# ORAL COMMUNICATIONS

# Sesquiterpenes as cancer multidrug resistance (MDR) modulators – facing a still unsolved problem

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Cancer patients who receive chemotherapy often experience resistance to a broad spectrum of chemotherapeutic agents. This phenomenon, called multidrug resistance (MDR), is the cause why many cancers fail to respond to chemotherapy [1].

Resistance to chemotherapy can have several causes [2], including the increased activity of drug pumps, the modulation of cellular pathways, the alteration and repair of target molecules. Together they build a complex network of cellular pathways and molecular mechanisms mediating an individual MDR phenotype [3]. Due to this phenomenon the efficacy of a given chemotherapeutic regimen varies from patient to patient [4].
One major form of MDR has been correlated with the presence of at least three molecular "pumps" that actively transport drugs out of the cell, causing a decrease in the intracellular cytotoxic drug accumulation. The most prevalent of these MDR transporters is the Pgp, a member of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily. Pgp has unusually broad poly-specificity, recognizing hundreds of compounds. Therefore, Pgp is a promising target for cancer therapy, and significant efforts have been focused on the development of effective reversal of the Pgp-mediated MDR [5].

Natural products have been the main source of drugs for humans during centuries, making them an excellent starting point for the development of new chemotherapeutical agents. In fact, many approved therapeutics as well as drug candidates are derived from natural sources. Thus, i. e. over 60% of the current anticancer drugs have their origin in one or another way from nature [6].

Over the last 30 years, a large number of secondary metabolites exhibiting a wide range of bioactivity have been isolated from the *Celastraceae* family. In the search for biologically active metabolites from species of this family, our group of investigation studied e.g. *Zinowiewia costaricensis* [7]*, Maytenus canariensis* and *Crossopetalum uragoga*. The most widespread and characteristic group of secondary metabolites from this plant family are the dihidro-β-agarofuran sesquiterpenes. They have attracted considerable attention from synthetic chemists and pharmacologists due to their complex structures and wide range of biological properties. On the basis of these properties, sesquiterpenes have been selected as *privileged structures*. As a part of an intensive investigation into active metabolites as reversal agents of the Pgp-dependent MDR phenotype, we have focused our efforts on the characterization of new sesquiterpenes as potential Pgp inhibitors [7, 8].

**References:**

[1] YUAN, H.; LI, X.; WU, J.; LI, J.; QU, X.; XU, W.; TANG, W. *Curr. Med. Chem.*, **2008**, *15*, 470–476.
[2] ALLER, S. G. ; YU, J.; WARD, A.; WENG, Y.; CHITTABOINA, S.; ZHUO, R.; HARREL, P. M.; TRINH, Y. T.; ZHANG, Q.; URBATSCH, I.; CHANG, G. *Science*, **2009**, *323*, 1718–1722.
[3] LAGE. H. *Cell. Mol. Life Sci.*, **2008**, *65*, 3145–3167.
[4] LAGE, H. *Proteomics Clin. Appl*., **2009**, *3*, 883–911.
[5] REYES, C. P.; MUÑOZ-MARTÍNEZ, F.; TORRECILLAS, I. R.; MENDOZA, C. R.; GAMARRO, F.; BAZZOCCHI, I. L.; NÚÑEZ, M. J.; PARDO, L.; CASTANYS, S.; CAMPILLO, M.; JIMÉNEZ, I. A. *J. Med. Chem.,* **2007**, *50*, 4808–4817.
[6] CRAGG, G. M.; NEWMAN, D. J. *Phytochem. Rev.,* **2009**, *8*, 313–331.
[7] MUÑOZ-MARTÍNEZ, F. ; MENDOZA, C. R. ; BAZZOCCHI, I. L.; CASTANYS, S. *J. Med. Chem*., **2009**, *48*, 4266–75.
[8] MUÑOZ-MARTÍNEZ, F. ; REYES, P. R. ; PÉREZ-LOMAS A. L.; JIMÉNEZ, I. A.; GAMARRO, F.; CASTANYS, S. *Biochim. Biophys. Acta*, **2006**, *1758*, 98–110.

## NOTES

# Metal complexes in the development of new antitumoral drugs

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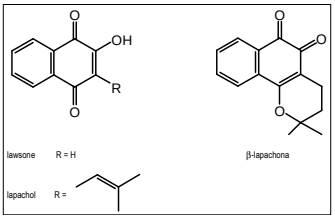
Transition-metal-based drugs are increasing its importance in the therapy of cancer and other diseases. The best example of these metal-based drugs is cisplatin (CDDP), one of the most used anticancer drug. Nowadays metal complexes have an enormous potential for applications in medicine and are present in many fields such as diagnostic agents, magnetic resonance imaging (MRI), enzyme inhibitors, radiopharmaceuticals, therapeutic agents, etc.

Metals are often used to organize in the space the organic ligands. They provide an expanded set of coordination geometries for the generation of molecular diversity. Furthermore, they can play an important role in modifying the pharmacological properties of known drugs.
Most of these metal-based drugs were discovered by serendipity or random screening. But lately they are being designed to interact with their molecular target, taking advantage of the properties inherent to the metal centre.
In these cases, there are two main ways of using the properties of the metal centre:

1. Organometallic mimics of enzyme inhibitors. In this case the metal organizes the organic ligands in three-dimensional space (structural role).
2. Redox activation of organometallic prodrugs. In this case, the role of the metal is not only structural; their redox properties are used to control their action in biological systems.

In our research group we are interested in the development of potential antitumoral molecules. We are especially interested in the obtention of several metal complexes containing natural naphthoquinone ligands. In this communication we will report the results obtained in the preparation of complexes of several naphthoquinonic ligands with some metals (Cu, Co, Zn, Mn, Pt, Ru …) with the aim of improving their biological activities.




Naphthoquinones used as ligands

**References:**

Kelland, L., *Nature Rew. Cancer*, **2007**, *7*, 573-584.
[2]Hambley, T.W., *Science*, **2007**, *318*, 1392-1393.
[3]Meggers,E., *Curr. Opinion Chem. Biol.*, **2007**, *11*, 287-292.
[3]Schatzschneider,U.; Metzler-Nolte,N. *Angew. Chem. Int. Ed.*, **2006**, *45*, 1504-1507.
[4](a) Ravelo, A.G., Estévez-Braun, A., Mesa-Siverio, D., Pérez-Sacau, E., Lacal, J.C., Ramírez-Molina, A., Báñez-Coronel, M. (PCT/EP2006/070276). (b) Jiménez-Alonso, S., Chávez, H., Estévez-Braun, A., Ravelo, A.G., Pérez-Sacau, E., Machín, F., **2008**, *J.Med. Chem.*, *51,* 6761-6772. (c) Jiménez-Alonso, S., Pérez-Lomas, A.L., Estévez-Braun, A., Muñoz-Martínez, F., Chávez, H., Ravelo, A.G.,
[5]Gamarro, F., Castanys, S., López, M., **2008**, *51*, 7132-7143.
[6]Gokhale, N., Padhye, S., Newton, C., Pritchard, R., **2000**, Metal-Based Drugs, *7,* 121-128.

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## NOTES

# Synthesis of Lauroxanes: Active Marine Toxins from Red Algae

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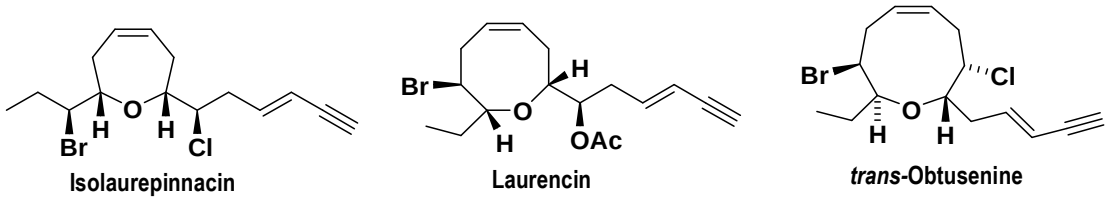
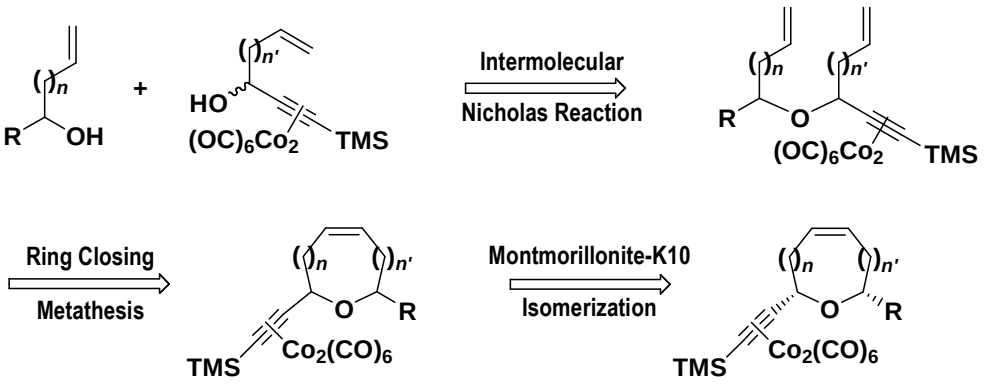
Red algae of the genus *Laurencia* produce a multitude of unique compounds, such a series of nonterpenoid C15 metabolites generally named *lauroxanes*, which exhibit a large number of biological properties including antitumor, antimicrobial, immunosuppressant, antifeedant, pesticide activity, etc. These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




**Scheme 1**

In this contribution we report on our current efforts on the formal synthesis of Isolaurepinnacin and Laurencin, in order to study their activity. 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**).




**Scheme 2**

**References:**

a) Yasumoto, T.; Murata, M. *Chem Rev*. **1993**, *93*, 1987-1909. (b) Faulkner, D. J. *Nat. Prod. Rep*. **2002**, *19*, 1-48

[2] (a) Ortega, N.; Martín, T.; Martín, V. S. *Org. Lett*. **2006**, *8*, 871-873. (b) Ortega, N.; Martín, T.; Martín, V. S. *Eur. J. Org. Chem*. **2009**, 554-563.

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## NOTES

# Strategies in mechanism elucidation of new antitumour compounds

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Proliferative disorders such as cancer are recognized as diseases of the cell cycle. It has generally been found that in tumour cells, the mechanisms that normally function to restrain cell division are defective, whilst those that promote division become more active [1]. The cell cycle regulation plays a critical role in malignant transformation and in the development of resistance to chemotherapy [2]. Within our program directed at the discovery of new antitumor agents, we have focused our attention in compounds that disturb the cell cycle. Our strategy to determine the possible mechanism(s) of action is based on a modular set of biological experiments. Herein, we exemplified this strategy with a set of representative families of Michael acceptors exhibiting antitumor activity.

The first step consists in the screening of pure compounds for growth inhibition and cytotoxicity using the SRB assay [3] against a panel of six to ten human solid tumour cell lines from diverse origin. The results on the biological activity allow us to classify the compounds according to their anticancer activity profile. From this data expressed as $GI_{50}$, a structure-activity relationship is drawn. The outcome of this first step is the selection of a lead for further testing. Then, we analyse cell cycle phase distribution by flow cytometry to determine if cell growth inhibition due to the lead compounds involves cell cycle perturbations. In addition to cell cycle disruption, we determine if the candidate is able to induce apoptosis. Further studies are performed by western blotting analysis of protein extracts of cells exposed to the lead drug at diverse dose-time schedules. After examination of protein expression, we might be able to point out the biological target involved in the interaction of the drug candidate.

In this communication we will show the results obtained and provide insights into the possible mechanism of chemotherapeutic action of new antitumour compounds.

**References:**

[1] Brooks, G.; La Thangue, N.B. *Drug Discov.Today* **1999**, *4*,455.
[2] Schwartz, G; Shah, M. *J. Clin. Oncol.* **2005**, 23, 9408.
[3] Miranda, P.O.; Padrón, J.M.; Padrón, J.I.; Villar, J.; Martin, V.S. *ChemMedChem* **2006**, 1, 323.

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## NOTES

## Why potency isn´t everything

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This short presentation will introduce the typical developmental stages of drug discovery and why drug development is generally a long and somewhat arduous exercise. We will also discuss the most common pitfalls with a particular emphasis on pharmacokinetics.

