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International Congress

Natural Anticancer Drugs

Olomouc, Czech Republic, June 30 – July 4, 2012

GUEST EDITORS

Jiri Gruz
Vladimir Krystof

Dear NAD2012 participants

It is a great pleasure for us to welcome on behalf of the Phytochemical Society of Europe (PSE, www.phytochemicalsociety.org) and the local organizing committee all scientists attending the congress **Natural Anticancer Drugs** (NAD2012). The stimulating tradition of the previous PSE conferences related especially to the International PSE Symposium on Natural Products in Cancer Therapy, 23-26 September 2008, Naples, Italy, will be further enriched with more than two hundred contributions by scientists coming from all over the world.

The main object of the NAD2012 congress is to promote the advancement of knowledge of the natural constituents with respect to their chemistry, biosynthesis, production, function, effects on human physiology and pathology of cancer, and the application of such knowledge in oncology. The conference is regarded by the PSE as the event which can draw the phytochemists and molecular oncologists together and it appears to be stimulating and beneficial. It can also enable a forum in which the young scientists can present their first work results.

The efforts of many people have made this conference possible and I thank them all, especially the members of the Organizing Committee. We would also like to thank all plenary lecturers for accepting the invitation to share with us their latest results and present a general overview of their area of expertise.

We are extremely grateful to our colleagues from Palacký University in Olomouc, City of Olomouc, Ludwig-Maximilians-Universität München (A. Vollmar), Teva Czech Industries s.r.o. (L. Cvak, B. Bíba, and L. Dvořák) and Novartis (D. Fabbro, P. Krastel). We also wish to express our gratitude to all other partners and sponsors of this congress.

We warmly invite you to make the most of this occasion to meet old friends, make new ones and, whatever stage you may be at in your career, to partake to the full in making this congress memorable by involving yourself in stimulating discussion and debate.

We wish you a wonderful scientific and personal experience at NAD2012 and a pleasant stay in Olomouc. We sincerely hope it will meet your expectations and we are looking forward to a very successful meeting.

Miroslav Strnad
On behalf of the Organizing Committee

CONTENTS

PL-1

Cell-targeted cytotoxics, a new generation of cytotoxic agents for cancer treatment

Christian Bailly

Institut de Recherche Pierre Fabre, Toulouse, France

A broad panoply of small molecules and proteins is available for the treatment of the many forms of solid tumors and blood cancers. A dozen kinases inhibitors have been approved over the past decade and a roughly similar number of approved monoclonal antibodies can be counted for the same period. In contrast, natural products and derivatives have become less popular in oncology, although there are still recent successes, such as for example the registration of (i) vinflunine in 2009 (in Europe) for the treatment of advanced bladder cancer and (ii) carbazitaxel in 2010, anovel semisynthetic taxoid as a second-line treatment for metastatic castration-resistant prostate cancer. Not only conventional cytotoxic agents, frequently combined with targeted therapeutics, remain largely employed in the clinic but there is also a continuous demand for novel cytotoxic drugs, safer and more potent, affecting more selectively cancer cells compared to normal cells. Here I will essentially illustrate the recent discoveries and development in the field of topoisomerase II inhibitors, with a new generation of promising molecules, including natural products and derivatives. The case of F14512, a polyamine-linked epipodophyllotoxin currently undergoing phase 1 clinical trials in onco-hematology, will be presented in more details. F14512 exploits the polyamines transport system (PTS) to selectivity accumulate in to cancer cell and efficiently induces topoisomerase II-dependent DNA strand breaks. Its potent cytotoxic activity and capacity to trigger apoptosis and senescence translate into a robust anticancer efficacy *in vivo*, exemplified using a range of human tumor xenograft models. F14512 exhibits a marked *in vivo* antileukemic activity against human AML models, established from patient samples, alone or in combination with cytarabine (AraC), supporting therefore the ongoing clinical development. This targeted cytotoxic agent is emblematic of the evolution of the field which now requires "smart molecules" designed for a selected population of cancer patients. Other examples of cell-targeted cytotoxic agents for cancer treatment will also be discussed.

PL-2

Immunoproteasome: current and future trends in drug development

Michael Groll

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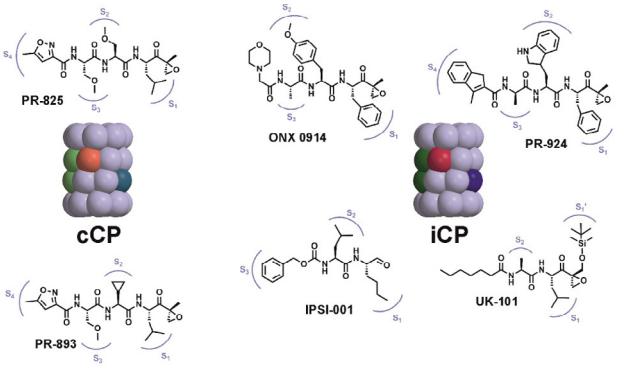
More than 90% of cell protein degradation is performed by the ubiquitin-proteasome pathway. The 20S proteasome is a core component of this machinery and regulation of its activity is particularly important for the vast amount of essential biological processes, such as cell proliferation, stress response, cell differentiation, cell death as well as antigen presentation. In the latter case, proteasomal cleavage products, which are short peptides and are normally further hydrolyzed to single amino acid by proteases escape this subsequent degradation and are, instead, selected and uptaken by MHC class I molecules for presentation to the immune system on the cell surface.



In this fine tuned proteasomal system a large variability of peptides are created, which ensures a fast and efficient immune response. Furthermore, substitution of constitutive proteasomal subunits with immuno-subunits results in a modified protein substrate cleavage pattern and, consequently, in the generation of new antigenic peptides. In our days, there is high demand in controlling pathogenic immune responses, targeting autoimmune disorders and addressing neurodegeneration diseases. Therefore, specific inhibition of the immuno-proteasome is a very promising approach to tackle this demanding mission.

PL-3

Natural products as starting points for the design of highly selective kinase inhibitors

Stefan Knapp

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Recent development in high throughput structural chemistry and parallel screening has enablesd kinome wide studies on inhibitor selectivity and binding modes. My group at the SGC is particularly interested in family wide comparison of kinase catalytic domains and contributed more than 50% of all novel kinase catalytic domain structures that have been deposited into the protein data bank during the past 7 years. Using this rich body of information we performed cross structural comparisons and were able to identify unique structural features that we utilized for the development of highly selective inhibitors. In addition, the generated protein reagents allowed us establishing a selectivity screening panel that covers all major kinase families. Large scale parallel screening identified a number of selective compounds that target novel kinases

that have been implicated in disease development but have not been extensively targeted. In particular natural product inhibitors provided excellent chemical starting points for the development of highly selective and cell active inhibitors. I will discuss in my talk recent progress targeting kinases involved in regulation RNA splicing as well as strategies targeting recruitment of kinases to signaling complexes using protein interaction inhibitors.

PL-4

Redox regulation of pro-inflammatory and anti-inflammatory signaling by some chemopreventive natural products

Young-Joon Surh

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The role of inflammation in carcinogenesis has been extensively investigated and well documented. Many biochemical processes, which are altered during chronic inflammation, have been implicated in tumorigenesis. These include shifting cellular redox balance towards oxidative stress, induction of genomic instability, increased DNA damage, stimulation of cell proliferation, metastasis and angiogenesis, deregulation of cellular epigenetic control of gene expression and inappropriate epithelial to mesenchymal transition. A wide array of pro-inflammatory cytokines, prostaglandins, nitric oxide, matricellular proteins are closely involved in premalignant and malignant conversion of cells in a background of chronic inflammation. Inappropriate transcriptional regulation of genes encoding inflammatory mediators, survival factors and angiogenic and metastatic proteins is the key molecular event in linking inflammation and cancer. Numerous studies have been reported with the global biochemical profiling technologies, such as DNA microarray, proteomics, metabolomics, transcriptomics, lipidomics, etc., to identify and characterize a series of critical molecules/changes in the inflammatory signaling. Inflammation, a major culprit of cancer, can modulate NF-kB, AP-1, Nrf2, HIF-1a, STAT3 and p53 tumor suppressor. The proper regulation of these redox-sensitive transcription factors and their regulators mediating pro- or anti-inflammatory signaling hence provides important strategy for the chemoprevention and treatment of cancer. This lecture will highlight the multifaceted role of inflammation in carcinogenesis in the context of altered cellular redox signaling and its modulation by some anti-inflammatory and antioxidative natural substances with chemopreventive potential.

ACKNOWLEDGEMENTS

This work was supported by the Global Core Research Center (GCRC) grant from the National Research Foundation (NRF), Ministry of Education, Science and Technology, Republic of Korea.

PL-5

25 years of protein kinases drug discovery: what did we achieve?

Doriano Fabbro

Expertise Kinase Platform, CPC, Novartis Pharma AG, Basel, Switzerland

Protein kinases act as molecular switches with remarkable plasticity and dynamics upon interaction with regulatory domains as well as modulators including small molecualr weight kinase inhibitors (KIs). Conformation, therefore, provides a conceptual framework to understand many aspects of kinase biology. Deregulation of protein kinase activity by mutation and/or amplification is associated with a variety of pathologies ranging from cancer to inflammatory diseases, diabetes, infectious diseases and cardiovascular and metabolic disorders. Pathologic kinase deregulation often shifts the conformation to an active "on" state, leading to constitutive signaling. Approximately one third of all protein targets under investigation in the pharmaceutical industry are protein or lipid kinases. At present about 150 kinase-targeted drugs are in clinical development and many more are in various stages of pre-clinical development. To date 16 small molecular weight kinase inhibitors and a handful of therapeutic antibodies have been registered (againts 30 distinct kinase targets) mainly in cancer indications. With one exception, all of these registered KIs are directed towards the ATP-binding site and display different selectivity, potency and pharmacokinetic properties. Protein kinases with gain of function mutations appear to be more sensitive (and sometimes resistant) to KIs compared to the wt variant. Unfortunately, protein kinases escape inhibition by either mutating key residues in their kinase domain or by pathway reactivation leading to a bypass of the targeted kinase. Thus, one of the major challenges in kinase drug discovery today, among others, is to understand the mechanisms of resistance to kinase inhibition which allows appropriate strategies to override these various types of resistances. Strategies for developing multiple KIs targeting different kinase sites and discovering synergistic inhibitor combinations will be discussed.

PL-6

Natural compounds as inhibitors of the 10 hallmarks of cancer

Marc Diederich

Hopital Kirchberg, LBMCC, Luxembourg, Luxembourg

Cancer is one of the most deadly diseases in the world. Although advances in the field of chemo-preventive and therapeutic medicine have been made regularly over the last ten years, the search for novel anticancer treatments continues. In this field, the natural environment, with its

rich variety of organisms, is a largely untapped source of novel compounds with potent antitumor activity. We focus here on selected compounds that act on the eight major hallmarks of cancer including self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replication, sustained angiogenesis and tissue invasion, metastasis, altered cellular metabolism and the evasion of immune destruction. Moreover, we identify compounds that interfere with the two enabling characteristics coined by Hanahan and Weinberg recently, namely inflammation and genome instability.

ACKNOWLEDGEMENTS

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PL-7

Antibody drug conjugates- new opportunities for natural products in oncology

Frank E. Koehn

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Natural products are an unsurpassed source of anticancer drugs. Yet, it remains difficult to develop natural product chemotherapeutics because of dose-limiting toxicity derived from their poorly-selective mechanism of action. As a result, many effective cancer agents cannot be dosed at concentrations high enough to eradicate the tumor. Function-blocking monoclonal antibodies, on the other hand are highly specific, but have shown only minimal antitumor efficacy against solid carcinomas, predominantly because of their limited potency. Antibody drug conjugates (ADC) offer a modality by which the cell-killing potency of a "small molecule" cytotoxin can be combined with the exquisite selectively of a monoclonal antibody (mAb) for delivery to the tumor. ADC's consist of a monoclonal antibody which binds a tumor-associated antigen (target), onto which one or more molecules of a potent cytotoxin (payload) are conjugated. In principle, the specificity of the mAb for the tumor cell target serves to deliver the cytotoxin to the tumor cell, and spares peripheral tissues from exposure to the cytotoxin. The overall design of the ADC approach is conceptually simple and elegant. However, the chemical, biochemical and cellular processes necessary for the mode of action are complex, interdependent, and challenging to monitor by assay. Natural products by virtue of their diverse, potent mechanisms of action, and privileged chemical scaffolds, are a promising source of payloads for the next generation of ADC's. Driven by the recent clinical success of calicheamicin antibody conjugates we have maintained an ongoing interest in isolating ADC payload cytotoxins, and in understanding the micro-organisms which produce them. In this talk, we describe results from our laboratory in this regard.

PL-8

Targeting cytoskeletal organization of cancer cells with natural product-based agents

Sergey Kozmin

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Small molecules that bind to monomeric or filamentous actin elicit their antiproliferative effects by impairing the ability of cells to progress through the cell cycle and undergo cytokinesis due to the defective actin cytoskeleton. Understanding the mode of action of such compounds expands our knowledge of actin biochemistry and provides opportunities for the development of new therapeutic agents. We are employing our extensive expertise in chemistry and chemical biology of actin-targeting natural products to assemble a series of new pharmacological agents that effectively inhibit tumor growth in vitro and in vivo.

ACKNOWLEDGEMENTS

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PL-9

Interfacial inhibitors: one of nature's paradigms for targeting macromolecular complexes

Yves Pommier

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Interfacial inhibitors belong to a broad class of natural products and synthetic drugs that are commonly used to treat cancers as well as bacterial and HIV infections. They bind selectively to interfaces as macromolecular machines assemble and are set in motion. The bound drugs

transiently arrest the targeted molecular machines, which can initiate allosteric effects, or desynchronize macromolecular machines that normally function in concert. Based on five archetypical examples of interfacial inhibitors: the camptothecins, etoposide, the quinolone antibiotics, the vinca alkaloids and the novel anti-HIV inhibitor raltegravir, we will discuss the common and diverging elements between interfacial and allosteric inhibitors and give a perspective for the rationale and methods used to discover novel interfacial inhibitors. Detailed information can be found in the reference below.

PL-10

Natural products as leads to anti-tumor agents

David J. Newman

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Over the timeframe of chemotherapeutic use (roughly mid-1930s to date), natural products from all sources, plant, microbe and marine, have been used either as direct drug sources, or as chemical scaffolds upon which to build novel drug candidates. Even today, approximately 20 years into the usage of combinatorial chemistry as a discovery tool, natural products in one guise or another still predominate as leads to anti-tumor drugs. This presentation will briefly cover the history of these drugs and then proceed to demonstrate how novel agents frorm previously unrecognized sources may well be the next frontier in natural product drug discovery, not only in anticancer treatments, but in other diseases as well.

PL-11

Genotoxic stress: mechanisms, disease relevance and emerging therapeutic interventions

Jiri Bartek

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Palacky University, Olomouc, Czech Republic
Institute of Molecular Genetics, Prague, Czech Republic

Cellular responses to genotoxic insults, including the mechanisms of DNA damage sensing, signaling and repair rely on multiple protein modifications that operate in concert with the phosphorylation network governed by the ATM/ATR kinases, collectively known as the DNA damage response (DDR). This lecture will summarize our recently published and unpublished data documenting the biological and pathophysiological role of the emerging ubiquitylation/deubiquitylation cascade, including the RNF8, RNF168, HERC2 and BRCA1 ubiquitin ligases, in DNA damage signaling, checkpoint pathways and repair in human cells. The data will also include results from high-throughput RNAi-based screens for novel DDR components and live-cell imaging of human cells to analyze the spatiotemporal orchestration of the key DDR pathways. Examples of natural substances that modify the signaling and effector pathways within the DDR network will be provided, including compounds that inhibit key DDR kinases and cyclin-dependent kinases (CDKs), the latter also required for proper genome maintenance. Emphasis will be on responses to ionizing radiation and replication stress, the latter identified in our laboratory as the key trigger of a DDR barrier against activated oncogenes and tumor progression. Unpublished data on a novel mechanism regulating RNF168, critical to limit cellular responses to ionizing radiation, will be discussed. In addition, our recent results exploitation of DDR defects in human pathology, with focus on a new radiosensitivity syndrome (mimicking ataxia-telangiectasia) caused by a homozygous defect of the RNF168 ubiquitin ligase, and on tumor-associated DDR aberrations as predictive markers to guide individualized cancer therapy, will be presented. The latter topic extends our concept of DDR as a biological anti-cancer barrier and selection for DDR defects that allow the nascent tumor cells to escape from the DDR-imposed checkpoints, thereby facilitating tumor progression and also affecting responses to standard-of-care genotoxic therapies as well as the emerging DDR-targeted drugs such as PARP inhibitors, or modulators of various DDR-related kinases.

PL-12

Harnessing nature's orphan ligands: enhanced processes for natural products drug discovery

Guy T. Carter

Carter-Bernan Consulting, LLC, 350 Phillips Hill Rd, New City, NY 10956 USA

Although major pharmaceutical companies have marginalized natural products over the last 20-30 years, the changing landscape of drug discovery now provides new opportunities for Nature's privileged structures. Screening for drug leads in phenotypic screens provides an ideal opportunity to realize the value of natural products. As Pharma strives to develop innovative new drugs, natural products will be increasingly valued as sources of novel leads. The conundrum traditionally has been translating the profound effects displayed by fascinating and novel secondary metabolites into serious candidates for drug development. The processes leading from an initial discovery through cycles of optimization, safety evaluations and ultimately selection for development are rigorous and demand adequate supplies of the natural product lead. The chances for a successful outcome of this process are greatly enhanced when material supply is not limiting and when structural diversification of the parent compounds is feasible. Advances in total synthesis (especially function-oriented syntheses) and biosynthetic technologies, offer new avenues for the medicinal chemical optimization of

biologically active secondary metabolites. Genetic manipulations of biosynthetic pathways now provide the means to expedite strain development as well as extending the range of scaffolds available for analogue generation. This presentation will highlight examples of enhancements to the traditional processes of natural products-based drug discovery that improve the chances of success.

PL-13

Natural product research as innovative source for new medications

Philipp Krastel

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Many – if not all – natural products play an important role in the survival of their producers in their natural environment. Their acceptors are, in the majority of the known cases, proteins transforming a chemical signal into a biological response. These accessory molecules have often been maintained during evolution and can be found across species barriers. Some of these proteins are involved in human disease pathways and may represent intervention points for medical therapy. Under this premise, the structural diversity and the biological bias of natural products are key motivations to apply them as a source in the search for new pharmacophores, or as tools for target identification. The availability of new techniques, like genome sequencing or improved analytical methods is changing natural product research substantially in recent years. Integration of these technologies into industrial natural product research enables efficient ways to find new chemotypes or targets as starting point for drug discovery projects. For example by use of genome sequencing the full biosynthetic potential of a single organism can be estimated and used for the generation of chemical diversity. The talk will highlight recent aspects in natural product research and will illustrate how biological diversity can be converted into chemical diversity as rich source for new medications. In addition it will be shown, how natural products can be used as probes for target identification.

0-1

Biosynthetic studies on the patellamides

Marcel Jaspars

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Prochloron sp. is the cyanobacterial symbiont of the ascidian *Lissoclinum patella*. Recent studies in our and US labs have shown that *Prochloron* sp is responsible for the production of the highly bioactive modified cyclic ribosomal peptides, the patellamides. The biosynthesis will be discussed including the mechanism needed for the processing enzymes to recognise the pre-peptide and the proteases involved in cleavage and formation of the macrocycle and their biotechnological applications.

ACKNOWLEDGEMENTS

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0-2

Glucan in cancer – magic bullet or big con?

Vaclav Vetvicka

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In the past decades, glucans have been called "biological immunomodulators" or "biological response modifiers" and sometimes "pathogen-associated molecular patterns". Unlike the majority of natural products, glucans retain their full biological activity even after rigorous purification. This characteristic allows us to evaluate and explain their biological and immunological activity on both a cellular and a molecular level. Polysaccharides in general, and glucans in particular, have a long history as immunomodulators. Only in the last decade, extensive research by numerous scientific groups has helped to reveal the extraordinary effects that glucan has on our immune system. Experiments done by a University of Louisville research group, led by Gordon Ross, focused on one particular receptor called complement receptor type 3 as a promising target of glucan. Subsequent detailed analysis of the interaction of human cells with glucans has demonstrated that the CR3 receptor is primarily responsible for both the binding and biological effects of glucans. CR3 is considered to be the most important receptor mediating clearance of opsonized immune complexes by the phagocytic system. Even general description of various fields with demontrated beneficial effects of glucan would fall way behind the scope of this talk. Cancer treatment, stimulation of cellular and humoral immunity, bone marrow support, anti-stress effects, lowering blood sugar and cholesterol, suppression of mercury poisoning, just to mention a few. With so many documented positive effects, glucan should be on a fast track to be approved as a drug. However, the situation is different. Since the 1980s, two types of glucan have been successfully used as traditional medicine for cancer therapy in Japan and China. In Japan, glucan is already licensed as a drug effective in cancer treatment. In addition, at least 28 clinical trials are currently under way in the United States as well as other countries such as Czech Republic, Turkey, Netherlands, France and Phillipines. The area covered by these clinical trials include cancer treatment, lowering cholesterol levels, stimulation of immunity and blocking stress. With respect to cancer, beneficial properties of glucan in cancer therapy have been recognized for centuries. Their proposed mechanism of action occurs mainly via stimulation of macrophages and priming of neutrophil complement receptor 3 for eliciting CR3-dependent cellular cytotoxicity of iC3b-opsonized tumor cells. Recent studies revealed that b-glucans may also promote antitumor T cell responses. Thus, b-glucan-mediated tumor immunotherapy may engage both innate and adaptive anti-tumor immune reponses to restrain tumor progression. Addition, much less studied role of glucan in cancer treatment is based on intersecting of immunomodulatory and physical properties of glucan. Recently, natural and chemically-modified glucans have been used for drug delivery in many forms: 1) release of drugs from a glucan gel matrix, 2) in combination with other materials to form suitable drug delivery systems, 3) as carriers in drug formulations consisting of gels, tablets and ingestible films, 4) as soluble and particulate conjugates, and 5) and as soluble and particulate glucans for encapsulation and delivery of macromolecules. This takes us back to the question: which, out of dozens if not hundreds of individual glucans on the current market, is the best one? From these data and from careful comparison of other studies, it is evident that all glucans are not created equal. All these facts make the answer to the original question "Is the glucan a magic bullet or big con?" difficult to answer. Clearly, a highly purified, characterized and biologically active glucan represents a potent tool and clearly deserve the potential to become a successful drug. At the same time, at least 50 percent of all commercial glucans sold today represent little more that sugar dust.

ACKNOWLEDGEMENTS

This work was supported by an NIH grant.

0-3

Colon cancer chemopreventive properties of auraptene and 4-geranyloxyferulic acid and their derivatives

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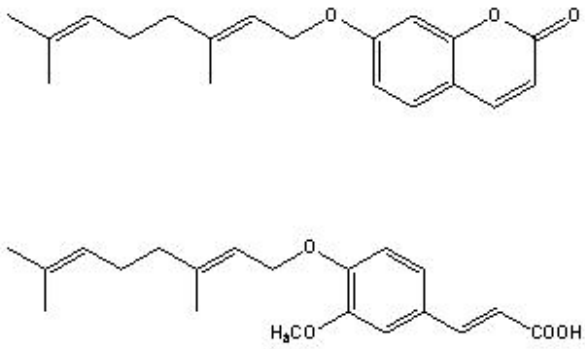
Dietary feeding cancer chemoprevention by natural products extracted from edible vegetables and fruits represents a novel challenging field of research in cancer therapeutic approaches. In this context we have been studying in recent years the pharmacological properties of prenyloxyphenylpropanoids, a rare class of plant secondary metabolites. Among these the most promising compounds were seen to be auraptene, a geranyloxycoumarin widespread in fruits (e.g. agrumes) of plants belonging to the Rutaceae family, and 4-geranyloxyferulic acid, an active principle extracted so far only from *Acronychia baueri* Schott, belonging to the same family.



Using the azoxymethane / dextrane sodium sulphate induce colon cancer models in mice, auraptene and 4-geranyloxyferulic acid, orally administered as such or in form of prodrugs (peptide derivatives or cyclodextrin inclusion complexes) were shown to be able to largely reduce the incidence of adenoma and adenocarcinoma, as well as to positively modify other biological parameters closely related to colon cancer growth and development. Results obtained in our studies indicate that both auraptene and 4-geranyloxyferulic acid may represent novel effective agents for cancer therapy and lead compounds for development of a novel class of dietary feeding chemopreventive compounds.

ACKNOWLEDGEMENTS

The collaboration of Prof. Takuji Tanaka from the Kanazawa Medical University, Ishikawa and The Tohkai Cytopathology Institute Cancer Research and Prevention, Gifu, Japan and his group, Japan was gratefully acknowledged.

0-4

Engineering biosynthetic pathways for antitumor compounds in microorganisms

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Natural products are an important source of bioactive compounds usually isolated from plants, animals or microorganisms. Microorganisms are producers of a large variety of biologically active natural products, many of which have clinical, veterinary or agricultural applications. Within microorganisms, actinomycetes are the most prolific microbial group in terms of production of bioactive compounds, being producers of approximately two-thirds of all bioactive compounds. Important and useful therapeutic drugs as antibiotics, antifungals, antiparasites or antitumor drugs and also compounds with application in agriculture such as insecticides or herbicides are produced by members of the actinomycetes family. Traditionally, pharmaceutical companies have developed screening programmes in the search for novel bioactive natural products and improving in production yields has been based on mutagenesis and selection programmes. The development of recombinant DNA has opened up the possibility of applying genetic manipulation to engineer biosynthetic pathways in order to generate novel derivatives with potential application. Thus, "combinatorial biosynthesis" has emerged as a new technology in which genes from different biosynthetic pathways are combined either in a producer organism or in a heterologous host. The recombinant strains, containing gene combinations "not previously found in nature", can produce novel derivatives from known bioactive compounds. This technology is especially useful when trying to introduce chemical modifications in bioactive compounds which are not amenable to chemical means. In this communication several examples will be presented on the application of combinatorial biosynthesis to generate novel derivatives from bioactive natural products. In addition, some examples will be shown on novel strategies using combinatorial biosynthesis to alter the glycosylation pattern in bioactive compounds by taking advantage of the existence of a certain degree of substrate flexibility of glycosyltransferases, in particular with respect to the sugar donor.

ACKNOWLEDGEMENTS

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0-5

Natural compounds inhibiting human topoisomerase I

Alessandro Desideri

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Eukaryotic topoisomerase I (Top1) is a monomeric enzyme that catalyzes the relaxation of supercoiled DNA during important processes including DNA replication, transcription, recombination and chromosome condensation. The catalytic cycle of the enzyme involves a nucleophilic attack of the active site tyrosine (Tyr723) on the DNA backbone resulting in a breakage of one DNA strand, allowing the free 5'-DNA substrate to rotate around the intact strand, a second nucleophilic attack, driven now by the 5'-hydroxyl DNA end, restores intact DNA and free enzyme. Human topoisomerase I is of significant medical interest being the only target of the antitumor drug camptothecin (CPT). CPT reversibly binds to the covalent intermediate DNA-enzyme, stabilizing the cleavable complex and reducing the rate of religation. The stalled topoisomerase I collides with the progression of the replication fork producing lethal double strand DNA breaks and cell death. Here I will describe how single mutations located far from the drug binding site are able to induce drug resistance and I will provide a structural-dynamical explanation for such a resistance using a combined experimental and computational approach. The mechanism of interaction between the enzyme and some "new" natural compounds will be also described.

ACKNOWLEDGEMENTS

This work has partly supported by The Italian Association for Cancer Research (AIRC).

0-6

Modulation of cellular stress responses by natural compounds in anticancer therapy

Yong Sang Song, Mi-kyung Kim

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During the tumorigenesis, cancer cells are frequently exposed to metabolic stress. As tumor grows larger, it outgrows its local blood supply leading to inadequate supply of oxygen, nutrients, and growth factors. In addition, inefficient ATP production through 'aerobic glycolysis' and accumulation of intracellular mutant proteins further make cancer cells more susceptible to metabolic stress. These cellular stress stimuli lead to disturbance in the protein folding capacity of the endoplasmic reticulum (ER) and subsequent accumulation of unfolded proteins in the ER, causing ER stress. Cancer cells respond to ER stress through induction of either unfolded protein response (UPR) or autophagy, both of which are suggested to contribute to cancer cell survival and progression. However, when ER stress is extensive and sustained, cell death is induced typically by apoptosis, as well as autophagic cell death. Since most normal cells are not subject to stress, modulation of these cellular stress responses have emerged as an alternative anticancer strategy. Recently, phytochemicals, which are natural compounds present in plants, have been demonstrated to exert their anticancer activity through the modulation of the cellular stress responses. Phytochemicals, such as curcumin, resveratrol and genistein, have been demonstrated to upregulate the expression of UPR components, like GRP78 and GADD153, to cause apoptosis in several cancer cell lines. Moreover, these compounds have also shown to induce autophagy, as evidenced by the formation of autophagosomes. In this presentation, the cellular stress responses in cancer cells, phytochemicals as a modulator of UPR and autophagy, and their therapeutic potentials in cancers will be briefly discussed.

ACKNOWLEDGEMENTS

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0-7

Deciphering the molecular mechanisms of action of interface microtubule targeting agents

Francois Devred

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Most commercialized anticancer drugs that target microtubules have been obtained from natural sources. Indeed, several structurally diverse compounds isolated from plants, marine flora or microorganisms have been purified and synthesized for their anticancer bioactivity. Among these, several molecules belong to classes of anticancer drugs which target the microtubule cytoskeleton either by stabilizing it or destabilizing it. To distinguish between these drugs and to understand in which conditions they are more likely to be efficient, it is crucial to fully characterize their interaction with tubulin. Indeed, knowing the molecular mechanism of action of each drug

that is already used in chemotherapy protocols will help to find strategies to circumvent resistance. In addition, understanding the molecular basis of their effects on microtubule cytoskeleton could help designing analogues with greater pharmacological activity and with fewer side effects. By taking examples of several interfacial microtubule targeting agents, we show how characterization of their binding to tubulin can be achieved combining biophysical and biochemical approaches. We also show how understanding of the complexitiy of these mechansims could lead to the discovery of new targets or biomarkers which will open new perspectives in anticancer strategies.

O-8

Searching for antitumor natural products using a combined *in vivo/in vitro* bioassay-guided approach

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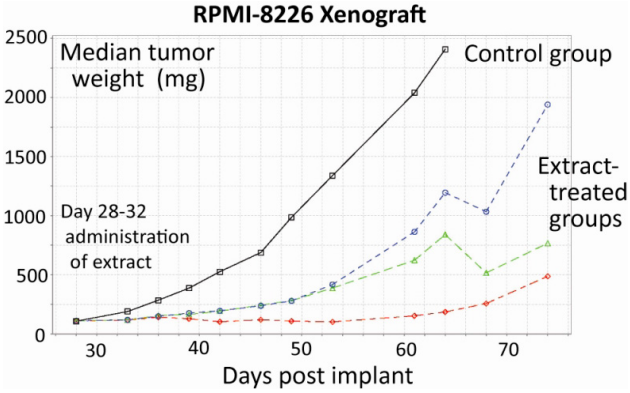
From ~2001-2006, our lab utilized molecular targeted screening of natural products extracts in the search for antitumor compounds. The work led to the finding of several new HIF-1 inhibitors, including a tubulosine analog from *Alangium longiflorum*, a camptothecin derivative from *Ophiorrhiza trichocarpon*, a strophanthoside glycoside from *Crossosoma bigelovii*, and a peptide HDAC inhibitor from *Burkholderia thailandensis*. As with many of the compounds that show activity in molecular targeted anticancer screens, *in vivo* development of these compounds failed to live up to the promise of the *in vitro* results. Problems involving drug absorption, distribution, metabolism and toxicity in animal models are well documented. In order to avoid the unforeseen obstacles in the progression from molecular targeted *in vitro* to *in vivo* testing, we have adapted an older methodology in which an extract is assayed *in vivo* using athymic nude and/or SCID mice prior to isolation and elucidation of biologically active components. Crude extracts are first screened at 100 ug/mL in the one dose NCI-60, and if cytotoxicity is evident, five dose testing is performed. Visual inspection of the dose response curves/mean bar graphs for highly susceptible tumor cell lines and COMPARE analysis are used to prioritize extracts for the *in vivo* queue. The maximum tolerated dose of the crude extract is determined, followed by IP, IV or SC administration of the extract into mice that have been implanted with hollow fibers containing tumor cells both IP and SC. If antitumor activity is observed, xenograft experiments are performed using the relevant cell/mouse model. Promising extracts are selected for bioassay-guided fractionation and purification/identification of the active principle(s). In the figure presented below, mice were implanted with the human leukemia cell line RPMI-8226 xenograft on day 1 and subsequently treated with extract on days 28-32 under three dosing schedules. Promising data as that shown below has encouraged us to decipher the chemistry of these extracts and to look for synergistic interactions of the extract components.



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O-9

Natural tubulin-targeting anticancer drugs with covalent mechanism

Jose Fernando Diaz

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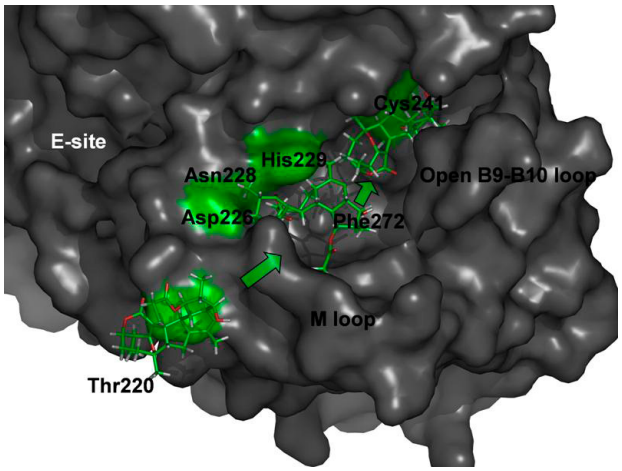
Microtubule-modulating agents modulate microtubule assembly by changing the assembly/disassembly properties of the GDP- and GTP-bound forms of tubulin. Among microtubule modulating agents, paclitaxel, docetaxel and vinca derivatives are very efficacious chemotherapeutic agents. Although they are widely used for the treatment of leukemia, breast, ovarian and lung cancer, their clinical administration is severely hampered by its low solubility (in the case of paclitaxel and docetaxel) and by the development of resistance due to overexpression of both the P-glycoprotein (P-gp) drug efflux pump. Thus, compounds

that are less susceptible to P-gp drug efflux may have novel pharmacokinetic and pharmacodynamic profiles, including the potential for oral administration. Tubulin modulators devoid of the problem of P-gp-mediated drug efflux are thus badly needed. A large number of compounds with microtubule modulating activity have been investigated in this context. Among these compounds, pironetin, cyclostreptin and zampanolide have novel mechanisms of action. They bind covalently to unassembled tubulin and microtubules (the latter only cyclostreptin and zampanolide), avoiding being pumped out of the cell and so they are able to overcome P-gp-mediated multidrug resistance (MDR).



Moreover, these compounds are useful tools to investigate the interaction of microtubule modulating agents with tubulin. Covalent labeling of proteins is a powerful tool that has been used extensively for identification of acceptor molecules in heterogeneous mixtures and in the selective labeling of receptor sites in biological systems. The labeling methods make use of the reactivity of one or more common functional groups in the active sites of protein molecules with reactive groups of ligands. This talk will cover recent research done in our group to characterize the interaction of cyclostreptin and zampanolide with their binding sites in unassembled tubulin and microtubules and their effects in tubulin structure and activity. These compounds label tubulin both at the luminal and the pore site of paclitaxel in microtubules at different residues, thus marking the pathway for leading from the pore where these compounds enter the microtubule to the luminal binding pocket.

ACKNOWLEDGEMENTS

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0-10

Exploiting the biosynthetic potential of myxobacteria using synthetic biotechnology

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Myxobacteria belong to the few established microbial sources for natural products exhibiting various bioactivities. In fact, myxobacteria can be described as multi-producers of natural products, as a single strain usually produces several different compound families. High throughput genome sequencing and in depth analysis of metabolite profiles reveal that an astonishing variety of novel compounds is still to be found. Most of these compounds are produced by giant multimodular assembly lines comprising polyketide synthases (PKS) and/or non-ribosomal peptide synthetases (NRPS). Understanding the mechanisms involved in their biosynthesis is the prerequisite for optimizing microbial production by genetic engineering. As most of the myxobacteria grow slowly, are difficult to handle and genetic modification has proven difficult, synthetic biotechnology approaches have been initiated and developed to transfer and express myxobacterial biosynthetic pathways in suitable host strains. In the presentation, some examples for the exploitation of the biosynthetic potential of myxobacteria will be given. This includes harvesting of genetic information for genomics guided discovery of biosynthetic pathways as well as developing molecular biological tools for the expression and modification of natural product biosynthetic pathways. Recently, we also included gene design and gene synthesis technologies in our approaches to construct a complete artificial pathway for the anticancer compound epothilone, an approach that will also be discussed in the presentation.

ACKNOWLEDGEMENTS

The work presented has only been possible due to the involvement of several individuals and collaboration partners. Funding by the German Research Foundation (DFG) and the German Federal Ministry of Education and Research (BMBF).

0-11

Structure-function studies of microtubule binding molecules

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Microtubules are key components of the cytoskeleton that are composed of α/β-tubulin heterodimers. Their dynamic behavior is essential for the regulation of diverse cellular functions including cell division, motility and intracellular transport. These properties make microtubules attractive targets for antimitotic drugs. The best-understood microtubule-targeted compounds bind to three regions on tubulin, which are known as the vinca, the colchicine, and the taxane site, respectively. In my presentation, I will give an overview on the current structural knowledge on microtubule-binding drugs and will further discuss the structure-function relationships of novel entities bound to tubulin as well as their implications for microtubule function.

ACKNOWLEDGEMENTS

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0-12

Synthesis and biological activity of saponin OSW-1 and its analogues

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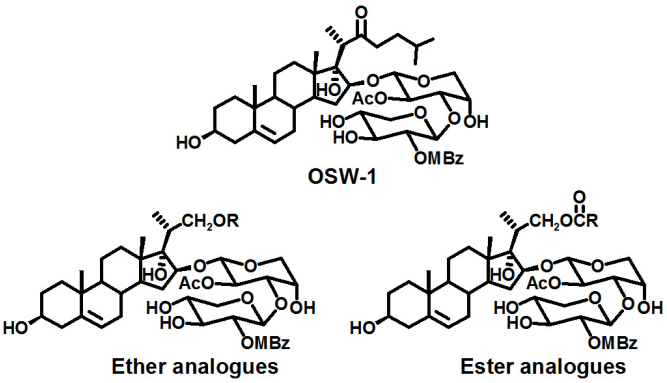
OSW-1 is a highly potent anticancer saponin isolated from *Ornithogalum saundersiae*. It shows high toxicity to several types of malignant cells, but it is also toxic to normal human cells. New OSW-1 analogues that are easier to obtain by chemical synthesis than the natural product have been designed. The synthesized ether and ester analogues proved slightly less active than OSW-1 but have a much wider therapeutic window, since they are less toxic to normal cells. The analogues induce apoptosis of mammalian cancer cells, just like OSW-1, in a concentration-dependent manner.



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0-13

Transcriptional responses to topoisomerase I-targeting drugs

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Nuclear DNA topoisomerase I (TOP1) relaxes DNA supercoiling generated by transcription and replication by producing transient TOP1-DNA cleavage complexes (TOP1cc). TOP1 has held a particular interest owing that it is the only known target of camptothecin (CPT), from which anticancer agents are derived. CPT and its derivatives bind at the TOP1-DNA interface and prevent the resealing of TOP1cc. As TOP1 religation activity is slowed down by the drugs, replication and transcription complexes "collide" with the TOP1cc, thereby generating irreversible TOP1cc and DNA damage. During replication, stalled TOP1cc are converted to DNA double-strand breaks by "replication run-off". The nature of transcription-mediated DNA damage and downstream cellular responses is less well characterized. We will review our recent works showing that cells rapidly respond to TOP1cc-mediated transcription block (1) by inducing the hyperphosphorylation of Rpb1, the largest subunit of RNA polymerase II, and promoting the Brca1-dependent degradation of TOP1; and (2) by producing DNA double-strand breaks and activating an ATM-dependent DNA damage response pathway by a mechanism that depends on R-loops (RNA:DNA hybrids). Lastly, we will discuss our recent observations indicating that these transcription-dependent DNA double-strand breaks tend to form selectively at sub-telomeric genes.

0-14

The novel tubulin antagonist pretubulysin exhibits vascular disrupting properties *in vitro* and *in vivo*

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Vascular-disrupting agents (VDAs), such as combretastatin A-4 phosphate (CA4P), act in contrast to classic anti-angiogenic agents on already established tumor blood vessels. VDAs have emerged as a novel promising class in anti-cancer treatment. We aimed to elucidate the vascular-disrupting potential of the new tubulin-depolymerizing agent pretubulysin (PT). PT is a synthetically accessible precursor of tubulysin, a myxobacterial compound that has recently been found to exert potent tumor cell death-inducing properties. In this study we focused on the action of PT on the endothelium *in vitro* and *in vivo* in comparison to the lead VDA CA4P. We investigated the effects of PT on key features of vascular disruption using human dermal microvascular (HMEC-1) and human umbilical vein endothelial cells (HUVECs): (i) PT induced a concentration-dependent disassembly of established endothelial tubes on Matrigel *in vitro* as well as in an *ex vivo* aortic ring model. (ii) PT rapidly increased endothelial permeability within 1 h, as measured by impedance sensing (xCELLigence, Roche) and Transwell® assays (tracer: FITC-dextran). (iii) Moreover, by immunocytochemistry and confocal microscopy, we found that PT leads to a disruption of microtubules and cell junctions (VE-cadherin, claudin-5), and to a strong induction of F-actin stress fibers. In all assays, both PT and CA4P showed comparable effectiveness in the concentration rage of 30-300 nM. Regarding cytotoxicity, PT-treated cells did neither undergo apoptosis (analysis of subdiploid DNA content) or necrosis (PI staining) within 24 h, nor reduce their metabolic activity (CellTiter-Blue® assay) after 1 or 24 h. Furthermore, after washing out of PT, HUVECs were even able to reassume their normal morphology. Moreover, we investigated the action of PT on established A-Mel-3 tumor blood vessels *in vivo* in a hamster dorsal skinfold chamber. First results suggested that PT (10 mg/kg IV, 2 h) decreases blood flow (red blood cell velocity) and vessel diameter as well as the density of functional vessels in the tumor, but not in neighboring healthy tissue. In summary, we could show for the first time that PT exhibits the typical features of a microtubule-targeting VDA *in vitro* and *in vivo*. The future challenge will be to figure out the therapeutical *in vivo* potential of PT using different tumor models as well as its underlying mechanisms.

ACKNOWLEDGEMENTS

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0-15

Natural products as leads for anticancer drug discovery

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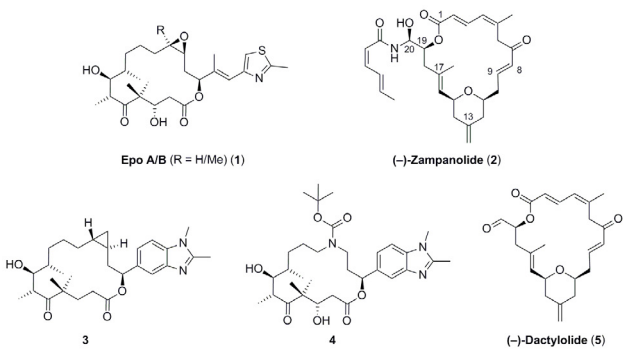
Natural products are a prolific source of lead structures for drug discovery and development and a significant fraction of current prescription drugs are either natural products themselves or have been derived from a natural product lead, either directly or indirectly. This contribution will discuss selected aspects of the chemistry and biology of the microtubule-stabilizing natural products epothilones A/B (1) and zampanolide (2), which are proven or potential leads for drug discovery in oncology. Epothilones are bacterial macrolides with potent microtubule-stabilizing and antiproliferative activity. At least 8 epothilone-type agents have entered clinical trials in humans and one of these (ixabepilone, Ixempra®) has been approved by the FDA in 2007 for clinical use in cancer patients. However, the structural diversity represented by this group of clinical compounds is rather limited, as they show little divergence from the original natural product leads. In contrast, our own research has explored the question, whether epothilones could also serve as starting points for the development of new structural scaffolds, or chemotypes, for microtubule-stabilization that might serve as a basis for the discovery of new generations of anticancer drugs. Within this conceptual framework we have elaborated a series of epothilone-derived macrolactones, whose overall structural features significantly deviate from those of the natural epothilone scaffold and thus define new structural families of microtubule-stabilizing agents. Key elements of our hypermodification strategy are the change of the natural epoxide geometry from *cis* to *trans*, the incorporation of a conformationally constrained side chain, the removal of the C3-hydroxyl group, and the replacement of C12 with nitrogen. So far, this has led to analogs 3 and 4 as the most advanced (i. e. the most rigorously modified) structures, both of which are potent antiproliferative agents with low nM activity against several human cancer cell lines *in vitro*.



