marked effects on cancer cells. Regrettfully however, their narrow therapeutic index has hitherto prevented their development as anti-cancer drugs. Preliminary data have indicated that bufadienolides might have a larger therapeutic window than that displayed by cardenolides. However to date, the bufadienolide cardiotonic steroids remain largely unexplored despite their structural diversity and great natural abundance. Notably, plants of the *Ranunculaceae* genus (mainly *Helleborus* species) are known to contain several bufadienolides i.e hellebrin, hellebrigenin, and helleborin.

In order to further explore the potentially greater therapeutic margin of bufadienolides, hellebrin and hellebrigenin were first isolated from *Helleborus* species in high yields. We then chemically explored position 19 of hellebrin and positions 3 and 19 of hellebrigenin to generate by hemi-synthesis new derivatives of these two natural products. The selection of potential novel lead compounds was experimentally guided; involving the MTT cytotoxicity assay on human and rodent cancer cell lines, a Na+/K+\text{-ATPase} inhibition assay and evaluation of maximum tolerated dose *in vivo*. In addition to marked cytotoxic/anti-proliferative activity against cancer cell lines, investigations have indeed revealed our bufadienolide derivatives to be less toxic *in vivo* suggesting the potential for a larger therapeutic window. Analysis of the structure-activity relationship notably of the hellebrigenin derivatives has revealed interesting compounds for further development and mechanism of action deciphering.
The aryltetralin lignan podophyllotoxin is used as precursor for semisynthetic derivatives like Etoposide or Teniposide which are used in the treatment of cancer. However, continued supply of podophyllotoxin is not compatible with the conservation of the wild Podophyllum plants. Therefore the identification of other sources of this rare natural lignan is required. Screening for rapid growth and high lignan yield showed that Linum species belonging to the Syllinum section are promising for exploitation in vitro [1].

In continuation of our research on lignans in Bulgarian Linum species [2,3], we have established several callus and suspension cultures from single sterile seedlings from L. elegans, endemic species in the Balkan area and checked for the occurrence of lignans. There is no report in the literature related to in vitro cultures from L. elegans. Here we report the identification of the 6-methoxypodophyllotoxin (MPTOX) and podophyllotoxin (PTOX) as the main lignans in the suspension from this endemic plant species belong to the Section Syllinum.

As a result of more than 2 years maintenance of the cultures, and optimisations of growth media, a stable growth and production of the both compounds was achieved. The amounts of MPTOX and PTOX were determined as aglycone after enzymatic hydrolysis with β-glucosidase. The presence was verified using HPLC. The contents of PTOX in suspension of L. elegans is 3.6 mg/g dry weight. The contents of MPTOX is 0.9 mg/g dry weight.

The antiproliferative action of the suspension extracts was tested against malignant cell lines (the chronic myeloid leukemia – derived cell lines K-562 and LAMA-84, the Hodgkin lymphoma-derived HD-MY-Z and the human urinary bladder carcinoma-derived EJ cells) with etoposide as a positive control. The tested extracts reduced the viability of tumor cells in a concentration-dependent manner, whereby their relative potency was comparable or even superior to that of the referent drug etoposide. The extract from suspension of L. elegans showed a moderate cytotoxicity to all tested cell lines with IC50 in the range from 0.015 to 0.802 µg/ml. The juxtaposition of the IC50 values show that notwithstanding the cell line and its respective cell type and origin the extract from L. elegans proved to exert the most prominent cytotoxic activity. Taken together our results give us reason to conclude that the extract from Linum elegans, due to the established profound cytotoxic potential, which may be directly linked to the higher content of PTOX.

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Activity-guided Isolation of Anti-cancer Compounds from *Arnebia euchroma* (Royle) Johnston (Boraginaceae)

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In traditional Chinese medicine, the roots of *Arnebia euchroma* (Royle) Johnston are used to treat cancer. In the search for the active principle, we extracted the roots successively with solvents of different polarities and examined the activity of the extracts against human CCRF-CEM leukaemia cells, human MDA-MB-231 breast cancer cells and human HCT 116 colon cancer cells. For the quantification of cell proliferation and viability, we used the XTT based colorimetric assay. The alkaloid vinblastine served as positive control. The extracts were tested at a concentration of 10µg/ml. Only the petroleum ether extract showed strong activity against the three cell lines (inhibition: CCRF-CEM: 98%, MDA-MB-231: 89%, HCT 116: 96%). To isolate the active compounds, the extract was fractionated by semi-preparative HPLC and six fractions were obtained. Five of the six fractions showed activity against the cancer cell lines. Using HPLC and proton NMR data, it could be shown that fractions 1 (not active), 2 and 5 were mixtures of several substances whereas fractions 3 and 4 were pure compounds. Fraction 6 was a mixture of two active substances which could be separated by semi-preparative HPLC. The IC\textsubscript{50} values of the isolated pure compounds were determined and, according to proton and carbon NMR data, the compound in fraction 4 could be identified as acetylshikonin or acetylalkannin (absolute steric configuration not yet determined, IC\textsubscript{50}: CCRF-CEM: 3.0µM, MDA-MB-231: 10.8µM, HCT 116: 8.8µM), the compound in fraction 6-1 as dimethylacrylshikonin (IC\textsubscript{50}: CCRF-CEM: 2.7µM, MDA-MB-231: 23.1µM, HCT 116: 19.6µM) and the compound in fraction 6-2 as epoxyalkannin (IC\textsubscript{50}: CCRF-CEM: 2.7µM, MDA-MB-231: 14.2µM, HCT 116: 10.6µM). The structure elucidation of compound 3 (IC\textsubscript{50}: CCRF-CEM: 2.8µM, MDA-MB-231: 20.3µM, HCT 116: 18.7µM) is in progress.
Green tea polyphenols have received much attention from the research community, nutritionists, and public due to their antioxidant, anti-inflammatory and chemopreventive properties. This study investigated the ability of green tea extract and its constituent polyphenols, (epigallocatechin-3-gallate, epicatechin, epicatechin-3-gallate, epigallocatechin) to suppress cell proliferation, induce apoptosis, and induce differentiation of a variety of neoplastic cell lines, including U-937 (histiocytic lymphoma), THP-1 (acute monocytic leukaemia), PC3M (adenocarcinoma), CHL-1*, A-375*, WM-226-4* (*melanoma).

Apoptosis was assessed using a combination of approaches, in cells treated with green tea extract (0.0073, 0.073, 0.73 mg/mL) or polyphenols for 24, 48, 72 and 96 hours. Detection of phosphatidylserine on the surface of apoptotic cells was undertaken using annexin V by flow cytometry. Changes in chromatin morphology, plasma membrane damage, and other morphological features of apoptosis were achieved using vital dyes. Internucleosomal cleavage was detected by gel electrophoresis of DNA, and caspase-3 activity was evaluated by live-cell imaging.

The level of apoptosis induced by green tea extract differed according to the cell line used; U-937 and HP6002 being most sensitive (72% apoptosis after 72 hours), and THP-1 least sensitive (62%). However all cell lines demonstrated apoptosis induction in a dose and time-dependent manner. Plasma membrane “blebbing” was prominent in all concentrations and times studied, whereas pyknosis, karyorrhexis and apoptotic laddering were more prevalent in cells treated for longer time periods (48-96 h) at 0.73 mg/mL. Flow cytometric analysis revealed the order of effectiveness of different polyphenols to induce apoptosis were EGC>EGCG>ECG>EC.

This study demonstrated that green tea induce high levels of apoptosis in a variety of cancer cells in vitro, suggesting a role in the treatment of cancer in addition to prevention.
Narciclasine: an Amaryllidaceae Isocarbostyril Alkaloid which Reveals Significant Anti-tumor Effects in Human Melanoma Models

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The rapid increase in the incidence of malignant melanoma has not been associated with improved therapeutic options over the years. Indeed, melanomas have proven to be resistant to apoptosis and consequently to most chemotherapy and immunotherapy.

The Amaryllidaceae family has long been known for its medicinal and toxic properties \cite{1}. Narciclasine was first isolated from different Amaryllidaceae species in 1967 \cite{2}. Despite rapid demonstration of anti-cancer activity, poor attention was paid to this class of compounds and to narciclasine in particular. However, recent work has shown that narciclasine can overcome the apoptosis resistance of several cancer types: i.e. prostate cancer \cite{3} and glioblastoma \cite{4}. The aim of the present study was to investigate whether narciclasine has therapeutic potential against melanomas.

