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Università di Catania

PHARMADAY 2022

IL DIPARTIMENTO INCONTRA LE AZIENDE

2^ EDIZIONE

Catania, 1 giugno 2022

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Dipartimento di Scienze del Farmaco e della Salute
Edificio 2, Città Universitaria, viale A. Doria, 6 - Catania

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Ringraziamo tutti i relatori, le aziende e gli enti per la loro partecipazione.

PROGRAMMA

Ore 8:30 Saluti istituzionali e introduzione al workshop

Prof. Francesco Priolo – Rettore dell'Università di Catania
Prof. Salvatore Baglio – Delegato alla Ricerca, Università di Catania
Prof. Rosario Pignatello – Direttore DSFS

Sessione 1 - Moderatori: prof. Francesco Pappalardo, prof. Agostino Marrazzo

9:00-9;30 Lecture: ***Modeling and Simulation in Medicine: state of the art, opportunities, and regulatory challenges***
(Prof. Marco Viceconti, Dip. di Ingegneria Industriale, Università di Bologna; Direttore Laboratorio di Tecnologia Medica, Istituto Ortopedico Rizzoli)

9:30-10:00 Lecture: ***Fondazione Ri.MED: Building a Mediterranean Translational Research Centre*** **(Dott. Alessandro Padova, Fondazione Ri.MED)**

10:00-10:15 ***Question time***

10:15-10:45 Sessione poster e Break

Sessione 2 - Moderatori: prof.ssa Agata Copani, prof. Salvatore Sortino

10:45-13:45 Presentazioni delle sezioni/gruppi di ricerca del dipartimento

1. **Innovative nanotechnology-based platforms for the controlled release of active molecules (A. Bonaccorso)**
2. **Neuroprotective effects of (±)-PPCC, a selective sigma-1 receptor agonist, in cellular models of Alzheimer's disease-like dysfunction (A. Caccamo)**
3. **Light and therapeutic applications: innovative strategies developed by the PhotoChemLab (A. Fraix)**
4. **Pivotal role of carnosine in the modulation of microglia and macrophagesactivity: multimodal mechanism of action and therapeutic potential in neurodegenerative disorders (G. Caruso)**
5. **A novel photothermal-responsive nanosystem for curcumin release (S. Petralia)**
6. **COMBINE Group –UNICT (V. Di Salvatore)**
7. **Porous bio-composite material based on natural fiber-Halloysite for gas adsorption and drug delivery (V. Patamia)**
8. **Promising new strategies for ophthalmic drug delivery (D. Santonocito)**
9. **EtnaSaffron (Crocus Sativus): analytical characterization and coloring power determination through ISO 3632/2003 standard (F. Sipala)**
10. **Effect of natural antioxidants on tissue transglutaminase overexpression induced by amyloid-β: biological, molecular and computational studies (G. Sposito)**
11. **MOR/DOR Dual-Target Approach: 2S-LP2Effects in Inflammatory and Neuropathic Pain (S. Spoto)**

12. New Brilliant Blue G Derivative as Tool in Retinal Surgery (A. Spadaro)

13:45-15:00 Sessione poster e Pausa pranzo

Sessione 3 - Moderatore: prof. Carmelo Puglia

15:00-15:30 **Lecture: *Tecnologia e territorio: approcci integrati per formulare eco-cosmetici Hi Tech* (Dr.ssa Elena Ghedini, Dip. SMN, Università Ca' Foscari Venezia; Co-Founder e Direttore Ricerca e Sviluppo di VeNice srl)**

Sessione 4 – Moderatore: prof. Giampiero Leanza

15:30-15:45 **Centro di Ricerca IMPRonTE (Centro di Ricerca Centro di ricerca per l'imaging molecolare, preclinico e traslazionale) (M. Gulisano)**
15:45-16:00 **Centro di Ricerca NANOMED (Centro di Ricerca in Nanomedicina e Nanotecnologia Farmaceutica) (C. Puglia)**
16:00-16:15 **Centro di Ricerca CERNUT (Centro di Ricerca in Nutraceutica e Prodotti Salutistici) (L. Vanella)**

Sessione 5 – Moderatore: prof. Filippo Caraci

16:15-17:00 **Gli Spin-off del DFSF**
Prof. M. Gulisano: BEEN
Dr.ssa G. Russo: MIMESIS
Prof.ssa G. Tempera: NACTURE

17:00-17:20 Sessione poster e Coffee break

17:20 **Tavola Rotonda: *La Terza Missione per l'Ateneo e il Dipartimento: l'importanza del confronto con le aziende* (Moderatori: prof.ssa Annamaria Panico, prof. Simone Ronsisvalle, dr.ssa Barbara Tomasello).**

Saluti finali

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RELAZIONI ORALI

IL 1

Modelling and Simulation in Medicine: state of the art, opportunities, and regulatory challenges

Marco Viceconti[a b]

[a] *Department of Industrial Engineering, Alma Mater Studiorum - University of Bologna (IT)*

[b] *Medical Technology Lab, IRCCS Istituto Ortopedico Rizzoli, Bologna (IT)*

For a long time, the paths of physics and biology diverged, but starting from the mid of the past century the two fields started to converge again. An important effect of this is that mathematical modelling, and later computer modelling & simulation entered in the toolchest of life science researchers. But for a while the use of computer model was strictly limited to a very specialistic research use. Only in the last 20 years the idea that computer models could be used in the clinical practice started to emerge, first as speculative hypothesis of some visionaries, but today as a solid reality with certified solutions and companies that commercialise them. In the seminar we will provide an historical overview of this journey, and briefly reflect of the state of the art of future perspective of two specific use cases: Digital Twins for healthcare, where computer models are used as clinical decision support systems, and In Silico Trials, where computer models are used in the derisking of new medical products.

Marco Viceconti

University of Bologna

Marco Viceconti is full professor of Computational Biomechanics in the department of Industrial Engineering of the Alma Mater Studiorum – University of Bologna, and Director of the Medical Technology Lab of the Rizzoli Institute. Before he was at the University of Sheffield, UK, where he founded and led for seven years the prestigious Insigneo Institute for in silico Medicine. Prof Viceconti is an expert in *In Silico Trials*, the use of subject-specific modelling to test new medical products.
He is one of the key figures in the *in silico* medicine international community: he founded the VPH Institute, an international no-profit organisation that coordinates this research community, and drove the creation of the Avicenna Alliance, which represent the biomedical industry interests in this domain. According to SCOPUS he published 368 papers (H-index = 52).

IL 2

Fondazione Ri.MED: Building a Mediterranean Translational Research Centre

Alessandro Padova[a]

[a] *Fondazione Ri.MED, Via Bandiera 11, 90133 Palermo*

The Ri.MED Foundation was established in 2006 with an international partnership between the Italian Government, the Region of Sicily, the Italian National Research Council (CNR), the University of Pittsburgh and the University of Pittsburgh Medical Center (UPMC).
The Foundation, based in Palermo, promotes, supports and leads biomedical and biotechnological research projects, with emphasis on the translation of innovative results into clinical practice.
Ri.MED is currently focused on the creation of the Biomedical Research and Biotechnology Centre, in Carini in the metropolitan city of Palermo, that will host 600 scientists. On the same campus, there will be the Ismett 2 hospital that will ensure truly translation research from bench to bedside.
Currently Ri.MED operates its research activities in satellite laboratories focusing on organ insufficiencies, infectious diseases, oncology, neurodegenerative and cardiovascular disease. Core lab facilities support the development of innovative therapeutic and diagnostic products:

- a drug discovery engine integrating HTS, drug design, medicinal chemistry, structural biology and biophysics and proteomics
- a regenerative medicine and immunotherapy team including a GMP factory for cell products totally integrated with Ismett
- a tissue engineering and bioengineering innovation hub

In preclinical and clinical research, strategic are the partnerships with the CNR, University of Pittsburgh, UPMC and IRCCS-ISMETT, which have yielded proprietary intellectual property resulting in several joint patent applications. The generation of intellectual property represents a fundamental intangible asset in the valorization of the Fondazione Ri.MED, with the perspective of developing an innovative model of sustainable research.

