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Marine-inspired synthetic glycolipids as new immunomodulatory substances in the treatment of cancer and neurodegenerative diseases

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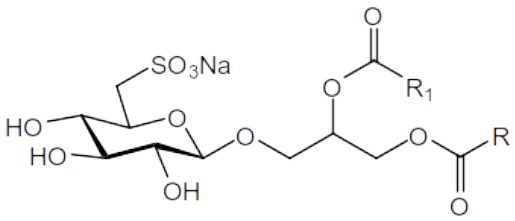
Glycolipids are primary metabolites present in all living organisms and characterized by interesting biological properties. In recent years, glycoglycerolipids and ceramides have attracted particular interest as either agonists of cellular receptors, e.g. Toll-Like Receptors (TLRs) and MHC-related glycoproteins of the CD1 family, or chemical entities for the development of anti-tumoral and immunological drugs.

In the last years, we have focused our study on the immunological properties of sulfoglycolipids, that have wide distribution in terrestrial and marine photosynthetic organisms. This communication summarizes our work about the synthetic preparation and development of this family of molecules and their analogs as novel immunomodulators. In this regard a representative example was a sulfoquinovosyl diacylglycerol named -SQDG18 (Sulfavant)[1] that prototypes a class of natural-derived glycolipids able to prime human DCs by a TLR2/TLR4-independent mechanism and trigger an efficient immune response in vivo against melanoma. Sulfavant induces maturation of DC with expression of MHC II molecules and upregulation of costimulatory proteins (CD83, CD86). Mice immunized with OVA associated to Sulfavant (1:500) produced a titer of anti-OVA Ig comparable to traditional adjuvants. In an experimental model of melanoma, vaccination of C57BL/6 mice by Sulfavant-adjuvanted hgp10 peptide elicited a protective response with reduction of tumour growth and increase of survival. The study of the mechanism of action of this molecule highlighted new cellular processes at the basis of the regulation and modulation of the immune response and which favor the rapid achievement of cellular homeostatic conditions following perturbative events.



β- 6'-Sulfoquinovosyl-1,2-O-diacylglicerols

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Structural characterization of crystalline materials (with different complexity) by Single-Crystal Diffraction Analysis

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Single-Crystal X-Ray Diffraction (SCXRD) is a powerful non-destructive analytical technique commonly considered as the main tool for the structural characterization of crystalline materials at the atomic level. For this reason, SCXRD is very useful for a wide range of disciplines such as chemistry, biology, materials science, or pharmacology in both academic and industrial settings.

The structure solution of small molecules is today considered a routine, if not trivial, process, that involves different steps such as crystallization, data collection, initial phasing and structure refinement. This process allows to provides detailed information about the atomic arrangement of crystalline compounds, including bond-lengths, bond-angles, and details of site-ordering. Its contribution is therefore fundamental to understanding the physicochemical properties of many substances and their relationships with the structure in crystalline materials.

In contrast, the crystal structure determination of macromolecules is a complicated, often still challenging, multi-step process, that involves the availability of different types of data (e.g. high resolution XRD data, SAD data, molecular models suitable for MR) and the combination of different tools (e.g. EDM techniques, efficient algorithms for substructure location and/or for phase extension, restrained least-squares procedures).

When single crystals of sufficient dimensions are not available, electron diffraction (ED) may be considered a useful alternative technique; unfortunately, the quality of the crystal structure models obtained by structure refinement is not comparable with that usually obtained by XRD.

IC researchers have a long tradition in the development of innovative crystallographic methods for the structure solution of molecules with different composition and complexity, by XRD as well as ED techniques. The core of these theories is implemented in automatic software (i.e. the SIR family), widely used by the scientific community.

This webinar aims at providing a brief overview of the technique, methods and strategies for a successful structure solution and some applications to real cases.

Molecular mechanisms linking obesityăto neurodegeneration: focus on neuroinflammation

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Neuroinflammation and chronic activation of the innate immune response are associated with the early onset of neurodegenerative diseases. The finding that obesity and metabolic disorder are accompanied by chronic low-grade inflammation, astrogliosis, and the release of pro-inflammatory cytokines, has fundamentally changed our approach to the underlying early causes of neurodegenerative disturbances. This is of special relevance, since that from 2019 the World Health Organization recommends focusing on modifiable risk factors in the prevention of Alzheimer's Disease (AD). Diet composition is certainly one of these factors, especially by considering the massive worldwide consumption of a high-calorie diet, rich in fats and sugars (i.e.ăHighăFatăDiet HFD - or Western diet), which induces multi-organ inflammation, including brain-blood barrier, adipose tissue, and gut-brain axis. In AD, synaptic loss and dysfunction are early and strongly correlated with cognitive impairment. Pre-fibrillar oligomeric beta-amyloid (Ab) and/or tau accumulate on synapses,and induce pathological synaptic dysfunction and loss. This communication provides an overview of our sequential studies linking obesityăto neuroinflammation and neurodegeneration by exploiting *in vitro* and *in vivo* studies in a mouse model of HFD-induced obesity, also extended to the transgenic microglia CX3C-eGFP strain. Functional studies of synapses (patch-clamp recording, calcium imaging) have been extended by time-lapse cell imaging, confocal microscopy, CLEM and TEM, biochemical assays, LC-MS / MS, and NMR analysis of the mouse brain, and associated with tests for memory and cognitive performance. Data revealing the impact of leptin (a pro-inflammatory cytokine) and its interplay with the endocannabinoids and the neuropeptide Orexin-A (OX-A) in the regulation of neurogenesis and Tau phosphorylation will be discussed to provide new molecular insights to prevent neuroinflammation and neurodegeneration.

