7th YCIC
Young Cancer Investigators of the Canary Islands

4th YBIM
Young Biomedical Investigators of the Macaronesia

Ex Convento de Santo Domingo
La Laguna, Tenerife, Spain
March 17 – 19th

10 Aniversario ICIC 2001-2011 Instituto Canario de Investigación del Cáncer

Biopharmac Medicinas Naturales Canarias

PROGRAMA MAC 2007 - 2013 Cooperación Transnacional

EXCMO. AYUNTAMIENTO DE SAN CRISTÓBAL DE LA LAGUNA

ICIC Instituto Canario de Investigación del Cáncer

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INSTITUO CANARIO DE INVESTIGACIÓN DEL CÁNCER
UNIVERSIDAD DE LAS PALMAS, UNIVERSIDAD DE LA LAGUNA

7TH MEETING YCIC
Young Cancer Investigators of the Canary Islands

4TH MEETING YBIM
Young Biomedical Investigators of the Macaronesia

March 17th-19th, 2011
Ex-Convento de Santo Domingo, Tenerife, Spain

Scientific Committee

Chairpersons:

Sergio Moreno, CIC (CSIC), Rafael Zárate, FICIC, Nicolás Díaz Chico, ULPGC, ICIC

Committee members:

Pedro C. Lara, Hosp. Dr Negrín, ICIC
Rafael Zárate, FICIC
Elisa Pérez Sacau, CEAMED SA, ICIC
Nicolas Díaz Chico, ULPGC, ICIC
Miguel Fernández Braña, CEU (Madrid)
Sergio Moreno, CIC (CSIC)

Local Organizing Committee

Rafael Zárate, FICIC
Elisa Pérez, CEAMED SA; ICIC
Nabil El Jaber, FICIC
Borja Guerra, FICIC

Supported by

BIOPHARMAC

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Fundación Canaria del Instituto Canario de Investigación del Cáncer (FICIC)

OBJECTIVES:

1. **Facilitate Young Biomedical Researchers to communicate their scientific results in a friendly and stimulating environment.**
2. **Encourage young and senior biomedical researchers to cooperate on the development of new projects.**
3. **Obtain the expert advice from the world-class invited biomedical scientists.**
4. **Start up a Biomedical Research Network in the Canary Islands that will increase the success of future projects.**
5. **Discuss a common strategy for Biomedical and Biotechnology development integrating all the universities and research institutions implicated.**

REGISTRATION

Participation as speaker in this Meeting will be by invitation from the Organising Committee. Participation with a poster is offered to any young biomedical investigator (<40 yrs), with preference to those working in Canaries, Azores and Madeira.

For those scientists presenting a poster, please be informed that these will be set up at the designated places at the commence of the meeting, and will be displayed throughout the entire meeting. The main authors will be standing before their posters for answering questions at the indicated time which will be informed at the moment of registration.

For the evaluation of the participants' work, a Scientific Committee has been set up:

Dr. Atanasio Pandiella (Univ. Salamanca)

Dr. Miguel Fernández Braña (Univ. San Pablo CEU)

Dr. Rafael Zárate Méndez (FICIC)

Dr. Elisa Pérez Sacau (CEAMED SA)

The criteria that will be evaluated include:

- Originality
- The innovative nature of the research
- Multidisciplinary
- Relationship with cancer
- Quality of presentation and defence

Please contact with:

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Thursday, March 17th, 2011	
Registration	15:30 -17:30
Informal Opening **Speaker: Sergio Moreno, Centro Investigación del Cáncer, Salamanca** **1st Symposium: NEW Bioactive COMPOUNDS** **Chairperson: Miguel Fernández Braña, CEU, Madrid.** **Miguel Xavier Fernandes (20´), CQM, Universidad da Madeira, Madeira** **Miguel Arruda (20´), Universidad de Las Azores, Azores.** Is the secret of longevity hidden in an unwanted invasive plant? Anticholinesterasic effect and antioxidant activity of *Hedychium gardnerianum* essential oils **KEYNOTE LECTURE (I)** **Invited speaker:** **Dr. Alicia Boto (45´),IPNA-CSIC, La Laguna, Tenerife** New Chemical Processes to Discover Drug Leads	18:15-20:00
Dinner	20:30

Friday, March 18th, 2011	
2nd Symposium: Molecular Biology and Biotechnology **Chairperson: Dr. Mario Díaz, ULL, ICIC** **Patricia Martín (15'), ICIC, ULPGC.** Biological activities of a novel series of compounds as DNA Topoisomerase II inhibitors **Belinda Rivero Pérez (15´), FICIC, ACIISI, HUNSC.** Detection of *BRCA1* and *BRCA2 mutations* in patients with high-risk for hereditary breast cancer in the Hospital Universitario Ntra. Sra. de La Candelaria (HUNSC). **Ina Hildebrandt (15'), FICIC, ACIISI.** Influence of alkylaminoethoxy side-chain size of novel tamoxifen derivatives with a methoxy terminal group on ER mediated transcriptional activity **Dr. Luis Alberto Henríquez (15'), ICIC, ULPGC** Radiation-induced apoptosis, initial DNA damage, and different genetic factors conform a complex system that influence normal tissue toxicity after radiotherapy **KEYNOTE LECTURE (II)** **Invited speaker:** **Dr. Raquel Marín (45´), ICIC, ULL** Estrogen receptors, as components of signaling platforms, are involved in neuronal preservation against Alzheimer's disease	09:00–11:00
1st Poster session (coffee break area)	11:00-11:30

3rd Symposium: Molecular Physiology and Tumor Genomics **Chairpersons: Dr. Sergio Moreno, Centro de Investigación del Cáncer, Salamanca** **Cristina Ramos Pérez (15'), ICIC, ACIISI, HUNSC.** Multiple basal cell carcinoma: monoclonal or polyclonal origin? **Jorge Marrero Alonso (15'), ICIC, ULL** FL-TX, the first fluorescent tamoxifen derivative: A biological evaluation **Ruymán Santana (15'), ICIC, ULPGC** Analysis of 17β-estradiol interplay with growth hormone in male rat hepatic transcriptome **Susana Beceiro (15'), ICIC, ULPGC** Regulation of dendritic cell chemotactic activity by LXR nuclear receptors **Dr. Cristina Bilbao (15'), ICIC, ULPGC** Double strand break repair components are frequent targets of microsatellite instability in endometrial cancer **KEYNOTE LECTURE (III)** **Invited speaker:** **Dr. Mario Díaz, (45´) ICIC, ULL** Androgens are powerful non-genomic inducers of calcium sensitization in visceral smooth muscle.	11:30-14:00
LUNCH	14:00 -15:30
4th Symposium: Molecular Biology and Tumor Genomics **Chairpersons: Dr. Leandro Fernández, ULPGC, ICIC.** **Dr. Germán Rodríguez (20´) ICIC, Josephine Nefkens Institute, Rotterdam.** Identification of hsa-miRNA target related proteins to tamoxifen response by miRCURY LNA™ microRNA Inhibitors coupled to pSILAC approach **KEYNOTE LECTURE (IV)** **Invited speaker:** **Dr. Atanasio Pandiella (45´), USAL, Salamanca** The Neuregulin/ErbB system in cancer: from the bench to the bedside	16:00–17:30
2nd Poster session (coffee break area)	17:30–18:00
5th Symposium: Tumor Genomics **Chairpersons: Dr. Nicolas Díaz Chico, ULPGC, ICIC.** **KEYNOTE LECTURE (V)** **Invited speaker:** **Dr. Isabel Tapia Páez (20´), Karolinska Institute, Novum, Sweden** Studies of DYX1C1 a dyslexia candidate gene involved in neuronal migration and possible role in Breast Cancer **KEYNOTE LECTURE (VI)** **Invited speaker:** **Dr. Manuel Perucho, (45´) Sanford-Burnham Medical Research Institute (SBMRI), La Jolla, California** Alteraciones Epigenéticas en genes ADAMTS de Metaloproteinasas: Posibles Biomarcadores de Tendencias Metastáticas en Cáncer Colorectal.	18:00-19:30

ICIC General Assembly	19:30-20:00
CLOSING DINNER	21:00

Saturday, March 19th, 2011

6th Symposium: Epidemiological Research and Bioactive molecules **Chairperson: Dr. Pedro C. Lara, ICIC, Hospital Dr. negrín, ULPGC.** **Dr. Pedro Lara (30'), ICIC, UI Hospital Dr Negrín, Las Palmas.** Major Vaults Protein (MVP): from multidrug resistance to DNA repair modulation **KEYNOTE LECTURE (VII)** **Invited speaker:** **Dr. Hugo Marsiglia (30´), Gustave Roussy, París** **KEYNOTE LECTURE (VIII)** **Invited speaker:** **Dr. Jose Lòpez Torrecilla (30´), H. Universitario Valencia** Prostate Cancer. Treatment Advance	09:00-10:30
3rd Poster session (Coffee break area)	10:30-11:00
CLOSING LECTURE **PhD Disertation** **Raquel Ramírez Moreno**	11:00-12:30
Activities: 10th anniversary of ICIC **ICIC 2011 Awards** **Closing Ceremony**	12:30

ORAL COMMUNICATIONS

New Uses for Old Drugs: The Discovery of P-Glycoprotein Inhibitors

Andreia Palmeira[1, 2, 3], Freddy Rodrigues[4], Emília Sousa[1, 2], Madalena Pinto[1, 2], Maria Helena Vasconcelos[3, 5] and **Miguel X Fernandes**[4]

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3 - Cancer Biology Group, IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Rua Dr Roberto Frias, 4200-465 Porto, Portugal
4 - Centro de Quimica da Madeira, Universidade da Madeira, Campus da Penteada, 9000-390 Funchal, Portugal
5 - Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Rua Anibal Cunha 164, 4050-047 Porto, Portugal

P-glycoprotein (P-gp) is one of the best characterized transporters responsible for the multidrug resistance phenotype exhibited by cancer cells. Therefore, there is widespread interest in elucidating whether existing drugs are candidate P-gp substrates or inhibitors. With this aim, a pharmacophore model was created based on known P-gp inhibitors and it was used to screen a database of existing drugs. The P-gp modulatory activity of the best hits was evaluated by several methods such as the rhodamine-123 accumulation assay using K562Dox cell line, and a P-gp ATPase activity assay. The ability of these compounds to enhance the cytotoxicity of doxorubicin was assessed with the sulphorhodamine-B assay. Of the 21 hit compounds selected *in silico*, 12 were found to significantly increase the intracellular accumulation of Rhodamine-123, a P-gp substrate. In addition, amoxapine and loxapine, two tetracyclic antidepressant drugs, were discovered to be potent non-competitive inhibitors of P-gp, causing a 3.5-fold decrease in the doxorubicin GI_{50} in K562Dox cell line. The overall results provide important clues for the non-label use of known drugs as inhibitors of P-gp. Potent inhibitors with a dibenzoxazepine scaffold emerged from this study and they will be further investigated in order to develop new P-gp inhibitors.

NOTES

Is the secret of longevity hidden in an unwanted invasive plant? Anticholinesterasic effect and antioxidant activity of *Hedychium gardnerianum* essential oils

Arruda M, Viana H, Rainha N, Baptista J, Rosa JS, Barreto MC

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Oxidative stress has been associated with the progression of chronic conditions such as cancer, aging and Alzheimer's disease. Essential oils from several plants have in their composition many compounds which have antioxidant properties and are also inhibitors of acetylcholinesterase. This may be the basis for the empirical use of essential oil mixtures to alleviate symptoms of Alzheimer's patients, whose deficit in acetylcholine may be countered in part by inhibitors of this enzyme.

Preliminary work on *Hedychium gardnerianum*, an invasive plant in the Azores archipelago, suggested that this plant may be an interesting source of compounds and/or compound mixtures which combine both anticholinesterasic and antioxidant properties. In this context *H. gardnerianum* collected on four different sites in S. Miguel island: Furnas (FU), Fogo (FO), Sete Cidades (SC) and Achada do Nordeste (AN). Essential oils from leaves were extracted by hydrodistillation and phytochemically characterized by GC/MS. Inhibition of acetylcholinesterase was assessed using a modification of the Ellman method and antioxidant activity was quantified by the DPPH radical scavenging assay. Principal Component Analysis (PCA) was carried out using XLSTAT Version 2011.1.03.

The essential oils presented a majority of sesquiterpene hydrocarbons (52.3 to 60.9%), followed by oxygenated sesquiterpenes (15.2 to 16.3%). PCA considered oils from FU and FO in one group and SC and AN in another group, mainly due to differences in the amount/ presence of (-)-□-Amorphene and □-Caracolene between the two groups. (-)-Cedreanol (15.2 to 16.3%), 3,4-Dimethyl-3-cyclohexen-1-carboxaldehyde (9 to 10.5%) and cadalin (5.0 to 6.4%) were present in all the essential oils.

All the essential oils presented high antioxidant activity, although to different extents, with EC_{50} values ranging between 8.5 and 31.1 □g/mL, which are interesting values when compared with standard compounds quercetin, trolox, ascorbic acid and BHT (3.1, 5.6, 10.3 and 31.0 □g/mL, respectively). All the oils were good acetylcholinesterase inhibitors, with IC_{50} between 1.03 and 1.37 mg/mL, lower than □-pinene, a known inhibitor of this enzyme (IC_{50} 1.43 mg/mL). There was no correlation between the inhibition of acetylcholinesterase and the antioxidant activity in the essential oils or between these activities and chemical composition.

Kinetic study of the type of AChE inhibition was also carried out for the same essential oils. As would be expected of complex mixtures such as essential oils, a mixed inhibition was detected, although the essential oil from AN was almost purely competitive, which is very interesting when one considers the potential therapeutic use of this oil.

These results seem to indicate that *H. gardnerianum* essential oil should be considered as a good candidate in the treatment or as a coadjuvant in the prevention of neurodegenerative diseases such as Alzheimer, possibly in aromatherapy, since it possesses both strong antioxidant properties and high anticholinesterasic activity. Therefore the characterization of action mechanisms of the compounds present in these oils and/or of compound mixtures which might present synergetic effects is being carried out in our research group.

NOTES

New Chemical Processes to Discover Drug Leads

Alicia Boto

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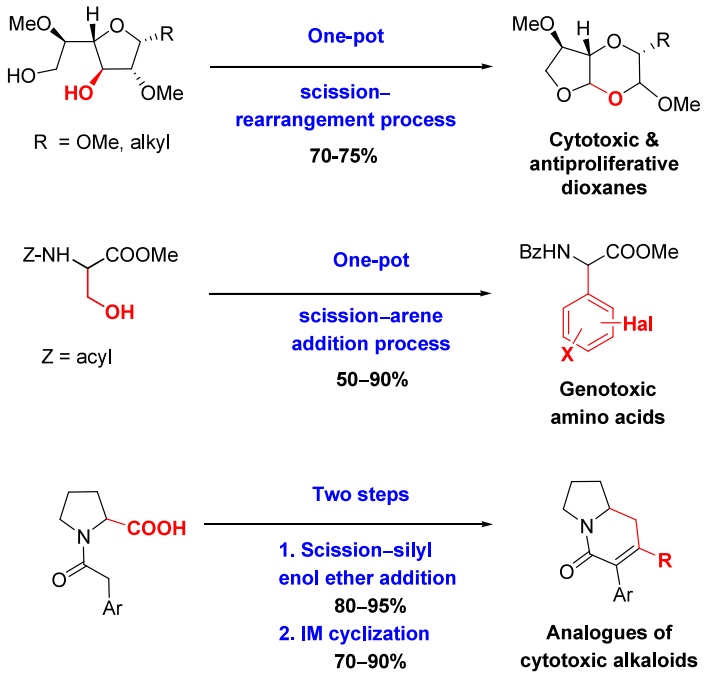
In sequential processes, several reactions take place in the same reaction vessel without the need to isolate the intermediate products. Therefore, these processes save reaction time and materials, and decrease the waste.