## NOTES

## RNA turnover pathways as novel targets for anti-cancer drugs

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Degradation of cytoplasmic mRNAs is an important aspect of the regulation of gene function in eukaryotes. Much of what is currently known about the underlying pathways of mRNA decay is derived from studies of the budding yeast *Saccharomyces cerevisiae*, in which mRNA turnover is initiated by deadenylation, followed either by decapping and 5'-3' degradation or by further 3'-5' exonucleolysis. The evolutionarily conserved Cid1 family of RNA nucleotidyl transferases (of which there are seven in human cells) are involved in several aspects of RNA metabolism, including RNA decay in the nucleus and cytoplasm. Our recent studies have shown that Cid1 itself, which we identified in the fission yeast model, acts as an RNA 3' terminal uridyl transferase in such a way as to target its cytoplasmic RNA substrates for degradation (Rissland and Norbury (2009) *Nat Struct Mol Biol* 16: 616-623). The human Cid1 orthologues ZCCHC6 and ZCCHC11 possess uridylyl transferase activities that have been implicated in the turnover of specific cytoplasmic mRNAs and in the regulation of microRNA biogenesis and activity. Recent data suggest that selective inhibition of these enzymes could be of therapeutic value, either as an adjunct to the widely used anticancer drug hydroxyurea (HU), or as a means of countering the Myc-dependent proliferation that characterises a wide variety of tumours. We have established robust *in vitro* assays for the identification of Cid1/ZCCHC6/ZCCHC11 inhibitors, and expect soon to be in a position to relate the structures of our early lead inhibitors to that of the target. Selective targeting of the distinctive biochemical activities associated with these RNA turnover pathways may ultimately lead to the generation of a distinctive new class of small molecule cancer therapeutics.

## NOTES

# Molecular prediction of normal tissue chemo-radiation-induced toxicity and response

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Radiotherapy is an important tool in the treatment of cancer. In the routine day-to-day practise of radiotherapy, treatment schedules are designed for the "average" patient with a given type of malignancy at a given site. Tumour control by radiation therapy requires the use of maximum dose that can be delivered while maintaining a tolerance risk of normal tissue toxicity. Adverse reactions due to x-ray exposure are a limiting factor for treatment success. Toxicity reactions could be acute or late toxicity, according to the Radio Therapy Oncology Group (RTOG) morbidity scoring system. RTOG classifies toxicity of patients into different levels: grade 1 (mild) to grade 4 (severe). Cancer patients exhibit large patient-to-patient variability in normal tissue reactions after radiotherapy. Prediction of individual sensitivity to radiotherapy could help to select the radiation protocol and to improve treatment results. During the last decade, a number of studies have supported the hypothesis that there is an important genetic component to the observed interpatient variability in normal tissue toxicity after radiotherapy. Even though, the genetic and molecular mechanisms of therapeutic radiation sensitivity is still poorly understood, and very little is known about the genetic variation possibly underlying inter-individual differences in normal tissue reactions when unselected cancer patients undergo radiotherapy. Many efforts have been employed to develop predictive tests applied to clinical practise. The eventual goal of predictive assays is to choose a treatment protocol that is optimal for each individual patient and that might give a better chance of cure than the conventional therapy. We present here the results and projects from our group related with molecular prediction of normal tissue chemo-radiation-induced toxicity and response. We have found an association between the constitutive gene expression profile of peripheral blood lymphocytes and the development of acute and late toxicity in locally advanced breast cancer patients. Determination of lymphocyte radiosensitivity by radio-induced apoptosis arises as a possible method for predictive test development. We observed that radiation induced apoptosis at different time points and radiation doses, fitted to a semi logarithmic model defined by a mathematical equation that gives an individual value of radiosensitivity and could predict late toxicity due to radiotherapy. Associations between common polymorphisms in DNA damage detection and repair genes and the development of adverse reactions to radiotherapy have been observed. Different projects related to this topic are ongoing in our group.

## NOTES

# Microsatellite instability predicts clinical outcome in radiation-treated endometrioid endometrial cancer

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Microsatellite instability (MSI) is the hallmark of cancer with DNA mismatch repair (MMR) deficiency and underlies 20-30% of endometrial cancer. Some in vitro studies suggest that radiation effects are modulated by the MMR system, however, little is known about the relationship between MSI and radiation response.

The aim of this study was to elucidate whether MSI predicts clinical outcome in radiation treated endometrioid endometrial cancer (EEC).

For that purpose a consecutive series of 93 patients with EEC treated with extrafascial hysterectomy and postoperative radiotherapy was studied. The median clinical follow-up of patients was 138 months, with a maximum of 232 months. Five quasimonomorphic mononucleotide markers (BAT-25, BAT-26, NR21, NR24, and NR27) were used for MSI classification.
Twenty-five patients (22%) were classified as MSI. Both in the whole series and in early stages (I and II), univariate analysis showed a significant association between MSI and poorer 10-year local disease-free survival, disease-free survival, and cancer-specific survival. In multivariate analysis, MSI was excluded from the final regression model in the whole series, but in early stages MSI provided additional significant predictive information independent of traditional prognostic and predictive factors (age, stage, grade, and vascular invasion) for disease-free survival (hazard ratio [HR] 3.25, 95% confidence interval [CI] 1.01–10.49; p= 0.048) and cancer-specific survival (HR 4.20, 95% CI 1.23–14.35; p= 0.022), and was marginally significant for local disease-free survival (HR 3.54, 95% CI 0.93–13.46; p= 0.064).

These results suggest that MSI may predict radiotherapy response in early-stage EEC.

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## NOTES

# COMBINATION OF RADIOTHERAPY AND NEW ANTITUMOR ACTIVE COMPOUNDS

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Several antitumor compounds are able to arrest cells at specific cell cycle checkpoints. G2/M is the most radiosensitive phase of the cell cycle. Our hypothesis is that the combination of new antitumor active compounds and radiation therapy would increase the therapeutic efficacy.

The main objectives of this study were to evaluate the dose-effect relationships for cytogenetic damage induced by our compound to the 3, 7 and 17 chromosomes and to analyse the type of interaction of the combined treatment of radiation and chemotherapy.

The *in vitro* experiments were performed in a panel of five human solid tumor cell lines. Propidium iodide was used to determine the percentage of cells at each cell cycle by flow cytometry. DNA fluorescent probe sequences homologous to specific regions on chromosomes 3, 7 and 17 were used to evaluate the cytogenetic damage by Fluorescence *In Situ* Hybridization (FISH). After six hours of incubation 96 well plates were irradiated with doses of 0,2,4,6,8 Gy at 0.5 Gy/min rate. The cell viability was measured with the sulforhodamine B assay and 10 different schedules of combination studies were designed. The dose-response interactions between the compound and radiation therapy were evaluated using the analysis method of Chou.

Compound inhibited, in a dose-dependent manner, the proliferation through G2/M phase arrest. The cell lines HBL-100 presented trisomy in the control sample and an increase in the number of the chromosomes 3,7 and 17 when the cells were exposed at 2μM and 4μM doses. Different interactions (antagonism, additivity and synergism) were found in the 10 schedules analysed.

The cytotoxic compound produces chromosome aberrations and arrests the cell cycle at the G2/M phase. The combination of radiation with chemotherapy provided schedules with synergistic interactions which open the way for further research.

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## NOTES

# Dioxin-like carcinogens through the intake of dairy products

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Dioxins and dioxin-like compounds (DL-compounds) are classes of environmental carcinogens characterized by exceedingly long half-lives in the environment. DL-compounds are organochlorine antropogenic contaminants that tend to bioaccumulate and biomagnificate in food-chain and living-beings.There are four kind of DL-Compounds: (1) Policlorinated - Dioxines (PCDD); (2) Policlorinated - Dibenzofuranes (PCDF); (3) Policlorinated Biphenyls (PCBs) and (4) Polibrominated Biphenyls (PBBs). Groups 1 and 2, are derivates of industrial activities such as the synthesis of certain industrial halogenated aromatics chemicals, by-products from other commercial processes and by-products of combustion, while substances included in groups 3 and 4 were made for a great variety of uses including transformers, condensers, paint and pesticide aditives etc.These chemicals tend to accumulate in animal and human tissues after exposure through dietary sources. In human beings and livestock, the milk is the major route of excretion of these compounds, and therefore 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. 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. 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

# "Cooperation between LXR and Caveolin-1 in regulation of cholesterol metabolism and inflammation"

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Previous studies have shown that LXRs nuclear receptors play important roles in macrophage functions, including cholesterol metabolism, inflammation and immune responses (Castrillo and Tontonoz, 2004; Joseph et al., 2004). These receptors have emerged as key regulators of cholesterol homeostasis, targeting genes of plasma membrane transporters like ABCA-1, thus promoting the cholesterol efflux pathway in macrophages (Coset et al., 2000; Wenkateswaran et al., 2000). So, LXRs would prevent the foam cell formation associated with the critical initial stages of the atherosclerosis disease.

Caveolin-1, which is responsible for the formation of caveolae, is also a high affinity, cholesterol binding protein (Murata et al., 1995). Some laboratories have investigated the role of caveolin-1in macrophages in relation to cellular cholesterol homeostasis and lipoprotein metabolism (Fielding and Fielding, 1997; Frank et al., 2006), but our knowledge in this direction is not totally complete. In addition to their effects on cholesterol metabolism, activation of the LXR by synthetic agonists has an inhibitory effect of inflammatory mediators such as COX-2, iNOs, IL-1b, IL-6, in macrophages *in vitro* in response to LPS activation or bacterial infection (refs. Castrillo et al., 200X). Recent works has demonstrated that activation of LXR can also prevent inflammation in macrophages not only by inhibition, but also through transcriptional activation of anti-inflammatory genes as ArgII (Marathe et al., 2006).

On the other hand, the studies carried out in the caveolin-1-defficient mice have pointed caveolin-1 an important role as an immuno-modulator protein. In macrophages specifically, caveolin-1 is up-regulated in response to microbial products and LPS (Wang et al., 2003; Lei et al., 2005), and to different apoptotic agents (Gargalovich and Dory, 2002). Other studies have linked the deficiency of caveolin-1 with the impairment of phagocytosis and the possible relevance of this protein in immunological homeostasis (Li et al., 2005). Nevertheless, is now accepted that the exact role of caveolin-1 with respect to innate immunity in macrophages is only just beginning to be understood.

Here, we provide novel additional data demonstrating that caveolin-1 participates and cooperates with LXR in the inflammatory immune responses and cholesterol metabolism functions in mouse peritoneal macrophages.

## NOTES

# 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 most industrialized 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.

**Acknowledgements:**This work was supported by grant FPI BES-2006-12056 and by project SAF2005-03270 from the Ministerio de Educación y Ciencia.

## NOTES

# Transcriptional activities of newly developed tamoxifen derivatives.

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According to data published by Consejería de Sanidad from the Gobierno Autónomo de Canarias, around 30% of cancer diagnosed in women corresponds to breast cancer; being one of the most frequent cancer among canarian women. Epidemiological studies predict that one occidental woman out of every ten will develop breast cancer in her lifetime. Approximately, two out of three women who develop this type of tumor have hormone-receptor positive breast cancer, so, consequently, can profit from hormonal therapy.

Tamoxifen (Tx) is a Selective Estrogen Receptor Modulator (SERM) which has been widely used on estrogen receptor-positive breast cancer treatment for more than 30 years, and, more recently, also on prevention of this disease. This drug has proven to be one of the most effective for the therapy, reducing, considerably, the mortality due to this disease. However, the existence of demonstrated undesirable side effects, such as the development of endometrial cancer, has encouraged physicians not to extend the treatment for more than five years in most of cases. 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 tamoxifen derivatives which maintain the triphenylethylene core but contain different lateral alkylaminoethoxy side-chains modifications. Five different carbonyl structures of tamoxifen have been assessed in this work: four carbamates (CTx) and a methoxy-tamoxifen derivative (MTx). Previous results from our laboratory showed that these novel derivatives were capable to reduce the proliferative effects of estradiol on the estrogen receptor-positive cell line MCF7 in a dose-dependent manner. We also have assayed these 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 agonistic effects of estradiol in a dose-dependent manner and, interestingly, this effect is achieved at pharmacological concentrations found on tamoxifen-treated patients. We have found significant differences at 3 μM between derivatives that were not observed in proliferative assays on MCF7 cells, however, estradiol induction levels, both in proliferative and ERα activation models, were not enough large to establish antiestrogenic dose-response curves. For this reason, we have assayed the transcriptional activity of these new derivatives on transitory 3xERE-luc transfected MCF7 cell line and T47D-KBluc cell line that stably express the estrogen-response luciferase reporter. Results fit well with those found on proliferative and ERα activation assays. All new compounds demonstrated a powerful antiestrogenic effect on transcriptional activity as Tx itself, with the exception of MTx that displayed one order of magnitude higher $IC_{50}$. In addition, an estrogenic synergic effect was observed only on MCF7 cells when 100 pM of estradiol and 100 nM of the triphenylethylene compounds were applied simultaneously; such cell line-dependent effects point to the existence of a structure-function relationship. On the other hand, Tx showed transcriptional activity induction itself in both cell lines. This effect only was weakly mimicked by MTx in both cell lines, and by two of the carbamates in the MCF7 cell line. Taken together, our results open a promising and alternative therapeutic window to long-term tamoxifen therapies.