Inhibition of 17β hydroxysteroid dehydrogenases from phytoestrogens: a combined kinetic and structural approach

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Steroid hormones act via specific receptors that activate gene transcription; hydroxysteroid dehydrogenases (HSDs) are responsible for pre-receptor regulation of steroid hormone activity. Indeed, 17β hydroxysteroid dehydrogenase (17β-HSD) modulate the biological potency of estrogens and androgens by reducing the (inactive) keto-form or oxidizing the (active) hydroxy-form at C17.

17β-HSDs are widespread among all organism, from vertebrates to bacteria. In humans, several different 17-HSDs have been identified that are related to the development of pathologies such as breast and prostate cancers, Alzheimers disease, polycystic kidney disease.

17β-HSD from the filamentous fungus Cochliobolus lunatus (17β-HSDcl) is the first, fully characterized fungal HSD. Its role in fungal metabolism is still not fully understood and recently it has been linked to the biosynthetic pathway leading to citoskyrin A, a potent in-vitro antibacterial. 17β-HSDcl has been proposed as a useful model system when studying the function of HSD enzymes belonging to the Short Chain Dehydrogenases superfamily. Indeed, by linking kinetics and structural data we have been trying to relate the structure and the function of 17β-HSDcl. Besides, by following the same approach we have investigated the inhibitory action of selected flavonols and isoflavones, which are known modulators of the endocrine system, in order to elucidate the structural determinants of their inhibitory action.

NMR spectroscopy for metabolomics: a powerful tool for biomarkers investigations

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Nuclear Magnetic Resonance (NMR) spectroscopy has long emerged as a powerful tool for understanding metabolic process in living systems. Over the last decade, metabolomics has been considered as a well-established approach to elucidate metabolism and its mechanism. Formally defined as the quantitative measurement of the multi-parametric metabolic response of living systems to pathophysiological stimuli or genetic modification, the term metabolomics has been put forward to describe the combined application of spectroscopy as high throughput screening and multivariate statistical approaches to investigate the multicomponent composition of biofluids, cells and tissues. The high applicability of the method is due to its ability to qualitatively and quantitatively characterize the chemical profile of low molecular weight metabolites (metabolome) present in any biological compartment as end products of the cellular regulatory pathways. Besides, the implementation of big data analysis and the elaboration of statistical models allow to easily visualize data trend and to predict sample classifications according to specific metabolite variation, which can be suggested as putative markers. Moreover, the advent of complex data integrations algorithms and network analysis allow assessing different -omics pathway involved, and possibly suggesting useful biological targets.

Here we present a general overview of how NMR spectroscopy can be applied to a wide range of samples and biological issues. We report some different applications, which investigated different matrices, such as exhaled breath condensates (EBC), used to study airway diseases, or serum to search for biomarkers in the Alzheimer disease, through a minimally invasive sampling.

The crucial role of stereochemistry in the inhibition of A amyloid growth and toxicity by Silybins

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The self-assembling of the amyloid β (Aβ) peptide into neurotoxic aggregates is considered a central event in the pathogenesis of Alzheimers Disease (AD). Based on the amyloid hypothesis much efforts have been devoted in designing molecules able to halt disease progression by inhibiting Aβ self-assembly. By combining biophysical, biochemical and computational techniques, we investigated the ability of four optically pure components of the natural product Silymarin (Silybin A, Silybin B, 2,3-Dehydrosilybin A, 2,3-Dehydrosilybin B), to inhibit A aggregation. Despite we demonstrated that all the investigated flavonoids prevent the formation of mature fibrils, our results showed that only Silybin B was able to halt the growth of small-sized protofibrils thus promoting the formation of large, amorphous aggregates. Our data suggest that Silybin B interacts mainly with the C-terminal hydrophobic segment $^{3}5$MVGGVV$^{4}0$ of Aβ40 and its conformation remains predominantly unstructured. By contrast Silybin A interacts preferentially with the segments $^{1}7$LVFF$^{2}0$ and $^{2}7$NKGAII$^{3}2$ of Aβ40 which shows a high tendency to form bend, turn and -sheet conformation in and around these two domains. Moreover in vivo studies in a transgenic C. elegans strain expressing human A, indicated that Silybin B is the most effective of the four compounds in counteracting Aβ proteotoxicity. This study underscores the pivotal role of stereochemistry in determining the neuroprotective potential of Silybins and points to Sil B as a promising lead compound for further development in anti AD therapeutics.