This presentation will introduce several sequential processes which allow the ***direct conversion*** of readily-available carbohydrate or amino acid derivatives into bioactive products, such as antiproliferative sugar-like dioxanes, cytotoxic amino acids, alkaloid precursors, acyclic nucleosides, azanucleosides, etc. These conversions take place under mild conditions and with moderate to excellent yields. The cytotoxic activities of these compounds will also be discussed.



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NOTES

Biological activities of a novel series of compounds as DNA Topoisomerase II inhibitors

Patricia Martín Rodríguez, Ana Estévez Braun, Grant McNaughton Smith, Ángel Gutiérrez Ravelo, Elisa Pérez Sacau, Patricia Quintana Espinosa, Sandra Jiménez Alonso, Leandro Fernández Pérez, Nicolás Díaz Chico

Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna; Departamento de Ciencias Clínicas, Universidad de Las Palmas de Gran Canaria; ICIC; CEAMED, s.a.

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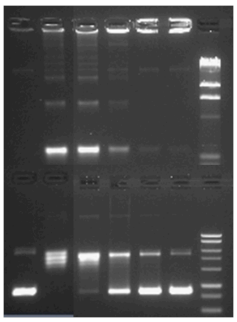
Topoisomerases are important targets in antitumoral chemotherapy. From a physiological point of view, these enzymes catalyze changes in the topology of the DNA molecule and facilitate events such as transcription, replication, and physical separation of sister chromatids in mitosis. (1) One point to understanding why topoisomerases are good antitumoral targets is because they are highly active in cells that are proliferating and therefore need to replicate and segregate the DNA. (1, 2, 3) To test whether compounds were active against human topoisomerase II, we employed the purified enzyme and a set of small molecules of circular DNA as substrates in a series of different in vitro experiments. The use of these small DNA molecules greatly facilitates the visualization of the topoisomerase reactions by simple electrophoretic means in the two assays used by us, decatenation and relaxation. 121 compounds were analyzed. 18 of them showed the ability to inhibit unless one of the enzyme function at 60□M; 6 of this 18 compounds inhibited both activities and 5 compounds exhibited inhibition in unless one of the enzyme function at 30□M. In addition, preliminary results of citotoxicity in different tumoral cell lines were obtained.
The results show that some of our compounds have the activity as DNA Topoisomerase II inhibitor. Moreover, some of them have citotoxic activities. Specifically, compounds derivated of Amonafide and compounds belonging to Naphthoquinones group are outlined as good candidates for further studies.



CM-000313 (*well 1,*vehicle without enzyme; *well 2,* vehicle with enzyme; *wells 3, 4, 5,* CM-000313 10μM, 30μM, 60μM; *well 6,* Suramina 20μM; *well 7,* MWM)

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1. McClendon AK, Osheroff N 2007 DNA topoisomerase II, genotoxicity, and cancer. Mutat Res 623:83-97
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3. Heck MM, Earnshaw WC 1986 Topoisomerase II: A specific marker for cell proliferation. J Cell Biol 103:2569-81

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NOTES

Detection of *BRCA1* and *BRCA2 mutations* in patients with high-risk for hereditary breast cancer in the Hospital Universitario Ntra. Sra. de La Candelaria (HUNSC).

Belinda Rivero-Pérez [1,2], Ana González-Hernández[1], Cristina Bilbao[2], Natalia Pérez-Rodríguez[3], Javier Dorta-Delgado[3].

Unidad de Investigación[1] y Servicio de Oncología Médica[3], Hospital Universitario Ntra. Sra. De Candelaria e Instituto Canario de Investigación del Cáncer (ICIC)[2]

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Breast cancer is the most common malignancy among women worldwide, and its incidence is increasing. The Canary Islands have the highest rate of breast cancer in Spain, and although factors like obesity or diabetes have tried to be related to breast cancer, the cause remain being unknown. It is currently estimated that 5-10% of all breast cancers are hereditary and attributable to mutations in several highly penetrant sysceptibility genes of which *BRCA1* and *BRCA2* are the most important. Several studies have determined that the penetrance of mutations in *BRCA1* and *BRCA2* genes is from 45-87% in breast cancer. *BRCA1* and *BRCA2* are considered tumor surppressor genes, associated with cell cycle regulation and DNA repair. The most frequent changes are nonsense and frameshift mutations and their detection is difficult due to the large size of these genes and their high heterogeneity.

The aim of our study is to optimize a method for the detection of *BRCA1* and *BRCA2 mutations*, for its routine application to the patients with high-risk for hereditary breast cancer in the Hospital Universitario Ntra. Sra. de Candelaria (HUNSC). This service tries to respond to the needs of those professionals who work with breast cancer patients in this hospital, evaluating, studying and counselling to the families with possible hereditary breast cancer syndromes. Also, we try to determine the recurring mutations in our population as well as the possible founder mutations.

In this moment, the optimization of the PCR amplification and sequencing protocols of the *BRCA1* and *BRCA2* exons and exon-intron boundaries is being performed in two anonymous DNA samples. Subsequently, a method for the detection of large deletions will be chosen and optimized. Up to now, the whole coding sequences and adjacent introns of the *BRCA1* gene have been successfully amplified and sequenced. The obtained sequences showed 100% homology with the *BRCA1* sequences deposited in the National Center for Biotechnology Information (NCBI). During the next months we are going to optimize the PCR amplification and sequencing protocol of *BRCA2*.

On a long-term, this project expects to put the Canary Islands on a level with other Spanish communities with regard to the service offered by multidisciplinary Genetic Counselling Units, in hereditary breast cancer and other hereditary cancer types.

NOTES

Influence of alkylaminoethoxy side-chain size of novel tamoxifen derivatives with a methoxy terminal group on ER mediated transcriptional activity

Ina Hildebrandt[1,2,3], Jorge Marrero-Alonso[1,2,4], Benito García Marrero[1,5], Alicia Boto[5], Tomás Gómez[1,2,4], Mario Díaz[1,2,4]

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(2) Laboratorio de Fisiología y Biofísica de Membranas, Departamento de Biología Animal; (3) Instituto Universitario de Bio-orgánica Antonio González (IUBO) & (4) Instituto de Tecnologías Biomédicas. Universidad de La Laguna (ULL)
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Tamoxifen (Tx) is a Selective Estrogen Receptor Modulator (SERM) with an extensive importance on antihormonal therapy targeted to estrogen receptor (ER) for breast cancer treatment from 70s up to now. However, it is widely recognized the incidence of undesirable side effects during long term therapies, which has encouraged physicians not to extend the treatment for more than five years in most of cases. Some of these undesirable effects, such as the development of endometrial cancer, are largely related to tissue-dependent estrogenic effects of Tx. In order to find novel SERMs that could be devoided of such tissue-dependent estrogenic properties, our group have synthesized four carbamates of tamoxifen (CTx) and another derivative with a methoxy terminal group (MTx). All of them have been evaluated using different *in vitro* and *in vivo* assays (unpublished data). Some of these results have been presented in previous YCIC meetings. Differences found on antiestrogenic transcriptional activity between CTx4 and MTx, both with a methoxy terminal group, have suggested that the position of the carbonyl group respect to the amine group could be related to the ER affinity, as well as to the potency of the effect. Results obtained in ER activation antagonism and antiproliferative assays fit well and support this theory. In this sense, members of the Laboratory of Membrane Physiology and Biophysics, in collaboration with the Institute of Natural Products and Agrobiology, have designed and synthesized novel MTx derivatives which differ in the alkylaminoethoxy side-chain size. The aim of this study was to evaluate the agonistic and antagonistic ER mediated transcriptional activity of these novel compounds on T47D-KBluc cell line, which stably express a luciferase reporter under the control of estrogen-response elements.

Supported by grants SAF2007-66148-C02-02, SAF2010-22114-C02-02 from MICINN and FICIC funds (Spain). I. Hildebrandt and J. Marrero-Alonso are hired by FICIC with funds from ACIISI (Spain) and FSE (EU).

NOTES

Radiation-induced apoptosis, initial DNA damage, and different genetic factors conform a complex system that influence normal tissue toxicity after radiotherapy

Luis Alberto Henríquez-Hernández [1], Pedro C Lara [1,2], Elisa Bordón [1,2], Fausto Fontes[1], Beatriz Pinar [1,2] Ruth Carmona-Vigo [2] and Marta Lloret [1,2]

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Patients treated with radiotherapy (RT) will develop clinical toxicity, and this may limit the efficacy of the treatment. Interpatient heterogeneity in normal tissue reactions varies considerably, yet the genetic determinants and the molecular mechanisms of therapeutic radiation sensitivity remain poorly understood. The prediction of the toxicity induced by RT could help to select the most appropriate treatment for each patient. Different assays have been developed to predict radiation injury and allow customization of RT protocols on an individual basis. Nonetheless, the transfer of knowledge to clinical practice is not yet successful. Radiation-induced lymphocyte apoptosis (RIA) was developed as a rapid tool for characterization of normal tissue radiosensitivity in several tumour locations by flow cytometry. In that way, patients with higher grades of late effects showed lower levels of RIA in peripheral blood lymphocytes (PBL). An association between DNA damage assays, quantifying the initial number of DNA double-strand breaks (DSB) induced by radiation, and radiation-toxicity has been reported. Thus, increasing numbers of radiation induced DSB were related to severe late toxicity in cancer patients. Recently, it has been reported an inverse association between DNA DSB and RIA in breast cancer patients, even at the genome level, where common canonical pathways and biological processes between both mechanisms have been observed. Moreover, patients who presented lower levels of initial DNA damage and higher levels of radiation induced apoptosis were at low risk of suffer severe late toxicity after RT. The variation in sensitivity of normal tissues and the consequent risk of developing late morbidity may be genetically determined. Few studies have been published with regard to radiation induced toxicity and gene expression profile. A relation between the constitutive gene expression profile of PBL and toxicity after RT has been recently reported, opening the possibility that the different constitutive expression levels of a selected group of genes would predict acute and late toxicity caused by RT. Finally, it has been reported that possession of variants in genes, the products of which play a role in radiation response, may be associated with the development of adverse effects after RT. There are several candidate genes that possess single nucleotide polymorphisms associated with radiation-induced sequelae, involved in DNA repair, apoptosis or cell growth mechanisms.

NOTES

Estrogen receptors, as components of signalling platforms, are involved in neuronal preservation against Alzheimer's disease.

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Summary of the work. Estrogen develops some crucial actions in the regulation of neuronal differentiation, synaptic plasticity, induction of neuronal survival, and regional neurogenesis in the adult that ultimately affect mood and cognitive processes. It has been demonstrated in the past that estrogen neuroprotection against Aβ-induced toxicity, a main hallmark of Alzheimer's disease neuropathology, is partially due to gene transactivation via a classical ERα-mediated mechanism. More recent reports have shown that estrogens can also interact with specific ERs located in close contact with the neuronal membrane (mER) to activate different, highly coordinated, signaling pathways that take place within a few minutes (named "alternative mechanisms"). The presence of neuronal membrane-related ERs similar to classical ERα has given raise to some controversy on the manner a hydrophilic molecule lacking transmembrane domains may be inserted into the plasma membrane, in order to rapidly interact with their natural molecular targets. This apparent paradox has been partially solved by the recent finding of mER present in neuronal *lipid rafts*, membrane microdomains with particular physico-chemical properties, where the receptor may be stabilize by its binding to raft resident molecules. In this order of ideas, our group has provided the first evidence of a raft-located ERα in murine septal and hippocampal neurons, and in human frontal cortex and hippocampus. In these membrane fractions as well as in microsomal fractions from different mouse brain areas, the receptor was found to physically interact with Cav-1 and with other proteins, such as a plasmalemmal VDAC (pl-VDAC) and the insulin growth factor-1 receptor, which may be part of this molecular complex at the neuronal membrane. In this signaling platform, caveolin-1 may be the pivotal anchoring protein that supplies stability for the integration and functionality of ERα, facilitating its associations with other signaling proteins in the raft microstructure. Interestingly, this complex appears disrupted in dystrophic neurites of amyloid beta plaques, probably facilitating beta amyloid-mediated cell damage.

Thus, ERs involved in plasma membrane actions in the brain appear to be multifaceted and versatile molecules that may be integrated in particular membrane microdomains where they may be differentially activated by estradiol and other extracellular signals. This fact may be crucial in the estrogen mechanisms developed by neurons to achieve neuroprotection, and it might be at the basis of the failure of estrogen replacement therapies supplied in post-menopausal periods, when the amount of membrane ERs may be decreased or absent.

This work is supported by grants SAF2010-22114-C02-01/02, and FICIC2009/18-11-2009.

NOTES

Multiple basal cell carcinoma: monoclonal or polyclonal origin?

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Basal cell carcinoma (BCC) is the most common type of skin cancer. It's considered malignant because it invades surrounding tissues but it rarely metastasizes or kills. In most cases it appears sporadically as a result of sunlight exposure in people over age 50, but there is a small percentage of people who develop multiple BCC at an early age. Some of them suffer from the Gorlin syndrome, which is known to be caused by carrying a mutated copy of the gene PTCH1. When there is inactivation or loss of the second copy of this gene the BCC develops. But there are also non-Gorlin patients who have multiple BCC with unknown origin.

The aim of this project is to analyze this last group of patients in search of signs of inactivation of the gene PTCH1, the main cause of the development of BCC in general, and to evidence if the origin of the multiple tumors is monoclonal (a skin-specific metastasis) or polyclonal.

NOTES

FL-TX, the first fluorescent tamoxifen derivative: A biological evaluation

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Tamoxifen (Tx) is a Selective Estrogen Receptor Modulator (SERM) extensively used on estrogen receptor-positive breast cancer treatment. However, clinical evidences demonstrate the incidence of undesirable side effects during chronic therapies. Pharmacological approaches had highlighted that some of these undesirable effects are related not only to tissue-dependent estrogenic effects of Tx, but to other mechanisms involving its interaction with different unidentified molecular targets. The development of fluorescent conjugates would help to unravel such cellular targets. In this sense, the first fluorescent tamoxifen derivative (FL-TX) has been synthesized and characterized by our group. The fluorescent derivative exhibited antiestrogenic activity in MCF7 cells transfected with 3xERE-luc reporter and T47D-KBluc cell line to similar levels than tamoxifen. Interestingly, unlike tamoxifen, FL-TX was devoid of agonist transcriptional ER-dependent activity within the same range of doses. Furthermore, FL-TX and tamoxifen were capable to antagonize the proliferative effect of estradiol in MCF7 cells at 3 μM concentration. FL-TX itself exhibited some cytotoxicity at lower doses than tamoxifen, although this toxicity was reversed in the presence of estradiol, at least at doses below 3 μM. In addition, *in vivo* assays confirmed that FL-TX was devoid of estrogenic uterotrophic effects in mice at least up to 1 mg/kg/day. However, FL-TX showed estrogenic uterotrophic agonism at higher doses, i.e. 10 mg/kg/day, in rats. On the other hand, the fluorescent derivative was less potent as antiestrogen than Tx itself *in vivo*. Using confocal microscopy experiments, we could demonstrate that FL-TX colocalizes with ER. Moreover, the percentage of colocalization of FL-TX-ER complex was similar to the percentage of competition of FL-TX binding by unlabeled estradiol, indicating the specificity of the ER-FL-TX binding. Finally, competition studies showed that the binding of FL-TX to the plasma membrane was totally antagonized by unlabelled tamoxifen, indicating the presence of plasma membrane binding sites specific for triphenylethylene compounds. Competition assays with nifedipine, a well known and specific L-type calcium channel blocker, indicated that these channels are side targets for triphenylethylene compounds at the plasma membrane; which are involved in the relaxing effect of tamoxifen and related derivatives that we have reported previously for duodenal and uterine smooth muscles (Díaz, 2002; Marrero-Alonso *et al.*, 2006).