**Acknowledgements:** This work has been possible thanks to the collaboration of Benito García Marrero and Alicia Boto from Instituto de Productos Naturales y Agrobiología (CSIC, Spain), and Leandro Fernández from Departamento de Ciencias Clínicas, ULPGC. I am very grateful to all my colleagues from Laboratorio de Fisiología y Biofísica de Membranas (Departamento de Biología Animal, ULL, Spain). Supported by research grant SAF2007-66148-C02-02 from Ministerio de Educación y Ciencia (MEC, Spain). J. Marrero-Alonso is FPU fellow from MEC (Spain).

## NOTES

# Analysis of molecular mechanisms involved in the regulation of SOCS2 transcription: potential role of SREBP and LXR signaling pathways

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Suppressor of cytokine signaling (SOCS) proteins negatively regulate cytokine and growth factor receptor signaling playing a critical role within immune, insulin or Insulin-like growth factor (IGF) -I pathways (1). Therefore, consistent with the importance of these cytokines and growth factors in human physiology, any SOCS imbalance could result in a broad range of pathologies (e.g., cardiovascular disease, insulin resistance, cancer or altered growth). The concentration of SOCS is constitutively low but it increases rapidly by the apparition of the different stimuli. Recently, human SOCS2 gene, a main regulator of somatic growth through modulation of the Growth hormone (GH)/IGF-I axis, has been cloned. A novel response element has been identified within the first intron of the human SOCS2 gene composed of an E-box followed by a tandem of Signal transducers and activators of transcription (STAT) 5b binding sites (2). GH-induced SOCS2 expression is regulated via activation of STAT signaling pathway (3). The presence of an E-box response element, that recognizes Sterol regulatory element-binding protein (SREBP) among other transcription factors, opens the possibility that SREBP may regulate the SOCS2 transcriptional activity via this specific region. In this work, we have explored the hypothesis that SREBP and/or Liver X receptor (LXR) α ligand T0901317, a potent activator of SREBP-1c expression, play any role on SOCS2 regulation. We have analyzed mRNA and SOCS2 promoter regulation by using qRT-PCR and cell transfection assays, respectively. Our results indicate that T0901317 inhibits GH-mediated SOCS2 mRNA expression *in vitro*. Additionaly, co-transfection with SREBP-1c or T0901317 treatment down-regulate GH-mediated SOCS2 transcription. These suggest than SREBP-1c and/or LXRα signaling are negative regulators of GH-dependent SOCS2 transcription. All these findings provide new insights into the transcriptional regulation of SOCS2 and the mechanism by which LXRα ligands may modulate GH physiology. *[This work was supported by MICINN SAF2006-07824, ICIC and ULPGC].*

**References:**

1. **Rico-Bautista E, Flores-Morales A, Fernandez-Perez L** 2006 Suppressor of cytokine signaling (SOCS) 2, a protein with multiple functions. Cytokine Growth Factor Rev 17:431-439
2. **Vidal OM, Merino R, Rico-Bautista E, Fernandez-Perez L, Chia DJ, Woelfle J, Ono M, Lenhard B, Norstedt G, Rotwein P, Flores-Morales A** 2007 In vivo transcript profiling and phylogenetic analysis identifies suppressor of cytokine signaling 2 as a direct signal transducer and activator of transcription 5b target in liver. Mol Endocrinol 21:293-311
3. **Flores-Morales A, Greenhalgh CJ, Norstedt G, Rico-Bautista E** 2006 Negative regulation of growth hormone receptor signaling. Mol Endocrinol 20:241-253

## NOTES

# Role of contamination by Persistent Organic Pollutants on Insulin Growth Factor levels and Bladder cancer: preliminary results

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Organochlorines are man-made chemicals that have shown the capacity to alter endocrine function and have been linked with several deleterious health effects, among them various types of cancer. The objective of this study is to assess the relationship between serum concentrations of persistent organic pollutants (POPs) and bladder cancer risk in a sample of cases and controls from the Hospital Universitario Insular de Gran Canaria (HUIGC), and also to assess the relationship between IGF-1 in serum and bladder cancer risk.

We have included both, case studies and hospital-based controls. We had serum extracted from 410 patients: 210 from new diagnosed cases of bladder cancer, admitted to the urology department of HUIGC for treatment of bladder cancer by transurethral resection of bladder (TURB). and 200 from hospital controls, that were selected among patients admitted with diagnosis of diseases HUIGC normally not associated with known risk factors for bladder cancer to exposure to POPs. Each participant answered a questionnaire with questions about demographic variables, habits and health. Results were individually matched by sex, age and area of residence.

We measured the serum concentrations of organochlorine pesticides and IGF-1. Gas chromatography/mass spectrometry was used to identify and quantify the analytes, including hexachlorobenzene, and 18 organochlorine pesticides and metabolites. IGF-I was analyzed in tube or microplate using a technique of enzyme immunoassay (ELISA) with the method developed by Diagnostic Systems Laboratory (Webster, Tx, USA). Our results showed that 4.4' DDE is present in 92% of samples and their concentrations vary according to having tumor, recidivations and not having tumor. The IGF1 and IGF-BP3 higher values in cases of high risk of progression and recurrence of bladder cancer in cases with lower risk of progression and recurrence. The levels are lower in the controls, these differences were statistically significant.

## NOTES

# Classification of Cell Death

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The death of a cell can be defined as an irreversible loss of plasma membrane integrity. Cell death is as important as cell proliferation for cell turn-over, and susceptibility to cell death is affected by a number of parameters that change with time. So far, different cell death types are defined by morphological criteria, without a clear reference to precise biochemical mechanisms. It is essential to replace morphological aspects with biochemical/functional criteria to classify cell death modalities. In this regard, apoptosis, a kind of cell death which is thought to be an important response to most chemotherapeutic agents in human tumor cells, can occur with or without, caspase activation. Moreover, autophagic cell death represents a type of cell death with autophagic vacuolization. This does not mean that cell death is executed by autophagy. Although clear definitions of cell death are difficult to be achieved, it is important to discriminate between dying as a process and death as an end point. Dying can occur by different mechanisms, each characterized by a number of criteria. However, not all criteria are needed to satisfy the definition. Dying in a cell population is a stochastic process, and at a given time, individual cells will be at different stages of the dying process. Therefore it is important to define the criteria used to assess a dying population.

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## NOTES

## Cdc14-1 release causes an intense and long-lasting DNA damage response as cells enter a new cell cycle.

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Sister chromatid non-disjunction during anaphase is a putative source of mitotic catastrophe that would lead to genetically aberrant daughter cells and cancer development. In budding yeast, inactivation of the conserved phosphatase Cdc14 blocks cells in telophase with non-disjunction of the ribosomal DNA (rDNA). Cdc14 can be successfully reactivated after the block which allows all the cells to enter a new cell cycle. However, more than 50% of the cells do this despite they fail to resolve and segregate the rDNA. Here, we have studied the fate of the cells after re-activation of Cdc14 and correlated this fate to the missegregation of the rDNA. Thus, we show that cells which failed in the rDNA segregation throughout the release delay cytokinesis and activate Rad52-dependent DNA damage response that begins once daughter cells have already entered the S-phase. Strikingly, the DNA damage response is massive, cumulative and long-lasting; suggesting that cells may inefficiently repair the expected one-ended breaks.

## NOTES

## Insights into kinase regulation and selective inhibition by large scale structural comparison

**Stefan Knapp**

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For more than 20 years protein kinases have been intensively pursued as targets for drug discovery, an effort that has resulted in 10 approved oncology drugs. Our limited knowledge of cellular signaling in cancer has strongly biased inhibitor development towards clinically validated kinases with little change in target selection during the past 10 years. Recently, large scale sequencing efforts and kinome wide knock-out studies have identified novel and structural diverse kinase targets with however largely unknown function. We recently initiated work to identify selective inhibitors for these potential targets that will be used to functionally annotate these kinases. Efforts in my laboratory and others led to a large body of structural data of kinases and their inhibitor complexes. I will discuss how this information can be used to develop strategies for the design of highly selective kinase inhibitors. In addition, data will be presented using these selective reagents to study the function of the kinase PIM1.

## NOTES

# CEAMED: An ICIC-born pharmaceutical company

**Nicolás Díaz-Chico**

*ICIC, ULPGC*

## NOTES

# Humate soil as new source of mycophenolic analogs

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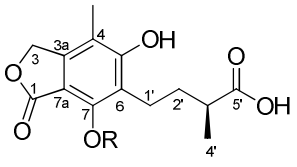
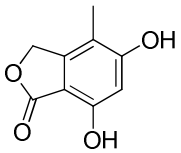
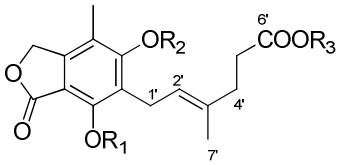
Humate soil is organic matter that has a positive effect on the physical, chemical, and biological reactions of the soil leading to an increase in soil fertility.[1] Humate is comprised of humic acid, fulvic acid, some minerals, bacteria and fungi which facilitate the development of deep root systems, enhance beneficial microbial activity and improve overall plant health and to resist diseases.[2]. From a particular humate collected in Cuba, NM, we isolated *Eupenicillium parvum*. The fungal culture was fermented on a batch scale and an acetone extract was obtained. Several separations using column chromatography (silica gel, Sephadex LH-20) and preparative TLC were performed to afford a new mycophenolic derivative, euparvic acid, and eight known mycophenolic acid derivatives. The structure of **1** was determined by interpretation of MS and homo- and hetero nuclear 2D NMR spectroscopic data, and confirmed by X-ray crystallography. The absolute configuration of **9** was determined via MPA ester derivatization. The compounds were tested in antibacterial and antifungal assays, and showed moderate activity.



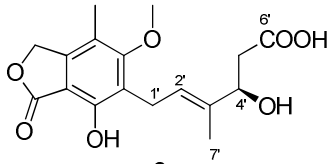
**1** R = H, Euparvic acid
**3** R = $CH_3$



**2**



**4** $R_1$, $R_3$ = H; $R_2$ = $CH_3$
**5** $R_1$, $R_2$, $R_3$ = H
**6** $R_1$ = Ac; $R_2$ = $CH_3$: $R_3$ = H
**7** $R_1$, $R_2$, $R_3$ = $CH_3$
**8** $R_1$ = Ac; $R_2$ , $R_3$ = $CH_3$



**9**

**References:**


- Six, J.; Frey, S. D.; Thiet, R. K.; Batten, K. M. *Soil Sci. Soc. Am. J*. **2006**, *70*, 555-569.
- Peña-Mendez, E.; Havel, J.; Patočka, J. *J. Appl. Biomed*. **2005**, *3*, 13-24.

## NOTES

# The Canary Islands CDC: An epidemiological study on cardiovascular diseases, diabetes and cancer

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## NOTES

## Can ageing be programmed in utero? Can you kill cancer cells by making them replicate faster?... and other DNA damage stories

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DNA damage is the source of procancerous mutations. In addition, recent evidence has also suggested that the reverse connection might also exist; namely that oncogenes can promote the generation of DNA damage. However, the nature of the damage that arises either by oncogenes or endogenously is still poorly understood. Our laboratory has centered its research in trying to understand how cells respond to "replicative stress", a type of DNA damage which arises unavoidably every time that a cell replicates its DNA, and which is mainly prevented by ATR and Chk1 kinases. Unfortunately, the essential nature of these kinases has limited their study, particularly at the organism level. In order to overcome these limitations, a significant part of our work in these years has focused in the development of cellular and animal models for the study of ATR/Chk1 function. The talk will give a general overview of some of our work in these last years, which has led to several discoveries such as the role of DNA damage during embryonic development on the later rates of ageing, or the counterintuitive concept that making cells replicate faster may be the way to specifically kill some types of cancer cells.