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Alessandro Padova, PhD

CEO, Ri.MED Foundation

Alessandro Padova serves since 2015 as Chief Executive Officer of Fondazione Ri.MED, a US-Italy public-private translational research initiative with responsibility of business administration including overseeing the Biotechnology Research and Biomedical Centre construction and the foundation scientific development. During the last 6 years, Fondazione Ri.MED has developed an integrated translational research engine encompassing drug discovery, regenerative medicine and tissue engineering delivering over 25 patents, mainly in partnership with University of Pittsburgh with a successful track record in technology transfer.
Over the last 25 years, Alessandro has had leading and executive roles implementing business development, management and R&D strategies with a successful track record at international level with appointments in several prestigious Italian, UK and US biotech companies such as Parke- Davis Neurosciences, Peptide Therapeutics plc, Medivir UK Ltd, Astex Technology Ltd and IRBM Science Park srl. He served as General Director of C4T S.C.ar.l. and Siena Biotech S.p.A., the latter an Italian clinical-stage biotech company with

a dedicated R&D research centre and over 150 employees with a diversified program's pipeline covering preclinical and clinical development including partnerships with Roche, Pfizer (Wyeth) and CHDI Foundations.

Over the years Alessandro has developed an extensive network at regional, national and international level with big pharma, international research foundations, public and private institutions and investors including key opinion leaders in relevant therapeutic areas.

Alessandro's expertise in the field has been recognised with several appointments in Board of Directors and Scientific Advisory Committee including a non-executive director appointment in TES Pharma Srl, Siena Exosomics S.p.A., Externatutics S.p.A. and Chairman of Rasna Therapeutics, Inc.

Alessandro obtained a PhD in Synthetic Organic Chemistry in 1994 at the Exeter University (UK) and has a strong interest in the integration of computer driven approaches in drug discovery, biomedical engineering and advanced therapeutic products. He co-authored several publications and patents.

IL 3

Technology and territory: integrated approaches to formulate Hi-Tech eco-cosmetics

Elena Ghedini

Ve Nice srl and CATMAT Lab, Department of Molecular Sciences and Nanosystems, Ca' Foscari University Venice and INSTM-RU Venice, Via Torino 155, 30172 Venezia Mestre, Italy

Renewable resources, their use and transformation for pivotal industrial processes, have a key influence on our everyday life and are very interesting potentialities for an ever-growing cosmetic industry. The market for new, natural and bioactive ingredients in cosmetics is, in particular, very large and increasing. Particularly appealing are ingredients derived from the biomass valorization, with attention to agri-food wastes. These biomasses are very rich in high value-added components, including essential oils, polyphenolic compounds, edible oils, pigments, dietary fibres, enzymes, etc.

Despite the great potential, the transformation of biomass into bio-metabolites and their introduction in a cosmetic formulation implies a multidisciplinary and integrated approach. The first issue to face is the optimization of an efficient and sustainable protocol to extract the bio-actives from the biomass. Moreover, it must be considered that the use of biomass derived functional ingredients involves several critical issues concerning their safety, their complexity in term of molecular composition and physical features (color, granulometry, smell). These issues can be faced only by the exploitation of Hi-tech formulative protocols. Precisely with this spirit in 2018 it was born, from the heart of CATMAT (research team of SMN Department of Ca' Foscari University), Ve Nice srl: innovative Start Up and Spin Off of Ca' Foscari University. The core business of Ve Nice is represented by design of chemical specialties and formulation processes, in the cosmetic sector in full accord with the paradigms of circular economy. The solid skills and expertise of Ve Nice, derived from years of research of CATMAT, have led to an international patent [1]. The invention regards a sustainable and scalable process to produce a carrier made of components of natural origin or products derived from biomasses, for the formulation of high-tech cosmetic products with drug delivery features. The synergy between technology and biomasses valorization are a key point in all the Ve Nice products.

The lecture presents the path which led to the foundation of Ve Nice: an effective example of technology transfer. The aim is to highlight the strengths but at the same time the issues encountered to face the market and to combine the researcher's vision with the entrepreneurial mentality.

[1]a) Brevetto italiano n. 102018000005118, "Formulato cosmetico a rilascio controllato di principi attivi" Inventrici M. Signoretto, E. Ghedini, F. Menegazzo.
b) International Patent Application, n. PCT/IB2019/053710, Inventors M. Signoretto, E. Ghedini, F. Menegazzo.

Elena Ghedini
University of Venezia Ca' Foscari

La Dott.ssa Elena Ghedini è una ricercatrice presso il Dipartimento di Scienze Molecolari e Nanosistemi dell'Università Ca' Foscari di Venezia. Laureata in Scienze dei Materiali a Padova, ha conseguito il Dottorato di Ricerca in Chimica Industriale presso il gruppo di ricerca CatMat, dove ha proseguito la sua carriera studiando materiali innovativi che trovano impiego in diversi ambiti, dalla catalisi alla cosmesi. È co-fondatrice dello spin off VeNice, acronimo di Naturale, Innovativo ed Efficace, che nasce dalla ricerca decennale nell'ambito dei sistemi a rilascio controllato di molecole biologicamente attive e che si occupa della formulazione di sistemi innovativi e green per la cosmesi.

COMUNICAZIONI ORALI

Innovative nanotechnology-based platforms for the controlled release of active molecules

Angela Bonaccorso [a], *Teresa Musumeci* [a,b,c], *Claudia Carbone* [a,b,c], *Rosario Pignatello* [a,b,c]

[a] *Dipartimento di Scienze del Farmaco e della Salute, Laboratorio di Drug Delivery Technology, Università degli Studi di Catania, Viale A. Doria 6, 95125 Catania.*
[b] NANO-*i, Centro di Ricerca in Nanotecnologie Oculari, Università degli Studi di Catania, viale A. Doria 6, 95125 Catania.*
[c] CERNUT, *Centro di Ricerca in Nutraceutica e Prodotti Salutistici, Università degli Studi di Catania, viale A. Doria 6, 95125 Catania.*

Nanomedicine is an emerging branch of nanotechnology focused on the application of materials in the nanoscale range as means for diagnostic tools or to deliver therapeutic agents in a controlled manner. It deals with the application of innovative platforms capable of improving the therapeutic efficiency of drugs and the diagnostic potential of imaging techniques in terms of specificity of action, sensitivity and reduction of adverse effects. These nanotechnology-based platforms are designed to promote the transport and release of diagnostic or therapeutic agents through biological barriers, to reach specific target sites, to mediate molecular interactions and to respond to local or external stimuli. Currently, a variety of nano-based pharmaceuticals have successfully entered the market and are used in clinical protocols, others are in various stages of preclinical and clinical phase studies, many others are still the subject of intense experimental research. Nanomedicine includes platforms of various nature, among which our team at the Laboratory of Drug Delivery Technology is particularly focused on polymeric and lipid nanoparticles, nanocrystals, nanogels and vesicular systems such as liposomes [1,2]. The choice of the more suitable nano-platform is closely related to the characteristic of the molecule under study, the selected administration route and the desired pharmacological scope. The physico-chemical (size, surface properties, shape, etc.) and technological (entrapment efficiency, drug release profile, stability, etc.) properties of such systems can be actually designed and tailored in relation to the specific purposes. Our know-how and skills are focused on the design and development of nano-platforms by using innovative methodologies (i.e. DoE) and their full characterization for the delivery of natural compounds (curcumin, berberine, essential oils); synthetic drugs (oxcarbazepine, NSAIDs, clozapine) and biological entities (peptides, antibodies, gene material) intended especially for brain, ocular and dermal targeting [3,4]. Furthermore, colon delivery of nutraceuticals and microencapsulation strategies of probiotics for functional foods product development can also be counted among our expertise [5-7]. In order to meet the demand for innovation and improve translational science, bridging the gap between basic research and applicable therapies, we continuously invest in the development of new industrially scalable technologies and production processes.