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NOTES

Analysis of 17β-Estradiol interplay with Growth Hormone in male rat hepatic transcriptome

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Since the somatomedin hypothesis was formulated in 1957, the roles of Growth Hormone (GH) in postnatal longitudinal growth in mammals have been extended to include important effects on metabolism and tissue maintenance and repair. Thus, other molecules involved in metabolism, such as estrogens, may modulate GH-regulated endocrine and metabolic functions in liver but the physiological basis of this interplay is not well understood. To explore this interplay we used both, adult hypothyroid-castrated (TXOX) male rat liver model to minimize the influence of endogenous hormones on the study; and DNA Microarray technology to have an overview on the effects of the treatments on the whole transcriptome. TXOX rats were treated with Estradiol Benzoate (EB) for twenty days before intermittent GH replacement during seven days. Hypothyroidism reduced body weight gain, circulating IGF-I, and mRNA levels of IGF-I and male-specific cytochromes such as CYP2C11 in liver, which were restored by GH replacement. However, in the presence of EB, GH was not able to restore these changes. In contrast, CYP2C12, a female differentiated gene, was induced by EB. EB also reduced circulating leptin but did not reverse the lipid phenotype caused by hypothyroidism (i.e., hypercholesterolemia and hypotrygliceridemia). EB provoked drastic changes in hepatic transcriptional profiling (up-regulated genes=382; down-regulated genes=290) particularly genes involved in lipid metabolism (i.e., PPAR pathway) and metabolism of xenobiotics by cytochrome P450. In the absence of EB, we identified 218 genes that were up-regulated by GH treatment, while 139 were down-regulated to the same extent. However, in the presence of EB, the number of GH-up-regulated and GH-down-regulated genes were reduced and practically inhibited, respectively. The number of biological processes regulated by GH treatment with significant representation in our set of genes was also significantly reduced in the presence of EB. A set of 84 genes were regulated in common by GH and EB. Taken together, this work highlights the influence of estrogens on liver transcriptome and its interplay with GH-regulated endocrine and metabolic functions in male which has a great relevance for lipid and drug metabolism.

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Regulation of Dendritic Cell Chemotactic Activity by LXR Nuclear Receptors.

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Inflammation is a local or systemic physiological response to tissue injury. It is therefore a protective reaction trying to restore tissue homeostasis. Inflammation constitutes an instigator and effector arm of our immune system and is crucial to protect against invading microorganisms. However, inflammation can cause detrimental effects, and contributes extensively to human diseases such as cardiovascular disease, Type-II diabetes, autoimmune diseases and cancer.

The Liver X Receptors (LXRs) are members of the nuclear receptor family of transcription factors. LXRs are endogenously activated by oxysterols and control the expression of genes important for cholesterol uptake, efflux, transport, and excretion in multiple tissues. Our recent work demonstrated that LXRs regulate diverse aspects of inflammatory gene expression in macrophages. These receptors negatively regulate the expression of inflammatory genes in response to external challenges. However, the role of LXRs in acquired immunity is still incompletely undestood. Dendritic cells (DCs) represent a unique system as sensors of pathogens, with the ability to efficiently activate naïve T cells. While the participation of LXRs in chronic processes, such as atherosclerosis, has been extensively studied in the last several years, few studies describe the role of LXR in DCs activtion and function. In this study we demonstrate that LXR activity contributes to the efficient migratory activity of DCs in response to chemotactic stimuli *in vitro* and *in vivo*. Transcriptomic expression analysis doesn´t prevent the correct differentiation of DC, although it affects the migratory capacity of these cells. DCs identified a discrete set of genes important for DC chemotaxis that are differentially regulated by LXR in DCs. These results indicate that LXR nuclear receptors are not only important for cholesterol homeostasis, but also regulate important aspects of leukocyte migration under physiological or pathological conditions.

NOTES

Double strand break repair components are frequent targets of microsatellite instability in endometrial cancer

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Aim: DNA double strand break (DSB) repair is a central cellular mechanism of the DNA damage response to maintain genomic stability. DSB components are frequently mutated in colorectal cancer with microsatellite instability (MSI). We investigated whether DSB repair is involved in endometrial cancer (EC) with MSI.

Methods: Mononucleotide microsatellite tracts of 14 genes of the DSB repair system were analysed in a series of 41 EC with MSI. Among these genes, the microcephalin 1 (MCPH1/BRIT1) has never been tested as target of MSI in tumour series.

Results: The most frequently mutated gene was DNAPKcs (n = 14, 34%) followed by RAD50 (n = 7, 17%), MRE11, ATR and BRCA1 (n = 6, 15%), and by CtIP and MCPH1 (n = 5, 12%). While DSB biallelic mutations were infrequent, a high proportion of tumours (n = 30, 73%) presented mutations at some component of the DSB repair pathway, and almost half of them showed alterations at two or more components. Tumours with mutations in two or more genes were significantly associated with advanced grade (p = 0.03) and vascular invasion (p = 0.02) and marginally associated with advanced stage (p = 0.07).

Conclusions: Our results suggest that in EC, the DSB repair is a relatively common mutational target of MSI and might contribute to tumour progression, and also that MCHP1 may be a novel target gene of MSI.

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NOTES

Androgens are Powerful Non-Genomic Inducers of Calcium Sensitization in Visceral Smooth Muscle

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Androgens (testosterone and 5-dihydrotestosterone, DHT) are recognized as genotropic inducers of a number of physiological functions mainly associated with the development of sexual characteristics. However, as in the case of estrogens, the number of studies evidencing androgen actions in non reproductive tissues has steadily grown over the past years. Here, we show that androgens acutely (~30 min) alter the frequency spectrum and potency of peristaltic activity and augment the amplitude agonist-induced contractile activity of intestinal smooth muscle. Maximal stimulation occurred at physiological concentrations of androgens (100 pM-10 nM) with EC_{50} values in the picomolar range. Androgen-induced potentiation was prevented by preincubation with androgen receptor (AR) antagonists but unaffected by pretreatment with cycloheximide plus actinomycine D, indicating that potentiation was mediated by ARs via a non-genomic mechanism.

The effects of androgens were mimicked by polyamines putrescine and spermine, and were completely blocked by inhibitors of polyamine synthesis. Likewise, androgens increase ornithine descaboxilase activity in intestinal tissues within the same time-course that contractile potentiation. Using ionomycin-permeabilized intestinal smooth muscle preparations under clamped low external calcium, we could demonstrate that androgens exert their effects by inducing a mechanism of sensitization to calcium and not by altering intracellular calcium homeostasis. Correspondingly, the potentiation of mechanical activity induced by androgens was accompanied by an increase in the phosphorylation of the regulatory myosin light chain (LC_{20}) within the same time-course than calcium sensitization and mechanical potentiation. The pursuit of potential signalling pathways linking androgen receptor activation with calcium sensitization revealed that mechanical potentiation of intestinal muscle by androgens involve activation of the Rho pathway, whose downstream effector, Rho-associated kinase (ROCK), is eventually responsible for displacement of the phosphorylation/dephosphorylation state of LC_{20} towards its phosphorylated form.

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NOTES

Identification of hsa-miRNA target related proteins to tamoxifen response by miRCURY LNA™ microRNA Inhibitors coupled to pSILAC approach

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During decades, tamoxifen has been the mainstay of hormonal therapy in both early and advanced ER-positive breast cancer patients (1). Approximately a half of patients with ER-positive and advanced disease do not respond to endocrine therapy or develop resistance. The biological mechanisms underlying intrinsic (de novo) and/or acquired tamoxifen resistance are clinically significant (2,3). It suggests that those mechanisms occur in breast tumors through differential gene expression and protein alterations (4,5). In breast cancer, altered miRNAs expression levels have been described (6,7). Recently, we analyzed by RT-PCR various candidate predictive miRNAs in 246 estrogen receptor (ER)-positive primary breast tumors of patients who received tamoxifen for advanced disease. Our results showed that either in univariate analysis and multivariate analysis, corrected for the traditional predictive factors, hsa-miRNA-30c, was significantly associated with benefit of tamoxifen treatment (*P*-value < 0.01) (8).

One of the most powerful ways to determining the function of a microRNA is by performing knockdown experiments. In such experiments, the phenotypic changes of cells transfected with antisense oligonucleotides are closely monitored to elucidate the biological role of the targeted microRNA. We would like to test the hypothesis whether; hsa-miR-30c is involved in tamoxifen response in luminal (ER+/PR-/ERBB2-) breast cancer cell lines (e.g. T47D and MCF-7). In addition, we would like to identify which targets are modulated by hsa-miR-30c using pSILAC, a proteomics based method which uses isotopic aminoacids to label proteins which change upon microRNA knockdown approach (10).

Finally, our data suggest that hsa-miR-30c can, by direct or indirect effects, tune protein synthesis from thousands of genes. Some of those need to be validated as tamoxifen predictive markers.

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NOTES

The Neuregulin/ErbB system in cancer: from the bench to the bedside

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The Neuregulins (NRGs) are a group of polypeptide growth factors that play critical physiological roles. Animals lacking NRGs die in utero due to heart disgenesia, and a role of NRGs in the control of peripheral nervous system homeostasis has also been demonstrated. In addition to these roles in animal development, deregulation of NRGs has been linked to frequent diseases such as cancer or schizophrenia. With respect to the former, preclinical studies have shown that overexpression of NRGs facilitates breast cancer cell proliferation, migration or invasiveness, and targeted expression of NRGs in the mammary gland of mice results in the appearance of adenocarcinomas. Moreover, increased expression of NRGs has been reported in different types of solid tumours, and their presence correlates with the response to some treatments used in the oncology clinic to manage breast cancer.

Most NRGs are biosynthesized as transmembrane precursors that may be released as soluble factors by the action of cell surface metalloproteases of the ADAM family. In their membrane-bound conformation the NRGs retain biological activity, indicating that cleavage of the transmembrane protein may not be critical for its activity. This conclusion is based on experimental systems in which uncleavable proNRGs are able to activate the NRG receptors on adjacent cells (in trans).

The NRGs act by binding transmembrane receptors of the ErbB family. In mammals, this family includes four members: EGFR (also termed HER1 or ErbB1), ErbB2/HER2/neu, ErbB3/HER3, and ErbB4/HER4. The NRGs were initially identified along searches for ligands of ErbB2 which demonstrated that NRGs could trigger tyrosine phosphorylation of ErbB2. Later, more detailed studies defined that NRGs indirectly activate ErbB2, as they act on ErbB3 or ErbB4, that upon NRG binding establish oligomeric complexes with other ErbB family receptors, including ErbB2.

Augmented expression of HER2 due to amplification is frequently found in breast cancer and is linked to worse patient outcome. Other studies showed increased functioning of the EGFR in lung, colon, brain, and head and neck cancer. These observations, together with the demonstration of a potent oncogenic role of HER receptors in preclinical models, established the bases for the development of agents that target HER receptors for the treatment of patients bearing tumours with high HER receptor functioning. Two types of anti-HER targeted agents have reached the oncology clinic. A first group includes antibodies that recognize the extracellular region of HER receptors. Trastuzumab represents the prototypical example of these biopharmaceuticals, as it was the first anti-HER treatment approved for patients with HER2+ metastatic breast cancer. A second type of anti-HER targeted therapy is represented by chemical tyrosine kinase inhibitors (TKIs). These are small cell-permeant molecules that interact with the kinase region of the receptors, neutralizing their enzymatic activity. Most of these inhibitors act on the ATP binding site within the intracellular kinase domain of the receptor, and are therefore considered competitive inhibitors. Depending on whether the interaction is covalent or not, these inhibitors have been divided into reversible or irreversible competitors. Examples of the former are offered by lapatinib, erlotinib or gefitinib. Irreversible HER receptor inhibitors are exemplified by canertinib, pelitinib or neratinib. We will review these concepts and also will show how combination of anti-HER receptors with some novel biological inhibitors may be of therapeutic relevance in breast cancers overexpressing HER2.

NOTES

Studies of DYX1C1 a dyslexia candidate gene involved in neuronal migration and possible role in breast cancer

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Seven dislexia candidate genes (DCGs) have so far been identified: *DYX1C1, ROBO1, DCDC2, KIAA0319, MRPL19, C2ORF3* and *DGKI*. Four of these genes have been implicated in either neuronal migration or axon and dendrite guidance. Even though some common biological mechanisms have been identified, relatively little is known about their function.

The *DYX1C1* gene was identified and cloned by our group in 2003 as the first candidate gene for dyslexia(*1*). The DYX1C1 protein does not show homology to other proteins with known function, it contains a p23 domain and three tetratricopeptide repeat domains which play role in protein-protein interactions. RNA interference studies in rats have shown that down-regulation of DYX1C1 and two other dyslexia candidate genes DCDC2 and KIAA0319 alter neuronal migration(*2-4*).

Interestingly, others have shown that DYX1C1 interacts with the carboxy terminus of Hsc70-interacting protein (CHIP)(*5*); which plays a role in the degradation of the estrogen receptors. Taking these facts into account we investigated the role of DYX1C1 in this process and found that DYX1C1 interacts and down-regulates the estrogen receptors alpha and beta(*6*). As a surprise finding, others have shown that DYX1C1 is up-regulated in breast tumors (*7*) and that it can be implicated in colon cancer(*8*). Therefore, we are planning to replicate and further investigate the role of DYX1C1 in the etiology of these diseases.

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NOTES

Alteraciones Epigenéticas en genes ADAMTS de Metaloproteasas: Posibles Biomarcadores de Tendencia Metastática enm Cáncer Colorectal.

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ADAMTS es una familia de proteinas secretadas que presentan el dominio catalítico típico de metaloproteinasas. Se han encontrado alteraciones en la expresión de los genes ADAMTS en algunos tipos de cáncer, aunque el papel que juegan en el proceso de carcinogénesis no está todavía claramente definido. En un estudio de las alteraciones en número de copias cromosómicas en tumores colorectales, descubrimos pequeñas delecciones en regiones que comprenden dos miembros de ésta familia génica, ADAMTS6 y ADAMTS14. En paralelo, realizamos el estudio de alteraciones epigenéticas, encontrando otro miembro de esta familia, ADAMTS19, frecuentemente hipermetilado en tumores gastrointestinales.

El descubrimiento independiente de alteraciones genéticas y epigenéticas en varios miembros de la familia ADAMTS fue el punto de partida para un estudio en más profundidad de estos genes en distintos tipos de cáncer. Resultados preliminares demuestran que ADAMTS19 está hipermetilado más frecuentemente en tumores gastrointestinales que en tumores de mama y ovario. En éstos últimos, existe una estrecha asociación entre la hipermetilación de ADAMTS19 y el fenotipo mucinoso lo que puede utilizarse como biomarcador de diagnóstico para diferenciar cánceres de ovario de metástasis de cánceres gastrointestinales con fenotipo mucinoso. Además, la metilación del gen ADAMTS19 puede tener valor predictivo de la tendencia a mestastasizar al ovario, pero no al hígado, de células de cáncer del colon y recto.

NOTES

Major Vaults Protein (MVP): from multidrug resistance to DNA repair modulation

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Vaults are evolutionary highly conserved ribonucleoproteins particles with a hollow barrel-like structure. The main component of vaults represents the 110 kDa major vault protein (MVP), whereas two minor vaults proteins comprise the 193 kDa vault poly(ADP-ribose) polymerase (vPARP) and 240 kDa telomerase-associated protein-1 (TEP-1). Additionally, at least one small and untranslated RNA is found as a constitutive component. MVP seems to play an important role in the development of multidrug resistance. This particle has also been implicated in the regulation of several cellular processes including transport mechanisms, signal transmission and immune responses. Vaults are considered a prognostic marker for different cancer types. The level of MVP expression predicts the clinical outcome after chemotherapy in different tumour types. MVP plays an important role in human malignancies and it is related with chemotherapy response and patient prognosis. Recently, new roles have been assigned to MVP including the association with the insuline-like growth factor-1, hypoxia-inducible factor-1alpha, or Ku70/80. Tumours overexpressing MVP showed low levels of Ku70/80 and Bax expression. Furthermore, low Ku70/80 expression was associated with upregulated Bcl-2, altered p53, and increased cell proliferation. Tumour progression and resistance to chemotherapy and radiotherapy may be activated through the suppression of Bax and upregulation of IGF1R, resulting in increased proliferation and reduced apoptosis caused by upregulation of Bcl-2 an altered p53. MVP seems to play a central role in radiotherapy response, and may be associated with radiotherapy resistance. In conclusion, MVP seems to be more than a multidrug resistance protein, and it has been proposed as a useful prognostic factor associated with radiotherapy response and resistance.