## NOTES

# Controverses in Oncology: Topoisomerase overexpression and Adriamicyn in breast cancer treatment

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## NOTES

# POSTER COMMUNICATIONS

# Docking study and preparation of flavonoid derivatives as estrogen receptor modulators

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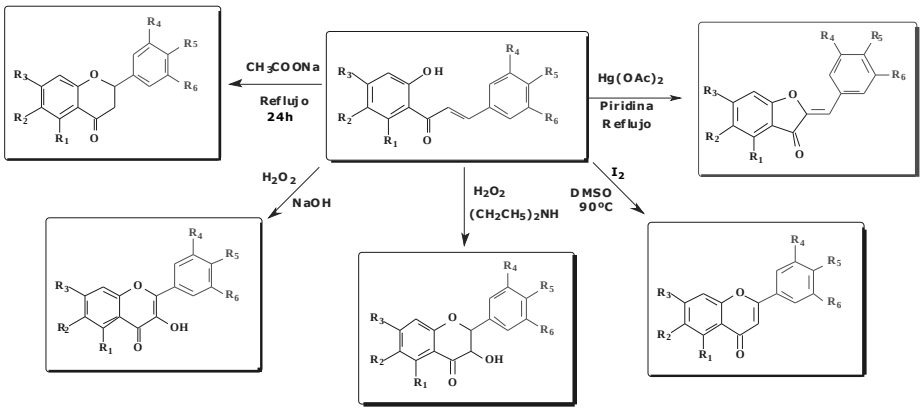
*Cyperus teneriffae* (Cyperaceae) is an endemic species to the Canary Islands which shows great capacity biosynthesizing secondary metabolites of biological interest such as aurones[1,2], flavanones[3] and flavonols[4] that are structurally similar to known estrogenic compounds.

Preliminary docking studies have shown that this type of molecules have important interactions into the binding pocket of the estrogen receptor  (ER).

In this communication we will report molecular docking of 4,6,3',4'-tetrametoxiaurone, 3'-hydroxy-4,6,4'-trimetoxiaurone, 3',5,5',7-tetrahydroxyflavanone, ombuine and tamaraxetin isolated from the root of *Cyperus teneriffae.* We will also report the synthesis and molecular docking[5] of a series of flavonoid analogues obtained by means of the classical Claisen-Schmidt reaction and oxidative cyclization of the corresponding one 2'-hydroxychalcones[6,7,8].




**References:**

1. Seabra, R.; Silva, A.; Andrade, P.; Moreira, M. *Phytochemistry*. **1998**, *48*: 1429-1432.
2. Seabra, R.; Andrade, P.; Ferreres, F.; Moreira, M. *Phytochemistry*. **1997**, *45*: 839-840.
3. El-Habashy, I.; Mansour, R.M.A.; Zahran,M.A.; El-Hadidi, M.N.; Saleh, N.A M. *Biochem. Syst. Ecol.* **1989**, *17*, 191-195.
4. Singh, N.B.; Singh, P.N. J. *Indian Chem. Soc*. **1986**, *63*: 450.
5. Norman, B. H.; Dodge, J. A.; Richardson, T I.; Borromeo, P. S.; Lugar, C W.; Jones,S A.; Chen, Keyue.; Wang, Y.; Durst, G L.; Barr, R. J.; Montrose-Rafizadeh, C.; Osborne, H. E.; Amos, R. M.; Guo, S.; Boodhoo, Amechand.; Krishnan, Venkatesh. *J.Med. Chem*. **2006**, *49*: 6155-6157.
6. (a) Somepalli Venkateswarlu,; Gopala K. Panchagnula,; Aditya L.; Gottumukkala, Gottumukkala V. Subbaraju. *Tetrahedron* **2007**, *63*: 6909-6914. (b) Dong, Xiaowu.; Liu, Tao.; Yan, Jingying.; Wu, Peng.; Chen, Ping.; Hu, Yongzhou. *Bioorg. Med.Chem*. **2009**, *17*: 716-726. (c) Maiti, Arup.; Cuendet, Muriel.; Croy, Vicki L.; Endringer, Denise C.; Pezzuto, John M.; Cushman, Mark. *J.Med. Chem*. **2007**, *50*: 2799-2806.

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## NOTES

# Cytotoxic Activity of new derivatives withanolides from Withaferine A isolated of *Withania aristata*

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Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. Thousands of herbal and traditional compounds are being screened worldwide to validate their use as anti-cancerous drugs[1].

The genus *Withania* (Solanaceae), are distributed in the East of the Mediterranean area, Macaronesian region and extend to south Asia. The genus 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 anticancer, antiinflammatory and immunomodulatory activities[2].
In the search for new cytotoxic natural compounds, the phytochemical analysis of the dichloromethane extract from the leaves of *Withania aristata* led to the isolation several withanolides with significant activity against human cancer. This work described the activity cytotoxic of eight new withanolides obtained by semisynthesis from known Withaferine A an abundant natural compound present in *Whitania* genus. In addition, the structures of these compounds were established from their physical and spectroscopic data.

The cytotoxic evaluation against three cancer cell line: HeLa (human carcinoma of the cervix), A-549 (human carcinoma of lung) and MCF-7 (human cancer breast) by colorimetric method were determined. Four of them shown significant activity ($IC_{50}$ <5 M) against HeLa and MCF-7 cells. A preliminary structure-activity relationship is discussed.

**References:**

1. Malik, F.; Singh, J.; Khajuria, A.; Suri, K. A.; Satti, N. K.; Singh, S.; Kaul, M. K.; Kumar, A.; Bhatia, A.; Qazi, G. N. *Life Sci*. 2007, *80*, 1525-1538.
2. Veleiro, A. S.; Oberti, J. C.; Burton, G. (2005) Studies in Natural Products Chemistry (part L), Bioactive Natural Products. Atta-Ur-Raman Ed., Elsevier Science Publishers, Amsterdam. 32: 1019.

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**NOTES**

## The role of cytoskeleton and membrane dynamics in HIV entry and infection.

**Barroso-González, J**.[1], García Expósito, L.[1], Barrero-Villar, M.[2], Cabrero, J.R.[2], Gordón-Alonso, M.[2], Álvarez-Losada, S.[3], Machado, J.D.[1], Muñoz-Fernández, M.A.[3], Sánchez-Madrid, F.[2], and Valenzuela-Fernández, A.[1]

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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. In this study, we show that HIV-1 virus promotes pore fusion formation and viral infection by inducing phosphatidylinositol 4,5-bisphosphate ($PIP_2$) production, during the first virus-target cell interactions. This process appeared to be mediated by the PI4P5-K Iα kinase. Hence, over-expression of wild-type PI4P5-K Iα increased HIV-1 Env-mediated $PIP_2$ production and enhanced viral fusion and replication, in permissive lymphocytes, whereas $PIP_2$ production and HIV-1 infection were both severely reduced in cells over-expressing the kinase-dead mutant D227A (D/A)-PI4P5-K Iα, or after knock-down of endogenous PI4P5-K Iα. Moreover, it was observed that X4-tropic HIV-1 viral fusion and infection required the activation of moesin, an actin adaptor protein of the ERM family that are activated in a $PIP_2$-dependent manner. HIV-1-gp120-induced CD4/CXCR4 association and clustering, occurred during early viral entry, and requires moesin-mediated plasma membrane-actin anchoring. Suppression of moesin, with dominant negative N-moesin or specific moesin silencing, impedes HIV-1-envelope-mediated F-actin reorganization, CD4/CXCR4 clustering and interaction, and inhibits HIV-1 entry and infection in lymphocytes. Remarkably, functional alteration of moesin, by specific silencing or a dominant-negative construct alters the trafficking of nascent endocytic clatrhin-coated vesicles, which accumulates near the plasma membrane carrying the TfR receptor. Therefore, we propose that HIV-1 virus promotes viral entry and infection by altering plasma membrane fluidity and dynamics, in a $PIP_2$-dependent manner, and reorganizing actin cytoskeleton to promote the recruitment, and direct interaction of viral receptors to favor early virus-cell interaction and fusion.

**References:**

1.- Barrero-Villar, M., Barroso-Gonzalez, J., Cabrero, J. R., Gordon-Alonso, M., Alvarez-Losada, S., Munoz-Fernandez, M. A., Sanchez-Madrid, F., and Valenzuela-Fernandez, A. (2008) *J Immunol* 181, 6882-6888

2.- Barroso-Gonzalez, J., Machado, J. D., Garcia-Exposito, L., and Valenzuela-Fernandez, A. (2009) *J Biol Chem* 284, 2419-2434

3.- Barrero-Villar, M., Barroso-Gonzalez, J., Cabrero, J. R., Gordon-Alonso, M., Alvarez-Losada, S., Munoz-Fernandez, M. A., Sanchez-Madrid, F., and Valenzuela-Fernandez, A. (2009) *J. Cell Sci. 122, 103-113.*

**Acknowledgements:**

Agustin Valenzuela-Fernandez. was supported by grants FIPSE 24508/05, FMM (Fundación Mutua Madrileña, Spain), FIS-PI050995 from the "Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo", Spain, and IDT-TF-06/066 and IDT-TF-06/063 from the "Consejería de Industria, Comercio y Nuevas Tecnologías del Gobierno Autónomo de Canarias", Spain, and "Fondo Social Europeo (FSE; RYC2002-3018)". Jonathan Barroso-González is supported by the FIS-PI050995 associated fellowship.

**NOTES**

# Cloning of Δ6-Desaturase gene in order to increase SDA content in *Echium acanthocarpum* hairy root cultures

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Stearidonic acid (SDA, 18:4 n-3) and gammalinolenic acid (GLA, 18:3 n-6) are polyunsaturated fatty acids (PUFAs), precursors of highly unsaturated fatty acids (HUFAs), Furthermore, HUFAs function as major nutrients, as part of cytoplasmatic membrane building elements and also play important roles in human health and nutrition , such as inflammatory response, brain development. In addition, these compounds aid to suppress the growth of some tumors and proliferation of breast cancer and melanoma . On the other hand, the current main dietary source of n-3 HUFAs is fish, but in view of the decline in fish stocks, searches for new suitable sources are being pursued employing higher plants . This would require in most cases the introduction of genes controlling each of the biosynthetic steps in the pathway. We focused our attention in the early steps of the pathway, which involves the introduction of a double bond at the Δ6 position controlled by a Δ6-desaturase enzyme. The Echium genus is of great interest because it constitutes one of the largest plant sources of SDA and GLA. We are interested in Δ6-desaturase cloning and overexpresion in E. acanthocarpum hairy roots in order to improve the fatty acid profile, specially the SDA content, which has been shown to share many of the biological effects of n-3 HUFAs . A Δ6-desaturase cDNA, whose enzyme shows specificity to the n-3 substrate, was isolated from Primula vialii and then cloned into the plant expression plasmid pGreen0029 and used for the transformation of Agrobaterium rhizogenes, subsequently employed for plant infection and hairy root induction. Four transgenic hairy roots lines were established and selected with kanamycin. Furthermore, the fusioned gene construct GFP-Δ6-desturase was also cloned in a similar manner and the establishment of these transgenic hairy roots is being conducted. We report on the development of these strategies and give the most relevant results achieved so far.

**References:**

[1]Siddiqui, R.A.; Harvey, K.; Stillwell, W. *Chemistry and Physics of Lipids*, **2008**, 47-56.
[2]Horrobin, D.F. *Lipid Res,* **1992**, 31: 163-194.
[3]Van Gool, C.J. *et al. Am. J. Clin. Nutr*, **2003**, 77: 943-951.
[4]Napier, J.A.; Beauodin, F.; Michaelson, L.V.; Sayanova, O. *Biochimie*, **2004**, 86: 785-793.
[5]Siddiqui, R.A.; Harvey, K.; Stillwell, W. *Chemistry and Physics of Lipids,* **2008**, 47-56.
[6]Donaldson, M.S. *Nutr. Journal*, **2004**, 3: 19-40.
[7]Chiou, S.Y. *et al. J. Biotechnol.*, **2001**, 85 247-257.
[8]Guil-Guerrero, J.L. *et al*, *Phytochemistry*, **2000[a]**, 53: 451-456.
[9]Guil-Guerrero, J.L. *et al*, *Phytochemistry*, **2000[b]** 56: 525-529. .
[10]Whelan, J. *J. Nutr.***2009**, 139: 5–10.

**Acknowledgments:** To CTM-2006-14279-c02-01 Project of Ministry of education and science, PI042005/067 Project of Canary Government and Instituto Canario de Investigación del Cáncer (ICIC) for financial support. ECS thanks FPU program (Ministry of education and science, SPAIN) for financial support.