[1] A. Bonaccorso et al. *Pharmaceutics.* **2020**, 12, 476; doi:10.3390/pharmaceutics12050476
[2] C. Carbone et al, *Colloids Surf B Biointerfaces*. **2020,** 186:110705. doi: 10.1016/j.colsurfb.2019.110705
[3] C. Carbone et al. *Pharmaceutics*, **2019**, 11(5), 231. doi: 10.3390/pharmaceutics11050231
[4] T. Musumeci et al. *Eur. J. Pharm. Biopharm.* **2018**; 133, 309. doi: 10.1016/j.ejpb.2018.11.002
[5] C. Curcio et al. *Pharmaceuticals (Basel)* **2020**,13(6),131. doi: 10.3390/ph13060131.
[6] C. Curcio et al. *Current Nutraceuticals* **2021**, 2(2). doi: 10.2174/2665978601999201126212614
[7] A. Bonaccorso et al. *AAPS PharmSciTech*. **2021**, 22(3), 123. doi: 10.1208/s12249-021-01996-x.

OTH OR02

Neuroprotective effects of (±)-PPCC, a selective sigma-1 receptor agonist, in cellular models of Alzheimer's disease-like dysfunction

Antonella Caccamo [a], Orazio Prezzavento [a], Barbara Tomasell [a], Roberta Ruppi [a],Giampiero Leanza[a]

[a] Dipartimento di Scienze del Farmaco e della Salute, Università degli Studi di Catania, Viale A. Doria 6, 95125 Catania.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss and accumulation in the brain of pathological proteins, this latter possibly resulting also from metabolic alterations linked to endoplasmic reticulum (ER) stress. The sigma-1 receptor is a trans-membrane protein of the ER which, when activated, stabilizes Ca^{2+} signaling between the ER and mitochondria, thus implementing cell responses to stress. Interestingly, sigma-1 receptors are widely distributed in brain areas affected by AD pathology and therefore representing a possible pharmacological target for the treatment of AD. The (±)-PPCC, a highly selective sigma-1 receptor agonist, exhibits remarkable anti-amnesic properties, as previously seen in a rat model of AD-like cognitive impairments. Here, the possible neuroprotective actions of the (±)-PPCC upon the stress-induced accumulation of pathological proteins were investigated in primary cultures of rat cortical neurons exposed to thapsigargin, an ER stress-inducing drug. Treatment with (±)-PPCC markedly reduced the thapsigargin-induced toxicity, thus improving neuron survival. Furthermore, the treatment with (±)-PPCC also reduced the expression levels of C99, the fragment precursor of β-Amyloid (Aβ), as well as of the transactive response DNA-binding protein of 43 kDa (TDP-43) phosphorylated at Ser409. Both effects were reversed by pretreatment with the sigma-1 antagonist BD1047. Taken together, the data indicate that (±)-PPCC may efficiently protect cortical neurons from the toxicity induced by thapsigargin and also normalize the expression levels of some proteins related to AD whose levels were altered as a consequence of thapsigargin-induced ER stress. Interestingly, these effects were consistently reversed by BD1047, therefore suggesting that they may take place via direct activation of sigma-1 receptors.

Light and therapeutic applications: innovative strategies developed by the PhotoChemLab

Aurore Fraix [a], *Adriana Graziano* [a], *Francesca Laneri* [a], *Cristina Parisi* [a], *Mimimorena Seggio* [a], *Salvatore Sortino* [a]

[a] *PhotoChemLab, Dipartimento di Scienze del Farmaco e della Salute, Università degli Studi di Catania, Viale Andrea Doria,6, 95125 Catania.*

The control of drug bio-activity is a crucial requisite in order to enhance the therapeutic efficacy and to reduce the potential drawbacks. To this end, the use of light as trigger of the biological effect is ideal, as photons do not influence physiological parameters such as pH and temperature, are easy to manipulate and allow an excellent control of the treatment in terms of location, time and dosage. The research activity of the PhotoChemLab mainly focuses on the design, fabrication and characterization of light-activatable molecular and supramolecular systems, and nanomaterials able to release non-conventional therapeutic species for antibacterial and antitumor applications. These therapeutic agents, namely nitric oxide (NO), singlet oxygen ($^1O_2$) and heat, share several desirable properties: i) are transient species that confine their activity close to their generation site, ii) are multitarget species able to interact with all biological components (DNA, lipids, proteins) and iii) do not suffer of multi drug resistance phenomena. Particular attention is paid to the development of multimodal systems able to release simultaneously more than one active species in association with fluorescence properties useful for intra-cellular tracking [1-4]. Our most recent and significant achievements will be presented in this contribution.

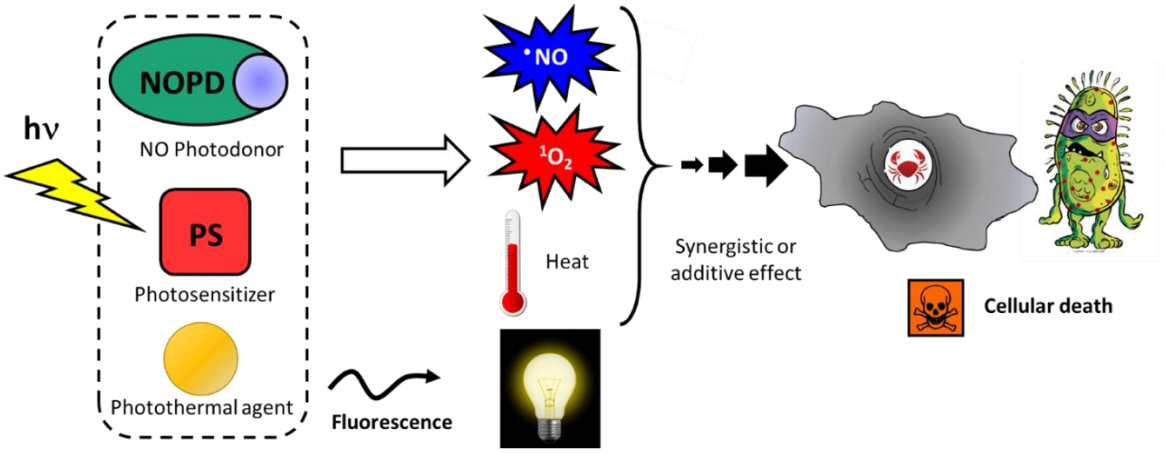

Figure 1: Schematic of the action of multimodal photo-activatable platform.

[1] A. Fraix, C. Parisi, M. Seggio, S. Sortino. *Chem. Eur. J.* **2021**, 27, 12714–1272.
[2] C. Parisi, M. Failla, A. Fraix, L. Menilli, F. Moret, E. Reddi, F. Spyrakis, B. Rolando, L. Lazzarato, R. Fruttero, A. Gasco, S. Sortino. *Chemical Science* **2021**, *12*, 4770-4776.
[3] A, Fraix, V. Kirejev, M. Malanga, E. Fenyvesi, S. Beni, M. B. Ericson, S. Sortino. *Chem. Eur.J.*, **2019,** *25*, 7091-7095.
[4] A. Fraix, C. Parisi, M. Failla, K, Chegaev, F. Spyrakis, L. Lazzarato, R. Fruttero, A. Gasco, S. Sortino. *Chem. Commun.* **2020**, *56*, 6332-6335.