NOTES

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Prostate Cancer. Treatment Advance

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Prostate cancer is the most common malignant tumor over 65 years in males, with a peak incidence at age 72. Death rates for this tumor gradually decreased since the FDA approval of the determination of PSA, although the incidence rate has not declined.
The confluence of early diagnosis and improved treatment has led to survival at 5 years has had an improvement of 35.7% over the past 25 years.
To this improvement has contributed radiotherapy techniques such as IMRT and IGRT and brachytherapy in the modalities of low and high dose rate. The combination with hormone therapy, in patients with high risk, has significantly increased survival rates at which we had with radiotherapy exclusively, which has introduced new strategies to improve both the intermediate as high risk patients.
The improvements of our current results are aimed at trying to characterize the risk groups to implement them more personalized treatments. In this direction are being investigated biomarkers that could determine the prognosis of patients with current standard treatments, early characterizing patients at high risk of failure.
The threshold of tolerance of tissues, with current doses of irradiation, is trying to improve to overcome the effect of irradiation with nano-particles or genes that contribute to improving both the local control and survival. All these topics are reviewed during the presentation.

NOTES

Doctoral thesis

Genetic and epigenetic changes in endometrial cancer

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Endometrial cancer (EC) is the most common pelvic gynecologic tumor in developed countries. It is usually diagnosed in early stages, but despite its good prognosis, approximately 20% of patients die from the disease. The prognosis and current treatment of EC is based on the use of clinicopathological variables such as grade, myometrial infiltration, histological type, etc. Along with these, have been described new molecular variables involved in the tumorigenesis and progression of this tumor type. Its use in clinical practice could be helpful to improve the classification of tumors, prognosis and treatment of patients.

The proposed objectives of this doctoral thesis were to estimate the incidence, the relationship with clinicopathological and molecular variables and survival of patients, of the following genetic and epigenetic changes: 1) mutations in the repeated tracts of EPHB2 gene and double strand breaks repair system components; 2) promoter methylation of EPHB2, EPHB4, SFRPs and PITX2 genes.

In conclusion, the repeated mononucleotides A9 tract in exon 17 of EPHB2 gene was altered in endometrial tumors with microsatellite instability (MSI). In gastrointestinal tumors was considered as MSI target gene, but not in EC due to their low mutational frequency.

In our series, double-strand breaks repair system genes with repeated mononucleotides tracts at coding regions were a frequent MSI mutational target. Simultaneously alteration in two or more double-strand breaks repair genes was associated with a worse condition of clinicopathological variables with clinical interest like undifferentiated histological grade, vascular invasion and more advanced stage.

In EC, the EPHB2 gene promoter was not methylated and EPHB4 methylation frequency was lower than those reported for colorectal tumors. However, it was associated with older patients and E-Cadherin gene methylation. In univariate analysis, EPHB4 methylation predicted a worse cancer-specific survival.

SFRPs genes promoter methylation was common in EC. The methylation percentages were higher in tumors than in healthy endometrial tissue for the genes SFRP1, SFRP2 and SFRP5, but not for SFRP4.The methylation of SFRP1, SFRP2 and SFRP5 genes was not related with clinicopathological variables studied or clinical outcome of patients. SFRP1 and SFRP5 genes methylation was positively associated with MSI. Furthermore, tumors with methylated SFRP1 had frequently mutated -Catenin and methylated APC genes. In univariate analysis, the co-methylation of SFRP1, 2 and 5 genes was related with advanced stage, myometrial infiltration, vascular invasion and short cancer-specific survival.

PITX2 gene promoter methylation level was higher in tumors than in healthy endometrial tissue and it was directly associated with MSI. The PITX2 gene methylation was a poor prognosis marker in the endometrial tumors group with diploid DNA content in both, univariate and multivariate analysis, which included the traditional clinicopathological variables.

NOTES

POSTER COMMUNICATIONS

IGF-1R expression predicts clinical outcome in oral carcinoma patients treated by surgery and radiotherapy

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Objectives: To assess the expression of IGF-1R in oral cavity squamous cell carcinoma (OCSCC) patients, to explore its relation to clinical and pathologic prognostic factors and its role in predicting clinical outcome.
Patients and Methods: One hundred and thirty one patients suffering from OCSCC were included in this study from July 1989 to April 2005. Follow-up was closed in May 2010. The mean follow-up for survivors was 110.26 ± 47.42 months. Patients were staged following the TNM classification. Patients with pathological stages I and II were referred to surgery. Patients in stages III and IV were referred to radiotherapy up to a mean dose of 62 Gy in 1.8-2 Gy fractions. IGF-1R expression was estimated by immunohistochemistry technique.
Results: IGF-1R was expressed in 101 patients (77.1%) and was related to pathological tumor grade (P=0.012). Tumor state and tumor grade were the most important prognostic factors for overall survival in multivariate analysis. IGF-1R expression was not a predictive factor in the whole series. In those patients presenting higher tumor stages (III-IV), IGF-1R expression was statistically significant for LDFS (P = 0.016), DFS (p=0.029), CSS (p=0.009) and OS (p=0.023).
Conclusion: Low IGF-1R expression is related with better long-term local control in patients suffering OCSCC patients in advanced stages of the disease (III-IV).

NOTES

Increased risk of breast cancer in women bearing a combination of long (CAG)n, (GGC)n and (TTTA)n repeats in androgen receptor and aromatase genes

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Microsatellites or simple tandem repeats are widely spread in the genome and may be located anywhere, including coding, regulatory or intronic regions. Some microsatellites show high levels of polymorphism. Variation in the length of these sequences may affect gene transcription, $_m$RNA stability, RNA splicing or protein structure and function.

Sex steroids hormones play an important role in breast cancer (BC) Some genes related to sex hormones metabolism like the aromatase enzyme (*CYP19*) or androgen receptor (*AR*) contain polymorphic microsatellites. The aromatase enzyme catalyses the conversion of androgens into estrogens in several tissues, including breast cancer. The intron 4 of *CYP19* contains the polymorphic microsatellite (TTTA)n. Androgens can act directly on breast cancer cells by binding to androgen receptors. Androgen receptor is present in the majority of breast cancer specimens and is a transcription factor which regulates the transcription of some genes related with diverse physiologic process as proliferation and cellular differentiation.

The AR gene contains in the exon 1 two polymorphic microsatellites (CAG)n and (GGC)n. Long (CAG)n repeats have been related to an increased risk of breast cancer, whereas the influence of (GGC)n and (TTTA)n repeats on breast cancer is still unclear. To investigate the possible association of the length of these polymorphic microsatellites with BC, a case–control study was carried out in 300 women with breast cancer patients at the Department of Oncology of the University Hospital Nuestra Señora de La Candelaria and 435 controls selected from the CDC Canary Island Cohort study Women with long medium allele for (CAG)n (average of both (CAG)n alleles > 22 repeats) have an increased risk of BC (OR = 1.51; CI95% = 1.08–2.16; p = 0.018). No differences were found for the medium allele of (GGC)n or (TTTA)n repeats when they were considered independently. When the length of (CAG)n, (GGC)n and (TTTA)n microsatellites was considered jointly women carrying longer medium alleles for the three microsatellites (CAG>22 repeats, GGC>=17 repeats y TTTA>7 repeats), showed the highest BC risk (OR = 2.89; CI95% = 1.28–6.53; p = 0.011).

Acknowledgements:To Instituto Canario de Investigación del Cáncer (ICIC)

NOTES

Synthesis and Molecular Modelling Analysis of Flavonoid Analogues as SERMs.

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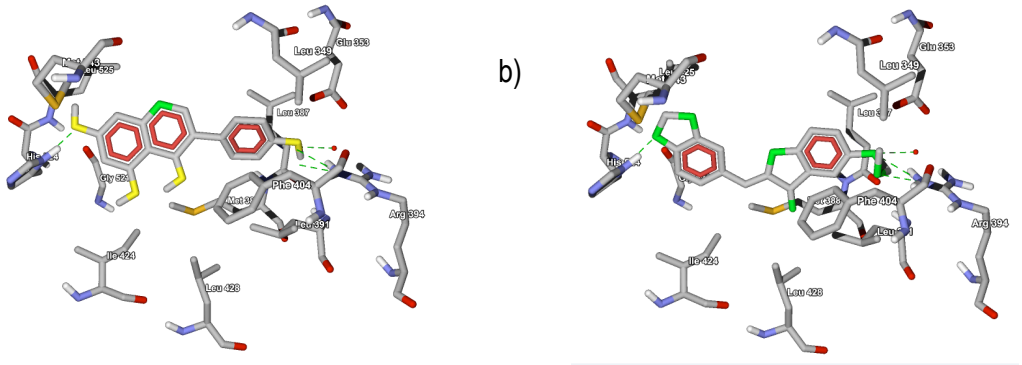
The endogenous steroid estrogen 17 β-estradiol (E_2) plays an important role in the development, growth, and function of a large number of tissues in both females and males[1,2]. The phytoestrogens are a group of naturally occurring compounds with estrogenic activity that are present in plants or that arise from bacterial or fungal metabolism that can mimic, and some cases antagonize, the effects of endogenous estrogen (E_2)[3]. In adults the phytoestrogens may have protective effects against certain forms of cancer, cardiovascular diseases and osteoporosis. Furthermore, some of them also prevent undesirable menopausal symptoms[4]. As a result of these potentially beneficial effects and as part of a project aimed at the development of selective estrogen receptor modulators (SERMs), in this communication we will report the synthesis of a series of flavonoid analogues through a biomimetic approach. Thus, the different types of flavonoids are obtained from oxidative cyclization of a 2'-hydroxychalcone type-precursor [6,7].
We will also report the key interactions of representative compounds into the binding pocket of estrogen receptors α/β.

a) b)



Acknowledgements: We thank to "Ministerio de Ciencias e Innovación" (Project SAF 2009-13296-C02-01) for financial support. A.A thanks to Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (CDCH, from UCV) for pre-doctoral fellowship.

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NOTES

In vitro activity of Statins against *Leishmania* sp.

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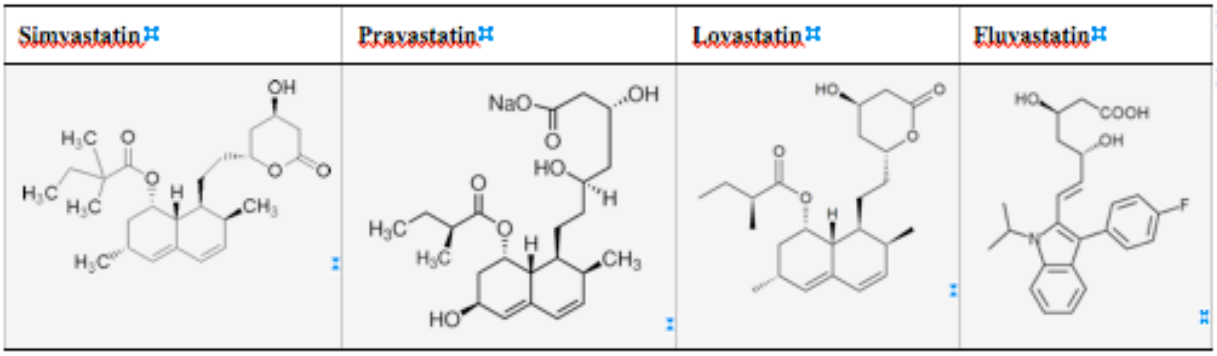
Statins are a family of lipid-lowering drugs widely used to control the cholesterol level and to prevent stroke and cardiac failure in patients at high risk of coronary artery disease. The mechanism of action is inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), the rate-limiting enzyme of the mevalonate pathway, which plays a central role in the production of cholesterol and others end products required for a number of essencial cellular functions. Recently, different statins have been used against some parasites such as Trypanosoma brucei, Schistosoma haematobium, Plasmodium falciparum and Toxoplasma gondii. Protozoa of the genus Leishmania are obligate intracellular parasites that are transmitted to the mammalian host by the bites of infected sand flies. In humans disease syndromes range from self-healing cutaneous lesions to debilitating mucocutaneous infections, subclinical viscerotropic dissemination, and fatal visceral involvement. Leishmaniasis has become an important emerging infectious disease in many developed as well as underdeveloped countries. The WHO estimates that 1.5 million ases of cutaneous leishmaniasis and 500,000 cases of visceral leishmaniasis occur every year in 82 countries. Estimates indicate that there are approximately 350 million people at risk for acquiring leishmaniasis, with 12 million currently infected. The current available treatments include pentavalent antimonials, amphotericin B, miltefosine, paramomicine and pentamidine. These drugs can be administered alone or in combination. However, combinations of these drugs even when their concentrations are not high, are normally highly toxic to the patient. Most of these treatments require several days of hospitalization because of its intravenous or parenteral way of administration. Nevertheless, the appearance of resistant strains to these active compounds is rising as a main problem in the current therapeutic measures against these parasites. In this work, a previosly developed colorimetric 96-well microtiter plate assay, based on the oxido-reduction of Alamar Blue® assay, was used, for the determination of four statins (Simvastatin, Pravastatin, Lovastatin and Fluvastatin) efficacy against Leishmania amazonensis and Leishmania donovani.



NOTES

Bioactive metabolites from the South American medicinal plant *Achyrocline satureioides.*

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Argentine folk medicine uses many medicinal species to counteract many diseases. *Achyrocline satureioides* (Lam.) D. C, a member of the family Asteraceae, is one of the most widely used species. In the last two decades, *A. satureioides* has been the subject of intense scientific research using both in vitro models and in vivo animal models, providing experimental evidence that extracts of this species have a broad spectrum of pharmacological and therapeutic properties. Among the properties registered so far, the most important activities are: antioxidant, antihyperglycemic, anti-inflammatory and immunomodulatory activity. Moreover several studies reported antiherpetic and anti HIV activity[1,2,3,4], cytotoxic effect on human hepatocellular carcinoma cell line, as well as antibacterial activity[1,2,3,4].

The determination of Minimum Inhibitory Concentration of bioactive plant extracts from *Achyrocline satureioides* showed a high activity against Gram-positive and Gram-negative pathogenic bacterial strains. In the present communication we will report the isolation and structural elucidation of metabolites from extracts of aerial parts of this species as well as the synthesis of some derivatives.

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Acknowledgements: To the "Ministerio de Ciencia e Innovación" (Projet SAF 2009-13296-C02-01) for financial support. C.N.C thanks the "CONICET-Consejo Nacional de Investigaciones Científicas y Técnicas" for the predoctoral grant.

