Poster Session

Short tandem repeat polymorphisms and breast cancer

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Microsatellites are widely spread in the genome and may lie in any of its regions, including coding , regulatory and intronic regions. Expansion or contraction of these sequences may affect gene transcription, mRNA stability, RNA splicing or protein structure and function. Some of them have been related with breast cancer by association studies. The purpose of this study was to investigate the role of four polymorphic microsatellites lying in different genes related to breast cancer : E2F-4 (transcription factor involved in gene transactivation during the G1-S transition of the cell cycle), EGFR (epidermal growth factor receptor), UGT1A1 (UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, including the chemotherapeutic drug irinotecan, into water-soluble excretable metabolites), and NOTCH4 (a receptor implicated in the NOTCH pathway and related to breast cancer (BC) development). To carry out this case-control study, blood samples of incident breast cancer patients have been collected at the Hospital Insular de Gran Canaria. Control samples were selected from Canary Island Cohort study (CDC). Data related to risk factors for BC (first degree family history of BC, age at menarche, menopausal status, oral contraceptive use, etc.) were obtained from each woman through a structured questionnaire and in-person interview. At this moment samples (cases and controls), are being genotyped to these four polymorphisms.

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NOTES

Synthesis and Structural Study of a □,ε-Peptide with a Beta Turn Conformation.

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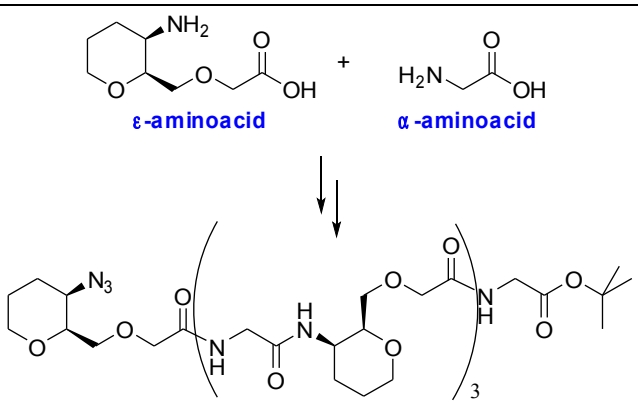
During the last years foldamers have show a particular interest in many scientifics fields including chemistry, materials and biological activities because of their capability to adopt specific compact conformation. Recently, have been development oligomers that can bind to specifics proteins surface sites, helping molecules acting against several cancer cell lines, where their activity has been diminished, and in some cases inhibited because of an over express of anti-apoptotic proteins.

Within our program direct to the search of new structural units suitable for cation recognition phenomena, we focused our attention on the *cis*-2-alkyl-3-oxy-tetrahydropyran unit, as a key motive for the design of new chiral cation receptors. We found that some cyclic oligomeric esters with this structural unit present a flat conformation, showing phenomena of self-assembling in the solid state. We will be described in this poster, the synthesis and studies of molecules equivalents to those esters but using amides, because these have the potential to create hydrogen bond, an useful tool in foldamers design.



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New benzofurans isolated from the root of the *Cyperus teneriffae*

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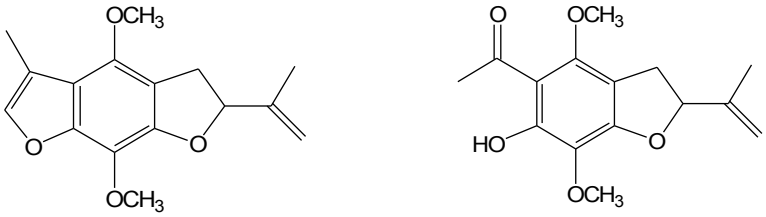
The benzofurans are a large group of structures that are of great interest because they exhibit a wide range of biological and pharmacological activities. For example some benzofurans are inhibitors of the induction of caspase-1[1], inhibitors peptidases related alzheimer[2] or inhibitor of angiogenesis[3,4]

In relation to the above mentioned, in this communication we report the isolation and structural characterization of the first benzofurans and phenol derivatives found in the root of the *Cyperus teneriffae*, a species belonging to the family Cyperaceae. This is the first phytochemical study of this species which shows great capacity biosynthesizing secondary metabolites of biological interest.



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Acknowledgements: To Ministerio de Educación y Ciencias (Project SAF 2006-06720) and FICIC (Pr. ResCancerBiotech FICIC-03/08) for financial support. A.A thanks the Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH, from UCV) for pre-doctoral fellowship.

NOTES

Preliminary studies of a novel fluorescent tamoxifen derivative

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Tamoxifen, a triphenylethylene antiestrogen, has been widely used as an adjuvant in the treatment of hormone receptor-positive breast cancer for over 20 years. However, numerous studies show that these triphenylethylene compounds interact with targets other than canonic estrogen receptors (ER), such as the ER located at the plasma membrane, antiestrogen-binding sites (AEBS), ionic channels and other cell membrane proteins as well as with intracellular pathways proteins. These interactions are usually related to the existence of adverse side-effects during chronic therapies.

The development of fluorescent conjugates on permeable and impermeable derivatives will help to unravel the cellular targets for tamoxifen, both at the plasma membrane and intracellular levels, giving us the possibility to obtain more specific and selective compounds to treat breast cancer with less undesirable side-effects.

In this sense, the first fluorescent tamoxifen derivative has been synthesized by our group using 7-nitrobenz-2-oxa-1,3-diazole (NBD) as fluorophore and maintaining the triphenylethylene core of tamoxifen which is essential to interact with estrogen receptors. The fluorescence emission and excitation spectra were determined by measurement spectrofluorimetry. Efficiency and viability of this fluorescent compound was assessed using confocal microscopy the estrogen receptor-positive cell line MCF-7. These assays were also carried out on SN56 cells, a murine cholinergic cell line from the basal forebrain, which also express estrogen receptors. Finally, competition studies showed that the fluorescent derivative binding at the plasma membrane could be readily antagonized by tamoxifen, indicating that the plasma membrane binding site is specific for triphenylethylene compounds.

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NOTES

INDUCTION OF CELL CYCLE ARREST AND APOPTOSIS BY NEW SYNTHETIC ALIPHATIC ACETOGENINS ANALOGS

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Phytochemicals present in fruits and vegetables are recognized as playing an important role in cancer prevention. Studies have shown that phytochemicals extracted from avocado selectively exhibit anticarcinogenic activity in cell culture studies. Although epidemiological studies show the health benefits of avocado, the cellular and molecular mechanisms of the phytochemicals responsible for cancer prevention are largely unknown. From the major chemical constitutients of avocados we have focused our attention on the so-called "aliphatic acetogenins". Representative examples of aliphatic acetogenins of avocado are shown in Figure 1.

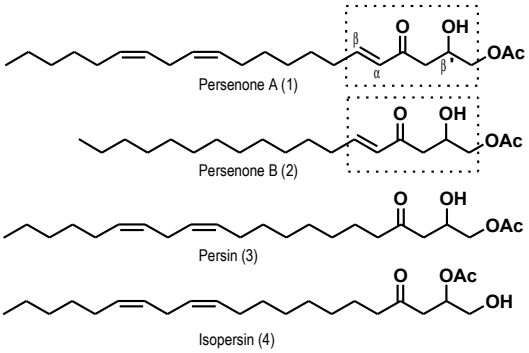

Figure 1. Chemical structures of natural aliphatic acetogenins from avocado

The objective of the present study was to evaluate the antiproliferative activity of a series of novel persenone analogs against six different representative human solid tumor cells. Cell proliferation was evaluated after 48 h of product exposure using the SRB assay. In addition, we examined cell cycle phase distribution by flow cytometry to determine if cell growth inhibition involves cell cycle changesSummary of the work.

NOTES

Simvastatin regulates the JAK/STAT signaling pathway in rat osteosarcoma cells

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Statins are extensively used as hipocholeterolemic drugs. They act by inhibiting the 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme in mevalonate biosynthetic pathway. Besides this well known effect, statins are capable to induce apoptosis and decrease viability and proliferation of several cancer cells. These effects can be reversed by mevalonate. On the other hand, proliferation and survival of several cancer cells and tumors are strongly dependent of Signal Transducer and Activators of Transcription (STAT) proteins. The physiological activation of STAT signaling pathway is rapid but transient in nature and its inactivation is mainly due to Supressors Of Cytokine Signaling (SOCS) proteins. Furthermore, STATs induce the expression of SOCS proteins which then act in a negative feedback loop to inactivate STAT. However, in cancer cells there exists an "aberrant" activation of STAT (mainly STAT3 and STAT5) due to 1) constitutive activation of tyrosine kinases and/or 2) "turning off" negative regulators of STAT like SOCS proteins. Thus, finding drugs to inhibit STAT or activate SOCS might be a potential strategy to develop anticancer drugs. In this work, we have studied the effects of simvastatin on osteosacoma cell line, UMR-106. Simvastatin induces apoptosis on UMR cells, inhibits STAT-mediated transcriptional activity after stimulating cells with FBS or GH, and increase expression of SOCS-3 and CIS genes in a time-dependent manner, two natural inhibitors of STAT signaling pathway. These findings suggest that simvastatin, and probably other statins, inhibit STAT signaling pathway through, at least in part, by induction of SOCS proteins.

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NOTES

Reversion of Human Pgp-dependent Multidrug Resistance by New Sesquiterpenes from *Celastrus vulcanicola*

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Multidrug resistance (MDR) is one of the main challenges in the chemotherapy of cancer and other important diseases[1,2]. In an intensive study of South American medicinal plants, herein we report the isolation, structure elucidation and biological activity of nine new and seven known dihydro-□-agarofuran sesquiterpenes from the leaves of *Celastus vulcanicola*. Their structures were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments. All the compounds have been tested on human MDR1-transfected NIH-3T3 cells, in order to determine their ability to revert the multidrug resistance phenotype due to P-glycoprotein overexpression. Six compounds from this series showed higher effectiveness to the classical P-glycoprotein modulator verapamil when reversing resistance to daunorubicin and vinblastine. The structure-activity relationships are discussed.

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NOTES

Hairy roots cultures of *Echium acanthocarpum* for the production of medicinally important fatty acids

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Highly unsaturated fatty acids (HUFAs) of the ω-3 and ω-6 series (ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid) play important roles in several pathologies, specially to prevent cardiovascular diseases, and to suppress the growth of some tumors and proliferation of breast cancer and melanoma[1]. Furthermore, stearidonic acid (SDA) and gammalinolenic acid (GLA), ω-3 and ω-6 are precursors of HUFAs. The marine fish stocks represent the predominant natural source of these n-3 HUFAs for humans, but in view of the decline in these stocks, alternative sources are being pursued, for instance, a plant source such as, *Echium* genus oils, which constitute one of the largest plant sources of SDA and GLA. A new approach in this area has been designed and achieved in our research group, based on the novel induction and establishment of hairy root cultures for fatty acids studies[2-3]. The results indicate, for the first time, that indeed the roots of *Echium* are also the site of biosynthesis of fatty acids together with the aerial parts[4]. Furthermore, it was demonstrated that fatty acid production was almost uniform during the culture period with the maximun production established around the last third stage of culture (rate between fatty acid production and biomass) with the most abundant fatty acids being LA (32-44% of total fatty acid, 3-5 mg FA/g DW/mg TL), followed by palmitic acid (21-26% of total fatty acid, 2.5-3.0 mg FA/g DW/mg TL) GLA (9-13% of total fatty acid, 600-1000 □g FA/g DW/mg TL), ALA , oleic acid and SDA. The double bond index (DBI) coefficient increased with culture time, showing values of DBI of 2.5-3.0 which indicate an unsaturation enrichment of the extracted oil. In order to increase the accumulation of SDA, the overexpression of Δ6 desaturase from *Primula vialii*[5], which converts □-linolenic acid into SDA, but not linoleic acid into GLA, is being attempted in transgenic *E. acanthocarpum* hairy roots.