Pivotal role of carnosine in the modulation of microglia and macrophages activity: multimodal mechanism of action and therapeutic potential in neurodegenerative disorders

Giuseppe Caruso [a,b], Claudia G. Fresta [c], Cristina Benatti [d], Nicolò Musso [c], Mariaconcetta Giambirtone [b], Margherita Grasso [a,b], Simona F. Spampinato [c], Sara Merlo [c], Annamaria Fidilio [c], Giorgia Spampinato [c], Filippo Drago [c], Giuseppe Lazzarino [c], Maria A. Sortino [c], Nicoletta Brunello [d], Claudio Bucolo [c], Blake R. Peterson [e], Fabio Tascedda [d], Susan M. Lunte [f,g], Filippo Caraci [a,b]

[a] *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy;* [b] *Oasi Research Institute—IRCCS, 94018 Troina, Italy;* [c] *Department of Biomedical and Biotechnological Sciences, University of Catania, 95125 Catania, Italy;* [d] *Centre of Neuroscience and Neurotechnology and Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy;* [e] *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA;* [f] *Ralph N. Adams Institute for Bioanalytical Chemistry, University of Kansas, Lawrence, KS 66047-1620, USA;* [g] *Department of Chemistry and Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047-1620, USA.*

Carnosine is a naturally occurring dipeptide widely distributed in mammalian tissues, existing at particularly high concentrations (mM) in muscles and brain. It is involved in many cellular defense mechanisms against oxidative stress and inflammation, including inhibition of amyloid-β (Aβ) aggregation and scavenging of reactive oxygen species (ROS). As part of the innate immune response, microglia and macrophages represent the cells primarily activated, especially under pathological conditions. In the context of Alzheimer's disease (AD), microglia exert a dual role: promote the clearance of Aβ *via* phagocytosis and increase neuroinflammation through the secretion of inflammatory mediators and ROS. In addition to microglia, peripheral macrophages play a role in Aβ clearance from the brain *via* its uptake and degradation.

The activity of carnosine was tested in an *in vitro* model of Aβ-induced neuroinflammation (BV-2 + Aβ oligomers (Aβo)) and an *in vitro* model of Aβ-induced peripheral inflammation (RAW 264.7 + Aβo). An ample set of conventional and innovative techniques such as ELISA and microchip electrophoresis coupled to laser-induced fluorescence was used to evaluate the protective activity of carnosine.

In our experimental model of Aβ-induced neuroinflammation, carnosine: 1) prevented cell death in BV-2 cells challenged with Aβo; 2) lowered oxidative stress by decreasing the expression of pro-oxidant enzymes and the relate ROS production; 3) decreased the secretion of pro-inflammatory cytokines, rescued IL-10 levels, and increased the expression and release of TGF-β1; 4) prevented Aβ-induced neurodegeneration in primary mixed neuronal cultures treated with Aβo, a neuroprotective effect mediated by TGF-β1.

In our experimental model of Aβ-induced peripheral inflammation, carnosine: 1) prevented cell death in RAW 264.7 macrophages treated with Aβo; 2) counteracted Aβo-induced apoptosis; 3) decreased oxidative stress by decreasing nitric oxide and total ROS levels as well as the production of peroxynitrite Aβo-induced; 4) enhanced macrophage phagocytic activity; 5) rescued the expression levels of fractalkine receptor CX3CR1.

Overall, our data suggest a novel multimodal mechanism of action of carnosine underlying its protective effects in macrophages and microglia and the therapeutic potential of this dipeptide in counteracting pro-oxidant and pro-inflammatory phenomena observed in different disorders characterized by elevated levels of oxidative stress and inflammation such as AD.

A novel photothermal-responsive nanosystem for curcumin release

Grazia Maria Letizia Consoli [a], *Maria Laura Giuffrida* [b], *Cristina Satriano* [c], *Teresa Musumeci* [d], *Giuseppe Forte* [d], *and Salvatore Petralia* [d]

[a.] *CNR-Institute of Biomolecular Chemistry, 95126 Catania, Ital,* [b] *CNR-Institute of Crystallography, 95126 Catania, Italy,* [c] *Department of Chemical Sciences, Laboratory of NanoHybrid Biointerfaces (NHBIL), University of Catania, 95125 Catania, Italy,* [d.] *Department of Drug and Health Sciences, University of Catania, 95125 Catania, Italy*

An innovative luminescent and photothermal-responsive nanosystem has been synthetized by a one-pot solvent- and reagent-free method. This novel nanomaterial is composed by a carbonized core and cross-linked chains of poly(*N*-isopropylacrylamide). It exhibits LCST behavior, high photothermal conversion efficiency and no toxicity on eukaryotic cells.
Proof of concept experiments confirmed an excellent thermo-induced curcumin release activity to be used for photothermal-controlled drug release. Molecular dynamic study evidenced the formation of hydrogen bonds between the two phenolic atoms of curcumin and the carbonyl oxygen atoms on the polymers terminations, The electronic energy value for the nanosystem/curcumin adduct was about 16.79 kcal/mol.

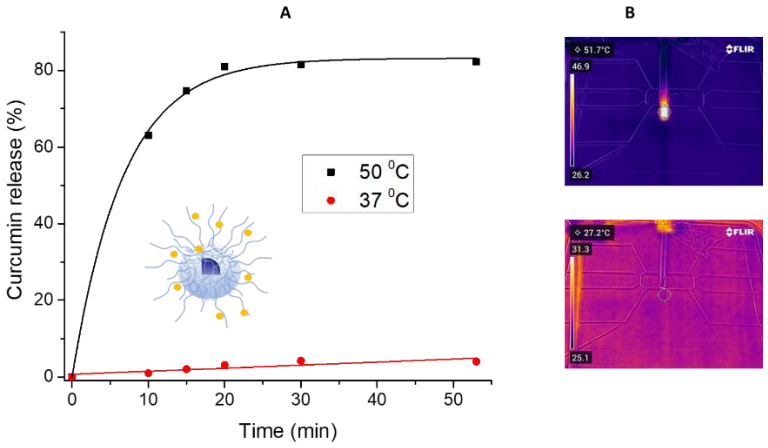

Figure A) Release of curcumin from the CPDs-PNM at 50 °C and at 37 °C, B) representative thermophotographs during the photothermal experiments.

[1] G. Granata, I. Paterniti, C. Geraci, F. Cunsolo, E. Esposito, M. Cordaro, A. R. Blanco, S. Cuzzocrea, and G. M. L. Consoli, Mol. Pharm. **2017**, 14, 1610.
[2] G. M. L. Consoli, M. L. Giuffrida, C. Satriano, T. Musumeci, G. Forte and S. Petralia A novel facile one-pot synthesis of photothermal-responsive Carbon Polymer Dots as promising drug nanocarriers. Chem Comm **2021** submitted.
[3] G. Consiglio, P. Di Pietro, L. D'Urso, G. Forte, G. Grasso, C. Sgarlata, D. Cossement, R. Snyders, and C. Satriano, *J. Coll. Interface Sci.*, **2017,** 506, 532.

COmputational Modeling in systems BIomediciNE (COMBINE) Group – UNICT

Giulia Russo [*], *Valentina Di Salvatore* [a], *Francesco Pappalardo* [a]

[a] *Dipartimento di Scienze del Farmaco e della Salute, Università degli Studi di Catania, Catania*

*giulia.russo@unict.it;valentina.disalvatore@unict.it; francesco.pappalardo@unict.it

Innovations in information and communication technology infuse all branches of science, including life sciences. Nevertheless, healthcare is historically slow in adopting technological innovation, compared with other industrial sectors. In recent years, new approaches in modelling and simulation have started to provide important insights in biomedicine, opening the way for their potential use in the reduction, refinement, and partial substitution of both animal and human experimentation.
The Research Group "*COmputational Modeling in systems BIomediciNE*" (COMBINE for short) of the University of Catania, Department of Drug and Health Sciences, deals with computational biomedicine and its main research activities are focusing on *In Silico trials*, vaccines and immunotherapies and precision medicine.
COMBINE group has developed the Universal Immune System Simulator (UISS) that is a computational framework that makes use of a multi-scale, multi-organ, three-dimensional agent-based simulator of the immune system, with an attached module able to simulate the dynamics of a biological pathway at the molecular level, named COmputational StrategieS for the analysis of Biological pAthways and moleculaR surface/binding (CrOSSBAR).
UISS was applied to several objectives and aims: *i)* to make predictions about the outcome of specific vaccination strategies; *ii)* to optimize vaccine dosage for the best result in terms of efficacy and minimization of adverse effects in a personalized fashion; *iii)* for vaccine formulation discovery (both at cellular and molecular level); *iv)* to optimize drug combinations for obtaining maximum efficacy, and *v)* to discover molecular and/or cellular targets for overcoming drug resistance.
In the context of in silico trials, COMBINE Group is developing innovative scientific and technological *In Silico trials* solutions for product design, development and assessment of drugs and other biomedical products. UISS computational modelling framework is able to simulate and predict the outcome of therapeutic strategies in a personalized fashion with the aim to deliver a validated in silico platform capable to integrate and empower the standard clinical trials for the testing of several treatments in patients. In this way, a virtual set of patients could complement a clinical trial (reducing the number of enrolled patients and improving statistical significance), and/or advise clinical decisions. UISS simulation platform potentially provides a level 3 simulation platform (i.e., each individual of the reference population will be simulated and represented using biological and physiopathological data coming from real subjects) able to achieve the necessary statistical power (eventually integrated by a number of virtual subjects) to offer an effective way to estimate disease progression even under specific treatments.
Today, challenges of continuing development of vaccines and/or immunotherapies for example the ones for COVID-19 pandemic represent an urgent need. As never before, the application of modeling and simulation can promptly design better vaccine prototypes, support decision making, reduce experimental costs and time, and ultimately improve success rates of vaccine and immunotherapies trials. In this context, UISS simulator was validated in full preclinical settings in predicting optimal dosage cancer vaccine administration and in the identification of the best adjuvant in vaccine formulation against influenza A and Human Papilloma Virus (HPV).