NOTES

Screening of novel antitumoral JAK-STAT inhibitors using human erythroleukemia (HEL) cells

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The Janus kinases (JAKs) are an important family of four cytoplasmic tyrosine kinases, JAK1, JAK2, JAK3 and TYK2. JAK2 is involved in cellular growth factor signalling and play an essential role in cytokine signal transduction through the phosphorylation of specific STAT proteins. Deregulation of JAK2 by chromosomal aberrations may contribute to leukemogenesis. The recent identification of an activating mutation of JAK2 (V617F) in >95% of polycythemia vera patients, and ca. 50% of essential thrombocythemia and myelofibrosis patients has raised much interest in the discovery and development of selective JAK2 inhibitors as a potential targeted treatment for these patients. The human erythroleukemia cell line (HEL) carrying JAK2 (V617F) mutation is a useful model for screening potential JAK2 inhibitors. HEL cells were used here to screen the antitumoral activity of 21 novel compounds with structural analogies with known JAK/STAT inhibitors. STAT5 phosphorylation was inhibited in more than 95% by 9 compounds and in 40-75% by 8 molecules. Most (n=19) products inhibited STAT3 phosphorylation in more than 50%, and 8 of them provoked (almost) completed absence of phosphorylation. Six compounds completely inhibited both STAT5 and STAT3. Most products decreased cell viability (MTT assay) with an IC_{50} < 5μM (n=10 compound tested), and 3 molecules, those that completely inhibited STAT5 and STAT3 phosphorylation, decreased the cell viability with the lower observed IC_{50} (around 1μM). In conclusion, our data suggest that 3 out of 21 tested novel products may deserve future studies to know its possible antitumoral capability. [This research has been supported by Ministry of Education and Science (SAF2009-13296-CO2-02) and ICIC]

NOTES

In vitro activity of Statins against *Acanthamoeba castellanii* Neff

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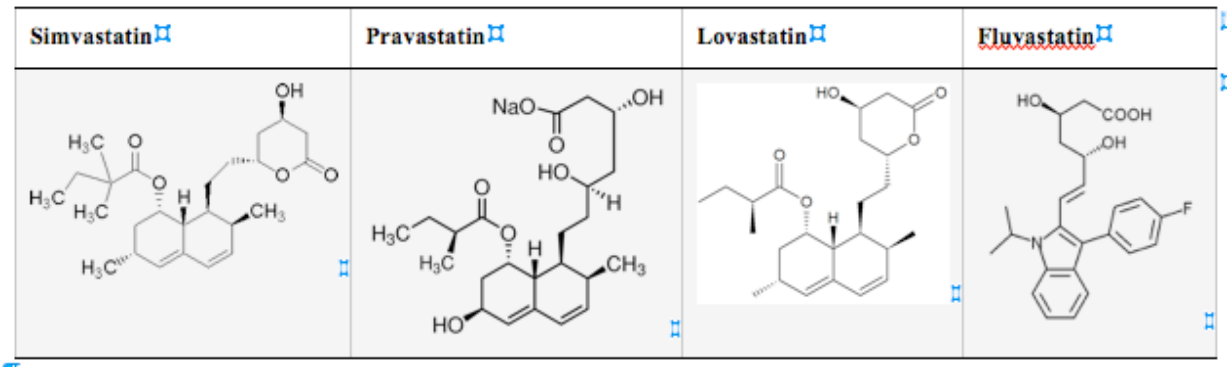
Statins are a family of lipid-lowering drugs widely used to control the cholesterol level and to prevent stroke and cardiac failure in patients at high risk of coronary artery disease. The mechanism of action is inhibiting the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA), the rate-limiting enzyme of the mevalonate pathway, which plays a central role in the production of cholesterol and others end products required for a number of essencial cellular functions. Recently, different statins have been used against some parasites such as Trypanosoma brucei, Schistosoma haematobium, Plasmodium falciparum and Toxoplasma gondii.

Free-living amoebae of the genus Acanthamoeba are ubiquitous protozoa that pervade the entire environment and include amphizoic strains that are pathogenic to humans and animals. These protozoa are opportunistic causal agents of a sight-threatening ulceration of the cornea called Acanthamoeba keratitis (AK), disseminated infections (mostly cutaneous and nasopharyngeal) and usually fatal Granulomatous Amoebic Encephalitis (GAE).

Present therapeutic measures for Acanthamoeba keratitis rely on topical applications of antimicrobials. However, the length of these treatments makes the process arduous and they are poorly effective against cystic stages of the protozoan, residual infection often remains even after treatment. No treatment against GAE has been established although therapeutic measures have been used with apparent effect as an adjunct to surgery. In this work, a previosly developed colorimetric 96-well microtiter plate assay, based on the oxido-reduction of Alamar Blue® Assay, was used, for the determination of four statins (Simvastatin, Pravastatin, Lovastatin and Fluvastatin) efficacy against Acanthamoeba castellanii Neff.



NOTES

Preparation of Nucleoside Analogues to Study Cytotoxic and Antiviral Activities

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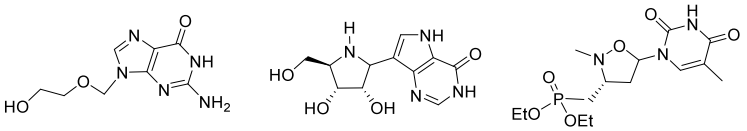
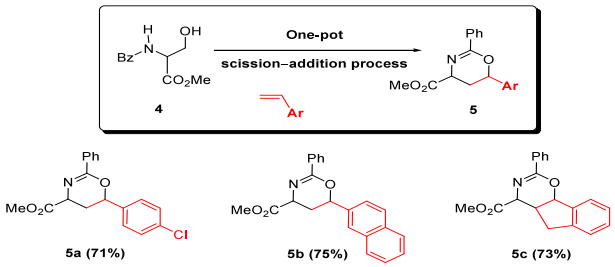
The preparation of nucleoside analogues has a great interest due to the pharmacological utility of these compounds,[1] which display antiviral,[1d,e] cytotoxic,[1f] antiparasitic,[1g] antifungal,[1g] and antibiotic activities.[1g] For example, acyclovir (**1**) is widely used for the treatment of herpes virus infections[1d,e]; immuncilin-H (**2**) inhibits the uncontrolled proliferation of T-cells[2]; and isoxazolidine **3** is a promising anti-HIV agent, comparable to AZT in potency but with low levels of cytotoxicity.[3]



acyclovir (**1**) immucilin-H (**2**) **3** antiviral (anti-HIV)

In this research line, our group has elaborated different derivatives, such as *N*- and *C*-acyclic nucleosides (analogues of acyclovir **1**), and *N*- and *C*-azanucleosides (analogues of immunocyline **2**).[4] In this work, we present some novel *C*-azanucleosides such as compound **5** (Scheme), prepared from simple substrates (amino acid derivatives **4**). These compounds could have either cytotoxic or antiviral activities; therefore, these products will be classified according to their cytotoxic and antiproliferative properties. Those with a significative cytotoxicity will be further studied as antitumorals, and those with low cytotoxicity will be tested as antivirals (to ensure a good therapeutic window).



In a first set of experiments, using breast cancer MCF-7 cultured cells, we have investigated the cytotoxic and antiproliferative properties of twelve nucleoside derivatives used at different doses (1nM, 10 nM, 1 mM, 5 mM, 10mM). Cell viability quantification demonstrated that no significant toxicity was observed at any of these concentrations, indicating that these derivatives do not cause any significant mortality in this cell line. These data suggest that these molecules do not have either any cytotoxic nor proliferative properties, and may therefore serve as potential antiviral compounds with therapeutical applications. The antiviral assays are presently in process.

Acknowledgements: This work was supported by Projects CTQ2009-0719 and FEDER (AB), SAF2010-22114-C02-01/02 (MD and RM) and FICIC-GI 18-12-2009 (MD and RM) from Plan Nacional I+D, MICINN, Spain. JAG is a JAE-postdoctoral fellow (CSIC) and CF holds a research fellowship from ICIC.

NOTES

Morphological Effects of Tamoxifen Derivate FL-TX on Mice Uterus

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Tamoxifen is one of the first selective estrogen receptor modulators (SERM) which has been used in clinical therapeutics as adjuvant for breast cancer since early 1980s. However, evidences have steadily grown that continual administration of therapeutic doses of this molecule can provoke some adverse health effects including the increased risk of uterine cancer. Therefore, the research of novel tamoxifen derivatives lacking these side effects may constitute a promising alternative therapeutical approach. In this sense, we have performed different uterotropic bioassays on immature Swiss CD-1 female mice (18-19 days old) subcutaneously injected for three consecutive days with 17b-estradiol (1 mg/kg/day), tamoxifen (1mg/kg/day), the first fluorescent tamoxifen derivative (FL-TX, 1mg/kg/day) and vehicle (3.3% ethanol in olive oil, 10 ml/kg/day). Following treatments, we compared different estrogen-responsive endpoints in the uterus: epithelial cell height from uterine horns as a parameter of hypertrophy; number of luminal epithelial cells as a parameter of hyperplasia; and gland number. Results demonstrated that, as compared with the uterotropic effects of either estradiol or tamoxifen, uterus of FL-TX treated mice did not show any significant increase in any of these morphometric endpoints. Instead, we observed similar uterotropic values than vehicle-treated animals. These data indicate that, unlike tamoxifen, FL-TX does not enhance estrogenicity in the mouse uterus, and suggest that this tamoxifen derivative might have potential properties as an alternative adjuvant in tamoxifen therapies.

Acknowledgements: This work was supported by grants CTQ2009-0719 and FEDER (AB), SAF2010-22114-C02-01/02 (MD and RM) and FICIC-GI 18-12-2009 (MD and RM). JM holds a research fellowship from FICIC with funds from ACIISI (Spain) and FSE (EU).

NOTES

How to write in English? (From a Spanish mind to an English paper)

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All of us, I mean, people working on science and/or research, to a greater or lesser extent, has to deal with an additional task: writing and presenting our results in English. However, not being considered within the scientific curricula in Spain, science students do not receive sufficient help with their English writing problems at university. So, there is a steadily increasing need to learn English writing.

This written communication attempts to provide non-native speakers of English with some pieces of advice in relation to writing and presenting skills, namely, those to help them improve coherence within paragraphs as well as between paragraphs (information structure and linking strategies), to tidy up their language and style in order to help their readers follow and understand their line of thought, to give arguments and to present data, and, ultimately, to create an effective conference presentation.

NOTES

ShK toxin domain in nematode metalloprotease has a pharmaceutical potential

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Steinernema carpocapsae is a nematode high virulent to insects. During the initial phase of parasitism the nematode excretes a set of proteins that have been characterized by our group and shown to have a role in infection. Interestingly some of these proteins present multifunctional functions that were attributed to the presence of different domains. Here we describe the K^+ channel-blocking toxin domain in a *S. carpocapsae* secreted metalloprotease. Based in EST sequences a 1,583 nucleotides cDNA encoding for a putative metaloprotease (Sc-NAS) was amplified from the parasitic stage. Sequence analyses predicted a zinc-binding motif and a methionine turn motif signatures for the astacin domain (Jing et al., 2009). With the catalytic domain, a small C-terminal domain (38aa) with six cysteines was identified, which is distinct from other those find in other nematode astacins. Instead, similar domains have been described in sea anemone toxins, named ShK, that are specific for potassium channels. The location of the three disulfide bridges, common to all members of this family, is also shown in Sc-ShK. Surprisingly, this *S. carpocapsae* domain shows closely structural similarity with the human 2K72 (that blocks K+ channels Kv1.6 and Kv1.3) than with the ShK toxin from the hydrozoan. Expression analyses indicated that Sc-ShK was up-regulated in parasitic stage and strongly induced in vitro by insect tissues, thus suggesting it has a role in the parasitic process. Recombinant ShK domain was produced in *E.coli* system, purified by affinity chromatography and used to functional assays. *In vivo* assays the recombinant ShK domain was able to paralyse insect larvae, causing tumescence and leading to the release of hemolymph by spiracles, indicating that recombinant Sc-ShK is active and probably play a role in the disturbance of insect ionic homeostasis. The voltage-gated K+ channel Kv1.3 sea anemone ShK is currently under evaluation for the development of biopharmaceuticals for the treatment of T-cell mediated autoimmune disorders such as the multiple sclerosis (Norton et al, 2004). The searches for ShK analogs have gained an increasing interest, such as 2K72 that blocks Kv1.3 with picomolar affinity and high selectivity (Rangaraju et al., 2010). The homology of Sc-ShK with the human 2K72 encourages our studies on the activity of Sc-ShK using BBMV and animal models to provide a picture in its potential as a pharmaceutical.

Jing Y, Toubarro D, Hao Y, Simões N. 2010. Cloning, characterization and heterologous expression of an astacin metalloprotease, Sc-AST, from the entomoparasitic nematode Steinernema carpocapsae. Mol Biochem Parasitol. 174:101-8.
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Rangaraju S, Khoo KK, Feng ZP, Crossley G, Nugent D, Khaytin I, Chi V, Pham C, Calabresi P, Pennington MW, Norton RS, ChandyKG. 2010. Potassium channel modulation by a toxin domain in matrix metalloprotease 23. J Biol Chem. 285:9124-36.

NOTES

Histo Blood Group Antigens and P16: Potential Biomarkers of Cervical Intraepithelial Neoplasia

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Expression of blood group related antigens and p 16 in normal, in displastic and tumoral uterine cervix from 44 hysterectomised women with carcinoma of the cervix was investigated; the results were correlated with patients´ABH phenotype and secretor status. We used an indirect immunoperoxidase technique and a panel of monoclonal antibodies directed against different blood group related antigenic specificities and p16.

Anomalous expression of blood group antigens in premalignant lesions from cervix was found. Partial loos of expression of blood group antigens in different grades of cervical intraepithelial neoplasia, and total loss of expression in CIN III and in infiltrating carcinoma of the cervix from secretor patients was revealed. This study investigates the loss of presence of blood group antigens, the expression of p 16 and the correlation between these two findings. We found anomalous expression of blood group antigens in the areas of CIN I, CIN II and CIN III of the cervix. In secretor individuals the normal exocervix was positive and showed the presence of these correspondent ABH antigens in all cases, but in some adjacent areas of CIN I and CIN II a partial loss of blood group antigen expression was found. This results correlated well with the grade of intraepithelial neoplasia. We have found overexpression of p 16 in cervical squamous cell carcinoma and diffrente grades of Dysplasias These changes were the opposite observed in the expression of blood group antigens son that both biomarkers showed a mirror-like staining pattern when they were applied on the same biopsy section. Alteration of blood group-related antigens and overexpression of p 16 might be useful in the diagnosis and prognosis of premalignant lesions and carcinoma of the cervix These finding herein described confirm the importance of these antigens as tumour biomarkers and they might be useful for the study of cervical carcinogenesis.

NOTES

Three component Nitro-Manich Reaction operating "on water". A convenient access to biological relevant 1,2-diamins.

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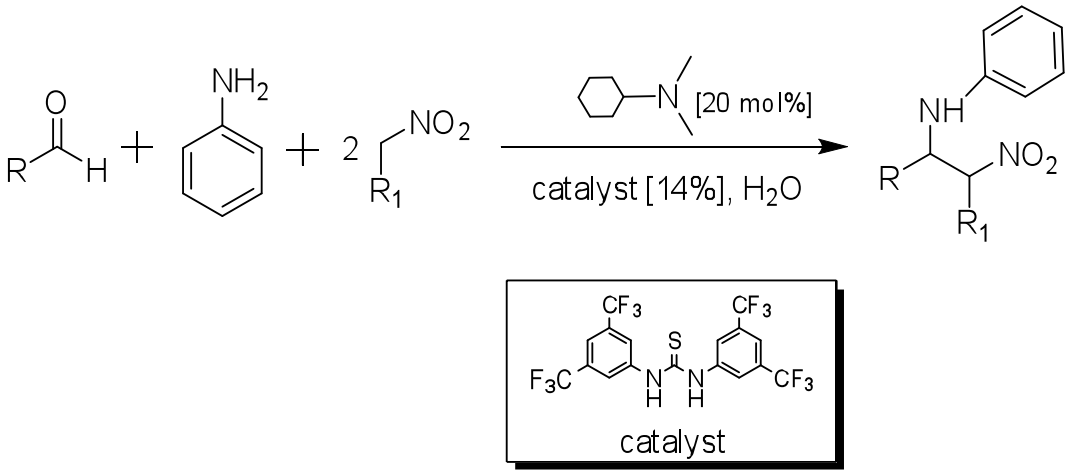
Herein we present an efficient multicomponent Nitro-Manich5 reaction with a wide scope in the aldehyde component. The manifold operates under "on water" conditions6 and involves the three-component reaction of aniline, an aldehyde and an alkyl-nitro in the presence of catalytic amounts of cyclohexyldimethylamine (Lewis base) and a thiourea-derivatived (catalyst). The presence of water and thiourea is essential for the reaction; whereas the water catalyzes the formation of the intermediate imine, the thiourea activates7 the nitro-anion by H-bonding formation.