Narciclasine was extracted and purified from narcissus bulbs. 10 melanoma cell lines, including established cell lines of mouse and human origin, as well as primary human melanoma cultures were used to evaluate the compound’s anti-cancer activity. The IC\textsubscript{50} of narciclasine determined by means of the colorimetric MTT assay was ~40nM for all cell lines. These results were similar to those obtained for apoptosis-sensitive cancer cell lines. Flow cytometric investigations revealed cell cycle blockage in the G2/M phase and induction of apoptosis only at higher narciclasine concentrations (200 to 500nM). These results were further confirmed by quantitative videomicroscopy which showed anti-migratory and cytostatic effects of narciclasine at the IC\textsubscript{50} value. As cytokinesis seemed to be impaired, the effects of narciclasine on the actin cytoskeleton were investigated and revealed major perturbations at concentrations around the IC\textsubscript{50} value, even after only two hours incubation. Moreover, highly apoptosis-resistant cancers seemed to be as sensitive to the compound as apoptosis-sensitive models. Given the lower sensitivity of normal cells to narciclasine (250 fold higher IC\textsubscript{50} \cite{3}), we believe the compound could be of great interest as a chemical scaffold to derive novel isocarbostyril derivatives for combating apoptosis-resistant migrating or metastasizing cancer cells.

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Oxidation Potentials and Radical-scaevenging Properties of Novel Mitochondrion-targeted Quercetin Derivatives

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We are developing redox-active, polyphenol-based molecules capable of concentrating into mitochondria to act as radical scavenging anti-oxidants or death-inducing pro-oxidants. Both oxidation-quenching and -enhancing activities would be useful in pathophysiological situations (e.g. neurodegeneration and cancer, respectively). Quercetin (3,3',4',5,7-pentahydroxy flavone) is a model polyphenol we have used to produce mitochondriotropic derivatives [1]. We have synthesized two different derivatives in which the 3-OH or the 7-OH of quercetin have been permanently linked via a short alkyl chain to the triphenylphosphonium moiety, a membrane-permeant cationic group.


We are developing redox-active, polyphenol-based molecules capable of concentrating into mitochondria to act as radical scavenging anti-oxidants or death-inducing pro-oxidants. Both oxidation-quenching and -enhancing activities would be useful in pathophysiological situations (e.g. neurodegeneration and cancer, respectively). Quercetin (3,3',4',5,7-pentahydroxy flavone) is a model polyphenol we have used to produce mitochondriotropic derivatives [1]. We have synthesized two different derivatives in which the 3-OH or the 7-OH of quercetin have been permanently linked via a short alkyl chain to the triphenylphosphonium moiety, a membrane-permeant cationic group.

If these compounds are to display the desired redox activity in vivo, the chemical modifications introduced should not significantly alter the oxidation potential and the reactivity vs radical species of the parent compounds. To verify this important point we are using cyclic voltammetry and in vitro tests of lipoperoxidation promotion or protection and free radical scavenging using 2,2'-diphenyl-1-picrylhydrazyl (DPPH\textsuperscript{•}). The two isomeric quercetin derivatives turn out to behave differently: while the oxidation potential of the 7-derivative is very close to that of quercetin itself, the 3-substituted molecule is oxidized at higher anodic potentials (ca. 0.15 V higher). This difference may be ascribed to the different stabilities of the two-electron, two-proton oxidation products in the two cases, and suggests that the compound bearing the substituent at position 7 may be more suitable for our purposes than the one substituted in 3.

Curcumin and resveratrol, two natural products with an antioxidant, antiinflammatory and antitumoral probed properties had been widely studied. Numerous works report results about their proapoptotic activity [1, 2]. Curcumin enhances Bax and p53 gene expression [3]. Resveratrol induces apoptosis through ROS-dependent mitochondria pathway [4]. So, the apoptotic effect of these two products is explained following different ways [5]. The aim of this study is to probe if both substances could act sinergically, improving their own antiproliferative capacity.

IC50 were calculated using MTT assay for each substance individually over HeLa and HT-29 tumoral cell lines. Later, 75-25%, 50-50% and 25-75% curcumin-resveratrol (IC50 concentration) mixtures were assayed. Growing percentage of HT-29 and HeLa cell lines treated with curcumin-resveratrol 75-25 %, was 13.28 % and 6.4 % respectively. These results confirm the synergic effect. In order to know the apoptotic mechanism of action, a preliminary immunocytochemical assay was performed, studying p53 and Bcl-2 gene expression in treated cells. Results show an increase in the antip53 binding and a diminu-

Further analyses are needed to understand the mixture apoptotic mechanism over these cell lines.

Cytotoxic Activity in the Benthic Diatom Cocconeis scutellum

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The benthic diatom Cocconeis scutellum affects the sex reversal of the protandric shrimp Hippolyte inermis [1] inducing selective apoptosis of the androgenic gland of the crustacean [2]. After evaluating the ecological role of the diatoms in the benthic environment, other possible activities were also evaluated on different systems.

With the aim to ascertain their pharmacological potential, the diatom extracts were administrated on several human cell lines originated by different kind of tumors [BT20 and MDA468 (breast cancer), LNCap (prostate tumor), BRG (Burkitt lymphoma) or by normal lymphocytes COR (EBV transformed lymphoblastoid B cell line)] to check their apoptotic activity. The apoptotic pathways induced by the diatom extracts by detection of cleaved caspases 3, 8 or 9 by Western Blot were investigated as well. The results of these experiments will be presented and discussed according to recent chemical analysis of the extracts of C. scutellum [3]. Finally, after further separation of the extract, fraction 3, the richest in polyunsaturated fatty acids, appears as the most active fraction in inducing apoptosis.

Fungal endophytes have a recognized role in promotion of plant growth and protection against biological adversities. To this effect they must be capable to establish compatible interactions with their hosts which are mediated to some extent by secondary metabolites. Biological activity of such compounds may go beyond a regulative role of the tripartite system plant - pathogen - endophyte, and their properties are more and more studied with reference to possible implications in human medicine. The finding that some widespread pharmaceuticals originally extracted by plants, such as taxol and camptothecin, are actually also produced by endophytic fungi has stimulated prospecting for these particular microorganisms within novel plant species and ecosystems, particularly in tropical forests. However, as it is thought that every plant species may host up to 100 endophytic microbial species [1], most of which are yet to be described, there is a quite obvious opportunity to better develop research in the field by also considering autoctonous forests more carefully. In this perspective we have started collecting endophytic fungi from forest trees in Campania, southern Italy. Among several strains so far isolated, we have found two yet unidentified strains whose culture extracts have showed notable antiproliferative and pro-apoptotic effects on a human tumor cell line (A549). Culture extract of isolate E7CF from European barberry (Berberis vulgaris) stimulated apoptosis at a concentration of 75-90 μg/ml, while the same effect was induced by culture extract of isolate E1AT from silver fir (Abies alba) at a slightly higher concentration (100 μg/ml). Further investigations are in progress with the aim to purify the exrolites responsible for the biological activity, and to study their antitumor properties more in depth.

Zanthoxylum armatum (DC), Rutaceae, is extensively used in south asian medicine as antiseptic, adstringent, carminative, stomachic, and anthelmintic drug [1]. In the present study, the potential anticancer activity of fruits of this plant was tested in vitro. The essential oils of the fruits are known to contain linalool, E-carveol, limonene and methyl cinnamate as main constituents [2]. Z. armatum dried fruits (Timur) were obtained in Kathmandu, Nepal, and extracted with ethanol. Proliferation assays were performed for four days and viable cells assessed using MTT (Biomedica, Vienna, Austria). Cell cycle analysis on ethanol-fixed and propidium iodide/RNAse A-treated cells was carried out by flow cytometry. RNA was extracted from treated and control cells and gene expression analysis performed using the human genome survey microarray V2.0 (32K, Applied Biosystems, Foster City, CA, USA).

Cytotoxic activities of dilutions of the extract were evaluated in a panel of leukemia, small cell lung cancer (SCLC), pancreatic and colon cancer cell lines. IC50 values ranged from 0.6 mg/ml for HL-60 leukemia cells to 1.8 mg/ml for drug-resistant NCI-H526 SCLC cells. COLO 205 colon cancer cells revealed S-phase arrest in response to Z. armatum extract, whereas the more chemosensitive HL-60 cells accumulated in G1/0 phase. Sensitive cells exhibited signs of mixed apoptotic/necrotic cell death as demonstrated by annexinV/ propidium iodide as well as toluidine blue staining of semi-thin cell-culture sections. Genome-wide expression analysis of extract-treated versus control COLO 205 colon cancer cells revealed autophagin, TRAIL receptor, protocadherin, cyclin C, prostaglandin receptor, metallothioneines, and S100 protein as the most markedly induced, and myeloperoxidase, RAS-GEF, ceramide kinase, translation/elongation factors and metallothioneine proteinases as the most downregulated genes.