## NOTES

# MELANOMA.
# TUMORS REGISTER HOSP. UNIVERSITARIO NTRA. SRA. DE CANDELARIA.

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Hospital tumours register (HTR) have the capacity of patients monitoring and could bring us information about the results of the oncologist attention.
For epidemiology they are less useful that population registers but clinically they are more precise and gives interesting evolutive information.
Besides they help us to the improvement and control of the assistance quality.

The Hosp. Univ. Ntra. Sra. De Candelaria Tumours Register has been working since 1980. Firstly they only includes the medical oncology neoplasm. Since 1993 we collect all the infiltrative and "in situ" ones (including the non-melanoma skin cancer) that have been diagnosed o treated at HUNSC.
The reference area of the hospital includes Santa Cruz, the south half municipalities of the island, La Gomera and El Hierro.; with a population about 566.430.
The neoplasm localization and morphology are codified by La Clasificación Internacional de Enfermedades para Oncología (CIE-O 2ª edición) and La Clasificación Internacional de Enfermedades (ICD 10ª edición)
Everyday we incorporate new patients who came to our service and also we have access to the Hospital Pathological Anatomy Service, making possible the updating of the old cases and registering the new ones.

We have worked updating those patients diagnosed of melanoma, included in the Tumours Register of Hosp. Univ. Ntra. Sra. De Candelaria, who have finished or stopped for years their reviews at medical oncology,
We also bring up to date the Register, using the centre information: radiological or laboratory test, hanging reviews, visit to urgencies, and also primary attention data base and melanoma Committee information. We have incorporated 443 cases of melanoma, diagnosed between January 1997 and November 2009.

**NOTES**

# The lupane-type triterpene 30-oxo-calenduladiol is a CCR5 antagonist with anti-HIV-1 and anti-chemotactic activities

**García Expósito, L.**[1,3], Barroso-González, J.[1,2,3], El Jaber-Vazdekis, N.[2,3], Machado, J.D.[1]; Zárate, R.[2,3], Ravelo, A.G.[2,3], Estévez-Braun, A.[2,3], and Valenzuela-Fernández, A [1,3].

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The existence of drug-resistant HIV viruses in patients receiving antiretroviral treatment urgently requires the characterization and development of new antiretroviral drugs designed to inhibit resistant viruses and to complement the existing antiretroviral strategies against AIDS. We assayed several natural or semi-synthetic lupane-type pentacyclic triterpenes in their ability to inhibit HIV-1 infection in permissive cells. We observed that the 30-oxo-calenduladiol triterpene, compound [1], specifically impaired R5-tropic HIV-1 envelope-mediated viral infection and cell fusion in permissive cells, without affecting X4-tropic virus. This lupane derivative competed for the binding of a specific anti-CCR5 mAb or the natural CCL5 chemokine to the CCR5 viral coreceptor with high affinity. 30-oxo-calenduladiol seems not to interact with the CD4 antigen, the main HIV receptor, or the CXCR4 viral coreceptor. Our results suggest that compound [1] is a specific CCR5 antagonist, since it binds to CCR5 receptor without triggering cell signaling or receptor internalization, and inhibits RANTES-mediated CCR5 internalization, intracellular calcium mobilization and cell chemotaxis. Furthermore, compound [1] appeared not to interact with the -chemokine receptors CCR1, CCR2b, CCR3 or CCR4. Thereby, the 30-oxo-calenduladiol-associated anti-HIV-1 activity against R5-tropic virus appears to rely on the selective occupancy of the CCR5 receptor to inhibit CCR5-mediated HIV-1 infection. Therefore, it is plausible that the chemical structure of 30-oxo-calenduladiol or other related dihydroxylated lupane-type triterpenes could represent a good model to develop more potent anti-HIV-1 molecules to inhibit viral infection by interfering with early fusion and entry steps in the HIV life cycle.

**References:**

1. - Inciardi, J. A., and Williams, M. L. (2005) *AIDS Care* 17 Suppl 1, S1-8
2. - Simon, V., Ho, D. D., and Abdool Karim, Q. (2006) *Lancet* 368(9534), 489-504
3. - UNAIDS. 2008. Report on the Global AIDS Epidemis
4. - Pohlmann, S., and Reeves, J. D. (2006) *Curr Pharm Des* 12(16), 1963-1973
5. -Aiken, C., and Chen, C. H. (2005) *Trends Mol Med* 11(1), 31-36
6. -Alakurtti, S., Makela, T., Koskimies, S., and Yli-Kauhaluoma, J. (2006) *Eur J Pharm Sci* 29(1), 1-13
7. - Barroso-González J, El Jaber-Vazdekis N, García-Expósito L, Machado JD, Zárate R, Ravelo AG, Estévez-Braun A and Valenzuela-Fernández A. (2009) *J Biol Chem*. 284(24):16609-20.

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## NOTES

# Hexaazatrinaphthylenes: synthesis, self-assembly properties and biological activities

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We have synthesized a novel family of water soluble π-conjugated hexaazatrinaphthylenes (HATNAs) dendritic architectures **G1**, **G2, G´3**, derivates **3a-e** and other analogues compounds using commercial available building blocks (**1** and **2**) by a short and efficient microwave assisted reaction[1] as depicted in the Figure 1. The **G1** properties and other compounds as nanomaterials have been studied by TEM, SEM, DSC, STM, XRPD and cyclic voltammetry. We will show physical characterization, as well as the study of some properties of these compounds, such as organogelating,[2] metal coordination[3] and potential biological activity.[4]

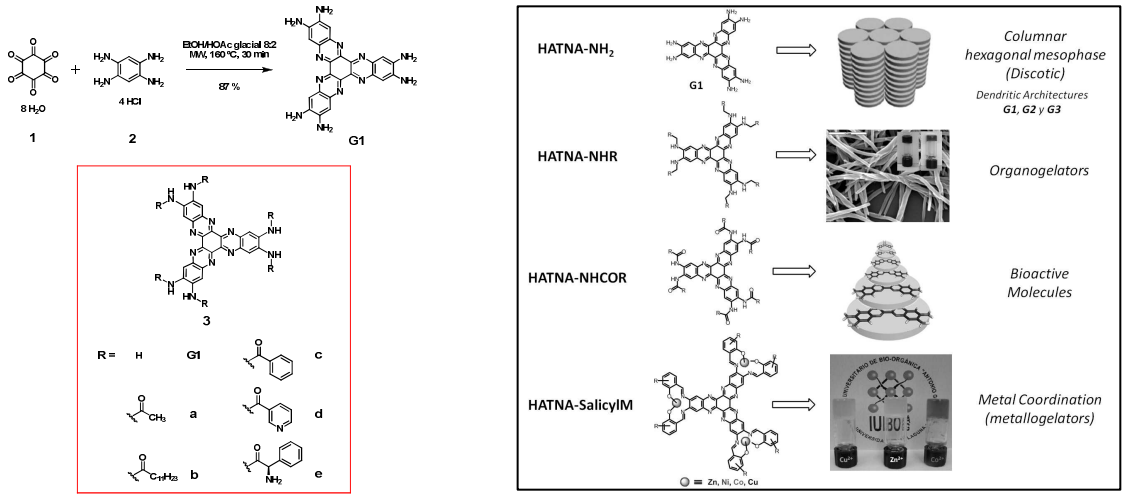


**Figure 1.** Synthesis of compound **G1** and amide-derivates (*left*). Representation of families of analogues-compounds with HATNA core (*right*).

Furthermore, preliminary studies in several compounds showed potential leishmanicidal activity and DNA intercalation.[5]

**References:**


- Velázquez D. G., Orive A. G., Arrieta, A. A., Sacau, E. P., Creus A. H., Ravelo A. G., *Chem. Comm.* **2009**, *(submitted).*
- Velázquez D. G., Orive A. G., Creus A. H., Ravelo A. G., *(sent)*
- Velázquez D. G., Orive A. G., Creus A. H., Ravelo A. G., (*manuscripts in preparation)*
- Preliminary results show endonuclease inhibitory activity.
- Results not published.


**Acknowledgements**: Financial support by MEC (SAF2006-06720 and CTQ2008-06017/BQU). DGV acknowledges Gobierno de Canarias for pre-doctoral fellowship.

## NOTES

## POLYMORPHIC MICROSATELLITES IN E2F-4, EGFR, AND NOTCH4 GENES AND BREAST CANCER

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Microsatellites or simple tandem repeats are abundant across genomes and some of them show high levels of polymorphism. Variations of these sequences, may affect gene transcription, mRNA stability, RNA splicing or protein structure and function. Some genes with an important role in breast cancer, like E2F-4 (transcription factor involved in gene transactivation during the G1-S transition of the cell cycle
, EGFR (epidermal growth factor receptor) and NOTCH4 (a receptor implicated in NOTCH pathway) carry polymorphic microsatellites. Some of them have been associated previously with breast cancer .
A case–control study, nested in the 'CDC of the Canary Islands' cohort, has been designed to evaluate the effect of these short tandem repeats polymorphisms on BC. Two hundred breast cancer patients at the department of oncology of Hospital Insular de Gran Canaria volunteered for this study. Three hundred women residents in Gran Canaria were selected as controls from CDC cohort .

**NOTES**

# Preparation and biological evaluation of -acyloxyamides as STAT-5 inhibitors. Structure-Activity relationships

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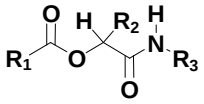
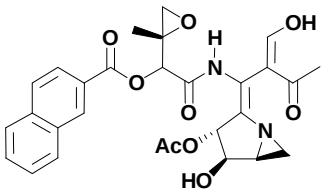
The -acyloxyamide moiety is present in numerous bioactive natural products.[1] An illustrative example is the antitumoral azinomicine.[2] The synthesis of compounds containing the -acyloxyamide unit results attractive from a pharmacological viewpoint.



**α–aciloxiamide**



**azinomicine**

On the other hand, one of the molecular alterations associated with tumour development is the constitutive or aberrant activation of the transcription factors STAT. This action mechanism, *i.e.*the inappropriate functioning of the Jak, Stat proteins or the silencing of their natural inhibitors (ej. SOCS), is frequently implicated in the tumour transformation of the cells induced by oncogenes, and in the malignant progression of multiplehuman cancers and other diseases. Recently, this pathway has been validated as an important target of therapeutic use in cancer.[3]

In this communication we will describe the preparation and STAT-5 inhibition of a set of α-acyloxyamides which show resemblance to the potent JAK2 inhibitors AG490 and the derivatives WP1066 and LS-104[3a]. We will also discuss some Structure-Activity relationships.

**References:** (a) Zhu, J.; Bienaymé, H. eds Multicomponent reactions, Chapter 5, 121-168, Wiley-VCH, Weinheim, **2005**; (b) Gore,V.; Hulme, C. *Current Medicinal Chemistry* **2003**, *10,* 51; (c) Semple, J.E.; Owens, T.D.; Nguyen, K.; Levy, O.E. *Org. Lett.*. **2000,** 2, 2769; (d) Banfi,L.; Guante, G.; Riva, R. *Chem. Commun.* **2000,** 985.

[2] Coleman, R. S.; Kong J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3538.

[3] (a) Wilks, A. F. *Seminars Cell. Dev. Biol.* **2008**, *19,* 319; (b) Wernig, G.; Kharas, M. G.; Okabe, R. et al. *Cancer Cell.* **2008**, *13*, 11; (c) Vertovse, S.; Manshouri, T.; Quintas-Cardama, A. et al. *Clin. Cancer Res.* **2008**, *14*, 788.

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**NOTES**

# Anti-HIV activity of lupane and sesquiterpene derivatives.

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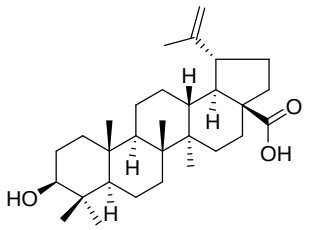
As the world enters the third decade of the AIDS (acquired immunodeficiency syndrome) epidemic, this pandemic has rapidly grown into the fourth leading cause of mortality globally.[1] The WHO estimated on 33 millions the number of people living with HIV (Human Immunodeficiency Virus) and, on 2 millions the AIDS deaths annually.[2] These dates and the existence of drugs-resistant HIV viruses in patients receiving treatment urgently requires the characterization and development of new antiretroviral drugs designed to inhibit resistant viruses and to complement the existing strategies against AIDS**.** Several pentacyclic triterpenes with lupane skeleton such as betulinic acid (1) or sesquiterpenes β-dihidoragarofuanes, like compound 2 represent a new class of potent and promising anti-HIV agents.[3] In this communication we will report the results of a set of the lupane and sesquiterpene derivatives have shown anti-HIV activity.