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NOTES

Microwave-assisted Diversity-oriented Domino Synthesis of Functionalized Nicotinic Acid Derivatives

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A survey of methodologies have been described for the general access to functionalized pyridine rings. Among them, modern (catalyzed) versions of the Bohlmann-Rahtz heteroannulation reaction involving b-aminoacrylates (conjugated enamines) and conjugated alkynes. Recently, Rodriguez and col. have reported a regioselective multicomponent synthesis of functionalized nicotinic acid derivatives. In spite of these advances, there is still a need for the implementation of novel diversity-oriented synthetic methodologies for the controlled access to these heterocycles with structural (functional) diversity and wide substitution patterns decorating the ring. Therefore, the use of commercially available or synthetically simple starting materials and bench-friendly and environmentally benign reaction processing are important reaction values that should also be appropriately taken into account by these strategies. We have described the microwave-assisted diversity-oriented synthesis of functionalized alkyl nicotinates, from propargyl vinyl ethers, via a complex and efficient domino manifold involving at least five distinct and discrete chemical steps: [3,3] propargyl enol ether rearrangement; (ii) 1,3-protropic isomerization; (iii) condensation; (iv) 6-aza-electrocyclization; (v) elimination. Furthermore, the propargylic platforms are rapidly and easily assembled from commercially available or simply accessed materials. The obtained alkyl nicotinates feature a maximum of two diversity points at the ring and one appended chemical handle for further elaboration (ester functionality).

The reaction is fast, economical, bench-friendly and environmentally benign. These practical advantages convert this approach in a good alternative to other well-known methods to rapidly generate libraries of functionalized nicotinate derivatives **6** to use in drug discovery programs.

Acknowledgements: This research was supported by the Spanish Ministerio de Ciencia e Innovación, the European Regional Development Fund (CTQ2008-06806-C02-02) and the Spanish MSC ISCIII (RETICS RD06/0020), FUNCIS (REDESFAC PI01/06) and the Fundación Instituto Canario de Investigación del Cáncer (FICI-G.I.N808/2007). G.M.-A. thanks Spanish MEC for a FPU grant. Authors thank technician Ms. Anna Jurado Varona for her experimental assistance.

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NOTES

Preparation of Benzimidazoles with Structural Complexity through a Domino Process.

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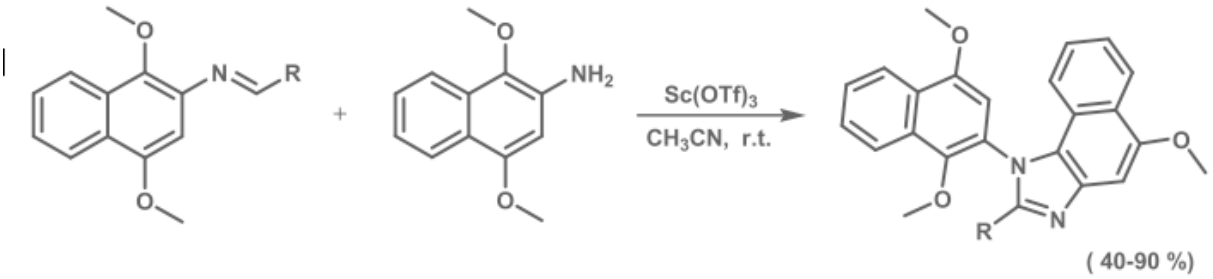
Heterocyclic compounds offer a high degree of structural diversity and opportunities for the discovery of new drug candidates because of their ability to bind to multiple receptors with high affinity and favorable pharmacokinetic properties. In particular, N-containing polycyclic structures have been reported to be associated with a wide range of biological activity.[1-4]

In this communication we describe the synthesis of a variety of disubstituted naphthoimidazoles using 1,4-dimethoxynaphthalen-2-amine and several imines in the presence of $Sc(OTf)_3$. The imines were formed from the mentioned 1,4-dimethoxynaphthalen-2-amine and aromatic aldehydes. The obtained naphthoimidazoles and their derivatives combine two privileged structures which convert them into potential bioactive compounds.



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NOTES

Taking advantage of the pathogenic molecules of *S. carpocapsae*

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Entomopathogenic nematodes have evolved sophisticated strategies to overcome host defences, to interact with the immune system and to interfere with essential host systems. We are trying to prove that they can be a valuable reservoir of biotechnological systems and tools. "Patho-biotechnology" is the rising approach that is used to describe the exploitation of pathogenic organisms, or their virulence strategies (e.g. immunomodulation and evasion, survival strategies), for beneficial applications both in industry and biomedical applications. *Steinernema carpocapsae* is an entomopathogenic nematode that lives associated with the bacterium *Xenorhabdus nematophila*. The first analysis of cDNA transcripts expressed in the parasitic phase of this nematode allowed the identification of two ESTs (Sc346 and Sc926) with homology to saposins-like proteins that we cloned and sequenced. Sc346 (GenBank Accession Nº HQ441750) was characterized as a poreforming protein based on sequence and structural analysis. It has a high gene expression at L4 stage of the nematode, suggesting its relation with the defence control of the nematode against the exponential growth of its symbiotic bacteria inside the parasitized insect. Sc926 (GenBank Accession Nº HM028668) was identified, by structural and sequence homology, as a prosaposin. Its high gene expression levels at the parasitic stage of the nematode raise the hypothesis that it has an important role in the insect parasitism, by perturbing the membranes of the insect intestinal cells thus facilitating the invasion of the host. In order to investigate the function of each protein, recombinants Sc346 and Sc926 were produced in *E.coli* system and purified by affinity chromatography. The potential functions of these two pathogenic proteins will be proved by functional assays. It's our conviction that they can be valuable tools for genetic engineering, biotechnology or even clinical applications.

NOTES

Synthesis and anti-inflammatory activity of *ent*-kaurene derivatives

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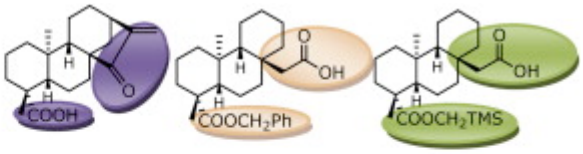
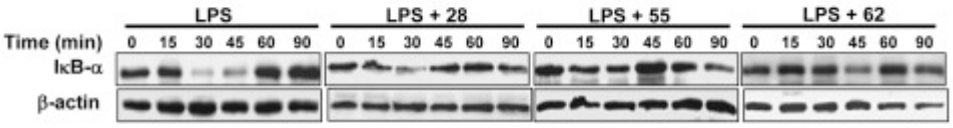
A series of kaurene derivatives (**1**–**63**) were prepared and evaluated for anti-inflammatory activity[1,2]. Thirteen of the tested compounds were able to inhibit NO production with an IC_{50} between 2 and 10 M. Compounds **11**, **12**, **14** and **23** showed low percentage of cell viability, whereas compounds **9**, **10**, **17**, **28**, **37**, **48**, **55**, **61** and **62** were non-cytotoxic at the concentration up to 25 M. Some structure–activity relationships were outlined. Compounds **28**, **55** and **62**, were selected as representative compounds and they potently inhibited the protein expression of NOS-2. We also determined that inhibition of NF-B activation might be the mechanism involved in anti-inflammatory effects of these kaurene derivatives. As expected, cytokines IL-6, IL-1, TNF- and IFN- were downregulated in the presence of compound **28**, **55** and **62** after stimulation with LPS. These results indicate that kaurene derivatives might be used for the design of new anti-inflammatory agents.





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Acknowledgements: To "Ministerio de Ciencia e Innovación" (Proyect SAF 2009-13296-C02-01) for financial support. IHF thanks the "Ministerio de Ciencia e Innovación" for the predoctoral grant.

NOTES

Synthesis and effects on cell viability of flavonols and 3-methyl ether derivatives in human leukemia cell lines

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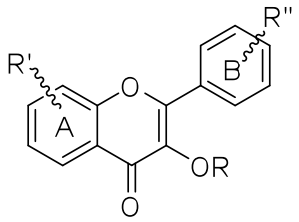
Polyphenolic compounds are of great current interest due to their possible anticancer activities. In previous studies with naturally occurring and semisynthetic phenyl-benzo-γ-pyrones, we showed that methylation of hydroxyl group at position C3 of this skeleton yields a compound with a higher antiproliferative activity in several cancer cell lines [1].

In this study we have synthesized 27 compounds with a phenylbenzo-γ-pyrone core structure (**1**) containing a hydoxyl or a methoxy group at position C3 and analyzed their cytotoxicity against HL-60 and the mitoxantrone resistant HL-60/MX1. A compound containing a halogen on position 4´of the B ring (2-phenyl group) was the most cytotoxic compound in both cell lines, with IC_{50} values of approximately 5 μM. The cytotoxic effects of the selected compound was accompanied by a concentration- and time-dependent appearance of apoptosis as determined by DNA fragmentation and sub-G1 ratio and associated with caspase-3 activity and poly(ADP-ribose)polymerase cleavage. The findings of this study suggest that the chemical synthesis of this kind of compounds might allow the discovery and development of novel anticancer agents.



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Acknowledgments: This work was supported by a Grant from the Ministry of Science of Innovation of Spain and the European Regional Development Fund (SAF2010-21380 to FE), a Grant from CSIC (Proyecto Intramural de Incorporación - 2007022) and Agencia Canaria de Investigación, Innovación y Sociedad de la Información (FEDER) (C200801000174).

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NOTES

Productive CD4-dependent HIV-1 fusion, entry and infection dynamically studied by Total Internal Reflection Fluorescence Microscopy in living cells.

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Total Internal Reflection Fluorescence Microscopy (TIRFM) can dynamically study, at the plasma membrane, the fate of internalization or export of different cargos or cell-surface molecules. In this work, we aimed to apply the TIRFM technology to the study of HIV-1 fusion and entry process, determining the function of Arf6-mediated membrane dynamics on HIV-1 early infection. TIRFM studies of the CD4-dependent HIV-1 uptake process were performed by using fluorescent HIV-1-Gag-EGFP viral particles in permissive TZMbl (CD4+/CXCR4+/CCR5+) cells, transiently expressing the fluorescent CD4-DsRed molecule together with one of the different Arf6-HA construct and the PH-ECFP probe. We first observed that TZMbl cell over-expressing the Arf6-Q67L and Arf6-T44N constructs presented accumulation of PIP_2-associated structures on plasma membrane where these mutants distributed. Indeed, we observed that wt-Arf6-, Arf6-Q67L- and Arf6-T44N-ECFP constructs did not co-localize with cell-surface CD4-DsRed, and that HIV-1 binding to CD4 did not promote co-distribution of virus-bound or free CD4 with Arf6 constructs. Arf6 mutants did not affect the formation or the trafficking of clathrin-coated structures (CCS). Our results indicated that alteration of Arf6-mediated PIP_2-membrane dynamics at regions of HIV-1/CD4 interaction, by over-expressing Arf6-Q67L-HA or Arf6-T44-HA mutant or Arf6 knock-down, prevented productive CD4-dependent HIV-1 uptake and infection (BlaM-Vpr virions), without affecting the first HIV-1/CD4 interaction. Therefore, we propose that TIRFM is a powerful and appropriate technology to study early HIV-1 infection events, which require Arf6-coordinated plasma membrane dynamics to promote viral fusion and entry, in a clathrin independent manner.

NOTES

Unraveling the role of DNA metabolism in chromosome segregation in *Saccharomyces cereviciae*

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Aneuplody is one of the main characteristics of cancer cells. Failure in sister chromatids segregation has been proposed as one of the main mechanisms leading to aneuplody. The causes that maintain sister chromatids linked at the time of anaphase are unknown. Here we present a yeast model in which we can follow chromosome segregation identifying both daughter cells just before cytokinesis. With this model we are investigating the role of different proteins involved in DNA damage repair, resolution of recombination intermediates or replication termination under conditions of incremental DNA damage. We will present the latest results of our study focused on the helicase superfamily.

NOTES

Cytotoxic Activity of Terpenes and Flavonoids Isolated from *Pulicaria burchardii* and *Pulicaria canariensis*

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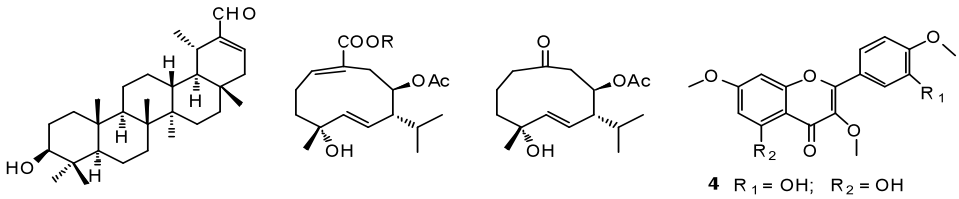
The *Pulicaria* genus (Asteraceae: Inuleae, Inulinae) consists of about 100 species distributed throughout Europe, North Africa, Canary Islands, Cape Verde and Asia, which are used as traditional herbal medicines [1]. In the Canary Islands this genus is represented by two species located in the eastern islands (Lanzarote and Fuerteventura): *Pulicaria burchardii* Hutch and *Pulicaria canariensis* Bolle, the latter having two endemic subspecies *canariensis* and *lanata* [2]. As a part of our continuing search for novel, plant-derived cancer chemotherapeutic agents and our systematic investigation of the composition of Canarian endemic plants [3], we have studied the constituents of two species of the *Pulicaria* genus. Here we report the isolation of two new compounds, the sesquiterpene (1*E*,5*E*)-8-acetoxy-4-hydroxy-7H-germacra-1(10),5-dien-14-oic (**2**) and a nor-sesquiterpene (5*E*)-8-acetoxy-4-hydroxy-7H-germacra-5-en-10-one (**3**), from *Pulicaria canariensis* ssp. *lanata* along with ten known compounds, including the flavonoid 5,3´-dihydroxy-3,7,4´-trimethoxyflavone (**4**). From *Pulicaria burchardii* we identified seven known compounds. The physical and spectroscopic data of the triterpenoid 3-hydroxytaraxaster-20-en-30-al (**1**) are reported. The structures of compounds **1**-**3** were determined on the basis of HR–MS, and 1D– and 2D– NMR studies. The structure of **2** was corroborated by crystal X-ray diffraction. Cell viability experiments reveal that the semi-synthetic flavonoid (**4b**) was the most cytotoxic compound against human leukemia cells and the cytotoxicity was caused by induction of apoptosis as determined by microscopy of nuclear changes.



1

2 R = H
2a R = CH_3

3

4 R_1 = OH; R_2 = OH
4a R_1 = OAc; R_2 = OH
4b R_1 = OAc; R_2 = OAc
4c R_1 = OMe; R_2 = OH
4d R_1 = OMe; R_2 = OAc

Acknowledgment. This work was supported by a Grant from the Ministry of Education and Science of Spain and from the European Regional Development Fund (SAF2007-62536) to F.E, Instituto Canario de Investigación del Cáncer to F.L. and Agencia Canaria de Investigación, Innovación y Sociedad de la Información (FEDER) (C200801000174) to J.T. F.L. was supported by JAE-doctoral Program from the Ministerio de Ciencia e Innovación.

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NOTES

Synthesis and cytotoxic activity evaluation of four novel spirostan saponins

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Saponins are triterpene or steroid glycosides that are commonly distributed in plants and some marine organisms. A lot of saponins have been obtained from natural sources and by chemical synthesis, displaying remarkable structural diversity and a wide spectrum of biological activities.[8] Spirostan saponins are the largest class of the steroidal saponins, which have spirostan aglycones and sugar substitutions primarily at the 3-OH group of the steroid unit. It was recently found that a quite common feature of spirostan saponins is their inhibitory activity against the growth of some tumor cell lines.[9] These saponins exerted their antitumor effect by inducing apoptosis in cancer cells.[10] Among a great number of naturally occurring spirostan saponins, dioscin (**Figure 1**) is best known to have these properties.