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NOTES

MVP expression is related to IGF1-R in cervical carcinoma patients treated by radiochemotherapy.

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Purpose:

To assess the expression of MVP in cervix carcinoma patients treated by radiochemotherapy, its relation to clinical and pathologic prognostic factors and its role in predicting clinical outcome. In adition the relation to IGF-1R expression in this cohort of patients will be explored.

Materials and methods:

Sixty consecutive patients suffering from localized cervix carcinoma were prospectively included in this study from July 1999 to December 2003. Follow-up was closed in November 2007. Patients were staged following the TNM classification. All patients received pelvic radiation (45-64.80 Gy in 1.8-2Gy fractions) followed brachytherapy and concomitant cisplatin at 40 mgr/sqm/week doses. MVP expression was studied by immunohistochemistry in paraffin-embedded tumour tissue.

Results:

MVP was expressed in 58 patients (96.7%) and no relation was found with clinicopathological variables. High MVP expression was related to high IGF1-R expression (p=0.023). Complete response after treatment was observed in 50 patients (83.3%). Clinical stage of the disease and clinical response to radiochemotherapy were the most important prognostic factors related to survival. High MVP and IGF1-R tumour expression was strongly related to poor local and regional disease free survival (p=0,006), distant disease free survival (p=0.050) disease-free survival (p=0,006), cause specific survival (p=0.007) in patients achieving a complete response.

Conclusion:

MVP and IGF-1R expression were related in clinical cervical tumours and confer reduced long-term local control in patients who achieved clinical complete response to radiochemotherapy.

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An Efficient Metal-Free, Three-Component Carbene-Based Manifold for the C2-Functionalization of Imidazole Operating "On Water"

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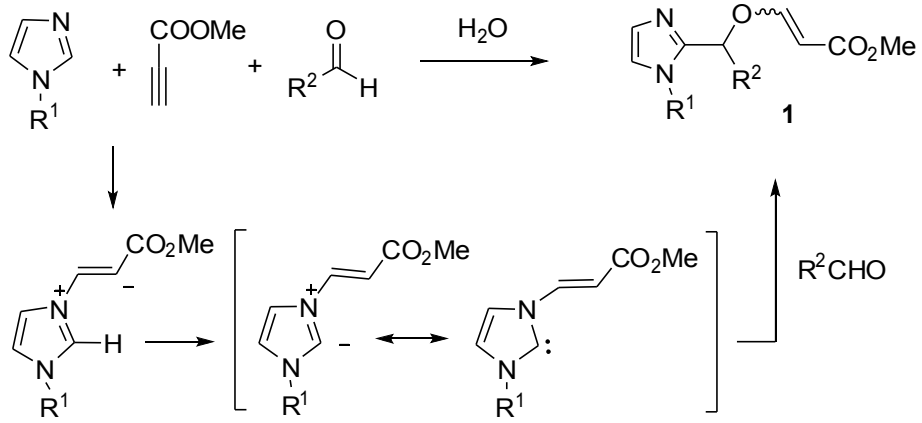
A nucleophilic carbene generated "on wáter" is the base of a new multicomponent manifold for the selective C2-functionalization of imidazole derivatives. The manifold operates under "on water conditions" and utilizes an N-protected imidazole, methyl propiolate and an aldehyde to construct C2-chain functionalized imidazoles **1**. These molecules are hybrids of imidazole (privileged structural motif)[2] and □-alkoxyacrylate (a synthetically relevant functionality).



The manifold is launched by the Michael addition of imidazole onto the propiolate to generate a zwitterionic imidazolyl-acrylate intermediate **2** which is converted into the nucleophilic carbene **3** (Breslow's carbene)[3] by a 1,4-hydrogen shift. Nucleophilic addition onto the aldehyde generates the C2-functionalized imidazole **1**.

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Maytenus spinosa, an Important Source of New Agarofuran Sesquiterpenes with anti-MDR Activity

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As part of our ongoing research program to isolate bioactive compounds focused on antitumoral products belonging to the Celastraceae family used in South America[1], *Maytenus spinosa* (Grisebach) Lourteig & O'Donell, was studied.[2] The EtOH extract of the leaves of *M. spinosa* was repeatedly chromatographed to yield the known triterpenes lupeol, epilupeol, betuline and lupenone together twenty agarofuran sesquiterpenos. Fifteen sesquiterpenes resulted new to the literature (i.e compounds 1, 2 and 3). Their structures were determined by means of ^{1}H and ^{13}C NMR spectroscopy studies, including ^{1}H-^{13}C heteronuclear correlation, (COSY, ROESY, HSQC and HMBC). The agarofuran sesquiterpenes are very interesting because they exhibit a broad range of biological activities (anti-MDR, anti-HIV, antifeedant…).[3] In this communication we will report the structural elucidation of the new compounds and the preparation of new agarofuran derivatives, the corresponding results of biological evaluation.

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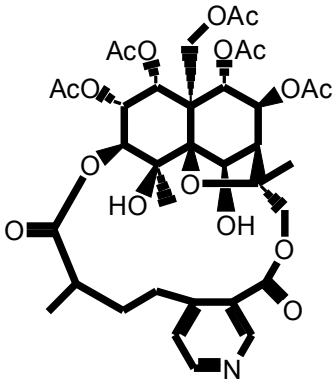

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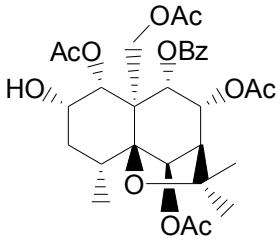

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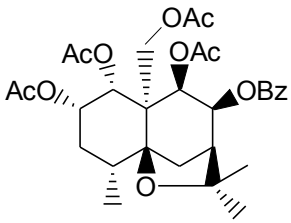

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Hypoxia downregulates Ku70/80 expression in cervical carcinoma tumors

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Hypoxia may lead to conditions that either cause increased spontaneous damage to DNA or inhibit DNA repair processes. Cells have several mechanisms to repair DNA damage before mutations appear. Non Homologous End Joining (NHEJ) repair-associated genes were also downregulated by hypoxia in one study , but no relation was found in other. Ku70/80 are key genes in the NHEJ repair pathway for radiation induced double strand breaks (dsb). In the clinical setting, Ku expression has been related to survival in patients treated by radiation, although results are rather controversial.

The aim of the present study was to assess the relation of the expression of the NHEJ repair protein Ku70/80 and tumor hypoxia in clinical cervical tumors. Angiogenesis and p53 protein expression were also studied in relation to hypoxia-induced tumor progression.

Forty-three consecutive patients were prospectively included in this study from July 1997 to September 2001. Patients suffering from localized cervix carcinoma, diagnosed and treated by definitive radiation at the Las Palmas Hospitals were included. Ku70/80 expression, tumor angiogenesis (CD-31 expression) and p53, was studied by immunohistochemistry Hypoxia may inhibits the NHEJ DNA repair through downregulating Ku70/80 expression and combined with an increased angiogenesis and altered p53 expression would be responsible for tumor progression in cervical carcinoma. Tumor oxygenation was measured by a polarographic probe system "pO2 Histograph" (Eppendorf AG, Hamburg, Germany). To determine tumor oxygenation, median pO2 and the percentage of pO2 values < 10 mmHg and < 5 mmHg were obtained from the pooled data for each individual.

In conclusion, hypoxia inhibits the NHEJ DNA repair through downregulating Ku70/80 expression combined with an increased angiogenesis and altered p53 expression. These mechanisms would be responsible for tumor progression in cervical carcinoma.

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NOTES

CDC14-dependent chromatid linkages lead to S-phase checkpoint activation and irreversible DNA damage responses.

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Chromosome segregation and cytokinesis are both regulated by the conserved phosphatase Cdc14 in late anaphase. Timely Cdc14 activation by FEAR network is a pre-requisite for full resolution and segregation of the ribosomal DNA locus (Torres-Rosell J, Machín F & Aragón L. Cell Cycle. 2005). Here we present data about the fate of cells after re-activation of Cdc14 very late in telophase. We show that most cells enter a new cell cycle and transit through a new G1 but then get arrested in S-phase. This arrest is a consequence of the irreversible activation of DNA damage checkpoints and correlates with the initiation of replication within the rDNA locus. Furthermore, correlation between failure in the rDNA resolution and activation of the DNA damage response is found. Finally, the age-related rDNA-specific replication fork block protein Fob1 worsens the DNA damage, a likely consequence of the worsening of the anaphase chromatid linkages that arise after Cdc14 inactivation.

NOTES

Ambiphilic allenes: Syntesis and Reactivity

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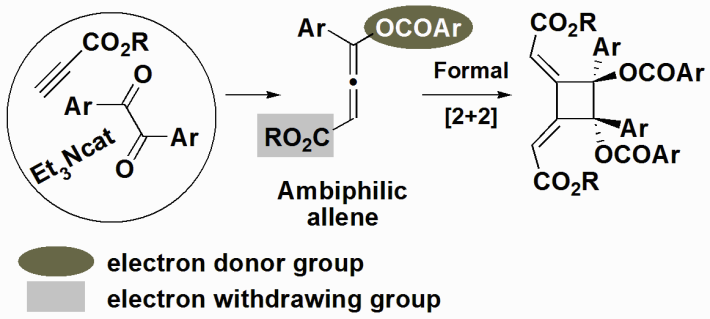
A novel and efficient organocatalytic synthetic manifold leading to ambiphilic allenes from readily available starting materials is described.[1]

The manifold involves the organocatalytic generation of a propiolate anion and a novel and highly effective rearrangement of a conjugated □-acyl propargylic alkoxide intermediate.

In the absence of any external chemical agents, these allenes perform a thermally-driven dimerization reaction to generate the corresponding fully-substituted cyclobutanes in a regio- and highly stereoselective manner.



Interestingly, the reaction network forms 2C-O and 4C-C bonds, while only breaking 2C-C bonds.

The in situ generation of these allenes is mild enough to be compatible with a large number of organic functionalities and it constitutes an excellent workbench for the discovery of new complexity generating processes. This issue is under study in our lab.

[1]Manuscript under editorial revision.

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Cytotoxic Activity of New steroidal lactones from *Withania aristata*

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The genus *Withania* (Solanaceae) is known for elaborating withanolides, which are steroidal lactone built on an ergostane skeleton of 28 carbons. Several of these substances have displayed various biological activities, such as cytotoxic, anticancer, antifeedant, antiinflamatory and immunomodulating activities[1]

Cancer is one of the major human diseases and causes considerable suffering and economic loss worldwide. Although considerable progress has been made in treating cancer, the incidence and mortality rate for most forms of cancers still remain very high. Therefore, further research is needed for the development of safe products for the prevention and treatment of human cancers. In this way, natural products are lead molecules for many of the drugs that are currently in use, and over 60% of anticancer drugs available in the market are of natural origin[2]. We have undertaken a systematic survey of the Canary Islands genera of *Withania* (Solanaceae)[3]. The phytochemical analysis of dichloromethane extract of the leaves of *W. aristata* led to the isolation of two new withanolides (W-1, W-2), in addition to five known (W-3-W-6). Their structures were determinated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments (COSY, ROESY, HMQC and HMBC), and chemical correlations.