In conclusion, the extract of fructus Z. armati induces cell cycle arrest and cell death in diverse cancer cell lines, involving increased expression of autophagin and TRAIL receptor, compatible with induction of an oxidative cellular stress response and autophagic/apoptotic cell death.

Genetic Engineering for Production of Plant Triterpenoid Saponins with Antiproliferative Activity

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Asteraceae family is a rich source of triterpenoid saponins. Some of them are reported to be effective against tumor cells [1]. Our previous studies evidenced that three new triterpenoid saponins (named astersedifoliosides) with an oleanane-type skeleton, isolated in \textit{A. sedifolius}, were able to arrest the growth of transformed thyroid cell line, KiMol, dose dependently [2]. \textit{AsOXA1} gene was isolated in \textit{A. sedifolius} through the homology with other plant genes encoding OSC enzymes. OSCs catalyze the cyclization of 2,3-oxidosqualene into tetracyclic and/or pentacyclic carbon skeleton for the biosynthesis of aglyconic portion of saponins. \textit{AsOXA1} gene was expressed in yeast mutant and the presence of triterpenic or pentacyclic compounds was investigated by GC and GC/MS analyses. A peak corresponding to beta-amyrin, identified by retention time and MS spectra superimposed to that of standard \textit{β}-amyrin, was found only in yeast cells expressing \textit{AsOXA1} [3].

To increase the production of astersedifoliosides, a vector for \textit{Agrobacterium} transformation was constructed with \textit{AsOXA1} under the control of the constitutive promoter \textit{CaMV}35S. Strains of \textit{A. tumefaciens} (EHA105 and LBA4404) with \textit{CaMV}35S::\textit{AsOXA1} have been used to transform different plant species including \textit{Aster caucasicus}, \textit{Medicago truncatula} as well as model plant \textit{Arabidopsis thaliana}. In this work, the results obtained from transformation experiments of \textit{A. caucasicus} and \textit{A. thaliana} will be presented. In the latter, saponins are absent, but it is known that different OSC genes are expressed and many triterpenes including lupeol as well as beta-aminyl are produced [4]. Following \textit{AsOXA1} overexpression, it is expected an increase in saponin and in triterpene production in \textit{Aster} and \textit{Arabidopsis}, respectively.

Screening for Compounds from Arctic and Sub-Arctic Marine Invertebrates with Anti-cancer Activities at MabCent-SFI

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MabCent-SFI is a centre for research based innovation hosted by the University of Tromsø, Norway, that focuses on the identification of bioactive compounds from Arctic and sub-Arctic marine organisms. MabCent-SFI covers the pipeline from sampling to bioassay screening and research with the identification of drug leads with potential commercial interests as the main objective.

The anti-cancer screening program at MabCent-SFI includes:

i) identification of compounds with cytotoxic or cytostatic activities towards cancer cell lines,
ii) screening for inhibitors of the NF-κB signalling pathway,
iii) search for compounds with kinase inhibitory capabilities.
In Portuguese Estremadura and Alentejo littoral there are several threatened and endangered Brassicaceae species. Their habitat is being progressively reduced due to construction, recreational activities, and invasion of land by seawater. Some are endemic and mostly they are unstudied. This is the case of Malcomia pátula ssp. gracilima [1] also designated as Malcomia lacera ssp. patula [2] that grows sparsely in beaches dunes and to the present has not been studied.

Brassicaceae plants are known to be rich in glucosinolates that, through myrosinase hydrolysis, originate isothiocianates. These compounds act as protectors against carcinogenesis induced by various carcinogens. Sulphorophane is considered the most potent in the aliphatic series of isothiocyanates [3]. Malcomia maritime glucosinolates were studied for anticarcinogenic activity [4]. Isothiocyanates also present high cytotoxic activity to cancer cell lines. We’ve previously demonstrated the cytotoxic effect of Tropaeolum majus L. benzylisothiocyanate to several human and murine tumor cell lines with a range of IC_{50} values from 0.86 to 9.4 μ and was relatively no toxic to mice (LD_{50} of 140 mg/kg) [5]. We’ve also showed the possibility of producing biotechnologically this compound, by suspended cell cultures cultivated in a 2 L bioreactor [6-8].

Cell cultures of Malcomia plants, collected in the dunes of Costa da Caparica beach, were initiated with the double purpose of inducing organogenesis to obtain a clone of plants to replant and calllogenesis to obtain cell lines optimized for glucosinolate production. To achieve these goals leaves and stems explants were inoculated in two different media (Murashige and Skoog and Gamborg B5) with different levels of sucrose and several growth regulators balances were essayed.

Cell differentiation and glucosinolate accumulation, were observed in vivo and in vitro plant tissues through histochemical analysis. These studies will progress by the HPLC quantification of glucosinolates and analysis of the cytotoxic activity of the correspondent isothiocyanates.

Natural Derived Hydroxylated Byphenyl Compounds as Potential Anti-melanoma Agents

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Dehydrodieugenol and dehydrodicresol, are C$_2$-symmetrical dimers of the natural eugenol and creosol, respectively. Both of them are hydroxylated biphenyls, conformationally flexible and manifest a stronger inhibitory effect on lipid peroxidation and scavenging ability for superoxide radicals compared with that observed in their monomers. Since dehydrodieugenol and dehydrodicresol seem to induce cytotoxicity with a lower level of toxicity in tumour cells, they may represent a promising tool to generate new hydroxylated biphenyls with enhanced biological activity. We prepared several hydroxylated biphenyl derivatives and tested them for their anti-proliferative activity on malignant melanoma (MM) cell lines, in order to select new anticancer compounds capable to inhibit melanoma cell growth toward the characterization of novel therapies against such an aggressive tumour. We found a good and selective antitumoral activity against MM cells in bromo-containing biphenyls and in their atropo-enantiomers being dibromo-dehydrodieugenol enantiomeric form (1)-(S) the most active with IC$_{50}$ ranging around 20-30µM [1]. Similarly, (aS)-(1)-dibromo-dehydrodicresol showed slightly higher IC$_{50}$ values (25-35µM) on MM cells [2]. Given the importance of the hydroxylated biphenyl unit, we have selected dehydrozingerone, a known phenolic natural product with antiinflammatory, antioxidant and antitumour promoting activities, as starting monomer to prepare biphenyl compounds with improved antitumour activity. Among these dehydrozingerone related biphenyls, the best activity was displayed by the $\alpha$, $\beta$-unsaturated keton which showed IC$_{50}$ around 1-2 µM on MM cells, without affecting normal fibroblast proliferation rate. The antitumour activity of such compound is being further investigated in order to evaluate its potentiality in inducing apoptosis as well as in interfering with the main molecular pathways involved in MM development and progression.

Targeted Derivatives of Paclitaxel

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Paclitaxel (PTX) is a diterpenoid taxane derivative, which is mainly used for the treatment of ovarian and breast cancers [1]. Paclitaxel and its administration solution Cremophor cause many undesirable side effects which can be reduced by targeted chemotherapy.

Targeted chemotherapeutic agent is a complex of an anticancer drug and a carrier molecule. Peptide hormone GnRH (Gonadotropin releasing hormone) and its derivatives were used as the carrier molecules, because GnRH receptors are expressed on various cancerous cells in higher concentration than on most normal tissues. This is reason why GnRH is suitable for a selective destruction of these cells [2].

GnRH was connected to Paclitaxel via linking molecules modifying its 2’-hydroxyl group. Many types of linkers were prepared, e.g. PTX-succinyl-triethylene glycol, PTX-succinyl-Phe-Phe-OH, PTX-maleimidobutyric acid [3-5]. All of these molecules possess an appropriate functional group for the peptide to be linked in the following step.

The products were purified by reversed-phase HPLC and analysed by ESI-MS. Products were tested on tumor cell cultures. The inhibitory effectiveness of these compounds was evaluated as IC₅₀.

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Cell Culture-based Assays Identify Edible Rainforest Fruits with Potential for Chemoprevention of Cancer

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There is increasing interest in dietary components from fruit and vegetables that contribute to reducing risk of cancer. Much of this work has concentrated on components isolated from a small number of well known, largely temperate, food plants. Australia's tropical rainforests are characterised by a diverse, ancient and unique tree flora of Gondwanan origin, many of which produce edible fruits. The aim of this work was to develop reliable assays (using cultured human cells) for discovery of new compounds and extracts from the edible fruit of these rainforest plants which may help prevent the onset of cancer.