On the other hand, some studies of saponins structure - activity relationships have demonstrated that both the aglycone and the sugar moiety play an important role in the biological activity.[11] Herein we report the design and synthesis of four novel spirostan saponins (**1-4**) employing dioscin as a lead structure. Saponins **1** and **2** contain the same dioscin trisaccharide but the aglycone has hydroxyl and/or carbonyl functionalities at different positions. The other dioscin analogues (**3** and **4**) wich have the same steroid include a different trisaccharide pattern.

Additionally, we have tested these compounds against human leukemia cell lines in order to study their cytotoxic activity.

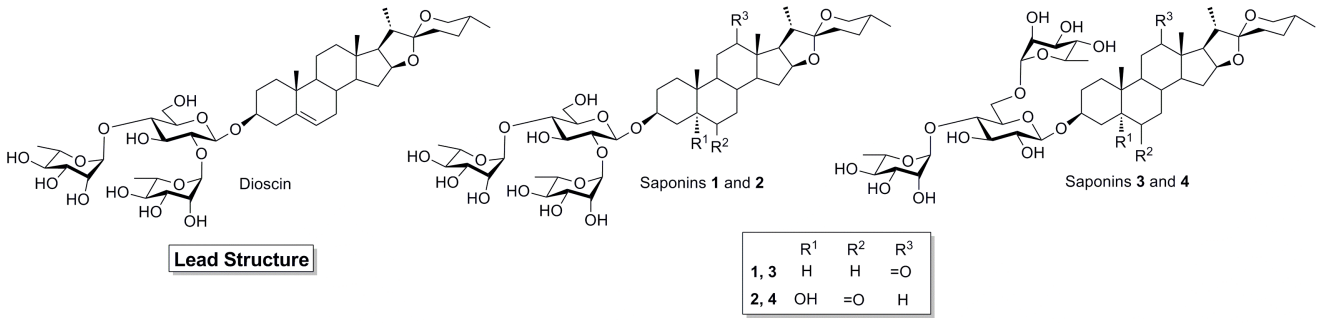

Figure 1- Dioscin as lead structure for the synthesis of four novel spirostan saponins (**1**, **2**, **3** and **4**)

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NOTES

HIV-1 requires Arf6-mediated membrane dynamics to efficiently enter and infect T Lymphocytes

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As the initial barrier to viral entry, the plasma membrane along with membrane trafficking machinery and cytoskeleton are of fundamental importance in the viral cycle. However, little is known about the contribution of plasma membrane dynamics during early HIV-1 infection. Considering that Arf6 regulates cellular invasion via several microorganisms by coordinating membrane trafficking, our aim was to study the function of Arf6-mediated membrane dynamics on HIV-1 entry and infection of T lymphocytes. We observed that an alteration of the Arf6-GTP/GDP cycle, by GDP-bound or GTP-bound inactive mutants or by specific Arf6 silencing, inhibited HIV-1-envelope-induced membrane fusion, entry and infection of T lymphocytes and permissive cells, regardless of viral tropism. Furthermore, cell-to-cell HIV-1 transmission of primary human CD4+ T lymphocytes was inhibited by Arf6 knock-down. Arf6 silencing or its mutants did not affect fusion, entry and infection of VSV-G pseudotyped viruses or ligand-induced CXCR4 or CCR5 endocytosis, both clathrin-dependent processes. Finally, we use non-replicative, X4- or R5-tropic HIV-1 viral particles containing BLaM-Vpr to study early viral fusion entry in Arf6 Knockdown CD4+ T lymphocytes. Our results show that specific Arf6 silencing significantly inhibited viral fusion and entry, regardless of viral tropism, without affect entry of VSV-G pseudotyped virus. Therefore, we propose that efficient early HIV-1 infection of CD4+ T lymphocytes requires Arf6-coordinated plasma membrane dynamics that promotes viral fusion and entry.

NOTES

Microwave Assisted Domino Rearrangement of Propargyl Vinyl Ethers: Synthesis of Functionalized Aromatic Compounds

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Propargyl vinyl ethers (PVEs) constitute a privileged group of small size, structurally simple, readily available, and densely functionalized scaffolds. Efforts from our group, and others, have revealed the synthetic potential of these platforms in accessing important heterocyclic cores. The key to the chemical reactivity encoded in these structures is the [3,3] propargylic sigmatropic rearrangement. In our laboratory we have already transformed these units into 1,2-dihidropyridines[1a] and nicotinic acid (3-pyridine carboxylic acid)[1b] derivatives. Now, a novel reactivity profile of propargyl vinyl ethers has been developed, which is controlled by the presence of a hydrogen atom at the homopropargylic position. This strategy has been conveniently used to construct multifunctionalized phenolic platforms, including salicylaldehydes, and the corresponding ketone derivatives.[2]

Acknowledgments: This research was supported by the Spanish and European MICINN RDF (CTQ2008-06806-C02-01 and CTQ2008-06806-C02-02), MSC projects ISCIII (RETICS RD06/0020/1046) FUNCIS (REDESFAC PI01 / 06). G. MA and LCM thank MEC for the FPU and FPI fellowships, respectively. The authors thank Ms. Ana Jurado for the preparation of starting materials.

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NOTES

Searching for cytotoxic activity in *Bacillus thuringiensis* isolated in São Miguel.

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Some *Bacillus thuringiensis (Bt)* isolates express crystals with proteins named parasporins that have cytotoxicity against specific human tumor cell lines. Usually parasporins are found to be non-insecticidal and non-hemolytic and preferentially kill human cancer cells. Thus we decide to screen *Bt* parasporal inclusion bodies with cytotoxic activity in São Miguel *Bt* collection. We found about 180 *Bt* isolates with bipyramidal shape, 45 presented spherical morphology and 23 were amorphous. Bacteria were grown on T3 medium for 3 days at 30ºC to obtain crystals that were isolated by centrifugation. To extract crystal proteins, the mixture was suspended in 50 mM Na2CO3 (pH 10.0) + 10 mM dithiothreitol + 1 mM EDTA at 37°C for 1 h and the supernatant was passed through a membrane filter for sterilization. Protein solutions (pH 10.0) were then treated with proteinase K (60 g/ml) for 90 min at 37°C. Phenylmethylsulphonyl fluoride was added to the solution to stop the proteolytic reaction. The cytotoxic essays were performed on a 96-well plate. Each well contained 2×104 HeLa or Vero cells incubated at 37°C for 16 h. 10 l protease-treated protein solution (0.6 mg/ml) was added to each well. After 24h incubation, cytotoxicity was assessed with a cell proliferation test using an MTT assay. So far we tested 20 isolates and 3 showed some cytotoxic activity against HeLa cells, one bipiramidal crystal (S8A), and two spherical (S27D, S35E), with 23%, 22% and 34% respectively. Differential cytotoxicity was observed in activated proteins from the bipyramidal crystal (S8A), these proteins preferentially target HeLa cells. These 3 crystals were screened for insecticidal activity against Lepidoptera and Coleoptera insects, and as expected did not presented any activity. Based in analysis of the SDS-PAGE profiles a protein with 57KDa emerged as a candidate to support cytotoxic activity. This protein is being prepared to be analysed by MS/MS. Amino acid sequences will be used to produce degenerate primers to identify the encoding gene. Concerning S8A proteins they are being purified by chromatography and fractions tested to cytotoxic activity.

NOTES

Socs2 Knockout mice are protected against hepatic steatosis in high fat diet but also are more insulin resistant.

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Socs2 a member of the Suppressor of Cytokine Signalling protein family acts as a negative regulator of the GH receptor. GH is the key regulator of the body size in mammals and is an important regulator of lipid and glucose metabolism, GH has diabetogenic action inducing insulin resistance. Socs 2 deficient mice are characterized by a 30-50% increase in body weight in the absence of increased circulating levels of GH or IGF-1 (1). Gigantism in Socs2 Knockout mice is dependent of endogenous production of GH, despite of the GH dependent gigantism observed in Socs2 Knockout, these mice do not show obvious alteration in insulin sensitivity (2) in contrast to GH transgenic mice which show marked insulin resistance (3). Because of the positive profile in relation to glucose control observed in Socs2 Knockout mice in comparision to GH transgenic mice, we decide to analyze the metabolic response of Socs2 Knockout mice to High fat diet. Our findings show that Socs2 Knockout mice are protected from liver steatosis induced by high fat diet. On the other hand, Socs2 Knockout mice show a more severe induction of insulin resistance upon High fat feeding compare to control mice.

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NOTES

Potential application of *Hedychium gardnerianum* on Alzheimer's Treatment

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Some species of Zingiberaceae have been the subject of a range of chemical and pharmacological investigations due to their significance in traditional medicine. The properties attributed to these plants are due to their richness in active compounds, such as terpenes and terpenoids. Cholinesterase inhibitors were introduced in the therapy of Alzheimer Disease in the 1990s. The hopes and interest raised by these drugs are well demonstrated by the 41,370 references listed by PubMed under 'Acetylcholinesterase inhibitors'. In particular, the scientific community is searching for novel acetylcholinesterase inhibitors displaying less secondary effects.

Previous studies on *H. gardnerianum* bioactivity showed that its essential oils had a higher content of -pinene,-pinene and -cadinol and exhibit a significant antimicrobial activity against *S. aureus* and *S. epidermis*. Antithrombin activity was also reported in extracts of the aerial part of this plant while the villosin, a labdane-type diterpene isolated from rhizome, showed a very high and selective cytotoxicity activity against NCI-H187 (small cell lung cancer).

As part of a study whose main objective is the discovery of potential commercial uses of the Azorean invasive species, the acetylcholinesterase inhibition (Anti-AChE) properties of the methanol and dichloromethane extracts from *H. gardnerianum* young leaves, mature leaves, stems, rhizomes, seeds and fruits collected near Furnas Lake were assayed. The best result was record to dichloromethane extract of mature leaves with IC_{50}= 0.74 mg/mL[6]. Anti-AChE was assayed using a modification of the Ellman method.

Continuing our study and using a Anti-AChE bio-guided assay methodology, the more active fraction (dichloromethane extract from mature leaves) was fractionated by its solubility in hexane and hexane:dichloromethane (1:1) and by column chromatography eluted with hexane:ethyl acetate in several proportions until a semi-purified fraction more active than the original extract (IC_{50}= 0.30 ± 0.04 mg/mL) was obtained. The obtained results show the potential use of *Hedychium gardnerianum* as AChE inhibitor.

Subsequently, the variation in Anti-AChE potency with geographic location was studied. *H. gardnerianum* were collected on the same location as previously and also on other three sites. Dichloromethane and methanol extracts, and essential oils from mature leaves were prepared, since these were where the highest activity was previously found. Interestingly, the Anti-AChE activity of these dichloromethane extracts was higher than in the plants collected in the first part of this study with IC_{50} between 0.28 and 0.41 mg/mL, respectively. The dichloromethane extract from Furnas shows the higher activity than the other three sites.

Finally, an attempt was made to characterize the inhibition type of the dichlomomethane extracts, since ideally reversible competitive inhibitors are preferable as therapeutic agents. As would be expected in mixtures, a mixed pattern of inhibition was detected in most of the cases, although the dichloromethane extract from the Furnas was almost truly competitive.

The obtained results show the feasibility of using *Hedychium gardnerianum* as an excellent source of acetylcholinesterase inhibitors. The active compounds responsible for this effect appear to be nonpolar in nature, as evidenced by their presence in dichloromethanic extracts.

NOTES

Synthetic Analogues of Phenalen-1-one

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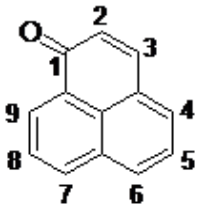
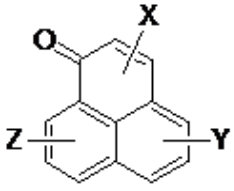
Phenalenone and derivatives are known to possess a range of biological activities including antifungal, antimicrobial and antibacterial, and as such the phenalenone core can be considered as a privileged platform. Interestingly, the use of phenalenone-based structures, as potential anticancer agents, has not been thoroughly investigated. We are therefore undertaking a rigorous SAR analysis of the phenalenone system as a new source of anticancer agents. In this communication we will discuss our approach and progress to date.



Phenalenone



Derivatized phenalenones

Acknowledgements: "Ministerio de Educación y Ciencia" (Proyect SAF 2009-13296-C02-01). MBF thanks the Canary Cancer Research Institute (Instituto Canario de Investigación del Cáncer)(ICIC) for fellowship .

NOTES

Characterization of a Serine Protease with Anti-CLOT Activity

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Steinernema carpocapsae is a nematode with two life phases, in the first it is free and lives in symbiosis with an enterobacteriaceae, in the second it is parasitizing insects that are killed within 48 hrs. This particular life style supports the recent proposal of this organism as a model for innate defence system. Recently in a transcriptomic analysis of the nematode entering the parasitic phase we identified a transcript with high identity with human plasmin and with an earthworm fibrinolytic protease. Based in the EST sequence we get a full cDNA that we named Sc-SP-1 and that we expressed in E. coli. Sc-SP-1 is a serine-protease with a pI of 8.7, a molecular mass of 27.3 kDa, a catalytic efficiency of 22.2x104 s-1 M-1 against N-Succinyl-Ala-Ala-Pro-Phe-pNA and is inhibited by chymostatin (IC 0.07) and PMSF (IC 0.73). Preliminary *in vitro* assays showed that Sc-SP-1 hydrolysis fibrin formed by the reaction of fibrinogen and thrombin in a Petri dish. Moreover Sc-SP-1 destroys clots in the insets, which is a close related system to the coagulation system in vertebrates. Clots in insects, like in vertebrates, have a double function - haemostasis and defence and are underlined by conserved mechanisms. Further work is planed to investigate fibrinolytic and/or thrombolytic activity of Sc-SP-1 to decide its potential in biotechnology and biomedicine.

NOTES

Cdc14-1 release causes a DNA damage repair response in budding yeast.

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Sister chromatids non-disjunction is one of the most important sources in the generation of spontaneous tumors. In the yeast *Saccharomyces cerevisiae*, temporal inactivation of the phosphatase Cdc14 leads to a telophase arrest with non-resolution of the chromosome bearing the rDNA array. Reactivation of Cdc14 allows cells to enter a new cell cycle, even though the 50 % of these cells have failed to properly resolve the rDNA array. Here, we have studied the fate of these cells and the effect of the failure in the resolution of a chromosome during mitosis. Cells perform cytokinesis and progress until a new S-phase, where a Rad52 response is detected. This response correlates with the missegregation of the rDNA array. Moreover, there is a Rad9-dependent block of the cell cycle in the new S-phase. Altogether, these results suggest that at least a double-strand break (DSB) arises as a consequence of entering a new cell cycle without resolving sister chromatids. These “one-ended” DSBs are detected and cells block their cell cycle while trying to repair the damage.

NOTES

Synthesis and antitumoral activity of 5-aromatic substituted naphthalimides

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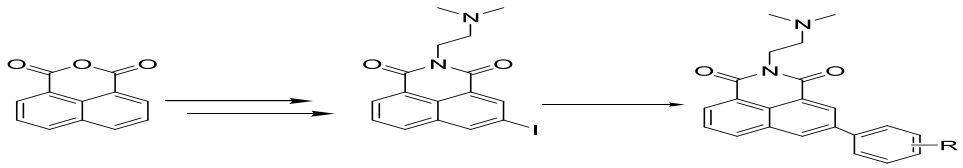
Naphthalimides constitute an important class of anticancer agents characterized by a high cytotoxic activity upon a variety of murine and human tumour cells.[1] Representative examples include compounds such as
monomeric naphthalimides (amonafide and mitonafide) and bisnaphthalimides (elinafide and bisnafide). Amonafide is currently being evaluated in a phase III clinical trial for the treatment of secondary acute myeloid
leukemia (sAML). Recent studies have demonstrated that amonafide inhibits Topo II catalysis prior to the formation of Topo II-DNA cleavable complexes, which suggests that amonafide induces less DNA damage than the classical Topo II drugs.