These compounds can be considered as representative examples of new chemotypes for microtubule stabilization. Zampanolide (2) has only recently been discovered to be a microtubule stabilizer and little is known so far about the SAR around this lead structure. We have reported a total synthesis of 2, which will be shortly reviewed in this presentation. In addition, the activity of a number of zampanolide analogs will be discussed; this will include (-)-dactylolide (5), which is the non-natural enantiomer of the natural product (+)-dactylolide. Like 2, 5 was found to bind to microtubules, albeit with lower affinity.

0-16

Identification of new G-quadruplex-targeting chemical scaffolds from natural sources

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More than 120 natural and semi-synthetic compounds from the NCI Natural Products Set II, the NCI Diversity set II, the NCI Mechanistic Diversity Set and compounds isolated from *Paulownia tomentosa* (*Paulowniaceae*), *Morus alba* (*Moraceae*), *Maclura pomifera* (*Moraceae*) and extracts from *Cuscuta* spp. (*Convolvulaceae*), *Achillea* spp. (*Asteraceae*) and *Maytenus macrocarpa* (*Celastraceae*) were evaluated for their G-quadruplex-binding ability. Fluorescence Resonance Energy Transfer (FRET) DNA melting assay was performed using an Opticon DNA Engine instrument and 96-well plate format. The human telomeric sequence FAM-d(G3[TTAG3]3)-TAMRA (F21T) was used along with the control DNA duplex hairpin sequence FAM-d[(TA)2GA(TA)4T6(TA)4TC(TA)2]-TAMRA. The stock solution of fluorescence-tagged DNA sequences (Eurogentec, UK) in water (20 μM) was diluted to 400 nM using FRET buffer (50 nM potassium cacodylate, pH 7.4) and annealed by heating at 85 °C for 5 mins followed by cooling to room temperature over 5 h. Fluorescence readings were taken at intervals of 0.5 °C over the range 30–100 min, with a constant temperature maintained for 30 s prior to each reading. Principal results: Thaspine (NSC 76022): F21T ΔTm 5 μM = 24.0 °C, ΔTm 1 μM = 13.2 °C; duplex ΔTm 1 μM = 1.7 °C; Solanine (NSC 35611): F21T ΔTm 5 μM = 10.7 °C, ΔTm 1 μM = 2.4°C; duplex ΔTm 1 μM = 0.0 °C; Daunorubicin (NSC 82151): ΔTm 5 μM = 27.9 °C, ΔTm 1 μM = 13.8 °C; duplex ΔTm 1 μM = 11.6 °C; Coralyne sulfoacetate (NSC 154890): ΔTm 5 μM = 42.6 °C, ΔTm 1 μM = 29.9 °C; duplex ΔTm 1 μM = 3.2 °C; Acetopapaverine (NSC 98542): ΔTm 5 μM = 36.7 °C, ΔTm 1 μM = 8.0 °C; duplex ΔTm 1 μM = 0.4 °C; and *Achillea wilhemsii* chloroform extract: ΔTm 50 μg/mL = 10.3 °C; duplex ΔTm 50 μg/mL = 1.2 °C. Three new chemical scaffolds have been identified as G-quadruplex stabilising agents. Solanine showed good stabilising activity at 5 μM concentration and good selectivity, but low activity at 1 μM concentration. Daunorubicin possesses good stabilising activity to G-quadruplex, but its selectivity is poor. The best molecule from this screening was found to be thaspine which falls within Lipinski Rule of Five for drug-likeness. Two compounds (coralyne sulfoacetate and acetopapaverine) have significant selective activity but have structural similarity to existing ligands. Very promising results showed *Achillea wilhemsii* chloroform extract. Pure compounds from that extract are expected to possess higher activity than the whole extract. Their isolation is the subject of on-going studies. Part of this research has recently been accepted for publication in *Bioorganic and Medicinal Chemistry Letters*.

ACKNOWLEDGEMENTS

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0-17

Development of new allosteric inhibitors based on a thermodynamic and structural analysis of VEGF receptor 2 dimerization

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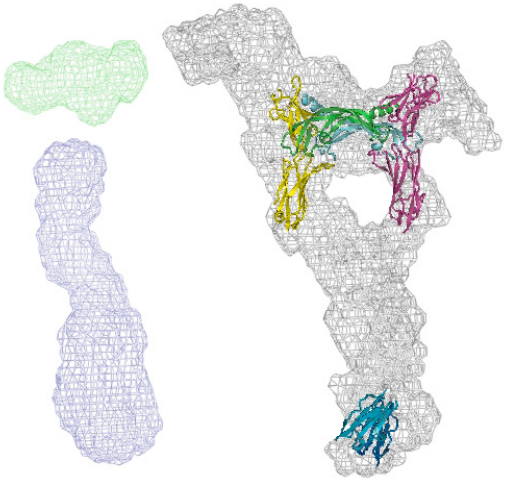
Vascular Endothelial Growth Factors (VEGFs) activate three receptor tyrosine kinases, VEGFR-1, -2, and -3, which regulate angiogenic and lymphangiogenic signaling.

The major mediator of VEGF signaling is VEGFR-2. The extracellular part of VEGF family receptors consists of seven immunoglobulin-homology domains (Ig-domains). It was shown earlier that domains 2 and 3 (D23) mediate ligand binding, while structural studies revealed homotypic contacts in the membrane-proximal Ig-domains 4 and 7. Ligand binding promotes receptor dimerization and instigates transmembrane signaling and receptor kinase activation. Isothermal titration calorimetry showed that the Gibbs free energy of VEGF-A, -C or -E binding to D23 or the full length ECD is dominated by favorable entropic contribution with enthalpic penalty. The free energy of VEGF binding to the ECD is 1.0-1.7 kcal/mol less favorable than for binding to D23. A model of the VEGF-E/VEGFR-2 ECD complex derived from small angle scattering data provided evidence for homotypic interactions in D4-7. We also solved the crystal structures of complexes between VEGF-A or -E with D23 which revealed comparable binding surfaces and similar interactions between the ligands and the receptor, but showed variation in D23 twist angles. The energetically unfavorable homotypic interactions in D4-7 may be required for proper orientation of receptor monomers and prevent ligand-independent activation of VEGFR-2. These interactions may thus prevent the deleterious consequences for blood and lymph vessel homeostasis arising from inappropriate receptor activation. Based on our structural analysis we mutated D4 and D7 of VEGFR-2 and found that these domains are not essential for receptor dimerization, but play an essential function in receptor activation. We therefore investigated the possibility to design new receptor-inhibitory molecules interacting with Ig-domains D4 or D7. We isolated designed ankyrin repeat proteins (DARPins) and single chain variable fragments (scFvs) interacting with D23, D4 or D7 of VEGFR-2. Binders specific for Ig-domains 2 and 3 inhibited ligand binding, receptor dimerization, and activation, while reagents specific for Ig-domains 4 or 7 blocked receptor signaling without interfering with receptor dimerization. These findings show that the membrane-proximal receptor domains D4 and D7 allosterically regulate VEGFR-2 activity and open new possibilities for inhibiting aberrant angiogenesis in disease.



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0-18

AXP107-11, a multi-targeted natural compound for cancer treatment

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AXP107-11 is a crystal re-engineered novel solid state form of the isoflavone molecule genistein with greatly enhanced biopharmaceutical properties. *AXP107-11* has superior dissolution rate and solubility profiles, translating into a markedly improved oral bioavailability of AXP107-11as compared to the parent from of genistein. *AXP107-11*was developed from bench to clinic in only 2 years showing that crystal re-engineering is a unique and feasible strategy for accelerated drug development. *AXP107-11* is an orally active, non-toxic, multi-targeted chemosensitizer that sensitizes human pancreatic cancer cells to gemcitabine and reduces metastasis in animal models of cancer. These properties of *AXP107-11* make the compound a promising new agent for the treatment complex and heterogeneous solid tumors, such as pancreatic cancer. *AXP107-11* is currently in Phase Ib/IIa clinical trials in combination with the first-line gemcitabine chemotherapy in patients with pancreatic adenocarcinoma. *AXP 107-11* has multiple mechanisms of action, including enzyme inhibition as well as selective estrogen receptor beta binding and activation. *AXP107-11* also has anti-inflammatory properties that could be beneficial in cancer treatment. Targeting more than one biochemical pathway (i.e. multi-targeted compounds) has the potential to reduce the risk for development of resistance of tumor cells to therapy, which is a major medical problem in management of cancer. The story behind the development of AXP107-11 and some clinical data will be presented.

0-19

New cytotoxic metabolites from the endophytic fungus *Stemphylium globuliferum*

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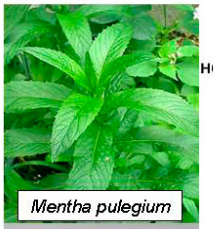
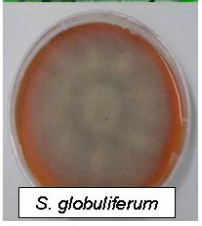
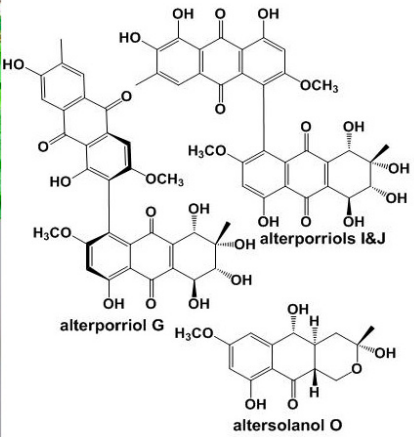
EtOAc extract of *Stemphylium globuliferum*, an endophytic fungus of *Mentha pulegium*, exhibited considerable cytotoxicity when tested *in vitro* against L5178Y cells. Chemical investigation of the EtOAc-extract yielded several new anthracene derivatives, including monomeric anthranoids and dimers belonging to alterporriol-type atropisomers. The structures were determined on the basis of one- and two-dimensional NMR spectroscopy, mass spectrometry and CD calculation. All compounds isolated during this study were tested for their cytotoxicity toward L5178Y mouse lymphoma cells.







Among the alterporriol-type anthranoid dimers, the mixture of alterporriols G and H (4/5) exhibited considerable cytotoxicity against L5178Y cells with an EC50 value of 2.7 μg/mL, whereas altersolanol A as well as altersolanol N were the most active monomers with EC50 values of 0.21 and 0.44 μg/mL, respectively.

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0-20

The diterpene glucoside fusicoccin inhibits tumor cell growth through stabilisation of 14-3-3 complexes

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Fusicoccanes are diterpenes with a characteristic 5-8-5 core ring structure produced by fungi, bacteria, liverworts and higher plants (for review, see Trends in Plant Science 2012, in press). Many fusicoccanes have biological activity and the best characterised fusicoccanes belong to the Fusicoccin-group. Fusicoccin is produced by a plant infecting fungus (Phompsis amygdali) and when it enters the plant cells it strongly activates the plasma membrane H^+-ATPases. The mode of action of Fusicoccin is rather unique, since it acts as a protein:protein stabilizer, in this case it stabilizes the interaction between the ATPases and a class of scaffold proteins: the 14-3-3 proteins. 14-3-3 proteins form dimers and their prime activity is binding to a wide range of cellular proteins in a phosphorylation dependent manner. The crystal structure shows that Fusicoccin binds in a pocket of the conserved 14-3-3 binding groove. Since 14-3-3 proteins form an ancient family of proteins with a conserved structure in fungi, plants and animals and in view of the important role of 14-3-3 proteins in cell proliferation and apoptosis, we tested whether Fusicoccin affects the growth of tumor cells. Ovarian tumor cells (OVCAR3) and lung cancer cells (A549) were strongly reduced in growth when Fusicoccin was applied together with Interferon-α (IFN-α). Further analysis showed that FC is tumor cell specific, and that IFN-aprimes the tumor cells for apoptosis induction by FC. Apoptosis was induced through up-regulation of the death receptor DR4 and activation of the TRAIL pathway. When Fusicoccin was applied to cells that are estrogen dependent (breast cancer cells; MCF7), estrogen stimulated growth was reduced in the low estrogen concentration range. In these cells we identified the Estrogen Receptor alpha (ERα) as a target for Fusicoccin. In analogy to the activation of the plant ATPase, Fusisocccin stabilizes the interaction between ERα and 14-3-3 proteins at the extreme C-terminus of ERα. This so-called F-domain is involved in receptor dimerisation and binding of 14-3-3 proteins to the F-domain reduces the estradiol stimulated dimerisation. Using an ERE-Luc assay we demonstrated that Fusicoccin reduces the transcriptional activity of ERα, what is probably caused by reduced dimerisation. Using a fluorescent anisotropy assay we showed that Fusicoccin enhances the affinity of the phosphorylated peptide derived from the ERα C-terminus and human

14-3-3 proteins. Co-crystallisation of this trimeric complex and X-ray diffraction at high resolution showed how the Fusicoccin molecule and the peptide interact in the 14-3-3 binding groove. So, the natural compound Fusicoccin acts as a small-molecule ligand that, in contrast to anti-estrogens, inhibits the ERα activity through binding outside the ligand binding domain (LBD), without an apparent effect on cofactor binding. Fusicoccin and related Fusicoccanes have potential as novel anticancer drugs and will be used as a lead to develop molecules that target tumor cell promoting signalling pathways.

0-21

The novel indirubin derivative 6BIO inhibits proliferation and metastasis of breast cancer cells

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Initially, indirubins were discovered as the active component of a well known traditional Chinese medicine. Since then, it was shown that natural indirubins and also its synthetic, cell permeable derivative 6BIO (6-bromoindirubin-3-oxime) display a remarkable potential to inhibit glycogen synthase kinase 3 (GSK-3) and cyclin-dependent kinases (CDKs) by interacting with the ATP-binding pocket of these kinases. By an inverse virtual screening approach phosphoinositide-dependent kinase 1 (PDK1) was proposed by us as a yet unknown target of 6BIO. Western blot analysis and confocal microscopy revealed that treatment of breast cancer cells with 6BIO resulted in a reduced phosphorylation of AKT, an important downstream target of PDK1. Since about 70% of all breast cancer carcinomas express moderate to high levels of activated PDK1, we investigated the impact of 6BIO treatment on different breast cancer cell lines by analyzing the anti-proliferative and cytotoxic effects as well as the influence on reduction of metastatic capabilities of these cells. As determined by crystal violet staining and xCELLigence experiments, incubation of SkBr3, MDA-MB-231, MCF7 and AKT overexpressing SkBr3 breast cancer cell lines with increasing concentrations of 6BIO led to a dose-dependent inhibition of proliferation. In addition, 6BIO treated breast cancer cells showed a strongly diminished long-term clonogenic survival rate. Furthermore, Nicoletti assays and Hoechst staining indicated that stimulation of breast cancer cell lines with high doses of 6BIO led to an induction of apoptosis. Cell treated with sub-toxic doses of 6BIO displayed a strongly reduced attachment capacity, as shown by adhesion assays on fibronectin and xCELLigence experiments. Scratch assays as well as chemotaxis assays revealed a significantly diminished migratory potential of 6BIO treated breast cancer cells compared to control cells. Furthermore, 6BIO significantly disrupted the invasion capacity of the cells. The impact of 6BIO treatment on the migration and invasion inhibition might be due to altered localization of the well-known migration associated molecules FAK and Rac1 within the cells, as shown by confocal microscopy. In summary, we were able to show that 6BIO inhibits PDK1 signal transduction in different breast cancer cell lines and reduces proliferation, clonogenic survival, attachment, migration and invasion of the cells. Thus, the indirubin derivative 6BIO might serve as a highly promising potential therapeutic agent for metastatic breast cancer cells.

0-22

From mayapples to human malignancies: etoposide as a topoisomerase II-targeted anticancer drug

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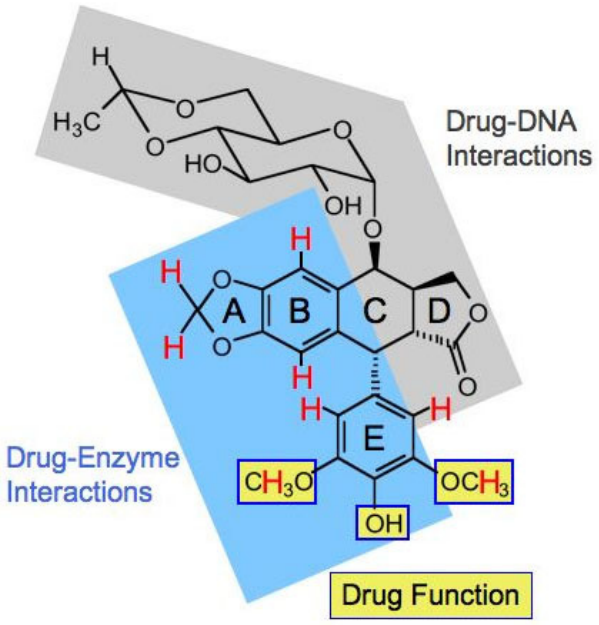
Etoposide (see Figure), which is derived from podophyllotoxin, is one of the first clinically relevant topoisomerase II-targeted anticancer drugs. The parent natural product is produced by *Podophyllum peltatum*, more commonly known as the mayapple or American mandrake plant. Podophyllotoxin has been used as a folk remedy for over a thousand years and is an antimitotic drug that acts by preventing microtubule formation. The clinical use of this compound as an antineoplastic agent was prevented by high toxicity, but two synthetic analogs, etoposide and teniposide, displayed increased antineoplastic activity and decreased toxicity. Further analysis revealed that neither drug interacted with microtubules but rather acted as topoisomerase II poisons (*i.e.*, compounds that increase levels of topoisomerase II-mediated DNA cleavage). Etoposide was approved for clinical use in the mid-1980's and for several years was the most widely prescribed anticancer drug in the world. The modification that converts podophyllotoxin to a topoisomerase II poison is the substitution of the 4'-OMe of the pendant E-ring to a 4'-OH. Numerous studies carried out in my laboratory have revealed considerable information regarding the mechanism of action of etoposide. The drug enters the ternary enzyme-DNA-drug complex primarily through interactions with topoisomerase II.



Etoposide increases levels of enzyme-mediated cleavage by inhibiting the ability of the enzyme to ligate DNA. It has no effect on the forward rate of DNA cleavage. Etoposide binds to topoisomerase II through interactions mediated by its A-, B-, and E-rings. Interactions with DNA in the ternary complex appear to involve the D-ring and the C-4 glycosyl moiety. Drug activity can be increased significantly if the C-4 group is substituted by moieties that display tighter binding to either topoisomerase II or DNA. Despite its important role in treating cancer, ~2-3% of patients treated with etoposide eventually develop specific acute myeloid leukemias (AML) that are characterized by chromosomal translocations involving the *MLL* gene at 11q23. Evidence suggests that a quinone metabolite of etoposide may play a role in the leukemic process and recent studies demonstrate that etoposide quinone displays a greatly enhanced activity towards human topoisomerase II.

0-23

Bufadienolide compound, cinobufagin, inhibits the expression of hypoxia-inducible factor 1 alpha subunit and cortactin in colon cancer cell lines

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Cancer has become one of the leading diseases causing high death rate worldwide. Our laboratory has been working on the development of anticancer therapies by targeting apoptotic signalling pathways and has focused on a range of traditional Chinese medicines which are primarily natural compounds, such as a bufadienolide compound, cinobufagin (CBF). CBF inhibits sodium/potassium ATPases and is a major component of traditional Chinese medicine Chansu, which has been widely used for treating heart failure and anti-inflammation in China for centuries. The initial cytotoxicity assays showed a high sensitivity of CBF in colon cancer cell line HCT116 and HT29 among several other human cancer cell lines. Interestingly, the subsequent examinations of CBF-induced apoptosis revealed that distinct intracellular pathways were activated in the two cell lines. The occurrence of mitochondrial permeabilisation was involved in apoptotic process of both cells. However, the addition of antioxidant N-acetyl-Lcysteine (NAC) only significantly countered the cell death in CBF-treated HCT116 cells but not in the treated HT29 cells. During apoptosis, CBF increased the activity of Caspase-3 in HCT116 cells but inhibited Caspase-3 in HT29 cells. Furthermore, the release of mitochondrial apoptosis-inducing factor (AIF) by CBF was found in HCT116 cells, which leads to Caspase-3 independent apoptosis. In contrast, such translocation of AIF was not observed in HT29 cell line. CBF also showed an inhibitory role in the protein expression of hypoxia-inducible factor 1 alpha subunit (HIF-1α) in both colon cancer cell lines. CBF-induced protein inhibition was not related to the transcription of HIF-1α mRNA, which was elevated overall after CBF treatment. Further investigations on HIF-1α intracellular distribution revealed that CBF inhibited HIF-1α activity at different points of hypoxia-regulated pathway between the two cell lines. HIF-1α protein expression was significantly inhibited in the fraction of organelle membrane in HCT116 cells, suggesting an interruption occurring in the nuclear membrane during the translocation of HIF-1α. On the other hand, CBF seemed to directly suppress the synthesis of HIF-1α in cytoplasmic fraction of HT29 cells and showed no impact on nuclear import and accumulation. In addition, CBF was found to inhibit the expression of cortactin (CTTN) that is required for cell migration. Immunocytochemistry showed a nuclear translocation of CTTN observed in HCT116 cells for the first time, which could be resulted from CBF treatment. Furthermore, the inhibition of CTTN was not only detected in HCT116 cell line but also in HCT116 implanted xenograft models. Nevertheless, the nuclear import of CTTN was not found in the tumour tissues of xenografts. In conclusion, our results strongly suggest that CBF could be developed as a therapeutic agent for colon cancer. This is due to its capacities in induction of apoptosis and inhibition of HIF-1α and CTTN protein expression.

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0-24

Mode of cell death induction by pharmacological V-ATPase inhibition

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Recently V-ATPase, a multisubunit proton pump, has come into focus as an attractive target in cancer cell invasion. However little is known about the role of V-ATPase in cell death and especially the underlying mechanisms remain mostly unknown. We used archazolid B, a macrolide of myxobacterial origin being a novel very potent inhibitor of the V-ATPase as an experimental drug as well as a chemical tool to decipher V-ATPase related cell death signaling. We found that archazolid induced apoptosis in highly invasive tumor cells at nanomolar concentrations while showing almost no cytotoxicity in non tumorus cells. Apoptosis was caspase-dependant and executed by the mitochondrial pathway shown by breakdown of mitochondrial potential, leakage of cytochrom c from the mitochondria and activation of caspase-9. Prior to apoptosis induction archazolid lead to the activation of stress responses such as autophagy and glycolysis. Autophagy was induced at concentrations that do not alkalize lysosomes and was shown by degradation of p62 or fusion of autophagosomes with lysosomes. Autophagy was found to be a survival mechanism at low concentrations but could not be accomplished at higher concentrations due to lysosome alkalization by V-ATPase inhibition. Archazolid also lead to glycolysis induction which is mediated by the hypoxia-inducible-factor-1 alpha and induced due to energy stress. This was shown by a decline of the ATP level and arrest of energy consuming processes (AMPK, mTOR, eIF2alpha). As silencing HIF1alpha increases apoptosis, glycolysis was suggested to be an adaptive mechanism. We conclude that archazolid leads to energy stress which activates autophagy and glycolysis and finally leads to apoptosis. We propose V-ATPase as a promising drugable target in cancer therapy caught up at the interplay of apoptosis, autophagy and cellular/metabolic stress

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0-25

Products of natural origin manufactured by TEVA Opava and especially paclitaxel

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TEVA factory in Opava, former Galena, is a traditional producer of natural products. While in the past, mainly herbal extracts were produced, since fifties of the last century the portfolio contained more and more natural chemical entities. Some of them were later on abandoned (tropane alkaloids, cardiotonic glycosides, semisynthetic steroids), the other are still produced. Thus the factory is now a world leading producer of ergot alkaloids (the whole spectrum of therapeutically used alkaloids, both natural and semisynthetic) and silymarin (milk thistle dry extract). In the nineties the portfolio of traditional herbal products (although in the case of ergot alkaloids can be this term misleading) was enlarged by natural products obtained by fermentation: cyclosporine and mycophenolic acid, later on, after connection with TEVA, tacrolimus. Also the portfolio of herbal drugs was widened by galanthamin and mainly paclitaxel. And it will be the natural anticancer drug paclitaxel on which example the actual situation will be demonstrated in the lecture. Discussed will be sourcing of raw material, manufacture and business aspects.

0-26

Inhibition of angiogenesis by pretubulysin and its derivates

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Tubulysins are microtubule disrupting tetrapeptides produced by myxobacteria. One tubulysin family member – Tubulysin A (TubA) – has recently been evaluated as a very potent anti-proliferative and anti-angiogenic agent *in vitro* and *in vivo*. However, time consuming myxobacterial fermentation processes only lead to small yields of TubA and its chemical synthesis so far is difficult due to complex side moieties. In contrast, a natural precursor of tubulysins – Pretubulysin (Prt) – lacks these complex side moieties and is more efficiently synthetically accessible than TubA. In the present work we characterized the anti-angiogenic potential of Prt and of 7 modified Prt-derivatives. We used human microvascular endothelial cells (HMEC-1) and human umbilical vein endothelial cells (HUVEC) in a set of *in vitro* angiogenesis assays: proliferation, migration (scratch assay), chemotaxis, tube formation, cell cycle analysis (flow cytometry with PI). Additionally, microtubule staining (immunocytochemistry) and *in vitro* tubulin polymerization were performed to compare the cellular data with tubulin effects. We also tested Prt in an *in vivo* xenograft model using HuH7 cells in SCID mice. Tumor size (volume and mass) and degree of vascularization (CD31 staining) were quantified. *In vitro*, Prt showed a similar inhibitory potential as TubA at key points of angiogenesis, such as endothelial cell proliferation (EC50 Prt 2.3 nM; TubA 1.2 nM); migration (EC50Prt 5 nM; TubA 3.5 nM); chemotaxis and tube formation (similar effects at 3 nM and 30 nM respectively). Furthermore, induction of cell cycle arrest and apoptosis occurred for Prt and TubA treated HMEC-1 at similar concentrations (EC50 2.4 and 2.9 nM after 48hrs). To find relevant relations between the Prt molecular structure and anti-angiogenic effects, we tested 7 modified Prt-derivatives in the same set of *in vitro* assays. Dependent on the kind of modification these compounds' antiangiogenic efficiency and their microtubule depolymerization influence decreased mildly (10-fold) to dramatically (1000-fold) in comparison to Prt. A dramatic drop of activity was caused by C-terminal truncation, opening of the N-terminal piperidine ring or replacement of the central thiazole ring by a triazole- or a phenoxyring. The *in vitro* tubulin polymerization assay suggests, that these different activities do not result from different cellular drug uptake or metabolism, but rather from different drug effects on tubulin. *In vivo,* Prt strongly inhibited mean tumor volume (control 169.63± 122.21 mm^3; Prt 9.35 ± 12.36 mm^3) and vascularization (control 221.4 ± 37.16 vessels/mm^2; Prt 70.6 ± 24.18 vessels/mm^2) in the HuH7 xenografts without causing weight loss or other obvious side effects in the treated mice. Prt is considerably chemically simplified, but still similarly active in comparison to TubA at blocking angiogenesis *in vitro* and highly promising as new anti-angiogenic agent *in vivo*. SAR studies on Prt derivatives could help to design even more simplified tubulysin-based anti-angiogenic compounds.

ACKNOWLEDGEMENTS

This work is done within the framework of the DFG Research Group FOR 1406 –myxobacterial compounds as therapeutic leads and chemical tools in cancer research.

0-27

Identification of new natural anti-cancer drugs by functional biochemical and cellular screenings of natural compounds isolated from marine organisms

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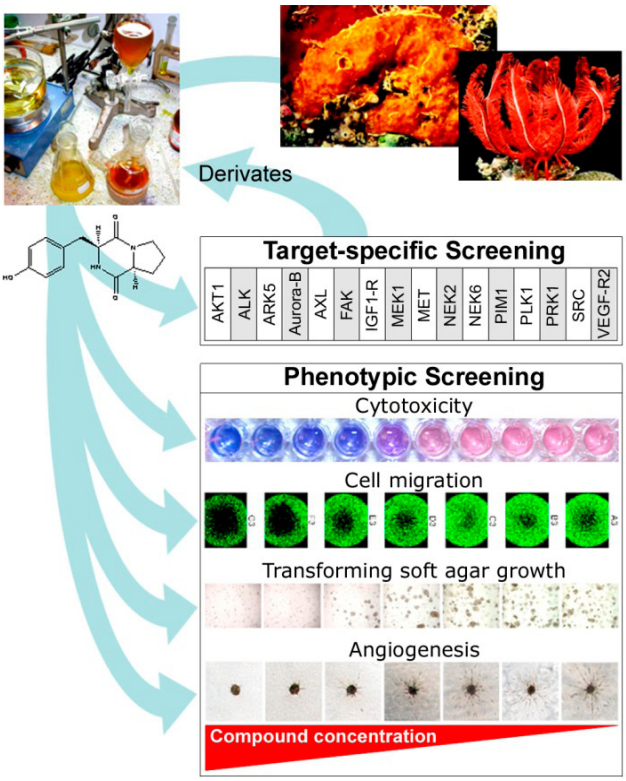
The broad biodiversity of marine organisms provides a rich source of new natural compounds, which can be used for the discovery of new bioactive lead structures. In this project we elucidated the anti-tumoral potency of 320 purified compounds isolated from marine organisms of the Chinese and Vietnamese coastal waters and the Chinese deep sea.



We performed in parallel two screening approaches. In a first target-driven approach we checked the inhibitory impact of the purified compounds on 16 recombinant protein kinases that have been shown to be relevant targets in various types of human cancer. Hits of this screening were further analyzed for their cellular potency in appropriate cellular phosphorylation assays. In a second target-independent, phenotypic-driven approach we addressed the impact on cell transformation, migration and angiogenesis, representing important cancer hallmarks. Furthermore we determined unspecific toxicity of the compounds in a counter assay, where the influence on the viability of human peripheral blood mononuclear cells from healthy donors was analyzed. Examples of indentified compounds will be presented, showing different inhibitory profiles in the various assays. The results demonstrate the validity of this parallel screening approach for the discovery of new anti-tumoral compounds.

0-28

The use of a combination of tamoxifen and doxorubicin synergistically to induce cell cycle arrest in BT483 cells by down-regulating cdk1, cdk2 and cyclin d expression

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Tamoxifen and Doxorubicin are used alone or in combination to treat breast cancer. Although these drugs have been utilized in combination, the advantage of their combination, in terms of therapeutic efficacy, still remains controversial. This study uses breast cancer cell lines to demonstrate the synergistic interaction between Doxorubicin and Tamoxifen, and to explain the CDK1 and CDK2 expression underlying the synergy. This study demonstrates that the combination of Doxorubicin and Tamoxifen significantly reduces the growth of ER-positive breast cancer cells and that this is driven primarily by the enhanced effect of the decreased protein expression of the CDK1, CDK2 and cyclin D. It is also proposed that selective modification of AKT inactivation, ERK and JNK activation probably contributes to the synergistic interaction. Overall, the findings suggest that a combination of Doxorubicin and Tamoxifen could be effective in the treatment of ER-positive breast cancers, so this combination warrants further investigation.

ACKNOWLEDGEMENTS

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0-29

Epigenetic modulation by the marine natural product largazole: from discovery to broad-spectrum therapy

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Histone deacetylase (HDAC) inhibitors represent a relatively new class of anticancer agents that target dysregulated acetylation of histone lysines. Since HDACs also have non-histone targets, inhibitors of this enzyme class may additionally modulate protein-protein interactions and subcellular localization affected by the acetylation

status. Certain HDACs are overexpressed and hyperactive in cancer cells, and suppression of these enzymes' activities provides superior selectivity over more traditional anticancer agents. To date, two HDAC inhibitors – vorinostat and romidepsin – have reached the market, with romidepsin being an actual natural product and vorinostat closely related to the natural product HDAC inhibitor trichostatin A. Over the past decade, several secondary metabolites with high structural diversity from microorganisms, marine sponges and cyanobacteria have been discovered to possess HDAC inhibitory activity and are currently at the clinical and preclinical stages. Our research group recently discovered the most potent natural class I HDAC inhibitor known to date, largazole, a cyclic depsipeptide from a marine cyanobacterium of the genus *Symploca*. Largazole possesses highly differential growth-inhibitory activity, preferentially targeting transformed over non-transformed cells. The intriguing structure and biological activity of largazole have attracted strong interest from the synthetic chemistry community to establish synthetic routes to largazole and to investigate its potential as a cancer therapeutic. Screening against the NCI's 60 cell lines revealed that largazole exhibits broad-spectrum activity, but is particularly active against several colon cancer cell types, including HCT-116. Enzyme inhibition strongly correlated with the growth-inhibitory effects. Comparative genome-wide transcript profiling revealed a close overlap of genes that are regulated by largazole, romidepsin and vorinostat. Stability studies suggested promising bioavailability of the active species, largazole thiol, which is released from the pro-drug largazole upon protein-assisted thioester hydrolysis. Largazole strongly stimulated histone hyperacetylation in a HCT-116 xenograft tumor model, showed efficacy in inhibiting tumor growth and induced apoptosis in the tumor. Apart from its anticolon cancer activity, we also found that largazole cooperated with dexamethasone to induce E-cadherin localization to the plasma membrane in triple-negative breast cancers *in vitro* and *in vivo*, and to suppress cellular invasion. This effect appeared to be due to increased association of E-cadherin with gamma-catenin rather than being mediated by transcriptional regulation. Aggressive cancers often express E-cadherin in cytoplasmic vesicles rather than on the plasma membrane, which may contribute to the invasive phenotype of these tumors. Since therapeutic strategies to restore the anti-invasive function of E-cadherin in cancers are not currently available, our studies provide the scientific basis for a new approach to treat highly invasive cancers. The lecture will provide an overview from the initial discovery of largazole to latest mechanistic and biological data, including these various promising *in vivo* anticancer activities.

ACKNOWLEDGEMENTS

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0-30

From bedside to bench: characterization of *in vitro* and *in vivo* anticancer properties of *Achyranthes aspera* (apamarg) leaf extract

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Pancreatic cancer (PaCa) constitutes nearly 2% of all the newly diagnosed cancers. On the contrary, 6% of all cancer related deaths are attributed to this particular cancer type. Currently approved chemotherapeutic agents GEMZAR and 5-FU extend survival marginally by up to five months. Therefore PaCa is a dreadful disease. This underscores the need to discover new drugs to combat PaCa. Ayurveda is the system of medicine practiced in India. It is a rich source of traditional pharmacopeia. *Achyranthes aspera* (Family: *Amaranthacea*) is one such plant used as a cure for PaCa. However, its anti-tumor properties have not been validated by current scientific approaches, thus remain anecdotal. We systematically investigated its anti-cancer properties *in vitro* and *in vivo*. Acetone extract of *A. aspera* leaf powder was dried under vacuum. The residue (LE) was suspended in methanol and assayed for bioactivity. We tested differential antiproliferative activity on a panel of human cancer cell lines, time and dose dependent *in vitro* anti proliferative activity on PaCa cell lines. We further examined its effect on global gene expression on PaCa cells *in vitro*. *In vivo* antitumor activity of LE was tested on athymic mice harboring human pancreatic tumor. We monitored LE toxicity in mice by recording changes in behavioral, histological, hematological and body weight parameters. PaCa cells were significantly more sensitive to LE and the cytotoxicity was dose and time dependent. LE was non-toxic to mice as indicated by hematological and biochemical parameters. Compared to the control set, IP administration of LE to tumor bearing mice significantly reduced tumor weight and volume. Global gene expression analyses of cultured human cancer cells treated with LE indicated developmental pathways like Notch was significantly affected by LE. In real time PCR assays we found changes in the transcripts of metalloproteases (MMP1 & 2), inhibitors

of MMPs (TIMP-2) and angiogenic factors (VEGF-A and VEGF-B). Real time PCR analyses in the tumor tissues harvested from control and test mice revealed LE significantly induced Caspase-3 mRNA (P<0.001) and suppressed expression of pro survival kinase Akt-1 (P<0.05). Immuno histo-chemistry (IHC) confirmed increases in caspase-3 and reduction in phosphorylated Akt levels in tumor harvested from treated mice. The IHC results are in agreement with RT PCR data. It confirms induction of apoptosis and interference with survival signals by LE. For the first time we systematically tested the anti-proliferative activity of *A. aspera*. The data support the use of *A. aspera* as anti cancer drug in Ayurveda. We believe that this plant holds promise of additional treatment options for PaCa patients.

ACKNOWLEDGEMENTS

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0-31

Embelin induces apoptosis in human glioma cells through inactivating NF-κB

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Aggressive tumor growth and diffuse tissue invasion are hallmarks of malignant glioma. Embelin is an active compound isolated as a novel XIAP inhibitor from the *Embelia ribes* to exhibit various medicinal effects including anti-inflammatory and anti-cancer activities. In the present study, we investigated whether embelin has a therapeutic effect in glioma. We found that embelin suppressed proliferation of glioma cells by inhibiting NF-κB, which is a crucial transcription factor associated with several human diseases including cancer and controls multiple genes involved in tumor progression such as a proliferation of tumor cells, but not that of normal immortalized human astrocytes. Embelin inhibited NF-κB activity by reducing nuclear translocation of p65 through decreasing phosphorylation and proteasomal degradation of IκBα in glioma cells. In addition, embelin induced apoptosis of glioma cells while p65 overexpression inhbibited embelin-induced apoptois in glioma cells. Taken together these results indicate that embelin could be a potent therapeutic agent for glioma via blocking aggressive glioma cell proliferation through inhibiting NF-κB activity.

ACKNOWLEDGEMENTS

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0-32

Anti-tumor toxins and their efficacy in combination with triterpenoid saponins

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Anti-tumor toxins are mostly fusion proteins comprising two functional domains: a high affinity ligand (growth factors or antibodies) that binds to cancer-associated antigens and a toxin component that eliminates the tumor cell after endocytosis. Although some anti-tumor toxins are in clinical trials or have been approved such as Ontak® (a fusion between diphtheria toxin (DT) and interleukin-2) severe side effects are still a great problem during treatment. In previous studies we have shown that the co-application of a particular anti-tumor toxin (SE) with a crude mixture of triterpenoid saponins augmented the efficacy of SE tremendously by concomitantly decreasing both its dosage and side effects. SE is composed of the *N*-glycosidase saporin and the human epidermal growth factor as a targeting ligand. For the application of anti-tumor toxins in cancer therapy it would be a pathbreaking step if the efficacy of arbitrary anti-tumor toxins could be enhanced by the use of triterpenoid saponins. For this reason we constructed different anti-tumor toxins varying in the toxic moiety and targeting the epidermal growth factor receptor (EGFR). Truncated variants of *Pseudomonas* exotoxin, *Diphtheria* toxin and ricin from *Ricinus communis* L., dianthin-30 from *Dianthus caryophyllus* L. and saporin-3 from *Saponaria officinalis* L. and human pancreatic RNase I, served as toxin moieties. A saponin was isolated from *Gypsophila paniculata* L., and all EGFR-targeted anti-tumor toxins were tested in the combination therapy *in-vitro* with this saponin. The saponin-mediated efficacy increase was investigated on NIH-3T3 cells, transfected with human EGFR. Especially dianthin-30- and saporin-3-based anti-tumor toxins benefited most from the combination with saponins. Saponins did not influence the plasma membrane permeability as evident in

FACS analysis. Surface plasmon resonance measurements pointed to an intracellular interaction of the toxin part of anti-tumor toxins and saponins. The overall effectiveness of the combined therapy was investigated in a syngeneic tumor mouse model in BALB/c mice with a saporin-3 based EGFR-targeted toxin and saponin. 7 out of 10 mice showed > 90% remission compared to a control group where 7 out of 8 mice had > 10 mm^3 tumors. Based on a NOAEL (no observed adverse effect level) of about 60 µg/treatment the saponin was well tolerated.

0-33

Resveratrol triggers different cell death pathways in cancer cell lines: apoptosis and autophagy

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Resveratrol, (trans – 3,4', 5 – trihydroxystilbene) (Res) is a phytoalexin which can cause apoptosis in many cancer cell lines. Res caused apoptosis in HeLa, the human cervical cancer cell line, in a dose and time – dependent manner. When working with an optimized dose of 60 µM, apoptosis was verified by an increase in the sub – G0 / G1 population, membrane phosphatidylserine externalization, TUNEL assay, activation of caspase-3 and PARP cleavage. The TUNEL positivity was observed from ≥ 24 h of Res Treatment. Also, cleaved PARP-1, a major apoptosis marker, showed increase at 12 h, and a more pronounced positivity (increase in PARP -1) at 24 h. The observations that levels of ß-catenin decreased appreciably only after 24 hrs (i. e. at 36 & 48 h), whereas Caspase-3 activation was noticeable from 12 h, raised the question of whether the ß-catenin degradation was caspase-dependent or not. We treated the cells with 60 µM Res along with caspase-3 inhibitor and found that ß-catenin levels did not change appreciably. At the same time in similarly – treated cells cleavage of PARP1 was also absent. The data indicate a caspase – mediated degradation of ß-catenin during Res induced apoptosis. We then probed the nature of caspase-3 independent cell death induced by Resveratrol. Also, we wanted to find out whether Res. –induced autophagy had a pro- , or, anti-survival effect on HeLa cells. Accordingly, cells treated with Res only or with (Res +caspase-3 inhibitor) were harvested & probed for LC-3, the well-known autophagy marker protein. In extracts of (Res +caspase-3 inhibitor) treated cells both LC-3 (I) & LC-3 (II) bands were present compared to absence of the LC-3 (II) band in only Res treated cells. Our studies with MCF-7 cells showed indications of autophagy upon Resveratrol exposure. We expect to provide further insights about the modes of cell death in both HeLa and MCF-7 cells after Res exposure.

0-34

Comparative bioactivity studies on pretubulysin and other antimitotics from myxobacteria

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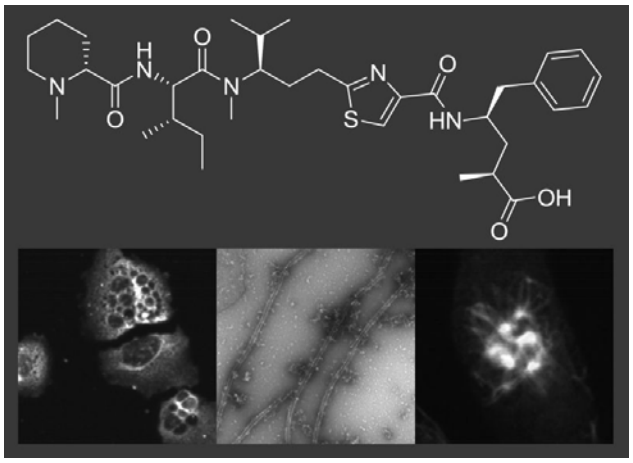
To date, six distinct compound classes that are produced by myxobacteria have been found to directly interfere with the eukaryotic cytoskeleton by either stabilizing/destabilizing microtubules or actin filaments. Of these, microtubule-targeting drugs have been the subject of concerted efforts to elucidate their biosynthesis, total synthesis, and semi-synthesis to improve their pharmacological properties and yields. Pretubulysin is a natural product produced by several myxobacterial strains in only minute amounts. It represents the first enzyme-free intermediate in the biosynthesis of tubulysins, which are assembled by a PKS/NRPS (polyketide synthase/nonribosomal peptide synthetase) hybrid system. Tubulysins are described as very effective microtubule-destabilizing agents with structural similarity to dolastatin-10 and with GI50 values against mammalian cells in the picomolar to low nanomolar range. Compounds of this class are already in advanced preclinical trial as anticancer and antiangiogenic agents. However, the isolation of tubulysins is tedious and includes fermentation and multiple chromatographic steps. These supply issues with naturally occurring derivatives were circumvented by the total synthesis of pretubulysin, which, in contrast to tubulysin, is synthetically accessible in gram-scale quantities. In the course of our initial studies we characterized pretubulysin and some of its synthetic precursors with regards to antimitotic effects, inhibition of cancer cell migration, and apoptosis induction in cancer cell lines.



We demonstrated that synthetically accessible pretubulysin exhibits only a minor reduction in anticancer activity relative to the parent compound tubulysin. With a synthetic route in hand, pretubulysin or its derivatives appear to be better-suited for the development of novel antimitotic agents in tumor therapy, including tumor-targeting constructs, because the supply issues commonly encountered in the preclinical development of pharmaceuticals from natural products have been circumvented.