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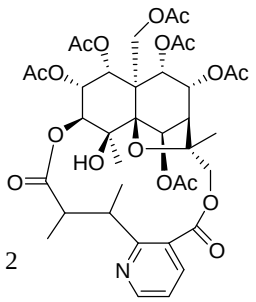


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**References:**

1. UNAIDS. AIDS epidemic update, December 2007.

2. Data published by WHO in December 2007:
http://www.who.int/hiv/data/2008_global_summary_AIDS_ep.png

[3]**a**. Qian, K.; Yu, D.; Chen, C.; Huang, L.; Morris-Natschke, S. L.; Nitz, T. J.; Salzwedel, K.; Reddick, M.; Allaway, G. P.; Lee, K. *J. Med. Chem..* 52, 3248-3258, (2009). **b**. Hoiuch,, M. *et al. J. Nat. Prod.* 69, 1271-1274 (2006). **c**. Barroso, J.; El Javer, N.; García-Expósito, L.; Zárate, R.; Ravelo, A. G.; Estévez-Braun, A.; Valenzuela, A. *J. Biol .Chem.* 284, 16609-16620 (2009).

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**NOTES**

# Biotechnology for the production of the anticancer compounds 22-hydroxytingenone and tingenone

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In the present study we report on an efficient CEAMED SA production system of nor-triterpene methylene quinones with high added value, using biotechnological tools of *in vitro* cultures from the Celastraceae species *Maytenus canariensis* (Loes.) Kunk & Sund. The Canary Islands spindle tree is a source of sesquiterpene and triterpene compounds, which have been reported to display important biological activities with high pharmacological and medical interest[1]. Regarding the nor-triterpene methylene quinones, 22β-hydroxytingenone and tingenone, antimitotic activity by inhibiting the polymerization of tubulin[2] and spectroscopically interaction with DNA[3] have been demonstrated. They also display antiparasitic and insecticidal activity against *Giardia intestinalis*[4], *Trypanosoma cruzy*[5], *Crithidia fasciculate*[6], *Cydia pomonella*[7], as well as antibiotic [8-9], antiinflammatory and antioxidant[10] activities. Furthermore, the 22β-hydroxytingenone has also been described as chemical precursor of patented hemi-synthetic triterpene derivatives of proven inhibitory activity of the cholino-kinase enzyme, an enzyme participating in a broad range of cancer processes[11]. These molecules display in their structures 5 stereogenic centres, making their chemical synthesis impractical; thus, in order to achieve a continuous production of these bioactive compounds, we established an *in vitro* culture system of *M. canariensis* cell aggregates, under different culture conditions and growth regulator and nutrient regimes, capable of producing very high yields in a short time span. The optimised system accumulated 370fold larger amounts of 22β-hydroxytingenone and 227fold larger amounts of tingenone than the native plant. The most relevant results on the production of these natural products are presented and discussed.

**References**

[1] González, A. G., Alvarenga, N. L., Ravelo, A. G., Jiménez, I. A., Bazzochi, I. L., Canela, N. J. & Moujir, L. *Phytochemistry* **1996**, 43:129-132.
[2] Morita, H., Hirasawa, Y., Muto, A., Yoshia, T., Sekita, S., Shirota, O. *Bioorganic & Medicinal Chem.* **2008,** Lett. 18: 1050-1052.
[3] Campanelli, A. R., D´Alagni, M., Marini-Bettolo, G. B. *FEBS* **1980**, Lett. 122: 256-260.
[4] Mena-Rejon, G. J., Pérez-Espadas, A. R., Moo-Puc, R. E., Cedillo-Rivera, R., Bazzocchi, I. L., Jiménez-Diaz, I. A., Quijano L. *J. Nat. Prod*. **2007**, 70: 863-865.
[5] Liao, L. M., Silva, G. A., Monteiro, M. R., Albuquerque, S. *J. Bioscience*. **2008**, 63:207-210.
[6] Goijman, S. G., Turrens, J. F., Marini-Bettolo, G. B., Stoppani, A. O. M. *Experientia* **1985**, 41: 646-648.
[7] Avilla, J., Teixido, A., Velázquez, C., Alvarenga, N., Ferro, E., Canela, R. *J. Agric. Food Chem*. **2000**, 48: 88-92.
[8] González, A. G., Jímenez, I. A., Ravelo, A. G., Coll, J., González, J. A., Lloria, J. Biochem. *System Ecol*.**1997**, 25: 513-519.
[9] Sotanaphun, U., Lipipun, V., Suttisri, R., Bavovada, R. *Planta Med.* **1999**, 65: 450-452.
[10] Jeller, A. H., Siqueira-Silva, D. H., Morais-Liao, L., da Silva-Bolzani, V., Furlan, M. *Phytochem.* **2004**, 65:1977- 1982
[11] World Intellectual Property Organization, WO 2007/077203 A2

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## NOTES

## Synthesis and Induction of Apoptosis Signaling Pathway of ent-Kaurene Derivatives

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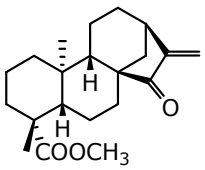
Thirty one *ent*-kaurene derivatives were prepared from kaurenoic acid, grandiflorenic acid, 15α-acetoxy kaurenoic acid and 16α-hydroxy kaurenoic acid. They were tested for their ability to inhibit cell growth in the mouse leukaemic macrophagic RAW 264.7 cell line. The most effective compounds were **12**, **20**, **21** and **23**. Similar effects were obtained in other human cancer cell lines such as Hela, HepG2 and HT-29, although RAW 264.7 cells were more sensitive.

The apoptotic potential of the most active compounds was investigated and they were able to induce apoptosis being compound **12** the best inducer. The caspase-3, -8 and -9 activities were measured [1, 2]. The results obtained showed that compounds **12**, **21** and **23** induce apoptosis via the activation of caspase-8, whereas compound **20** induces apoptosis via caspase-9.
Inmunoblot analysis of the expression of p53, Bax, Bcl-2, Bcl-x1 and IAPs in RAW 264.7 cells was also carried out. When cells were exposed to 5 μM of the different compounds, expression levels of p53 and Bax increased whereas levels of antiapoptotic proteins such as Bcl-2, Bcl-x1 and IAPs decreased.
In conclusion, kaurene derivatives (**12**, **20**, **21** and **23**) induce apoptosis via both the mitochondrial and membrane death receptor pathways, involving the Bcl-2 family proteins [3]. Taken together these results provide a role of kaurene derivatives as apoptotic inducers in tumor cells and suggest their potential application as antitumor agents.



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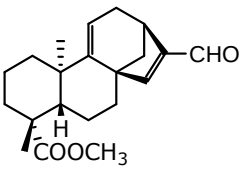

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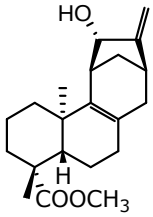

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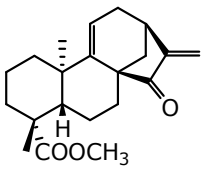

**23**

**References:**
1. Enari, M., Nagata, S. (1996) Nature 380:723-26
2. Slee, E.A., et al. (1999) Cell Death Differ 6:1067-74
3. Li, H. et al. (1998) Cell 94: 491-501

**Acknowledgements:** To "Ministerio de Ciencia e Innovación" (Project SAF 2006-0.720) and FICIC (Pr. ResCancerBiotech FICIC-03/08) for financial support. IHF thanks the "Ministerio de Ciencia e Innovación" for a predoctoral fellowship FPI.

## NOTES

# Tertiary skipped diynes: a pluripotent building block for the modular and diversity-oriented synthesis of nitrogen heterocycles1

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The development of Diversity-Oriented Synthetic methodologies (DOS)2 to construct libraries of small molecules to explore the chemical space is a current topic in modern organic synthesis. This approach requires the use of building blocks bearing a functional group or an array of interconnected functional groups expressing a polyvalent reactivity profile (multiple reactivity). Tertiary skipped diynes3 have a pluripotent reactivity profile, that can be expressed using different reagents in multiple reactions for the generation of structural diversity.

A recent report from this lab has shown that these diynes are efficient precursors of chain functionalized tetrasubstituted pyrroles4 via an efficient microwave assisted domino reaction with primary amines. In this communication we report the extension of this methodology in the use of this C7 pluripotent array of organic functionalities for the regioselective domino synthesis of chain functionalized fully substituted pyrazoles using N-substituted hydrazine derivatives (R2NHNH2). The mechanism and scope of the process will be discussed.

**References:**

Tejedor, D.; López-Tosco, S.; García-Tellado, F. Manuscript under preparation.
Tejedor, D.; González-Cruz, D.; Sántos-Expósito, A.; Marrero-Tellado, J. J.; de Armas, P.; García-Tellado, F. *Chem. Eur. J.* **2005**, *11*, 3502-3510.
(a) Tejedor, D.; López-Tosco, S.; González-Platas, J; García-Tellado, F. *J. Org. Chem.* **2007**, *72*, 5454-5456. (b) Tejedor, D.; López-Tosco, S.; Cruz-Acosta, F.; Méndez-Abt, G.; García-Tellado, F. *Angew. Chem. Int. Ed.* **2009**, *48*, 2090-2098. (c) Tejedor, D.; García-Tellado, F. *Chem. Soc. Rev.* **2007**, *36*, 484-491.
Tejedor, D.; López-Tosco, S.; González-Platas, J; García-Tellado, F. *Chem. Eur. J.* **2009**, *15*, 838-842.

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## NOTES

# FL-TX: A novel fluorescent derivative of tamoxifen

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It is widely recognized that tamoxifen, a Selective Estrogen Receptor Modulator (SERM) extensively used on estrogen receptor-positive breast cancer treatment, leads to undesirable side effects during chronic therapies. Numerous studies have shown that some of these effects may be exerted through acute non-genomic mechanisms. In this sense, we have shown that both, tamoxifen and novel tamoxifen salts derivatives synthesized in our laboratories, rapidly and reversibly inhibited (in a dose-dependent manner) the contractile activity of mouse duodenal and uterine smooth muscles likely through the interference with L-type calcium channels activity (Díaz, 2002; Marrero-Alonso et al., 2006).

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, raising the possibility of designing more specific and selective compounds with less undesirable side-effects. In this sense, the first fluorescent tamoxifen derivative has been synthesized by our group using low molecular weight fluorophore, but maintaining the triphenylethylene core of tamoxifen, which is essential to interact with estrogen receptors. Efficiency and viability of this fluorescent compound were assessed by confocal microscopy on estrogen receptor-positive cell lines MCF-7 and murine-derived neuronal SN56 cells. The fluorescent derivative exhibited antiestrogenic activity in MCF-7 transfected with 3XERE-luc reporter and T47D kbluc cell line to similar levels than tamoxifen. Interestingly, unlike tamoxifen, FL-TX was devoid of transcriptional ERα-dependent activity within the same dose range. In confocal microscopy experiments, we could demonstrate that FL-TX colocalize with ERα. Moreover, the percentage of colocalization of FL-TX-ERα complex was similar to the percentage of competition of FL-TX binding by unlabeled estradiol, indicating the specificity of the ERα-FL-TX binding. Finally, competition studies showed that the binding of fluorescent derivative at the plasma membrane could be readily antagonized by unlabelled tamoxifen, indicating the presence of plasma membrane binding sites specific for triphenylethylene compounds.

**References:**

Díaz M. (2002). Triphenylethylene antiestrogens-induced acute relaxation of mouse duodenal muscle. Possible involvement of calcium channels. *European Journal of Pharmacology*. 445(3):251-260.

Marrero-Alonso J., García Marrero B., Gómez T. and Díaz M. (2006) Functional inhibition of intestinal and uterine muscles by non-permeant triphenylethylene antiestrogen derivatives. *European Journal of Pharmacology*. 532(1-2):122-134.