With this background, we decide to synthesize new naphthalimides analogs based on an extension of the structure through an aromatic ring. Thus, we obtained a set of 18 aromatic substituted naphthalimides at C-4, C-5 and C-6. The best antiproliferative activities were achieved with the 5-substituted derivatives.
In this communication we present an efficient synthesis for 5-aromatic substituted naphthalimides from the commercial naphthalic anhydride and by Pd-catalyst Suzuki reaction[1] of 5-iodonaphthalimide. We also report the results of Topoisomerase II inhibitory activity.



Acknowledgments: To the Ministerio de Ciencia e Innovación (MICINN) for financial support (Proyecto SAF 2009-13296-C02-01). PQE thanks CONICYT for the predoctoral grant.

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NOTES

Serum Insulin Growth Factor-1 (IGF-1) and IGF Binding Protein-3 in bladder cancer patients: COPIURO Study

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INTRODUCTION
The insulin-like growth factor-I (IGF) has been described as a pro-carcinogenic, mitogenic and anti-apoptotic peptide. In fact, IGF-I has been involved in the development of prostate, breast, lung, colorectal and cervical cancer. Nevertheless this growth factor could be playing a role in the proliferation of other tumour cells, such as urothelial cells in bladder cancer tumours.

OBJECTIVE: To evaluate the putative role played by insulin-like growth factors in patients suffering of bladder cancer.

METHODOLOGY: In this study, we compare serum levels of IGF-I and Insulin-like binding protein 3 (IGF-BP3) between bladder cancer patients (cases; n =162) and non-bladder cancer patients (controls; n = 138) enrolled in a hospital-based case-control study. Serum values of IGF-I and IGF-BP3 were determined by a commercially available immunoassay method.

RESULTS:
Mean IGF-I was higher in cases (106.2 ng/ml) than in controls (102.6 ng/ml). Mean IGFBP-3 was also higher in cases (3.2 mcg/ml) than in controls (3.0 mcg/ml), although differences did not reach statistical significance.
However, when the sample was stratified by age, both IGF-I and IGF-BP3 were significantly higher in cases than in controls ($p<0.05$) in the group of subjects between 51-70 years (mean IGF-I values of 113.3 and 93.9 ng/ml, respectively; and mean IGF-BP3 values of 3.5 and 2.8 mcg/ml, respectively).

CONCLUSION: Patients with bladder cancer, show high values of serum IGF-I and IGF-BP3, especially in the age-group with highest bladder cancer incidence (51-70 years). Such results seem to indicate that Insulin-like growth factors may contribute to the development of bladder tumours and should be taken into account in the study of this type of tumours.

NOTES

Production of sponge cytotoxics: Developing farming structures and stress induced biotransformation

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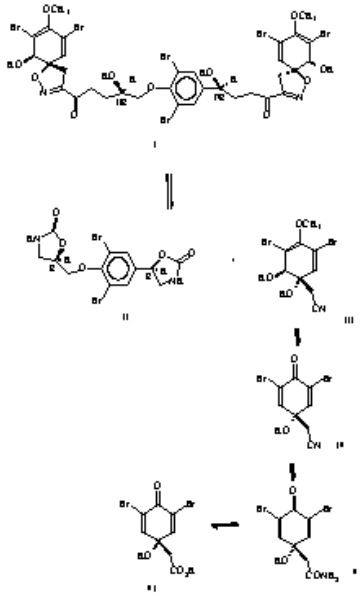
The marine sponge *Verongia aerophoba* from Canary Islands is a rich source of bromotyrosine derived cytotoxics.[1] To obtain enough of the bioactive compounds for application in human therapy, sponges have to be cultured.[2] Before sponge mariculture is accepted as a commercially viable method of supplying bioactive metabolites, it must be demonstrated that adequate production of sponge biomass and metabolite is possible.[3] In this study we provide recent data related with both aspects. Sponge growth rates have been measured in terms of projected area, and the production of three main metabolites (11R,17R-*epi*-Fistularin-3 **I**, Verongiolide **II** and Verongic Acid **VI**) in diferent culture conditions in fish farm effluents have been measured by HPLC (reverse phase, MeOH/H_2O as eluent). These results show that the bioprocess is feasible. The progresses towards quantification of cytotoxic factors (Aeroplysinin 1 **III** and dienone **V**) and antileukemic (Verongionitrile **IV**) also will be presented.



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NOTES

Prostate cancer survival 1998- 2008. Tumours Register Hospital Universitario Ntra. Sra. de La Candelaria

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Hospital Universitario Nuestra Señora De Candelaria (HUNSC) Tumours Register has been working since 1980. Firstly they only included the medical oncology neoplasm. Since 1993 we collect all the infiltrative, central nervous system 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 city, the south half municipalities of the island, La Gomera and El Hierro.; with a population around 548.223.
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 come 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 prostate cancer, included in the Tumours Register of HUNSC. For that, we have used the hospital information: radiological or laboratory test, hanging reviews, visit to urgencies, palliatives care unit; and also external sources: primary attention data base, and The National Death Index, in order to obtain more exactly survival curves.

We have incorporated 2692 urological cancer patients diagnosed between January 1998 and December 2008, corresponding 1398 of those cases to men with prostate cancer.

NOTES

Reaction of 2,5-dihydroxy-3-undecyl-[1,4]benzoquinone with enaminones. Easy access to dihydropyridin- and dihydropyran embelin derivatives.

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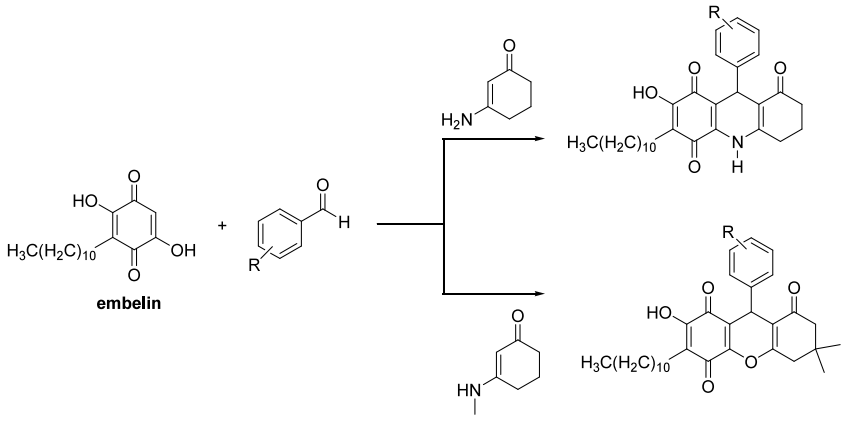
Quinones are an important class of natural compounds that show a wide range of applications in medicinal chemistry. The rearrangement 1,4-benzoquinonic appears in many compounds with pharmacologic importance and it can be considered a privileged structure[1]. One of the most simple 1,4-benzoquinonic compound isolated from natural sources is embelin. Embelin is isolated as the main secondary metabolite from *Embelia ribes*[2]. Recent studies have shown that embelin is a fairly potent, nonpeptidic, cell-permeable inhibitor of XIAP (X-linked inhibitor of apoptosis protein), and it represents a promising lead compound for designing an entirely new class of anticancer agents[3]. These antecedents justify the interest for the preparation of new embelin derivatives[4].

In this communication, we report the synthesis of dihydropyridin- and dihydropyran-1,4-benzoquinone systems through a multicomponent reaction from embelin, aromatic aldehydes and cyclic enaminones. The type of enaminone used (primary or tertiary) is key in the formation of the corresponding oxygenated or nitrogenated ring.



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NOTES

Cytotoxic Metabolites from *Maytenus retusa*

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Celastraceae species have been used for centuries throughout South America and China as insect repellents and insecticides and for the treatment of several diseases, such as stomachaches, fever, rheumatoid arthritis and cancer[1]. One of the most representative genera widely used in folk medicine is the *Maytenus* genus[2].

With the aim of obtaining new bioactive compounds, the first phytochemical study of *Maytenus retusa* was carried out, isolating seven new terpenes (**1**-**7**) and thirty six known compounds[3]. Their structures were determined by 1D and 2D spectroscopic studies. The isolation of 21-oxopristimerine (**2**) is relevant from a biogenetic point of view since it has been proposed as a key intermediate in the conversion of pristimerine into tingenone.

Some of these compounds were tested against tumoral cell lines HL60 and MCF7, showing IC_{50} values ranging between 0.2 and 4.7 μM.

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NOTES

Evaluation of methyl esters of flavonoids in HL-60 cells

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Flavonoids are polyphenolic compounds of great interest due to their possible anticancer activities. We have synthesized and analyzed the cytotoxicity of twenty methyl esters of flavonoids in the human leukemia cell line HL-60. Two methyl esters, which were selected for additional experiments (SME), displayed cytotoxic activities determined at 72 h by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. SME arrested HL-60 cells at G1 phase of the cell cycle, which was associated with the accumulation of cyclin D_1 and $p21^{Cip1}$. The cytotoxic effects of SME were accompanied by the concentration- and time-dependent appearance of DNA and nuclear fragmentation, increase in the percentage of sub-G1 cells, processing of pro-caspases-9, -8, -6 and -3 and poly(ADP-ribose)polymerase cleavage. The pan-caspase inhibitor z-VAD-fmk and the cell permeable caspase inhibitors z-LEHD-fmk and z-IETD-fmk significantly reduced the percentage of hypodiploid cells, suggesting that caspases are involved in SME-induced cell death. Pretreatment of cells with the specific mitogen-activated extracellular kinase 1/2 inhibitor PD98059, together with SME, resulted in an important enhancement of cell death.These results might have important clinical implications for the use of SME in combination with MEK 1/2 inhibitors as potential therapeutic agents.

This work was supported by a grant from the Ministry of Science and Innovation of Spain and from the European Regional Development Fund (SAF2010-21380).

NOTES

Industrial production of cytotoxics: single cell ergosterol peroxide obtained from the facultative marine fungus *Paecilomyces variotii*

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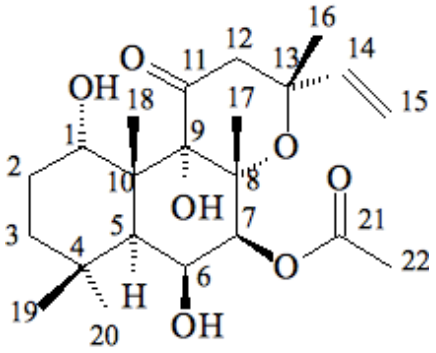
The marine environment has been shown to be a rich source of natural products. Preliminary data have demonstrated the potential of marine fungi to produce useful bioactives but the majority of these microbes remain uncultured. In this work, 5, 8-epidioxy-22E-ergosta-6,22-diene-3-ol (*ergosterol peroxide* **I**), a known bioactive metabolite was isolated from *Paecilomyces variotii* fungus that was grown in a marine static fermentation system, at pilot plant scales. Ergosterol peroxide completely inhibits growth and induces apoptosis of HL60 cells at a concentration of 25 M[1]. Also, suppresses inflammatory responses in RAW264.7 macrophages and growth of HT29 colon adenocarcinoma cells[2]. Finally, exhibits inhibitory effects on human breast adenocarcinoma MCF-7 cells by inducing cell apoptosis[3]. In this communication, the authors will present the recent advances obtained in the laboratory towards the industrial production of this high-added value product, which in recent years has become so interesting for the pharmaceutical industry and biotech companies[1,2,3].



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A practical method for large-scale production of Forskolin, an immune system enhancement compound from *Coleus forskohlii*

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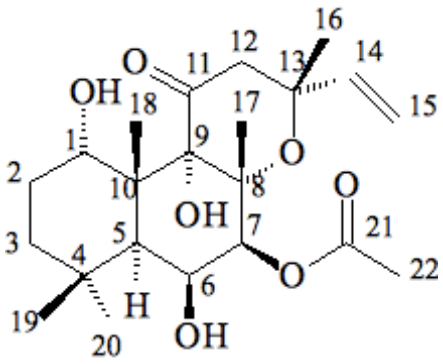
Forskolin I (7-□-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-ene-11-one) and derivatives are known bioactives metabolites from the roots of *Coleus forskohlii*, a traditional Ayurvedic herb. *Coleus* has been used for a variety of conditions and exhibits potent immune system enhancement by activating macrophages and lymphocytes. The effects of the diterpenes isolated from *C. forskohli* have been intensively researched *in vitro*, animal, and human clinical studies and its relevance is corroborated by the 10,000 papers published in the last decade. Forskolin and 1-deoxyforskolin were found to be active against HIV (NL4-3)[1]. Also, forskolin is a potent platelet aggregation inhibitor and has been examined for its effects on tumour-induced human platelet aggregation and pulmonary tumour colonization in mice. In fact, forskolin 2 □M strongly inhibits the melanoma cell-induced human platelet aggregation[2]. These findings raise the possibility that forskolin could prove of value in the clinic for the prevention of cancer metastasis. In this communication, an advanced and optimized procedure will be presented. The authors will explain a sensible and effective method for the large-scale separation of forskolin at laboratory scale. The new method is based on a previous crystallization of the crude extract from *C. forskohlii* followed by a simple normal-phase flash chromatography. At this time the procedure is being sized to industrial scale in order to produce this high-added value product under the framework of the Canarian Sustainable Industrial Development project (DISCan-2007).



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NOTES

QSAR Studies of P-glycoprotein Inhibitors

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The resistance to anticancer agents represents the major obstacle for successful cancer chemotherapy [1]. There are several mechanisms of multidrug resistance (MDR), but the most important is the mechanism associated with expression of P-glycoprotein (P-gp). P-gp is a transmembrane glycoprotein overexpressed in most cancer cells found to be resistant to therapeutic agents. This glycoprotein effluxes antitumor drugs through ATP-dependent process and is thereby associated with treatment failure in cancer [1, 2, 3]. Therefore, one of the most promising strategies to overcome drug resistance is to find compounds that inhibit P-gp activity [1].

Recent studies have showed that some diterpene compounds, such as jatrophanes and lathyranes, isolated from *Euphorbia* species are promising MDR reversing agents in cancer cells [2]. The P-gp inhibitory activity of these compounds is assessed as fluorescence activity ratio (FAR). Using these results we applied QSAR (Quantitative Structure-Activity Relationship) using a Heuristic method. After performing QSAR studies, we obtained several correlations between molecular descriptors and biological activity of these compounds for the two concentrations under study (4 and 40 g/mL), these models were statistically valid and showed high prediction ability (R^2 for test set between 0.534 and 0.791).

Considering the molecular descriptors (relative number of carbon atoms and benzene rings, for example) involved in the obtained QSAR models, it is possible to modify compound structures in order to increase their biological activity for this specific target.

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BIOPHARMAC – MAC/1/C104

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Pharmacophore-based screening for the discovery of P-glycoprotein inhibitors

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P-glycoprotein (P-gp), is an ATP-dependent which efflux a broad panel of structurally and functionally diverse compounds, which range from low molecular weight compounds such as cyclosporines, up to lipids. Overexpression of this protein may result in multidrug resistance and is a major cause of the failure of cancer chemotherapy and diminished efficacy of antibiotics and antiviral agents. [1] Many compounds exhibit P-gp inhibitory activity, like flavonoids, either natural or synthetic, are known to exhibit diverse biological activities, in particular, many compounds exhibit antitumor activities. [2] We employed a pharmacophore screening to retrieve molecules with P-gp inhibitory activity from a database of compounds. Following standard procedures, we apply other filters to enrich the pool of retrieved compounds with hit compounds that, in later stages, will not drop out of the drug discovery process.

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