ACKNOWLEDGEMENTS

TBA.

O-35

Topoisomerase IIβ (TOP2B) and a model for *MLL* rearrangements

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Chromosome translocations are associated with the development of leukaemia. The mixed lineage leukaemia (*MLL*) locus at 11q23 is associated with therapy related acute leukaemia (t-AML) and also with neonatal leukaemia. The translocation breakpoints lie within a break point cluster region that has a DNase I hypersensitive region and a cryptic promoter, suggesting that transcription may be involved in the mechanism of generation of *MLL* translocations. Based on this and various other lines of evidence including the link between topoisomerase II drugs and t-AML we proposed a model for generation of *MLL* translocations. In this model topoisomerase IIbeta (TOP2B) mediated DNA breaks induced during transcription are the causative DNA double stranded breaks. We further postulated that these breaks occurred within transcription factories where multiple genes are in close proximity, thus facilitating breaks on separate genes to become misrepaired. This model has testable predictions, the genes that become translocated such as *MLL* and AF9 should be transcribed within the same transcription factory some of the time, and more often than control genes that have not been reported to be translocated. In the absence of TOP2B there should be fewer breaks in the *MLL* locus in response to t-AML inducing agents such as etoposide and in the absence of TOP2B there should be fewer chromosome aberrations such as micronuclei in response to etoposide. We have recently published data supporting these testable predictions with etoposide. RNA FISH confirmed that transcription of *MLL* and its frequent translocation partners *AF9* and *AF4* do co localised more often than control genes. DNA FISH utilising a clinically used *MLL* break apart probe demonstrated that in the absence of TOP2B there were fewer breaks in the *MLL* locus, but genome wide DNA DSBs measured by gamma H2AX did not reduce in the absence of TOP2B. Etoposide induced chromosome aberrations assayed by counting micronuclei were reduced in the absence of TOP2B. Further work is required to determine if this TOP2B dependent mechanism applies to dietary components such as flavonoids, which have been suggested to be a cause of *MLL* translocations seen in neonatal leukaemia.

ACKNOWLEDGEMENTS

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O-36

Indolequinone derivatives inhibit TNFα-induced NF-κB activation in human leukemia *via* inhibition of NF-κB-DNA binding activity

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Indolequinone derivatives have been shown to be potent antitumor agents. So far, two mechanistic targets were identified, underlining their properties: NQO1 and thioredoxin reductase (TrxR). Nevertheless, there are still indications pointing out that there should be additional mechanisms implicated in order to elucidate their growth inhibitory effects on cancer cells. In the present study, we identified novel molecular targets responsible for their

final antitumor effect. As aberrant NF-κB activity is typically linked to high TNFα concentrations in patients with myelodysplastic syndromes and because this high level of TNFα is believed to be responsible for leukemic properties, we used this model to study potential effects of indolequinones on NF-κB in human leukemia cell lines. Excessive production of TNFα by the tumor itself and by the tumor microenvironment is well documented. Many cancer cells constitutively secrete TNFα, which appears to contribute directly to oncogene activation, DNA damage and tumor growth. We were interested to see how indolequinones interfere with the classical pathway of pro-inflammatory signaling through the TNFα-induced NF-κB transcriptional pathway. Our study for the first time revealed indolequinone derivatives as potent inhibitors of the TNFα-induced NF-κB pathway. However, involvement of indolequinones in the NF-κB pathway is not upstream as the TNFα-induced NF-κB cascade was successfully initiated. We provide the evidence indicating that indolequinones have to act further downstream, in the nucleus, affecting either binding of NF-κB proteins to DNA or by stimulation of transcriptional function of NF-κB. NF-κB inhibition potential of indolequinones may contribute to their total growth inhibitory effects on cancer cells. Moreover, preferential targeting cancer cells by indolequinones makes them promising safe drug component in cancer treatment.

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0-37

The mechanism of action of elisidepsin involves a direct hit on the plasma membrane

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Elisidepsin (PM02734, Irvalec®) is a synthetic compound whose structure is closely related to that of Kahalalide F isolated from the indopacific mollusk Elysia rufescens. Although it has entered clinical trials its mechanism of action is still debated. It has been observed that it induces nuclear fragmentation, damage of lysosomal membranes and necrotic cell death. On the molecular level the expression of ErbB proteins in general, and ErbB3 in particular, has been shown to correlate with elisidepsin sensitivity and knockdown of fatty acid 2-hydroxylase (FA2H) expression is known to give rise to elisidepsin resistance. We have used several cell lines whose ErbB1-3 expression level varied by several orders of magnitude and compared their sensitivity to elisidepsin. We found that the IC50 value was not significantly altered by ErbB protein expression. In addition, RNA interference-mediated inhibition of ErbB3 expression did not shift the IC50 either. Although these results convincingly show that ErbB proteins do not modify elisidepsin sensitivity, we observed that it did exert effects on the distribution and association of ErbB receptors. Fluorescence resonance energy transfer (FRET) experiments showed that the homoassociation of ErbB2 and ErbB3 was decreased by the drug. In addition, elisidepsin induced the redistribution of ErbB3, an exogenous (GPI-anchored GFP) and an endogenous (PLAP, placental alkaline phosphatase) raft-associated protein from the plasma membrane to intracellular vesicles. We applied fluorescent membrane probes to detect the alterations in the structure of the plasma membrane. Elisidepsin increased the fluidity (revealed by decreased fluorescence anisotropy) and decreased the hydration (revealed by elevated generalized polarization of Laurdan) of the plasma membrane within a minute of drug administration. These changes are characteristic of liquid-ordered (raft) domains. The above results suggest that the primary target of elisidepsin is the lipid membrane whose structure and composition is influenced by a multitude of factors. One of them is the partial oxygen pressure known to affect the efficiency of lipid hydroxylation. We found that hypoxic conditions significantly decreased the sensitivity of elisidepsin of cell lines which were medium-sensitive to the drug (IC50 ~ 10 mM) without influencing highly sensitive ones. Additionally, there was strong correlation between the sensitivity of cell lines and their fatty acid 2-hydroxilase expression confirming previous suggestions that the enzyme plays a crucial role in the mechanism of action of elisidepsin. Altogether our results suggest that the primary target of elisidepsin is the plasma membrane and that alterations in membrane composition may significantly alter the sensitivity of cells to the drug.

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0-38

Evaluation of effect on cell cycle, necrosis and antiproliferative activity *in vivo* of argentatin B

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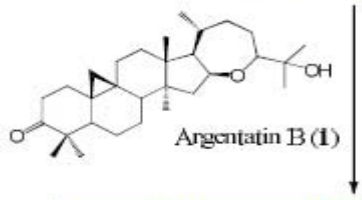
Previously we had demonstrated the antiproliferative activity (AA) of argentatin B (1) against several human cancer cell lines, however its action mechanism is not known. Now we wish to report the AA of 1 on RKO (colorectal) human cancer cell line and the plausible mechanism of 1 in this cell line. In addition the *in vivo* AA of 1 on nu/nu mice inoculated with PC-3 human cancer cell line was also evaluated. Treatment of the RKO cancer cells with different concentrations of 1 leads to a dose-dependent reduction in cell proliferation when evaluated by FDA assay. A biochemical marker of apoptosis is the caspase 3 activity however 1 at 15, 25 and 35 μM doses evaluated at 6, 12, 24, and 48 h did not increase the activity of this enzyme. It is known that cleavage of PARP is also a biochemical maker of apoptotic processes. Nevertheless the results showed that 1 at 15, 25 and 35 μM doses for 12, 24, 48, and 72 h did not induce cleavage of PARP. Another marker of apoptosis is the exposure of phosphatidylserine (PTS) at the cell surface. The cells treated with 1 at 35 mM doses during 48 h did not exposed PTS. Even more the results showed that 23% of total cell was positive to PI. The loss of membrane integrity could be evaluated by measuring the release of lactate dehydrogenase (LDH). Our results showed that 1 induced 17 and 23% the release of LDH at 24 and 48 h respectively. Conversely, we decided to analyze the cell cycle of RKO cells treated with different doses of 1. The results showed that at 24 and 48 h the proportion of cells in G1 phase increased in a dose-dependent manner, whereas the cells in S and G2/M phases decreased simultaneously. Additionally the DNA content and the percentage of cells in every phase of the cycle were evaluated.



Assay	TRV	Decrease of tumor growth %
Control	433.52	
125 mg/Kg (1)	289.44	33%
250 mg/Kg (1)	258.33	59%
500 mg/Kg (1)	386.56	11%
Cis-platin 40 mg/Kg	274.69	36%

The data showed that the percentage of cells in G2/M and S phase decreased significantly at the 15, 25 and 35 mM doses. Six-week-old athymic mice were utilized for the evaluation *in vivo* of the putative AA of 1. The animals were injected subcutaneously in the back with PC-3 (1×10^6) cells. After the installation of tumor, groups of five mice received weekly different doses of 1 dissolved in sesame oil. Our results showed that the animals treated with 250 mg/Kg dose/week of 1 for 3 weeks showed a slower growth, about 59%, of the tumor volume with respect to those inoculated with PC-3 cells but only received the vehicle. In short 1 induce cytotoxicity by necrosis cell death and G1phase cell cycle arrest and it showed AA *in vivo*. Although 1 showed a relative low activity as anti-proliferative compound there are some considerations that make 1 feasible to be studied for example it constitute 10% of the by-product obtained from the industrial process to acquire rubber from *P.argentatum* then 1 could be available in large quantities. Additionally we had showed that 1 is not genotoxic or cytotoxic to lymphocytes in proliferation.

ACKNOWLEDGEMENTS

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0-39

Development of a novel class of anti-tumor agents based on the ω-3-epoxide of eicosapentaenoic acid

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Epidemiological and experimental studies have found that ω-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) decrease tumorigenesis and metastasis, whereas evidence suggests that dietary ω-6 PUFAs may enhance tumor growth. In cells PUFAs undergo biotransformation to a range of potent eicosanoid mediators. Thus, cytochrome P450 enzymes oxidize ω-3 and ω-6 PUFA at each olefinic bond to produce a series of isomeric epoxides. Epoxides of the ω-6 PUFA arachidonic acid stimulate mitogenesis and the rate of tumor cell migration. In contrast, the 17,18-epoxide formed at the unique ω-3 bond in EPA, which is absent in ω-6 PUFA, decreased cell proliferation; the isomeric EPA epoxides formed at alternate olefinic bonds did not inhibit proliferation. We hypothesized that the ω-3-epoxy-EPA structure may be a scaffold for the development of novel anticancer drugs. Fully saturated analogues of ω-3-epoxy-EPA containing 20-22 carbon atoms were synthesised in a novel six-step procedure. Construction of the saturated alkyl chains was achieved using alkyl-alkyl Negishi cross coupling reactions catalysed by the recently developed N-heterocyclic carbene PEPPSI-iPr. Wittig reactions were used to generate the ω-3 olefinic bonds with excellent regio- and cis-selectivity. Subsequent epoxidation of the olefins with m-chloroperoxybenzoic acid afforded the desired cis-epoxides, whose structure and isomeric purity was confirmed by ^{1}H- and ^{13}C-NMR, GC-MS and elemental analysis. The C20-epoxide inhibited the proliferation of aggressive MDA-MB-231 breast cancer cells (MTT reduction; IC50 17 ± 7 μM after 48 h of treatment), while the C21 and C22 analogues were two and four-fold less potent. From flow cytometry the C20-epoxide promoted the accumulation of cycling cells in G1 phase in a time- and concentration-dependent manner, and also increased the proportion of cells in sub-G1 phase (to 123 ± 6% and 175 ± 24% of control after 24 and 48 h of treatment, respectively, with 10 μM C20-epoxide), suggestive of late apoptosis. Consistent with these findings, the C20-epoxide activated apoptosis, as evidenced by increased caspase-3 activity (EC50 21 ± 1 μM at 48 hr) and increased FITC-Annexin V staining. From immunoblotting, increases in the active form of caspase-3 and poly (ADP-ribose) polymerase cleavage as well as the proapoptotic Bax/Bcl-2 ratio were observed in C20-epoxide-treated cells. The C20-epoxide was also found to decrease the migration of MDA-MB-231 cells to 73±6% of control which tested at a concentration of 10 μM. Taken together, the C20-epoxide, a saturated analogue of the naturally occurring ω-3-epoxide of EPA, has emerged as the prototype of a novel class of anticancer agents that activate the intrinsic pathway of apoptosis and impair the migratory potential of MDA-MB-231 breast cancer cells.

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0-40

Improving taxane production in cell suspension cultures of *Corylus avellana l.*

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Paclitaxel (Taxol® BMS), an effective plant-derived antitumorous agent, is being successfully used to treat different cancer processes. The main natural source of Taxol is the inner bark of several *Taxus* species, but it accumulates at a very low concentration (about 0.02% of dry weight) and the cost of its extraction is extremely high. The growing demand for Taxol and the difficulty in increasing its production by genetic engineering has prompted a search for new sources of taxanes. It has recently been announced that Taxol and related taxanes can be extracted from the angiosperm *Corylus avellana L.*, with production taking place in shells, leaves and cell cultures of hazel plants, although at low levels. The main aim of the present work was to increase taxane production in *C.avellana* cell suspension cultures. The *C. avellana* cell suspension cultures growing in MS media with 2,4-dichlorophenoxyacetic acid (2,4-D) 1 mg/L and benzylaminopurine (BA) 0.5 mg/L were elicited with methyl jasmonate (100 μM) or coronatine (1 μM). Fresh and dry weights of the cells were measured to determine the growth capacity of cultures, and taxane production was analyzed by HPLC-DAD and HPLC-MS. The studied cell suspensions were found to produce taxanes in low quantities. The addition of the elicitors to the cell cultures increased production but reduced growth. With the aim of further enhancing taxane production, 64 different culture media combining 2 basal media (B5 and MS), 2 sources of sugar (sucrose and sucrose plus fructose), 2 types of auxins (2,4D and NAA at 2 concentrations), and 2 types of cytokinins (KIN and BAP, at 2 concentrations) were studied. We used a fractional factorial design to reduce the study to 32 media, and subsequent studies revealed the best combination for increasing cell growth and taxane production. The main advantage of using hazel cell cultures for taxane production is that hazel is widely available, has considerable capacity for growth and is easier to cultivate

in vitro than yew. After the addition of the elicitors methyl jasmonate or coronatine to the cultures, although growth was reduced, their capacity to produce taxanes was dramatically enhanced, especially in the case of coronatine. The highest total taxane production was found after 14 days of elicitation with coronatine, the total taxane levels being more than 14 times higher than in control conditions. After media optimization, we analyzed the taxane production in the optimal conditions. We can conclude that *C.avellana* cell cultures have potential as a commercial source of taxanes for use as therapeutic agents or new precursors for semi-synthetic production.

ACKNOWLEDGEMENTS

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O-41

Mitosis with damaged DNA (checkpoint adaptation) in cancer cells treated with clinically relevant concentrations of camptothecin or other genotoxic agents

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The majority of cancer treatments are based upon mechanisms that damage DNA, such as topoisomerase I and II inhibitors, DNA alkylating agents, and radiation. Cancer cells respond to DNA damage by activating the DNA damage checkpoint pathway. Human cancer cells can escape the DNA damage checkpoint and enter into mitosis even though they still have damaged DNA; a process known as checkpoint adaptation. The DNA damage checkpoint and checkpoint adaptation are controlled by protein kinases and phosphatases, which may be valuable pharmacological targets. We developed an experimental model of checkpoint adaptation in which we treat human colon cancer cells (HT-29) with clinically relevant concentrations of camptothecin (CPT). During 30 to 72 h post-treatment nearly 100% of the cells asynchronously displayed a rounded morphology, suggesting they had entered mitosis. Rounded cells were collected by mechanical shake-off and compared to adherent, interphase cells that still were under a DNA damage checkpoint. The rounded cells were positive for phospho-ser10 histone H3, cyclin B1, and had high Cdk1 activity. Importantly, they were also positive for histone gamma-H2AX and displayed comets in the alkaline comet assay. These data indicate that the cells had undergone checkpoint adaptation. Mitotic cells with damaged DNA are 100 x more sensitive than interphase cells to apoptosis inducers such as ABT263. Although the majority of cells that undergo checkpoint adaptation die, 1-2% survive with genomic changes such as an altered karyotype and micronuclei. We found similar results using HeLa, M059K and U2OS cells treated with CPT, cisplatin, etoposide or DNA alkylating agents. We found that Checkpoint kinase 1 (Chk1) protein levels are similar in cells before and after checkpoint adaptation. By contrast, Chk1 dephosphorylation on ser345 coincides with checkpoint adaptation. The proportion of affected cells and the timing of checkpoint adaptation can be altered with chemical inhibitors of key protein kinases such as Chir124 (Chk1), CR8 (Cdks), and BI2536 (Plk1). Currently, the most effective compounds to drive cells into mitosis after a genotoxic treatment are PP2A phosphatase inhibitors. Our data provide insight into the biochemical steps required for checkpoint adaptation, and provide a rational approach to evaluate and improve genotoxic therapeutic agents.

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O-42

Effects of archazolid on leukocytes

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Monocytic leukocytes are a major component of the innate immunity and protect the host against pathogenic microorganisms. In chronic inflammatory diseases and cancer, however, monocytes may promote the persistence of an inflammatory milieu and thus differentially contribute to all phases of the cancer process and to disease progression. Archazolid is a novel antitumor agent, firstly isolated from cultivated myxobacteria *Archangium gephyra.* It belongs to the class of v-ATPase inhibitors and potently suppresses cell growth of various mammalian cancer cell lines with IC50 values in the nanomolar and even subnanomolar range. In view of the growing link between inflammation and cancer, we here analysed the immunomodulatory effect of archazolid in human blood monocytes. Archazolid markedly reduced the amount of the pro-inflammatory cytokines TNFα, IL-8 and IL-6 in the milieu of monocytes stimulated with LPS for 4 (TNFα, IL-8) or 18 h (IL-6), with significant effects

starting at concentrations of 1 to 10 nM. Similar effects were observed for other v-ATPase inhibitors, i.e. bafilomycin and apicularen. Archazolid did not reduce monocyte viability within 24 h up to concentrations of 1 μM, indicating that the suppressive effects on cytokine release were not due to cytotoxicity. Interestingly, archazolid did not reduce mRNA transcription (e.g. for IL-8), instead caused a progressive intracellular accumulation of the translated cytokines, suggesting a perturbation of the classical mechanisms of protein transport and/or secretion. In fact, archazolid increased the intracellular vesicular staining for IL-8, as observed by immunofluorescence microscopy, though it caused only minor morphological changes at the level of the endoplasmic reticulum exit sites and Golgi. Interestingly, archazolid increased the release of IL-1β, which is not exported through the classical endoplasmic reticulum-to-Golgi export, and thus showing opposite effect of non-classical secretory pathways. In the light of these findings, we propose a key role for v-ATPase in the release of inflammatory cytokines and that the inhibitory effects of archazolid on this pathway may contribute to its antitumor potential, in particular for inflammation-triggered cancers. In this respect, archazolid may represent a novel type of natural anticancer agent combining direct anti-tumoral effects with immunomodulatory properties.

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0-43

Quantitative proteomics analyses to elucidate the mode of action of englerin a, a promising anti-renal cancer natural product

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Natural products offer tantalisingly specific biological effects, but how they work is often difficult to understand. Their complex chemical structures are difficult to recreate for medical purposes. Englerin A, an extract from the stem bark of Tanzanian small tree, *Phyllanthus engleri*, is a small molecule with remarkable activity against renal cancer cells and is now accessible to total synthesis. However, biological mode of action adopted by englerin class of compounds remains unknown. One route to obtain such information is to recognize upstream and downstream regulatory effects exerted by the drug through protein expression profiling followed by thorough bioinformatics analysis of regulated protein clusters. Here we used advanced mass spectrometry-based SILAC (stable isotope labelling amino acids in cell culture) approach for system-wide protein expression profiling and bioinformatics analysis of such data to capture englerin A-affected pathways. We identified ~230 regulated proteins from over 4,000 quantified proteins in response to englerin A treatment in renal cancer cell lines. Gene ontology analysis of those regulated proteins indicated that 'RNA processing' proteins were enriched in the up-regulated cluster whereas 'cellular component organization' proteins in the down-regulated cluster. In-depth pathway analysis showed enrichment of 'DNA-damage response' proteins in the up-regulated cluster whereas 'tissue remodelling and wound healing' proteins in the down-regulated cluster. Moreover, to investigate whether englerin A affects phosphorylation that plays vital roles in signalling pathways in cancer, we carried out SILAC-based phosphoproteomics analysis for englerin A-treated cells. The preliminary analysis of over 6000 quantified phosphorylation sites revealed more down-regulated phosphorylation sites suggesting that englerin A may have either selected kinase inhibitory or phosphatase activating action. Motif analysis showed that the respective kinases for those sites are involved in cell cycle and apoptosis. Altogether our data demonstrate that englerin A targets biological pathways in cancer, perhaps through protein phosphorylation signalling. Further analysis, which is in progress, may reveal protein targets of englerin A as well as its biological mode of actions that would lead to the evaluation of therapeutic values of this promising anti-renal cancer natural product.

ACKNOWLEDGEMENTS

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0-44

Fungal anticancer drugs from the sea: new sesquiterpenoids from a marine-derived strain of *Penicillium* nov. sp. for osteosarcoma treatment

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Osteosarcoma is a relatively uncommon cancer which principally affects children, adolescents and young adults and for which no satisfactory treatment is currently available. As marine environment represents a rich reservoir of original bioactive compounds it could be an

interesting source to search for new anti-osteosarcoma drugs. Actually, five marine molecules have been approved as anticancer drugs and sixteen others are currently in clinical trials. Among marine organisms, fungi of the genus *Penicillium* are known to produce a wide range of bioactive metabolites including around 300 cytotoxic compounds already described. Herein we present a study on secondary metabolites active against osteosarcoma and produced by a marine-derived strain of *Penicillium*, belonging to a new species, and isolated from a natural sample of seawater gathered on the Loire estuary. Bioguided fractionation of a crude extract led to the isolation of a new sesquiterpenoid compound, ligerin, related to fumagillin. Fumagillin and its derivatives are a family of antiangiogenic MetAP2 inhibitors with a potential therapeutic use. Among them, TNP-470 was the first one to enter phase I and phase II clinical trials for the treatment of solid cancers. Its clinical development had to be stopped due to its toxicity, leading to the search for new candidates. Bioactivity of ligerin on osteosarcoma cells was evaluated using flow cytometry and time-lapse analyses highlighting a significant decrease of the cell proliferation speed and an effect on cell mobility. Antiproliferative activity against osteosarcoma cell lines was comparable to that of doxorubicin, currently used in treatment of osteosarcoma but was more specific, with a weaker activity on non-tumor cell lines. In an *in vivo* osteosarcoma murine model, ligerin did not induce noticeable toxicity and showed an antitumoral effect. At the same daily subcutaneous dose than TNP-470, 60% of the ligerin-treated mice exhibited a tumor volume equivalent to that of mice treated with TNP-470, but contrary to these last ones, they did not exhibited any significant loss of weight. Bioactivity of semisynthetic ligerin analogs will also be presented. Further investigations of the metabolome of the studied fungal strain in order to search for new ligerin derivatives are currently on-going. This work confirms that marine-derived strains of *Penicillium* are a promising source of new anticancer compounds.

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O-45

Effects of classical and experimental taxanes in cell line models

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Background and Aims: Classical taxanes (Paclitaxel and Docetaxel) have been successfully used as single agents or in combination in chemotherapy of breast, ovarian, head and neck, lung, and prostate carcinomas. The response rate is generally 30 to 50%. Taxanes strongly bind to β-tubulin and promote polymerization of microtubuli causing formation of irregular structures such as asters and irregular bundles, block progression through the M phase and causes cell death. *E*nhanced metabolism e.g. by cytochromes P450, efflux by ABC transporters or variability of β-tubulins may cause failure of therapy by taxanes. The aim of our study is to identify factors and mechanisms causing taxane-resistance. Classical taxane-sensitive (MDA-MB-435) and resistant (NCI/ADR-RES) cell line models were employed to study differences in mechanism of action of paclitaxel *vs.* experimental Stony Brook Taxanes (SBT), e.g. SBT-1216. Cytotoxicity, cell cycle analysis, apoptosis, transport of taxanes and their metabolism were followed in these models by standard techniques of cell biology. We have shown that the observed 1000-fold lower sensitivity to paclitaxel in NCI/ADR-RES cells compared with MDA-MB-435 cells corresponded to P-glycoprotein (ABCB1) overexpression. Correspondingly, the paclitaxel uptake was up to 20-fold lower in the resistant NCI/ADR-RES cells than in the sensitive MDA-MB-435 model. In comparison with paclitaxel the uptake of SBTs was 1.2- to 3.8-times lower in the MDA-MB-435 cells and 1.5- to 6.5-times higher in NCI/ADR-RES cells. Flow cytometric analysis, after propidium iodide staining, showed that all SBTs caused G2M block of the cell cycle similar to paclitaxel, but at concentrations by order of magnitude lower in resistant models. Both SBT-1216 and paclitaxel activated caspase-3, caspase-9, caspase-2 and caspase-8 in sensitive as well as resistant cells. In contrast to human liver microsomes, both cell lines showed negligible metabolism of SBT-taxanes. In contrast with paclitaxel, the sensitivity of MDA-MB-435 cells to SBTs was comparable to that of NCI/ADR-RES cells. Virtually absent taxane metabolism in cell models did not explain the observed differences in their individual efficiency and higher cytotoxic effects than paclitaxel. Furthermore, our study showed that experimental SBTs efficiently kill the resistant tumor and sensitive cell lines by mechanism dependent on effect on G2M arrest and/or apoptosis. Structural modifications of SBTs resulting in their decreased transport by P-glycoprotein most probably caused their higher efficiency than paclitaxel in multidrug resistant NCI/ADR-RES tumor cells. SBTs seem to be promising experimental drugs for classical taxane-resistant tumors.

ACKNOWLEDGEMENTS

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0-46

Synthesis around pretubulysin – a promising anticancer compound

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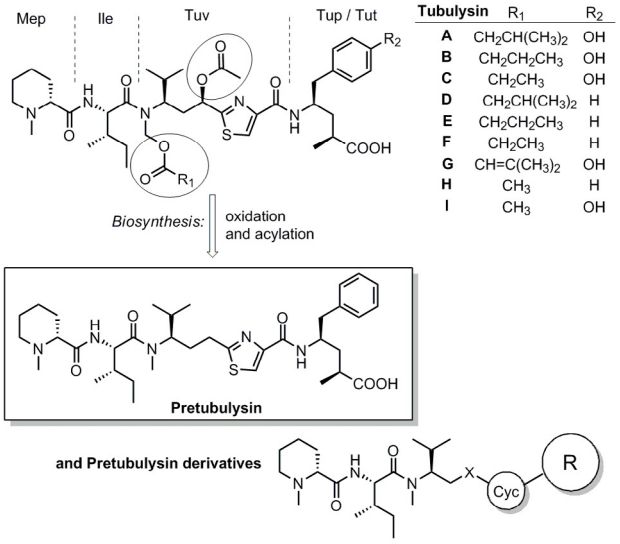
The Tubulysins are a family of natural products found in myxobacteria (e.g. *Angiococcus disciformis* An d48 and *Archangium gepyhra* Ar315) with exceptional high antimitotic effect on cancer cells in picomolar range by interfering with microtubule dynamics. Structurally, they are tetrapeptides consisting of natural L-isoleucine (Ile) and three unnatural amino acids, D-*N*-methylpipecolic acid (Mep), tubuvaline (Tuv) and tubuphenylalanine (Tup) or tubutyrosine (Tut) respectively.



A polyketide synthase – nonribosomal polypeptide synthetase (PKS/NRPS) multienzyme 'assembly line' is responsible for their biosynthesis. For our synthetic studies we chose Pretubulysin, a late-stage biosynthetic precursor of Tubulysins, which is missing functionalities like the acid and base labile *N,O*-acetal and the configurationally labile acetoxy group in the middle part. Therefore Pretubulysin is synthetically easier accessible while sustaining high activity. We were able to synthesize several derivatives of Pretubulysin with variations in the middle Tuv-part and/or the *C*-terminal Tup, e.g. click chemistry allowed us to synthesize a small library of triazole-Pretubulysins. With our 'Synthesis around Pretubulysin' we were able to gain deeper insights in the biosynthetic pathway and to provide sufficient material for biological evaluations.

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0-47

The antitumoral cosmomycin D, elucidation of the biosynthetic cluster and inference of biological actions

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The biosynthetic gene cluster of the cosmomycin D, an antitumural anthracycline has been elucidated by mining the genome of the producer strain *Streptomyces olindensis.* 14 ORF's where previously described and the remind boundaries genes where predicted by AntiSMASH, FGENESH and manual means; where predicted proteins where analyzed for functionality by Blast and Pfam search. The cluster is one of the largest between anthracyclines producers with 41 ORF's, and 43129 nucleotides. The average size of the ORF's is 1051 nucleotides, being the ORF 17 the bigger with 3574 nucleotides, corresponding to a protein of 761 a.a., a putative Uvr-like protein involved in resistance to cosmomycin D, homologous to that of *drr*C gene. The smaller ORF is number 53 with a size of 223 nucleotides, codifying for a protein of 84 a.a., corresponding to a minimal acyl carrier ketosynthase. The main genetic functions found on the cluster allow classify the genes and predict hypothetical funtions: genes involved in the aglicone synthesis (16), regulators (2), glycosiltransferase (3), sugars (9), resistance (3), and unknown functions (7) like *cos*Y that doesn't appear to have an apparent function. Cosmomycin D is biosynthesized by a PKS type II fashion way where a complex of single genes called minimal PKS (*cos*E, *cos*C and *cos*B) are responsible for the early building of the polyketide backbackbone starting with the condensation of a propionate-CoA and nine acetates coming from malonil-CoA. Finally, the whole biosynthesis process ends on a linear tetracyclic polyketide backbone of the type 7,8,9,10-tetrahydro-5,12-naphtacenoquinone, with two trissacharide chains formed by L-rhodinose, 2-deoxy-L-fucose and L-rhodosamine, attached at C-7 and C-10 by the glycosyltransferases. The biological activity of cosmomycin D was evaluated against acute myeloid leukemia (AML) cell line HL-60, killing the cells, in low dose-dependent manner when compared with doxorubicin. Cosmomycin D proved also to be more toxic in an equimolar basis against Juket cells than doxorubicin. Cosmomycin D triggers the intrinsic apoptotic pathway, and also its potent antitumor activity has been associated with its ability to modulate BCL-2 protein family members.

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0-48

Effect of benzo[c]phenanthridine alkaloids on melanoma cells

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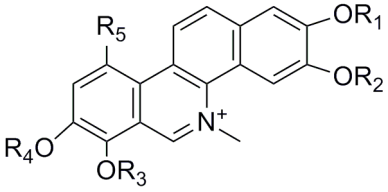
Over the last decades, cutaneous melanoma has shown increasing incidence rates in Caucasian populations. Since it is notoriously resistant to conventional chemotherapy, seeking for new drugs affecting melanoma growth is important. Benzo[c]phenanthridine alkaloids (BAs) are natural compounds that represent a small group of isochinoline alkaloids occurring in many plant species. BAs are under intensive investigation not only for their antiproliferative but also colour properties. We have compared the effects of five BAs – sanguinarine (SA), chelerythrine (CHE), chelidonine (CHLD), sanguilutine (SL), chelilutine (CHL) on human malignant melanoma cell lines with functional and non-functional p53 protein. All tested alkaloids exhibit strong anti-proliferative activity. CHL, CHE and SA induced apoptosis, which was probably mediated by decreasing levels of anti-apoptotic proteins (Bcl-xL, Mcl-1, XIAP) and was accompanied by mitochondrial membrane potential decrease as well as caspase-3 and PARP cleavage. In addition, the toxicity of SA, CHE, CHLD and CHL was significantly reduced by z-VAD-fmk (pan-caspase inhibitor) preincubation, while SL toxicity was not. Although all alkaloids caused DNA damage, which was demonstrated by induction of H2AX phosphorylation, none of the tested alkaloids stabilized p53 and their toxicity in cells with non-functional p53 was comparable to wild type cells. Because SL induced another, caspase independent, type of the cell death we studied SL effects in more details. During the first hour of SL treatment, we have observed vacuolization of cytoplasm indicating autophagy activation; lately necroptotic cell death was detected. Autophagy was confirmed by immunofluorescence detection of dotted GFP-LC3 protein distribution. Addition of autophagy inhibitors (3-methyladenine, Bafilomycin-A1 and LY294002) to A-375 cells decreased the cell viability upon SL treatment indicating that autophagy promotes survival of SL-treated cells. Oppositely, necrostatine, specific inhibitor of necroptosis, completely reversed antiproliferative effect of SL.



	R_1	R_2	R_3	R_4	R_5
sanguinarine	$-CH_2-$		$-CH_2-$		-H
chelerythrine	$-CH_2-$		$-CH_3$	$-CH_3$	-H
sanguilutine	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-OCH_3$
chelilutine	$-CH_2-$		$-CH_3$	$-CH_3$	$-OCH_3$

Our results indicate that BAs could effectively treat tumours, which have lost wild type p53, including malignant melanoma. These results show that the individual BAs induce various types of the cell death, including apoptosis, autophagy and necroptosis. Only SL kills tumour cells by induction of caspase independent cell death, necroptosis, which is accompanied by autophagy. Under SL treatment autophagy served as cell rescue mechanism, while necroptosis as cell death mechanism. Either both these processes could be activated simultaneously or necroptosis follows autophagy. The ability to induce caspase independent cell death rank SL to compounds with potential for the treatment of apoptosis resistant tumour cells.

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0-49

Synthesis, structure elucidation and biology of palmerolide c and chamuvarinin

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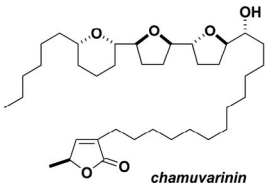
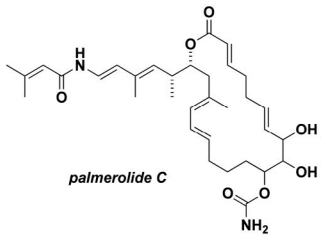
Nature provides an armada of structurally diverse secondary metabolites with unique and often unexplored biological modes of action. Combined with their molecular architectures natural products continue to provide the inspiration to develop practical synthetic routes and methods not only to aid further biological evaluation, but to provide confirmation of their complete structure and as platforms for the development of lead compounds in the quest for new generation pharmaceuticals. This talk will discuss our recent endeavours in relation to the synthesis and structure elucidation of the marine macrolide palmerolide C, which displays activity towards human melanoma, and the synthesis and biological evaluation of

chamuvarinin, a unique acetogenin found in plant extracts used in traditional West African medicine.





0-50

Flavonoids contents, antioxidant and anticancer activities of 72 species of ferns from China

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Ferns are well-known traditional Chinese medicinal herbs and extensively used to treat skin tumefaction, to protect the liver and to treat hepatitis, being also used as antipyretics. Large numbers of ferns such as Dryopteris erythrosora, Cyrtomium fortune, and Coniogramme aponica, occur in China. The major identified constituents in fern plants are flavonoids, alkaloids, and terpenoids. Among them, flavonoids in ferns have attracted great interest. The flavonoids contents, DPPH free radical scavenging activities, FRAP and anticancer effects on carcinomic human alveolar basal epithelial cell line A549 of 72 ferns from China were investigated. The dry ferns material was extracted with 50% ethanol for 5 h at room temperature for twice. The total flavonoid contents in ferns extracts were ranged from 0.094% to 12.15%. *Woodwardia magnifica* Ching et P. S. Chiu appeared highest total flavonoid contents. The total flavonoid contents of 14 species of ferns were beyond 5.0%. The antioxidant activities of these ferns extracts showed a significant reciprocal proportion to the total flavonoids contents. Moreover, the ferns with higher contents of total flavonoid showed more obvious inhibition effects against A549 cell line. *Selaginella frondosa* Warb exhibited strongest inhibition against A549 cell line. Flavonoids are of great interest for their bioactivities, which are basically related to their anti-oxidative properties. The relationship between the antioxidant potential and anticancer activity of these ferns were found. We further used the HPLC-MS (ESI) and HPLC-UV to identify the flavonoids in these ferns.

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0-51

Green tea extract EGCG induces cell death in chronic lymphocytic leukemia cells through the regulation of PI3K and proteasome activity

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Chronic lymphocytic leukemia (CLL) represents the most common leukemia of adults in the western hemisphere. The disease is characterized by a clonal expansion of leukemic B cells which typically co-express CD19 and CD5. A major advance in understanding the biology of CLL has been achieved in the recent years. However, the disease remains incurable and relapse often occurred after treatment with the current treatment with immunochemotherapy. Therefore, new therapeutic modalities are urgently needed. There is accumulating evidence that green tea extract EGCG [(-)-epigallo-3-catechin-gallate] may exert a preventive effect or a direct anti-tumor activity in several tumor types and clinical trials with EGCG are already ongoing. Therefore, the aim of this study was to evaluate the effect of EGCG on the viability of CLL cells and understand the possible molecular mechanism of action of this compound. Peripheral blood mononuclear cells (PBMC) of 12 CLL patients (60-98% CD19+/CD5+) were exposed to a wide range of concentrations of EGCG (0,1 – 200μM) and cell viability was evaluated by cell titre blue assays after 1, 2 and 3 days. The results demonstrated a dose and time dependent decrease in the cell viability after the exposure to EGCG with an IC50 ranging between 50-80μM. A moderate variation in the response to EGCG was observed between patients demonstrating the heterogeneity of the disease. FACS analysis using annexin V / propidium iodide (Anx/PI) staining showed that EGCG increased the percentage of early apoptotic cells (Anx+/PI-) and late apoptotic/necrotic cells (Anx+/PI+). Using RT-PCR, we observed a downregulation in the mRNA expression of the catalytic domain p110a and the regulatory domain p85 of phosphoinositide 3-kinases (PI3K) as well as Bcl-2 and Mcl-1 at high concentrations of EGCG. Western blot analysis demonstrated a decrease in the phosphorylation (i.e. inactivation) of Akt at

pThr308 residue as well as de-phosphorylation (i.e. activation) of the tumor suppressor PTEN at pSer380 residue. In parallel, an induction of PARP cleavage was observed. Furthermore, proteasome assays showed that EGCG effectively inhibits the chymotrypsin-like activity within 4 h of incubation in parallel to induction of early apoptosis. In conclusion, these data demonstrate that EGCG induces cell death in leukemic CLL cells through a mechanism which may involve the inactivation of PI3K/Akt signaling cascade and inhibition of proteasome activity. The results also point to a potential therapeutic effect of EGCG in CLL which warrants further evaluation.

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There is no conflict of interests.

0-52

Marine natural and semisynthetic fumagillin derivatives as anti-osteosarcoma agents

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In a previous study, bioguided fractionation of an extract of a marine-derived *Penicillium* strain belonging to a new species led to the isolation of ligerin. This chlorinated sesquiterpene derivative of fumagillin exhibited a specific antiproliferative activity against osteosarcoma cell lines and *in vivo* antitumor activity in a murine model. In order to select the best therapeutic candidate in the chemical series of these sesquiterpenoids, two strategies were followed: isolation of naturally occurring analogs or semisynthesis of ligerin derivatives. Natural structural analogs were searched by LC-HRMS/MS investigation of the metabolic fingerprints of four morphologically different strains belonging to the same new *Penicillium* species cultivated on various media. Using HRMS/MS modelization of the sesquiterpene core, some original metabolites could be identified in bioactive crude extracts, allowing to envisage their targeted purification. Semisyntheses of derivatives were also performed by substitutions conducted on the side chains of ligerin, i.e. the halogen atom and/or the acidic moiety. Bioactivity of these compounds was evaluated by *in vitro* assays on murine and human osteosarcoma cell lines.

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0-53

In vitro assessment of gap junctional intercellular communication (GJIC) as a tool for identification and characterization of chemopreventive activity of phytochemicals

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Gap junctional intercellular communication (GJIC) is critical for sustaining tissue homeostasis by maintaining a proper balance between cell proliferation, apoptosis and differentiation. Alterations of GJIC have been associated with different pathologies including cancer. While tumor promoters and oncogenes inhibit GJIC, tumor suppressor genes and several chemopreventive chemicals were demonstrated to upregulate or restore GJIC. To demonstrate that assessment of GJIC is a valuable *in vitro*-biomarker for identifying the chemopreventive effects of phytochemicals; we evaluated the efficacy of selected cancer chemopreventive phytochemicals (resveratrol, quercetin, curcumin and silibinin) in preventing the inhibition of GJIC induced by various environmental toxicants including known tumor promoters: 12-O-tetradecanoylphorbol-13-acetate (TPA), lindane, fluoranthene, perfluorooctanoic acid (PFOA), and pentachlorophenol (PCP). WB-F344 rat liver epithelial cells were pretreated with a phytochemical for 30 min and then exposed to an inhibitor of GJIC for 15 min. GJIC was measured using a scrape loading/dye transfer assay. All the selected phytochemicals were able to prevent inhibition of GJIC from at least one of the tested toxicants/tumor promoters. The cells pretreated with resveratrol maintained functional GJIC after exposure to TPA, lindane, fluoranthene and PFOA, whereas quercetin prevented GJIC inhibition induced by TPA, lindane, PFOA and PCP. Curcumin and silibinin blocked GJIC inhibitory effects of lindane. Thus, the protective effects of phytochemicals on GJIC in the employed experimental model do not seem to be general, but rather GJIC-inhibitor specific. The specificity of the observed protective effects could be related to different modes of action of individual phytochemicals as well as to different mechanisms of GJIC-inhibition of the tested toxicants/

tumor promoters. TPA and lindane were shown to inhibit GJIC through a MEK1/2-dependent mechanism, fluoranthene through a phosphatidylcholine-specific phospholipase C (PC-PLC)-dependent mechanism, PFOA through both MEK1/2- and PC-PLC-dependent mechanism, and PCP through MEK1/2- and PC-PLC-independent mechanism. Our study indicates that GJIC may be a common cellular target affected by various phytochemicals with cancer chemopreventive activity, but these chemopreventive effects act through different molecular and biochemical mechanisms.

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0-54

Chemosensitizing effects of natural substances in docetaxel resistant prostate cancer cell (PC3-TxR)-relation to Pgp inhibition

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Docetaxel (Dtx) is a standard chemotherapeutic agent for the treatment of androgen independent prostate cancer. However, development of resistance to Dtx by prostate cancer cells invariably occurs with continuous therapy. Thus identifying agents with chemosensitizing effect to overcome chemoresistance to Dtx could be an important therapeutic development. One well known mechanism of resistance by the cancer cells is over-expression of Pgp, an efflux transporter that pumps intracellular docetaxel to extra-cellular site. Identifying Pgp inhibition may be a potential useful probe for identifying chemosensitizing agents since Pgp inhibition can be rapidly determined using a fluorescent detection assay. Previously we have observed that an herbal extract, *Tripterygium wilfordii* (TW) can possess both strong chemosensitizing and Pgp inhibition (PgpI) effects, in a dose dependent manner. Thus we investigated the potential of Pgp inhibition as predictor for chemosensitizing effect (CE) of a given substance. The PgpI and CE were determined for 6 natural substances (Tripterygium wilfordii ethanol extract and its 5 major chemical components) and 2 well known Pgp inhibitors, PSC833 and cyclosporine A (CsA) at concentrations that are considered safe (i.e. about 50% IC50 which was determined separately).Pgp inhibition was measured by flow cytometry using accumulation of the fluorescent Pgp substrate (daunorubicin 5 μM) in Pgp over expressing K562/DOX leukemia cells. The cells were grown in RPMI 1640 medium with 10% FBS. Aliquots of cell suspension were incubated, in the presence or absence of the natural substances or Pgp inhibitor together with daunorubicin for 60 min at 37°C. A known Pgp inhibitor, PSC 833 (10 μM) was served as positive control (100% inhibition). Chemosensitizing effects from the natural substances or Pgp inhibitors were determined using proliferation inhibition assay with Sulforhadamine B (SRB)in PC3-TxR cells (resistant to Dtx). After seeding in 96-well plates and incubated for 24 h at 37°C in a humidified, 5% CO2 atmosphere, 7 Dtx concentrations with or without combination of natural substance or Pgp inhibitor (in triplicate wells per concentration) were incubated for 72 h at 37°C. After the cells were fixed, washed and air dried, the cells were then stained with SRB. The protein bound SRB was resolubilized in 10mMTris-HCl (pH 8.0) and optical density read at 565 nm with control cells (without Dtx) set at 1. Dose–response curves for% inhibition was plotted using GraphPad Prism 5 software. The IC50 of Dtx or drug (D), and its combination with the natural substance or Pgp inhibitor (IC50D, and IC50CD respectively) were obtained. CE was defined as IC50D/IC50CD. At equivalent PgpI of about 14%, CE values for TW, CP6, PSC838 and CsA were 3.3, 3.7, 7.5 and 2.9 respectively. Based on the present study, PgpI does not appear to predict CE, which varies considerably with different substances at equivalent safe concentrations. The differences in these CE values from the individual substances may reflect different mechanisms which could be important consideration for in vivo efficacy.