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## NOTES

# Synthesis of 1,2-Dihydropyridines via a Domino Reaction: Propargyl- Claisen Rearrangement – Isomerization – Amine Condensation – Cyclization.1

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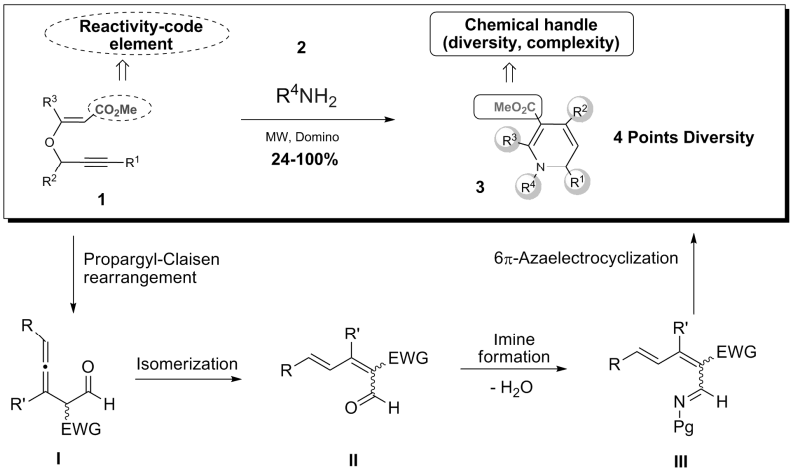
An efficient domino approach leading to 1,2-dihydropyridines from readily available propargyl vinyl ethers is described.[2]

Propargyl vinyl ethers (**1**), which are easily prepared from propargyl alcohols and the corresponding alkynoates in the presence of catalytic amounts of appropriate tertiary amines of phosphines,[2] undergo a thermal propargyl-Claisen rearrangement leading to conjugated dienals (**II**) after the isomerization of the allenic intermediates (**I**). Primary amines (**2**) are capable of converting the dienals into azatrienes (**III**) which are readily converted into the final 1,2-dihydropyridine products (**3**). This sequence of reactions is conveniently carried out under microwave irradiation or conventional heating in a single step and with excellent yields. Key to this transformation is the presence of an ester or sulfone at the vinyl functionality.




This methodology has been extended to the synthesis of substituted nicotinic acid derivatives.

**References:**


- D. Tejedor, G. Méndez-Abt, F. García-Tellado, *Chem. Eur. J.* **2009**, DOI: 10.1002/chem.200902140.
- a) D. Tejedor, A. Santos-Expósito, G. Méndez-Abt, C. Ruiz-Pérez, F. García-Tellado, *Synlett* **2009**, 1223-1226; b) J. Inanaga, Y. Baba, T. Hanamoto, *Chem. Lett*. **1993**, 241-244; c) E. Winterfield, *Chem Ber.* **1964**, 97, 1952-1958; d) R.E. Ireland, P. Wipf, J.-N. Xiang, *J. Org. Chem*. **1991**, *56*, 3572-3582.


**Acknowledgements:** This research was supported by the Spanish Ministerio de Ciencia e Innovación, the European Regional Development Fund (CTQ2005-09074-C02-02) and the Spanish MSC ISCIII (RETICS RD06/0020/1046 and RD06/0020/0041), CSIC (Proyecto Intramural Especial 200719), FUNCIS (REDESFAC PI01/06 and 35/06) and the Fundación Instituto Canario de Investigación del Cancer (FICI-G.I.N808/2007). G.M.-A. thanks Spanish MEC for a FPU grant.

## NOTES

# Secondary metabolites from *Convolvulus floridus*

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*Convolvulus floridus* is a Convolvulaceae, endemic to the Canary Islands[1]. It has been reported the isolation of alkaloids and coumarins, with antioxidant, anti-inflammatory and antitumoral activity from plants of this family[2,3,4].

In this communication, we will report the isolation and structural elucidation of several coumarins and some phenolic compounds from the aerial parts of *C. floridus.*

**References:**
1. Eich, E., Ed. (2008). Solanaceae and convolvulaceae - secondary metabolites. Springer. Heidelberg-Germany
2. Gupta, P. Et al. (2007). Chem.Pharm.Bull. 55: 771-775.
3. Liu, J. Et al. (2007). Phytochemistry. 68: 1775-1780.
4. Kummalue, T. Et al. (2007). Journal of Ethnopharmacology. 110 : 439-443.

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**NOTES**

# Synthetic studies to potential antitumoral substituted piperidines

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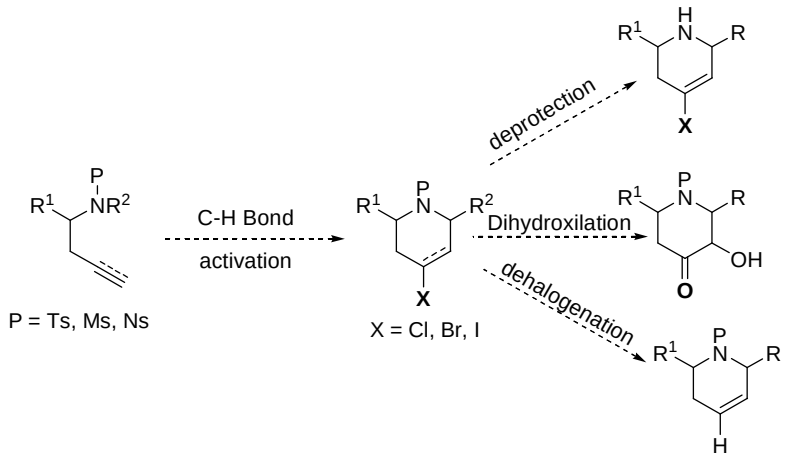
The piperidine ring is widely distributed throughout Nature, e.g. in alkaloids and are an important scaffolds for drug discovery, being the core of many pharmaceutically significant compounds.[1] The syntheses of these types of compounds have been extensively studied in the development of new drugs containing six-membered ring heterocycles. Among existing methodologies, the Prins cyclization has emerged as a powerful tool in the synthesis of this type of heterocycles.

Recently, we described the direct aza-Prins cyclization between □□□-unsaturated tosylamines and aldehydes using catalytic amounts of inexpensive, environmentally friendly and stable iron (III) species to obtain 6-membered azacycles in good to excellent yields (**Scheme 1**).

Herein, we describe a study about C-H bond activation in the six-membered ring cyclization and further derivatization.

(**Scheme 1**).




**References:**

(a) Fodor, G. B.; Colasanti, B. Alkaloids: Chemical and Biological Perspectives. Ed. S. W. Pelletier, Wiley, New York **1985**, vol. 23, p. 1-90.
(b) Baliah, V.; Jeyarama, R.; Chandrasekaran, L. *Chem. Rev*. **1983**, *83*, 379-423.

[2]Watson, P. S.; Jiang, B.; Scott, B. *Org. Lett*. **2000**, *2*, 3679-3681.

[3]Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Chem. Rev*. **2003**, *103*, 893-930.

[4](a) Caderas, C.; Lett, R.; Overman, L. E.; Rabinowitz, M. H.; Sharp, M. J.; Zablocki, J. *J. Am. Chem. Soc*. **1996**, *118*, 9073-9082. (b) Metais, E.; Overman, L. E.; Rodríguez, M. I.; Stearn, B. A. *J. Org. Chem*. **1997**, *62*, 9210-9216. (c) Dobbs, A. P.; Guesné, S. J. J.; Hursthouse, M. B.; Coles, S. J. *Synlett* **2003**, *11*, 1740-1742. (d) Dobbs, A. P.; Guesné, S. J. J.; Martinove, S.; Coles, S. J.; Hursthouse, M. B. *J. Org. Chem*. **2003**, *68*, 7880-7883. (e) Hanessian, S.; Tremblay, M.; Petersen, F.W. *J. Am. Chem. Soc*. **2004**, *126*, 6064-6071 and references cited therein. (f) Pastor, I. M.; Yus, M. *Curr. Org. Chem*. **2007**, *11*, 925□957.

[5](a)Carballo, R. M.; Rodríguez, M. L.; Ramírez, M. A.; Martín, V. S.; Padrón, J. I. *Org. Lett*. **2006**, *8,* 3837□3840. b) Miranda, P. O.; Carballo, R. M.; V. S. Martín, Padrón, J. I. *Org. Lett.* **2009**, *11*, 357-360.

## NOTES

## Cdc14-1 release causes an intense and long-lasting DNA damage response as cells enter a new cell cycle.

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Sister chromatid non-disjunction during anaphase is a putative source of mitotic catastrophe that would lead to genetically aberrant daughter cells and cancer development. In budding yeast, inactivation of the conserved phosphatase Cdc14 blocks cells in telophase with non-disjunction of the ribosomal DNA (rDNA). Cdc14 can be successfully reactivated after the block which allows all the cells to enter a new cell cycle. However, more than 50% of the cells do this despite they fail to resolve and segregate the rDNA. Here, we have studied the fate of the cells after re-activation of Cdc14 and correlated this fate to the missegregation of the rDNA. Thus, we show that cells which failed in the rDNA segregation throughout the release delay cytokinesis and activate Rad52-dependent DNA damage response that begins once daughter cells have already entered the S-phase. Strikingly, the DNA damage response is massive, cumulative and long-lasting; suggesting that cells may inefficiently repair the expected one-ended breaks.

**NOTES**

# Synthesis of new cytotoxic naphthalimide derivatives

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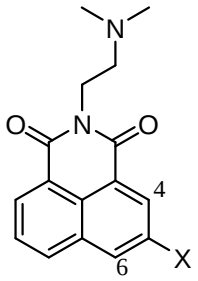
Naphthalimides are a class of compounds known to have high antitumoral activity against both murine and human tumor cells. These compounds bind to DNA inhibiting the action of topoisomerase II, being the most out-standing amonafide (1) and mitonafide (2)[1]. However, they present some undesired side effects.[2] Several series of naphthalimides have been synthesized, mainly carrying out modifications on the nitrogenated side chain and on the aromatic rings.

With these antecedent we decide to explore the preparation of new naphthalimides with an extension of the conjugation through a not fused aromatic ring, in the 4, 5 or 6 positions. In this communication we will report some preliminary biological results.




***mitonafide*** *(X:$NO_2$)*
***amonafide*** *(X:$NH_2$)*

**References**:
[1] M.F. Braña, M. Cacho, A. Gradillas, B.Pascual-Teresa , A. Ramos. *Curr. Pharm*. Des. **2001**, 7, 1745.
[2] A. Awada , R. Thödtmann, M.J. Piccart, J. Wanders , A.H.G.J. Schrijvers, I.M. Von Broen, AR. Hanauske. *Eur. J. Cancer.* **2003**, 39, 742.

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**NOTES**

# PITX2 methylation as a novel prognostic factor in endometrial cancer

**Ramírez-Moreno R**[1, 2], Rodríguez-González FG[1, 3], Santana-Farré R[1, 4], Bilbao C[1, 4], Torres S[1, 2], Martens JW[3], Napieralski R[5], León L[1, 6], Falcón O[1, 7], Schmitt M[5], Foekens JA[3], Fernández-Pérez L[1, 4], Díaz-Chico BN[1, 2], Díaz-Chico JC[1, 2].

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Paired-like homeodomain transcription factor 2 (PITX2) is a member of the PITX homeobox family involved in pituitary-specific gene regulation and left-right patterning during embryonic development. Methylation status of PITX2 gene promoter is a validated prognostic marker for outcome prediction in breast and prostate cancer patients. Our aim was to study the PITX2 methylation in endometrial cancer (EC) to elucidate the possible association with clinical-pathologic variables and patient's outcome.

PITX2 methylation was estimated by quantitative methylation real-time polymerase chain reaction (QM-PCR) (Epigenomic) in a series of 201 EC patients and 40 healthy endometrial tissue controls (CT).

PITX2 methylation level in EC was significantly higher than in CT (p=0.03). When PITX2 methylation in EC was categorized in quartiles, the higher quartile cut-off value (6.58) was higher than the maximum value observed in CT (5.96). PITX2 methylation status in EC (dichotomized by using the higher quartile cut-off value) was unrelated to the clinico-pathological variables. Univariate survival analysis showed that patients with high PITX2 methylation had a worse outcome (recurrence as end-point), either in the whole series (RR: 1.42, 95% CI: 1.01-2.00; p=0.047), in the endometrioid (EEC) group (RR: 1.61, 95% CI: 1.11-2.34; p=0.011), and in the EEC with a (near)diploid DNA content tumors (EEC+DIPLOID) group (RR: 1.86, 95% CI: 1.25-2.79; p=0.002). In multivariate analysis, including traditional prognostic variables, high PITX2 methylation was associated to a poorer recurrence-free survival in the EEC+DIPLOID) group of patients (RR: 1.59, 95% CI: 1.05-2.40; p=0.0028).

This is the first study of PITX2 methylation in EC. Our results suggest that PITX2 methylation is higher in EC than in CT, and that high PITX2 methylation is associated to a worse disease-free survival in patients with EEC+DIPLOID tumors.