The compounds have been tested for their cytotoxic activity, against HeLa, Hep-2, A-549, MCF-7 and vero cell lines, its derivatives exhibited IC_{50} ranging from 0.6 to 20 □g/ml against all the cell lines used.

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Acknowledgements We are indebted to the DGES (CTQ2006-13376/BQU) and FICIC (01/2007) projects for financial support. G.G.LL. thanks to the Gobierno Autónomo de Canarias for a fellowship

NOTES

Synthesis of bioactive □acyloxyamide through a new domino reaction

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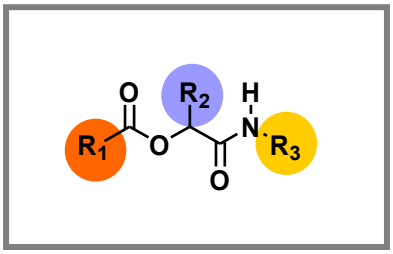
In the last years the domino reactions have emerged as valuable tools in organic synthesis for the development of bioactive molecules[1]. Such importance is due to the capacity of these reactions to cover a wide "chemical space" in only one event of reaction. Through this type of reactions it is possible to obtain the formation of molecules with diversity and structural complexity from simple reagents in a sequence (involving several transformations), without isolating intermediates, nor changing conditions of reaction nor adding additional reagents.

One of the research lines of our group is based on the synthesis of bioactive products by means of domino reactions[2]. In this communication we will report the results obtained in the development of a new □-acyloxyamide with three structural diversity points. This core is of great interest since it is present in numerous bioactive natural products[3].



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Comparison of different isolation methods to measure small non-coding RNAs in fresh frozen and formalin-fixed paraffin embedded (FFPE) specimen

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During the past years evidence has accumulated that the expression of microRNAs (miRNAs) is associated with cancer progression. MiRNAs are small RNA molecules encoded in the genomes of most eukaryatic organisms. These evolutionary highly conserved, ~21-mer non-coding RNAs regulate the expression of genes mostly by binding to the 3'-untranslated regions (3'-UTR) of specific mRNAs. Due to their small size several technical challenges are associated with the accurate measurement of miRNAs. In addition, the analysis of nucleic acid in FFPE specimens is challenging because due to fixation and embedding conditions, the nucleic acids are usually heavily fragmented and chemically modified. In this study we will discuss different isolation methods to measure small non-coding RNAs in fresh frozen and FFPE specimen. **Methods:** We have measured miRNAs isolated by various protocols from fresh frozen and FFPE human breast cell lines and tissues, before and after applying specific enrichment procedures for small RNA species. In isolated RNA fractions, miRNAs were quantified real time by a multiplex RT Taqman based method (Taqman microRNA assay from Applied Biosystems). **Results:** 1) Our experiments showed significant variation in mature miRNA expression dependent on the miRNA isolation method. 2) Use of standard purification columns resulted in selective removal of small RNAs. 3) Especially for the lower expressed miRNAs gain in sensitivity could be achieved in a uniplex compared with a multiplex RT reaction. **Conclusion:** Our study identifies several sources of technical bias in measuring mature miRNAs and we propose a solid methodology to access miRNA levels in fresh frozen and FFPE human specimen.

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NOTES

Neurotensin, neurotensin receptor type 1 and β-catenin expression in tumoral and healthy endometrial tissue

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Área de Biología Celular . Universidad de La Laguna. Spain

Presence of peptides receptors in tumoral cells, as a molecular target for diagnosis and therapy of several cancers, has been extensively studied. One of these peptides is Neurotensin (NT), which is involved in various tumors. Our aim was to study the expression of this peptide and its receptor type 1 (RNT1) in tumoral and healthy endometrial tissue as it has not been reported their expression in this tissue until now.

β-catenin is a binding and signaling protein in healthy epithelial tissues, but it has been observed inside nucleus in several cancer types, so it is used as diagnostic marker. Knowing this –apart from that one of the gene that mutates in endometrial cancer is precisely β-catenin gene–, our work aim was to study β-catenin sub-cellular expression and its relationship with NT and RNT1 too.

In this work we used immunohistochemistry techniques with primary antibodies against NT, RNT1 and β-catenin. Samples –healthy and tumoral endometrial tissues biopsies– were donated by Hospital Universitario de Canarias.

Most striking results are that different NT, RNT1 and β-catenin expression and co-expression patterns seem to be related with tumor infiltration degree. Moreover, we obtained unpublished results for endometrial cancer cell biology: first time detection of β-catenin inside endometrial adenocarcinome cells nucleus, and its co-expression with NT and RNT1 inside tumoral cells nucleus

Relationship between NT, RNT1 and β-catenin sub-cellular changes and healthy and tumoral endometrial tissue, could be useful as prediction markers or early detection too

Acknowledgements: Unidad de Anatomía Patológica del Hospital Universitario de Canarias.

NOTES

New kaurene derivatives with anti-inflammatory and antitumoral activity

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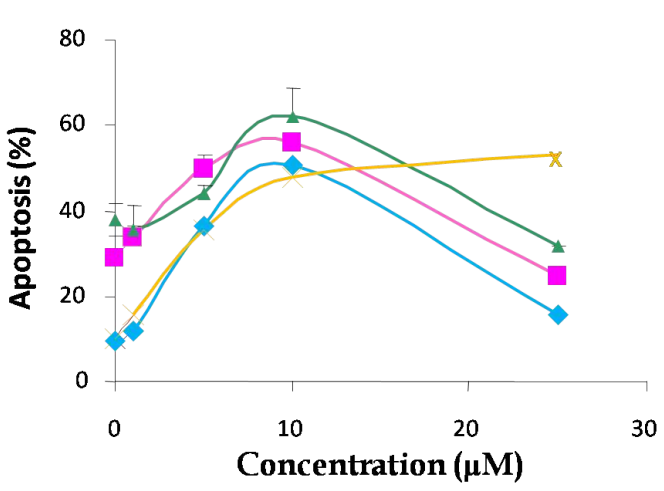
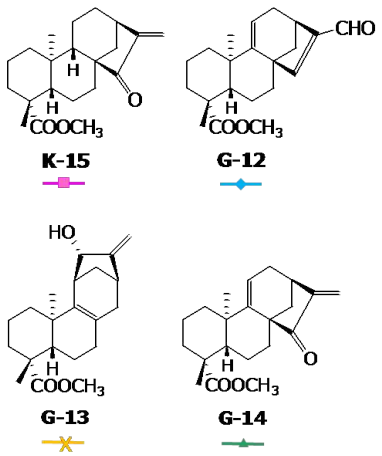
More than a century ago Rudolf Virchow demonstrated the presence of leukocytes in tumors and suggested that tumors arise at sites of chronic inflammation and that inflammation mediators, by enhancing cell proliferation, may serve as tumor promotores[1]. The protein called NF-κB has implicated an inflammation-induced. NF-κB comprises a family of inducible transcription factors that serve as important regulators of the host immune and inflammatory response[2] promoting the expression of specific cellular genes involved in host defense such as pro-inflammatory cytokines, chemokines, cell adhesion molecules and inducible nitric oxid. NF-κB provides a mechanistic link between inflammation and cancer[3].

Kaurane diterpenes have been isolated from numerous medicinal plants, which have been used for treatment of cancer and inflammation[4]. Under the investigation focused on the development of the new agents with anti-inflammatory and/or antitumoral activity, we have prepared several kaurene derivatives.

In this communication we will report the transformations carried out on the natural diterpenes kaurene and grandiflorenic acid and the corresponding bioactivity. We will also show some structure-activity relationships.





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PI4P5-Kinase Iα is required for efficient HIV-1 entry and infection of T cells

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HIV-1 envelope (Env) triggers membrane fusion between the virus and the target cell. The cellular mechanism underlying this process is not well known. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is a second messenger that binds, through its phosphorylated headgroup, to a variety of effector molecules and regulates their function and cellular localization. PIP_2 is known to be important for the late steps of the HIV-1 infection cycle by promoting Gag precursor protein $Pr55_{Gag}$ localization to the plasma membrane during viral assembly, but it has not been implicated in early stages of HIV-1 membrane-related events: viral attachment and entry to tha target cell. In this study, we show that binding of the initial HIV-1 Env-gp120 protein induces PIP_2 production related to the plasma membrane in permissive lymphocytes through the activation of phosphatidylinositol-4-phosphate 5-kinase (PI4P5-K) Iα isoform. Overexpression of wild-type PI4P5-K Iα increased HIV-1 Env-mediated PIP_2 production and enhanced viral replication in primary lymphocytes and CEM T cells, whereas PIP_2 production and HIV-1 infection were both severely reduced in cells overexpressing the kinase-dead mutant D227A (D/A)-PI4P5-K Ialpha. Similar results were obtained with replicative and single-cycle HIV-1 particles. HIV-1 infection was also inhibited by knockdown of endogenous expression of PI4P5-K Iα. These data indicate that

PI4P5-K Iα-mediated PIP_2 production is crucial for HIV-1 entry and the early steps of the viral cycle in permissive lymphocytes, before viral assembly and egress.

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Antagonist effects of novel tamoxifen derivatives on ERα activation

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According to data published by Consejería de Sanidad from the Gobierno Autónomo de Canarias, one out of every three types of cancer diagnosed in women corresponds to breast cancer; being one of the most frequent cancer among canarian women (around 30%). On the other hand, mortality and incidence rates in the Islands are higher than those in the rest of Spain.

Tamoxifen, a Selective Estrogen Receptor Modulator (SERM), is widely used on estrogen receptor-positive breast cancer treatment. In spite of the existence of demonstrated undesirable side effects, this drug has proven to be one of the most effective for the therapy, reducing, considerably, the mortality due to this disease. Such undesirable effects justify the development of novel compounds exhibiting a more selective profile as SERM. In this way, we have synthesized and tested different permeable and impermeable tamoxifen derivatives which maintain the triphenylethylene core but contain different lateral alkylaminoethoxy side-chains. Previous results from our laboratory showed that permeable derivatives were capable to reduce the proliferative effects of estradiol on the estrogen receptor-positive cell line MCF-7 in a dose-dependent manner. In this work, we have assayed these novel derivatives to assess their effects to antagonize the ERα activation mediated by 17β-estradiol by mean of a nuclear receptor ERα ELISA assay. Our results indicate that these derivatives were capable to reduce the agonist effects of estradiol on ERα, also in a dose-dependent manner. Interestingly, we have found significant differences at 3 μM between derivatives that were not observed in proliferative assays on MCF-7 cells. In this sense, we have found that some of the new derivatives displayed a more powerful antiestrogenic effect on ERα activation than tamoxifen itself.