0-55

Plant cell factories: industrial revolution or green revolution?

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Plants have traditionally been a major source of medicines. Currently still about a quarter of all prescription medicines used in the developed world are derived from plants; if we only consider c the anticancer compounds, over 60% are either directly extracted from plants, or are analogues or derivatives of plant extracts (e.g. Taxanes, Epipodophyllotoxines, Combretastatins, Vinca alkaloids, Camtothecins). In an effort to become less dependent of seasonal variation, or weather conditions, the last two decades of the twentieth century saw an increasing interest in plant cell and tissue cultures. The general idea was to get plant cell suspension cultures to grow in fermenters, similar to those that were used in the pharmaceutical industry for the microbial production of antibiotics, special enzymes, or of recombinant proteins. Conditions in a fermenter could be tightly controlled, and production

of plant derived medicines could continue all year round. Although some success stories were reported, a general drawback of seemed to be that the ability to accumulate secondary products diminished in undifferentiated cell cultures. Apparently, some form of tissue specialization is required for efficient production of plant secondary metabolites. Hairy root cultures addressed this problem; these were plant tissue cultures that could be grown in vitro under tightly controlled conditions. However, hairy roots cultures required development of novel types of fermenters since the classical stirred tank fermenters would grind up the roots. A number of hairy root fermenter systems have been reported working at sizes up to pilot-plant scale. However, at present there are no examples of commercial production of plant derived medicines by hairy root cultures. Interestingly, several examples have been reported in which human recombinant proteins were produced via hairy root fermentation. Since hairy roots grow in a contained system, and do not have the ability to pollinate, these GM-plants give little cause for environmental concern. In a parallel development, there has been a growing interest in the development of non-food (industrial) crops. GM plants producing pharmaceuticals were one of the areas expected to have great commercial potential. Production of biomass in the field is much cheaper than production in bioreactors. However, recent field trials with GM-crops have met with considerable resistance by environmentalists. When considering the production of anticancer compounds, it is important to keep an eye on developments that may go on beyond the field of biotechnological production. Political and societal changes may hamper further development of biotechnological solutions. On the other hand, improvements in agronomy may make classical breeding of medicinal crop plants an increasingly more attractive option for drug production.

O-56

Myxobacteria; a source of unusual antitumor agents

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Though for many years, the myxobacteria were thought of as a "strange type of microbe", only of interest to a small group of microbial physiologists, due to the pioneering work of Reichenbach and Hoefle, came the recognition that these bacteria contained a cornucopia of gene clusters that synthesize a large number of potent secondary metabolites. This presentation will cover some of the earlier work on the epothilones and the successes, both direct and indirect, from these earlier discoveries as potential antitumor agents and then will continue with a discussion of the future for molecules and genes from these organisms.

O-57

Target discovery malaysia: sampling and bioassaying of bioactive compounds

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Target Discovery Malaysia is a novel joint venture between the state of Perak, Malaysia and private investors involved in the biotagging, sampling, bioassay-guided fractionation and identification of natural products from the Malayan rainforest and marine environments. Sampling will be followed by identification and reference sample deposition prior to extraction. Plant and fungal sources will have an initial priority but also animal toxins will be tested. Initial bioassaying will focus on anticancer and antibacterial properties, the anti-cancer priority is obvious, as 50% of all anti-cancer drugs approved by regulatory authorities in the EU and USA until 2004 are natural products or derivatives of natural products. Natural products as that they can be viewed as a population of "privileged structures" selected by molecular evolution to interact with a wide variety of biological targets. The number of unique protein architectures ("folds") is much smaller than the number of protein families predicted by sequence similarity, and the receptive binding space is focused into clusters of "superfolds. Natural products, by virtue this of molecular co-evolution with cellular constituents and as messenger molecules between organisms and eth environment, have been selected to preferentially bind to these "superfolds" and thus provide validated starting points as candidate drugs. The presentation will discuss the background and issues relating to the bioprospecting of the of the rainforest environment as a source of novel anti-cancer drugs in general as well as the unique approach selected by *Target Discovery Malaysia* to investigate the potential of the 130 Milllion-year old Malayan rain forest as a source for novel anti-cancer lead structures.

O-58

Nrf2 and HO-1 as the targets for chemoprevention and anti-cancer therapies

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Nuclear factor erythroid-2 related factor 2 (Nrf2) is a master of cellular response against oxidative and electrophilic stress. Among numerous genes activated by Nrf2 there is heme oxygenase-1 (HO-1) an enzyme degrading heme to carbon monoxide, iron and bliverdin. Besides removal of

prooxidant heme the HO-1 is playing, however, an important role as the mediator of cells differentiation, proliferation and survival. It regulates tumor growth by affecting tumor initiation, growth and metastasis as well as the angiogenesis which promotes tumor development. Our recent studies have elucidated that Nrf2 and HO-1 can affect the growth of skin carcinoma, melanoma, rhabdomyosarcoma and lung cancer. In this talk the current results on the cross-talks between Nrf2, HO-1 and microRNAs in progression of lung cancer will be particularly discussed.

O-59

Tumour vascular damaging effects of tubulin-binding combretastatins

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The tumour vasculature is an attractive target for therapy because the provision of oxygen and nutrients by a single vessel supports the survival of many tumour cells, as well as providing a major route for metastatic spread. This presentation will describe the development of tubulin binding combretastatins as tumour vascular disrupting agents (VDAs), highlighting mechanisms of action and resistance. 17 combretastatins were isolated from the South African bush willow, combretum caffrum, by Professor Bob Pettit of Arizona State University. Their chemical structure is similar to the classical tubulin-binding agent, colchicine. CA1 and CA4 were synthesized and found to be moderately active against a variety of tumour cells but their main anti-tumour activity resided in their ability to disrupt tumour blood vessels in vivo, causing a rapid and extensive shut-down of blood flow in the majority of solid tumour types tested pre-clinically. Both compounds are less toxic than colchicine because of more favourable binding kinetics. More soluble phosphorylated prodrugs of CA4 and, later, CA1 (CA4P and CA1P) were synthesized for pre-clinical use. CA4P and CA1P are being developed by OxiGene as fosbretabulin and Oxi4503 respectively. Both are rapidly de-phosphorylated in vivo to the active forms, binding beta-tubulin and resulting in disruption of microtubules. For CA4P at least, this disruption activates RhoA-ROCK signaling to cause phosphorylation of myosin light chain (MLC) and thus re-modelling of the actin cytoskeleton of endothelial cells, which is associated with an increase in permeability of endothelial cell monolayers, translating into an increase in permeability of tumour blood vessels to macromolecules in vivo. Blood flow shut-down occurs selectively in tumour blood vessels, which is most likely associated with abnormal features of the tumour microcirculation such as high vascular permeability and interstitial fluid pressure, heterogeneous blood flow and fragility of the blood vessel wall. Extended blood flow shut-down results in extensive tumour cell necrosis. However, innate resistance is observed in some tumour types, as well as in the peripheral rim of even the most sensitive tumours. Acquired resistance also arises from hypoxia and hypo-glycaemia induced by vascular shut-down and the influx of immune cells such as macrophages. Thus, as with other vascular-targeted strategies, the main potential of VDAs for clinical use resides in their combination with conventional treatments. The primary toxicity is, unsurprisingly, cardiovascular but with a different profile from that associated with anti-angiogenic agents such as bevacizumab (Avastin). A better understanding of predictive factors and resistance mechanisms should help to identify suitable patients for VDA treatment and optimize combination treatments. Several combretastatin analogues have been developed, including the Sanofi compound, ombrabulin, which is now the most advanced tubulin-binding VDA in development. Currently, ombrabulin is in Phase III study in sarcoma with cisplatin and Phase II study in ovarian cancer with carboplatin and paclitaxel, as well as in Phase II study for non-small cell lung cancer (NSCLC) with taxane and carboplatin. CA4P (fosbretabulin) is in Phase II study in ovarian cancer with bevacizumab, with a Phase III study in anaplastic thyroid cancer planned. Various other combretastatin-like compounds are at earlier stages of clinical development.

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O-60

Antimetastatic action of archazolid

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Growing evidence suggests that in metastatic cancer cells the vacuolar (H^+)-ATPase plays a major role in cancer progression and metastasis. The V-ATPase is a member of ATP-dependent proton pumps supposingly overexpressed

in highly invasive cancer cells. It is involved in cellular processes like trafficking and endocytosis. Reported is a potential inhibitory effect of V-ATPase inihibition on tumor cell migration. However, a precise signaling pathway remains still undefined. Here, we show that pharmacological or genetic interference with V-ATPase significantly reduces migration of invasive tumor cells *in vitro*. Importantly, the V-ATPase inhibitor Archazolid markedly abrogates tumor dissemination in a syngeneic mouse 4T1 breast tumor metastasis model. More precisely, Archazolid treatment impairs directional motility resulting in defects on two master players in cell migration, the epidermal growth factor receptor (EGFR) and the Rho-GTPase Rac1. The spatially-restricted localization of the EGFR to the leading edge is disturbed by Archazolid treatment. Furthermore, Archazolid treatment or silencing of V-ATPase abrogated also the activation of Rac1, as well as Rac1-dependent dorsal and peripheral ruffles by inhibiting Rab5-mediated endocytotic/exocytotic trafficking of Rac1. Taken together, our results show that Archazolid effectively decreases metastatic dissemination of breast tumors by impairing the trafficking and spatially-restricted activation of EGFR and Rho-GTPase Rac1, which are pivotal for directed movement of cells. Thus, our data discloses unexpected mechanisms of V-ATPase action in tumor dissemination.

O-61

Narciclasine as a novel potential weapon to combat brain cancers

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Narciclasine is a plant growth regulator whose anti-tumor effects have been known for millennia from folk medicine extracts of *Narcissus* bulbs. It displays potent *in vitro* anti-tumor activity below 100 nM in the NCI 60 cancer cell line panel. It is far less toxic to normal than to cancer cells. The compound is pro-apoptotic at high concentrations (>1μM) to cancer cells of epithelial origin, i.e. carcinoma cells, but not to glioma cells. In carcinoma cells, narciclasine pro-apoptotic activity relates to its activation of the initiator caspases of the death receptor pathway. In glioma cells, which are naturally resistant to apoptosis, narciclasine impairs the organization of the actin cytoskeleton at concentrations which are anti-proliferative (IC50 values of 30-90 nM). Same features are observed in melanoma cells that are also naturally resistant to apoptosis. The actin cytoskeleton is implicated in both cell proliferation (cytokinesis) and cell migration (including the metastatic process). Treatment of glioma cells with narciclasine at 50-100 nM causes an increase of cells exhibiting unpolarized morphology with strong stress fibers and focal adhesions all around the cells, suggesting that the disassembly of stress fibers and focal adhesions was attenuated, rendering the cells therefore more adherent to their substratum and less invading to neighboring tissues, including the brain parenchyma. Narciclasine-induced effects on the actin cytoskeleton occur through activation of Rho GTPases. Narciclasine also impairs protein syntheses in cancer cells through the inhibition of the eEF1A elongation factor (another Ras-like-GTPase), a feature that in turns also impact on the actin cytoskeleton organization. Narciclasine appears more active in an intracranial xenograft model of human non-small-cell lung cancer (NSCLC) than in the same model orthotopically grafted into the lungs of mice. Taxol was ineffective in this intracranial model and the differences in activity between the two compounds may reflect the ability of narciclasine to more readily cross the blood-brain barrier. In the same manner, the treatment of human glioblastoma orthotopic xenograft- and melanoma metastasis orthotopic xenograft- bearing immunocompromized mice with non-toxic doses of narciclasine significantly increases the survival of these mice. Narciclasine anti-tumor effects are of the same magnitude than temozolomide, the drug associated with the highest therapeutic benefits in treating glioblastoma patients. Lycorine, a narciclasine's congener display similar effects, but with about two logs of weaker activity. In conclusion, specific and selective targeted delivery approaches should be envisaged to move narciclasine in pre-clinical trials, and hopefully in clinical trials for patients with brain tumors, including gliomas and brain metastases.

O-62

Endophytic fungi as sources of new bioactive metabolites

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This presentation gives an overview on some of our recent results on new bioactive compounds from endophytic fungi that inhabit higher plants and live with their host plants in a mutualistic relationship. Endopyhtes are known to produce anticancer metabolites such as paclitaxel, camptothecin and others that were hitherto only known from plants. In addition these fungi are prolific sources of other structurally new metabolites that are of interest due to their cytotoxic or antibiotic properties which provoke strong interest in these compounds as lead structures for biodiscovery. In our group the focus is on endophytic fungi from extreme habitats such as Mangrove swamps and from medicinal plants. Fungi are selected for in depth investigations based on antibiotic and/or cytotoxic activities of their crude extracts followed by bioactivity guided isolation of the active principles. Compounds isolated are structurally divers and include peptides and depsiteptides, alkaloids and polyketides such as dimeric and monomeric

anthraquinones and xanthone derivatives. Several recent examples of ongoing projects such as new cytotoxic pyrrocidine derivatives that act as Nf kappa B inhibitors will be presented.

0-63

From wortmannin to modern PI3K inhibitors – applications in cancer, inflammation and metabolic control

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The fungal product wortmannin has been identified as the first nM inhibitor of phosphoinositide 3-kinase (PI3K). Mechanistic studies have revealed that wortmannin covalently binds to a conserved lysine (Lys 802 in PI3Kα; Lys 833 in PI3Kγ) within the ATP-binding pocket of PI3Ks and PI3K-related protein kinases (PIKKs), such as the target of rapamacin (TOR), DNA-dependent protein kinase (DNA-PK), Ataxia Telangiectasia Mutated (ATM), and ATM and Rad3-related (ATR). Wortmannin has been widely used to explore PI3K signaling, shows in vivo toxicity, limited biological stability but can provide prolonged PI3K inhibition. Wortmannin has early on been recognized as an anti-inflammatory compound. A mechanistic connection between inflammation and PI3K activity could finally be established using genetically modified mice without a functional PI3Kγ catalytic subunit, providing a basis for the development of strategies for chronic inflammatory conditions such as rheumatoid arthritis, atherosclerosis and allergic responses. Novel insights in localized, PI3Kγ-mediated PtdIns(3,4,5)*P*3 production and an PI3Kγ-adapter-specifc role of Ras are currently exploited to modulate cell-specific PI3K activation in inflammatory, proliferative and metabolic disease. Wortmannin has been instrumental to spur PI3K research, and has promoted the dissection of PI3K and PIKK signaling, which is now at a transition point entering therapeutic applications.

0-64

Ecdysteroids as modulators of multi-drug resistance: structure-activity relationship study

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Ecdysteroids are herbal analogs of the insect molting hormone, that, among other beneficial bioactivities, can act as mild anabolics in mammals without affecting their steroid hormone system. Our recent studies demonstrated that these compounds can modulate resistance to doxorubicin in cancer cells that over-express the ABCB1 transporter. Ecdysteroid diacetonides such as 20-hydroxyecdysone 2,3;20,22-diacetonide (1) and some acetates decrease the resistance of the cells to doxorubicin, while polar ecdysteroids such as turkesterone can increase it. These studies provided the basis for the synthesis of new ecdysteroid diacetonides, with the objective of finding new compounds with improved activity. 20-hydroxyecdysone (20E), derived from Cyanothis sp., was purchased at 93% purity. As side-products from the synthesis of 1, three novel ecdysteroid diacetonides, derivatives of the accompanying constituents, have been obtained. Moreover, acetonide formation of 20E or its 20,22-monoacetonide was also performed by using methyl-ethyl ketone instead of acetone, in order to obtain further five new apolar derivatives. Studies were conducted using L5178 mouse T-cell lymphoma cell line (non MDR) and its sub-cell line transfected with pHa *MDR*1/A retrovirus, overexpressing the human ABCB1 efflux pump (MDR cell line). The effect of the compounds on the intracellular accumulation of rhodamine 123 was tested by using flow cytometry. Antiproliferative activities were determined by the MTT assay. Combination studies with doxorubicine were done by using the checkerboard microplate method and the MTT colorimetric assay, and the results were evaluated by using the CompuSyn software. Flow cytometry measurements revealed that six of the new compounds were able to increase the intracellular accumulation of rhodamine 123 by the MDR mouse lymphoma cells. Combination Indices at 50% of growth inhibition showed that the new ecdysteroid diacetonides, like the ones tested before, significantly reduce the resistance of the cells to doxorubicin. Although the mechanism by which edysteroids modulate resistance is still to be clarified, the newly obtained results extended those of our previous SAR study and provided

further valuable information on the possible apolar groups at positions 2, 3, 20 and 22, in order to obtain ecdysteroid derivatives with strong MDR modulator activities.

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P-1

Chemical composition of the plant *Punica granatum* l. (pomegranate) and its effect on heart and cancer

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In this report, the chemical composition and pharmacological properties of Punica granatum L. (Punicaceae) have been reviewed. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity.

P-2

Phytochemical screening, antibacterial, and *invitro* cytotoxic evaluation of *Cichorium intybus* root extracts indigenous to Iraqi Kurdistan

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The phytochemical screening in the root of *cichorium intybus* had led to detection of alkaloids, carbohydrates, cardioactive glycosides, steroids, and phenolic compounds. Antibacterial evaluation of petroleum ether, 80% ethanol, and ethyl acetate root extracts at three different concentrations against three gram positive and five gram negative bacteria using agar well diffusion method was carried out, all bacterial strains found to be susceptible for ethyl acetate extract with lowest MIC value (15.625 mg/mL) against *Bacillus spp,* and *Staphylococcus aureus*. TLC agar overlay bioautography method resulted in identification of three constituents from ethyl acetate extract with antibacterial activities chlorogenic acid, caffeic acid and kaempferol of which caffeic acid showed the lowest MIC value (0.15 mg/mL) against *Escherichia coli,* and *Proteus spp. In vitro* cytotoxic activity of 80% ethanol and ethylacetate extracts at concentrations 0.2, 0.4, 0.5, 0.6, 0.8, 1 mg/mL against human lung adenocarcinoma epithelial cell line (A549) at time intervals 24, 48, and 72h showed significant decrease in cell viability with increase in concentration of extracts. IC50 for 80% ethanol extract was recorded as (0.7033±0.0472) on 72hr, and for ethylacetate extract as (0.96±0.02) and (0.566±0.01527) on 48 and 72h respectively.

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P-3

Black tea polyphenols reverses epithelial-to-mesenchymal transition and suppresses cancer invasion in human oral cancer cells

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Metastasis, the major cause of cancer death, is a multistep process involving cell adhesion and proteolytic degradation of the extracellular matrix, essential to achieving cell motility. Epithelial to mesenchymal transition (EMT) has been considered essential for cancer metastasis, a multistep complicated process including local invasion, intravasation, extravasation, and proliferation at distant sites. The purpose of the present study was to characterize the effects of black tea extracts (BTE) on cell invasion, motility, and proteinase expression, while the impact on p-focal adhesion kinase (p-FAK), p-Src, and p-paxillin activities were also examined to explore the underlying mechanism for the involvement of BTE in tumor cell invasion and metastasis. To examine the inhibitory effect on the cell invasion and adhesion, Transwell invasion assay and cell-matrix adhesion assay were performed. We examined the effect of BTE on factors of cancer metastasis and EMT by Western Blot. We treated squamous cell carcinoma-4 (SCC-4) cells with various concentrations of BTE, and then subjected cells to gelatin zymography and casein zymography to investigate the expression of matrix metalloproteinases (MMPs) and urokinase-type plasminogen activator (u-PA). To evaluate the effects of BTE on the MMP-2 and u-PA promoter, we performed a transient transfection with the pGL3-MMP-2 and pGL3-u-PA promoter and analyzed the luciferase activities. Herein we provided molecular evidence associated with the antimetastatic effect of BTE, which contained polyphenols including gallic acid, gallocatechin, epigallocatechin-3-gallate, and epicatechin-3-gallate, in an oral squamous cell culture system by showing a nearly complete inhibition on the invasion (P<0.001) of SCC-4 cells via a reduced activities of MMP-2 (P<0.001) and u-PA (P<0.001). BTE exerted an inhibitory effect on cell migration (P<0.001), motility (P<0.001), spread and cell-matrix adhesion (P<0.001). Promoter luciferase analysis revealed that BTE inhibits the transcription of MMP-2 and u-PA. We performed Western blot to find that BTE could induce up-regulation of epithelial marker such as E-cadherin and inhibit the mesenchymal markers such as snail-1, vimentin, which

promote cell invasion and metastasis. BTE inhibited p-focal adhesion kinase (p-FAK), p-Src, and p-paxillin, indicating the anti-EMT effect of BTE in oral squamous cell carcinoma. Taken together, these results suggested that BTE could reduce the invasion by reversing EMT in human oral cancer cells.

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P-4

Anticancer effect of the water extract of *Wasabia japonica* on HepG2 cells in vitro and in vivo

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Wasabia japonica (WJ) has been used as an important spice in Japanese foods. WJ is a member of the Brassicaceae family. Previous studies indicated that high consumption of Brassica vegetables lowered colon cancer risk. Our previous study indicated that the water extract of fresh wasabi roots (WJE) induced a strong cytotoxic effect toward HepG2 cells in a dose- and time-dependent manner that was probably via induction of apoptosis. In this study, we further identified the mechanisms of the liver cancer cell-killing effect of WJE. The effect of WJE on the early stage of apoptosis was detected by annexin V-FITC/PI double stain. The portion of annexin V-FITC positive/PI negative distribution of HepG2 cells was increased by WJE in a dose- and time-dependent manner. The results of flow cytometry of 4\', 6-diamidino-2-phenylindole for mitochondria membrane potential showed a significant elevation at 72 h after the treatment indicating the involvement of the mitochondria-dependent pathway. Western blotting of apoptosis related proteins was performed to identify the mechanisms. An increase in the protein levels of cleaved-caspase-3 and 9 (pro-apoptotic proteins), p-JNK, p-p38, and a decrease in the protein level of Bcl-2 (an apoptosis inhibitor) were observed. In vivo, the xenograft model was used to determine the anti-cancer effect of WJE. The growth of the xenografts was delayed when the animals were treated with WJE. Furthermore, the tumors were significantly shrunk by treating the animals with a combination of WJE and anticancer drug Everolimus. These results suggested that the cytotoxicity of WJE in HepG2 cells was exerted by inducing apoptosis. The outcomes of animal study further support the application of WJE as an adjuvant for chemotherapy of liver cancer.

P-5

Enhancing effect of mulberry water extracts on the toxicity of paxlitaxel via inducing mitotic catastrophe in bladder cancer TSGH cells

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Mulberry fruit has been reported to contain a wide range of polyphenols and have chemopreventive activities. However, little is known regarding the effect of mulberry fruit on anticancer efficacy as an adjuvant of anti-cancer drug. In this study Human bladder carcinoma TSGH 8301 cells were used as a model to investigate the enhancing effect of mulberry water extract (MWE) on the cytotoxicity of paclitaxel (3nM) in vitro. The results of MTT assay demonstrated that the anti-survival effect of MWEs synergized with paclitaxel. Cell cycle analysis showed that the treatment of MWE at a dose of 250 μg/mL combined with paclitaxel for 24 and 48 h induced apoptosis in TSGH cell, while at doses of 500, 750, 1000, and 1500 μg/mL led to G2/M phase arrest. The results of Western blotting analysis confirmed that the combined treatment for 24 h induced an accumulation of phosphorylated cyclin B1 levels and a stimulation of Cdc2/cyclin B1 kinase complex activity, *witch are* the chief cell-cycle factors for the G2 to M phase transition. *The level of* Bcl-2, an anti-apoptotic protein, was decreased when MWE was at 250 μg/mL, and then increased as MWE increased. DAPI nuclear staining showed that the combined treatment of high doses of MWE and paclitaxel caused multinucleation in TSGH cells, a cellular morphology of genome instability that could lead to mitotic catastrophe (MC) cell death. The deregulation of Aurora A which is required for centrosome separation and formation of a bipolar spindle was known to result in multinucleation that constitutes the most prominent morphological traits of mitotic catastrophe. The level of phosphorylated Aurora A was induced by the exposure of MWE together with paclitaxel for 24 h in TSGH cells. *These data* provided the first evidence that mulberry water extracts enhanced the anticancer efficacy of paclitaxel by inducing mitotic catastrophe in vitro, indicating that MWE could be used as an adjuvant for cancer chemotherapy in the future.

P-6

A stimulatory effect of *Cassia occidentalis* on melanoblast differentiation and migration

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In vitiligo, the active melanocytes in the epidermis are totally missing, whereas melanoblast cells in the outer root

sheath of hair follicles are not affected. In an attempt to find potent repigmenting agents for vitiligo therapy, pod extracts of Cassia occidentalis was found to be effective in inducing differentiation and migration of mouse melanoblast cell line. Methanolic extract redissolved in DMSO at 12.5 lg/mL was found to cause 3.5- to 3.8-fold melanin induction in melb-a melanoblast cells after 4 days in treatment medium. In addition it induced the tyrosinase activity and altered melb-a cell morphology. Transwell migration assay showed the potential of this herbal candidate to induce direct migration of treated cells. To the best of our knowledge, this is the first report investigating the effect of Cassia occidentalis on the differentiation and migration of melanoblast cells. The findings of present study are significant in designing preclinical and clinical studies on the efficacy of C. occidentalis as a stimulant for skin repigmentation in vitiligo.

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P-7

Tryptophan metabolism: can a non-natural anticancer drug enhance the production of a natural one?!

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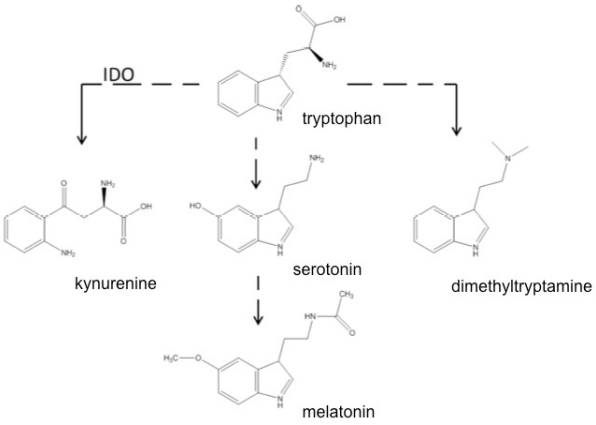
1-Methyl-tryptophan (1-MT) is a competitive inhibitor of the IFN-γ induced enzyme indoleamine-2,3-dioxygenase (IDO). IDO is the rate-limiting enzyme of tryptophan metabolism by the kynurenine pathway. As this enzyme is related to tumor escape from immune system, 1-MT is proposed as an adjuvant anticancer molecule. Tryptophan can be also metabolized by two other pathways, one that lead to serotonin and melatonin production and the tryptamine pathway. By inhibiting IDO, and consequently kynurenine pathway, this study aimed to observe metabolic interrelationships among different tryptophan metabolism pathways.



An unique result was obtained: 1-MT enhanced the expression of some of the enzymes involved on melatonin (MLT) synthesis and the production on human melanocytes, fibroblasts and melanoma SK-MEL-19 and 147 lines. The antiproliferative effect of melatonin in tumor cells is well-recognized. Due to our findings, we tested the ability of melatonin to diminish melanoma lines proliferation and migration. The effect observed for melatonin was comparative to 1-MT in the clonogenic and scratch test assays. Perspectives are to observe 1-MT effect on serotonin pathway in *in vivo* models of melanoma.

ACKNOWLEDGEMENTS

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P-8

Tryptophan metabolism on cancer: the expression and effects of tryptamine pathway on melanoma and glioblastoma cell lines

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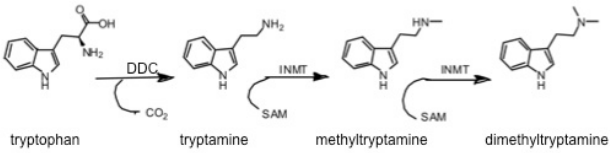
Tryptamine (TRY) and dimethyltryptamine (DMT) are indolic alkaloids originated from the decarboxylation of tryptophan (TRP) and represents the lesser-known route of degradation of this amino acid (DMT path). TRP is mainly degraded by kynurenin pathway (KYN path) and serotonin pathway (SER path) resulting in compounds that participate in many physiological and pathological processes such as inflammation and cancer. In cancer, KNY path can contribute to tolerance phenomena and immune escape of tumor, and SER path produce metabolites that have immunomodulatory effects and can control tumor growth. Given the importance of different biochemical pathways of TRP metabolism in tumors

and the lack of information about tryptamines effects, we investigated the impact of TRY and DMT on tumor cell migration and proliferation.



Caption:
DDC: aromatic L-amino acid decarboxylase
INMT: indole N-methyltransferase
SAM: S-adenosyl-methionine

Melanoma cell lines SK-Mel 19 and SK-Mel 147 and glioblastoma cell lines A172 and T98G were cultured with TRY and DMT (100 mM) for scratch and clonogenic assays. TRY and DMT showed an antimigrative action on melanoma but not on glioma cells. SK-Mel 19 and SK-Mel 147 was very senstitive to TRY and DMT as measured by the clonogenic assay. It is noteworthy that RT-PCR analysis showed that SK-Mel 147 and A172 cells express some of enzymes that are responsible for the TRP conversion to TRY and DMT (*ddc* and *inmt* genes). The role of TRY and DMT on tumor biology is still unclear but our findings about DMT path in tumor progression and invasiveness give new ideas on tumor metabolism study.

ACKNOWLEDGEMENTS

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P-9

Prostate apoptosis response-4 inducing cancer cells death via coordinating apoptosis and autophagy pathways in hypopharyngeal carcinoma

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Hypopharyngeal carcinoma (HPC) is usually poorly distinguished and hard to carry out early diagnoses because of the asymptomatic in patient. Besides, the highest metastasis rate of HPC among all head and neck cancers resulting in poor prognosis and high mortality rate. Therefore, definition of a useful biomarker for prognosis after anticancer treatments of HPC patient is quite important. Two self-destructive processes, autophagy and apoptosis, are important for inducing cancer cell death and have a functional relationship via complicated cross-talking signals which remain unclear. Prostate apoptosis response-4 (Par-4) has been shown as a positive-regulator in apoptotic signaling and also a tumor suppressor protein in most cancers. Par-4 is regulated and enhanced by various proteins then leading to different localizations which differentially sensitize cancer or normal cells to diverse apoptotic stimulus. However, the role of Par-4 in autophagy process remains largely obscure. In the present study, we firstly demonstrated Par-4 induction triggered autophagy in HPC cells. By knocking down or ectopic expressing Par-4, Par-4 not only sensitized HPC cells to chemotherapy drugs (5-FU and cisplatin) or x-ray irradiation treatment but also sufficiently leading to apoptosis and autophagy. The autophagic flux was activated by Par-4 and contributed to cell death due to increasing transition from autophagosome to autolysosome phase. By treatment of purified nature compounds A or B, we further demonstrated Par-4 induction sensitized cancer cells to these compounds and resulting significant cell death. Our functional studies further revealed that Par-4 coordinated apoptosis and autophagy through impeded PKC/p62 complex formation, then down regulating NF-κB transcriptional activity, and/or antagonized Bcl-2 anti-apoptosis and -autophagy abilities. The systemic analysis also illustrated multiple signaling pathways which involving in apoptosis and autophagy were simultaneous influence by Par-4 incitation. Finally, our clinical analysis manifested that Par-4 enhancement raised the prognosis of HPC patients with chemo-radiotherapy which is consistent with our previous in vitro study of Par-4 activity. Taken together, Par-4 may be a chemo-sensitizer in clinical investigations which sensitize cancer cells to death. It is worth to further investigate the therapeutic potential of natural chemopreventive agents or chemotherapeutics by verifying the enhancement of Par-4 expression level after the drug treatments. Based on the powerful death-inducing function through both apoptosis and autophagy in cancer cells, Par-4 may provide a useful focus for the development of cancer-selective therapeutics.

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National Science Council, Taiwan, Tzu Chi University, Taiwan, Hualien Tzu Chi medical center, Taiwan.

P-10

Cytotoxic effect and apoptosis induction of aqueous extract of *Agrostemma githago L.* seed on gastric (AGS) cancer cell line

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Agrostemma githago L. ,a well known toxic member of the Caryophyllaceae is a wild plant. this study was designed to examine possible cytotoxic effect of freeze-dried aqueous extract of *Agrostemma githago* seed on the cancer cell line of AGS (human gastric carcinoma). The aqueous

extract of the seed of *Agrostemma githago* was prepared and freeze-dried. AGS cancer cell line was treated by the extract and incubated for 24, 48 and 72 h. Cytotoxicity was examined by MTT assay. EB/AO staining was used for apoptotic cell detection. The IC50 values were 69.95, 36.46 and 15.85 mg/mL for AGS cell line after 24, 48 and 72 h respectively.. The EB/AO staining showed an increase in apoptotic cells. The results of current study showed that the freezed-dried aqueous extract of the seed of *Agrostemma githago* has cytotoxic effect on gastric cancer cell line by means of apoptosis. It seems that several compounds are possibly responsible to cytotoxic effect of the extract.

ACKNOWLEDGEMENTS

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P-11

Evaluation of the cytotoxicity of *Satureja spicigera* and its main compounds

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Satureja spicigera (Lamiaceae) grows wildly in North-West of Iran. In this study, bioassay guided isolation and identification of the main compounds has been reported using various chromatographic methods and comparison of their spectral data with those reported in the literature. Brine Shrimp Lethality and four cancerous cell lines HT29/219, Caco 2, NIH-3T3 and T47D were used for cytotoxicity evaluations. From the aerial parts of *S. spicigera*, nine known compounds including two flavanone, 5,7,3',5'-Tetrahydroxy flavanone (8) and 5,4'-dihydroxy-3'-methoxyflavanone-7-(6''-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (9), one dihydrochalcone, nubigenol (7), together with thymoquinone (1), thymol (2), carvacrol (3), beta-sitosterol (4), ursolic acid (5) and oleanolic acid (6) were identified. Among the isolated chalcone and flavanones, compound 8 was effective against *Artemia salina* larva (LC50= 2 μg/mL) and only the compound 9 demonstrated IC50 value of 98.7 μg/mL on the T47D (Human, Breast, ductal-carcinoma). Other compounds did not show significant inhibition of the cell growth.

ACKNOWLEDGEMENTS

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P-12

Synthesis and characterization of an emulsified nanoparticles containing HPV 16 E7(49-57) peptide and CpG oligodeoxynucleotides for immunotherapy of cervical cancer

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Human papillomavirus (HPV) is the most common sexually transmitted infection and is responsible for over 90% of cervical cancer cases. HPV-16 has been identified in approximately 50% of all human cervical tumors. The viral proteins E6/E7 had been found to not only inactivate p53 and pRb but also modify cell cycle therefore to be oncoproteins. Based on these reasons, HPV-16 E6/E7 proteins are ideal targets for developing immunotherapy against cervical cancer. HPV16 E7-derived cytotoxic T lymphocyte (CTL) epitope 49–57 (RAHYNIVTF) has been shown to induce effective T cell responses to eradicate established HPV16-induced tumors in mice. Peptides as small drugs offer several advantages, such as high specificity, low toxicity and no accumulation in organs. But peptide-based immunotherapies have faced limited clinical success caused by the relatively low immunogenicity in human. In the present study, we want to increase the immunogenicity of HPV16 E7(49-57) peptide by using microencapsulation technology. With the aim of enhancing the immunotherapy potency, we design and optimize an emulsified delivery system containing selected oil, phosphate buffered saline (PBS) and an amphiphilic copolymer PEG-b-PLA. The latter comprises hydrophilic PEG and hydrophobic PLA, both are selected because of their biocompatibility and bioresorbability. The formulated emulsion shows good stability at least 6 months at room temperature and more than one year at 4°C. *In vitro* stimulating dendritic cells by PEG-b-PLA and the corresponded emulsion demonstrate no activation of dendritic cells. To further evaluate the adjuvanticity of emulsion, we load HPV-16 E7(49-57) peptide to the emulsion to immunize mice inoculated TC-1 (HPV-16 E6/E7 and c-Ha-Ras co-transformed) tumor cell as an immunotherapy model study. We give tumor inoculated mice a single dose of HPV-16 E7(49-57) peptide alone or coupling with emulsion and further monitor tumor size and the survival rate. In other hand, we also carry out single-cell suspensions prepared from immunotherapy treated mouse spleen and re-stimulated by peptide *in vitro* to compare T-cell responses in different formulations. Our *in vivo* studies show a single dose of peptide coupling with emulsion can

slow the increasing of tumor size with respect to peptide alone. Surprisingly, tumor elimination as well as T cell responses are enhanced after the peptide formulated with CpG/emulsion. These results indicate that the biodegradable copolymer PEG-b-PLA formulated emulsion may be an efficient injectable delivery system for peptide drug to treat cervical cancer. Moreover, formulating HPV-16 E7(49-57) peptide with CpG/emulsion offer effective specific T cell response and therapeutic effect which may be helpful in developing cancer immunotherapy in the future.

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P-13

New highly complex alkaloids from the sponge-associated fungus *Aspergillus* sp.

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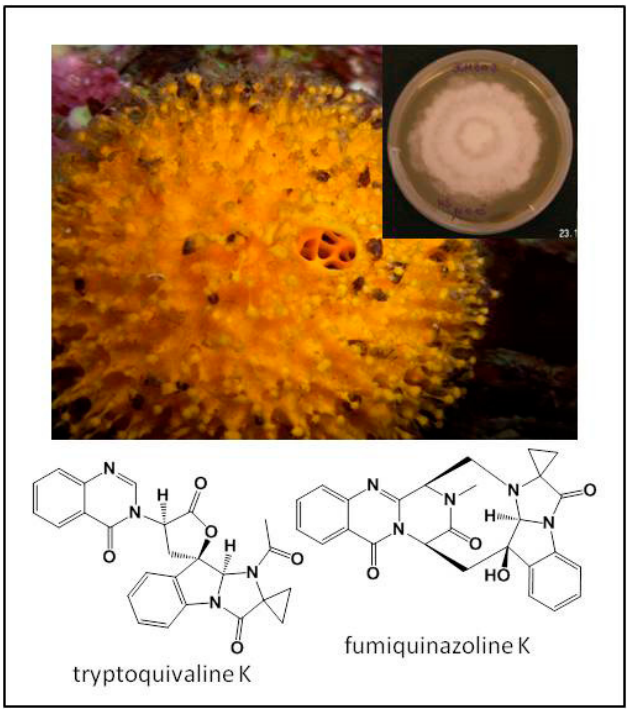
Seven new alkaloidal metabolites including tryptoquivaline K and fumiquinazolines K-P, together with six known compounds, were isolated from the fungus *Aspergillus* sp. obtained from the Mediterranean sponge *Tethya aurantium*. The highly complex structures of the new compounds were unambiguously determined by extensive 1D and 2D NMR and mass spectral analysis, and their absolute configuration by CD calculations. All new compounds were found to incorporate a 1-aminocyclopropane-1-carboxylic acid residue, which has only been rarely discovered in nature. The core scaffold of the isolated compounds may be biosynthetically derived from anthranilic acid, tryptophan, alanine and the rare 1-aminocyclopropane-1-carboxylic acid involving a trimodular nonribosomal peptide synthetase.



Upon testing the compounds for their cytotoxic activity by the MTT method, the structurally related known fumiquinazoline J, which was also isolated in this study, exhibited pronounced cytotoxic activity against mouse lymphoma cells (L5178Y) with an IC50 value of 3.7 μM, and moderate activity against human ovarian cancer (A2780) and human Philadelphia chromosome-positive chronic myelogenous leukemia (K562) cell lines with IC50 values of 18.5 and 15.0 μM, respectively. The remaining investigated compounds showed weak or no activity in this assay.

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P-14

Regulation of the kynurenine pathway by tryptamines: directing tryptophan metabolism and impacting tumor growth

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Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting step in the kynurenine pathway. It is an interferon- γ (IFN-γ)-induced tryptophan-degrading enzyme and participates in the mechanism of tumor immune tolerance. Thus, to restore the anti-tumor immunity of the host, IDO inhibition has been considered as a strategy for anticancer therapy. The aim of this study was to identify whether metabolites originated from competitive routes of tryptophan metabolism, such as the serotonergic or tryptamine pathways, have some inhibitory action on IDO activity. Serotonin, melatonin, tryptamine (TRY) and *N, N*-dimethyltryptamine (DMT) were tested. Only TRY and DMT modulate the activity of recombinant IDO, as classical non-competitive inhibitors, with a Ki of 156 μM and 506 μM, respectively. The inhibitory activity was also observed on constitutively expressed or IFN-γ-induced IDO in A172 human glioma cell line. In conditions that TRY and DMT did not affect A172 cell viability and proliferation, they increased in approximately 50% the cytotoxic activity of peripheral blood mononuclear cells (PBMC) in co-cultures assays. We conclude that IDO inhibition by TRY and DMT contributed to a more effective action of PBMCs against tumor cells. Thus, tryptamine route, through its metabolites, may contribute to the endogenous regulation of the kynurenine pathway and should be considered in proposals that aim to identify new inhibitors of IDO.

P-15

Cherry and cactus pear natural extracts for colon cancer therapy – in *vitro* evaluation of chemopreventive and chemosensitization effects

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Epidemiological data suggest that ingestion of bioactive compounds from fruits and vegetables, such as polyphenols and terpenes, may contribute to reduce the incidence of cancer in humans. The mechanisms by which these compounds inhibit tumourgenesis is widely described and include attenuation of tumour angiogenesis, induction of cell cycle arrest and promotion of apoptosis. Sweet cherries (Prunus avium) and cactus pears (Opuntia ficus indica) have been reported to be rich sources of perillyl alcohol, flavonoids, phenolic acids and betaleins, which are already identified to exhibit *in vitro* and *in vivo* chemopreventive effect against several types of cancers. The aim of this work was to develop natural chemotherapeutic agents derived from cherries and cactus pears and evaluate their effectiveness in a colon cancer cell model. All extracts were produced with clean technologies from crop residues, namely Saco cherry culls and cactus pear juice wastes. Cherries were processed by high pressure extraction with CO2 and ethanol and perillyl alcohol- rich product (*cherPOH*) was obtained. The antiproliferative activity of this extract was evaluated using HT29 cells. The results obtained showed that cherPOH presents higher antiproliferative effect than fresh fruits (150-fold improvement). Moreover, cherPOH induced cell cycle arrest in a different checkpoint (G1 phase) than doxorubicin (G2/M phase). This suggests that cherPOH can be used in combination with chemotherapeutic drugs to enhance the inhibition of tumor survival. The extraction process was further optimized in order to obtain a natural ingredient with improved potency (32-fold); the effective dose value -ED50 after 24h of incubation was 0.2mg/mL. Betalain and flavonoid rich-extracts (*oBET* and *oFLAV*) were developed from different species of Opuntia (*Opuntia robusta* and *Opuntia ficus indica)* using hydroalcoholic or high pressure extractions. Both extracts inhibited HT29 cell growth in a time and dose dependent manner (ED50 values: *oBET* -9.6mg/mL; *oFLAV* – 12.3 mg/mL), probably by generating ROS at a cellular level. Cell cycle arrest was also analyzed and the two extracts exhibited different responses, which could be related with the distinct composition of samples: *oBET* induced cell cycle arrest into G1 phase whereas oFLAV promoted similar distribution in all cell cycle phases. Furthermore, *oFLAV* was the only extract capable to reverse chemoresistance in

a drug-resistant HT29 cell population. When resistance cancer cells were pretreated with oFLAV, their sensitization to the drug increased up to 3 times. The results, presented in this study, showed that phytochemical-rich extracts from cherries and cactus pears are promising natural chemotherapeutic and chemosensitization ingredients for colon cancer therapy.