Presently, UISS for Tuberculosis (UISS-TB), a specific disease module of UISS capable to reproduce the dynamics of the immune system affected by TB and predict the outcome of a real clinical trial under the administration of specific interventions, is going to be evaluated through the Qualification Advice process at the European Medicines Agency as potential novel methodology to speed up and improve the process of getting new drugs on the EU market.

The underlying concept of precision medicine, in which health care is individually tailored on the basis of the variability of person's genes, lifestyle and environment, is not new. This approach will allow doctors and researchers to predict more accurately which treatment and prevention strategies will be the best for each patient and for a particular disease. Recently, thanks to the availability of large-scale biological databases containing information of many samples and phenotypes, of genes and drugs, and of detailed epidemiological data, the analysis and integration of such large information into predictive models, can be helpful to build testable *In Silico* models as evidence-based predictors to guide future clinical practice.

UISS computational framework is also capable to improve new knowledge in the field of molecular networks and systems biology, allowing the identification of optimal therapeutic targets associated with genomic and proteomic biomarkers. In this context, UISS *In Silico* solution can help in the design of new drugs that minimize the side effects and maximize the therapeutic response; then, in the identification of diagnostic and prognostic biomarkers, and in the design and dynamic improvement of personalized therapies.

The continuous research and development in COMBINE Group is supported by several European funded projects within the context of specific competitive research and innovation programmes, boosting the creation and a better dispersion of improving knowledge and increasing technologies.

The multidisciplinary context in which *In Silico* medicine and COMBINE Group act can potentially facilitate biomedicine research and make it more powerful and effective.

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Porous bio-composite material based on natural fiber-Halloysite for gas adsorption and drug delivery

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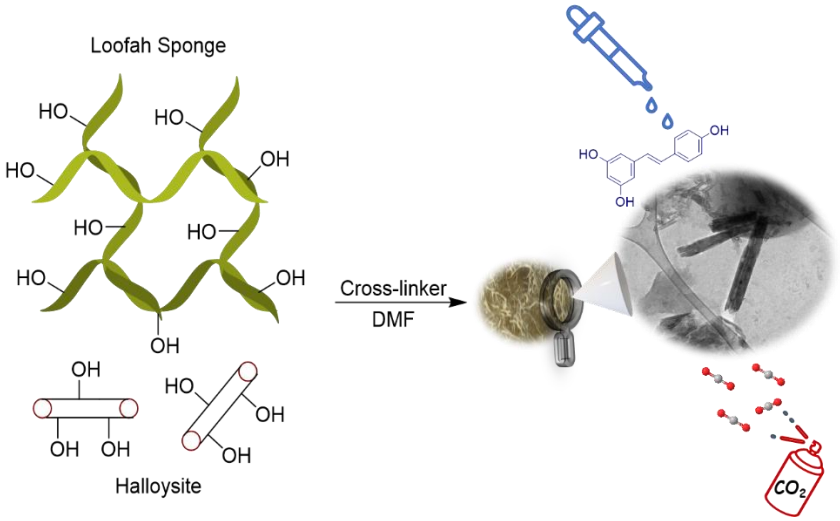
The advancement of materials science and technology has led researchers to look at nature to find new materials with high performance and low cost. Among these, the loofah sponge has been widely used as a natural material in industrial applications, thanks to its polyporous structure and light consistency.[1] This work aims to functionalize the loofah fibers easily with halloysite, a clay mineral of the kaolin group,[2] to improve its adsorption performance and mechanical properties. Two different crosslinkers were used for functionalization, and the new composites were characterized by FT-IR, TGA, SEM, HR-TEM. The as-obtained green composites were applied for the carbon dioxide capture studies, showing superior adsorption capacities than the single components, loofah and halloysite, and materials currently used in industry, such as BEA and MOR zeolites. Furthermore, besides being able to be reused for several cycles of carbon dioxide adsorption, the new composites have proved to be excellent candidates for the delivery of resveratrol, maintaining its anticancer activity. The results underline the synergistic effect of functionalization in increasing the adsorption properties compared to the starting materials and the possibility of using this eco-sustainable and low-cost porous system in various fields such as gas adsorption and drug delivery.



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Promising new strategies for ophthalmic drug delivery

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The advent of nanotechnology has made important innovations in the field of biological research and clinical practice. In particular, Lipid-based Nanocarriers (LNCs) can be exploited to modify the release of drugs and to improve the stability of the drug over time, avoiding its rapid degradation. LNCs are colloidal carrier systems composed by a biodegradable lipid matrix (GRAS) and they are divided into a first (Solid Lipid Nanoparticles - SLNs) and a second generation (Nanostructured Lipid Carriers - NLCs). The composition of these nanoparticles, along with their characteristics, make them ideal for the carrying/delivery of sensitive bioactive compounds, protecting them against chemical degradation and also facilitating their application in various administration routes [1,2]. The encapsulation of drugs into these nano-carriers increases their solubility, stability, cell uptake, specificity, tolerability, and therapeutic index. Therefore, these carriers are suitable for almost all routes of drug administration, including the ophthalmic route. The advent of these nanocarriers has made important innovations in the field of ophthalmology; in particular, NLC are able to increase tolerability and interaction with the ocular mucosa [3]. In previous works by our group, palmitoylethanolamide (PEA)-loaded nanostructured lipid carriers (NLCs) were developed to enhance the ocular bioavailability of this drug [4]. Pharmacokinetic studies showed that the retinal levels of PEA were significantly higher in the group treated with a PEA-NLC formulation versus an aqueous suspension of PEA. Moreover, it was able to significantly inhibits retinal tumor necrosis factor (TNF-α) levels in streptozotocin-induced diabetic rats, suggesting that the novel ophthalmic formulation may be useful for the treatment of retinal diseases, such as diabetic retinopathy [5]. Therefore, NLCs demonstrated their ability to deliver high levels of lipophilic drugs to the back of the eye after topical ocular administration.
In order to investigate how the LNCs interact with the ocular mucosa and reach the posterior eye segment, we have formulated lipid nanocarriers (LN-ODAF) that were designed to bear a traceable fluorescent probe [6]. The chosen fluorescent probe was obtained by a conjugation reaction between fluoresceinamine and the solid lipid excipient stearic acid, forming a chemically synthesized adduct (ODAF). *In vivo* results pointed out that after ocular instillation, LN-ODAF were concentrated in the cornea (two hours), while at a longer time (from the second hour to the eighth hour), the fluorescent signals extended gradually towards the back of the eye. From the results obtained, LNCs demonstrated a efficient carriers of an active pharmaceutical ingredient (API) involved in the management of retinal diseases.