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NOTES

Cytotoxic and Antimalarial Alkaloids from *Pancratium canariense*

Juan C. Cedrón,[1,2] Ana Estévez-Braun,[1,2] Ángel G. Ravelo,[1,2] Matías López,[1] Leticia G. León,[1,3] Juan M. Padrón,[1,3] David Gutiérrez,[4] Ninoska Flores[4]

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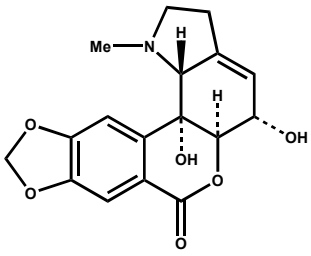
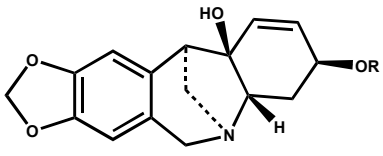
The Amaryllidaceae alkaloids are a selected group of secondary metabolites produced exclusively by plants of this family.[1] These alkaloids are known because of their bioactivity, such as antitumoral, antiviral, acetylcholinoesterase inhibitors, antimalarial, etc.[2] For example, pancratistatin is a narciclasine-type alkaloid with important cytotoxic activity against several tumor cell lines and less toxicity than other anticancer drugs such as paclitaxel or etoposide.[3]

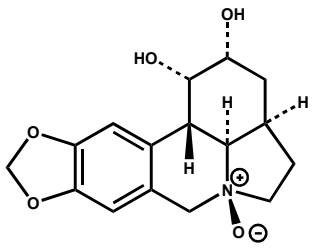
Pancratium, the most distributed genus from the Amaryllidaceae family along the Mediterranean area, has an endemic species in the Canary Islands: *Pancratium canariense*. From the bulbs of *P. canariense* we have isolated and elucidated 16 alkaloids.[4] Four of them resulted new to the chemical literature, and were named Pancratinine A, B, C and D. Their structures were determined by 1D- and 2D-NMR and X ray studies.



Pancratinina A



R=Me: Pancratinina B
R=H: Pancratinina C



Pancratinina D

The alkaloids were tested for antimalarial and cytotoxic activity. The results pointed out that alkaloids with a haemanthamine-type skeleton showed important activity against *Plasmodium falciparum* F32 and several human tumour cell lines

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NOTES

Moesin regulates the trafficking of nascent Clathrin-Coated Vesicles

García-Expósito*[1,3], Jonathan Barroso-González*[1,3], José-David Machado[2,3] and Agustín Valenzuela-Fernández[1,3] (*authors that have working equally).

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Clathrin-coated vesicles are responsible for the trafficking of several internalized biological cargos. We have observed that the endogenous F-actin-linker moesin co-distributes with constitutive components of clathrin-coated structures. Total internal reflection fluorescence microscopy studies have shown that short interference RNA of moesin enhances the lateral movement of clathrin-coated structures and also provokes their abnormal clustering. The aggregation of clathrin-coated structures has also been observed in cells over-expressing N-moesin, a dominant-negative construct unable to bind to F-actin. Only over-expressed moesin constructs with an intact phosphatidylinositol (4,5)-bisphosphate-binding domain co-distribute with clathrin-coated structures. Hence, this N-terminal domain is mostly responsible for moesin/clathrin-coated structure association. Biochemical approaches together with total internal reflection fluorescence microscopy comparative studies, between intact cells and plasma-membrane sheets, indicate that moesin knock-down provokes the accumulation of endocytic rab5-clathrin-coated vesicles carrying the transferrin receptor. The altered trafficking of these endocytic rab5-clathrin-coated vesicles accounts for a transferrin receptor recycling defect that reduces cell-surface expression of the transferrin receptor and increases the amount of sequestered transferrin ligand. Therefore, we propose that moesin is a clathrin-coated vesicle-linker that drives cargo trafficking, acting on nascent rab5-clathrin-coated vesicles by simultaneously binding to clathrin-coated vesicle-associated phosphatidylinositol (4,5)-bisphosphate

and actin cytoskeleton. Hence, functional alterations of moesin may be involved in pathological disorders associated with clathrin-mediated internalization or receptor recycling.

Acknowledgements: This work was supported by grants FIS-PI050995 (Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain), FIPSE-24508/05 and FIPSE-24661/07 (Fundación para la Investigación y Prevención del SIDA en España), IDT-TF-06/066 (Consejería de Industria, Comercio y Nuevas Tecnologías del Gobierno Autónomo de Canarias, Spain) and ULL-06-1500 (Universidad de La Laguna, Tenerife, Spain). Agustín Valenzuela-Fernández and José David Machado were supported by "Fondo Social Europeo (FSE; RYC2002-3018 and JCI2005-1042, respectively)". Jonathan Barroso-González and Laura García-Expósito are supported by the FIS-PI050995 and FIPSE-24661/07 associated fellowships, respectively.

NOTES

CELL CYCLE EFFECTS OF MICHAEL ACCEPTORS

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The interaction of antitumor drugs with cellular targets involves various types of chemical binding, of which covalent binding is one of important strategies in designing effective anticancer molecules. The anticancer compounds employing covalent binding, structurally diverse, belong to many chemical classes, including quinones, mustards, nitrosourea, alkylsulfonate, pyrrolo bcnzodiazepines, and others.

Recent evidences suggest that antineoplaslic alkylating compounds, quinones, mustards, cisplatin, alkylsulfonate bind directly to various celular nucleophiles. Most of these compounds contain, or acquire by cellular metabolism, electrophilic centers, and some are Michael acccptos in a broader sense. A group of representative natural cytotoxic compounds containing Michael acceptor are naphthoquinones such as menadione and shikonin

analogues, cytotoxic quassinoids, sesquiterpenoid benzoquinones, and others. Mitomycin, meanwhile, is transformed by reductive activation into a conjugated dienone as a Michael acceptor.

Chemosensititvity tests of several synthetic Michael acceptors were performed using the SRB, assay [1] against a representative panel of human solid tumor cell lines. Cell cycle analysis and Annexin V staining confirmed that these new Michael acceptors induce apoptosis in all cell lines. The results were confirmed by DAPI staining.

References: **1**. Miranda, P. O.; Padrón, J. M.; Padrón, J. I.; Villar, J.; Martín, V. S. *ChemMedChem* **2006**, *1*, 323

Acknowledgements: This research was supported by the Spanish MEC, co-financed by the European Regional Development Fund (CTQ2005-09074-C02-01/BQU), the Canary Islands Government, the Spanish MSC-ISCIII FIS (RD06/0020/1046), and the Fundación Canaria de Investigación y Salud (PI 1/06 and PI 35/06). L.G.L. thanks the Spanish MSC-FIS for a postdoctoral contract. M.C.V.-H. thanks the Spanish MSC for a postdoctoral CIBERER U740 contract. J.M.P. thanks the Spanish MEC-FSE for a Ramón y Cajal contract.

Apoptotic effect of withanolides isolated from *Withania aristata* on human cervical cell line

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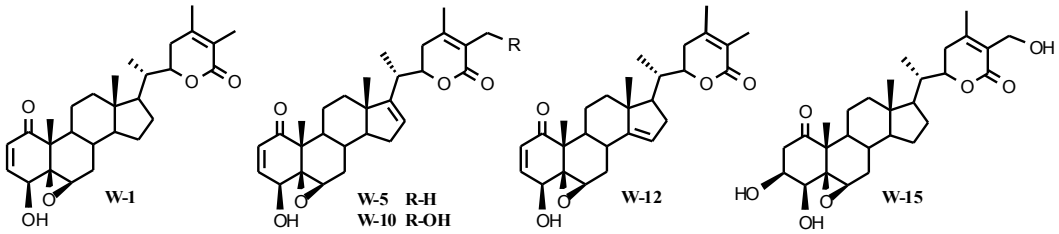
Plants from the genus *Withania* (Solanaceae), are distributed in the East of the Mediterranean area, Macaronesian region and extend to south Asia [1]. The therapeutic potential of *Withania* species has been attributed to the presence of withanolides, a group of naturally occurring C-28 steroids built on an ergostane skeleton in which C-22 and C-26 oxidized to form a δ-lactone [2].

A preliminary cytotoxic evaluation of the dichloromethane extract of leaf from *W. aristata* distributed for the more occidental island of the Canary Archipelago, revealed a potent

activity (IC_{50} 12,2 µg/mL) against HeLa cell line. Phytochemical studies of this extract give to the isolation of several withanolides and in the present study we report the antiproliferative activity of five of them against HeLa cells and the ability of induction apoptosis. Three of the compounds exhibited the caspase-3 activation and the morphologic evaluation by fluorescent staining (Hoechst 33342 and BCECF) revealed modification of cell shape, chromatin condensation and blebs formation and Annexin V stained cell showing cytoplasmic membrane flipping typically detected in early apoptotic cells. Also the preliminary structure-activity relationship is discussed.



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Acknowledgements: We are indebted to the DGES (CTQ2006-13376/BQU) and FICIC-GI nº05/2008 projects for financial support. Thanks to the Universidad de Los Andes for a fellowship.

Introduction of cancer pharmacogenetics in canary popolution

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Cancer is a complex disease influenced by environmental, epigenetic and genetic factors. The Canary Islands surpass the rate of Cancer (i.e., breast, colorectal) of most European Union countries. The disease has important genetic and enviormental components, most of them are still unkown. The Pharmacogenetic is a relative newborn science which investigates the influence of the individual genetic charge in response to drug treatments. Often, the same treatment has different responses in different people and this could be explained by genetic individual factors. In the case of cancer, pharmacogenetic acquires more importance because the chemotherapy is an aggressive, long-length and expensive treatment; and should be improved for to obtain the best clinical results and to avoid adverse effects sometimes very hawkish for the patience. We present here a project that introduces pharmacogenetic tools applied to breast and colorectal cancer in the canary population.

Fluoropyrimidines (5-FU) and taxol derivatives are two of the most common drugs against breast cancer. It is known that the efficacy of those treatments is conditioned by genetic factors as single nucleotide polymorphism (SNP) and single tandem repeats (STRs). The mechanism of action of 5-FU is the inhibition of Thymidilate Shynthase (TS). This inhibition occurs after the formation of a complex which includes fluorodeoxyuridine monophosphate (FdUMP), TS and 5-10 methilentretahydropholate (CH2-FH4). The existence of CH2-FH4 depends of the enzyme Methilen-tatrahydropholate reductase (MTHFR). TS have a STR polymorphism that consists in the repetition of 28 bp in the promoter region of the gen. The normal status is 2/2 or 2/3. Being 3/3 is associated with best response to 5-FU therapy. MTHFR present a C677T SNP; in this way people who possess TT form have been associated to better response to 5-FU. MDR1 is a gene that codifies P-glicoprotein (Pgp). This protein is involved in chemotherapy resistant process. MDR1 is a high polymorphic gene, and there exists a SNP involved in chemotherapy treatment (C3435T). T allele correlates with low levels of Pgp, and this is correlated with a best response to taxol and cyclophosphamide. Finally, a polymorphism in p53 codon 72 gene (Arg/Pro) is considered a predictive factor in neoadyuvant therapy with antraciclines. A patients Pro/Pro has minor response to neoadyuvant therapy, and is in relation with poor clinical diagnosis. In this work we have selected 135 patients with breast cancer, 48 of them treated with neoadyuvant therapy, from Oncology Service of Hospital Insular de GC. Patients have been treated with different chemotherapy protocols, but all of them have 5-FU as a common drug. 305 control samples were selected, matched in age and local distribution. Because the allele distribution of polymorphism differs in function of the population, the first aim of this work was to determinate polymorphism distribution in the canary population, as well as to explore significant differences between cases and controls. A second important aim was to establish a possible relation among gene polymorphisms, clinical status and outcome. The project is over the first aim. Chi square test was used in the statistical analysis using GraphPad Prism 4 software. Until now, we have analyzed TS, MDR1 and p53 polymorphisms in case and control samples. The frequency of these genetic variations are: TS 28bp STR, (2/2) 0.33, (2/3) 0.44, (3/3) 0.23 in controls and 0.24, 0.51 and 0.25 respectively in cases (p=0.1234). MDR1 C3435T, (CC) 0.28, (CT) 0.54, (TT) 0.18 in controls. 0.26, 0.52 and 0.22 respectively in cases (p=0.5596). p53 codon 72 Arg/Pro, (Arg/Arg) 0.56, (Arg/Pro) 0.34, (Pro/Pro) 0.10 in controls. 0.54, 0.40 and 0.06 respectively in cases (p=0.3060). Currently, we are working in the MTHFR polymorphism as well as over a creation of a complete database that includes information about the tumour, treatment and clinical outcome. With all data on hand we will start the second stage of the project

Acknowledgements: The Oncology Service from Hospital Insular de Gran Canaria (Fátima Guillén and Elia García). Dra. Cristina Bilbao Sieyro. Dra. Ruth Zárate Romero and Dr. Jesús García Foncillas from Hospital Universitario de Navarra. Ana González Hernández and Dr. Antonio Cabrera de León (CDC Project). Work supported by Consejería de Industria del Gobierno de Canarias, ICIC and FUNCIS.