P-16

Bioactivity guided isolation of anticancer constituents from leaves of *Juglans regia*

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Cancer is one of the most eminent human diseases which have encouraged researchers worldwide to discover new anticancer agents. Plants have historically been used in treating cancer. Juglans genus (family Juglandaceae) includes several species and is widely distributed throughout the world. Juglans regia L. (Walnut) is its well-known member. Walnut leaves contain several therapeutically active constituents. In this experiment, the leaves of Juglans regia L. have been extracted with n-hexane, chloroform, ethylacetate and then with methanol. A bioactivity-guided approach based on MTT assay for growth inhibition and flowcytometry for cell cycle arrest study was taken to identify the active compounds in CHCl3 soluble fraction. From this active fraction, seven compounds 7-Hydroxy-3,5,4\'-trimethoxyflavone, 5-Hydroxy-3,7,4\'-trimethoxyflavone, Lopeol, Daucosterol, 4-Hydroxy- α-tetralone, β-Sitosterol and Regiolone have been isolated and examined for their dose–response effect on the viability of MCF7(human breast adenocarcinoma), T47D (human ductal breast epithelial tumor), BHY (human oral squamous cancer) and NIH3T3 (mouse embryonic fibroblast) cells. Based on MTT assay, all of the seven examined compounds inhibited growth of both oral and breast human cancer cells (IC50 range= 9-74 μM). Among seven tested compounds only two compounds had lower cytotoxicity in normal cell line which one of them was a new natural compound in this genus. These two compounds significantly arrested cell cycle progression in MCF7 cells. Taken together, this finding suggests that these two compounds may be useful in cancer treatment.

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P-17

Anticancer effects of phenolic compounds

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The most plentiful antioxidants in the diet are polyphenols. Polyphenols exhibit many biologically significant functions, such as protection against oxidative stress and degenerative diseases. They also modulate different signaling pathway such as glutathione biosynthesis, nuclear factor-kappa B (NF-κB) activation. In this research, anticancer effects of different phenolic compounds were investigated. Prevention of cancer is one of the most documented biological properties of the polyphenols. The effects of polyphenols on human cancer cell lines are protection and reduction the number of tumors or their growth. Anticancer effects of polyphenols have been monitored at different tissues, including mouth, stomach, duodenum, colon, liver, lung, mammary, and skin. Polyphenols affect the metabolism of pro-carcinogens by modulating the expression of cytochrome P450 enzymes which prompt their activation to carcinogens. The absorption of polyphenols could then stimulate these enzymes for their own detoxification effects and thus, stimulate our defenses against toxic xenobiotics. Polyphenols can also stimulate apoptosis of tumor cells and can suppress the angiogenesis, therefore decrease the growth of tumors. Many polyphenols, such as quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid, resveratrol, or curcumin, showed protective effects in some cancerous models by different mechanisms. Resveratrol is another polyphenol which have been reported as strong antiproliferative agent. Quercetin showed anticancer effects by blocking EGFR tyrosine kinase activity. Also, quercetin inhibits the formation of diolepoxide 2(DE2) and B[a] p activation. EGCG (*Epigallacto catechin gallate)* which exists in green tea inhibits the activity of telomerase. Phenolic compounds can induce various biochemical processes for prevention of cancer. Some polyphenols cause apoptosis in cancerous cells, and suppress growth and proliferation of various types of tumor cells.

P-18

Molecular mechanism inhibiting human hepatocarcinoma cell invasion by 6-shogaol and 6-gingerol

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We previously demonstrated that 6-shogaol and 6-gingerol, two active compounds in ginger (*Zingiber officinale*), possess anti-invasive activity against highly metastatic hepatoma cells. The aims of this study were to evaluate the inhibitory effect and molecular mechanism underlying the transcription and translation of MMPs and uPA in Hep3B cells as well as the anti-angiogenic activity of 6-gingerol and 6-shogaol. By gelatin zymography and luciferase reporter gene assays, we found that 6-gingerol and 6-shogaol regulate MMP-2/-9 transcription. Moreover, 6-gingerol directly decreased expression of uPA, but the 6-shogaol-mediated decrease in uPA was accompanied by up-regulation of PAI-1. 6-Gingerol and 6-shogaol concentrations of ≥ 10 μM and ≥ 2.5 μM, respectively, significantly inhibited the phosphorylation of MAPK and PI3K/Akt signaling, the activation of NF-kB, and the translocation of NF-kB and STAT3. Incubation of 6-gingerol or 6-shogaol with HUVECs or rat aortas significantly attenuated tube formation. 6-Shogaol and 6-gingerol effectively inhibit invasion and metastasis of HCC through diverse molecular mechanisms, including inhibition of the MAPK and PI3k/Akt pathways and NF-kB and STAT3 activities to suppress expression of MMP-2/-9 and uPA and block angiogenesis.

ACKNOWLEDGEMENTS

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P-19

Cytotoxicity effects of *Anthemis nobilis* (roman chamomile) leaves extract in oral human cancer cell line

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Currently, natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents. Plants have historically been used in a wide range of diseases such as cancer and are recognized for their ability to produce secondary metabolites. In this regard, Roman chamomile (Anthemis nobilis) has been used to treat cancer. The current study was designed to evaluate the antiproliferative activity of total ethanol extract from leaves of *Anthemis nobilis* L. The total phenolics and flavonoids content of this extracts were also determined to obtain further information on the correlation between the contents of phenolic compounds and antiproliferative effects. Antiproliferative activity was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and flowcytometry methods against human oral cancer (BHY) cell line. The total phenolics and flavonoids were determined by Folin-Ciocalteu and aluminum chloride colorimetric methods. Our present study has shown that ethanol extract has potent antiproliferative activity and also induces cell cycle arrest after 24 h treatment. The results obtained herein indicate that Roman chamomile extract may contain effective compounds which can be used as a chemotherapeutic agent.

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P-20

The cytotoxic effects of methanolic extract of *Teucrium persicum*

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Teucrium persicum is an endemic plant of Iran that its pharmacologic properties have not been previously investigated. In this study we report that the methanolic extract, isolated from aerial parts of this plant dramatically inhibits cell proliferation of human cancer prostate PC-3 cells with the IC50 value of 150 µg/mL. The cytotoxic effects of *Teucrium persicum* are comparable very well to those of known anticancer drugs such as Doxorubicin, Imatininb and Carboplatin. *Teucrium persicum* extract also inhibited proliferation of T47D (breast) and SW480 (colon) cancer cells. The extract at 50µg/mL and 150µg/mL concentration values decreased proliferation of T47D cells by 55% and 65% respectively and the same concentrations inhibited SW480 cell proliferation up to 80%. We are currently investigating the mechanism(s) of cell proliferation inhibitory effects of *Teucrium persicum* and the results will be presented in this conference.

P-21

Modulatory effects of curcumin in conjunction with piperine on benzo(a)pyrene-mediated dna adducts and biotransformation enzymes

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The antigenotoxic effects of curcumin alone and with piperine on benzo(a)pyrene-diol epoxide DNA adducts (BaPDE-DNA adducts), and carcinogen biotransformation enzymes was investigated in liver and lung of mice. Male Swiss albino mice received curcumin (100 $mgkg^{-1}$ body weight) and piperine (20 $mgkg^{-1}$ body weight) separately as well as in combination orally in corn oil for seven days as pretreatments and thereafter 2 h, BaP (125 $mgkg^{-1}$ body weight) was administered orally in corn oil. A single dose of BaP to normal mice increased the activities of ethoxyresorufin o-deethylase (EROD), pentoxyresorufin o-dependentylase (PROD) and levels of BaPDE-DNA adducts in both the tissues. Quinone reductase (QR) activity was also elevated in the BaP treated group in both liver and lung when compared with normal control group but no significant change was assessed in glutathione S-transferase (GST) activity. Pretreatment of curcumin and curcumin plus piperine before administration of a single dose of BaP significantly decreased the activities of EROD, PROD and the level of BaPDE-DNA adducts with consequent increase in QR and GST activities. The study clearly indicates that curcumin when given in combination with piperine is more effective in modulating BaPDE-DNA adducts (liver and lung), and activities of EROD (liver) and GST (liver and lung).

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P-22

Ameliorative effects of green and white tea against benzo(a)pyrene induced oxidative stress in Balb/c mice

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Benzo(*a*)pyrene (BaP) is one of the polycyclic aromatic hydrocarbons (PAH) classified as carcinogenic to humans by International Agency for Research on Cancer. Green tea (GT) and white tea (WT) is known to exert their protective effects by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system. A single dose of BaP (125 mg/kg, orally) increased the levels of lipid peroxidation (LPO) and decreases endogenous antioxidants such as superoxide dismutases (SOD), glutahione reductase (GR), catalase (CAT), glutathione-s-transferase (GST) and glutathione (GSH) significantly which indicate imposition of oxidative stress in pulmonary and hepatic tissues. Pretreatment with green and white tea for 35 days before single dose of BaP tends to normalize the depleted levels of GR, SOD, CAT, GST and GSH content and increased LPO level in both hepatic and pulmonary tissues. The percent DNA in comet tail, BaPDE-DNA adducts and 8-oxo-dG levels reflected the decreasing pattern of DNA damage from BaP treated group to the groups that received pretreatment with GT and WT. Our study concludes that both green and white tea is effective in combating BaP induced oxidative insult and DNA damage.

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P-23

The myxobacterial compound chondramide shows anti-angiogenic and anti-migratory potency

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Tumor induced angiogenesis as well as metastasis are crucial steps in the progression of cancer. During migration, the key step in these processes, high plasticity of the cytoskeleton, especially of actin filaments, is required. In our study, we target the actin cytoskeleton by the myxobacterial compound Chondramide (Ch) isolated out of *Condromyces crocatus*. This cyclodepsipeptide binds actin at the phalloidin binding site, stabilizes fibrous actin and, thus, could be a potent inhibitor of cell migration. Here, we investigate the anti-angiogenic and anti-metastatic potential of Ch by analyzing its functional impact on endothelial and cancer cell motility. For our work, we use HMECs (human microvascular endothelial cell) as endothelial cell line and the metastatic breast cancer cell line MDA-MB-231. Ch shows an anti-proliferative effect on HMECs and MDA-MB-231 cells in a nanomolar range (EC50 values of 82 nM or 61 nM, respectively). The migration of HMECs and MDA-MB-231 was inhibited by Ch in a concentration dependent manner as shown in Scratch Assay or Boyden Chamber Assay, respectively. In the context of angiogenesis, the tube formation of HMECs on Matrigel was diminished at same concentrations like migration (100 nM – 300 nM). Interestingly, at a concentration of 100 nM Ch, HMECs were able to adhere and form first intercellular contacts. However, later maturation of tubes involving condensation of cell-cell-contacts to tubes was inhibited. Further, we tested Ch on its potency as a tube disrupting agent. For this purpose, a Tube Disruption Assay on Matrigel as well as impedance sensing (xCellingence™, Roche) were performed, in which Ch exhibited disrupting properties depending on concentration. To investigate the intracellular processes responsible for the decrease of migration and tube formation, treated cells were analyzed via fluorescent staining (Rhodamin-Phalloidin, γ-Tubulin, LAMP-1) and confocal microscopy. We observed that HMECs and MDA-MB-231 cells lose normal cell shape above a certain concentration of Ch (100 or 200 nM, respectively) forming globular cells. At lower concentrations, cells maintain normal cell shape, however, they exhibit juxtanuclear f-actin aggregates. These knots are located close to the centrosome, do not overlap with lysosomes and persist over 48h. This phenomenon could indicate an aggregate formation as an escape response of the cells, like known for other actin targeting drugs. Summarizing, we show that Ch reveals promising properties to inhibit cell motility of endothelial and tumor cells as well as potency for tube disruption. In the future, we will test Chondramide in different tumor mouse models to further evaluate its therapeutical potential *in vivo*.

P-24

Edible algae-derived methanolic extracts has anti-oral cancer effect through apoptosis and Ros pathways

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The biological function of methanolic extracts of the edible red algae (MERA) on oral squamous cell carcinoma cells (OSCC) was evaluated. We found that MERA has growth inhibition effects on OSCC cell in the dose-response manner (P<0.05). MERA-treated OSCC cells displayed the significant increase of sub-G1 population and annexin V-FITC intensity in dose-response manner (P<0.001). g-H2AX intensities of MERA-treated OSCC cells were significantly increased in dose-response manner (P<0.05). The intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) of MERA-treated OSCC cells was significantly increased and decreased in dose-response manner, respectively (P<0.05). Our results suggest that edible algae-derived MERA has potential therapeutic effect against OSCC through apoptosis and ROS pathways.

P-25

Time to start with the dietary antimetastatic agents

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Metastases develop when cells break away from the primary tumour to colonise the distant organs. It is the worst outcome of the tumour and therefore, prevention of metastasis has moved to the centre of clinical attention. Incidence of metastatic disease may be minimised by dietary manipulation, however, the question remains when to start the intake of antimetastatic nutritional components. It is traditionally considered that dissemination of malignant cells from the primary tumour to secondary sites is a late-stage event during the tumour progression, occurring only after the ontogenesis of cancer has proceeded to the full malignancy in the primary tumour environment. However, several lines of recent evidences

indicate that initiation of metastasis may begin much earlier in tumorigenesis than previously thought and dissemination of cancerous cells can start already long before the manifestation of the first symptoms and diagnosis. Therefore, the hypothesis is proposed in this abstract that natural health products with antimetastatic properties could not only be a reasoned approach to inhibit the progression of metastases in the diagnosed cancer patients, but much farther : various dietary agents which suppress hypoxic stress and acidosis, control angiogenesis, promote integrity of extracellular matrix, protect endothelial lining, avoid platelet aggregation, and support immune system should be a certain, conscious and constant part of the everyday diet for all people. For instance, it is well known that without angiogenesis the tumour expansion is limited only to 1-2 mm exhibiting no threat to the life. However, so a minimal malignancy reveals no clinical symptoms or complaints, remains usually undetected in this early stage and may start to spread to the distant regions. Therefore, a rational and reasonable consumption of natural antimetastatic agents acting through the different intracellular pathways should be a certain part of the regular diet of all people, probably leading to prevention or retarding the early dissemination of possibly existing tumours, keeping the disease under control, and potentially saving/prolonging the lives. Cancer is avoidable (at least, in part) by chemopreventive strategies and properly and in due time applied dietary changes remain the key players in tumour progression.

P-26

Evaluation of the antioxidant effect for primulaceae family-derived extract against hydrogen peroxide-induced DNA damage in oral cancer cells

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Primulaceae family-derived extract (PDE) is derived from a herb plant and its antioxidant effect and cell function is unclear. We tested the possible antioxidant ability to protect the reactive oxygen species (ROS)-induced DNA damage. Using plasmid conformation assay, we found that PDE/H2O2 treatment for 30 min at 37 °C leaded to decrease the relaxed/linear forms and the supercoiled form is recovery in a dose-dependent manner. It suggests that the PDE has antioxidant effect upon H2O2-treated plasmid DNA. When tested in culture cells, the cell viability of PDE-treated oral cancer cells Ca9-22 was analyzed by MTS assay to examine their cellular response against H2O2. Because several reviews had summarized that the antioxidant has benefit in physical concentration but has a damage effect in severe concentration, we will explore the dosage effect of PDE in the cellular level. The cell cycle, apoptosis, DNA damage, ROS, and mitochondrial membrane potential of PDE-, PDE/H2O2-, or H2O2-treated Ca9-22 cells were analyzed by flow cytometry. Therefore, the nature of PDE against H2O2-induced DNA damage will be explored for a potential chempreventative agent.

P-27

Archazolid a induces anoikis in highly invasive breast cancer cells: a first report on underlying mechanisms

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The vacuolar-ATPase (V-ATPase), a proton pump located at the membrane of acidic organelles, has recently come to focus as a promising cancer target. In tumor cells it was found to be expressed on the plasma membrane where it regulates the extracellular pH of metastatic cancer cells to facilitate migration and metastasis. A prerequisite for metastasis is the ability to overcome detachment induced cell death (Anoikis). It occurs mainly due to the loss of anchorage-dependent survival signals by Integrin-extracellular matrix (ECM) interaction and can be executed by both the intrinsic and extrinsic apoptotic pathway. Anoikis resistant cancer cells establish several mechanisms to circumvent Anoikis like a constitutive activation of Integrin downstream signaling. As V-ATPase inhibitors have shown to prevent migration and invasion of tumor cells we propose that the inhibition of the V-ATPase could induce Anoikis related pathways in Anoikis resistant cancer cells and impede metastasis. We used the novel V-ATPase inhibitor Archazolid A, a macrolide of myxobacterial origin, to investigate the induction of Anoikis related pathways in Anoikis resistant, highly metastatic breast cancer cells (Skbr3). As effects of Archazolid A on Skbr3 cells had to be investigated in an ECM independent state, Skbr3 cells were cultivated on PolyHEMA coated plates to keep them detached. Cells were treated with increasing concentrations of Archazolid A for various periods of time and analyzed for apoptosis and Integrin signaling. Apoptosis was significantly induced in Anoikis resistant Skbr3 cells by 10 nM Archazolid A after 48 h. In a soft agar assay treatment with Archazolid A (1 nM, 10 nM, 24 h) significantly reduced the ability of Skbr3 cells to form colonies concentration-dependent.

Previous results showed that Archazolid A (10 nM, 24 h) leads to an increase of Integrin β1 on the cell surface of adherent cells and simultaneously inhibits its internalization and localization at the leading edge as shown by FACS analysis, immunocytochemistry and confocal microscopy. Furthermore, using an antibody which only recognizes integrin β1 in its active state, FACS analysis revealed that the level of active Integrin β1 on the cell surface of floating cells was significantly decreased when cells were treated with Archazolid A (10 nM, 24 h). This data suggest a role of Integrin in Archazolid A induced Anoikis. To this end, western blot experiments confirmed that the phosphorylation of focal adhesion kinase (FAK), a direct downstream protein of Integrin-receptors, was reduced. Furthermore, the impact of Archazolid A treatment on Caspase-8 was analyzed as Caspase-8 is known to be involved in Anoikis related cell death via Integrins. Archazolid A treatment (10 nM, 48 h) leads to an activation of Caspase-8 measured by a fluorometry based Caspase-activity assay. To more deeply analyze Anoikis signaling we investigated BIM expression – a major player in Anoikis – and found an upregulation and translocation to the mitochondria after 5 h of treatment with 10 nM Archazolid A. We suggest that the V-ATPase inhibitor Archazolid A is a promising compound for treatment of Anoikis resistant, metastatic cancer cells since it induces cell death via impairment of the Integrin β1 survival signal, activation of Caspase 8 and induction of Anoikis related pathways.

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P-28

Cytotoxicity of CSCI-1 in human breast cancer cell

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Cancer stem cells are a subset of cancer cells that initiate the growth of tumors. Cancer stem cells can also be found in established cancer cell lines, albeit in low frequency. They can be enriched by culturing cancer cells in the serum-free tumorsphere culture condition. Since the cancer stem cells have been reported to be resilient to common chemotherapeutic drugs in comparison to the regular cancer cells, screening for compounds selectively targeting cancer stem cells may provide a more effective therapeutic strategy.

P-29

The role of quercetin in the treatment of androgen receptor negative prostate cancer cells and estrogen receptor negative breast cancer cells

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Loss of p27 and overexpression of S phase kinase-associated protein 2 (Skp2), an ubiquitin ligase subunit targets p27 for degradation by the ubiquitin-proteasome system, are correlated with tumor progression in many cancers, such as prostate cancer, breast cancer, and lung cancer. In this study, we found quercetin downregulate the oncoprotein Skp2 inPC3, DU145 and BT483 cells at *24 hour* of *treatment*, but not MDA-MB-231 cells. However, combination treatment for 24h with *Doxorubicin and* quercetin significantly and synergistically enhanced growth inhibition and Skp2 down-regulation in BT483 and MDA-MB-231 cells. However, the down-regulation of Skp2 was not always correlate with the up-regulation of p27, suggesting that quercetin -dependent Skp2 down-regulation can influence cell growth in several ways. Taken together, quercetin is capable of inducing cell cycle arrest through a different extent in prostate and breast cancer cells. Finally, we also demonstrated that quercetin and EGCG inhibit growth of human prostate carcinoma cells through different mechanisms. Both quercetin and EGCG suppress the expression of Skp2 in androgen-independent human prostate carcinoma PC3 cells. However, EGCG treatment results in apoptosis via caspase 3 activation, but quercetin did not induce apoptosis in PC3 cells. Our data suggested the novel molecular mechanisms of prostate and breast cancer growth inhibition by quercetin and implicated their potential therapeutic applications.

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P-30

Beta-caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation

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Both PI3K/AKT/mTOR/S6K1 and mitogen activated protein kinase (MAPK) signaling cascades play an important role in cell proliferation, survival, angiogenesis, and metastasis of tumor cells. In the present report, we investigated the effects of β-caryophyllene oxide (CO), a sesquiterpene isolated from essential oils of medicinal plants such as guava (*Psidium guajava),* oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) clove (Eugenia caryophyllata), and black pepper (*Piper nigrum* L.) on the PI3K/AKT/mTOR/S6K1 and MAPK activation pathways in human prostate and breast cancer cells. We found that CO not only inhibited the constitutive activation of PI3K/AKT/mTOR/S6K1 signaling cascade; but also caused the activation of ERK, JNK, and p38 MAPK in tumor cells. CO induced increased reactive oxygen species (ROS) generation from mitochondria, which is associated with the induction of apoptosis as characterized by positive Annexin V staining, loss of mitochondrial membrane potential, release of cytochrome c, activation of caspase-3, and cleavage of PARP. Inhibition of ROS generation by N-acetylcysteine (NAC) significantly prevented CO-induced apoptosis. Subsequently, CO also down-regulated the expression of various downstream gene products that mediate cell proliferation (cyclin D1), survival (bcl-2, bcl-xL, survivin, IAP-1, and IAP-2), metastasis (COX-2), angiogenesis (VEGF), and increased the expression of p53 and p21. Interestingly, we also observed that CO can significantly potentiate the apoptotic effects of various pharmacological PI3K/AKT inhibitors when employed in combination in tumor cells. Overall, these findings suggest that CO can interfere with multiple signaling cascades involved in tumorigenesis and used as a potential therapeutic candidate for both the prevention and treatment of cancer.

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P-31

Antiproliferative and apoptotic effects of fractionated extracts of *Picralima nitida* on human breast cancer cell line

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Picralima nitida has widely varied applications in Nigeria folk medicine. Many herbalists have claimed to use the leaves, seed and stembark as treatment for various fevers, hypertension, jaundice, gastro-intestinal disorders and for malaria. To establish the proximate analytic parameters and to scientifically evaluate the cell proliferative and apoptotic effects of the crude and fractionated extracts of the plant using breast cancer cell line. The proximate analysis of the crude powdered drug was carried out according to standard methods. The antiproliferative and apoptotic effects of the crude methanolic extract (50 µg/mL) and fractionated extracts (10 µg/mL) were subjected to in vitro evaluation using breast cancer cell line (MCF-7) measured by flow cytometry. The crude powdered plant material has a high moisture content of 21.23% and a total ash value of 9.92%. Among the fractions, the chloroform fraction demonstrated a significant (P<0.001) antiproliferative activity against eostrogen receptor positive breast cancer cell line (MCF-7). The proliferative phase (G2 + S) was decreased by 15% in comparison to the negative control. A significant (P<0.001) apoptotic effect was also observed for the chloroform fraction. Other fractions did not show significant inhibition of proliferation and apoptosis of MCF-7. The root bark extract of *Picralima nitida* especially the chloroform fraction possesses good anticancer activity and can be potentially used as natural anticancer agent, particularly against breast cancer.

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P-32

The degree of redox imbalance influences the chemotherapeutic response of malabaricone-a in leukemic cells vs. solid tumors

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Intrinsically higher basal levels of reactive oxygen species (ROS) enhances cancer cell proliferation and survival, which can potentially be halted by supplementary oxidative assault when provided by anti-cancer compounds. The objective of this study was to elucidate whether generation of oxidative stress by a plant derived diarylnonanoid, malabaricone-A (MAL-A) influenced its cytotoxicity in leukemic cell lines and solid tumor cell lines. The cytotoxicity of MAL-A in leukemic cell lines (U937, MOLT3, CCRF CEM and K562) and solid tumor cell lines (MCF7, A549 and HepG2) was measured by a MTS based cell viability assay, changes in levels of ROS and non protein thiols by flow cytometry using CM-H2DCFDA and CMFDA respectively. Apoptosis was determined by Annexin-V (phosphatidylserine externalization), 10-N-nonyl acridine orange (cardiolipin peroxidation) along with cell cycle analysis by flow cytometry. Concomitantly, activity of caspases and Glutathione peroxidise (GPx) was determined colorimetrically. The cytotoxicity of MAL-A in leukemic cell lines was higher than in solid tumor cell lines, IC50 (mean ± SEM) being 8.70 ± 4.0 vs. 47.51 ± 10.7 μg/mL. Leukemic cell lines had higher basal levels of ROS as compared to solid tumor cell lines and MAL-A increased these basal levels in leukemic cells by 24.92 fold whereas the increase in solid tumors was only 6.3 fold. In leukemic cells, preincubation with an anti-oxidant, N-acetyl-L-cysteine attenuated the cytotoxicity of MAL-A, suggesting that redox imbalance contributed susbtantially towards its cytotoxicity. This did not hold true for solid tumors as N-acetyl-L-cysteine failed to decrease the cytotoxicity of MAL-A, suggesting that ROS partially accounted for MAL-A induced cell death. However, at an IC50 concentration of MAL-A, the initial events of apoptotic cell death were comparable in both cell types, which included peroxidation of mitochondrial cardiolipin, externalization of phosphatidylserine, decreased GPx activity and arrest of cell cycle progression. The redox imbalance induced by MAL-A was greater in leukemic cells than solid tumors which possibly accounted for its 4.4 fold higher cytotoxicity. However, the initial events of apoptosis mediated by MAL-A was comparable in both groups. As MAL-A triggered a more favourable chemotherapeutic response in leukemic cell lines than solid tumor cell lines, it suggests that compounds capable of triggering a redox imbalance are more likely to be effective in leukemias.

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P-33

Evaluation of antioxidant activity of the three species of *Ziziphus* and their fractions

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Today, there is a great attention on natural antioxidants as chemopreventive agents. In the present study, the antioxidant activity of the three species of *Ziziphus* was investigated. Each crude extract was loaded on resin as stationary phase and eluted by water, water:ethanol (1:1) and ethanol, respectively. The *in vitro* antioxidant activity was evaluated by nitric oxide, hydroxyl and DPPH radical scavenging analysis. It was found that all of the extracts have remarkable antioxidant activitiy. Water:ethanol fraction of the samples showed potential free-radical scavenging activity. In the DPPH radical scavenging assay, the IC50 's of the water:ethanol fractions of *Ziziphus jujuba*, *Z. mauritiana and Z. spina christi* were 140.24±2.21, 115.8±1.07 and 174.34±2.52 μg/mL, respectively. The IC50 of standard quercetin was 61.43±0.98 μg/mL. In the nitric oxide radical scavenging activity assay, crude extract of *Z. mauritiana* showed highest antioxidant activity (IC50= 143±9.3 μg/mL). The water:ethanol fractions all of the samples exhibited the greatest hydroxyl radical scavenging effect at concentration of 1 mg/mL. Based on the results of this study, we conclude that active fraction can be effectively used as a preservative and chemopreventive agent.

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P-34

The butanol fraction of guava (*Psidium cattleianum* Sabine) leaf extract suppresses MMP-2 and MMP-9 expression and activity through the suppression of the ERK1/2 MAPK signaling pathway

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The leaf extract of guava (Psidium cattleianum Sabine) has traditionally been used for the treatment of diarrhea and diabetes in East Asia and other countries. Recently, the leaf extract has been employed in the therapy of cancer, bacterial infections, and inflammation in experimental models. However, the exact mechanisms of how guava leaf extract inhibits tumor metastasis and invasion are still unknown. In the present study, we investigated in detail the molecular mechanism(s) responsible for the potential antimetastatic and antiinvasive effects of the butanol fraction of guava leaf extract (GBF). Interestingly, we observed for the first time that GBF suppressed both matrix metalloproteinases (MMP)-9 and MMP-2 expression and activity in part through the downregulation of the ERK1/2 activation in lung cancer cells. Also, importantly, the major components of the GBF were identified as d-glucuronic acid, quercetin 3-glucuronide, loganin, and xanthyletin by LC-ESI-MS/MS. Collectively, our data indicate that the guava leaf could reduce the metastasis of lung cancer cells and therefore suggest that it could be advantageously used to control the metastatic process.

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P-35

Chondramide a inhibits cancer cell growth by the induction of apoptosis and senescence

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The actin cytoskeleton is a crucial component to maintain cellular homeostasis. It is important in processes like muscle contraction, trafficking of cellular compartments, mitosis, invasion and migration. Thus, actin-binding compounds like Chondramide A (Chon A), a cyclic depsipeptide of myxobacterial origin could be developed as potential anticancer therapeutics. Aim of this study was to characterize antitumoral effects of Chon A in mammary cancer cells in vitro. In fact we could show that Chon A inhibits proliferation of two breast tumor cell lines MCF-7 and MDA-MB-231 in a low nanomolar range. The long term survival of these cell lines was impaired by Chon A as the clonogenic survival was also decreased by about 60% (MDA-MB-231) and 80% (MCF-7), respectively. To proof if the observed effects are due to actin overpolymerization, the Triton-X 100 soluble fraction was analyzed for its actin content and showed a time (24h – 72h) and dose dependent decrease, which suggested that Chon A overpolymerized actin to its filamentous form that is not soluble in the Triton-X-100 fraction. Clarifying the mechanisms underlying the antiproliferative effects of Chon A, we examined whether Chon A induces apoptosis and/or premature cellular senescence. Annexin V/PI costaining revealed a concentration dependent apoptotic cell death induced by 500 nM Chon A in both cell lines. Cleavage of PARP, a known substrate of caspase-3, detected by western blotting, supported the induction of apoptosis by Chon A, too. The induction of cellular senescence was cell line specific, as MCF-7 showed an increased β-galactosidase activity, compared to MDA-MB-231 which did not display this hallmark of senescence. The cdk-inhibitor p21, which is important for senescence induction, was also increased with Chon A 500 nM treatment in MCF-7 cells. These results suggest, that the inhibition of growth in MDA-MB-231 is only due to apoptosis induction, whereas in MCF-7 cells both processes apoptosis and senescence are involved. Furthermore, phosphorylation of histone γH2A.X, an established sign for DNA damage, treated with Chon A was observed in both cell lines. To this end, Chon A induces pronounced cell cycle arrest in G2-phase (40% versus 20% in untreated cells). Our investigations reveal that Chon A is a potent inhibitor of breast cancer cell proliferation¸ via induction of apoptosis and premature cellular senescence. Induction of senescence has not yet been reported for actin – binding drugs. Thus, further experiments will focus on the

mechanism underlying senescence induction. One major tool will be PCR and micro-array technologies to possibly find new and valuable players and targets in senescence and growth arrest signaling.

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P-36

Evaluation of cytotoxic effects of *Cistus salviifolius* with alamar blue and MTT assays on HepG2 cells

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The genus *Cistus* has a widespread utilization in Turkish folk medicine. Beside many pharmacological activities, its cytotoxic effects were featured on various carcinoma types including ovarian, lung, central nervous system, breast, column, leukemia carcinomas. Previous cytotoxicity studies are mostly subjected on *C. creticus*[1,2] and there is no study on *C. salviifolius*. The aim of this study is to evaluate cytotoxic potential of *C. salviifolius* which is naturally occuring Mediterranean plant in Turkey. The *n*-hexane, MeOH, water and *n*-BuOH extracts (200, 100, 50, 25, 10, 5 µg/mL) of *Cistus salviifolius* were treated with HepG2 cells for 3, 6, 9, 12, 24, 48 h and Alamar Blue fluorometric and MTT colorimetric assays were performed. Most of the extracts especially MeOH extract of the plant reduced the cell viability within the 24 h. The cytotoxic potential of *C. salviifolius* were investigated and expressive cytotoxic activity were found compared with controls. Secondary metabolites which is responsible for this activity and the act of mechanism should be evaluated with further studies.

P-37

Anacardic acids as inhibitors of quorum sensing-controlled virulence factors of *Chromobacterium violaceum* and *Pseudomonas aeruginosa*

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An emerging problem associated with the indiscriminate use of antibiotics is the selection of resistant bacteria with higher tolerance levels against broad-spectrum antibiotics. The utilization of compounds, which inhibit quorum sensing pathways (IQS), may be a useful alternative to antibiotics since quorum sensing system (QS) controlled phenotypes are considered as a new target for antimicrobial chemotherapy. QS modulates the expression of genes involved in processes of survival, virulence and pathogenicity, such as swarming, biofilm formation and secretion of virulence factors. The IQS molecules do not cause a direct inhibition of microbial viability, but instead repress cell-density dependent phenotypes like the production of virulence factors and biofilms. As result, the bacterium does not develop resistance and the immune system eliminates the infection. Recent investigations have focused in discovering new agents derived from natural products, to handle the bacterial pathogenesis by means of IQS. The discovery of new non-toxic, broad spectrum IQS is important for combating bacterial infections caused by susceptible and antibiotic resistance strains. Traditional Mexican Medicine uses a wide variety of plants in the treatment of bacterial infections. *Amphipterygium adstringens* (Schltdl) Standl (Anacardiaceae) is an endemic tree of Mexico, where is commonly known as "Cuachalalate". The stem bark is used in the form of extracts, infusions or pulverized, to alleviate different ailments of human beings, e.g. stomach cancer, ulcers, gastritis and infections. In this work, we tested the hexane extract (HE) of the stem bark of *A. adstringens* and an anacardic acids mixture (AAm) isolated from the same source, for their ability to inhibit QS-regulated behaviors in the in model system with *Chromobacterium violaceum* bacterium. An effect dose-response was observed in the inhibition of production of the violacein in the cultures by HE, with a 71.7% with a concentration of 6 mg/mL and reaching a maximum effect of 91.6% with 55 mg/mL. For the case of the AAm, also the effect was observed, showing an inhibition of the pigment of 39.9% with a concentration of 2 mg/mL and reaching a maximum effect of 94% with 166 mg/mL. The

HE like the AAm, did not show an antimicrobial effect in *C. violaceum*, which evidence that the inhibition of the violacein production is due to an inhibition of the QS. We also investigated the inhibition of quorum sensing-controlled virulence factors production on the pathogenic bacteria *Pseudomonas aeruginosa*. Our results show that the AAm inhibits the production of the three virulence factors in a dose response manner without affecting the growth, being the maximum inhibitions observed of 75%, 86% and 91% for elastase (with 500 mg/mL), pyocyanin (with 200 mg/mL) and rhamnolipids (with 500 mg/mL) respectively. The anacardic acids are molecules that have been identified in diverse plant species and antibiotic activity has been demonstrated on different bacterial species, nevertheless, our results indicate that also they present a mechanism that involves IQS. This same activity is presented by the EH, which confers a new property to stem bark to fight the infections.

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P-38

Plasmid/polymer nanocomplex: non-viral gene therapy approach for cancer treatment by IL-12

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Recently gene therapy by different cytokines has been introduced as a new strategy for cancer treatment. Among different cytokines, interleukine-12 (IL-12) shows variety of immunomodulatory and antitumor effects. These effects make it as a promising candidate for cancer treatment. Severe toxicity of systemic administration of IL-12 lead to engage local administration of this cytokine in tumor site. Although, delivery of IL-12 gene by viral vectors is effective, safety of this method is uncertain and the other methods (e.g. gene gun and electroporation) are expensive and have less efficiency. Cationic polymers emerge high transfection efficiency to deliver IL-12 gene into tumor site because of higher gene capacity and less limitation on the size of the plasmid in comparison to viral vectors. Successful delivery of plasmid into the nucleus leads to expression of IL-12 which in turn leads to activation, maturation and differentiation of immune cells. Electrostatic interactions between the polymer amine groups and the phosphate groups of plasmid encoding IL-12 lead to DNA condensation and forming nanoparticles (polyplexes). Polyplex formation protects the plasmid from enzymatic degradation. The structure of cationic vectors has been modified to improve the interaction between the polymer and nucleic acid, delivery of nucleic acids into specified cells and reduce toxicity and immunogenicity of the polymers. To achieve efficient cancer immune therapy by IL-12 more experience in various human cancerous cells should be done.

P-39

Embelin inhibits growth and induces apoptosis through the suppression of AKT/mTOR/S6K1 pathways

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Akt/mTOR/S6K1 signaling cascades play an important role both in the survival and proliferation of tumor cells. In the present study, we investigated the effects embelin (EB), identified primarily from the *Embelia ribes* plant, on the Akt/mTOR/S6K1 activation pathways in human prostate cancer cells. We found that EB exerted more significant cytotoxic and suppressive effects of Akt and mTOR activation against androgen-independent PC-3 cells as compared to androgen-dependent LNCaP cells. Moreover, EB suppressed the constitutive activation of Akt/mTOR/S6K1 signaling cascade, which correlated with the induction of apoptosis as characterized by accumulation of subG1 phase, positive Annexin V binding, down-regulation of antiapoptotic (bcl-2, bcl-xL, survivin, IAP-1, and IAP-2) and proliferative (cyclin D1) proteins, activation of caspase-3, and cleavage of PARP. We also observed that EB can significantly enhance the apoptotic effects of a specific pharmacological Akt inhibitor when used in combination and also caused broad inhibition of all the three kinases in Akt/mTOR/S6K1 signaling axis in PC-3 cells. Overall, our results clearly demonstrate that EB inhibits multiple signaling cascades involved in tumorigenesis and can be used as a potential therapeutic candidate for both the prevention and treatment of prostate cancer.

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P-40

The anti-inflammatory effect of *Nuphar lutea* thioalkaloids

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The use of plant extracts to alleviate inflammatory diseases is centuries old and continues to this day. In previous published work we found that *Nuphar lutea* L. SM leaf and rhizome extracts (NUP) inhibit Nuclear Factor kappa B (NF-kB) and exhibit anti-cancer and cytotoxic properties. NF-kB is a transcription activation factor which plays roles in inflammation, immune reactions, carcinogenesis and apoptosis. Based on this information we decided to investigate whether NUP presents anti-inflammatory properties as well. An active fraction containing a mixture of dimeric sesquiterpene thioalkaloids was purified and 6-hydroxythiobinupharidine and 6- hydroxythiobinuphlutine were identified as a major component. We found that NUP protected mice from LPS-induced lethal toxic shock. We asked whether the effect of NUP is correlated with the expression of different pro- and anti-inflammatory cytokines in sera or in thioglycolate induced macrophages. The results show that NUP treatment decreased the expression of pro-inflammatory and increased the expression of anti-inflammatory cytokines. The beneficial anti-inflammatory effect of NUP (given IP) was tested also in a dextran sulfate sodium salt (DSS) induced colitis model in mice. NUP- treated mice achieved a lower Disease Activity Index (DAI). Intestine shortening, erosion of the lamina propria mucosa, disappearance of glandular epithelium and inflammatory cell infiltration as measure of disease, were partially prevented in NUP-treated mice. Over all, these results suggest that NUP can be used as an anti-inflammatory agent and allows further investigation of its mechanism of action.

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P-41

Towards the sustainable and continuous *in-vitro* production of plant derived anticancer compounds

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Medicinal plants have been used for the past millennium to treat various conditions, and more recently especially in the oncology market. Despite an increasing interest in the translation of traditional knowledge of medicinal plants into clinical drugs, progress has been quite slow since the discovery of Paclitaxel and Camptothecin in the 1970s. One aspect which could explain the limited number of complex molecules entering clinical trials and reaching the patient is the restricted supply chain of the plant raw material, thus limiting the availability of Active Pharmaceutical Ingredients (API). Still today, most of the raw materials needed for the extraction of the active ingredients are harvested from cultivated or wild plant populations, posing a threat to the bioavailability of certain medicinal plants and strong variability in the yield of API. We have developed a new *in-vitro* propagation method based on the use of temporary immersion bioreactors that allows for the rapid and abundant generation of a leafy-biomass from medicinal plant cell cultures. We are also developing cryopreservation protocols to fulfil regulatory issues. This technology provides a unique opportunity for the sustainable production of complex APIs which require plant cell differentiation.

P-42

Anticancer activity of natural cytokinins

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Plant hormones cytokinins have been shown to have anticancer activity both in vitro and in vivo. This study presents the systematic analysis of the relationship between the chemical structure of cytokinins and their cytotoxic effects against a panel of human cancer cell lines. Our results confirm the cytotoxic activity of several cytokinins, including isopentenyladenosine, cis-zeatin riboside, kinetin riboside, benzyladenosine and topoline ribosides. The most potent compound was ortho-topolin riboside, with low micromolar IC50 values. The potency of ortho-topolin ribosed was confirmed against NCI60, a standard panel of 60 cell lines with diverse histopathological origins. Importantly, its selectivity is different from the

patterns of a set of established anticancer drugs, suggesting its unique mechanism of activity.

P-43

Antiproliferative effects of eupatorin, an active constituent of extract of *Orthosiphon stamineus*

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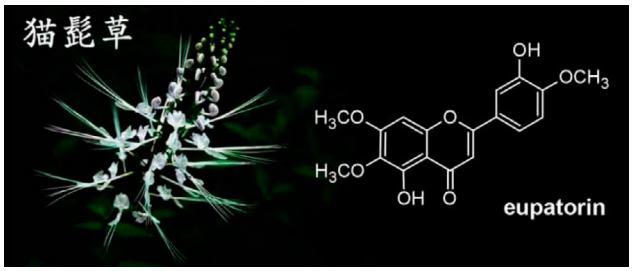
Orthosiphon stamineus is a medicinal herb widely used for many centuries in folk medicine in South East Asia for treatment of various disorders. Extracts of its leaves consist of a wide range of compounds, including terpenoids, organic acids such as rosmarinic and caffeic acid, or flavonoids. The polymethoxylated flavonoids sinensetin, eupatorin and 3′-hydroxy-5,6,7,4′-tetramethoxyflavone have been previously found to be dominant compounds in the chloroform fraction of such extracts.



We investigated the antiproliferative properties of eupatorin in human cell lines. Eupatorin reduced the number of viable cancer cells in the culture with IC50 values in micromolar range. Its antiproliferative effect was linked to arrest in the G2/M phase of the cell cycle, followed by cell death with marks of mitotic catastrophe. The mechanism of antiproliferative and proapoptotic properties of eupatorine are probably caused by its nonspecific inhibition of many protein kinases.

P-44

Anti-proliferative and pro-apoptotic effects of methanolic extract of *Caesaipinia bonduc* and its fractions in estrogen-sensitive human breast adenocarcinoma cell line (MCF-7)

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Caesalpinia bonduc is a plant that belongs to the family *Caesalpiniaceae* and it is used for the treatment of various diseases including Diabetes, asthma, filarial and inflammatory condition. The present study was conducted to determine proximate analysis, apoptotic and antiproliferative effect of the plant against human breast cancer cell line (MCF-7) in an in vitro model. The methanolic extract was fractionated using Petroleum ether, chloroform and ethyl acetate in this order. The MCF-7cells were treated with each extract at concentration of 10 µg/mL for 48 h and DMSO serving as control. The percentage of cells in the various phases was determined by DNA flow cytometer after staining the cells with propidium iodide. Proximate analysis indicated that the dried leaf contained Ash 9.09%±0.01, moisture 10.54%±0.01, Acid insoluble 2.85%±0.01 and water insoluble 5.38% ±0.03, the high Ash content is suggestive of a rich mineral content. The results obtained from the flow-cytometer analysis showed that chloroform fraction was the most active fraction again MCF-7 cells with 32.86% decrease in proliferative phase. The amount of apoptotic cells were calculated based on the appearance of the cells in sub G1. The result showed that there were no significant difference (P>0.05) between percentage of apoptotic cell in treated groups and control group. The finding suggests that the mechanism of cell death is probably through a different mechanism rather than the direct induction of apoptosis in tumor cell. The finding also showed that at 10 µg/mL *Caesalpinia bondus* extract induces antiproliferative effect on MCF-7 cells by arresting the cell cycle at the G2/m phase to prevent the cells transition from G2 to M phase, thereby contributing less to cell division rather than DNA synthesis.