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Etna Saffron (Crocus Sativus): analytical characterization and coloring power determination through ISO 3632/2003 standard

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Phytocostituents are bioactive compounds contained in every plant and responsible of plant's protection from microorganisms and predators. They are responsible of plant's color, smell and taste and they can have potential biological effects. Phytocostituents content and bioavailability varies according to the soil and the type of cultivation, the season, the species and the method of conservation.
Saffron is a bulbous annual plant, widely used for its antioxidant, anticancer, antidepressant, and antiseptic properties. *Crocus Sativus* is the only crocus species to have a certain economic importance due to the presence of volatile compounds that give the aroma, non-volatile active components and active ingredients such as crocin, picrocrocin and safranal.
The aim of this work is to search for the presence of phytocostituents in the Etna *Crocus Sativus* received from the Capizzi Vincenzo Farm. In particular, the carotenoids present are determined both by studying the coloring power through the ISO 3632/2003 standard and by using analytical instrumentation UPLC-Ms-Ms (third-party analysis).
The results of the study demonstrate that Etna *Crocus Sativus* contains crocetine with a high coloring power, safranal with an odorous power, picrocrocin with a bittering power and other carotenoids such as lycopene, crocin, beta-carotene and zeaxanthin. In particular, the presence of crocetine is here demonstrated by using both analytical techniques, while the presence of the other carotenoids is here confirmed exclusively by mass spectrometry.

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Effect of natural antioxidants on tissue transglutaminase overexpression induced by amyloid-β: biological, molecular and computational studies

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Alzhèimer Disease (AD), one of the most common neurodegenerative diseases, is characterized by progressive neuronal loss and accumulation of proteins, including Amyloid-beta (Aβ), a neurotoxic protein. It is known that tissue transglutaminase (TG2), an ubiquitary calcium-dependent protein, is involved in protein aggregation in AD. Previous our studies showed that Aβ(1-42) and its fragments Aβ(25-35) and Aβ(35-25) induced an overexpression of TG2 and its isoforms on Olfactory Ensheathing Cells (OECs), a glial population of the olfactory system that express neural stem cell markers, including Nestin. In the last years growing attention rose on neuronutraceutics and their effect on mental health. Among these molecules, we focused our research on indicaxanthin, and astaxanthin, natural compounds that were able to cross the blood–brain barrier. In this study, the effect of indicaxanthin or astaxanthin pre-treatment on TG2 and its isoform expression levels exposed to Aβ(1–42) or by Aβ(25–35) or Aβ(35–25) on OECs was assessed. Furthermore, we evaluated thei effect on the expression levels of Vimentin and Glial Fibrillary Acid Protein (GFAP). The percentage of cell viability and the apoptotic pathway activation were also evaluated. Since Nestin, a marker of neural precursors, is co-expressed in pluripotent stem cells with cyclin D_1, a marker of cellular proliferation, the effect of indicaxanthin and astaxanthin pre-treatment on their expression levels was also tested. In addition, the production of total reactive oxygen species (ROS) and superoxide anion ($O_2^{\cdot-}$), were assessed. In parallel, docking studies were performed to obtain informations relative to the interaction between the indicaxanthin or astaxanthin and TG2.
We found that indicaxanthin or astaxanthin pre-treatment was able to reduce TG2 overexpression and its isoforms, decreasing total ROS and $O_2^{\cdot-}$production and GFAP and Vimentin expression levels. In addition, they inhibited apoptotic pathway activation and induced an increase in the Nestin and cyclin D_1 expression levels. Docking results showing that indicaxanthin and astaxanthin were able to prevent the TG2 change conformation induced by Aβ.
Our data demonstrated that indicaxanthin or astaxanthin pre-treatment stimulated OECs self-renewal through the reparative activity played by TG2. Therefore, indicaxanthin and astaxanthin might represent an innovative mechanism to contrast TG2 overexpression in AD.

MOR/DOR Dual-Target Approach: 2S-LP2 Effects in Inflammatory and Neuropathic Pain

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Inflammatory and neuropathic pain are the most common forms of persistent pain [1]. Despite their benefit in acute pain, MOR agonists have a limited application in chronic pain due to their side effects [2]. In this scenario, a novel therapeutic strategy could be represented by the development of MOR/DOR Dual-Target compounds, supported by the co-expression of both receptors in areas involved in pain modulation [3]. Starting from LP2, a MOR/DOR agonist [4], it was investigated the pivotal role of the stereocenter at the N-substituent of its 6,7-benzomorphan scaffold and thus the (2S)-N-2-methoxy-2-phenylethyl-6,7-benzomorphan (2S-LP2) was synthesized [5]. Radioligand competition binding assay showed a lower inhibition constant (Ki) for 2S-LP2, especially on MOR (K_i=0.5 ± 0.03 nM) and DOR (K_i=2.59 ± 0.05 nM), than both K_i values of 2R-LP2 and LP2, indicating an improved binding profile for 2S-configuration [5]. Results obtained in the mouse tail-flick test demonstrated a better antinociceptive effect for 2S-LP2 together with higher potency as observed by lower value of ED_{50}= 0.6 mg/kg i.p. (0.4-0.8, 95% C.l.) when compared to 2R-isomer (ED_{50}= 1.8 mg/kg i.p.) (1.2-2.8, 95% C.l.) and to LP2 values (ED_{50}= 0.9 mg/kg i.p.) [4]. Based on these previous findings, our aim was to evaluate the antinociceptive effect of 2S-LP2 in animal models of inflammatory and neuropathic pain. Considering the adaptability of 6,7-benzomorphan scaffold, we also tested, through radioligand competition binding assay, the affinity of 2S-isomer for sigma-1 receptor, which is involved in analgesic opioid activity modulation [6]. Our results show a K_i of 112.72 ± 13.49 nM for sigma-1 receptor compared to sigma-2 receptor (K_i=1013.91 ± 191.31 nM). In the mouse formalin test, 2S-LP2 (0.5-0.7 mg/kg, i.p.) exhibited a significant analgesic effect antagonized by naloxone (3 mg/kg s.c.) pretreatment, an opioid antagonist. In Chronic Constriction Injury (CCI) model, 2S-LP2 maintained its antiallodynic effect at dose of 0.7 mg/kg i.p, antagonized by naloxonazine (10 mg/kg i.p.) and naltrindole (3 mg/kg i.p.), MOR and DOR selective antagonists, respectively. Taken together, these results indicate 2S-LP2 as new promising drug for the treatment of different chronic pain states.

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New Brilliant Blue G Derivative as Vital Dye in Retinal Surgery

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Our study was aimed at assessing the retinal binding of a new synthetic Brilliant Blue G (BBG) derivative (pure benzyl-Brilliant Blue G; PBB) ophthalmic formulation, to improve vitreoretinal surgery procedure [1-3]. Protein affinity of the new molecule was evaluated in vitro (cell-free assay). Furthermore, an ex vivo model of vitreoretinal surgery was developed by using porcine eyes to assess the pharmacological profile of PBB, compared to commercial formulations based on BBG and methyl-BBG (Me-BBG). PBB showed a higher affinity for proteins ($p < 0.05$), compared to BBG and Me-BBG. In vitro studies demonstrated that the high selectivity of PBB could be related to high lipophilicity and binding affinity to fibronectin, the main component of the retinal internal limiting membrane (ILM). In Fig. 1 is illustrated a simplified model of the passive diffusion of molecules from the ILM to the retina (A, Diffusion of a molecule with low affinity to ILM proteins; B, Diffusion of a molecule with high affinity to ILM proteins).