High exposure level to dioxin-like carcinogens through intake of commercial milk from the Canary Islands market (Spain)

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Organochlorine compounds (OCs) are a class of environmental carcinogens characterized by exceedingly long half-lives in the environment. These chemicals tend to accumulate in animal and

human tissues after exposure through dietary sources. As, in human beings and livestock, the milk is the major route of excretion of these compounds, milk intake, in human populations, has been used as a surrogate for the assessment of human exposure to these highly lipophilic environmental carcinogens. There is a growing interest in organic products as a safer and environmentally friendly type of food, but OCs are not residues of products currently used on the crops but ubiquitous contaminants. A comparison of residue levels of environmentally persistent contaminants between organic and conventional milk was undertaken. Ten commercial brands of organic milk and seventeen of conventional milk were collected from supermarkets of the Canary Islands (Spain) from December 2007 to April 2008, for characterization of OCs residue levels. Gas chromatography/mass spectrometry was used to identify and quantify the analytes, including 25 dioxin-like polychlorinated biphenyl (PCB) congeners, hexachlorobenzene, and 18 organochlorine pesticides and metabolites. Our results showed that there were no statistical differences between both types of milk and that all the samples showed some degree of chemical contamination. Hexachlorobenzene was detected in 100% of the samples. Of the 18 OC pesticides measured, a mean of 9 residues per sample was detected in conventional milk samples (range 4-14), and an average of 7 residues per sample (range 5-9) in organic milk. The pesticide transchlordane was present in 100% of samples, and the main metabolite of DDT, 4,4'-DDE in more that 80% of samples. Also 85% of samples had residues of any of the hexachlorocyclohexane isomers. With respect to the presence of residues of dioxin-like PCBs in milk, 100% of the conventional and organic milk samples showed detectable levels of some of the analysed congeners. The contribution of mono-ortho and non-ortho PCBs (dioxin-like PCBs) to Toxicity Equivalents (PCB-WHO-TEQ) in milk from canarian markets was calculated as 0.48 pg/ml for conventional milk and 0.47 pg/ml for organic milk. Due to the high consumption of this product in the Canary Islands (according to the data obtained from the Canary Islands Nutritional Survey), the PCB-WHO-TEQ intake from milk was estimated, resulting in a mean intake of 11.9 pg/kgbw/week. Having into account that EU has set the maximum TEQ exposure limit as 14 pg/kgbw/week (considering the contribution of all sources - PCDDs, PCDFs, non-ortho PCBs, and mono-ortho PCBs – and all foodstuffs), in Canary Islands population only milk-associated PCBs-intake represents nearly 85% of this limit. This intake was independent of the type of milk consumed, conventional or organic. The absence of statistical differences between organic and conventional OCs residue levels reveals the fact that the contamination of food by OCs is inadvertent to the consumer because it becomes from environmental sources. In conclusion, having into account that the entire population of these Islands is subjected through the diet to this alarming situation, it is very important to articulate the appropriate measures to diminish the still very high level of environmental contamination by these environmental carcinogens.

NOTES

Synthesis of Oxacycles of different sizes using Iron (III) as catalyst

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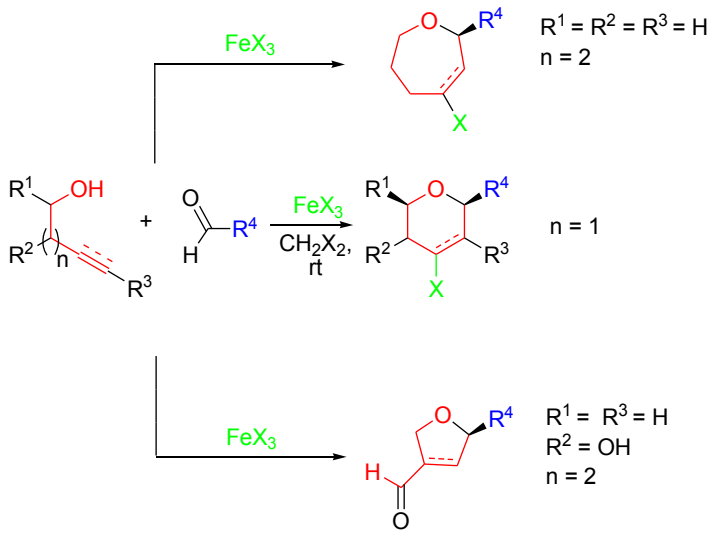
During the past two decades the Prins cyclization has emerged as a powerful tool in the synthesis of tetrahydropyran rings, and it has been successfully used in the synthesis of natural product and medicinal chemistry.[1] Usually, this reaction involves the construction of six membered ring heterocycles using double bond as nucleophilic species. Prins cyclizations of alkynes, using homopropargyl acetals, have also been reported but this methodology has attracted much less attention. We have recently developed in our laboratory, the alkyne Prins cyclization to obtain dihydropyrans and tetrahydropyrans.[2]



In this communication we will discuss the different factors that lead, in a simple way, to 5, 6 and 7 member rings, using the Prins cyclizations as key reaction and substoichiometric iron (III) salts as catalyst.

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Acknowledgements: This research was supported by the Ministerio de Educación y Ciencia of Spain, co-financed by the European Regional Development Fund (CTQ2005-09074-C02-01/BQU), FICIC, the Spanish MSC ISCIII (RETICS RD06/0020/1046) and the Canary Islands Government. M. P. supported by the Programme Alban, the European Union Programme of High Level Scholarships for Latin America, scholarship nº E07D402567AR.

Investigating *Thevetia* Genus as a source of new anticancer drug

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The species *Thevetia ahouia* and *T. peruviana* are ornamental trees of the family Apocynaceae, commonly distributed in many tropical countries.

The genus *Thevetia* is well known as a source of cardenolide glycosides, whose basic chemical structures consist of a steroidal (cyclopentanoperhydrophenanthren) ring, 3□- and 14□–hydroxyl groups with the glycoside function at C-3, and an □,□-unsaturated five-membered lactone ring attached to 17□

Cardiac glycosides are a class of natural product that are traditionally used to increase cardiac contractile force in patients with congestive heart failure, but they are also reported as being active against a wide range of cancer types.[1,2].

In the course of our phytochemical study to search for anticancer agents from higher plants, eight organic sub-extracts of the epicarp and seed from *Thevetia ahouia* and *T. peruviana*, containing mainly cardenolide glycosides, were screened for *in vitro* anticancer activity against MCF-7 (carcinoma of breast), HeLa (carcinoma of cervix) and A-549 (carcinoma of lung) human cancer cell lines.

Five of the sub-extracts were active against the three cell lines assayed, showing IC_{50} between 0.03 and 15 µg/mL. The most active was the dichlorometane sub-extract from the seed of *T. peruviana* with values IC_{50} ranging from 0.03-0.41 µg/mL, higher than mercaptopurine used as a positive control.

It has been demonstrated that cardenolide glycosides produces apoptosis and this effect is mediated through inhibition of Na^+, K^+, ATPase, release of mitochondrial cytochrome *c*, activation of caspase cascade, and Poly (ADP-ribose) polymerase (PARP) cleavage.[3]

Further investigations should be performed to determine the structure of the cardenolide glycosides responsible for the anticancer effect of *Thevetia ahouia* and *T. peruviana.*

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Antitumor-promoting effects on Epstein-Barr virus of sesquiterpenes dihydro-β-agarofuran

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Species of the Celastraceae family have a long history of use in traditional medicine, especially in Asia and Latin America. Sesquiterpene esters, based on the dihydro-β-agarofuran skeleton, are considered to be chemotaxonomic indicators of this family and they have attracted considerable interest on account of their wide range of biological activities. These data along with their structural characteristics have led them to be considered as "privileged structures"[1].

Inhibition of the tumor promotion stage in the multistage of chemical carcinogenesis has been regarded as a promising strategy for cancer chemoprevention. In the search for cancer chemopreventive agents, the inhibition of Epstein-Barr virus early antigen (EBV-EA) induced by the tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) has been developed as a primary screening test [2].

As part of an intensive study of the bioactive metabolites from species of Celastraceae, we report herein the antitumor-promoting effects on EBV-EA in Raji cells of twenty sesquiterpenes, with a 1,2,3,6,8,15-hexasubstituted 4β-hydroxydihydro-β-agarofuran skeleton, isolated from Crossopetalum tonduzii. Five of the compounds showed strong inhibitory activity (90-96 % inhibition at 10 mol ratio/TPA), higher than that of β-carotene, which is known as a typical antitumor promoter.

The structure-activity relationship (SAR) study suggests than the presence of an acetate group at C-1 plays an important role in the inhibitory activity, whereas the nicotinate moieties decrease the activity.

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NOTES

Implication of liver X receptors (LXRs) in the resolution of inflammation and autoimmunity

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The Liver X Receptors (LXRs) are members of the nuclear receptor superfamily of transcription factors that play central roles in the transcriptional control of lipid metabolism and inflammation. Activation of LXRs promotes the expression of genes involved in cholesterol homeostasis and inhibits the expression of inflamatory genes. LXR signalling is important for the innate immune response against intracellular bacteria. Thus, the study of LXR function in macrophages is unraveling previously unrecognized links between innate immunity and lipid metabolism. Chronic inflammation and disregulation of the immune mechanisms are currently underlying many human diseases. If unchecked at the resolution of immune responses, production of cytokines leads to chronic inflammation and autoimmunity. For example, systemic lupus erythematosus (SLE) is a chronic inflammatory disease with unknown etiology in which the immune system turns its defenses upon self-elements such as chromatin. SLE is characterized by lymphocyte expansions, hypergammaglobulinemia and renal damage. Nephritis is caused by deposition of DNA-specific autoantibody complexes, activation of the complement cascade and subsequent infiltration of T cells and macrophages that amplify the local inflammatory response that results in eventual renal failure. Here we describe that mice lacking LXRs manifest an age-

dependent breakdown in self-tolerance and develop autoantibodies and autoimmune glomerulonephritis. We will also discuss the potential implications of LXR-dependent pathways in the protection against autoimmune disease and the potential mechanisms involved. Our results demonstrate that LXRs are important players in the protection against autoimmune disease and suggest that pharmacological activation of LXR could be a therapeutic alternative to ameliorate inflammatory disregulation in autoimmune disease.