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P-45

Antiproliferative and apoptotic activities of methanolic leaves extract and various fractions of *Cola lepidota* K. Schum (Sterculiaceae) against estrogen receptor positive (ER +) breast cancer cells

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To evaluate scientifically the antiproliferative and apoptotic effects of the methanolicextracts and other fractions of stem *Cola lepidota* using breast cancer cell lines in order to validate its ethnopharmacological usage in Nigeria folklore medicine as herbal remedy for cancer related ailments. The powdered leaves of the plant were extracted exhaustively with methanol using soxhlet apparatus and concentrated to dryness in vacuo. The methanolic (CLL-M) extract was further partitioned into three fractions using different analytical grade (Sigma-Aldrich, Germany) solvents: petroleum ether (CLL-P), chloroform (CLL-CH) and ethyl acetate (CLL-EA) in increasing order of polarity. Antiproliferativeand apoptotic effects of the crude methanolic extract (50 µg/mL) and fractions (10 µg/mL) were subjected to in vitro evaluation using breast (MCF-7) cancer cell line by the flow cytometric method using DMSO as control. Bright field images after 48 h treatment of the MCF-7 cells with the solvent (0.01% v/v DMSO) only and with 50 µg/mL of methanolic and chloroform fractions revealed significant reduction of cell growthby decreasing proliferation rate and causing enhanced cell death (apoptosis) or a combination of these two mechanisms in contrast to the DMSO control. This was not only observed by the distribution of the cell cycle phases but also visible under bright field microscopy. Both treatments caused higher detachment rates of the MCF-7 cells so that the cells became rounded and lost their adhesion to the culture dishes. In contrast to the well spread cells under control conditions, cells treated with the methanolic and chloroform fractions were smaller and showed some morphological changes. Especially the chloroform fraction caused apoptotic alterations of the MCF-7 cells presumably because of cell dehydration, an early event in apoptosis, had been triggered (Darzynkiewicz et al. 1997). Both the methanolic fraction (CLL-M), and ethyl acetate (CLL-EA) fractions of the leaves and chloroform (CLL-CH) fractions of of *Cola lepidota* were efficient in inhibiting breast cancer cell line proliferation(arrest cell proliferation at the (G2+S) phase) in non-cytotoxic concentrations. This lends credence to the ethno-medicinal uses of the plant, and could therefore be potential sources for pharmacologically active products suitable for development as chemotherapeutic and chemopreventive agents. Fraction CLL-CH demonstrates significant induction of apoptosis pathway.

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P-46

Derivatives of natural 2,3-dehydrosilybin as angiogenesis modulators

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Angiogenesis is a complex physiological process through which formation of new blood vessels occurs from pre-existing ones. Its role is essential in ovulation, embryonic growth, placenta development, wound and injury healing and especially in growth and metastases of most solid malignancies. Excessive or insufficient angiogenesis is connected with many different human diseases (e.g. macular degeneration, rheumatoid arthritis, psoriasis, cancer, infertility, ulcers, heart diseases, obesity, teratogenesis). Plant kingdom is a rich source of biologically active natural compounds and up to date, many phytochemicals have been tested for antiangiogenic properties. Flavonolignan silybin, isolated from milk thistle (*Silybum marianum*), is a popular flavonoid possessing multiple biological activities operating at various cell levels including anticancer and antiangiogenic properties. We have recently demonstrated an influence of galloyl moiety in position 7 of silybin resulting in increase of its biological (antiangiogenic) activity (*1*). In the present study, a minor constituent of milk thistle extract (2,3-dehydrosilybin, DHS) and a series of its methylated and di-methylated derivatives were tested for antiangiogenic activity in a variety of *in vitro* tests with human umbilical vein endothelial cells (HUVEC). Primary screening was performed using MTT cytotoxicity

and wound healing migration tests. Subsequently, capillary-like tube formation of HUVEC on Matrigel and cell proliferation were evaluated. In HUVEC cell migration test, minimal inhibitory concentrations around 10 μM were observed for all these compounds except 3,7-dimethyl-DHS, where only partial inhibition was observed at 50 μM. Similar results were obtained using Matrigel tube formation inhibition test with significant inhibition by DHS already at 5 μM (93.7 ± 2.0% of control). In contrast, 3,7-dimethyl-DHS was virtually without effect up to 30 μM and quite slight (although significant) effect was observed for 3-methyl-DHS (70.3 ± 11.8% at 20 μM). Finally, all the compounds tested inhibited HUVEC proliferation with IC50 values 5.6 ± 0.28, 4.1 ± 0.45, 5.1 ± 0.27, 7.4 ± 0.14, 4.4 ± 0.34, 7.3 ± 0.66, 5.3 ± 0.11, 2.7 ± 0.32 μM for DHS, 3-methyl-DHS, 5-methyl-DHS, 7-methyl-DHS, 20-methyl-DHS, 3,7-dimethyl-DHS, 3,20-methyl-DHS and 7,20-methyl-DHS, respectively. From these results, we can conclude that both 3- and 7-hydroxy groups are required for antiangiogenic activity of 2,3-dehydrosilybin.

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P-47

New natural compounds of *Dionaea muscipula* as anticancerous agents

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Since centuries, natural compounds are used in traditional medicines. Recent studies showed that such substances are able to block the appearance and the development of many diseases such as cancers. The discovery and characterization of new molecules will permit to fill out the drug library of new therapeutic molecules that needs to become greater due to the emergence of new diseases and the appearance of resistance towards conventional treatments. The Venus Flytrap (*Dionaea muscipula* Solander Ex Ellis) is the most well known carnivorous plant. Inherent from the United States, this plant catches and digests small preys with an active trap. It was already described that this plant contains many therapeutic molecules previously described in other plants like myricetin, quercetin, plumbagin and ellagic acid but any specific natural compound has been discovered till today in *Dionaea muscipula*. The aim of this project is to discover and to characterize new and/or specific therapeutic molecules issued from *Dionaea muscipula*. We prepared methanolic leaves extracts of *Dionaea muscipula*. These extracts were shown to be uptaken by K562 cells and to be cytotoxic toward five different leukaemia cell lines (HL-60, Jurkat, K562, Raji, U937) and four adherent cancer cell lines: A549 (lung), MDAMB-231 (breast), PC-3 (prostate), SAOS-2 (bone) but have no effect on HT-29 cells (colon). Purification of the extract by preparative HPLC-MS has shown that the extract contains 7 major compounds and that among these different substances, at least one is unknown and exerts cytotoxicity towards K562 cells. Analysis of cell death by fluorescence microscopy after Hoechst/propidium iodide staining showed that this cytotoxic fraction induces cell death mainly by necrosis. Preliminary results showed that this fraction was till now unknown as therapeutic substance and will be identified by NMR.

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P-48

Extracts of *Acalypha alopecuroidea*: extraction, fractionation and anticancer activity

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Our previous work demonstrated the anti-inflammatory, anti-proliferative and pro-apoptotic activities of *Acalypha alopecuroidea*, an endemic plant in parts of Central America, used by Native Americans in traditional medicine. This study describes preparation of extracts from shoots, leaves and inflorescences, their purification by solid phase extraction, and fractionation by high-performance liquid chromatography. The anticancer activity of the extracts and each of the fractions was

monitored by measuring their cytotoxic effects on malignant human cancer cell lines. The root extracts displayed the highest cytotoxicity of the tested extracts, for which IC50 values against the CEM cell line were less than 0.4 mg/mL (70% ethanol extract) and 0.9 mg/mL (methanol:tetrahydrofuran extract), respectively. The root extracts exhibited strong cell cycle inhibitory activity and induced caspase-dependent apoptosis.

P-49

Cinnamic acid as a novel inhibitor of COX-2 expression

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Inflammation is considered a cancer-promoting factor. Cyclooxygenase-2 (COX-2), the inducible form of the family of cyclooxygenases is an important mediator of inflammation, which has been found to be constitutively expressed in many forms of cancer including breast, colon or prostate. A number of studies show that COX-2 is stably expressed since the early pre-neoplastic stages. This encourages us to consider COX-2 as a potential target in chemoprevention as well as in the treatment of cancer. Synthetic inhibitors of COX-2, which target its enzymatic activity, are the only clinical strategy to counteract COX-2. However, these compounds present severe side effects, a fact that limits their prolonged intake, like requested in chemoprevention or during anti-cancer treatment. An alternative strategy to target COX-2 functions is at the level of its gene expression. A number of studies show that several natural compounds including curcumin, resveratrol or apigenin preferentially target COX-2 expression without showing toxicity. Our study analyses of the effect of cinnamic acid, a natural compound derived from *Cinnamonium cassia* on COX-2 expression during carcinogenesis, with the final perspective to evaluate its potential in chemoprevention. For our chemopreventive purposes, we have used the non-carcinogenic breast cell line MCF10A, stimulated with the phorbol ester 12-phorbol myristate 13-acetate (PMA), which typically induces COX-2. We show a reduction of induced COX-2 expression after treatment with different concentrations of cinnamic acid (1-50 mM). This regulation takes place at both mRNA and protein levels. The results show that cinnamic acid is efficient in reducing the stability of COX-2 mRNA even when used at the lowest concentration tested (1mM). Moreover, an impact on p38 and Akt activation was observed. The concentrations used do not show any toxicity. This encourages us to further investigate the potential of cinnamic acid as a new COX-2 targeting agent and to evaluate its impact on cancer cell models that stably express this enzyme.

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P-50

Diet supplemented with UA or EGCG confer protection against DNA damage in colonocytes

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Diet is an important factor in colorectal cancer. High fat diets are considered a risk factor for the development of colon cancer as they increase the content of bile acids in the colon. Bile acids have shown to induce the formation of reactive oxygen and nitrogen species, and these, in turn, induce DNA damage. On the other hand, diets rich in fruits and vegetables have shown preventive effects on colon cancer. A recent study from our lab showed chemopreventive effects of natural compounds *in vitro* by protection against oxidative DNA damage and stimulation of DNA repair. In this study, we evaluated the effects of *in vivo* consumption of two natural compounds, (ursolic acid (UA) and epigallocatechin gallate (EGCG)), and a bile acid, deoxycholic acid (DCA), on DNA damage in colonocytes and lymphocytes isolated from Fischer 344 rats. These compounds were provided in the diet and administered daily for two weeks. Endogenous DNA damage (strand breaks, oxidized and alkylated bases) was evaluated using the Comet assay. Also, H2O2 and MMS were used, *ex vivo*, to investigate the potential of our natural compounds to protect against oxidative and alkylating damage, respectively. This study demonstrated that endogenous DNA damage in colonocytes was slightly higher than in lymphocytes. UA and EGCG decreased the levels of endogenous DNA damage in colonocytes, while

in lymphocytes, only UA had preventive effects. There was a significant increase of DNA damage with H2O2 treatment when compared with endogenous DNA damage in colonocytes, while treatment with MMS showed a tendency to increase DNA damage but was not significant. UA protected against both types of induced DNA damage, while EGCG only protected against H2O2-induced damage. According to the literature, DCA induces DNA damage *in vitro*, however after two weeks of *in vivo* DCA treatment, increase of endogenous DNA damage in colonocytes or lymphocytes was not observed in this study. UA and EGCG protected colonocytes and lymphocytes against DNA damage. These results suggest that UA can protect DNA from both endogenous and induced DNA damage in both cell types. EGCG was found to protect only against endogenous and H2O2-induced DNA damage in colonocytes. Further studies are undergoing to verify the potential of these compounds on induction of DNA repair systems, specifically base excision repair, mismatch repair, and direct repair by O^6-methylguanine DNA methyltransferase.

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P-51

Lysophosphatidic acid increased cell proliferation and colony formation in human prostate cancer PC-3 cells

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Prostate cancer is the most common form of cancer in men and the second leading cause of cancer deaths in men. Several chemotherapeutic drugs have been shown to be potentially effective in the patient with prostate cancer. Docetaxel, estramustine and mitoxantrone are used for prostate cancer treatment. Recently, many phospholipid mediators have received much attention because of their various biological activities. Lysophosphatidic acid (LPA) (1- or 2-acyl-sn-glycerol 3-phosphate) is one of the most interesting phospholipid mediator with multiple biological functions in various human diseases. In spite of its simple structure, it evokes various cellular responses including cellular proliferation, prevention of apoptosis, cell migration, cytokine and chemokine secretion, smooth muscle contraction and neurite retraction in various cell types. Prostate cancer cells as the other cancer cells are able to secrete LPA and to use it to regulate cell proliferation and migration. In the present study, we investigated the effects of LPA, docetaxel, estramustine and mitoxantrone on proliferation, colony formation and apoptosis of PC-3 cells. In this study, PC-3 cells were treated with LPA, docetaxel, estramustine, mitoxantrone, docetaxel + LPA, estramustine + LPA, and mitoxantrone + LPA. We chose 24 h incubation time, which is the most increased population of PC-3 cells. Cell proliferation assay kit was used to determine the cell proliferation of all groups. Colonies were fixed in absolute methanol and stained with 1% crystal violet and colony formation observed in these PC-3 cells. Apoptosis was detected by flow cytometric annexin V binding assay. Lysophosphatidic acid treatment increased cell proliferation of PC-3 cells compared to control group. Treated cells with docetaxel + LPA, estramustine + LPA and mitoxantrone + LPA increased cell proliferation of PC-3 cells compared to docetaxel, estramustine and mitoxantrone groups respectively. Lysophosphatidic acid increased colony formation. Docetaxel, estramustine and mitoxantrone decreased colony formation. Docetaxel + LPA, estramustine + LPA and mitoxantrone + LPA increased colony formation compared to docetaxel, estramustine and mitoxantrone groups respectively. Lysophosphatidic acid inhibited docetaxel, estramustine and mitoxantrone induced apoptosis. The data demonstrated that LPA increased the percentage of cell viability. Treating PC-3 cells with docetaxel, estramustine and mitoxantrone increased the percentage of apoptotic cells. In conclusion, LPA significantly increased cell proliferation and colony formation of PC-3 cells. Treated PC-3 cells with docetaxel, estramustine and mitoxantrone decreased cell proliferation, colony formation, and induced apoptosis. The combination of LPA with docetaxel, estramustine and mitoxantrone can promote the proliferation, colony formation of PC-3 cells. Lysophosphatidic acid protects PC-3 cells against docetaxel, estramustine and mitoxantrone induced apoptosis.

P-52

Antiproliferative effects of the *Holodiscus discolor* (Pursh) Maxim. leaves and flowers infusions

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Holodiscus discolor (Pursh) Maxim., (Rosaceae), called cream bush or ocean spray, have had a wide fulfillment in traditional medicine of indigenous peoples in Pacific Northwest, particularly in treatment of viral and skin diseases. Seeds have been used in the treatment of black measles, smallpox, chicken pox and as a blood purifier.

Bark has been used as a tonic, eyewash and in ointments on the burns. Leaves and flowers have been used against influenza, sores and diarrhoea. Since creambush has been used in various skin diseases at most topically, we decided to study antiproliferative/cytotoxic effects of flowers and leaves water extracts on human skin carcinoma cells (A 431). Extracts of the flowers (HDF) and leaves (HDL) were prepared according to Czecho-Slovac Pharmacopoea IV (PhBs IV, 1987). Dried powdered material (10 g) was extracted with water and both extracts (100g each) were lyophilized (yield: 22,07% HDF; 18,70% HDL). The time (24h, 72h) and dose (1 – 150 μg/mL) dependent growth inhibitory effects were studied using two tests indicating different cell compartments injury: 1/ LDH assay for cell membrane integrity, (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega) measuring LDH release via damaged cell membrane and 2/ MTT assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega), which is dependent on the mitochondrial dehydrogenases from living cells. The results from dose response curves are expressed by values ED50 and by max. effect (%) inhibition of cell proliferation and max. LDH release. Longer time of exposure (72 h) resulted in lowered ED50 values for both extractsP and tests applied, when significant higher effectiveness of HDL (vs HDF) was present. The extent of maximal effect on both, intracellular/mitochondrial level and plasma membrane level was nearly identical. The A431 cells are more prone to mitochondrial injury (time dependent increase of harmful effect) than to the damage of membrane integrity (time independent effect).

extract	test	ED_{50} (μg/ml)		max. effect (%)	
		24 h	72 h	24 h	72 h
HDF	MTT	67.9 ± 1.3[a]	50.1 ± 1.2*[b]	47.6 ± 2.7	29.7 ± 3.0*
	LDH	109.4 ± 1.0[c]	70.5 ± 1.0*	17.0 ± 3.7	18.6 ± 0.5
HDL	MTT	41.2 ± 1.1[a]	31.9 ± 1.3*[b]	45.3 ± 3.2	26.2 ± 4.4*
	LDH	90.4 ± 1.1[c]	71.9 ± 1.0*	21.4 ± 4.6	17.7 ± 0.3

ACKNOWLEDGEMENTS

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P-53

Selective induction of apoptosis by the isolated compounds from *Foeniculum vulgare* Mill. on human cancer cells

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South Africa has a wealthy supply of plants (about23 500species of higher plants) together with a high degree of endemicity (36.6%) in the indigenous South African flora, of which 4000 plant taxa are ethnomedicinally used and approximately 500 species are used in traditional medicine by an estimated 70% South Africans on a regular basis. These plants are used either separately or in combination. Few data and scientific information exist for ethnomedicinally or traditionally medicinal plants used in South Africa. Nowadays, extensive interest is given to natural products especially plant derived natural products that show various pharmacological properties (including cytotoxic) and cancer chemo-preventative effects. Therefore, South Africa has huge potential in identifying novel compounds to treat many diseases. Seven plants belonging to the Asteraceae, Apiaceae, Ebenaceae, Euphorbiaceae, Hypoxidaceae, and Alliaceae families were selected for the present study. These plants (*Artemisia afra*, *Centella asiatica*, *Euclea natalensis*, *Euphorbia ingens*, *Foeniculum vulgare*, *Hypoxis hemerocallidea*, and *Tulbaghia violacea*) were selected because they are used by a traditional healer, in Cape Town, as a mixture which he gives to his cancer patients. The ethanol extracts of seven plant species (ethnobotanically selected) were also tested for their cytotoxicity, assayed by the XTT assay, against four human cancer cell lines at concentrations ranging from 0.78 to 100 μg/mL. Of all the ethanol extracts, *Foeniculum vulgare* was found to have the best activity on HeLa cells, which exhibited an IC50 value of 19.97± 0.048 mg/mL. Therefore, it was selected for isolation of the bioactive principals. The extract of *Foeniculum vulgare* was fractionated using column chromatography with hexane and ethyl acetate at different ratios as eluent. Two known compounds, '4-methoxycinnamyl alcohol' and 'syringin' were isolated. The IC50 values of '4-methoxycinnamyl alcohol' and 'syringin' were found to 7.82 ± 0.28 mg/mL and 10.26 ± 0.18 μg/mL respectively on HeLa cells. Both compounds were tested for their cytotoxicity against U937 cells and also on peripheral blood mononuclear cells. At the concentrations of 10 and 100 mg/mL '4-methoxycinnamyl alcohol' showed similar cell proliferation as that of the positive control 'cisplatin'. 'Syringin' however, had much lower cytotoxicity on the U937 cells than '4-methoxycinnamyl alcohol'. IC50 was found to be 91.14 ± 0.63 mg/mL. Both 'syringin' and '4-methoxycinnamyl alcohol' were not cytotoxic at concentrations of 1 and 10 μg/mL on the PBMCs as compared to cisplatin. '4-Methoxycinnamyl alcohol' was selected based on its activity on the cancer cells, for further investigation with regard to its mechanism of action. On gel electrophoresis it did not showed a typical ladder pattern, instead a characteristic smear resulted which indicated necrosis. The ethanol extract of *F. vulgare* warrant further investigation to be considered for their potential as anticancer agents. Even though a large number of molecules exhibit anticancer activity *in vitro*, only few are able to induce anticancer activity without killing normal cells in clinical trials. There is however, a large gap between *in vitro* and *in vivo* studies. Therefore, new strategies are needed for discovering new anticancer agents that validate their efficacy and safety.

ACKNOWLEDGEMENTS

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P-54

Bigelovii a, a new nortriterpene saponin, exhibits potent antitumor activities on HL-60 cells

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Salicornia L. (Chenopodiaceae) is a genus of annual apparently leafless halophytic herbs that have articulated, succulent stems. *Salicornia bigelovii* was evaluated as an oil seed crop and seasoned vegetable with direct seawater irrigation (Glenn et al., 1991). Although the economic value of this plant has already been discovered and developed, its medicinal value has been ignored for a long time inChina. Investigation of characteristic constituents of Salicornia bigelovii Torr. led to isolation of three known oleanane-type triterpenoid glycosides and four 30-nortriterpenoid glycosides with two of them new. All compounds were isolated for the first time from Chenopodiaceae. Thus all the compounds were evaluated for their cytotoxicity and a new 30-nortriterpenoid glycosides, named Bigelovii A, showed moderate activity against four cell lines, HL-60 (promyelocytic leukemia), MCF-7 (breast carcinoma), HepG2 (liver carcinoma) and A549 (lung carcinoma),with IC50 values of 6.18, 78.08, 13.64 and >100 μM (Wang et al, 2012). Apoptosis and necrosis are two typical types of cell death (Wu et al, 2010). Apoptosis is characterized by several biochemical criteria such as internucleosomal DNA cleavage, caspase signaling activation, and the release of intermembrane mitochondrial proteins (Thornberry and Lazebnik, 1998). Using HL-60 cell line as a model of cancer cells, we found that treatment of Bigelovii A rapidly induced cell death. Bigelovii A -induced cell death in these cells displayed typical characteristics of apoptotic cells including membrane blebbing, cell condensation, chromatin condensation by hoechst staining, as well as an early biochemical marker of apoptosis, externalization of phosphatidylserine on the plasma membrane by Annexin V/PI binding. Flow cytometry confirmed that treatment with Bigelovii A increased the fraction of cells with hypodiploid DNA and that this effect was concentration- and time-dependent. In order to elucidate the apoptosis pathway, we investigated the effect of Bigelovii A on the level of Bcl-2 family by RT-PCR and Western blotting. Bigelovii A down-regulated the expression of anti-apoptotic gene bcl-2 and bcl-xl while it had no obvious influence on bax. Besides, Colorimetric assay and western blotting suggested that Bigelovii A -induced apoptosis was through the caspase-mediated pathway, by activation of caspase-3. Furthermore, a lactate dehydrogenase (LDH) release test suggested that an Bigelovii A-cholesterol interaction led to the rearrangement of the lipid bilayer and to subsequent cell membrane impairment. Taken together, these findings demonstrate that the new nortriterpenoid glycosides Bigelovii A may exhibit cytotoxic activity against HL-60 cells by inducing apoptosis via bcl-2 and bcl-xl suppression, caspase activation and also membrane permeabilisation.

P-55

Anti-melanoma activity of isomalabaricane triterpenes

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Melanoma is one of the most malignant cancers that causes over 70% of the mortality by skin cancers in Caucasian populations, but effective treatment is limited by its rapid metastasis, low response rates and fast development of resistance to chemotherapy. New modalities and more potent chemotherapeutic agents are urged to attenuate this highly fetal disease. About 50% of proteins in melanoma cells are synthesized and glycosylated in the rough endoplasmic reticulum (ER) before they are secreted to the Golgi complex and target organelles. Glycosylation is an enzymatic process through which an oligosaccharide is conjugated to a protein for proper physiological events. Aberrant glycosylation not only interferes with protein maturation but also initiates ER stress and unfolded protein responses, and triggers cell death. Disruption of glycosylation has been a novel therapeutic strategy for melanoma. In a continuing search for bioactive natural products, it was found that isomalabaricanes, a small class of rearranged triterpene metabolites obtained from marine sponges, inhibited the growth of melanoma cells by an induction of abnormal protein glycosylation, ER stress and cell death.

P-56

The 1,8-naphthalimidic derivatives of uridine as a potentially selective intercalators

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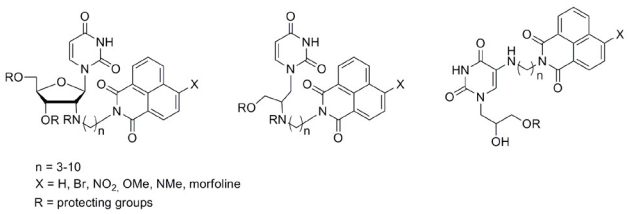
The derivatives of 1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione (1,8-naphtalimide) demonstrates good DNA-targeting anticancer activity. Bisnaphthalimides Elinafide and Bisnafide consisting of two naphthalimide residues connected by an aminoalkyl linker have been reported to inhibit topoisomerase II by acting as the bisintercalators in the major groove of DNA. The most active mononaphthalimides Amonafide and Mitonafide have also been reported to inhibit topoisomerase II. However, the development of Mitonafide was stopped in phase II of clinical

tests, the Amonafide completed clinical phase I and II trials. Amonafide and Mitonafide differ only in the substituent at C-5, having an NH2 and a NO2 group, respectively.



The potency of the antitumor properties of the naphthalimide derivatives made their interesting as potent modifications of the natural nucleosides. The connection of the intercalator moiety to the one of the natural nucleic acid component or its part, can effect in increasing of its bioavailability and selectivity towards with the target. The synthesis of three series of the uridine derivatives will be discussed (Figure). The naphthalimide moiety will be attached to the uridine and its acyclic analogs by amino group placed at the 2' or 5 carbon atom. To the connection of the intercallator moiety to the nucleoside we will use short aminoalkyl linkers.

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P-57

Isolation, identification and quantification of cytokinin nucleotides by high performance liquid chromatography and capillary electrophoresis

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Several naturally occurring riboside members of the plant hormone family of cytokinins as well as some of their synthetic derivatives showed cytotoxic properties against various human cancer cell lines. The mechanism of action is still not fully understood, though. We present here a survey of methods used for isolation of intracellular metabolites from cytokinin riboside treated cancer cells and their subsequent identification and quantification. Trichloroacetic acid is used for protein precipitation and metabolite extraction. The combination of high performance reversed-phase liquid chromatography with tandem mass spectrometry and/or quadrupole time-of-flight mass spectrometry is the main tool for new metabolites identification. The effect of the cytokinin riboside treatment on the energy state of the cells (ATP : ADP : AMP ratio) is monitored by capillary electrophoresis with diode-array detection.

ACKNOWLEDGEMENTS

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P-58

Screening of new bulgarian microalgal strains for antitumor activity

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During the last few decades there has been a renewed interest in natural products with anticancer activity. Recent trends in drug research from natural sources have shown that microalgae and cyanobacteria are promising organisms to furnish novel biochemically active compounds. Although a large number of strains have been shown to possess antitumor activity, the microalgal and cyanobacterial potential is still largely unexplored. The aim of our study was to evaluate and compare the abilities of seven new Bulgarian microalgal strains, belonging to Cyanophyta and Chlorophyta, to induce growth inhibition on HeLa tumor cell line. The cytotoxic effect of extracellular (cell-free culture liquids and exopolysaccharides) and intracellular substances (crude water and ethanol extracts; fatty acid mixtures) was evaluated, using MTT test. All investigated strains showed growth inhibitory activity of at least two tested extracts/constituents. The crude hot water extract of *Gloeocapsa* sp. R-06/1 and ethanol extracts of *Chlorella* sp. R-06/2 and *Gloeocapsa* sp. R-06/1 led to about 80% decrease in HeLa cells viability. The most active cell-free culture liquid was from *Synechocystis* sp. R10. The exopolysaccharides, isolated from *Gloeocapsa* sp. R-06/1 were seven times more active (IC50 = 24.4 μg/mL) than these of *Synechocystis* sp. R10. The free fatty acid mixtures from *Chlorella* sp. R-06/2, *Synechocystis* R10 and *Coelastrella* sp. showed the most potent anti-tumor activity (IC50 < 15 μg/mL) and these from *Scenedesmus incrassatulus* R-83 and *Gloeocapsa* sp. R-06/1 were slightly less active (IC50 < 30 μg/mL). In

conclusion, the most promising strains for further study are the thermal cyanoprocaryotes *Synechocystis* sp. R10 and especially *Gloeocapsa* sp. R-06/1, which showed the strongest inhibition of tumor cell growth and produced wider range of bioactive compounds. Our results justify the efforts of screening microalgal strains of local habitats.

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P-59

Studies on the secondary metabolites from the formosan soft coral *Paralemnalia thyrsoides*

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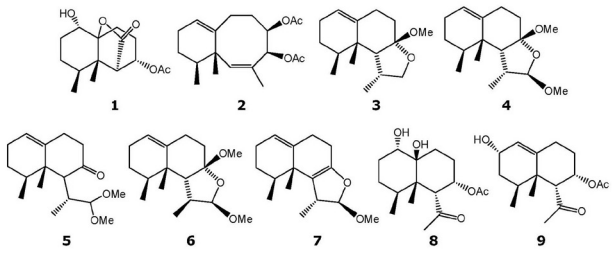
Soft corals of the genus *Paralemnalia* have been found to be a rich source of sesquiterpenoids of nor-nardosinane, nardosinane, neolemnane, aristolane, eremophilane, and related skeletons. First investigation on the chemical constituents of this coral collected at San-Shen-Tai has led to the isolation of nine new compounds (1–9), including one with dinor-nardosinane (novel skeleton)(1), one neolemnane (2), five nardosinanes (3–7), two nor-nardosinanes (8 and 9)5 along with twenty-three known compounds.



The structures of these compounds were determined on the basis of their spectroscopic analysis (^{1}H NMR, ^{13}C NMR, 1H–1H COSY, HSQC, HMBC, IR and HRESIMS) and by comparison of the physical and spectral data with those of the related known compounds. The relative stereochemistry and assignments of 1H NMR chemical shifts were determined by NOESY and coupling constants. The absolute stereochemistry of 1 was determined by application of the Mosher's method. Compounds 1–9 exhibited significant cytotoxic activity against A549, HT-29, P-388 cancer cell lines.

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P-60

A study on the evaluation criteria mandatory for anti-cancer drug approval based on clinical data

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According to [Regulation on Drug Product Authorization, Declaration, & Review], if no alternative drug product or therapy is available or if it is difficult to perform therapeutic confirmatory clinical study of a specific indication because the number of patients for clinical studies in Korea and/or foreign countries is very few (e.g., cholangiocarcinoma or other cancer with lower incidences as listed in statistical data on cancer published by the MHWFA), data on therapeutic exploratory clinical study may be submitted, in lieu of data on therapeutic confirmatory clinical study. But we don\'t have prepared the specific guideline to evaluate clinical study of anti-cancer especially for cancer with lower incidences. In this study, we analyzed the recent guidelines concerning clinical study of anti-cancer from national and international, the detailed cases and instruction made up for the guideline by discussion with practitioners. This guideline contains specific explanation of design of clinical study, criteria for subjects selection, primary endpoint, range of the primary endpoint. This guideline would be able to increase the reliability for the result of clinical study of cancers with lower incidences. To related personnel, it can help to perform through the scientific and systematic ways. By establishing the domestic guideline, we are expected to improve the reliability on domestic clinical study and international harmonization.

ACKNOWLEDGEMENTS

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P-61

A new oleanan type saponin from *Bellis perennis* through antitumoral bioassay-guided procedures

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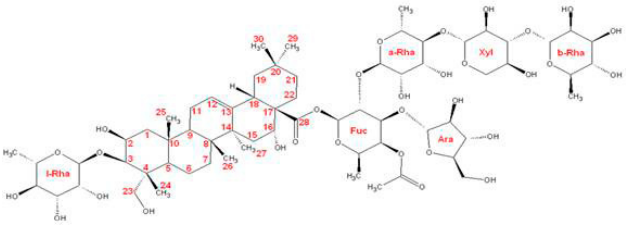
Bellis perennis (common daisy) has been used in the treatment of common cold, stomachache, eye diseases, eczema, skin boils, gastritis, enteritis, diarrhea, bleeding, rheumatism, inflammation, and infections of the upper respiratory tract in traditional medicine. Antitumoral activity of butanol extract of flowers of *B. perennis* was evaulated by using Potato Disc Tumor Induction Bioassay.



Through bioassay-guided fractionation and isolation procedures a novel saponin 3-*O*-α-rhamnopyranosyl polygalacic acid 28-*O*-{α-rhamnopyranosyl-(1→3)-β-xylopyranosyl(1→4)-α-rhamnopyranosyl-(1→2)-[α-arabinofuranosyl-(1→3)-4-*O*-acetyl-β-fucopyranoside]} (1) was isolated from the active fraction (93% inhibition). The structure elucidation of the isolated compound was accomplished by spectroscopic methods (1D- and 2D-NMR, and ESI-TOF-MS). Camptothecin was used as positive control for tumor induction bioassay.

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P-62

Antitumor activity of 9-deazapurines resembling to myoseverin

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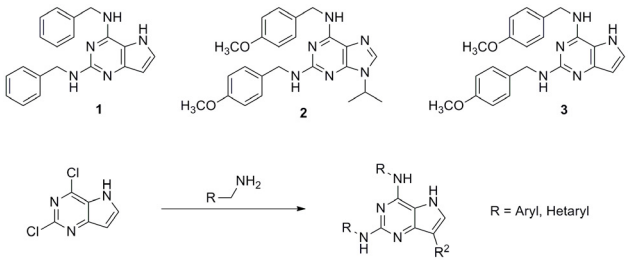
Recently we identified a potent antiproliferative activity of a series of 2,6-diamino-9-deazapurines. Among them, 2,6-bis(benzylamino)-9-deazapurine (1) showed IC50 on CCRF-CEM at 4.0 μM. We were interested, if the antitumor activity could be improved by an approximating the candidate molecules close to the structure of myoseverin (2) – a trisubstituted purine derivative which inhibits the microtubule assembly.



Microtubule interfering agents are generally known by their antitumor potential. When we prepared the derivative bearing the same substituent at amino groups as myoseverin – 2,6-bis(4-methoxybenzylamino)-9-deazapurine (3) – the IC50 on CCRF CEM retained in the same rank (10 μM), but the solubility in water was improved. However, the further approximation to myoseverin by adding an alkyl substituent to the position 9 decreases the water solubility. A short SAR study will be presented. Despite to the resemblance to myoseverin, the mechanism of action remains unclear and should be further investigated.

ACKNOWLEDGEMENTS

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P-63

Cytotoxic effects of peptides from enzymatic hydrolysates of some medicinal plant proteins

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To search for cytotoxic peptides derived from medicinal plants, crude protein extracts obtained from 22 medicinal plants were hydrolyzed with a proteolytic enzyme. All hydrolysates showed different degrees of hydrolysis and antioxidant activities. The hydrolysates were then screened for cytotoxic activity against three human cancer cell lines including KB, oral cavity epidermoid carcinoma; MCF7, breast adenocarcinoma and NCI-H187, small cell lung carcinoma using the Resazurin microplate assay (REMA). Hydrolysates from eight plant species were cytotoxic against indicated cancer cell lines. Hydrolysate prepared from bark of *Acacia catechu* (L.f.) Willd showed the highest cytotoxicity against all tested cancer-cell lines with highest selectivity to the MCF7- breast cancer cell. The results of this study suggest that *Arcangelisia flava* Merr. protein hydrolysate is a good source of natural anticancer agent.

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P-64

Potential anticancer properties of lupane-type saponins

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Lupane-type triterpenoids are natural products found worldwide in vegetables, fruits and plant species exhibit promising anti-inflammatory, anti-HIV and antitumour activities. The synthesis of lupane-type saponins i.e., sugar derivatives, seems to be a good way to improve their ability to enter target cells via interactions with mannose receptors and increase their water solubility, thus providing a convenient drug delivery strategy. We evaluated *in vitro* cytotoxic activity for parent triterpenoids (lupeol, betulinic acid, betulin and their acetylated derivatives) as well as their mono-and trisaccharide derivatives and mono- and trimannosides. We compared anticancer activity of tested analogues against normal human BJ fibroblasts and cancer cell lines of various histopathological origins: the T-lymphoblastic leukemia CEM, breast carcinoma MCF-7 and cervical carcinoma HeLa.

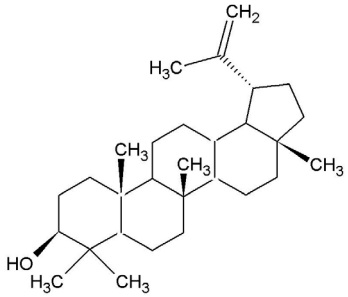

Fig. 1. Structure of lupeol.

The results showed that selected lupane-type saponins (derivatives of lupeol, betulinic acid and betulin) can inhibit the growth of various cancer cell lines at micromolar concentration, despite having limited effects on normal human fibroblasts.

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P-65

Occurrence of quaternary benzo[c]phenanthridine alkaloids in *Stylophorum lasiocarpum* (Oliv.) fedde

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For a long time, quaternary benzo[c]phenanthridine alkaloids (QBAs) are known to exhibit diverse biological effects. A great deal of attention is devoted to the main alkaloids sanguinarine (SA) and chelerythrine (CHE) and their cytotoxicity and induction of apoptosis. These alkaloids also display important anti-proliferative activities against cancer cell lines and have been discussed as potential cytostatic drugs for cancer treatment. In the last years also the other QBAs have received increased attention and new information regarding their biological activities has been published. Anti-proliferative and anti-microtubular activities of sanguilutine (SL), sanguirubine (SR), chelilutine (CL) and pro-apoptotic effects of sanguirubine, chelirubine (CR) and macarpine (MA) has been described. Recently, the anti-proliferative activity of SA, CHE, SL and CL on human malignant melanoma cell lines has been published. The all above mentioned results justify the continuation in the research of QBAs that is dependent on the isolation of alkaloids from plants sources. SA and CHE are isolated mainly from the species *Sanguinaria canadensis* or *Macleaya cordata* and are also available commercially. On the other hand, the minor QBAs SL, SR, CR, CL and MA are present only in several plant species and in significantly lower quantities. One of these species is *Stylophorum lasiocarpum* (Oliv.) Fedde (Papaveraceae) that esspecially in the roots acummulates several minor QBAs. It is a biennial herb native from Central Asia that is possible to grow also in Central Europe. This work was focused on study of alkaloid content in this plant during the vegetation period and the comparison of their production in one-year and two-year old plant with regard to the content of QBAs. Fluidized bed extraction was used for extraction of the plant material. Samples were analyzed by HPLC method on reversed phase with UV detection. The content of alkaloids was expressed as a percentage of dry aerial part or roots. Protoberberine alkaloid coptisine was the main alkaloid in aerial part and roots of the plant. The second main alkaloid was protoberberine stylopine. The content of QBAs in aerial part was very low. The alkaloid content in roots is generally higher than in aerial part. In one-year old plant, the quantity of benzophenanthridines changed during vegetation period. The content of SA (0.24%), CR (0.68%) and MA (0.36%) culminated in September. The amounts of CHE (0.02-0.06%) and CL (0.006-0.03%) varied in samples originating from different harvest but no significant alteration was observed during the vegetation period and between one-year and two-year old plant. Roots of two-year old plant are also abundant source of SA (0.41%), CR (0.53%) and MA. High amounts of MA (0.30-0.49%) were detected troughout vegetation season from May to October with culmination in July. As the mass of two year roots is significantly higher, it can be concluded that roots of two-year old culture *Stylophorum lasiocarpum* can be considered as a potencional source for isolation of the rare minor benzophenanthridine alkaloids CR and MA.

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P-66

Tubulysins and tubugis, new antimitotic compounds and their apoptosis inducing activity

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Tubulysins are highly cytotoxic compounds produced by myxobacteria which are even more potent than vinca alkaloids, a group of microtubule destabilizing agents currently used for the treatment of various cancers. Having synthesized a new generation of tubulysin analogues, termed tubugis these compounds show comparable biological activity to cancer cell lines like the tubulysins, but provide better accessibility and stability. Based on their outstanding activities the question arose whether there are further targets of these compounds. The purpose of this study was the biological evaluation of apoptosis effects induced in response to treatment with either tubulysin B or tubugi 1. Using Colo320, HT-29 (colon adenocarcinoma) and PC-3 (prostate adenocarcinoma) cell lines as model systems the cytotoxic activities of these compounds were measured via XTT-based proliferation assays over a time period of 72h. Microtubule depolymerizing properties were assessed by performing microtubule polymerization assays followed by confocal immunofluorescent analysis in PC-3 cells. Induction of apoptosis and cell cycle progression were monitored by flow cytrometry and the former validated by measuring caspase-3 and -8 activities, respectively. Taken together the tubugis revealed similar biological activities like the tubulysins in all three cell line systems with GIC50-values in the nano- to picomolar

scale. Tubulysin B as well as tubugi 1 exhibit microtubule depolymerizing activity in cells and in the polymerization assay. Both substances induce apoptosis in Colo320 cells by activating caspase-3 but not caspase-8 suggesting that the induction of apoptosis is mediated via the intrinsic apoptosis pathway. Encouraged by these promising findings further studies towards the development of tubulysins and their new derivatives as alternative anticancer drugs to the established vinca alkaloids will be performed.

P-67

Anticancer activity of 22-deoxo-23-oxa analogues of saponin OSW-1

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OSW-1 is the most active of saponins which were isolated from bulbs of *Ornithogalum saundersiae*, a perennial grown in southernAfrica. OSW-1 is weakly toxic toward normal cells but inhibits the growth of various types of cancer cell lines and is 10-100 times more potent than clinically applied anticancer agents, such as cisplatin and taxol. The aim of study relates to the synthesis of a series of OSW-1 analogues that are easier to obtain by chemical synthesis than the natural product and their cytotoxic properties are maintained. We prepared 22-deoxo-23-oxa analogues of OSW-1 with ether or ester moieties in the side chain, the structures of analogues were fully confirmed by spectroscopic methods. The anticancer activity of the new OSW-1 analogues was evaluated in vitro using eight cancer cell lines of different histopathological origins and normal human fibroblasts. The OSW-1 derivatives were substantially (3 – 360 fold) less toxic towards normal BJ human fibroblasts than towards malignant cell lines. The results suggest that the new 22-deoxo-23-oxa analogues of OSW-1 are slightly less active than OSW-1 but also less toxic to normal cells and induce concentration- and time-dependent apoptosis of mammalian cancer cells with caspase-3 activation.

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P-68

Computational study of aurora kinase/inhibitor complexes: implications for modeling and scoring

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Aurora kinases (AK) are eukaryotic serine/threonine protein kinases. AK are nuclear proteins which are essential regulators of mitosis. AK are over-expressed in diverse solid tumors thence have become attractive anti-cancer targets. We aimed at Aurora A, which is one member of family aurora kinases. Several Aurora A kinase inhibitors are in the preclinical or clinical phase of cytostatic drug development. We chose a series of about double dozen pyrazol based ligands, for which the experimental values of IC50 were determined by Coumar et al. and put through a computational study. Our approach employs a novel quantum mechanics based scoring procedure developed in the laboratory of prof. Hobza. It is based on the PM6 semi-empirical method extended about empirical corrections for the dispersion interactions and hydrogen-bonds. The procedure of the ligand-protein affinity determination includes not only the interaction energy calculation but also accounts for the solvent effects, deformation energy and the entropy effects. The coverage of all these terms drives the score close to the thermodynamic value of the bonding free energy of the protein-ligand complex. Therefore, the calculated score correlates well with the experimental IC50 values.

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P-69

Screening of red microalgal polysaccharides for antitumor activity

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The spectrum of a physiological activity of the carbohydrates that build algal cell walls is quite extended. They exhibit a significant biological activity and valuable physicochemical properties. The efforts of scientists are aimed at creating anti-tumor products of natural origin, which

have high biological activity, low toxicity and possess a broad spectrum of therapeutic activity. This study was designed to determine the anti-proliferative and apoptotic properties of polysaccharides (PSHs) from red microalgae *Dixoniella grisea* (DixPSH) and *Porphyridium cruentum* (PorphPSH) as well as to elucidate the mechanism of their action on two permanent human tumor cell lines – MCF-7 (breast adenocarcinoma) and HeLa (cervical carcinoma) and on primary culture from Graffi myeloid tumor in hamsters. Inhibition of cell proliferation was determined by MTT assay. Cell apoptosis was examined with double staining method with acridine orange and propidium iodide and with DNA fragmentation assay. Both PSHs decreased the tumor cell proliferation in a dose-dependent manner *in vitro*. Characteristic morphological signs of apoptosis were observed when the cells were treated with PSHs. Further analysis using agarose gel electrophoresis showed that both algal PSHs caused nuclear DNA fragmentation, which is a hallmark of *apoptosis.* On the other hand, the PSHs applied at a concentration equivalent of cytotoxic strongly stimulate the proliferation of bone marrow cells derived from Graffi tumor-bearing animals. It is known that sulfated PSHs, such as those derived from *Dixoniella grisea* and *Porphyridium cruentum* bind a broad range of proteins on cell surface. As a result they can influence the proliferation, differentiation, apoptosis and metastasis of tumor cells. Our studies indicate that both algal PSHs DixPSH and PorPSH may be a promising alternative to synthetic substances as a natural compound with high immunostimulating and antitumor activities.

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P-70

Nuclear receptors for steroid hormones in breast and prostate cancer cell lines after treatment with plant hormone derivatives

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The nuclear hormone receptor gene superfamily encodes structurally related proteins that regulate transcription of target genes. These macromolecules include receptors for steroid and thyroid hormones, vitamins, as well as different "orphan" receptors of unknown ligand. Ligands for some of these receptors have been recently identified, showing that products of lipid metabolism such as fatty acids, prostaglandins, or cholesterol derivatives can regulate gene expression by binding to nuclear receptors. Nuclear receptors act as ligand-inducible transcription factors by directly interacting as monomers, homodimers, or heterodimers with the retinoid X receptor with DNA response elements of target genes, as well as by "cross-talking" to other signaling pathways. Brassinosteroids (BRs), polyhydroxylated sterol derivatives with close structural similarity to animal and insect steroid hormones, are plant growth regulators representing a group of newly-discovered agents with relatively wide-ranging effects in plants. Molecular and cellular effects of natural BRs and their synthetic derivatives were examined in different human cancer cell lines and in primary endothelial cells in vitro. Natural and synthetic BRs caused growth inhibition, cell cycle arrest and initiation of apoptosis in many different cancer cell lines. The inhibition of proliferation, migration and tube formation of human endothelial cells by BRs was demonstrated. These effects are associated with antiangiogenic activity of BRs. Based on a structural domain similarity between steroids and BRs, the brassinosteroid cytotoxic activity could be, at least partially, related to brassinosteroid-steroid receptor interactions.