Assays using a range of normal and tumour human cell lines, and exposure to DNA-damaging agents, were developed for screening for chemopreventative activities. Growth in these assays was quantified at 5 to 7 days after treatment by comparing protein levels. Morphological observations of cells were also made and cell cycle arrest and apoptosis were assessed by flow cytometry. Extracts that showed selectivity for tumour cells compared to normal cells were then profiled for antioxidant activity and for inhibition of NO production. Quercetin and resveratrol were used as a model compounds in these assays.

Extracts of a range of edible fruits from Australian tropical rainforest trees were tested using the above methods, with a number showing good selectivity against human tumour cell lines. One of these fruits (EB902) was found to have very strong antioxidant activity (ORAC and total phenolics), as well inhibiting LPS induced production of NO in both RAW and murine microglial cells. Activity-guided fractionation of EB902 demonstrated the presence of a range of active components with differing hydrophobicity that contributed to the overall chemopreventative activity in the extract. These results (a) support the use of complex plant extracts as dietary chemopreventatives and (b) demonstrate the opportunity for discovery of new chemopreventative nutraceuticals from novel plant sources.
Enantioselective Synthesis of Aryltetraline Lignans Analogues of the Antitumoral Podophyllotoxin

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Aryltetralin lignans are lead substances for the semi-synthetic anticancer derivatives etoposide, teniposide and etopophos \cite{1}. They are derivatives of the aryltetralin lignan lactone podophyllotoxin which includes four contiguous chiral centers contained within a stereochromically unstable trans-fused tetrahydronaphthalene lactone skeleton \cite{2}.

These compounds are potent chemotherapeutic agents for a variety of tumors including small cell lung carcinoma, testicular cancer, and malignant lymphoma. They interact with tubulin and block mitosis to one that induced a block in late S or early G2 by interacting with topoisomerase II.

A synthetic route based on the enzyme-catalyzed cyclization of synthetic intermediates to analogs of the podophyllotoxin family is being explored \cite{3}.

Methyl sinapate ($R^* = \text{CH}_3$) was oxidized to give dihydrobenzthalene structures. Reactants were:

a) hydrogen peroxide and horseradish peroxidase (HRP) as the catalyst \cite{4}.
b) oxygen and N,N'-Ethylenebis(salicylideneiminato) Cobalt(II) [Co(II) salen] as the catalyst \cite{5}.

Its important biological role stimulated interest in developing efficient stereocontrolled syntheses.

In the HRP-catalyzed reaction the enantioselectivity after cleavage of the chiral auxiliaries $R^*$ was in the range 18-84%. Ab-initio calculations on the intermediate quinomethides have been used to explain the observed stereoselectivity in the oxidative phenol coupling of ferulate derivatives to phenylcoumarans \cite{6}. The same approach has been used to predict the stereochemistry of the oxidative phenol coupling to aryltetralines \cite{7}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Schematic representation of the enantioselective synthesis of aryltetraline lignans analogues.}
\end{figure}

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Nobiletin (3',4',5,6,7,8-hexamethoxyflavone) and Tangeretin (4',5,6,7,8-,pentamethoxyflavone) are polymethoxylated flavones found specifically in the fruit peels of citrus depressa consumed around the world. The presence of these flavonoids serves possibly as a natural defence against pathogenic fungi. These citrus flavonoids have a broad spectrum of biological properties such as anti-cancer and anti-inflammatory activities. They have also been described as health-promoting dietary supplements as they are extremely safe and associated with low toxicity and thereby making them excellent candidates for chemo-preventative agents. Many journal papers have shown that polymethoxylated flavonoids including nobiletin and tangeretin as being more potent inhibitors of tumour cell growth than hydroxylated flavonoids. Whilst the biological activities of nobiletin and tangeretin have been widely reported, its metabolic profile has been rarely studied. As Nobiletin and tangeretin is a major constituent in citrus fruit flavouring, extracts or citrus fruit juice, the in vitro human cytochrome P450 (CYP1) metabolism study of these compounds was investigated. Due to sample limitations for isolating and characterising an individual metabolite, two possible nobiletin metabolites and one tangeretin metabolite preparation were attempted in a similar multi-step organic synthetic route: 3'-hydroxy-5,6,7,8,4'-pentamethoxyflavone (3'-demethylnobiletin) and 3',4'-dihydroxy-5,6,7,8-tetramethoxyflavone (3',4'-des(demethyl)nobiletin) and 4'-hydroxy-5,6,7,8,-tetramethoxyflavone (4'-demethyltangeretin) respectively. Thus by comparing in vitro metabolic profiles of metabolite mixtures with the synthesised standard compounds, a major and a minor metabolite may be identified. The metabolite synthesis involves chalcone synthesis by Claisen-Schmidt condensation between substituted 2'-hydroxyacetophenone and hydroxyprotected benzaldehyde in alkaline medium, followed by cyclisation to the flavone. The intermediates prepared were chemically characterised by infrared (IR), mass spectra (MS) and nuclear magnetic resonance (1H & 13C NMR). This study is currently in progress.
**In vitro Anticancer Activity of Aloe Emodin Involves G2/M Arrest and Inhibition of Metastasis in Colon Cancer Cells**

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Aloe emodin (AE), a naturally occurring hydroxyanthraquinone is mainly found in leaves of *Aloe vera*. It has been demonstrated to exhibit antitumor activity in several tumor cells [1-4], however, little is known about its activity against colon cancer cells. Since aloe is widely used a dietary supplement and as well as a component in the traditional medicinal practice for treating colon tumors, a study to understand its mechanism of action in colon cancer cells was done. Different colon cancer cell lines like SW 620, SW 480, WiDr and RKO were used to assess its apoptosis inducing ability and to find out a moderately cytotoxic concentration. Among this, WiDr cells showed moderate sensitivity to AE and was chosen for our further experimental evaluations. We demonstrate that AE induced growth inhibition is in a concentration and time dependent manner. Treatment with AE resulted in a reduction in cell size, compromised membrane integrity, nuclear condensation and a positive Annexin/Propidium Iodide staining followed by loss of mitochondrial membrane potential and strong TUNEL positivity. Additionally, treatment with AE resulted in inhibition of the cell cycle, specifically a block at G2/M phase when analyzed using flow cytometry. These results were suggestive of growth inhibitory and apoptosis inducing ability of AE. Since many compounds target multiple signaling events simultaneously, we analyzed the role of AE in controlling experimental angiogenesis and metastasis. It is known fact that active angiogenesis and migration is a prerequisite for tumor metastasis and cancer progression and inhibition of tumour cell migration has been considered as an attractive therapeutic alternative for the suppression of cancer cell invasion and metastasis. Results showed convincing evidence that aloe emodin was able to inhibit cancer cell migration (by wound healing and transwell migration) and angiogenesis which agreed with the recent report [5]. Non toxic concentration of aloe emodin effectively reduced the PMA induced migration and angiogenesis in vitro. Dynamics of various Matrix Metalloproteinasess (MMPs) and signalling molecules involved with AE treatment were also analysed. Taken together, our data indicates that AE at higher concentrations show antiproliferative and apoptotic activity and at lower concentration show anti angiogenic and anti metastatic in human colon cancer cells. Hence AE may be considered as an eligible candidate for antitumor therapy in future.


Potential Role of Organic Sulfur Compounds from *Allium* Species in Cancer Prevention and Therapy

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Scientific search for new therapeutic agents is strongly supported by epidemiological studies about medicinal plants. Hence if beneficial effects on human health have been found, detailed studies are performed to identify active ingredients of plants in order to develop new drugs. In this context organic sulfur compounds (OSCs), especially allylsulfides, were elucidated to be the main compound class of *Allium* species (e.g. garlic, onion) responsible for anticancer and chemopreventive activities [1-3]. In this respect, potency of garlic formulations and of different OSCs were shown as well in epidemiological studies in men as in extensive preclinical studies in vitro and in vivo. Plants’ ingredients were active against a huge set of diverse cancers derived from different tissues such as prostate, gastrointestinal tract, liver, breast, lung, skin, brain and blood [3]. The effects of the compounds are exerted by different mechanisms affecting multiple cellular pathways and being strongly based on interactions with cellular proteins, DNA or oxidative stressors [4] finally leading to an induction of cell death, e.g. via apoptosis or inhibition of proliferation.

However mode of action of these OSCs is not yet fully understood and can therefore just be explained in part by their chemical reactivity, comprising both chemical bonds and other types of interactions with cellular contents, such as redox-reactions.