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NOTES

New approaches for the synthesis of lauroxanes

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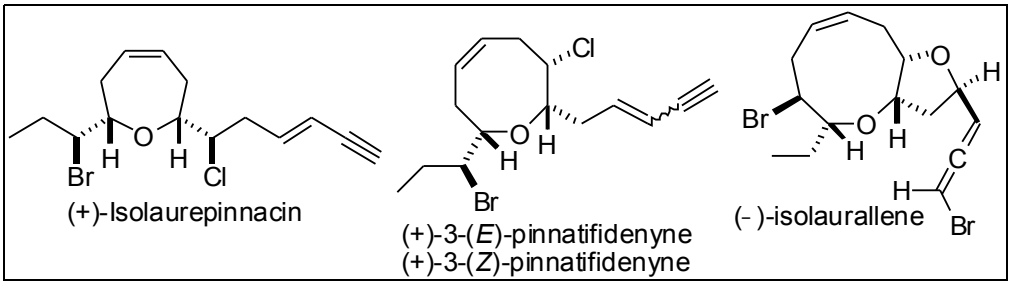
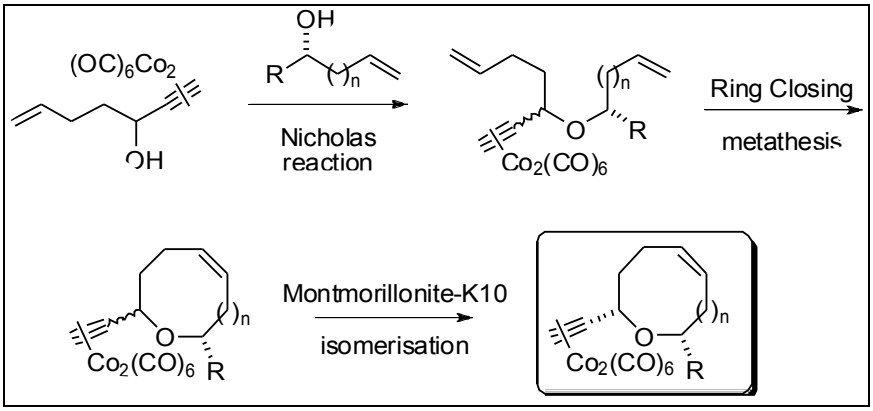
Red algae of the genus *Laurencia* produce a multitude of unique compounds, such a serie of nonterpenoid C15 metabolites generally named *lauroxanes*, which exhibit a large number of biological properties including antitumor, antimicrobial, immunosuppressant, antifeedant, pesticide activity, etc.[1] These natural products present polysubstituted cyclic ethers with a defined stereochemistry in the substituents and a ring size on a range from five to nine members (**Scheme 1**).



Scheme 1

In this contribution, we report on a new methodology for the synthesis of highly substituted medium sized cyclic ethers. Our strategy is based on an intermolecular Nicholas reaction between a propargylic-$Co_2(CO)_6$ cation and a secondary alcohol as nucleophile to form a linear ether, followed by a ring closing

metathesis to obtain the cyclic ether, and finally, an isomerization promoted by Montmorillonite K-10 in order to get the *cis* stereochemistry founded in most of the *lauroxanes* (**Scheme 2**).[2]



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Synthesis of new naphthalimide and bis-naphthalimides derivatives with potential antitumoral activity

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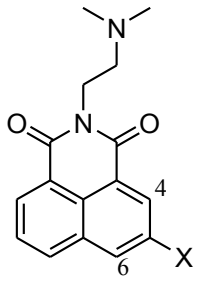
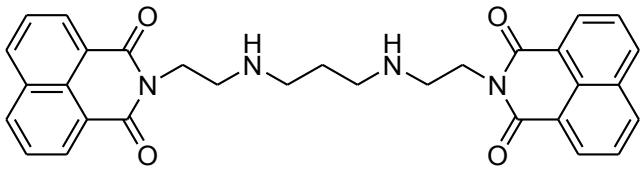
http://www.icic.es

Naphthalimides are a class of compounds with high antitumor activity upon a variety of murine and human tumor cells. These compounds bind to DNA by intercalation of the chromophore and two of them, mitonafide and amonafide, have been used in clinical trials.[1]

The therapeutic properties of these lead drugs were improved by designing bisintercalating agents, formed by the union of two naphthalimides groups by means of an alkylamine group. The best example of these compounds is elinafida[2]. Actually the majority of the synthesized derivatives present substituents at the position 5 or 6. [3]

In this communication we will report the synthesis of new naphthalimides and bis-naphthalimides, derivatives including those with substituents at the unusual position 4. Based on modelling studies, these compounds, promise to have a good antitumoral activity.





Mitonafide X:NO_2
Amonafide X:NH_2

Elinafide

References: **1**. Braña, M. F.; Ramos, A. *Current Medicinal Chemistry Anti-Cancer Agents* 2001,*1(3),*237-255.Naphthalimides as anti-cancer agents: synthesis and biological activity. **2**. Braña, F. M.; Castellano, J. M.; Morán, M.; et al. *Anti-Cancer Drug Design* 1993, *(8),* 257-268. Bis- Nphthalimides: a new class of antitumor agents. **3**. Brana, M. F.; Ana Gradillas, Angel Gómez, Nuria Acero, Francisco Llinares, Dolores Muñoz-Mingarro, Cristina Abradelo, Fernanda Rey-Stolle, Mercedes Yuste, Joaquín Campos, Miguel Á. Gallo, And Antonio Espinosa. *J. Med. Chem*. 2004, *47*, 2236-2242. Synthesis, Biological Activity, and Quantitative Structure-Activity Relationship Study of Azanaphthalimide and Arynaphthelimide Derivatives.

Acknowledgements: To the Ministerio de Educación y Ciencia (proyecto SAF 2006-06720) e ICIC for financial support.

Initial DNA damage is inversely related to radiation induced apoptosis.

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Background: Radiation induced normal tissue damage is the most important limitation to the delivery of a high potentially curative radiation dose. Late toxicity in patients treated by radiation therapy has been related to increased radiosensitivity of the lymphocytes as shown by increased number of dsb/Gy/DNA unit and reduced radiation induced apoptosis.The aim of the present study was to analyze if there is an inverse correlation between initial damage estimated by the number of dsb induced by a given radiation dose and the apoptotic rates observed.

Patients and methods: 26 patients treated of locally advanced breast carcinoma that were included in the present study, after given informed consent.

Flow cytometry

Lymphocytes were extracted with a density gradient centrifugation (Linfoprep, Gibco, Life Technologies). Peripheral lymphocytes from 4 healthy controls and the 26 breast cancer patients were irradiated with doses of 0, 1, 2 y 8 Gy. Data were analysed by the BD CellQuest program, with a Macintoch software (Apple Computer, Inc., Cupertino CA), calculating early and late apoptosis levels.

DNA initial damage analysis:

In short, mononuclear cells were isolated and mixed with 1% ultra-low-melting-point agarose, to obtain 250 □l plugs. The plugs were put in ice 1 hour before and during irradiation, which was performed using a ^{60}Co source (dose rate approximately 1.5 Gy/min). The irradiation doses used were 5, 10, 15, 30, 25, 30, 35, 40, 45 Gy, with a 0 Gy control tube. After irradiation, the plugs were placed in ice-cold lysis buffer and a pulse dose electroforetic nalysis was performed.

Results: Radiation induced apoptosis was increased by radiation dose and a strong correlation was found among RIA results at different doses.

Mean dsb/Gy/DNA unit was 1,70±0,83 median 1,46(0,63-4,08). When dsb values were segregated in two groups (the lower third against the two upper thirds of the distribution), an inverse correlation was found, reaching statistical signification for RIA induced at 1 Gy at 24 hours (p=0.029). A similar trend was found for RIA at 2 (p=0.050) and 8 Gy (p=0.066).

Conclusions: Cells from Ataxia Teleangiectasia and "sensitive population patients" may be genetically defective in the DNA damage pathway and may be unable to undergo apoptosis after Radiation-Induced DNA damage and may die by mitotic catastrophe.

Serum levels of insulin-like growth factor-I in relation to organochlorine pesticides exposure

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Context: Insulin-like growth factor 1 (IGF-I) and organochlorine pesticides (OCs) have been involved in the pathogenesis of several diseases like cancer, diabetes and growth disorders.

Objective and Design: The potential relationship between the serum levels of various OCs and serum IGF-I was investigated in adults (176 men and 247 women) from a representative sample of the general population of the Canary Islands (Spain).

Results: After adjustment for potential confounders, which include body mass index, age, and IGF-binding protein-3 (IGFBP-3), IGF-I levels were significantly lower in the 247 women who showed detectable levels of *p,p'*-DDD (a DDT-metabolite) than in women who presented non-detectable levels of this pesticide (p=0.030), specially in 36-50 years old women. A similar negative relationship was also found between IGF-I and aldrin (a non-DDT-derivative) in women (p=0.049). In the group of 176 men, aldrin seemed to exert a similar negative effect on IGF-I (p=0.046) and this effect was clearly significant in the oldest group (51-65 years) (p=0.009). A non-linear dose-response curve was observed between Total Cyclodienes Body Burden (TC; sum of aldrin, dieldrin and endrin) and IGF-I in men (p=0.024). These findings suggest that OCs could modulate the IGF-system in a way that is highly influenced by gender, age and by chemical or combination of chemicals implicated. Such circumstances may contribute to the development of a number of diseases related to IGF-I and should be taken into account in public health decisions.

NOTES

Hypoxia downregulates Ku70/80 expression in cervical carcinoma tumors

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Hypoxia may lead to conditions that either cause increased spontaneous damage to DNA or inhibit DNA repair processes. Cells have several mechanisms to repair DNA damage before mutations appear. Non Homologous End Joining (NHEJ) repair-associated genes were also

downregulated by hypoxia in one study , but no relation was found in other. Ku70/80 are key genes in the NHEJ repair pathway for radiation induced double strand breaks (dsb). In the clinical setting, Ku expression has been related to survival in patients treated by radiation, although results are rather controversial.

The aim of the present study was to assess the relation of the expression of the NHEJ repair protein Ku70/80 and tumor hypoxia in clinical cervical tumors. Angiogenesis and p53 protein expression were also studied in relation to hypoxia-induced tumor progression.

Forty-three consecutive patients were prospectively included in this study from July 1997 to September 2001. Patients suffering from localized cervix carcinoma, diagnosed and treated by definitive radiation at the Las Palmas Hospitals were included. Ku70/80 expression, tumor angiogenesis (CD-31 expression) and p53, was studied by immunohistochemistry Hypoxia may inhibits the NHEJ DNA repair through downregulating Ku70/80 expression and combined with an increased angiogenesis and altered p53 expression would be responsible for tumor progression in cervical carcinoma. Tumor oxygenation was measured by a polarographic probe system "pO2 Histograph" (Eppendorf AG, Hamburg, Germany). To determine tumor oxygenation, median pO2 and the percentage of pO2 values < 10 mmHg and < 5 mmHg were obtained from the pooled data for each individual.

In conclusion, hypoxia inhibits the NHEJ DNA repair through downregulating Ku70/80 expression combined with an increased angiogenesis and altered p53 expression. These mechanisms would be responsible for tumor progression in cervical carcinoma.

Acknowledgements: Authors wish to thank Ms Araceli Caballero for the statistical advice. This work was subsidized by grants: FIS 1035/98, 0855/01. Bordón E and Fausto Fontes were supported by an educational grant from the Instituto Canario de Investigación del Cáncer (ICIC)

Severe hypoxia induces chemoresistance in clinical cervical tumours through MVP overexpression

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Hypoxic tumours are resistant to chemotherapy and this effect seems to be mediated by upregulation of Major Vault Protein mediated by HIF-1 in studies preformed in vitro.