This study is focused on the effects of natural BRs and their synthetic analogues to localization and expression of nuclear steroid hormone receptors in hormone-sensitive and hormone-insensitive breast and prostate cancer cell lines compared with control untreated cells. Investigation of the mechanisms of action of BRs in human cancer and endothelial cells using cellular and molecular techniques indicated the possible involvement of steroid receptors in BR action. Understanding the mechanisms of nuclear receptor action will enhance our knowledge of transcription and hormone influences on disease and facilitate the design of drugs with greater therapeutic value.

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P-71

Natural compounds with antiproteasomal and anticancer activities

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Inhibition of protein degradation is one of strategies for suppression of uncontrolled proliferation of cancer cells. Proteolytic degradation is in cells mainly ensured by proteasome and its inhibition by bortezomib showed benefit in clinical use for the treatment of multiple myeloma and mantle cell lymphoma. We set up a cell-based screening method based on rapid proteasomal degradation of green fluorescence protein (GFP) fused to a short degron for identification of new proteasome inhibitors. We screened the library of hundreds natural compounds that previously showed anticancer activities. We identified some hits and then we studied their anticancer activities, antiproteasomal inhibition and mechanism of cell death. We monitored the accumulation of polyubiquitinated proteins in treated cells by indirect immunochemical methods. We also analyze levels of proteins with high turnover (e.g. MDM-2) or proteins whose expression is controlled by ubiquitin ligases (cyclins, p27 or p21). Some of the compounds showed to kill cancer cells we therefore evaluated the mechanism of cell death (autophagy, apoptosis) by enzymatic cellular assays or immunoblotting.

P-72

Advanced protein-ligand scoring function based on semiempirical quantum chemical method

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Free energy estimators are mostly referred to as scoring functions in the drug-design community. The score stands for the binding free energy or for some generalized quantity describing the ligand potency. Previously, we designed a scoring function based on the semiempirical quantum mechanical (SQM) PM6-DH2X method and applied it to three types of P-L complexes, namely the HIV-1 protease (PR), cyclin-dependent kinase 2 (CDK2) and casein kinase 2 (CK2) binding to series of inhibitors. The score consist of the interaction free energy, the correction for desolvation free energy, the change of the conformational free energies of the protein and ligand upon binding and the entropy change. The most accurate up-to-date methods are used for the respective terms thus offering a balanced and reliable scoring function. Each of the terms has a clear physical meaning and these terms are not adjusted/weighted by any means (fitting parameters) to the experimental data. Construction of the scoring function from the physically meaningful terms is a significant feature since it allows us to gain a deeper insight into the nature of the P-L binding. In the three above-mentioned applications of our SQM-based scoring function, the interaction free energies and desolvation free energies represented the dominant term of the score. The P-L interaction is calculated using the corrected SQM PM6 method that allows us to treat quantum effects present in the P-L binding such as proton transfer or halogen bond. Halogen bond is a noncovalent interaction between heavier halogens (Cl, Br, I) and electronegative elements (or electron donors) like oxygen and nitrogen. The binding is mainly due to the electrostatic interaction between positively charged sigma-hole (i.e. a region with a positive electrostatic potential) at the top of the halogen atoms and negatively charged electronegative atoms.Halogen bonds are claimed to play an important role in P-L binding and surprisingly large number (about 40%) of new inhibitors contains halogens.

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P-73

Understanding the potency of competitive CDK2 inhibitors using quantum chemical scoring

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Abnormal proliferation mediated by disruption of the normal cell cycle mechanisms is a hallmark of virtually all cancer cells. Compounds targeting cyclin-dependent kinases (CDK) and inhibiting their kinase activity are regarded as promising antitumor agents. Analogs of the natural purine-based substrate, ATP, can act as competitive CDK inhibitors. Indeed, series of compounds have been prepared with an increased affinity as compared to the natural substrate. To understand this stronger binding in structural and energy terms, we investigate a series of CDK2 inhibitors using two available crystal structures with the aid of quantum-chemistry based scoring function. The scoring function uses semiempirical quantum chemical method, PM6, augmented with dispersion and hydrogen-bonding corrections (PM6-D3H4). This method reliably describes different types of non-covalent interactions and is thus generally applicable to both natural and synthetic compounds. The scoring function is constructed as a sum of physical terms, i.e. interaction free energy including solvation effects, the interaction entropy, and the change of the conformation free energy of the ligand and protein upon binding. The scoring function has already been succesfully applied to series of HIV protease, CDK2 and CK2 inhibitors. In this study, the use of this scoring function allows us to rationalize the affinities of the individual compounds and serves us for further rational design.

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P-74

9-norbornyl-6-chloropurines – novel carbocyclic nucleosides with cytostatic and antiviral activity

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9-norbornyl-6-chloropurines (NCP) have been reported as novel anti-enterovirus compounds. Some of them display pronounced cytotoxic properties suggesting also their potential as anticancer drugs. In this work we explored several putative metabolic pathways of NCP aiming to better understand their mechanism of action. Metabolism was studied at the level of whole cells as well as purified enzymes. In order to reach maximal sensitivity, [^{3}H]-labeled NCP was used in most metabolic assays. The effects of the compounds on apoptosis and nucleic acid synthesis were also assessed using standard flow-cytometric protocols (annexin V-FITC/PI stain, BrU and BrdU incorporation). A major metabolite of NCP was identified as a glutathione conjugate (NCP-GS). The identity of the product was confirmed using authentic standards as well as by HPLC/MS analysis. Buthionine sulfoximine-pretreated cells prevented the metabolite formation and increased cytotoxicity of NCP, we therefore assume that the compound is predominantly active in its parent, unmetabolized form. NCP-GS was formed both in the microsomal and cytosolic fraction of rat liver homogenate as well as in the cells (CCRF-CEM, HepG2). Non-enzymatic conjugation with GSH occurred only marginally, majority of the metabolite can be attributed to the activity of glutathione-*S*-transferase (GST). Purified human GST was used to assess kinetic parameters for NCP conjugation, which were 1.9 mM (Km) and 1.2 $\mu mol.mg^{-1}.min^{-1}$ (Vmax). NCP induced moderate caspase-3 activation but the mode of cell death appeared to be rather non-specific (most cells were both annexin V and PI positive). The effect of NCP on DNA and RNA synthesis as monitored by BrdU and BrU incorporation was weak and appeared to be specific for the bi-chlorinated analogue. More studies are needed to decipher the mechanism of the cytotoxic activity of NCP and determine their usefulness as antitumor agents.

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P-75

Advanced protein-ligand scoring: successful prediction of cyclin-dependent kinase inhibition

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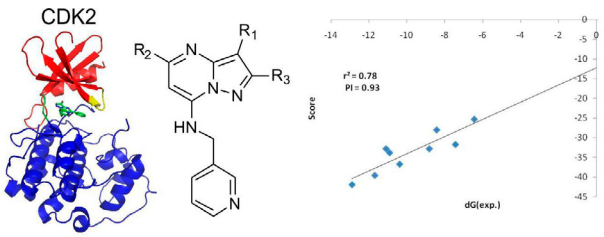
A novel scoring function based on semiempirical quantum mechanics (SQM) has been applied to complexes of cyclin-dependent kinase 2 (CDK2) with the pyrazolo[1,5-*a*]pyrimidine-based inhibitors (compounds 1-9) and their bioisosteres (compounds 10-12) to explain their potency in structural and energy terms. To this aim, we used two independent SQM methods (two corrected PM6 variants and DF-TB-DX) in a fast SQM/MM setup.



With only one X-ray structure available (CDK2/1, we examined two approaches to obtaining the structures of complexes (for compounds 2-9): i) building of modifications coupled with a short force-field molecular dynamics and ii) docking. The complexes were subsequently optimized and scored. All three SQM/MM methods gave good correlation with experimental binding data (r^2 of 0.60–0.78, predictive index of 0.83–0.93) for the building protocol, while the docking (and rescoring) approach failed (r^2 = <0.01 and PI = <0.1). Similarly, no correlation was found for MM-only scoring. In addition, the effect of flexible Lys33 in the active site of CDK2 on the scoring was investigated for the bioisostere series. Inclusion of the Lys33 flexibility qualitatively improved the description (r^2 = 0.86, PI = 0.80) and the results could be further refined by a three-layer QM/SQM/MM method (r^2 = 0.99, PI = 1.0). The later protocol was also applied to the whole series (1-12) which also gave encouraging results (r^2 = 0.73, PI = 0.87). The structures of the optimized complexes were used to rationalize the weak and strong affinities of the compounds. In conclusion, our method and protocol is applicable to congeneric series of CDK2 inhibitors with the aim of finding more potent compounds. In conclusion, our method is applicable to congeneric series of CDK2 inhibitors with the aim of finding more potent compounds.

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P-76

Synthesis and evaluation of affinity-based p38 MAP kinase probes

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The p38 MAPK is an important activator for a number of signaling pathways. Hence, it is involved in various disorders such as ovarian cancer, rheumatoid arthritis, Crohn's disease and psoriasis.Due to the low abundance of MAPKs, especially the p38 family classical tools for proteomics are not useful to investigate those proteins. During the past years affinity based proteomics was successfully applied to investigate low abundant proteins in complex mixtures on the one hand and for evaluation of protein inhibitors on the other. In order to get a better understanding of the p38 kinases and their signaling pathways, small molecules suitable for inhibitor affinity chromatography need to be synthesized. This method employs synthetic probes which contain a reversibly binding inhibitor immobilized to a solid phase. Low abundant proteins can be captured from crude material and identified via LC-MS-MS analysis. For the investigation of the p38 MAPK family a *Smithkline Beecham* derivative was integrated into our probe. It was immobilized and used for pulldown experiments with placenta lysate. To investigate low abundant kinases such as the p38 family as well as to evaluate for example kinase inhibitors, powerful probes for inhibitor affinity chromatography are required. Our current focus is therefore the synthesis of kinase probes suitable for inhibitor affinity chromatography.

P-77

Modified cryptophycins as precursors for tumor-targeting anticancer drug conjugates

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Progress in the therapy of cancer can be achieved with the use of tumor-targeting anticancer drug conjugates. Those substances contain a targeting group that preferably addresses the tumor cells, and therefore, is able to reduce the overall toxicity and raise the specificity for rapidly proliferating cancer cells. Derivatives of cryptophycins, which are cytotoxic cyclic depsipeptides, were synthesized by total synthesis. Such derivatives provide different conjugation sites e.g. to attach them to fluorescently labeled peptides for internalization studies.

P-78

Isolation of potentially cytotoxic *c*-geranylated flavonoids from *P. tomenstosa*

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Polyphenols as natural compounds are secondary metabolites of many plant species. The biological activity of polyphenols is varied and is often modified by presence of different substitution on basic skeleton. In the present time, our attention is focused on the group of prenylated polyphenols. These compounds arise from crossover of different biosynthetic pathways, one of them represents terpenoids. There are many possibilities of prenylation; and the type of prenyl connection and modification affect the biological activity of modified polyphenolic compound. Prenylated polyphenols show wide spectrum of biological effects, including antioxidative, antiphlogistic, antimicrobial, anticancerogenic and estrogenic. Their influence on metabolism of sugars and lipids is also described. *Paulownia tomentosa* (Thunb). Steud. (Paulowniaceae) is a fast growing plant and is known for its high quality wood. Besides this, it is used in traditional Chinese medicine to relieve bronchitis, asthmatic attacks and for reducing phlegm. It is also used for hair and scalp regeneration and for treatment for bacterial infections. When tested *P. tomentosa* showed hypotensive effect. Previously a number of phenolic compounds, including prenylated flavonoids have been reported from *P. tomentosa* fruits, flowers and leaves. All of them have modified prenyl side chain at C-6 of the flavonoid skeleton. Using several chromatographic steps including column chromatography on silica, preparative HPLC on reversed phase and preparative TLC, we successfully isolated several tens of flavonoid compounds. With help of different spectrophotometric methods (UV/Vis, IR, CD) and NMR and MS analysis we carried out structural elucidation of these compounds. According our analysis, these compounds belong to the group to the C-6 geranylated or prenylated flavonoids with different type of geranyl, prenyl and flavonoid ring B substitution. These compounds will be used for screening of cytotoxic activity on different types of cancer cell lines; potentially interesting compounds will be used for elucidation of mechanism of cytotoxic activity.

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P-79

The cucurbitacins e, d and i: investigation of their cytotoxicity and of their biotransformation in man

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Cucurbitacins are a class of natural compounds found mainly in plants of the family *Cucurbitaceae*. They are highly oxygenated tetracyclic triterpenoids known for their numerous potential pharmacological effects as anti-inflammatory, analgesic, hepato-protective, anti-HIV, antioxidants and antimicrobial activities. The purpose of the work was to compare the cytotoxicity of three cucubitacins I, D, E and that of glycosyl-cucurbitacins on the chondrosarcoma Sw1353 cancer cell line and to investigate their biotransformation in man. All the cucurbitacins tested showed a very high cytotoxicity after 12h even at 1 or 4 μM where almost 100% of the cells were apoptotic as observed by DNA fragmentation (tunnel assay). However, cell mortality (MTT assay) was more than 90% after 46h of incubation with all cucurbitacins at concentrations of 1 to 100 μM. No mortality was observed at 0.1 μM. Cucurbitacin E was readily hydrolyzed by human and rat hepatic microsomes and in rat plasma, leading to cucurbitacin I. The kinetics of the esterase reaction in human liver microsomes was Km 22 ± 6 μM, and Vmax = 571 ± 49 nmol/mg proteins/min. A very low hydrolysis rate was detected in human liver cytosol; however no detectable hydrolysis occurred in human plasma. On the other hand,

the three cucurbitacins were glucuronidated at a very low extent. No other major reaction (sulfation, hydroxylation, dehydrogenation, acetylation and glucosylation) occurred. Altogether, these results show that cucurbitacins I, D and E were stable molecules and present potent cytotoxic activity under our experimental in vitro conditions in man and rat.

ACKNOWLEDGEMENTS

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P-80

Archazolid reduces experimental breast cancer metastasis *in vivo*

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Mortality of cancer is rarely the consequence of primary tumors, but implication of tumor metastases. V-ATPase is an ATP-dependent proton pump that is overexpressed within the plasma membrane of various malignant tumor types and suspected to enhance the process of tumor metastasis. The myxobacterial compound Archazolid is a V-ATPase inhibitor. In previous experiments was shown that Archazolid reduces cell migration *in vitro* at low nanomolar concentration. Based on this result, we tested the effect of Archazolid on tumor metastasis *in vivo* in a syngeneic mouse model. Luciferase expressing breast cancer cells (4T1-Luc) were injected intravenously into Archazolid pretreated or untreated BALB/c mice. The pretreatment was 1mg/kg Archazolid, given intravenously 24 h and 4 h before tumor injection. Tumor development was monitored by *in vivo* bioimaging using an IVIS Lumina system. Eight days after tumor inoculation, the bioluminescence signals of the control mice were significantly stronger than the signals of the Archazolid pretreated mice. The tumor burden of the lungs was significantly higher in the control group which was reflected by the bioluminescence signals and the weights of the lungs. This demonstrates that Archazolid has the potential of impairing the metastatic process of 4T1 tumor cells *in vivo*. In addition, even though Archazolid had a strong effect on the tumor cells, there were no signs of other toxicity during the animal experiment. As the metastasis of 4T1 cells in BALB/c mice closely mimics human breast cancer, these results could be very promising concerning the further research of new natural anticancer drugs.

ACKNOWLEDGEMENTS

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P-81

The antitumoral V-ATPase inhibitor archazolid induces extracellular accumulation of procathepsin B

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The antitumoral myxobacterial macrolide Archazolid inhibits V-ATPase action by binding to the V0 c subunit. V-ATPase is an ATP dependent proton pump, controlling the intracellular pH and is present in various intracellular compartments including endosomes, lysosomes and secretory vesicles. Many cancer types are expressing V-ATPase on their plasma membranes, enabling them to lower the pH of the extracellular matrix, supporting invasion and metastasis. V-ATPase inhibitors are thus studied for their potential in cancer therapy. As secretion processes are of major importance for invasion, we analyzed changes of the secretome profile of breast cancer cells (SKBR3) upon V-ATPase inhibition. A proteomic approach was carried out to study alterations in protein abundance in the extracellular medium (ECM). Using the iTRAQ method we quantified relative changes in protein abundance during Archazolid treatment. Surprisingly, and counterintuitively to the antitumoral action of Archazolid, numerous lysosomal proteins were identified in the secretome of Archazolid treated cells. Secretion of such proteins is commonly seen as prometastatic. One of the identified proteins was Cathepsin B. Validation of the secretome data by western blot indicated that primarily the inactive proform of Cathepsin B accumulates within in the ECM already at the low concentration of 0.5 nM Archazolid, which is observed at 10 to 24 h. Intracellular proCathepsin B protein levels are constant, whereas levels of mature active Cathepsin B are lost within 10 h of treatment. The effect was found to be specific for V-ATPase inhibitors, but was not observed with other antitumor agents. We speculate that block of processing lysosomal proteins into bioactive forms may contribute to the antitumoral activity of Archazolid.

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P-82

Comparison of docking results of CDK2 inhibitors

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Fifteen structurally diverse CDK2 inhibitors were used in redocking study as a test suite for comparison of several docking programs. The geometries of inhibitors were taken from corresponding X-ray structures (1AQ1, 1E1X, 1H1P, 1H1S, 1OGU, 1PKD, 1PXJ, 1PXL, 1PXM, 1PXN, 1PXP, 2A4L, 2EXM, 2FVD, 2X1N). Inhibitors were redocked into the crystal pose positions (see Figure 1) with several docking programs (Autodock 4.2, Autodock Vina, DOCK 6, OEDocking). The influence of the protein structure flexibility was estimated by cross docking between different CDK2 crystal structures. The predicted binding energies were further compared to experimental values (data taken from Ref 1).

P-83

Standardized plant extracts in the treatment of cancer

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In this project three plant extracts are evaluated for their potential use in the treatment of cancer: *Chelidonium majus* (Papaveraceae), containing benzophenanthridine alkaloids; *Steganotaenia araliacea* (Apiaceae), known in African traditional medicine for its antitumoral activity and containing lignans such as steganacine; and *Gloriosa superba* (Liliaceae), traditionally used in India and containing colchicine and related alkaloids. The extracts are analyzed and standardized for these constituents. The hypothesis is that a combination of various active principles in an extract may have more beneficial effects than the pure substances, due to synergism and the presence of prodrugs such as glycosides. Cytotoxicity (IC50, μg/mL) of 80% EtOH extracts was determined against MDA-MB-231 WT (breast cancer), PANC-1 (pancreatic carcinoma) and HT-29 (colon adenocarcinoma) using the sulforhodamine B assay. After 24 h of incubation, IC50 values of 73.8 ± 11.5 μg/mL, 20.7 ± 1.0 μg/mL, and 20.6 ± 2.5 μg/mL, respectively, were observed for *C. majus*; 165.5 ± 8.0 μg/mL, 64.0 ± 3.3 μg/mL, and 68.7 ± 3.9 μg/mL for *S. araliacea*; and 0.33 ± 0.05 μg/mL, 0.13 μg/mL and 0.12 μg/mL for *G. superba*.

ACKNOWLEDGEMENTS

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P-84

Morinda citrifolia travels from Polynesia to the entire world

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Traditional cultures have been using fruit, bark, leaves and roots of native plants for food or for medicinal purposes for a very long time. In ancient Mexico, native people had a great tradition and knowledge about medicinal herbs; this knowledge was enhanced by other sources from different cultures; In the case of NONI, it grows in the Mexican Pacific Ocean coast, because of this it is important to study noni fruit growing in Mexico. Noni (*Morinda citrifolia L.*) is an evergreen tree that grows throughout the tropics. It is a tree of Polynesian origin. It has a long history of use as a medicinal plant in parts of southeast Asia, Polynesia and Australia, and is considered to be the second most important medicinal plant in the Hawaiian Islands. The leaves, roots, bark end fruits have all been used medicinally to treat a wide range of ailments: The plant has been reported to have broad effects including anti-cancer activity. In this work, we describe the study of non polar and polar extracts from the seeds. Plant materials was fresh noni fruit, the seeds were washed, dried and ground to powder and extracted with hexane and methanol. The oil obtained from hexane was studied by gas chromatography. Total phenolic compounds were estimated using Folin-Ciocalteu reagent (FCR). Stearic acid: (34.93%). Butyric acid: (34.73%). Content of phenolic compounds was 44.9 micrograms / mL. Phenolic compounds found and described in noni fruit include: damnacanthal which has been described as having anticancer properties. The potency of some of the compounds found in seeds, mainly phenolic structures, could provide scientific basis for the health benefit claims regarding *Morinda citrifolia* fruits in folk medicine and warrant further studies to assess their potential as effective natural remedies. The present study suggests that consumption of noni fruit, including the seeds might have potential health effects, however, further investigation on toxicity of the seeds, needs to be carried out before it can be recommended as a natural antioxidant.

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P-85

Solid phase synthesis and antiproliferative activity of new netropsin analogues

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Our investigations are concentrated on carbocyclic analogues of netropsin and distamycin, with benzene in place N-methylpyrrole rings. These analogues showed the antiproliferative activity against breast cancer cell lines. The carbocyclic lexitropsins built with at least three benzene units inhibited the topoisomerases DNA activity and amidolytic activity of proteolytic enzymes such plasmin or urokinase. Here we present solid-phase synthesis of netropsin analogues. It was started by connecting four aromatic amine-nitro compounds to polystyrene grains with Wang linker. Received in this way, immobilizing on grains, compounds with nitro aromatic group were reduced to obtain derivatives with free amine groups. The next steps were reactions of acylation by 3-nirobenzoyl chloride. Reduction of the nitro groups of obtained compounds was carried out, as previously, with use of the dihydrate of tin (II) chloride in DMF. 4-dimethylaminobutyric acid was used as a modified terminus. The activated N,N-dimethylamino butyric acid was added to intermediate products and final resin-bond analogues of netropsin were obtained. Cleavage by 50% trifluoroacetic acid in dichloromethane gave the desired compounds – four new analogues of netropsin. New compounds were investigated to determine their antiproliferative activity against MCF-7 and MDA-MB-231 breast cancer cells. This procedure is simple and general. The presented method in the field of netropsin structures modification gives the expectation that it will be obtained the compound with required activity; witch will be able to be applied as medical agent in anticancer therapy.

ACKNOWLEDGEMENTS

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P-86

Effect of dibenzocyclooctadiene lignans on multidrug resistant promyelotic leukaemia cells

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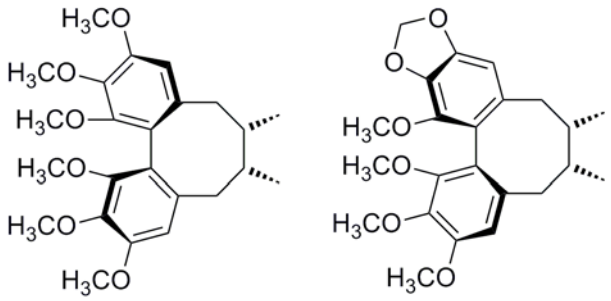
Dibenzocyclooctadiene lignans (DBL) are active compounds of *Schisandra chinensis* fruit, which is widely used in traditional Chinese medicine especially for treatment of lung, heart, and kidney afflictions as well as a tonic and antitussive. It was found that dibenzo[a,c]cyclooctadiene lignans possess a broad range of biological effects, including hepatoprotective and antiviral properties. Recently, dibenzocyclooctadiene lignans are discussed as compounds that have the potential to overcome multidrug resistance. The ability of cancer cells to be cross-resistant to structurally and functionally unrelated anti-cancer drugs is known as multidrug resistance. Cancer multidrug resistance is one of the major causes of chemotherapy failure. Over-expression of the ATP binding cassette members (ABC-transporters) is responsible for most cases of clinical cancer multidrug resistance. One of the current challenges of anticancer therapy research is uncovering compounds, which can act as MDR modulators and co-administer them with anticancer drugs to make treatment more effective and to minimize drug side-effects. In this study we used multidrug resistant promyelotic leukaemia cells overexpressing MDR1 (P-glycoprotein), the most common member of ABC transporters family (HL60/MDR). The ability to overcome multidrug resistance was examined in the panel of dibenzo[a,c]cyclooctadiene lignans, schizandrin, gomisin A, gomisin N, angeloylgomisin H, tigloylgomisin P, deoxyschizandrin-dicarbaldehyde, wuweizisu C, and *S*(-)- and *R*(+)-enantiomers of deoxyschizandrin, γ-schizandrin and gomisin J. The lignans were isolated from *Schisandra chinensis* seeds or prepared semisynthetically. We observed that resistant HL60/MDR was nearly hundred times more resistant to doxorubicin than the parental line HL60; although both cell lines were similarly sensitive to lignans treatment, indicating that the lignans are not exported from the resistant cells. Using doxorubicin accumulation assay we demonstrated that all lignans significantly enhanced the accumulation of doxorubicin in drug resistant cells.



R-(+)-deoxyschizandrin *S*-(–)-gomisin N

The results were comparable or even higher than Verapamil used as a positive control. Comparing enantiomers of individual lignans, we observed higher effect of *R*(+)-γ-schizandrin. On WST and PI- exclusion assay we demonstrated that the lignans enhanced cytotoxic effect of sub-toxic concentrations of doxorubicin. Deoxyschizandrin and Gomisin N were selected for further studies because of high activity in accumulation assay. Deoxyschizandrin and gomisin N had no effect on the cell cycle; however, when combined with sub-toxic doses of doxorubicin, they induced cell cycle arrest in the G2/M phase, what is typical for toxic doses of doxorubicin. The results proved the ability of DBL to overcome MDR resistance in P-glycoprotein overexpressing HL60 cell, due to the increasing doxorubicin accumulation inside the cells. DBL represents substances promising for treatment of multidrug resistant cancer.

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P-87

Anticancer effect of citrus limonoids: review of the available evidence

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Citrus byproducts management (about 350-420 mila ton/year) represents an environmental and economic problem. Pulp, pulp wash and yellow water are hard-to-digest and relatively resistant to microbial degradation due to their high content of compounds with antimicrobial activity (ascorbic acid, limonoids and polyphenols). Limonoids are no toxic and safe for use on human nutrition, even at high concentrations, as confirmed by their use in organic crops productions as natural extract from Rutaceae and Meliaceae vegetal families. Epidemiological studies have suggested an active role for citrus fruit in cancer prevention. The protective effect might be related to the content of vitamin C and limonoids. Limonoids are highly oxygenated triterpenoid present in large amounts in juice and citrus juice processing byproducts or in seeds. Biochemical composition and concentration of limonoids in citrus fruits or juices may be influenced by extraction methods, processing and storage which may affect their efficiency as biologically active compounds. Their extraction and recovery could be a good expedient to increase waste bio decomposition and to produce compounds with potential pharmacological proprieties and activities. The yearly waste production of citrus fruits (about 350 miles ton/year) could provide 140 ton of extracted limonoids. In vitro evidences showed that limonoids inhibit proliferation of breast cancer and human neuroblastoma cells. Moreover limonoids could induce detoxifying enzymes in liver and inhibiting formation of chemically-induced neoplasia in the oral cavity, stomach, small intestine, colon, lung and skin in mouse and hamster models. As overall results from cellular and animal models support anticancer properties for limonoids. However evidence regarding the antineoplastic effects of citrus limonoids in humans is lacking. Long-term clinical trials are needed to establish the link between consumption of citrus and reduced cancer risk in large populations. The available evidence regarding the anticancer activity of citrus limonoids will be reviewed and critically discussed.

P-88

Anticancer activity of lignans from *Dryopteris fragrans* (l.)Schot

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Many lignans exert potent antitumor activity. However, lignans from *Dryopteris fragrans* (L.) schot, a traditional medicinal herb in Northeast China, has not been reported previously.

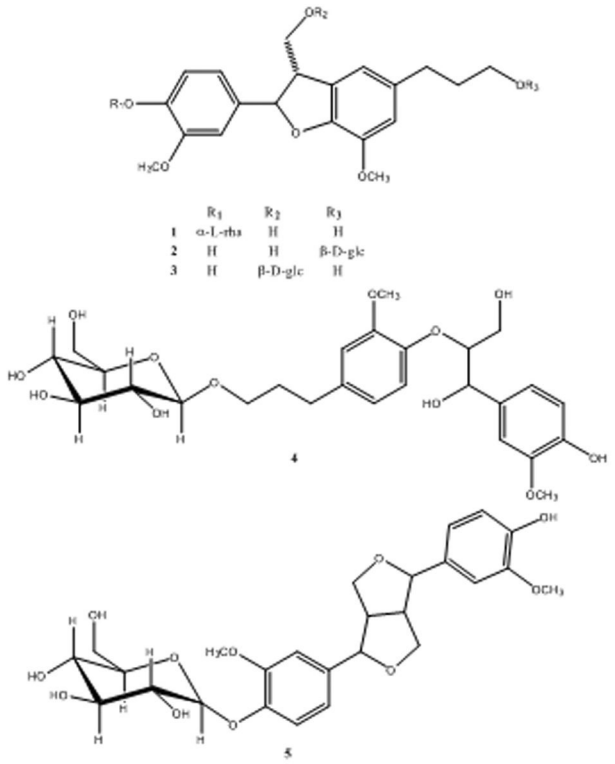

Fig. 1 Structures of lignans from *D. fragrans*

In this study, five lignan glycosides were isolated from the aqueous extract of *D. fragrans*, including (7R, 8S)-dihydrodehydrodiconiferyl alcohol-4-*O*-α-L-rhamnoside(1), (7S, 8R)-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucoside(2), (7S, 8R)-dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucoside(3), 8-*O*-4′-neolignan-9′-*O*-β-D-glucopyranoside(4), (+)-pinoresinol-*O*-β-D-glucopyranoside(5). Their structures were elucidated by various spectroscopic data such as NMR and CD spectra. Bel-7402 cells were treated with lignan glycosides and cell viability was measured by using MTT assay. All of them showed significantly anti-proliferative effect in human liver cancer Bel-7402 cells.

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P-89

Effect of peanut sprout extract on neurocytotoxicity in N18-RE-105 cells

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Neurodegenerative conditions such as the Alzheimer and Parkinson diseases, are associated with the production of reactive oxygen species and resultant oxidative stress. Glutamate is the major excitatory neurotransmitter of the central nervous system and may induce cytotoxicity through persistent activation of glutamate receptors and through oxidative stress mechanisms. On the basis of this information, we established a screening system using N18-RE-105 cells to identify therapeutic agents that can protect cells from glutamate toxicity. During the course of our screening program, we recently isolated an active compound, 8,13-dihydroxy-9,11-octadecadienoic acid (PSE-1), from peanut sprouts, which prevents glutamate-induced cell death. The chemical structure of PSE-1 was identified using spectroscopic methods and by comparison with the value in the literature. The antioxidant and neuroprotective effects of PSE-1 were evaluated using the oxygen radical absorbance capacity assay, Comet assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazolium bromide reduction assay, the lactate dehydrogenase release assay, a morphological assay and Hoechst 33342 staining. The results of the assays demonstrate that PSE-1 has neuroprotective effects and that PSE-1 could be a new potential chemotherapeutic agent against neuronal diseases.

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P-90

Possible involvement of clusterin protein in EGCG/GTE effects evoked in COLO 205 colon adenocarcinoma cells

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Epigallocatechin gallate (EGCG) is the compound of green tea extract (GTE) with reported ability to inhibit cell proliferation and induce apoptosis in cancer cells. It has been shown that EGCG affects different cellular signaling pathways, among them the up-regulation of clusterin (CLU) protein expression, which might be responsible for its proapoptotic action. CLU is a multifunctional chaperon protein involved in numerous processes in mammalian cells. The *CLU* transcription is complex and generates two protein forms differentially localized and therefore playing opposite roles in the cells. It is assumed that secretory form of CLU (sCLU, 80 kDa) exerts protective effect, whereas nuclear CLU (nCLU, 50 kDa) expression is a hallmark of apoptosis. The aim of our study was to verify whether EGCG/GTE could overcome the resistance of colon adenocarcinoma COLO 205 cells to TNF-alpha-induced cell death or initiates intrinsic apoptosis. Surprisingly, we found that EGCG [10-400 μM] and GTE [10-400 μg/mL] dose- and time-dependently stimulated COLO 205 cell viability (6, 12, 24 and 48 h). Both compounds did not sensitize COLO 205 cells to TNF-alpha. Western blot (WB) analysis revealed that EGCG [100 mM] and GTE [100 mg/mL] induce sCLU expression in the 12th hour of the experiment. To identify the molecular mechanism responsible for EGCG- and GTE-induced CLU expression, different metabolic inhibitors were used (STS, PMA, LiCl, AD, CHX and SB 216763). WB analysis has shown that only STS [1 μM] abrogated EGCG- and GTE-dependent CLU expression. GSK-3beta inhibitor, LiCl [20 mM], enhanced EGCG- and GTE-dependent effects with significant upregulation of CLU level observed at 6th hour of treatment. Astonishingly, AD [20 ng/mL] administration did not inhibit LiCl-induced effect. Conversely, another GSK-3beta inhibitor, SB 216763 [10 μM] delayed and extended EGCG- and GTE-induced CLU expression levels up to 48th hour of treatment. Similar effect was exerted by PMA [100 nM] treatment, whereas CHX [1 μg/mL] attenuated CLU expression until 48th hour of treatment. Accordingly, EGCG/GTE stimulated viability of COLO 205 cells and this effect was accompanied by elevated CLU protein level. We also investigated the expression of CLU in COLO 205 cells treated with verapamil [100 uM] L-type calcium channel blocker and a well-known antiarrhythmic drug, used mostly in cardiac diseases. We found that in this case verapamil induced severe macroautophagy, leading to necrosis. At the same time CLU was downregulated, as shown on WB analysis and electron microscopy observations. This data suggests the dual role of CLU in both cell death and survival mechanisms in COLO 205 cells.

P-91

New cytosine derivatives as inhibitors of dna methylation

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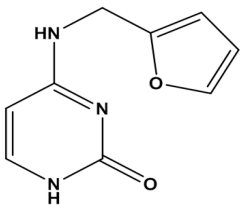
Neoplastic transformation is associated with alteration in DNA methylation, includes both global hypomethylation and gene specific hypermethylation. Great effort has been directed on towards development of novel strategies that can change the inappropriate gene methylation pattern in cells and thus, to redirect cell fate in human cancers. It is believed that the creation of conditions for restoration of the pattern of epigenetic modification that is proper for every organism is an opportunity to reverse cancerogenesis. DNA cytosine methylation catalyzed by DNA methyltransferase 1 (DNMT1) is an epigenetic route to gene expression regulation and development. Changes in methylation pattern lead to carcinogenesis. Inhibition of DNMT1 activity could be a good strategy of safe and efficient epigenetic therapy.



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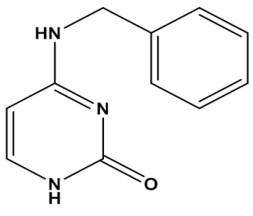

II

We present a novel group of cytosine analogs as inhibitors of DNA methylation, new methods of their synthesis and their effect on *in vitro* reaction of DNA methylation. Inhibitory activity of each compound was analyzed in *in vitro* DNA methylation reaction catalyzed by DNA methyltransferase from *Spiroplazma*. Cytosine derivatives were divided into three groups according to modification at exocyclic amino group. 4-N-furfurylcytosine (I) and 4-N-benzylcytosine (II) (Ki70 and 10 μM, respectively) were further modified. The best obtained inhibitors were

4-N-furfuryl-5-azacytosine, 4-N-benzyl-5-methylcytosine, 4-N-furfuryl-5-methylcytosine with Ki 0.7, 3.6 and 15 μM, respectively. These compounds cause a much greater reduction in the level of DNA methylation in cancer (HeLa) than in normal (HEK293) cells. Derivatives substituted with aliphatic chains at 4-N acted as uncompetitive inhibitors. Almost all of analyzed compounds inhibit DNA methyltransferase activity in the competitive manner.

ACKNOWLEDGEMENTS

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P-92

Salicylate and aspirin combined with PI3Ks inhibitor LY294002 or with natural pro-apoptotic agent gossypol promote melanoma cell death

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Currently available anticancer drugs do not improve the prognosis of metastatic melanoma. Salicylate (SA) and acetyl salicylic acid (aspirin, ASA) were found to suppress growth of a number of transformed cells, including prostate and colon cancer cells. In our study, we tested the direct effects of SA and ASA on three metastatic melanoma cell lines (A-375, G-361 and Mel-Juso). The treatment with SA or ASA at plasma-attainable levels was not toxic for the melanoma cells. In combination with LY294002, an inhibitor of phosphatidylinositol 3-kinases (PI3Ks), the viability of melanoma cells dramatically decreased. The proportion of dead cells in melanoma cell cultures also increased when SA and ASA were combined with Gossypol, an inhibitor of the antiapoptotic members of the Bcl-2 family. SA and ASA have both the ability to inhibit the production of pro-inflammatory prostaglandins. However, unlike SA, the ASA's specific mechanism of action is attributed to its unique ability to acetylate cyclooxygenase (COX) enzymes that involved in prostaglandin biosynthesis. SA does not have this capacity to acetylate. The levels of cell death induced by the combination of SA or ASA with LY294002 or with Gossypol were similar, indicating that the increase in cell death cannot be explained solely by the inhibition of the COX activity and probably involves an alternative cellular target.

ACKNOWLEDGEMENTS

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P-93

Influence of membrane positioning on the drug selectivity of cytochrome P450s

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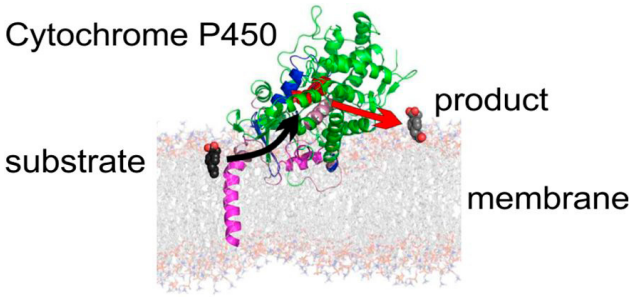
Cytochromes P450 (CYPs) are enzymes involved also in the metabolism of drugs with huge impact in the drug dosage and toxicity. Human CYPs are membrane-anchored proteins located mostly in endoplasmic reticulum or mitochondria. The structure of the CYP catalytic domain has rather conserved fold according to X-ray experiments. However, position of CYP on membrane still remains debatable. Recently, our group and others have published models of membrane anchored CYP2C9 and CYP3A4, based on atomistic molecular dynamics simulations. Here, we extend the set of models of membrane-anchored human CYPs. The set enabled us to identify common features and variations between individual CYPs on membranes. We have further computed membrane positions of typical substrates and products by a recently developed method. Moreover, we have evaluated membrane positions of enzyme's active site access/egress paths, which were calculated by a recently released MOLE*online* 2.0 software.



We have shown that the positions of openings of the substrate access and product egress channels corresponds the free energy minima of substrates and products, respectively. Finally, the depth and orientation of CYPs' membrane penetration is in agreement with known substrate preferences. This information can be further used in the prediction of the possible metabolism of new drug in the pipeline of the drug development.

ACKNOWLEDGEMENTS

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P-94

Computational structure-based approach to selectivity enhancement of purine-based inhibitors of cyclin-dependent kinases

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Enhancement of selectivity of purine-based ATP-competitive inhibitors to specific cyclin-dependent kinases (such as CDK5 or CDK9) may present a more powerful strategy to combat cancer than the classical CDK2 inhibition. Experimental data on CDK inhibition by a purine-based inhibitor roscovitine and its analogues can serve as a base for a structure-based SAR. However, the availability of crystal structures of CDK-inhibitor complexes is limited, both from the protein and ligand side. Fortunately, computational procedures can be used for modeling of the missing complexes: i) docking or building of inhibitor modifications can be performed for the inhibitors,and ii) for the proteins, homology modeling based on sequence alignment of human CDKs is carried out. Binding affinities of inhibitors to CDK enzymes are estimated using the quantum chemistry (QM)-based scoring function, which employs the corrected semi-empirical QM method, PM6-D3H4. This method reliably describes different types of non-covalent interactions and is thus generally applicable to both natural and synthetic compounds. The scoring function is constructed as a sum of the interaction free energy, interaction entropy, the change of conformational free energy of both ligand and protein. The scoring function has been developed in our laboratory and has successfully been applied to study the inhibition of HIV protease, CDK2 and CK2. In summary, we present a computational approach which opens way to a rational structure-aided design of novel CDK-specific inhibitors which may be based on natural products.

P-95

Biosynthesis of oleanolic and ursolic acids by *Salvia tomentosa* Mill. plant cell suspension in shaking flasks and stirred tank bioreactor

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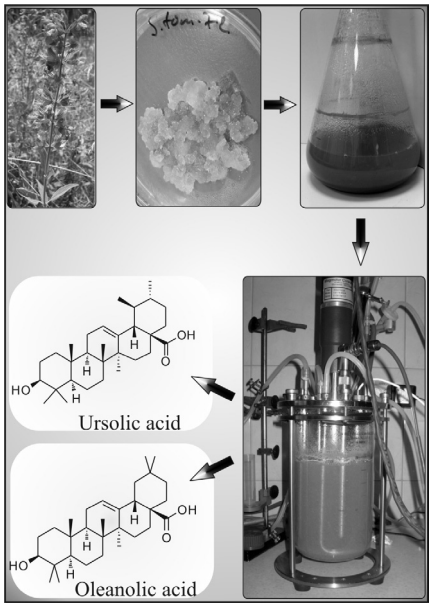
Ursolic (UA) and oleanolic (OA) acids are natural triterpenoids with remarkable anticancer activities. The *in vitro* cultivation of plant cell suspensions is a prospective alternative for production of anticancer substances under controlled conditions, overcoming the problems associated with field cultivation of plants. The aim was development of *in vitro* biosynthetic process for ursolic and oleanolic acids by cell suspension from the Bulgarian rare and protected *S. tomentosa* Mill. plant, based on its cultivation in shaking flasks and stirred tank bioreactor. *Shake flasks experiment. S. tomentosa* suspension was grown in a Linsmayer-Skoog (LS) nutrient medium, supplemented with 30 g/L sucrose and 0,2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D). The cultivation was conducted in 100 mL Erlenmeyer flasks with 20% (v/v) 10-days old suspension as inoculum, on a shaker (11.6 rad/s), at 26 °C in darkness. *Bioreactor cultivation*. The suspension was grown in 3-L stirred tank reactor (agitation speed 100 rpm, 30% of dissolved oxygen, at 26 °C, in darkness). LS medium of the same content and inoculum as mentioned above were used. *HPLC and GC/MS analysis*. Trietrpenoids were quantified by Waters HPLC with UV detector. Sugar assimilation was monitored by Shimadzu HPLC with refractive index detector. GC/MS profile was established by Agilent gas chromatograph 7890 MSD 5975C inert (EI 70 eV), HP-5 ms column. During shake flasks stage the accumulated dry biomass (ADB) reached its maximum (ADB=6.61 g/L) at the 11 day of the cultivation. The maximum biosynthesis: 15.52 mg/L for OA and 45.19 mg/L for UA were achieved at the same day as well. Due to aggregates formation during the bioreactor cultivation, homogenous sampling was hampered. For better process evaluation, measuring of specific oxygen uptake rate was included (SOUR). The highest SOUR value [3.094 μM/(L.min)] was achieved at day 11 of the cultivation, when 4.68 g/L ADB, 12.29 mg/L OA and 44.02 mg/L UA were achieved. The presence of the triterpenic acids was confirmed by GC/MS analysis. Cell suspension cultivation

of *S. tomentosa*, producing anticancer triterpenoids was successfully performed in shaking flasks and stirred tank bioreactor.



Although decreased biomass yield in the bioreactor, the yields of target metabolites were in comparable levels. This observation indicates that the stirred tank bioreactor have suitable design for future scaling up of the cultivation process. To the best of our knowledge, this is the first report for *in vitro* cultivation of *S. tomentosa* Mill. cell suspension for production of bioactive oleanolic and ursolic acids.