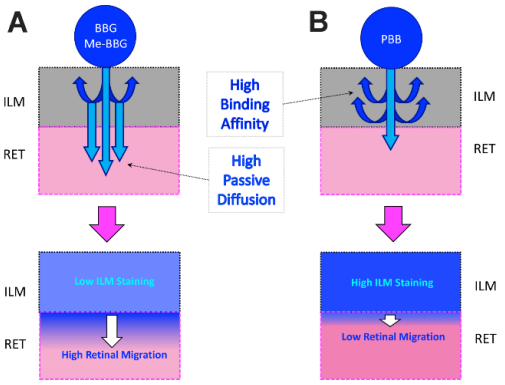

Fig. 1

The PBB staining capabilities were evaluated in porcine eyes in comparison with BBG and Me-BBG. Forty microliters of each formulation were slowly placed over the retinal surface and removed after 30 s. After that, ILM peeling was carried out, and the retina collected. BBG, Me-BBG, and PBB quantification in ILM and retina tissues was carried out by HPLC analysis. PBB levels in the ILM were significantly ($p < 0.05$) higher compared to BBG and Me-BBG formulations. On the contrary, PBB showed a much lower ($p < 0.05$) distribution in retina (52 ng/mg tissue) compared to BBG and Me-BBG, in particular PBB levels were significantly ($p < 0.05$) lower.
Therefore, the new synthetic Brilliant Blue derivative (PBB) showed a great ILM selectivity in comparison to underneath retinal layers. In conclusion, these findings had high translational impact with a tangible improving in ex vivo model of retinal surgery, suggesting a future use during surgical practice.

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COMUNICAZIONI POSTER

Sicilian wild cabbages as a source of nutraceuticals with a potentially wide range of biological activity

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The Brassica genus is one of the 51 genera of the Brassiceae tribe belonging to the *Brassicaceae* family, order Brassicales, including 37 different species, some of which are very important from an agronomic point of view. Modern Brassica crops (*Brassica oleracea* L.) are the result of the domestication of wild populations of *B. oleracea* and other species with the same diploid genome (2n = 18). The Mediterranean is one of the centres of origin of the genus; in fact, about ten wild taxa of this group are endemic to Sicily. The scientific literature reports that vegetables belonging to the Brassicaceae family, in addition to their not negligible nutritional values, are a good source of secondary metabolites with different biological activities (1), such as carotenoids, phenolic compounds and glucosinolates, a class of biologically active compounds characteristic of this family (2). Despite the few studies, most of the Sicilian wild taxa of *Brassica* sect. *Brassica* is a richer and more varied source of phytochemicals than the cultivated varieties of *B. oleracaea*. Interesting are our recent results on the biological activities of *B. villosa* subsp. *drepanensis* in an *in vitro* model of inflammation. Therefore, the present research aimed to evaluate the qualitative and quantitative phytochemical profile and some biological effects of *B. incana* subsp. *raimondoi*, an endemic species of Castelmola (Messina, Sicily), in an *in vitro* model of hepatic steatosis. The total phenolic, flavonoid and condensed tannin contents of the extract were determined spectrophotometrically, resulting in equal to 38.12 ± 0.50 mg GAE/g extract, 8.45 ± 0.60 mg QE/g extract and 4.70 ± 0.07 mg CE/g extract, respectively. HPLC phytochemical profile analysis confirmed the presence of several flavonoids and characteristic glucosinolates. The extract showed a good radical scavenging activity (DPPH test, IC50 = 1.33 ± 0.02 mg/ml) and a mild reducing power whereas no chelating properties were detected. No toxicity was found for the extract against brine shrimp larvae (*Artemia salina* Leach) and no cytotoxic effects were highlighted in human hepatocytes (HepG2) and fibroblasts (HFF-1). In an *in vitro* cell system of oxidative stress assessing cellular ROS levels and glutathione amount, the antioxidant activity of the extract was evaluated. The obtained data showed that *B. raimondoi* extract was able to counteract oxidative stress induced by H_2O_2 treatment on HepG2 cells, significantly decreasing ROS levels increasing the amount of intracellular glutathione. It is currently under examination the effects of the extract in an *in vitro* model of steatosis induced by oleic acid with the evaluation of markers related to the main mechanism of action in lipid hepatocyte metabolism (PPAR G, FAS, FABP4) and cellular inflammation (NF-kB, TNF alpha, Il-1 beta). These results suggest the health-promoting properties of *B. incana* subsp. *raimondoi* and its potential as a nutraceutical source.

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Delivery of memantine conjugated peptides by β-CD supramolecular inclusion complexes

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Memantine (Mem) is an uncompetitive, moderate-affinity N-methyl-D-aspartate (NMDA) antagonist believed to protect neurons from excitotoxicity. It works by blocking the pathological effect due to high levels of glutamate which can lead to neuronal dysfunction.
Mem is currently used as symptomatic approach to treat the cognitive impairment in Alzheimer's Disease (AD).
AD represents the most common form of dementia and belongs to the group of neurodegenerative disorders characterized by progressive loss of neurons in the central nervous system[1]. The primary histopathologic lesions of Alzheimer's pathology are amyloid plaques, neurofibrillary tangles (NFTs) and neuronal loss. Mature plaques consist of a central amyloid core with surrounding degenerating neurons affected by the toxic effect of the Aβ peptide.
A rational pharmacological approach for prevention of amyloid formation would therefore be to use drugs that specifically interfere with Aβ self-interaction and aggregation.
Previous studies suggest that amino acid residues within or close to $A\beta_{16–20}$ peptide are important for the adoption of the correct β-pleated sheet structure of Aβ[2]. It was also demonstrated that short peptides incorporating $A\beta_{16–20}$ can function as ligands that bind to Aβ and inhibit the formation of amyloid fibrils[3].
Despite great scientific efforts, beside Mem and few other symptomatic drugs, at the moment there are no effective pharmacotherapeutic options for prevention and treatment of AD.
We designed and characterized a peptide-based system formed by the Memantine and some Aβ fragments acting in principle as "stabilizers" of the monomeric form of Aβ.
An important aspect to be taken into consideration for the use of these conjugated systems in the pharmacological field is related to their poor solubility in aqueous environment. This problem can be solved with the help of β-cyclodextrins. In fact, the high affinity of compounds analogous to adamantane as memantine for the hydrophobic cavity of β-cyclodextrin is known[4].
The study of the interaction between the peptide conjugates and amyloid was conducted with the use of experimental techniques such as CD and Th-T fluorescence.
Others experiments were managed *in vitro* in order to test the potential toxicity of these systems and *in vivo* to assess short- or long-term recognition memory in mice with the Novel Object Recognition (NOR) test.

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Nutraceuticals: new frontier for drug delivery application

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Nowadays alternative medicine is attracting increasing interest in the scientific research; especially essential oils (EOs) have been the focus of various studies since they have a great variety of health benefits, such as antioxidant, anti-inflammatory, antimicrobial, regenerative and anxiolytic activities. Despite these interesting advantages, there are many drawbacks related to their low stability that can be overcome through the encapsulation in drug delivery systems (DDS). DDS are demanded by pharmaceutical, agricultural, cosmetic, nutraceutical and food industries because of their great potential toward the active agents delivered: protection from the environment improving their stability; masking of their organoleptic properties; improvement of compatibility in co-administration; enhancement of their transport, solubility, absorption and bioavailability; provision of controlled release over time and delivery to the target site. Recently, microencapsulation techniques were also studied by our research group to obtain functional food, and specifically alginate microcapsules loaded with *Lacticaseibacillus rhamnosus* GG were produced through ionotropic gelation technology. It has demonstrated to be a potential tool to preserve the viability of probiotic when delivered into drink such as orange juice [1]. Moreover, biomasses were successfully encapsulated into DDS, as natural super-antioxidants: in particular, Astaxanthin extracted from *Haematococcus pluvialis* was delivered into NLC, while Phycocianin obtained from *Spirulina* was encapsulated into microcapsules, in order to be administered orally and/or topically. Our research group also succeeded in producing Nanostructured Lipid Carriers (NLC) using both lab-scale (Phase Inversion Temperature method) and scalable (High Pressure Homogenization) preparation methods, in order to encapsulate EOs from the Mediterranean Area (such as *Rosmarinus officinalis* L., *Lavandula* × *intermedia* "Sumian", *Origanum vulgare* subsp. *Hirtum*,*Thymus capitatus* and *Mentha piperita*). In particular, the EO was used as intrinsic oily component of the lipid matrix, and its presence not only allowed to exploit the beneficial effect related to the specific properties of each oil (i.e. anti-inflammatory and antioxidant activity [2][1]), but also improved the stability of the nanoparticle structures, providing a great long-term stability [3] and enhancing the effectiveness of the co-administered drug. EO-loaded NLC have been proposed as a potential platform in the treatment of neurodegenerative diseases [4], but also in co-delivery with ferulic acid for the treatment of wound healing [3].