The aim of the present study was to assess the relation of the expression of the Major Vault Protein and tumor hypoxia in clinical cervical tumors. Forty-three consecutive patients suffering from localized cervix carcinoma already described elsewhere. MVP expression was studied by immunohistochemistry in paraffin-embedded. P53 and angiogenesis estimated by CD-31 staining were obtained from our files.Tumor oxygenation was measured by an Eppendorf device following standard criteria as previously described . To determine tumor oxygenation, tumor hypoxia data were reanalyzed for detecting cases of severe hypoxia and the percentage of pO2 values < 2.5 mmHg, were obtained from the pooled data for each individual.

MVP expression was considered low (negative/slightly positive) in 23 cases and high (strongly positive) in 20 cases. Mean vascular density (MVD) was 49.62±33.98% (median 41%, range 0-160).P53 expression showed a mean value of 39.15 ±27.62% (median 35%, range 0-92%). Mean tumor hypoxic fraction <2.5 mmHg (HF2.5) values were 35.89±26.80 (median 35.20%, range 0-91.30%).

High MVP expression was related to severe hypoxia as determined by higher hypoxic fractions HF (2.5) (45.82±28.00%) compared to low MVP expressing tumours (27.26±22.96%) (p=0.022) (Figure 1a). Tumours overexpressing MVP also showed increased angiogenesis (65.41+38.38) compared to low expressing cases (35.89+22.55) (p=0.003) (Figure 1b). MVP expression was independent of P53.

In this study we show for the first time that severe tumor hypoxia upregulates MVP expression in clinical cervical tumors, confirming previous preclinical studies about the role of hypoxia in favoring increased chemoresistance. In the clinical setting, upregulation of MVP by hypoxia is of critical relevance as chemotherapy is currently a standard treatment for those patients. Whether in clinical tumours this chemoresistance can be reverted by HIF-1 inhibitors deserves to be studied.

Acknowledgements: Authors wish to thank Ms Araceli Caballero for the statistical advice. This work was subsidized by grants: FIS 1035/98, 0855/01. Bordón E and Fausto Fontes were supported by an educational grant from the Instituto Canario de Investigación del Cáncer (ICIC).

MVP expression is related to IGF1-R in cervical carcinoma patients treated by radiochemotherapy

Marta Lloret, Pedro Carlos Lara; Elisa Bordón ; **Fausto Fontes**; Agustin Rey; Rosa Maria Apolinario, Orlando Falcón Bernardino Clavo

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Purpose:

To assess the expression of MVP in cervix carcinoma patients treated by radiochemotherapy, its relation to clinical and pathologic prognostic factors and its role in predicting clinical outcome. In adition the relation to IGF-1R expression in this cohort of patients will be explored.

Materials and methods:

Sixty consecutive patients suffering from localized cervix carcinoma were prospectively included in this study from July 1999 to December 2003. Follow-up was closed in November 2007. Patients were staged following the TNM classification. All patients received pelvic radiation (45-64.80 Gy in 1.8-2Gy fractions) followed brachytherapy and concomitant cisplatin at 40 mgr/sqm/week doses. MVP expression was studied by immunohistochemistry in paraffin-embedded tumour tissue.

Results:

MVP was expressed in 58 patients (96.7%) and no relation was found with clinicopathological variables. High MVP expression was related to high IGF1-R expression (p=0.023). Complete response after treatment was observed in 50 patients (83.3%). Clinical stage of the disease and clinical response to radiochemotherapy were the most important prognostic factors related to survival. High MVP and IGF1-R tumour expression was strongly related to poor local and regional disease free survival (p=0,006), distant disease free survival (p=0.050) disease-free survival (p=0,006), cause specific survival (p=0.007) in patients achieving a complete response.

Conclusion:

MVP and IGF-1R expression were related in clinical cervical tumours and confer reduced long-term local control in patients who achieved clinical complete response to radiochemotherapy.

Acknowledgements: The authors wish to thank Ms Araceli Caballero for statistical advice. This work was funded by the following grants: FIS 1035/98, 0855/01. E. Bordón and Fausto Fontes received an educational grant from the Canarian Cancer Research Institute (Instituto Canario de Investigación del Cáncer) (ICIC).

Major vault protein (MVP) affects NHEJ repair and apoptosis through Ku70/80 and BAX downregulation in cervical carcinoma tumors

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Purpose: We investigated the relationship between major vault protein(MVP) expression, the nonhomologous end-joining(NHEJ) repair gene Ku70/80 and related genes involved in the regulation of apoptosis and proliferation, to shed light on the possible causes of genetic instability, tumor progression and resistance to oncological treatment in clinical cervical cancer.

Patients and methods: One hundred sixteen consecutive patients with localized cervix carcinoma were prospectively included in this study from July 1997 to December 2003. Patients were staged according to the TNM classification. Forty patients had stage I disease, 45 stage II and 31 stage III-IVA. Most patients showed squamous tumors(98 cases) and grade II(52 cases) and III(45 cases) carcinomas. MVP, Ku70/80, IGF-1R, BAX, BCL-2, p53 and Ki67 expression was studied by immunohistochemistry in paraffin-embedded tumor tissue.

Results: Tumors overexpressing MVP(65/116 cases) showed low levels of Ku70/80 (P=0.013) and BAX expression(P<0.0001). Furthermore, low Ku70/80 expression was strongly related to suppressed BAX(P<0.001) and related to a lesser extent to upregulated BCL-2(P=0.042), altered p53(P=0.038) and increased proliferation (P=0.002).

Conclusion: We hypothesize that an early regulatory mechanism favors homologous or NHEJ repair at first, mediated by vaults along with other factors yet to be elucidated. If vaults are overexpressed, NHEJ repair may be suppressed by several mechanisms, with resultant genomic instability. These mechanisms may be associated with the decision of damaged cells to survive and proliferate, favoring tumor progression and reducing tumor response to oncological treatment through the development of resistant cell phenotypes. Further clinical studies will be necessary to test this hypothesis.

Acknowledgements: The authors wish to thank Ms Araceli Caballero for statistical advice. This work was funded by the following grants: FIS 1035/98, 0855/01. E. Bordón and Fausto Fontes received an educational grant from the Canarian Cancer Research Institute (Instituto Canario de Investigación del Cáncer) (ICIC).

Serum levels of insulin-like growth factor in young people in relation to level of contamination by DDT-derivative pesticides

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Insulin-like growth factor 1 (IGF-I) serum levels in adults have been related to the pathogenesis of several diseases. Recently, it has been suggested that IGF-I peak levels at puberty could determine IGF-I levels in adulthood. Because environmental contaminants, such as DDT-derivative pesticides (OC-DDTs), could influence serum levels of IGF-I in youngsters, a cross-sectional study of the association between serum levels of OC-DDTs and IGF system was developed in 159 samples from young people living in the Canary Islands (Spain). A negative correlation between the levels of the DDT-metabolite *p,p'*-DDD and IGF-I serum values, as well as a positive one with IGFBP-3 serum levels was evident in boys (13-19 years) (p = 0.002 and p = 0.040, respectively). Additionally, multivariate tests were used adjusting for confounding variables (age, height, and weight) and stratified by gender and age: IGF-I levels were significantly lower in boys (12-19 years) who showed detectable values of *p,p'*-DDD, than in boys with undetectable levels of this OC (p=0.019). Furthermore, in this multivariate model, a non-linear dose-response curve was observed between Total DDT Body Burden (sum of the six DDT-derivatives measured) and IGF-I in boys (13-19 years) (p=0.045). These findings suggest that OC-DDTs could modulate the IGF-system in a way that is highly influenced by gender and age. Improvements in our understanding of exogenous determinants of the IGF-system may provide new insights into the role played by environmental contaminants in IGF-related diseases.

NOTES

DISCan-2007 Project.
An example: Cytotoxic factors of *Verongia aerophoba*.

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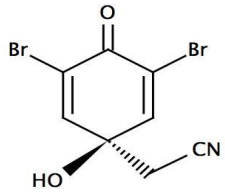
It's about to dynamize the sustainable industrial developement in the autonomous comunity of Canary Islands. We expect to mobilize the knowlegde pools and businessmen to set up or expand companies in Canary Islands and Northwest Africa. The keywords are in the sentence "STANDARD of LIVING of the citizen: WELLNESS". This is the sector in which, according to economists, is going to be the capital in the next decades[1]. The products to develop and sell are summarized next: Nutraceutical (minerals, oligoelements, fibers, unsaturated fats, high biological value proteins), Medicaments (from natural sources), Cosmetics (nutritious and protectives), Biomedical instruments, Environmental health. Some of our projects go straight to the cancer field, so we expect to produce –by means of mariculture of producing organisms- and to market new cytotoxic factors, like those produced by the marine sponge *Verongia aerophoba* from the Canary Islands[2].

Besides isolation and identification of known metabolites[2] (Aplysterol, Aplysterol acetate, Verongic acid, Fistularin-3, Fistularin-1, Verongiolide) we are going to present our works of *in situ* cultures of above-mentioned sponge, as well the isolation and identification of a new substance that we called **Verongionitrile** and assigned, using spectroscopic methods, structure I. This compound displayed an IC_{50}=10 µM against HL60 human leukemia cell lines, it stops cell cycle in 62M phase and it fragments procaspase-3 enzyme (this enzyme participates in apoptosis).



I.- Verongionitrile

References: **1**.The New Wellness Revolution: How to Make a Fortune in the Next Trillion Dollar Industry (second edition), Paul Zane Pilzer. 2002. **2**.Productos con actividad biológica de la esponja marina *Verongia aerophoba* de las Islas Canarias. Disertación para la obtención del diploma de estudios avanzados. Facultad de Ciencias del Mar. Universidad de Las Palmas de Gran Canaria. Julio, 2008.

Acknowledgements: The authors would like to thank to the technician Carmen I. Hernández Robaina by her experimental collaboration.

NOTES

Tumors register at Hospital Universitario NTRA SRA de Candelaria

Cruz Dorta, Raquel; Vilar Mesa, Mª Concepción; Dorta Delgado, Javier

Instituto Canario de Investigación del Cáncer. Group of oncología médica: nuevas estrategias terapéuticas para el cáncer. Hospital Universitario Ntra. Sra. de Candelaria.

INTRODUCTION

Hospital Tumours Register has the capacity of patients monitoring and could bring us information about the results of the oncologist attention.

Working at the Tumour Register of Hospital Universitario Ntra. Sra. Candelaria, last year, we have tried to actualize the information of population diagnosed of breast cancer, controlled in that tumour register, in a ten years period: January 1996 – December 2006, in order to know the global survival.

METHOD

We have worked updating those patients diagnosed of breast cancer, included in the Tumours Register of Hosp. Univ. Ntra. Sra. de Candelaria, who have finished or stopped for years their reviews at medical oncology. Everyday we incorporate new patients who came to our service and also we have access to the Hospital Pathological Anatomy books. We also bring up to date the register, using the centre information: radiological or laboratory test, hanging reviews, visit to urgencies, and finally, if we don't have any new information from that, we try to contact personally with them or their relatives.

We have incorporated 2.254 cases of breast cancer, diagnoses between January 1996 and December 2006.

This year we have continue keeping update the breast cancer cases of our Register, verifying that the survival curves adjust to the European reality of the moment; at the same time that we go on with other locations.

Acknowledgements: FICIC, ACAETCA.