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P-96

The anticancerogen effects of melatonin, quercetin and luteolin on HepG2 cell lines

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Melatonin (an indolamine, endogen hormone responsible from the circadian rhythm), Quercetin and Luteolin (two common flavanoids found in many natural products) are mainly antioxidant molecules which might have also pro-oxidant effects depending on the cellular oxidative status and doses. In this preliminary study, we investigated the effects of these molecules on cell viability on HepG2 hepatocarcinoma cell lines. We determined the cytotoxic effects of Melatonin (5uM-5000uM), Quercetin (5uM-1000uM) and Luteolin (5uM-1000uM) in a dose and time dependent manner (24h, 48h and 72h) on Hepatocarcinoma (HepG2) cell lines. Melatonin reduced the cell viability over 1000 uM dose in the all time courses. Quercetin reduced the cell viability over 50uM and its effects has started in the first 24 h. And its highest effect was at 200 uM after 48 h. Luteolin has reduced the cell viability at a percentage of%20 in between the lower doses (5uM-500uM), and over these doses it has shown to protect the cells probably reflecting that at higher doses its antioxidant effects might overcome its prooxidant effects. As a result, these prelimnary findings suggest that these natural compounds should have been further investigated to determine their optimal dose and time course for their anticancerogen effectiveness.

ACKNOWLEDGEMENTS

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P-97

A possible self protection mechanism in *Catharantus roseus* against vinca alkaloids

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Cancer is the second frequent disease and the third cause of death in Iran. The anti-microtubular agents; taxanes from yew and vincristine and vinblastine from vinca have been shown to be the most promising classes of anticancer drugs used for cancer therapy in Iran. Vinca (*Catharantus roseus*) alkaloids inhibit microtubules polymerization by interacting with the subunit(s) of the α/β tubulin heterodimer resulting in G2-M arrest, suppression of cell proliferation, and apoptosis. To scale up their production by biotechnological approaches, we have characterized the α/β tubulin gene family of *Catharantus roseus* in order to find out the possible self protection mechanism exist naturally in vinca which cause different resistance properties against Vinca (*Catharantus roseus*) alkaloids. Based on the results from β tubulin gene family sequencing (manuscript under preparation), characterization of α-tubulin gene family in *Catharanthus roseous* aimed in this investigation. Specific primers were designed using the α-tubulin sequence provided in NCBI. The α-tubulin genes were isolated, amplified and sequenced. The preliminary results showed a 25 base pair deletion in α-tubulin gene. The data will be utilized for target engineering of the plant to enhance the amount of alkaloids.

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P-98

Visible light enhances the anti-tumor effect of curcumin in a xenograft tumor model

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Curcumin, a dietary pigment from the plant Curcuma longa, inhibits cell proliferation and induces apoptosis in different cell lines. The therapeutic benefit is hampered by a very low absorption after trans-dermal or oral application. Recently, we have demonstrated that curcumin offers the described effects also at low concentrations (0.2 to 1 μg/mL) when applied in combination with UVA or visible light. To show the efficacy of this combination in vivo we used a xenograft tumor model with A431 tumor cells injected subcutaneously in the flanks of NMRI nude mice. Consequently, we investigated the effect of curcumin and visible light on tumor growth and studied targets involved in proliferation and apoptosis by immunohistochemical staining and Western blotting. The treatment consisted of peritoneal injection of 0.5 mg curcumin dissolved in 1% methylcellulose, 0.5 mg curcumin dissolved in 1% methylcellulose combined with 20 min irradiation with visible light, 1% methylcellulose (control), and 1% methylcellulose/visible light twice a day. Tumor volume was measured after 12 and 17days. Only curcumin/light treatment evoked a significant tumor growth inhibition compared to the control group. The average tumor volume at day 12 in curcumin/light treated mice was reduced by approximately 70% in comparison to the control group. The tumor volume reduction of the curcumin injected but non-irradiated group was not significant versus control, whereas the curcumin/light group also showed a significant difference versus this group. The relative ratio between tumor volumes of the different groups was similar after 17 days. There was no effect of the various treatments on body weight. To determine the proliferation rate in tumors, immunohistological sections were stained for Ki 67 which is expressed in all phases of the cell cycle except G0. Tumors isolated 24 hafter treatment showed a significant decrease in Ki 67 positive cells when treated with curcumin/light. Corresponding to the data on tumor growth, curcumin/light treatment inhibited proliferation by about 70%, whereas light and curcumin alone had no effect on Ki 67 expression. Furthermore, we tested whether a combination of light and curcumin triggers apoptosis. Thus, tumors were stained with bisbenzimide to observe the formation of apoptotic bodies. We found a significant 4-fold increase of apoptotic nuclei in tumor tissue of curcumin-injected and irradiated mice after 24 hr. Curcumin alone and irradiation without curcumin treatment did not show significant effects on the formation of apoptotic bodies. The effect on apoptosis was further confirmed by Western blot analysis showing enhanced activation of caspases-9. Vice versa inhibition of extracellular regulated kinases (ERK) 1/2 and epidermal growth factor receptor (EGF-R) was observed which may aid to inhibition of proliferation and induction of apoptosis. In summary, the present findings suggest a combination of curcumin and light as a new therapeutic concept to increase the efficacy of curcumin in the treatment of cancer.

P-99

Comparative bioactivity studies on different *Plantago* species

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The genus *Plantago* L. (Plantaginaceae) is represented by 21 species in the flora of Turkey. *Plantago* species are used externally to the treatment of wound, abscess and acnes, internally to the treatment of diabetes, urinary infections, cancer, common cold and viral infections in Anatolia. Several effects are described for the genus *Plantago* such as antitumoral, antiinflammatory, antibacterial, analgesic, antispasmodic and hepatoprotective. Earlier investigations performed on *Plantago* species resulted in the isolation of mainly iridoid glucosides, some phenylethanoid and flavonoid glycosides, caffeic acid derivatives, polysaccharides and lipids. In the present study, five *Plantago* species; *Plantago major* L.subsp. *major*, *Plantago major* L. subsp. *intermedia* (GILIB.) LANGE, *Plantago lagopus* L., *Plantago scabra* MOENCH and *Plantago holosteum* SCOP., were tested for their radical scavenging activities using 2,2-diphenyl-1-picryl hydrazyl (DPPH), superoxide (SO), nitric oxide (NO) and 2,2\'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals spectroscopically. Cytotoxic activites of these extracts were also investigated against HEP-2 (Human Epidermoid Carcinoma) and RD (Human Rhabdomyosarcoma) cell lines using 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assay. Water extract of *P. major* subsp. *major* showed the highest radical scavenging and cytotoxic activity comparing the other species. On the other hand, while total phenolic content of *P. major* subsp. *major* was found as 103.65 mg/g dry extract, the highest total phenolic content was found as 200.75 mg/g for the root extract of *Plantago holosteum* L by Folin-Ciocalteau method. Further researches will be performed on *P. major* subsp *major.*

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P-100

Drosera capensis and *Camelia sinensis* extracts protect DNA from ionizing radiation through the elimination of ROS

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Ionizing radiation causes DNA damage either by direct interaction between ionizing particles and DNA molecule or preferentially by an indirect mechanism through reactive oxygen species (ROS) generated by ionization of the water molecules surrounding the DNA. Compounds with antioxidant activity can potentially neutralize DNA damaging effect of ROS on DNA in case they sustain their antioxidant properties inside living cells and can diffuse to the close vicinity of chromatin. We used various *in vitro* antioxidant capacity assays, such as ORAC and TPC, and identified extracts from *Camelia sinensis* and *Drosera capensis* from a larger set of plant extracts as potentially strong antioxidants. These extracts we further tested in respect to their ability to protect DNA from IR-induced damage in living cells. For this we optimized and automated ultra-sensitive imunofluorescence-based method of DNA damage quantification in human cells after exposition to different types of clastogenic insults including low doses of gamma radiation. We found that cells treated by the plant extracts before irradiation showed significantly lower signal of γ-H2AX, a well established marker of DNA double strand breaks. In parallel, we confirmed that the extracts reduced the accumulation of ROS inside living cells, suggesting that the DNA-protecting effect was likely due to the ROS scavenging effect.

P-101

Anticancer properties of the new carrots from Ispica

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The chemical-physical and nutritional characteristics of \"New Carrot from Ispica" were studied. In particular we focused on β-carotene content. \"New Carrot from Ispica" obtained Protected Geographical Indication mark (PGI) in 2010; the main characteristics are reported inside the production\'s technical policy. This characteristics are related to the carbohydrate content (higher than 5 g/100g FM) and to the β-carotene (higher than 4 mg/100 g FM), depending of harvest time. Forty six samples of PGI carrot (*Daucus carota* L. sativus arcangeli) from two cultivar *Excelso* and *Dordogne* were analysed. The total carotenoids content was between 4.5 and 11.5 mg /100g FM. These are a bit higher than found by Dutta et al. (2005), who reported β-carotene content of fresh carrots to be 8.4 mg/100 g. This is interesting from medical point of view. The carotene, in fact, is converted in Vitamin A and stored inside the liver. The recent researches reported that foods rich in β-carotene, help in the reduction of lung cancer's risk and also the risk of some cancers of the oral cavity. Furthermore, the β-carotene is also essential for a proper growth and for the repair of body tissues. The β-carotene is also essential for the protection of the mucus's membranes of mouth, nose and throat. Thereby reduce the susceptibility of infections and plays as antioxidant against the damaging effects of free radicals. It prevents also the "night blindness", "weak-eyesight" and ultimately is strongly helpful in the formation of bones and teeth.

P-102

Cytotoxic and radical scavenging activities of acteoside and calceorioside B

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Phenylethanoid glycosides are naturally occurring compounds of plant origin and are structurally characterized with a hydroxyphenylethyl moiety in which a glucopyranose is linked through a glycosidic bound and esterified by a cinnamic acid moiety. To date several hundred compounds of this type have been isolated from medicinal plants and further pharmacological studies *in vitro* or *in vivo* have shown that these compounds possess a broad array of biological activities. Acteoside is one of the important phenylethanoid glycoside and also called kusaginin or verbascoside. It is isolated from different dicothiledone species mainly distributed in Asia. Up to date, different biological activities were reported for acteoside including antibacterial, antioxidant, antitumor, antiviral, anti-inflammatory, neuroprotective, hepatoprotective, immunomodulatory, and enzyme inhibitory actions. In this study, acteoside and its desrhamnosyl derivative calceorisode B which were isolated from the active fraction of *Plantago lagopus* L. were investigated for their cytotoxic activity against human cancer cell lines, Hep-2 (human epidermoid carcinoma), RD (human rhabdomyosarcoma), and MCF-7 (human breast adenocarcinoma) using MTT method. Strong cytotoxic activities of verbascoside and calceorioside A against HEP-2, RD and MCF-7 cell lines were determined between the concentration of 35-65 μg/mL and RD cell line was found the most sensitive cancer cell line for the tested phenylethanoid glycosides. In addition, acteoside and calceorioside showed strong radical scavenging activities against DPPH, nitric oxide and superoxide radicals comparable to that of reference compounds BHA, ascorbic acid and quercetin. Close cytotoxic and radical scavenging activities of verbascoside and calceorioside A indicated that rhamnose substitution did not affect the tested bioactivities of these phenylethanoid glycosides.

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P-103

Isolation of P-glycoprotein inhibitors from *Schisandra chinensis*

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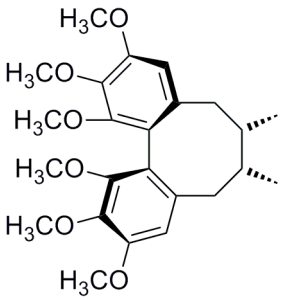
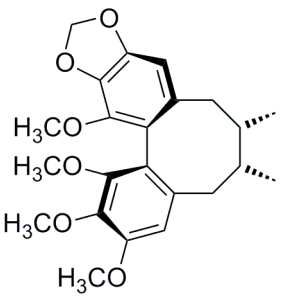
Schisandra chinensis (Schisandraceae) is a well-known medicinal plant in traditional Chinese medicine. The fruits and seeds have been used for centuries as a tonic and antitussive. Many studies have indicated that the active ingredients are dibenzo[a,c]cyclooctadiene lignans displaying hepatoprotective, antiviral and anticancer properties. Recently was found that dibenzocyclooctadiene

lignans inhibit ATP binding cassette (ABC) transporters, P-glycoprotein and multidrug resistance-associated protein 1 (MRP1), which export drugs out of the cancer cells. Over-expression of the ABC transporters is responsible for most cases of clinical cancer multidrug resistance. In our previous study, we have isolated nine dibenzo[a,c] cyclooctadiene lignans, schizandrin, gomisin A, gomisin N, gomisin J, angeloylgomisin H, tigloylgomisin P, deoxyschizandrin, γ-schizandrin and wuweizisu C from seeds of *Schisandra chinensis* and lignans were examined for their effect on doxorubicin-resistant human lung carcinoma COR-L23/R cell line over-expressing MRP1. We have found that two lignans, *R*-(+)-deoxyschizandrin and *R,S*-(±)-γ-schizandrin at relatively non-toxic concentrations enhanced the accumulation of doxorubicin in COR-L23/R cells and restored the cytotoxic action of doxorubicin on drug-resistant cells (Slaninova et al.: Toxicology in Vitro 23, 2009, 1047). In order to obtain more effective lignans, the methanolic extracts of *Schisandra chinensis* seed and stem were screened for their effect on accumulation of doxorubicin in promyelotic leukaemia cells HL60/MDR overexpressing P-glycoprotein. Accumulation of doxorubicin, a P-glycoprotein substrate, inside the living cells was analyzed by flow cytometry. Verapamil, an inhibitor of P-glycoprotein, was used as a positive control. The methanolic extract of both seed and stem increased intracellular doxorubicin accumulation. Activity-guided fractionation of both methanolic extracts on SPE cartridges Supelco LC 18 showed that active compounds were present in the fraction rich in lignans.



R-(+)-deoxyschizandrin



R-(+)-γ-schizandrin

The lignan fraction originated from the seeds was further purified by semi-preparative HPLC on a C18 column to give three subfraction with higher ability of doxorubicin accumulation than that of deoxyschizandrin. Further separation of one of these fractions on a semi-preparative C18 column provided two new lignans, which accumulated doxorubicin inside the HL60/MDR cells more effectively than well-known P-glycoprotein inhibitor verapamil or deoxyschizandrin.

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P-104

Cytotoxic activities of mannich bases of chalcones against human hepatoma and breast cancer cell lines

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Mannich ketones are well documented as potent cytotoxic agents. The bioactivity of Mannich ketones may be due to the alkylating ability of α,β-unsaturated ketones that are liberated *in situ* following deamination. Various studies on Mannich ketones have been reported from our laboratory but toxicities associated with this class of compounds have discouraged us to pursue them further. The unwanted toxicities associated with the Mannich ketones may be due to the rapid decomposition to form a reactive enone, which is sequestered indiscriminately with cellular nucleophiles. This has prompted our interest in chalcones, which carry an α,β-unsaturated keto motif and are associated with diverse biological activities and are often devoid of undesirable toxicity. We were very interested in studying 4′-hydroxychalcones due to their effectiveness as cytotoxic, antitumour agents. Cytotoxicity may be influenced by the nature of the amino group (pKa), shape and size of the amines dramatically to alter bioactivity. In our study, the reaction of various 4′-hydroxychalcones (Series A) with paraformaldehyde and several secondary amines [morpholine (Series B), pyrolidine (Series C), N-methylpiperazine (Series D), dimethylamine (Series E), diethylamine (Series F), dipropylamine (Series G)] led to the formation of a novel series of 4′-hydroxy-3′-substituted aminomethylchalcones (Series B, C, D, E, F and G). Compounds were obtained by Mannich reaction. Except B8, E1, and F1, the other 45 compounds synthesized and to be presented here were reported for the first time. The in vitro cytotoxic activities of Series A-G were tested against the human hepatoma cells (Huh7) and breast cancer cells (T47D) and compared with the precursor 4′-hydroxychalcones (Series A). These cell lines were selected because hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world and it ranks at third place in the list of cancer-related mortality,and breast cancer is the leading cause of cancer death among women with approximately a million new cases each year. MTT test was applied for the evaluation of cytotoxic activity. Despite there are some therapy options such as surgery, chemotherapy, and radiation therapy in the treatment of cancers, several side effects and drug resistance to chemotherapeutic agents are often encountered problems in the course of therapy. Therefore, it is necessary to develop novel approaches and discover

new drug candidates that can be used in the treatment of HCC and/or breast cancer/s. Synthesized Mannich bases displayed more cytotoxic activity than the precursor 4-hydroxychalcones against Huh 7 cell line from 1.17 times to >30 times in 34 Mannich bases among 48 and from 1.18 times to >25 times in 33 Mannich bases among 48 against T47D cell line. 39 compounds against Huh 7 cells, and 23 compounds against T47D cells among 48 Mannich bases showed more potent cytotoxicity than the reference compound 5-Fluorouracil, 5-FU. Our results suggest that preparation of Mannich bases of chalcones to develop new anticancer drug candidates is a useful modification to increase the cytotoxicity.

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P-105

Benzophenanthridine alkaloids and 2-arylbenzofuran neolignans from roots of *Zanthoxylum capense* and their cytotoxicity study

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The genus *Zanthoxylum*, comprises of approximately 250 species and is well known for its ethno pharmacological uses among Rutaceae family. Previous studies have demonstrated that plants belonging to this genus are rich sources of biologically active compounds, such as alkaloids, aliphatic and aromatic amides, coumarins, as well as lignans. *Zanthoxylum capense* (Thunb.) Harv. is a medicinal plant indigenous to Zimbabwe, South Africa, and Mozambique. Traditional healers use the decoction of its roots for snakebites, and the decoction of its root barks to treat tuberculosis, paralysis, and relief of toothache. However, until date there have been relatively few phytochemical studies on this species. During our search for bioactive compounds from the methanolic extract of *Z. capense* roots, thirteen compounds belonging to 2-arylbenzofuran neolignans and benzophenanthridine alkaloids, were isolated from MeOH extract of roots of African medicinal plant *Zanthoxylum capense*. The structures of these compounds were established by spectroscopic methods, namely 1D and 2D NMR (^{1}H, ^{13}C, DEPT; COSY, HMQC, HSQC, HMBC, NOESY), MS, IR, and UV. The structures of the known compounds were confirmed by the comparison of NMR spectroscopic data with those of reported in the literature. The cytotoxicity of these compounds was evaluated in HCT116 colon carcinoma cells by MTS assay. Three 2-arylbenzofuran neolignans and three benzophenanthridine alkaloids displayed significant cytotoxicity to HCT cells in a dose dependent and/or a single specific dose manner, showing comparable results to the positive control, 5-fluorouracil.

ACKNOWLEDGEMENTS

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P-106

Nor-β-lapachone against NQO1-overexpressing human prostate tumor cells

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In recent years, nor-β-lapachone, the inferior homolog of β-lapachone (a DNA repair inhibitor), has been recognized as important prototype with activity against cancer cells devoided of cytotoxicity in non-tumor cells. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a reductive enzyme that is important for the activation of many bioreductive quinones. Thus, differential levels of NQO1 in tissues, including tumors, can provide a target for an enzyme-directed approach to cancer therapy. Herein, we aimed to evaluate the role of NQO1 on the cytotoxicity of nor-β-lapachone using the prostate DU-145 tumor cells (NQO1-overexpressing). Cytotoxic potential of nor-β-lapachone was evaluated by the MTT assay, and apoptosis and free radicals were observed by flow cytometry. Also, comet assay was performed to evaluate the DNA strand breaks induced by nor-β-lapachone. For all experiments, cells were treated in the presence or absence of dicoumarol (NQO1 inhibitor). Nor-β-lapachone showed significant cytotoxic activity (IC50 2.98 μM) after 24 h exposure. In order to determine the mechanisms involved in the cytotoxicity, cells were treated with increasing concentrations (1, 2 and 4 μM) of nor-β-lapachone during 4 h. After exposure, apoptosis signals, DNA damage and free radicals production were observed. Coadministration of dicoumarol (50 μM) abrogated nor-β-lapachone

derivatives-mediated cytotoxicity and downstream apoptotic end points. In summary, NQO1 may be a pharmacologically exploitable target for therapy against certain tumors using lapachone compounds. Our results demonstrate that NQO1 is a key intracellular determinant for nor-β-lapachone in human prostate epithelial cancer cells.

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P-107

Methylated-resveratrol derivatives display different IC50 and cause different cellular responses in comparison with trans-resveratrol on lymphoma cell lines

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Resveratrol (E-RSV) is a natural polyphenol well characterized for its several beneficial properties. These span from the chemopreventive action to the antioxidant activity, the promotion of tissue differentiation and the antiproliferative effect in several tumoral experimental models. Some mono- and di-methylated derivatives have been synthesized in order to improve RSV natural antitumoral activity. We investigated the activities of some new RSV-methylated derivatives on two lymphoma cell lines. Here we show the antiproliferative activity, the cell cycle changes and the morphological modifications induced by the treatment with three original compounds: 4'-hydroxy-4-trans-methoxystilbene (MC40); 4'-hydroxy-3,5-trans-dimethoxystilbene (MC43); 3,4'-trans-dimethoxysilbene (MC149). We first determined the antiproliferative activity of E-RSV and we found that Toledo showed an IC50 of 11.6 micromolar while L-428 an IC50 of 27 micromolar after 48 h treatment. E-RSV did not induce any dramatic change inToledo cellular morphology but determined a significant change on the cell cycle. Specifically, E-RSV caused the accumulation in the S phase after 10 micromolar and the accumulation in G0/G1 starting at 25 micromolar. The sub-G1 peak increased in a dose-dependent fashion becoming statistically different at 10 micromolar. At variance with what observed inToledo cells, L-428 showed the appearance of the apoptotic bodies starting at 25 micromolar E-RSV. The three methylated derivatives displayed very different IC50 values onToledocells with MC40 being the most powerful showing an IC50 of 0.9 micromolar. Fluorescence microscopy showed that MC40-treated nuclei have a granular appearance. The cell cycle evidenced an accumulation of cells in the G0/G1 phase after 10 micromolar and the significant increase of the sub-G1 peak starting at a concentration of 5 micromolar. Interestingly, MC43 (a di-methylated- derivative) had an IC50 of 5.2 micromolar and determined a bi-phasic effect. The treatment with 5 micromolar determined the accumulation in the G2/M phase and the increase of DNA content whereas the treatment with 25 micromolar caused the cells to accumulate in the G0/G1 phase. The cell morphology was profoundly affected by MC43 in that polyploid cells appeared starting at 1 micromolar and became very abundant at 5 micromolar. On the contrary, MC149 did not seem to inhibitToledogrowth and the cell morphology did not show significant modifications. Our results represent the first characterization of these three RSV-derivatives in a model of lymphoma cells. The observed decrease in the IC50 after MC40 treatment and the changes in the cell cycle profile when compared to the cells treated with E-RSV suggest a different mechanism of action. AnnexinV/propidium iodide staining experiments are currently being performed in order to quantify the apoptosis onset and the possible necrosis at higher concentrations.

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Chalal M., Vervandier-Fasseur D., Delmas D., Meunier P. and Casali B.

P-108

Natural anticancer compounds of vitamin A and vitamin D origin act through their cognate nuclear receptors

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Natural compounds (retinoids, rexinoids) of the vitamin A origin and their biologically active derivatives are involved in a complex arrangement of physiological and developmental responses in many tissues of higher vertebrates. Both retinoids and rexinoids are either natural or synthetic compounds related to retinoic acids that act through interaction with two basic types of nuclear receptors: retinoic acid receptors (RARalpha, RARbeta and RARgamma and retinoid X receptors (RXRalpha,

RXRbeta and RXRgamma) as retinoid-inducible transcription factors. Thus, the retinoid receptors are considered to be ligand-activated, DNA-binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanism responsible for transcriptional responses in target genes. They exert both beneficial and detrimental activity; they have tumour-suppressive activity but on the other hand they are teratogenic. Retinoids inhibit carcinogenesis, suppress premalignant epithelial lesions and tumour growth and invasion in a variety of tissues. Natural and synthetic retinoids have therapeutical effects due to their antiproliferative and apoptosis-inducing effects. They are known to cause redifferentiation or to prevent further dedifferentiation of various neoplastic tissues. VDR is the only nuclear receptor protein that binds the biologically most active vitamin D metabolite of natural origin, 1α,25-dihydroxyvitamin D3 (calcitriol, "vitamin D" hormone), with high affinity. 1α,25-dihydroxyvitamin D3 is known to exert tumour-suppressive activity and its biologically active derivatives may have therapeutical exploitation due to their antiproliferative and apoptosis-inducing effects. We have evaluated effects of 13-*cis* retinoic acid and other biologically active compounds of natural origin on mammary gland tumour development and tumour progression. The expression of the retinoid/rexinoid nuclear receptor subtypes has been studied by the RT-PCR or EMSA techniques. In conclusion, experimental approaches based on studies of functional nuclear receptors for biologically active compounds – ligands for specific transcription factors might thus enhance therapeutical potentialities and bring positive results in the treatment of a variety of neoplasias.

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P-109

Kinetin riboside for CLL cells – a new proapoptotic agent

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Chronic lymphocytic leukemia (CLL) is characterized as a hematological neoplasm involving mainly deregulated apoptosis. A strong difference between patients in the disease progression, response to anti-cancer therapy, and outcome is a serious problem in the curing of this type of leukemia. Thus searching for new treatment options reflects to be important challenge for design effective personalized therapy strategies. Kinetin riboside [(6)-furfuryladenosine] belongs to derivative of plant hormones – cytokinins that displays impact usually on cell cycle reflecting the disturbances in cell signaling. The aim of our *ex vivo* studies was to evaluate the apoptosis induction efficacy of kinetin riboside in peripheral blood mononuclear cells (PBMCs) in comparison with normal ones obtained from blood of leukemic patients or healthy donors, respectively. We have also monitored the induction and progress of apoptosis in PBMCs exposed *ex vivo* to kinetin riboside in respect to chemo/immunotherapy used in hematological clinics to cure of this type of leukemia, i.e. purine analogs combined with active form of cyclophosphamide – mafosfamide, i.e. CM (cladribine + mafosfamide), FM (fludarabine + mafosfamide), and additionally to CM combined with monoclonal antibody – rituximab, RitCM. Studies were performed by using cell viability and rate of apoptosis assay, differential scanning calorimetry (DSC), and proteolysis of apoptosis marker – PARP-1. The strong differences in cell viability between leukemic and normal mononuclear cells exposed to kinetin riboside was registered. A strong diminution in CLL cell viability from 24 hrs of leukemic cells exposed to kinetin riboside was observed. While, only slight marginal decrease of normal PBMCs was noticed. The decrease of viable cell level was accompanied by the strong decrease of transition at 95±5°C in thermal scans of nuclear fraction preparations and proteolytic cleavage of PARP-1. Summarizing, we present a new multidirectional approach for the identification of anti-CLL agent – kinetin ribosiede indicating its high proapoptotic potential. Importantly, this natural plant small molecular compound reflects selective activity towards CLL cells.

P-110

Synergistic anticancer action of vitamins C and K3: *in vitro* and *in silico* studies

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Malignant glioma is the most common primary CNS tumour and it is an incurable one – it usually causes death within 2 years after conventional therapies. The development of more potent and less toxic compounds represents one of the major goals to overwhelm the poor outcome of patients with glioblastoma. The aim of this work was to study the cytotoxic action of vitamins C and K3 and

their mixture on rat glioma C6 cells *in vitro* and the plausible mechanism of the synergism of their anticancer action. The cells were grown in monolayer culture in 60-mL flasks at 37 °C and 5% CO2 in a water-jacketed incubator IR AutoFlow NU-2500E (NuAire, Plymouth, MN, USA). The culture medium consisted of Dulbecco's modified Eagle's medium (Sigma-Aldrich, Chemie GmbH, Steinheim, Germany) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 1% L-glutamine (Sigma-Aldrich). The cytotoxicity of vitamins C and K3 alone as well as cell sensitivity to combined treatment with vitamin C and vitamin K3 were estimated from the reduction of the cell viability. Cell viability was determined by means of a colony-forming assay. The concentration of vitamin C required to reduce cell survival by 50% was 0.3 mM for rat glioma C6 cells. Vitamin K3 killed 50% of cells at the concentration of 7 mM. Treatment of cells by both vitamins at the ratio of 100:1 (VC:VK3) greatly enhanced their cytotoxicity towards rat glioma C6 cell line. The vitamin K3 concentration required to kill 50% of cells was reduced from 7 to 1.4 mM, that is, about 5 times. The vitamin C concentration required to kill 50% of rat glioma C6 cells was also reduced – from 0.3 to 0.14 mM. It is assumed, that the synergistic anticancer effect of the ascorbate/menadione combination is likely explained by the redox-cycling that occurs between these compounds – vitamin C significantly increases the rate and product yield of the K3 redox reaction. However, the detailed theoretical analysis of this system has not been done yet. Therefore, the theoretical quantum-chemical analysis of the dynamic electron transfer processes within the complexes containing various forms of vitamins C (*L*-ascorbate, two forms) and K3 (menadione, one form) has been carried out. Optimization of the ground state complex geometry was provided by means of *GAUSSIAN03* package using HF/6-311G and HF/6-311G(2df.2pd) basis. Simulation of the intermolecular electron transfer (IET) was done using NUVOLA package [4], in the framework of molecular orbitals (MO) expressed as linear combination of atomic orbitals (AO). Rate of IET *k* was calculated using Fermi Golden rule. Optimized structure of the vitamins complex have been determined and the results of simulations allow us to create the model of reaction pathway. It can be concluded that the cytotoxic action of the mixture of vitamins C and K3 at the ratio of 100:1 is synergistic towards rat glioma C6 cells.

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P-111

Analytical method for the determination of 6-thioguanine and 6-methylmercaptopurine in erythrocytes for monitoring pediatric patients after azathioprine therapy

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6-mercaptopurine (6-MP), its pro-drug azathioprine (AZA) and 6-thioguanine (6-TG) are active substances which have been used in chronic gastrointestinal diseases like inflammatory bowel disease as well as in some kind of cancer e.g. acute lymphoblastic leukemia for many years. Unfortunately, the effectiveness of the treatment has several limitations associated with metabolism of these compounds and also with some genetic aspects. While 6-TG as a product of one metabolic pathway ensures an immunosuppressive effect, 6-methylmercaptopurine (6-mMP), which is formed in the second metabolic pathway, shows strong hepatotoxic properties in high concentration. Due to wide inter-individual differences in AZA and 6-MP metabolism among patients receiving identical doses of these agents, the monitoring of erythrocyte levels of 6-thioguanine nucleotides and 6-methylmercaptopurine nucleotides has been proposed as a useful clinical tool for assessing the treatment efficacy and toxicity. The method involves a simple treating procedure based on deproteinisation by perchloric acid followed by acid hydrolysis and heating for 60 min at 100°C in order to release the free bases. The 6-mMP derivate which is formed during acid hydrolyses and 6-TG was analyzed using high performance liquid chromatography with photo-diode array detection at 302 nm and 344 nm respectively. For the gradient elution the ammonium acetate/ formic acid/ acetonitrile buffer with C18 pre-column and column were used. The aim of this work was to validate a simple analytical method for the monitoring of pediatric patients during azathioprine therapy.

P-112

Glycerolipids from the egg masses of the mediterranean mollusca *Aplysia depilans*: anticancer activity against breast cell lines

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Aplysia egg masses are soft and gelatinous noodle like strands not physically protected from antipredatory attacks and exposed to bacterial infections. Despite their bright colour (from yellow to orange) and their highly nutritive value (rich in proteins and polysaccharides), the egg masses of sea hare were rejected by predators and showed strong antibacterial activities against marine and terrestrial pathogens. These observations suggest the existence of chemical defences responsible of the deterrence and the antibacterial effects. *Aplysia depilans* egg masses, collected from the gulf of Tunis, were chemically analysed: extraction, isolation and structural elucidation (1D and 2D NMR, MS), which led us to identify several glycerolipids. These metabolites may contribute in the chemical defences of the egg masses. All the metabolites were evaluated for their in vitro toxicity against three human tumor cell lines: HT29 (colon carcinoma), A549 (lung carcinoma) and MDA-MB-231 (Breast). Only two glycerolipids showed a selective and potent cytotoxicity against breast tumor cell lines at concentrations below 10 μM.

P-113

Geraniol inhibits tumor cell proliferation *in vitro* and *in vivo* by posttranscriptional regulation of HMGCR

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Plant isoprenoids are widely known as nontoxic natural compounds that inhibit cell proliferation with selectivity against tumor cells. Isoprenoids are also potent suppressors of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), the enzyme catalyzing the main rate-limiting step of the mevalonate pathway and cholesterol synthesis in mammalian cells. HMGCR inhibition decreases the elevated cholesterol levels widely required by rapidly growing cancer cells, inhibits the prenylation of certain growth-regulatory proteins that play a key role in controlling cell proliferation, and induces apoptosis. Geraniol, an acyclic isoprenoid monoterpene, occurring in the essential oils of several aromatic plants, is thought to represent a new class of chemotherapeutic agent. In order to evaluate the antiproliferative property of geraniol *in vitro* and *in vivo*, and to understand the mechanisms by which this monoterpene inhibits cell growth, we studied its effects on human- lung epithelial (A549) and human-hepatoma cell lines (HepG2) as well as on A549 implanted in nude mice fed with 50-75 mmol.GOH/kg diet for 21 days. Viability and proliferation of cells incubated with 200-600 μM geraniol were determined by trypan-blue–dye-exclusion cell and by the MTT assay. Apoptosis was determined by TUNEL assay and caspase-3 activity. HMGCR expression was analyzed by real-time RT-PCR, Western blots, and [^{14}C]HMG-CoA-conversion radioactivity assays. Lipid synthesis was assessed by incorporation of [^{14}C]acetate. Our results showed that 200-600 μM geraniol inhibits cell proliferation in both cell lines. HMGCR-mRNA expression was significantly increased in liver cells but HMGCR-protein levels and enzymatic activity, along with cholesterol synthesis, became significantly decreased. These results showed that geraniol down-regulates the enzyme by a posttranscriptional mechanism. *In vivo* assays geraniol significantly reduced tumor growth and significantly enhanced apoptosis of tumor cells. Treated host-mice also showed a decreased in HMGGR-protein levels and cholesterogenesis. The relevance of this work resides in the finding that geraniol both decreases HMGCR levels and inhibits tumor cell proliferation *in vitro* and *in vivo* suggesting that geraniol has a great potential as a natural drug against cancer.

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P-114

Benzofuran neolignans as inhibitors on enzime-based drug targets of tumor cell lines by docking analyses

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Research on anti-cancer agents through exploration for sources of novel cytotoxic substances has led to novel medical advances. Benzofuran compounds are common

metabolites in plant and microorganisms, possessing a wide-range of activities. However, benzofuran-based structures are considered as important moieties for anticancer drugs development, mostly due their action on enzyme-based drug targets such as kinase, topoisomerase and ribonucleotide reductase, among others, constituting in good targets for numerous tumor cell lines. As part of our research on anticancer agents, several plant-derived dihydrobenzofuran and benzofuran neolignans were evaluated *in vitro* against a set of tumor cell lines in order to observe the cytotoxic activity through cell viability. Benzofurans were found to be the most potent cell inhibitors against HeLa tumor cell line. In addition, for structure-activity relationship purposes, Autodock Vina was used to dock the most stable conformers from DFT-level optimized structures of test compounds within the active site of enzyme-based drug targets of antitumor cell lines such as aromatase, thymidylate synthase, human peptide deformylase, kinase and topoisomerase and ribonucleotide reductase. Good correlations were found between *in vitro* activities and docking. Most stable conformer of benzofuran 1 was found to exhibit the best correlation. Benzofuran neolignans might be considered as good candidates for structural optimization leading natural product-based anticancer drugs.

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P-115

Electrophoretically mobile *Saponaria officinalis* saponin as a highly synergistic enhancer of tumor specific toxicity for saporin-EGF: *in vitro* and *in vivo* evaluation

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Targeted toxin therapeutics is hindered by poor intracellular uptake, limited stability and non-specific immune stimulation. To address these problems, ligand-targeted toxins in combination with saponins at a low dose have been adapted and tested *in vivo* in the present work. We used a targeted toxin Saporin (Sap3)-EGF (Epidermal Growth Factor) in combination with an isolated saponin m/z 1861 (SO-1861) from *Saponaria officinalis* roots. The saponin had a relative electrophoretic mobility of 0.64. *In vitro* evaluation confirmed a more than million-fold enhancement in the concentration of toxin needed to induce death of TSA-EGFR cells. The dosages of targeted toxin required was highly reduced (0.00001 nM with SO-1861 to 10 nM without SO-1861) and there was a highly synergistic effect. *Ex vivo* hemolysis assay showed no or very less hemolysis (< 10%) up to 100 µg/ mL for SO-1861. In the acute toxicity studies SO-1861 was found to be non-toxic at a dose of up to 100 µg/ treatment. The toxic effects were evaluated by histo-pathological examination and testing of different blood paramters. No toxic effects in aspartate aminotransferase/alanine aminotransferase ratio was observed confirming hepatic safety of SO-1861. The levels of creatinine and C-reactive protein were also similar to control group thus ruling out damage to kidney or secondary inflammatory responses. *In vivo* studies in syngeneic tumor model over-expressing EGFR was done at two dosages , (a) 30 µg/treatment of SO-1861 + 0.1 µg Sap3-EGF and (b) 15 µg/treatment of SO-1861 + 0.3 µg of Sap3-EGF. A > 95% reduction (P< 0.001) in tumor volume was observed in the group b, and > 90% reduction (P< 0.005) in group a. There was a drastic reduction in the previously reported toxic side-effects of targeted toxin Sap3-EGF alone as the combination was evaluated below NOEL for indvidual components.

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P-116

Synthesis of functionalized porphyrinoid compounds to increase and optimize antiproliferative effects in neuroendocrine tumors

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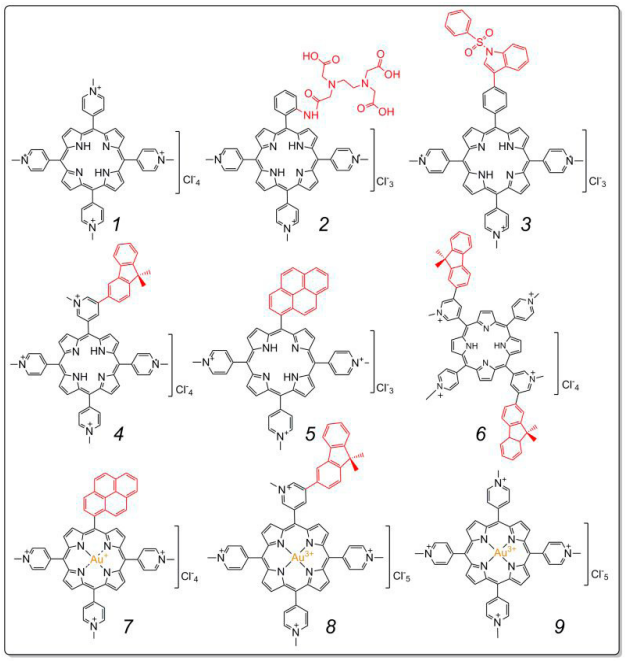
Cationic porphyrins represent an expanding class of compounds, which have several applications in biology, medicine and catalysis and have been studied from the viewpoint of their role as DNA cleavers, G-Quadruplex DNA stabilizers and downregulators of proto-oncogenes (RET, c-Myc, Ras, ERK etc.). Recent studies about the interaction of cationic porphyrins and their derivatives with telomeric DNA have moved porphyrin compounds into the spotlight of an alternative anticancer strategy. The tumoristatic effects of novel completely water soluble symmetric and asymmetric non-metalated and metalated porphyrinoid compounds were evaluated on two cancer types: a) small intestinal neuroendocrine tumor cells (SI-NETs), and b) medullary thyroid carcinoma cells

(MTCs). In addition, the intracellular mechanism was elucidated. The tumoristatic effects could be selectively altered on specified neuroendocrine tumors by specific chemical modification of the porphyrinoid compounds.



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P-117

Influence of oral echium oil (omega-3 fatty acid) supplementation on weight loss in (pre)cachectic head and neck cancer patients: the nutriom trial

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Cancer cachexia is a complex metabolic process responsible for 20-40% of all cancer deaths. Head and neck (H&N) cancer patients are nutritionally vulnerable because of tumour localisation, association of etiological factors with nutritional deficits and the intensive treatment. Recently, omega-3 fatty acids have gained interest for their potentially beneficial effects on weight maintenance in cancer patients undergoing intensive treatment. Multicentric, placebo-controlled trial. Newly diagnosed non-metastatic (stage I–IVB) head and neck cancer patients (≥18 or older) eligible for curative primary or adjuvant radiotherapy with or without systemic treatment will be randomised between omega-3 fatty acid supplementation (echium oil) or placebo. Primary objective: prevention of therapy-related weight loss. Secondary objectives: determination of beneficial effects of omega-3 FA supplements on body weight and composition, and quality of life in general; to define dropout and compliance to nutritional supplements; to establish feasibility & variability of BIA, DXA & JAMAR® as objective measurement tools of body composition & strength; identification of clinical risk factors of cachexia; evaluation of the use & reliability of different validated nutritional screening tools in this population; and identification of potential biomarkers for therapy-induced cachexia. The early detection of cachexia in cancer patients is very important. Omega-3 FA supplementation may be useful in the nutritional supportive care of H&N cancer patients, to reduce weight loss and potentially improve quality of life. This trial is currently recruiting patients.

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P-118

A bioassay directed fractionation approach to discover novel bioactive organobromines from marine sponges and algae

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Organobromine compounds (OBrs) are known to be either synthetic or natural and have been used in a number

of industrial processes as additives or final products. One such synthetic class of compounds, brominated flame retardants (BFRs), is found in various trophic levels of the food chain and has recently emerged as a contaminant of concern for human and animal health. In addition to the synthetic compounds, there are at least 1500 naturally occurring brominated compounds that have been identified in marine and terrestrial organisms. Several of these novel compounds have been shown to have bioactive effects ranging from anti-bacterial, anti-viral, anti-inflammatory, to anti-cancer. The purpose of this project is to identify novel organobromines found in marine organisms including sponges and algae, to optimize current extraction and bioassay-directed fractionation techniques, and to assign bioactive properties to these compounds. Samples were extracted from homogenized red algae, *Porphyra haitanensis*, and *Gracilaria lemaneiformis* using an accelerated solvent extraction system with DCM/Hexane (1:1) and hexane/MTBE (1:1). The crude organic extract was assayed for cytotoxicity and anti-proliferation properties in the Hek293Tcell line. Preliminary results indicated cytotoxicity and a decrease in cell proliferation at 1x concentration of the crude organic extracts. Investigation of additional properties of this crude extract is underway. A bioassay directed fractionation approach will be employed for further isolation/purification of bioactive fractions. Relatively pure bioactive fractions will be used for structural elucidation and quantification using mass spectrometric instrumental analysis. A high resolution gas chromatography mass spectrometry (HRGC-MS) system will be used for structural analysis with volatile fractions, and liquid chromatography mass spectrometry (LC-MS/MS), and quadrupole time of flight mass spectrometry (Q-TOF-MS) will be used for non-volatile fractions.

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Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic properties and impairs mitochondrial transcription and biogenesis

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Vitamin E analogue α-tocopherol succinate (α-TOS), exhibits selective anti-tumour effect that is dependent on its interaction with mitochondrial complex II (CII), particularly its proximal ubichinone-binding site(Qp). Binding of α-TOS to Qp results in generation of reactive oxygen species (ROS), activation of the Mst1 kinase and FoxO1 leading to induction of Noxa and subsequent apoptosis of cancer cells. Mitochondrial targeting of α-TOS via addition of triphenyl phosphonium generates a novel compound termed MitoVES (mitochondrially targeted vitamin E succinate), which retains anti-cancer selectivity, accumulates within mitochondria and exhibits significantly higher potency in apoptosis induction of cancer cells. The molecular mechanism of MitoVES induced apoptosis is similar to that of α-TOS, but the induction takes place at much lower doses owing to efficient accumulation at the interface of mitochondrial matrix and inner mitochondrial membrane, a place of CII localization. Importantly, MitoVES shows a anti-cancer activity *in vivo* using the *c-neu* mouse model of spontaneous breast cancer, where it significantly suppresses tumour growth and neovascularisation. Additionally, application of MitoVES at lower doses inhibits mitochondrial transcription (particularly that of D-loop transcript) and biogenesis leading to the block of cell proliferation but not apoptosis induction. These changes are subject to ongoing research but are probably related to inhibition of cell respiration connected with drop in mitochondrial membrane potential and ROS generation. In conclusion, the combination of cancer selectivity, anti-angiogenic and anti-proliferative properties makes MitoVES a very effective and potent agent against cancer cells *in vitro* and *in vivo*, showing the importance of mitochondria as a target site of cancer cells and opening a new paradigm in cancer treatment.

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