Besides some organic sulfur compounds were shown to possess selective activity against cancer cells in comparison with their healthy “counterparts” [4-6]. This is quite surprising due to the wide activity/reactivity range of the compounds and could be explained by different levels and patterns of protein expression, by different surviving mechanisms or by different redox-levels of healthy and cancer cells.

Overall properties of the compounds are quite favourable for ongoing drug development studies. Besides plants containing OSCs could be considered as functional food due to their beneficial effects on health.


Lignans in Leaves of Forsythia Species, Cultivars and its in vitro Cultures

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Plant phenolic compounds possess significant pharmaceutical properties. Nowadays a number of lignans are in the focus of research due to their various types of biological activity. These molecules exhibit antitumor effects against various types of cancer [1], as well as anti-HIV [2], anti-inflammatory [3], antioxidant [4] and neuroprotective properties [5].

These lignans (arctigenin, matairesinol, pinoresinol and phillygenin) occur in the highest quantities in Forsythia species and cultivars. Therefore, it is of great interest to compare the lignan content of the cultivars in order to establish cell cultures from the best cultivars.

In the present work leaves of four species and thirteen cultivars of Forsythia plant were analyzed for aglycone lignan profile (HPLC). The proportion of lignans varied in the leaves. The major lignan was arctigenin in every cultivar, the highest amount of which (99.7 mg/g) was found in Forsythia ovata ‘Robusta’. This was extremely high compared to other Forsythia species [6].

In vitro cell cultures were established from Forsythia ovata ‘Robusta’ and from other four species and cultivars with high lignan content such as Forsythia suspensa, Forsythia x intermedia ‘Golden Nugget’, F. i. ‘Karl Sax’ and F. i. ‘Week End’ on B5 medium. The determination of lignans in cell cultures is in process.

The Relation of Medicinal Plant Recipes from Thai Lanna Medicinal Plant Recipe Database in Anti-inflammatory Activity to Anti-cancer Action

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Introduction: An association between the development of cancer and inflammation has long been appreciated. Inflammation is a critical component of cancer which arises from sites of infection, chronic irritation, and inflammation [1]. At present, cancer is treated by operation, radiation, chemotherapy, immunotherapy and the combination of many treatments. For inflammatory symptoms and cancer, various modern medicines have been used to treat these diseases. Many drugs such as steroids and cytotoxic drugs are effective but have serious side effects. Thus, alternative medicines were taken an interesting for cancer treatment. As known, traditional medicines have long been used by the Thai people in the Lanna of northern Thai region. There are many Lanna Thai medicinal plant recipes which have anti-cancer and anti-inflammatory activities that have been popularly used by the people in the 7 provinces of the northern Thailand. The total numbers of recipes were 11,130 with 17 recipes having anti-inflammatory activities searched from the database. Besides, anti-cancer activities of these recipes have been investigating for the relationship to anti-inflammatory activities [2].

Material and Method: The anti-inflammatory activity of the selected recipes was determined by a hind paw edema test on both sexes of Spraque Dawley rats using prednisolone acetate as a standard anti-inflammatory agent and carrageenan as an edema inducer [3, 4]. The recipes were extracted using the method as described in the recipes.

Result and discussion: Three Thai Lanna medicinal plant recipes (No. 346, 896 and 717) showed significant anti-inflammatory activity (\(p<0.01\)) with the maximum edema inhibition activity of 94.45% at 2.03 mg/kg of recipe No. 717. Moreover, plants in recipe No. 717 were Dregea volubilis, Psophocarpus tetragonolobus, Quisqualis indica, Senna occidentalis, Fragraea fragrans and Caryota bacsonensis which gave bioactive compounds with anti-cancer and anti-inflammatory activities. There have been reported that the extract of these compounds represented the anti-cancer action in many cancer cell lines such as KB [5], P388 leukemia, B16 melanoma [6] cell lines.

Conclusion: This information will be used for further research and development of modern anticancer medicines.

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High dose chemotherapy for cancer is often accompanied by undesirable side effects. Photodynamic therapy (PDT) is an alternative modality that targets tumour tissue and exhibits relatively fewer side effects. PDT uses visible light to selectively irradiate only the tumour site to activate photosensitisers that produce cytotoxic reactive oxygen species. Though effective, PDT is limited to small tumours that allow adequate penetration of light for activation of photosensitisers. A combination of PDT and cytotoxic drugs may potentially lead to additive or synergistic interactions that would allow reduced doses of cytotoxic drugs and greater utility of PDT without compromising overall clinical efficacy. Hypericin is a naturally occurring napthodianthrone with good potential as a photosensitiser for PDT. In this study, the combined effect of hypericin-PDT and doxorubicin was examined in the oral squamous carcinoma (OSCC) cell lines HSC-2, HSC-3, HSC-4. The dose-response effects of doxorubicin or Hyp-PDT alone and in various combinations based on the checker-box model, were evaluated using the 3-(4,5-dimethylthiazol)-2,5-diphenyltetrazolium bromide (MTT) assay to quantify cell death. Synergistic, additive or less than additive cytotoxic effects were determined from isobolographic analyses of dose response curves. Doxorubicin in combination with Hyp-PDT exhibited additive or synergistic cell killing in all three cell lines. Interestingly, synergistic effects occurred only when low doxorubicin doses were combined with high hypericin doses. As a conclusion, this study demonstrated that it is possible to use lower doses of chemotherapeutic agents in combination with PDT to obtain similar or greater cytotoxic effects compared to single agents alone.
Cytotoxic Activity of C-Geranyl compounds from Paulownia tomentosa Fruits

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Newly discovered 5,7-dihydroxy-6-geranylchromone (1) was isolated from Paulownia tomentosa fruit and subsequently characterized. The structure of the isolated compound was explained on the basis of extensive NMR experiments including HMQC, HMBC, COSY, and NOESY, as well as HR-MS, IR, and UV. Its cytotoxicity (1) was evaluated using plant cell model represented by tobacco BY-2 cells. The other phyto constituents previously isolated from P. tomentosa [1] were similarly evaluated together with the known flavanones. The cytotoxicity (human erythro-leukaemia cell line K562) and activity on erythroid differentiation of compounds have also been evaluated. Acteoside (2) was determined to be the most toxic of the compounds tested at BY-2 cells, diplacone (6) at K562 cell line. Some aspects of the relationship between the flavanone skeleton substitution and the metabolic activation necessary for a toxic effect are discussed.

Cytotoxic Activity and Synergic Action of Schizandra chinensis Lignans

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While focusing on isolation and biological activity of dibenzocyclooctadiene lignans from Schizandra chinensis, we isolated and identified 12 lignans, namely schizandrin, deoxy-schizandrin, γ-schizandrin, gomisin J, gomisin A, gomisin N, tigloyl gomisin P, wuwizisu C, gomisin D, rubrisandrin, tigloylgomisin F and tigloylgomisin B. We consequently investigated in vitro antiradical activity with help of DPPH, ABTS+ and inhibition of the Fenton reaction, and cytotoxicity and synergic action on BY-2 cell line. A simple tree step method using column chromatography on silica (benzene:acetone) and gradient elution of MeOH/MeCN and H2O on reversed phase r was used for isolation of lignans. Fractions acquired from column chromatography were separated on preparative column with elution of MeOH/H2O, than on semipreparative column with help of MeCN/H2O. Compounds were identified on the base of UV, IR and 1H and 13C NMR analysis; HR-MS and CD measurement. Antiradical assays were performed on Synergy HT multiplate reader using routine colorimetric methods. Cytotoxicity of compounds tested was compared with the standard cytotoxic compounds camptothecin and cis platine. Compounds tested showed different degree of antiradical and cytotoxic activity in relation to the structural parameters.

Dibenzocyclooctadiene skeleton

Phytochemistry and Biological Activity of a Taxifolin Isolated from *Larix sibirica* Ldb. Growing in Mongolia

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*Larix sibirica* Ldb. (Pinaceae), a deciduous tree, is 20-30 m in height and distributed in Khubsugul, Khentei, Khangai, Mongol daurian, Khovdo, Mongolian Altai, Depression of great lakes, Dzungarian Gobi regions of Mongolia [1]. *L. sibirica* is used in Mongolian traditional medicine for externally dimpled skin diseases (dermato-invasion) and itching [2]. A predominant flavonoid had been isolated from Ethyl acetate fraction and was purified by recrystallization in methanol and was identified as a taxifolin through the methods of EI-MS, $^1$H, $^{13}$C NMR and 2D H-H, 2D C-H.