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**NOTES**

# Mitotic arrest induced by propargylic enol ethers

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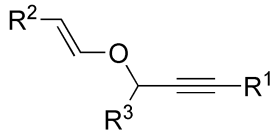
Cancer is widely accepted as a cell cycle disease in continue high unmet medical needs. The division cycle is an evolutionarily conserved progress used by all eukaryotic cells to control growth and division. Normal cellular proliferation is ordered and tightly controlled by a series of regulatory mechanisms that either permit or prevent cell-cycle progression through each phase and thus play an important role in maintaining the balance between "old" and "new" cells within an organism [1]. Progression through the cell cycle is controlled by several proteins that represent attractive drug targets for therapeutic intervention [2]. The objective of this study was to evaluate the antiproliferative activity of a set of 27 propargylic enol ethers derivatives in cancer cells.




The antiproliferative activity was performed against the solid tumour cell lines SW1573 (lung) and HBL-100 (breast) using the SRB assay [3]. Additionally, cell cycle phase distribution was investigated by flow cytometry and protein expression by western blotting. The results showed $GI_{50}$ values between 0.24–2.8 μM for the most active compound and let us to differentiate the derivatives as function of their activity profile. Cell cycle studies and protein expression showed the ability of the compound to induce mitotic arrest with the activation of BubR1 by phosphorylation

**References:**

[1] Brooks, G.; La Thangue, N.B. *Drug Discov.Today* **1999**, *4*,455.
[2] Collins, I.; Workman, P. *Nature Chem Biol.* **2006**, *2*,689.
[3] Miranda, P.O.; Padrón, J.M.; Padrón, J.I.; Villar, J.; Martin, V.S. *ChemMedChem* **2006**, *1*,323.

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**NOTES**

# High expression of hsa-miR-30a-3p, hsa-miR-30c and hsa-miR-182 predict favorable outcome on tamoxifen treatment in patients with recurrent breast cancer

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Altered miRNAs expression levels have been described in breast cancer (BC) and reported to be associated with metastasis, prognosis and treatment response, suggesting that miRNAs play an important role in BC. We have explored the association of selected miRNAs and tamoxifen clinical response.

In a series of 246 ER+ recurrent BC patients treated with tamoxifen, five selected miRNAs, hsa-miR-30a-3p, hsa-miR-30c hsa-miR-182, hsa-miR-187 and hsa-miR-422a, were quantified by real time PCR.

Univariate logistic regression analysis, using log-transformed continuous variables, showed that high levels of hsa-miR-30a-3p (odds ratio [OR]: 1.51, 95% confidence interval [95% CI]: 1.16-1.96; $P$ = 0.002), hsa-miR-30c (OR: 3.87, 95% CI: 2.16-6.93; $P$ < 0.001), and hsa-miR-182 (OR: 1.53, 95% CI: 1.09-2.16; $P$ = 0.013), were associated with clinical benefit of tamoxifen therapy. In multivariate analysis, including traditional predictive factors, of the miRNAs tested, only hsa-miR-30c was significantly associated with clinical benefit (OR: 3.14, 95% CI: 1.61-6.12; $P$ = 0.001). In order to assess the progression free-survival (PFS) time, miRNA expression levels were categorized in quartiles. In analogy to their relationship with clinical benefit, the same three miRNAs were also associated with longer PFS: hsa-miR-30a-3p (hazard ratio [HR]: 0.51, 95% CI: 0.34-0.76; $P$ = 0.001), hsa-miR-30c (HR: 0.47, 95% CI: 0.31-0.70; $P$ < 0.001), and hsa-miR-182 (HR: 0.57, 95% CI: 0.37-0.86; $P$ = 0.008). Global testing using available global gene expression data significantly associated the 3 predictive miRNAs with differential gene expression of HER-2, Rac-1 and Ceramide signaling pathways.

This study shows associations between hsa-miR-30c, hsa-miR-30a-3p and hsa-miR-182 expression levels and clinical benefit to treatment with first-line tamoxifen for recurrent BC and describes pathways putatively involved in these associations. Assessment of these miRNA levels and their pathways in primary tumors could help to improve treatment strategies for patients with recurrent ER+ breast cancers.

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**NOTES**

# Efforts Towards Total Synthesis of Teurilene by Biomimetic Strategy

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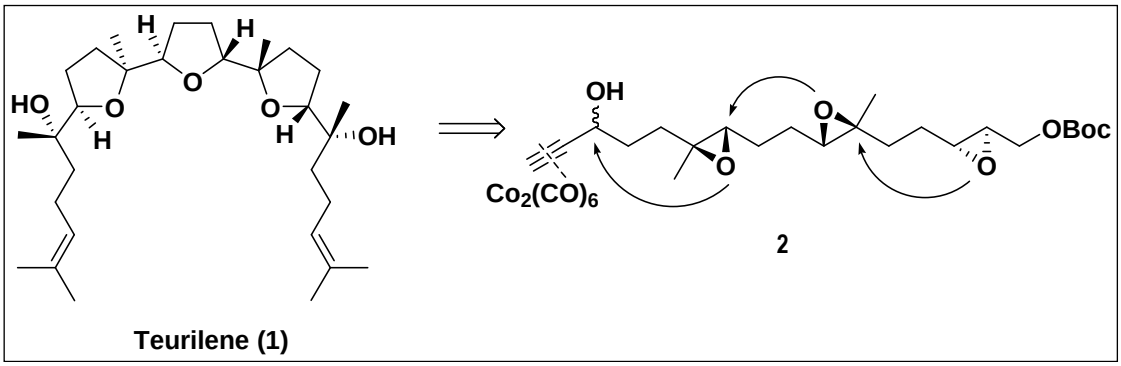
Teurilene (**1**) is a cytotoxic triterpene polyether against KB cells, which has been isolated from the red algae *Laurencia obtusa*. Teurilene is characterized by a link of three tetrahydrofurans in the center of the molecule and a beautiful arrangement of eight asymmetric carbons for $C_s$ symmetry.

In this contribution, we report on the approach to the synthesis Teurilene based on a biomimetic strategy. As depicted in **Scheme 1**, the fused tetrahydrofuran rings will be obtained by the formation of a Nicholas carbocation from a $Co_2(CO)_6$-alkyne complex **2** which would bring a cascade reaction where the epoxides act as intramolecular nucleophiles. All the stereocenters of the molecule will be defined previously with a Sharpless asymmetric epoxidation.




**Scheme 1**

**References:**
Suzuki, T.; Suzuki, M.; Furusaki, A.; Matsumoto, T.; Kato, A.; Imanaka, Y.; Kurosawa, E. *Tetrahedron Lett.* **1985**, *26,* 1329.
[2]Crisóstomo, F. R. P.; Martín, T.; Martín, V. S. *Org.Lett.* **2004**, 565.

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**NOTES**

# Betuletol 3-methyl ether, an inhibitor of tubulin polymerization with antimitotic activity in human leukaemia cells

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Flavonoids are polyphenolic compounds that are ubiquitously in plants and display a vast array of biological activities. Among them, Betuletol 3-methyl ether (BME) inhibits cell proliferation in human tumor cell lines and induces apoptotic cell death in human leukemia cells, including those that over-express the anti-apoptotic proteins Bcl-2 and Bcl-$x_L$. Competition assays indicate that BME binds to the colchicine-binding site of tubulin and inhibits the polymerization of microtubules. In addition, BME treatment resulted in a concentration-dependent accumulation in $G_2$/M phase of the cell cycle in HL-60 cells. Specifically, cells were blocked in the M phase and this event was associated with induction and phosphorylation of cyclin B1, accumulation of $p21^{Cip1}$, and also activation of Cdk1 via dephosphorylation of phosphoT14-Y15. Our findings suggest BME may be of benefit in potential treatment of cancer, either as a single agent or as a complementary therapy in combination with other anticancer agents.

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**NOTES**

## Production of cytotoxic factors by mariculture of *Verongia aerophoba*

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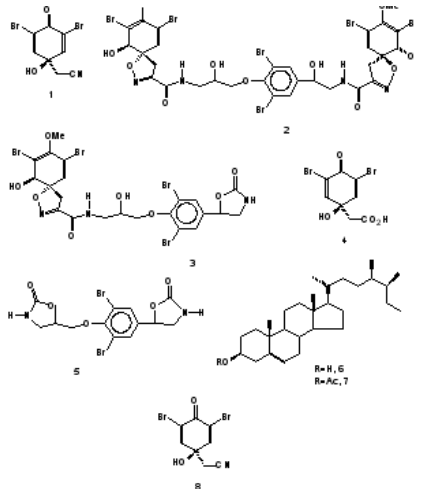
From the marine sponge *Verongia aerophoba* collected in the Canary Islands were isolated eigth compounds.[1] Six of them are bromotyrosine derivatives, a new compound we called verongionitrile (**1**) and other known metabolites fistularin 3 (**2**), fistularin-1 (**3**), verongic acid (**4**), verongiolide (**5**) and the dienone (**8**). The other two are steroids: aplysterol (**6**) and its acetate (**7**). Verongionitrile (**1**) displayed cytotoxic activity with an IC50 of 10 µM against HL60 human leukemia cell lines, it stops cell cycle in G2M phase and it fragments procaspase-3 enzyme. Besides verongionitrile (**1**) are known other *Verongia aerophoba* metabolites with cytotoxic and antibiotic properties such as fistularin-3 (**2**)[2] and other compound not isolated in our work: aeroplysinin-1 (displays cytostatic[3] and citotoxic[2] activity, inhibition of EGRF kinase[4] and antiangiogenic activity[5]). Because of the high pharmaceutical value of this metabolites some of them are now in the market of fine chemicals, but the biomass of the sponge cannot be collected from wild without threatening natural populations. In order to solve this supply problem, we started in situ cultures of the sponge *Verongia aerophoba* with successful initial results. The explants (fragments) of the sponge were placed into mesh bags of a submerged structure. In a few weeks, explants showed high survival and growth: the wounds were healed, new oscules were formed, the tissue grew over the mesh and some new branches could be observed. It´s needed more research to set up optimal growth conditions and to begin a clonal selection of the best producing organisms to scale-up this project up to an industrial level



**References:**

1) Ferriol, P. (2008). Productos con actividad biológica de la esponja marina *Verongia aerophoba* de las Islas Canarias. Disertación para la obtención del Diploma de Estudios Avanzados. Universidad de Las Palmas de Gran canaria. ISBN: 978-84-691-5853-1.

2) Teeyapant, R. et al. (1993). Antibiotic and cytotoxic activity of brominated compounds from the marine sponge *Verongia aerophoba*. Z. Naturforsch. 48, 939

3) Kreuter, M.H. et al. (1989). Cytostatic activity of aeroplysinin-1 against limphoma and epithelioma cells. Z. Naturforsch. (C) 44, 680

4) Kreuter M.H. et al. (1990). Inhibition of intrinsic protein tyrosine kinase activity on EGF-receptor kinase complex from human breast cancer cells by the marine sponge metabolite (+)-aeroplysinin-1. Comp. Biochem. Physiol. B 97, 151

5) Quesada A.R. et al. (2002). Aeroplysinin-1 and related compounds for the treatment of angiogenic diseases. PCT Int. Appl., p. 14

## NOTES

# Induction of G2/M phase arrest and apoptosis by the flavonoid Tamarixetin on human leukaemia cells

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Flavonoids are natural occurring polyphenolic compounds which display a remarkable spectrum of biological activities and they are among the most promising anticancer agents. We have investigated the effect of Tamarixetin on viability of four human tumor cell lines (HL-60, U937, Molt-3 and SK-MEL-1). This flavonoid inhibited proliferation in a dose- and time-dependent manner and blocked cell cycle progression at G2-M phase of the cell cycle and was associated with the induction of the cyclin-dependent kinases inhibitor p21Cip1/Waf-1. Cells incubated with Tamarixetin for three days and then incubated in flavonoid-free medium for the same period of time were unable to resume proliferation. Tamarixetin treatment induced apoptosis which was associated with cytochrome *c* release, cleavage of caspases and of poly(ADP-ribose) polymerase. Competition assays demonstrated that Tamarixetin binds to tubulin in the colchicine binding site. Although the antiproliferative effect of Tamarixetin is associated with an increase in the intracellular level of reactive oxygen species, this did not seem to play a pivotal role in the apoptotic process since different antioxidants (Trolox and *N*-acetyl-L-cysteine) were unable to provide cell protection. Pre-treatment of cells with desipramine was associated with a significant decrease in cell death triggered by Tamarixetin, suggesting that the activation of acidic sphingomyelinase is important in the mechanism of action of this flavonoid. The sensitivity of leukaemic cells to Tamarixetin suggests that this compound and related flavonoids might be considered as lead compounds for the development of chemotherapeutic strategies.

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**NOTES**