NOTES

Synthesis and SAR of piperidines and tetrahydropyridines

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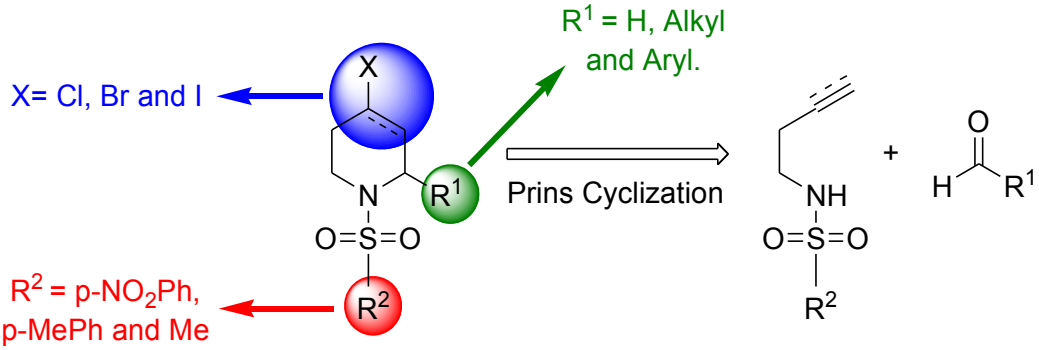
Piperidines, aliphatic six-membered nitrogen-containing heterocycles, are among the most promising therapeutic agents for a wide variety of diseases. The development of new and efficient methods for the preparation of structurally diverse piperidine derivatives is desired for the drug-discovery process.

The piperidines and tetrahydropyridines were prepared by means of the so-called aza-Prins-type cyclization.[1] This fast, simple, and versatile method is based on the consecutive generation of a □-unsaturated-iminium ion and further nucleophilic attack by the unsaturated carbon–carbon bond. In this pr mines



give tetrahydropyridines. Thus, we have prepared the set of piperidines and their corresponding analogs tetrahydropyridines.

In this communication, we will present the optimization, as cytotoxic agents, of a series of 2-alkyl-4-halo-piperdines and tetrahidropyridines, which possess a balanced pharmacological profile against a panel of representative human solid tumor cells: A2780 (ovarian), SW1573 (non-small cell lung) and WiDr (colon cancer).[2] Structural changes were used to probe structure-activity relationships, which lead to piperidine and tetrahydropyridine derivatives with a remarkably overall profile.

Referencias: 1. Carballo, R. M.; Ramírez, M. A.; Rodríguez, M. L.; Martín, V. S.; Padrón, J. I. *Org. Lett*. 2006, *8*, 3837-3840. **2**. León, L. G.; Carballo, R. M.; Vega-Hernández M.C.; Martín, V. S.; Padrón, J. I.; Padrón, J. M. *Bioorg. Med. Chem. Lett.* 2007**,** *17*, 2681–2684.

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Complex interplay of estradiol with growth hormone and thyroid hormones to regulate gene expression of suppressor of cytokine signaling in liver and kidney from hypothyroid male rats

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GH is major regulator of body growth and composition. SOCS proteins act as negative regulators of GH signalling. Recently, E2 was demonstrated to induce SOCS2 synthesis and inhibit GH-Jak2-Stat5 signalling in liver and kidney cells. To evaluate the role of E2 on

Thyroid Hormone- and/or GH-regulated SOCS in vivo this work used orchidectomized-hypothyroid (TX) male rats to minimize the influence of internal hormones on treatment. TX rats were treated 3 weeks with E2 (50□g/kg; sb; 5 days per week) before hormonal replacement with GH, T3, or T3 plus GH during 7 days. We analyzed body growth, serum lipids, and steady-state levels of SOCS mRNA expression in relation to cited hormones. TX resulted in an over 2-fold increase in serum cholesterol (Cho) and 2-fold reduction in serum triglyceride (Tg) levels. Unlike GH, T3 treatment normalized Cho levels. However, Tg levels were further reduced after GH treatment. E2 did not modify the effects of TX and hormones on serum lipids levels. In liver, but not in kidney, TX significantly reduced SOCS2 mRNA levels. In contrast, TX induced SOCS3 expression in both tissues and this effect was reversed by T3 treatment but not by GH. Unlike T3, GH superinduced SOCS2 mRNA levels in liver. However, the effect of GH on SOCS2 was inhibited in the presence of E2. In kidney, GH did not affected SOCS2 mRNA levels but enhanced E2 effect on this gene. E2 increased SOCS2 mRNA levels but not SOCS3 both in liver and kidney. Interestingly, whereas E2 enhanced T3 effect on SOCS2 this estrogen antagonized T3 effect on SOCS3 in both tissues. These results suggest that E2, GH and T3 play a complex interaction to maintain physiological levels of SOCS in liver and kidney.

Acknowledgements: This work is supported by Ministerio de Educación y Ciencia (SAF2006-07824) and FUNCIS (PI 60/05).

NOTES

Design and Synthesis of a Novel Series of Pyranonaphthoquinones as Topoisomerase II Catalytic Inhibitors

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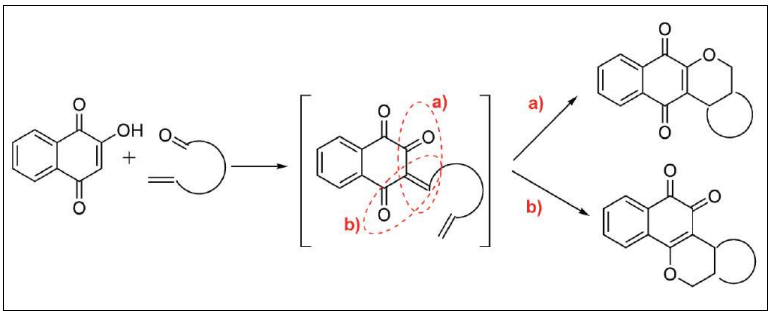
On the basis of previous pharmacophore modeling studies of naphthoquinones derivatives, we have designed and synthesized a new set of pyranonaphthoquinones. These compounds were obtained through a direct and
highly efficient approach based on an intramolecular domino Knoevenagel hetero Diels-Alder reaction from lawsone (2-hydroxynaphthoquinone) and a variety of aldehydes containing an alkene. The synthesized pyranonaphthoquinones were evaluated against the a-isoform of human topoisomerase II (hTopoIIa). Among the 11 derivatives studied, we found that six of them act as catalytic inhibitors of the enzyme in vitro. These six derivatives strongly preclude the enzyme from decatenating or relaxing suitable substrates. Finally, we correlate their active/inactive status with docking studies of these novel compounds into the ATPase domain of hTopoIIR .



Acknowledgment.: This work has been partly funded by the Spanish MEC (Project SAF 2006-06720) and ICIC (Instituto Canario de Investigación del Cáncer) to A.E.-B. and by FIS (Project PI06/1211) to F.M. Ramon y Cajal program (Spanish Ministry of Science & Technology) contributes to the financial support of F.M. S.J.-A. thanks the MEC for a predoctoral fellowship.

New Metal-Quinonic Complexes with Potential Antitumoral Activity

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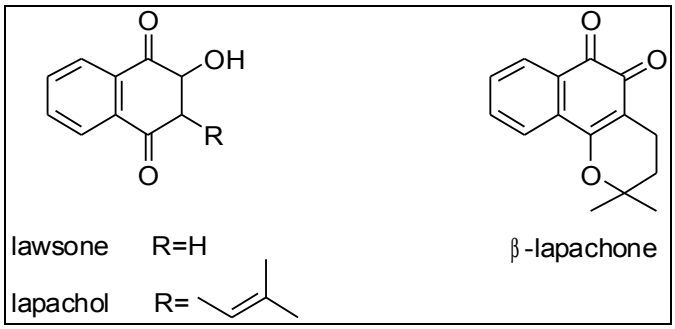
Quinones constitute a group of great interest due to their many biological activities[1], such as cytotoxic, antibacterial, antifungal, antiparasitic, anti-inflammatory, antimitotic and antimalarial. The quinonic core is a selected chemical entity[2] and it is considered by the NCI as an important biologically validated starting point to develop new bioactive molecules with good levels of citotoxicity[3].

In our research group we are interested in the development of potential antitumoral molecules[4], so with this goal we are especially interested in the obtention of several metal complexes containing quinone ligands. In this communication we will report the results obtained in the preparation of complexes of several quinonic ligands with some metals (Cu, Co, Zn, Mn, Pt, Ru …) with the aim of improving their biological activity[5].

The quinones used as starting material are commercial available quinones such as 2-Hydroxy-naphthoquinone (lawsone), 4-Hydroxy-3-(3-methylbut-2-enyl)-naphthalene-1,2-dione (lapachol) and 3,4-Dihydro-2,2-dimethyl-2H-naphtho(1,2-b)pyran-5,6-dione (β-lapachone).



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From conjugated tertiary skipped diynes to chain-functionalized tetrasubstituted pyrroles.[1]

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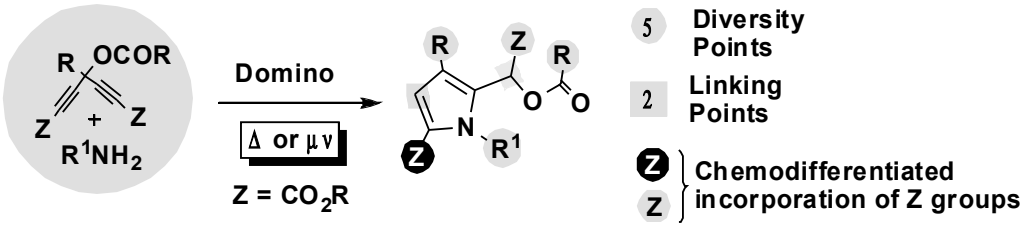
Symmetry breaking of *meso*-1,4-diyne scaffolds via nucleophilic amine addition onto one of the two equivalent alkynoate units affords chain-functionalized tetrasubstituted pyrroles. In particular, tetrasubstituted pyrroles can be considered as hybrid scaffolds comprising a structurally privileged pyrrole ring and a natural-occurring α-hydroxy acid motif featuring five points of functional diversity and two points for complexity generation. The synthetic manifold is triggered by the nucleophilic addition of a primary amine on the alkynoate function and operates following a domino construction principle which entails an aza-Michael addition, a 5-endo-digonal cyclization and a [3,3]-sigmatropic rearrangement. The reaction can be performed under conventional or microwave heating conditions; whereas the former is faster (30 min), the latter is slightly more efficient. The mechanism and the scope of this process will be discussed in the poster.



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Identification of novel genes regulated by liver X receptors in macrophages

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Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous components in the large arteries and is currently the leading cause of morbidity and mortality in mostindustrialized countries. It is now considered that atherosclerosis is a chronic inflammatory disease as well as a disorder of lipid metabolism. As modulators of both lipid metabolism and immune responses, macrophages play a central role in the atherogenic process. Multiple environmental factors contribute to the development of the disease, including hypercholesterolemia, hypertension and diabetes. The liver X receptors (LXRα and LXRβ), members of the nuclear receptor superfamily, are transcriptional regulators of cholesterol metabolism and determinants of atherosclerosis susceptibility. Our recent work has identified LXRs as lipid-dependent regulators of inflammatory gene expression that may serve to link lipid metabolism and immune functions in macrophages. Activation of LXRs with natural or synthetic ligands promote the expression of genes involved in cholesterol homeostasis and inhibit the expression of inflammatory genes induced by external insults.

This crosstalk, between macrophage inflammatory pathways and LXR signaling, points to these nuclear receptors as attractive therapeutic targets for pharmacological intervention. In an effort to investigate new connections between immunity, atherosclerosis and nuclear receptor signaling, we analyzed microarray experiments obtained from WT and LXR-deficient mice as well as macrophage cell lines expressing LXR isoforms. Our data points to selective gene expression patterns controlled by LXR isoforms that are important in inflammation and lipid metabolism.

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