Phase II detoxification enzymes are known to be responsible for detoxification and elimination of activated carcinogens suggesting that induction of these enzymes is an important biomarker for chemoprevention. We tested a quinone reductase (QR) activity by taxifolin in HCT 116 colon cancer cells [3]. Taxifolin induced significant QR activity and showed high chemoprevention index (CI) 5.75. To identify the target genes regulated by taxifolin, DNA microarray was performed with a 3K human cancer chip containing 3096 human genes associated with carcinogenesis. Significant analysis of microarray (SAM) revealed 428 differentially expressed (DE) genes as statistically significant. Among them, 65 genes were up-regulated including important chemopreventive enzymes such as NQO1, GSTM1 and TXNRD1, and 363 genes were down-regulated in the presence of 60 M taxifolin. Since the enzymes up-regulated contain antioxidant response element (ARE) in common, we hypothesized that taxifolin modulates chemopreventive genes through activation of the ARE. Transient transfection experiments using ARE-ER-CAT, and XRE-ER-CAT demonstrated that taxifolin activated ARE only but not xenobiotic response element (XRE) indicating that taxifolin is a monofunctional inducer. Taken together, taxifolin acts as a potential chemopreventive agent by regulating genes via an ARE-dependent mechanism.


We have investigated isoquinoline alkaloids from genus Thalictrum [1-7], Berberis [8], Hypecoum [9-11] and Papaver [12-13]. *P. pseudocanescens* M.Pop. distributed in Khentei, Khangai, Mongol Altai, Gobi Altai (Ih-Bogda uul) regions of Mongolia [14]. The plants of genus *Papaver* L., rich in various types of alkaloids, are well known as remedies in Mongolian and Tibetan traditional medicine used for headache caused by nervous disorders, hemi-headache, contracted blood and bile, pulmonary fever, discharge of blood, dyspepsia, spermatorrhoea, painful menstruation, plentiful whites, ectropion of rectum, acute and chronic inflammation of stomach, gastralgia [15].

The phytochemical investigation of the species *P. pseudocanescens* of Mongolian origin resulted in the isolation and identification of eight alkaloids. Alborine, mecambridine and mecambridine methohydroxide are retroprotoberberines [16]. Flavinantine, 8,14-dihydromurine and 8,14-dihydroflavinantine are promorphinanes [12, 13]. Amureninsine is an alkaloid of isopavine type and O-methylarmepavine is of benzylisoquinoline type [12]. O-methylarmepavine has been found for the first time in the genus *Papaver* and the promorphinanenes are new alkaloids for the species.

The antiviral activity of the alkaloids against the replication of viruses representing different taxonomic groups was studied. The compounds showed minor or weak dose-dependent antiviral effect against the replication of HRV-14, poliovirus type 1 and CV-B1.

Synthesis and Antiangiogenic Activity of Resveratrol Analogues

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Angiogenesis, that is the formation of new blood vessels, is of crucial importance in tumor growth and metastasis. Thus, inhibition of angiogenesis is an innovative and promising approach to cancer therapy. Some angiogenesis inhibitors are small molecules of natural origin; among them, \textit{E}-resveratrol (\textit{E}-3,5,4'-trihydroxystilbene, 1), originally isolated from \textit{Veratrum grandiflorum}, is one of the main polyphenols present in red wine. The first studies on 1 were focused on its possible role in preventing cardiovascular heart diseases (CHD) and on its cancer chemopreventive properties. In recent studies the simple resveratrol derivative \textit{E}-3,5,4'-trimethoxystilbene 2, proved far more active than 1 both as antiproliferative and antiangiogenic agent. These observations prompted us, in continuation of our studies on resveratrol analogues, to synthesize further methoxystilbenes as new antiangiogenic agents. The trimethoxystilbene 2 and the tetramethoxystilbenes 3 and 4 were synthesized by a Wittig-like reaction. Subsequently, we employed a mild treatment of substrate 2 with \textit{m}-CPBA at r.t. to obtain two hydroxylated methoxystilbenes (5 and 6). Analogously, a similar protocol was applied to the tetramethoxystilbenes 3 and 4 to obtain respectively the hydroxylated analogues 7, 8 and 9, 10 (Scheme 1).

Scheme 1. Reaction of methoxystilbenes 2, 3 and 4 with \textit{m}-CPBA.

Among these resveratrol analogues, we selected the substrate 2 as reference compound and the new resveratrol analogues (5, 7 and 9) for an evaluation of their antiangiogenic properties employing porcine aortic endothelial cell line (AOC) in a fibrin gel assay. The results are presented.
Brassinosteroids Induce G1-phase Cell Cycle Arrest and Apoptosis in Human Breast and Prostate Carcinoma Cells

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The study of plant-derived compounds with effect at the molecular level has become an important approach in the selection of new agents with antitumour activity in humans. Brassinosteroids (BRs), polyhydroxylated sterol derivatives with close structural similarity to animal and insect steroid hormones are plant growth regulators \cite{clouse1998, clouse2002} representing a group of newly-discovered agents with relatively wide-ranging effects in plants \cite{nemhauser2004}. Based on the structural motifs, one putative explanation for their strongly cytotoxic effect is their binding to steroid receptors.

In this study, we characterized the effect of natural BRs (28-homocastasterone and 24-epibrassinolide) on cell growth and apoptosis in human hormone-sensitive and hormone-insensitive breast and prostate carcinoma cells. The aim was to identify the processes associated with apoptosis induction and hormone-independent status in these cancer cells. The agents inhibited cell growth in all cell lines and resulted in alterations on the cell cycle progression and levels of cell cycle related proteins. Using flow cytometry, we found that BRs can disturb cell cycling in breast and prostate cancer cells. The results showed that treatment with either 28-homocastasterone or 24-epibrassinolide induced blocks in the G1 phase of the cell cycle in the MCF-7, MDA-MB-468 and LNCaP cell lines, associated with decreased expression of cyclin D1 and pRb phosphorylation and induction of cyclin kinase inhibitors p21\textsuperscript{Waf1/Cip1} and p27\textsuperscript{Kip1}. In hormone-dependent cells, BR treatment led to induction of apoptosis and resulted in alterations of localization and expression of the steroid hormone receptors (ER-\textalpha, ER-\textbeta, AR).

Based on our data, the effect of BRs can be compared to the effect of antagonists to steroid hormone receptors. Our results suggest that tested BRs are promising leads for the development of a new generation of potential anticancer drugs.

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\cite{clouse1998, clouse2002, nemhauser2004}

Saponins are a large family of steroid or triterpenoid glycosides, widely distributed in plants and in some marine organisms, in which hydrophilic mono- or oligo-saccharides are attached to a hydrophobic sapogenin backbone (1). They have received considerable attention because of their diverse, promising biological and pharmaceutical properties, including antitumor, antiviral, antifungal, antiinflammatory and other activities (2). They have also been shown recently to have significant effects on plant growth.

The aim of our study relates to saccharide lupane derivatives and their use for inhibition of hyperproliferation in mammalian cells and for treating proliferative diseases in mammals, especially in anticancer therapy. We developed a convenient approach for synthesizing derivatives of the saponins lupeol and 3-O-acetyl-betulinic acid bearing mannose and 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranose moieties. Lupeol and 3-O-acetyl-betulinic acid were mannosylated with tetra-O-benzoyl- or tetra-O-acetyl-α-D-mannopyranosyl trichloroacetimidates. Deesterification, followed by regioselective dimannosylation of unprotected monosaccharides with 2 equiv. of tetra-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate selectively yielded O-3,6-linked trimannosides. The cytotoxic activity of selected lupane-type saponins (derivatives of lupeol, betulinic acid and betulin) towards normal human fibroblasts and various cancer cell lines was also compared (3).

**In vitro**-cytotoxicity of Seco-entkaurenes from *Croton caracasana*

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Recently we published the isolation of the new seco-entkaurenes 1 and its methyl ester 2 from *Croton caracasana* flowers [1]. Now we wish to report the **in vitro** cytotoxic evaluation of these two new compounds against seven human cancer cell lines using the MTT test. The compounds were evaluated for cytotoxicity against: human prostate carcinoma cell line PC-3, human breast carcinoma MCF-7, human colon adenocarcinoma cell line LoVo, human colon carcinoma cell line X-17, human uterus cell line HeLa, the leukemia cells U937, K562, Jurkat E6.1, Jurkat JCam1.6 and on human normal fibroblast, at concentrations ranging from 5 to 25 μg/mL. The results indicated that these compounds display high cytotoxicity against all tested cancer cell lines (IC\textsubscript{50} range 1.85-5.70 μM) when compared with untreated cells. The cytotoxic effects against the Jurkat cells of the Caracasine acid (1) are noteworthy.

![Caracasine (1)](image1.png)  
![Caracasine acid (2)](image2.png)

Isolation of Anticancer Active Fractions from the Roots of Acalypha alopecuroides

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The genus Acalypha (Euphorbiaceae) contains about 450 species of perennial shrubs and trees as well as annuals growing in tropical and subtropical areas with a few representatives in temperate zones [1]. Two thirds of the known species are distributed in America, from southern United states to Uruguay and nothern Argentina. Extracts of some Acalypha species are used in Central America as traditional medicines to treat various diseases, inflammations and tumours. To date only a few representatives have been investigated phytochemically [2,3].

For this reason we started to study the cytotoxic activity of Acalypha extracts. The roots of Acalypha alopecuroides Jacq. were collected in the botanical garden of the Institute for Ethnobiology and Maya Research, Playa Diana, San José/Petén, Guatemala. Lyophilized pulverized roots were extracted in methanol-tetrahydrofuran (1:1) with addition of an antioxidant. The extract was purified by solid phase extraction (SPE) and yielded six fractions. The biological activity of these fractions was examined against human breast adenocarcinoma cell line (MCF-7) and human leukemic lymphoid cells (CEM) by Calcein AM cytotoxicity assay in 96-well plates. Cytotoxic active fraction was further fractionated by two dimensional HPLC separation. Only three biologically active fractions were detected, isolated and subjected to NMR and MS analyses. The results of NMR and MS studies confirmed a multicomponent composition of the active fractions. Structure elucidation of individual substances is now in progress.

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A Safety Study of Oral Cranberry Products Administration to Rats

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Cranberries (Vaccinium macrocarpon) have long been used by humans as a preventive measure for urinary tract infections and maintenance of gastrointestinal health. Fruits contain vitamin C, dietary fiber, glucose and fructose, flavonols, anthocyanins, proanthocyanidins, and organic acids. Paeonidin-3-glucoside and paeonidin-3,5-digalactoside predominate in the anthocyanins [1].

We evaluated the safety of three commercial cranberry products NUTRICRAN®90S (a spray-dried cranberry powder, 1.4 % proanthocyanidins), HI-PAC 4.0 (a spray-dried cranberry powder, 4.75 % proanthocyanidins) and PACRAN® (100 % cranberry solids, 1.5 % proanthocyanidins) in a 100-day study in rats. The animals (n=24) were randomized to 4 groups (6/group): Group 1, control; Group 2, 1500 mg NUTRICRAN®90S/kg feed; Group 3, 1500 mg HI-PAC 4.0/kg feed, and Group 4, 1500 mg PACRAN®/kg feed. The animals consumed ad libitum either the standard or experimental diets and had free access to water. The health of animals was checked daily, feed consumption and body weight were monitored. The concentration, homogeneity and stability of cranberry polyphenols in the prepared diets were periodically analyzed by LC-MS. The microstructure of heart, ileum, kidney and liver, hematological, clinical chemistry, and oxidative stress parameters were evaluated.

Conclusions: The oral administration of cranberry products to rats did not affect major organ function. Nevertheless, the level of plasma alkaline phosphatase was significantly decreased in Group 2, the antioxidant capacity in liver tissue in Groups 3 and 4, and GSH reductase and glutathione levels in Group 4. No other statistically significant alterations in any parameter between control and treated animals were observed. This study opens the gate for further safety studies of cranberries in humans.

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Megastigmane Glycosides from *Laurus nobilis* L. Leaves Induce Apoptosis in Human Melanoma Cell Lines

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Malignant melanoma is a highly aggressive tumour which frequently resists chemotherapy, therefore the search for new agents for its treatment is of great importance. In this study, individual constituents of *Laurus nobilis* L. leaves, previously isolated by our group [1] were assessed for their cytotoxic effect on several human melanoma cell lines. After a preliminary screening two compounds were selected and their anti-cancer potency against human melanoma cell lines was investigated. Megastigmane glycosides (MGs) greatly suppressed proliferation of three human melanoma cell lines: A375, WM115 and SK-Mel-28 in a time and concentration-dependent manner. We further investigated MGs mechanism of action using A375 as a representative cell line model. The MGs-induced inhibition of human melanoma cell proliferation was due to the induction of apoptosis as demonstrated by detection of caspase-3 activity. In melanoma, constitutive activation of NF-κB confers tumour survival capacity and escape from apoptosis [2]. Thus we hypothesized that the MGs-induced induction of apoptosis could be associated with suppression of NF-κB activation. As hypothesized, MGs treatment resulted in a time and concentration-dependent inhibition of constitutive NF-κB DNA-binding activity. Induction of apoptosis by MGs treatment was further confirmed by the demonstration of cleavage and consequently inactivation of poly (ADP ribose) polymerase (PARP-1), which represents a well-known marker of this process [3]. Nevertheless, this event could be directly correlated to the inhibition of NF-κB-DNA binding activity. In fact, it has been widely demonstrated that unmodified PARP-1 negatively down regulates formation of NF-κB-DNA complex via its physical association with the transcriptional factor [3].

Induction of apoptosis by MGs in human aggressive melanoma cell lines has high pharmacological value and NF-κB inhibition is considered a very promising strategy to improve the fight against cancer.

Cactus Pear Fruit Extract and its Indicaxanthin Component Induces Apoptosis in Human Melanoma Cell Lines through Inhibition of Nuclear Factor-κB

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Malignant melanoma is a highly aggressive tumour which frequently resists chemotherapy and has therefore stimulated an active search for new therapeutic agents. Cactus pear (Opuntia ficus indica, L. Mill) fruit has recently been acknowledged as a source of bioactive compounds able to act as antioxidants in vitro and to modulate redox-sensitive cell pathways involved in inflammatory processes [1]. In this study, we assessed the effects of a cactus pear fruit extract from the yellow cultivar, and of one of its components, the betalain pigment indicaxanthin, on three human melanoma cell lines: A375, WM115 and SK-Mel-28.

Both indicaxanthin and the whole extract strongly inhibit cell proliferation in a time and concentration-dependent manner. To further investigate their mechanism of action, we used A375 as a representative cell line model. The inhibition of A375 cell proliferation was due to the induction of apoptosis as demonstrated by detection of caspase-3 activity. In melanoma, constitutive activation of NF-κB confers tumour survival capacity and escape from apoptosis [2]. Thus, a relationship between the cactus pear fruit extract-induced induction of apoptosis and the suppression of NF-κB activation was hypothesized. Both cactus pear fruit extract and indicaxanthin treatment resulted in a time and concentration-dependent inhibition of constitutive NF-κB DNA-binding activity. Induction of apoptosis was further confirmed by the observed cleavage and consequently inactivation of poly (ADP ribose) polymerase (PARP-1), which represents a well-known marker of this process [3]. This event could be related to the inhibition of NF-κB-DNA binding activity. In fact, it has been widely demonstrated that unmodified PARP-1 negatively down regulates formation of NF-xB-DNA complex via its physical association with the transcriptional factor [3].

Induction of apoptosis by cactus pear fruit extract in human aggressive melanoma cell lines may have pharmacological value, since NF-κB inhibition is considered a very promising strategy to improve the fight against cancer.

In vitro and in vivo Antioxidant Activity of Lipophilic Hydroxytyrosyl Esters

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Hydroxytyrosol 1 is a natural polyphenolic antioxidant [1]. Its radical scavenger activity, derived from the catechol hydroxyls, protects cells against oxidative stress preventing their degeneration and reducing risks connected with aging pathogenesis [2] (atherosclerosis, arthritis, cardiovascular disease, etc.). Furthermore, 1 has been proved to be effective against proliferation and differentiation of human malignant tumor cells [3].

In spite of these highly interesting properties, hydroxytyrosol is not used as an antioxidant additive in foods and cosmetics because its high instability in the air makes it difficult to obtain and to store. Recently, we have developed a protection/deprotection procedure to obtain 1 as stabilized acetone 2, directly from the glycoside oleuropein [4] present in high amount in the olive tree. However, hydroxytyrosol suffers from a low solubility in fats that has hampered its use as an antioxidant additive in fatty foods and cosmetic creams. Its esters derivatives 5 could solve this problem [5].

On these bases, in order to prepare hydroxytyrosyl esters with increasing lipophilicity, we successfully tested the methyl orthoformate protected hydroxytyrosol 3 [6] as starting material to obtain the protected esters 4 and, after selective deprotection, the esters 5 in high yields. These latter lipophilic derivatives have been tested for their antioxidant activity by both in vitro and on cells experiments. In vitro experiments have been carried out either in hydrophylc and water/oil emulsion environment by using the ABTS assay, while DCF fluorescent probe assays have been used to measures changes in the intracellular level of ROS.

Preliminary Results on the Effects Induced by *Citrus bergamia* juice on HepG2 Human Hepatoma Cell Line

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Beneficial effects exerted by juices of different *Citrus* species are well documented [1], but few studies have been carried on bergamott (*Citrus bergamia* Risso), a small tree growing almost exclusively in Calabria Region (Italy). Bergamot juice (BJ), a by-product of *C. Bergamia* transformation industry, is the object of many studies aimed to characterize its composition and its pharmacological properties [2]. In consideration of the growing interest concerning the antiproliferative activities of several fruit juices and vegetable extracts, the aim of the present study was the evaluation of the effects of BJ on HepG2 human hepatoma cells.

HPLC analyses were carried out to characterize flavonoid and coumarinic profile of BJ. A toxicity test with *Artemia* nauplii was carried out to verify the eventual toxicity of BJ. The effect of increasing concentration BJ (from 0.5 to 5.0%) was tested on HepG2 cells, studying cell vitality and morphology, cell cycle and some biomarkers of programmed cell death. Results show that:

- naringin, neoerictrin and neohesperidin are the main components of the flavonoid fraction of BJ, as observed by HPLC analyses. In particular, two psoralens, such as bergapten and bergamottin, were detected in the coumarinic fraction of BJ;
- tested BJ concentrations do not exert toxicity in *Artemia* nauplii used as lethality bioassay, as we previously observed [3];
- HepG2 cells exposed for 48 hours to BJ concentrations ranging from 0.5 % to 5.0 % show a significant decrease of vitality (P<0.05), respect to untreated control, in a dose-time dependent manner and a variation in cell morphology, as we already demonstrated both in human and fish hepatoma (RTH-149) cell lines [3]. Even if we observed, after BJ treatment, variations in cell cycle and biomolecular markers (p53, Bcl-2, Bcl-xL), we can hypothesize, but cannot confirm at this stage of research, the activation of apoptotic event.

Further researches are necessary to verify the mechanisms of cell death and to individuate the components of BJ involved in these effects.

A 90-day Safety Study of Isomerized Hop Extract in Rats

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After CO\textsubscript{2} supercritical extraction of hop (\textit{Humulus lupulus} L. variety Aognus) cones for brewing purposes, the residue still contains economically interesting amount of several biologically active components namely flavonoid phytoestrogens xanthohumol (XN), isoxanthohumol (IXN) and 8-prenylnaringenin (8-PN). The objective of this study was to evaluate the safety of the isomerized hop extract (IHE) obtained from hop cones waste, in comparison with CO\textsubscript{2} hop extract, in rodents.

The rats consumed ad libitum either the standard diet (control Group 1) or diets with IHE containing 100 mg XN + IXN/kg feed (Group 2) or 1000 mg XN + IXN/kg feed (Group 3), and 1000 mg CO\textsubscript{2} hop extract/kg feed (Group 4) for 90 days. The diets were prepared monthly and periodically analyzed by HPLC to confirm concentration, homogeneity and stability. The health of the animals was checked daily and body weights were monitored twice a week and prior to sacrifice.

After 90 days the rats were sacrificed and organ and blood samples were collected. The content of XN, IXN and 8-PN was determined in plasma and feces by LC-MS. Histological examination of heart, kidney, liver and ileum was performed. In plasma the hematological and clinical chemistry parameters (bilirubin, urea, creatinine, total proteins, AST, ALT, GMT, ALP, cholesterol), antioxidant capacity, total SH-groups, and products of lipid peroxidation were determined. Products of lipid peroxidation, GSH, SOD, catalase, and GPx activities and DNA single strand breaks were measured in blood. In liver lipid peroxidation, GSH, SOD, catalase and GPx activities, and total amount of cytochrome P450 were evaluated.

Conclusions: Isomerized hop and CO\textsubscript{2} hop extracts did not affect major organ functions. However, both affected the antioxidant status of rats. We found lowered level of GSH in Group 3 or 4, decreased GPx activity in Groups 2, 3 and 4, and significantly increased ALT activity in Group 4. Body weight of animals in Group 3 and 4 was insignificantly decreased in comparison to control rats. For the use of IHE in dietary supplements further safety studies are needed.

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Constituents of *Schisandra verruculosa* and their Cytotoxic Effect on Human Cancer Cell Lines

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In the continuation of our search for new biologically secondary metabolites from genus *Schisandra*, *S. verruculosa* Gagnap is the only species found in Thailand. 1, 2- Bis-(4-hydroxy-3-methoxyphenyl)-3-hydroxypropan-1-one (evafolin B, 1), 1-(4-hydroxy-3-methoxy-phenyl)-3-hydroxypropan-1-one (2), abscisic acid (3), 4-hydroxybezaldehyde (4), 4-hydroxybenzoic acid (5), methyl 4-hydroxybenzoate (6), methyl 3,4-dihydroxybenzoate (7) and vanillic acid (8) were isolated from its stem wood. All the compounds were evaluated for their antitumor, antiproliferative and antioxidant activities. Only compound 7 exhibited moderate effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung), SF-286 (CNS) and on the mitogenic response of human lymphocytes to phytohemagglutinin. Compound 7 also showed a strong scavenging activity for DPPH free radical, only slightly less than ascorbic acid.

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Chemoprevention is a novel approach of pharmaceutical research which seeks to inhibit progression of cancer. Development of dietary compounds as potential cancer chemopreventive agents is desirable due to their low toxicity, safety and general acceptance as food supplement. In this study, bioactive compounds from the chloroform extract of Coleus tuberosus tubers were isolated based on bioassay-guided fractionation. A short term in vitro assay of Epstein Barr virus early antigen (EBV-EA) activation induced by tumour promoter phorbol 12-myristate 13-acetate (PMA) and sodium n-butyrate in Raji cells was used to detect the anti-tumour promoting effects of combined fractions from Coleus tuberosus chloroform extract. Two columns were conducted to screen for the active anti-tumour promoting compounds from the chloroform crude extract. These two columns yielded fractions A1-A12 and F4 with strong inhibition rate of more than 90% at 50μg/ml, while maintaining high cell viability of more than 80%. Preliminary analysis of the fractions using gas chromatography-mass spectrophotometry (GC-MS) showed presence of triterpenic acid compounds. Current work is still in progress to isolate and identify the active anti-tumour promoting compounds from these active fractions.
Photodynamic therapy (PDT) is now a well recognized modality for cancer treatment. It is based on administration of a photosensitiser followed by its in situ excitation at an appropriate wavelength [1]. It eradicate tumor by causing damage to the cancer cells, damage to the tumor vasculature and by engaging the immune response system via the formation of reactive oxygen species (ROS). Unlike conventional chemotherapy, PDT-treated tumors do not develop treatment-specific resistance and since only areas exposed to light are targeted, there is no systemic toxicity. To date, Photofrin®, Foscan® and Levulan® are the only PDT drugs approved for treatment of certain forms of cancer. These drugs, as well as most of the others under clinical and preclinical investigations are synthetic or semi-synthetic compounds based on a cyclic tetrapyrrole core and many of these suffer from limitations that include long photosensitivity in patients and slow clearance from the body. Realizing the potential of PDT, our laboratory embarked on a screening program to search the Malaysian biodiversity for novel structures that may be developed into clinical PDT agents with more ideal drug properties. This paper describes our study on one hundred and fifty-five extracts from 93 terrestrial species of plants in Peninsula Malaysia. These plants which can be classified into 43 plant families are diverse in their type of vegetation and their natural habitat in the wild, and may therefore harbour equally diverse metabolites with potential pharmaceutical properties. The extracts were assayed to determine their in vitro photo-cytotoxicity by means of a MTT cell viability test against human leukaemia cell-line HL60 [2] and thirty of these extracts were able to reduce the in vitro cell viability by more than 50% when exposed to 9.6 J/cm² of a broad spectrum light at 20 µg/mL. To the best of our knowledge, this is the first example of systematic screening of natural extracts for photo-cytotoxic activity. Six of the PDT-active samples, from the most readily accessible plant materials during re-collection, were further subjected to bioassay-guided fractionation to isolate four photosensitisers, all of which are based on the pheophorobide-a and -b core structures. Our results suggest that the main photosensitisers from terrestrial plants are likely to be those based on the cyclic tetrapyrrole structure, and further detailed examination of the remaining active extracts combined with dereplication strategies will be necessary to effectively identify novel photosensitisers.

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