As an evidence of these studies, our research group has achieved an all-round expertise in the development and characterization of drug delivery systems. Firstly, a statistical and mathematical approach called Design of Experiments is performed to obtain an optimized formulation, considering different parameters that could affect the quality of the system. Characteristics of the obtained platforms to be firstly analysed are mean particle size, polydispersion index and zeta-potential by Photon Correlation Spectroscopy; pH and osmolarity. Moreover, physical stability could be assessed using Turbiscan Ageing Station or accelerated stability studies by Climatic Chamber, while thermotropic parameters are analysed through Differential Scanning Calorimetry. Drug loading and encapsulation efficiency are measured using UV-vis Spectrophotometer or HPLC and *in vitro* drug

release profile through Franz-type diffusion cells. Relating to the administration route, eventual *in vitro* studies could be performed in order to analyse potential mucoadhesive properties of the systems.

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Development of 5-fluorouracil and heme oxygenase 1 inhibitor mutual prodrugs as an innovative strategy to develop novel anticancer agents

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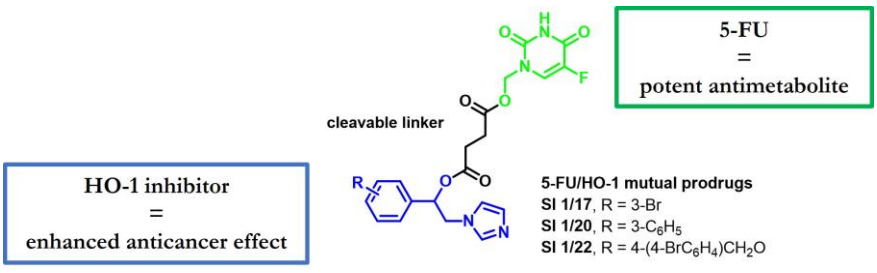
Overexpression of the heme oxygenase 1 (HO-1) levels in cancer cells has been linked to tumor growth, aggressiveness, and resistance phenomenon, suggesting the use of HO-1 inhibitors as a promising pharmacological approach in cancer chemotherapy [1].
5-Fluorouracil (5-FU) is a well-known antimetabolite drug largely employed as a first-line antineoplastic agent for the treatment of several types of cancer; however, its therapeutic efficacy is mitigated by low drug-like properties and severe side effects. Therefore, 5-FU-based mutual prodrugs have been developed for both improving biological activity and achieving targeted delivery to cancer tissues [2].
With this in mind, we synthesized novel mutual prodrugs of 5-FU and HO-1 inhibitors conjugating the two active pro-moieties (i.e., 5-FU and azole-based HO-1 inhibitors) through a succinic group that served as a cleavable linker [3]. *In vitro* enzymatic stability studies showed a suitable enzymatic hydrolysis rate in porcine esterase solution for the new hybrids. MTT assay was performed to evaluate the cytotoxicity effects of the new compounds towards DU145 human prostate and A549 lung cancer cells. Specifically, the newly synthesized 5-FU/HO-1 mutual prodrugs showed a similar or higher effect on cell viability compared to the reference compounds (5-FU and imidazole-based derivatives) or their combination. Moreover, the most potent compounds **SI 1/20** and **SI 1/22** were selected for more in-depth biochemical studies in DU145 cells. As a result, both compounds were able to increase the ROS production in a time and dose-dependent manner, an effect which was accompanied by the reduction of expression levels of HO-1. Finally, **SI 1/20** and **SI 1/22** showed to inhibit the enzymatic activity in intact DU145 cells, suggesting that these molecules can cross the cellular membrane. Altogether these promising results support our strategy and the further development of 5-FU/HO-1 mutual prodrugs.



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NLC as promising strategy for the treatment of ophthalmic tumor

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Uveal melanoma (UM) represents one of the most common malignant tumor of the eye, with mortality in 50% of patients in one year **Errore. L'origine riferimento non è stata trovata.**. It was demonstrated the involvement of both σ receptors, which modulate proliferation and angiogenesis, suggesting a possible use of σ_1 antagonist in the treatment of this diseases, together with HDAV inhibitors, which seem to play an important role as adjuvants. In particular, σ_1 receptor antagonists (±)-MRJF22, (S)-(–)-MRJF22 and (R)-(+)-MRJF22 – prodrugs of (±)-haloperidol metabolite II conjugated with valproic acid – showed interesting antiangiogenic effect in previous studies [1]. However, due to the physiological difficulties related to ophthalmic administration, nanoencapsulation into drug delivery systems represents a potential solution to efficiently reach the target site. Therefore, the aim of our work was the encapsulation of (S)-(–)-MRJF22 and (R)-(+)-MRJF22 into nanostructured lipid carriers (NLC), selected for their advantages in ophthalmic application [3], thus obtaining S-NLC and R-NLC, respectively. The preliminary physical-chemical characterization of the compounds by Differential Scanning Calorimetry (DSC) allowed assessing the thermal behaviour of the compounds, demonstrating that the temperature used in the NLC preparation method did not affect the molecules. NLC prepared with Softisan and Isopropyl myristate were produced using TRIS buffer pH 7.2-7.4 as aqueous phase, thus guaranteeing physiological pH and osmolarity values (respectively 7.2 and 0.260 mOsm/kg) and confirming their suitability with the intended administration route. NLC showed homogeneous (PDI=0.230) small particles (100-150 nm) with almost neutral zeta potential (-5 mV). *In vitro* cytocompatibility studies were performed on human UM 92-1 cells using blank NLC, confirming a good cell viability at all tested concentrations. The obtained results suggest that the prepared NLC are suitable for the ophthalmic delivery of (R)-(+)-MRJF22 and (S)-(–)-MRJF22 enantiomers as a promising treatment for UM. Further studies could be performed to assess in vitro drug release and proliferation on UM 92-1 cells.

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Hybrid MOR agonist/HDACi molecules as potential antinociceptive multi-target drugs: design, synthesis, and biological evaluation

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Persistent pain is a common clinical symptom of many diseases and many patients are still inadequately treated[1]. Despite existing different analgesic drugs, nowadays, opioid analgesics continue to be the hallmark of therapy against acute and chronic pain. Opioid analgesic effects are predominantly mediated by mu opioid receptor (MOR) whose activation also elicits side effects. Given the impossibility to segregate the analgesic effect from side effects arising from MOR activation, recently the medicinal chemistry approach focused on the development of multitarget drugs. This approach derives from the clinical practice of multitarget therapy or polypharmacology that consists in the co-administration of two or more drugs acting with different mechanism of action upon different targets. Even if MOR represents the main target involved in pain modulation, new molecular targets in pain transmission have been identified such as the histone deacetylase (HDAC) enzyme. Several evidence suggested that epigenetic regulation are involved in development and maintenance of chronic pain. It has been experimentally demonstrated that nerve injury or inflammatory conditions increase the expression of HDAC and that histone deacetylase inhibitors (HDACi) attenuate both neuropathic and inflammatory pain. Moreover, the hypoacetylation state of histones H3 and H4 contributes to the decreased expression of MOR in the dorsal root ganglion (DRG)[2]. For these reasons, dual-target MOR agonist/HDACi molecules could represent a good strategy used in clinical practice for the management of persistent pain conditions. Multitarget MOR agonist/ HDACi compounds have been designed recurring to the "merging" approach in which the two target pharmacophores are combined in the same central core [3]. The multitarget compounds have been synthesized through both classical and/or microwave-assisted synthetic methods. Intermediates and final compounds have been appropriately purified through flash chromatography. The structural characterization of the compounds has been determined by ^{1}H-NMR and ^{13}C-NMR. *In vitro* their affinity profile *versus* opioid receptors have been performed through competition binding assays, with [^{3}H]DAMGO, [^{3}H]Delthorphin II and [^{3}H]U69,593 on MOR, delta opioid receptor (DOR) and kappa opioid receptor (KOR), respectively. Some assayed compounds showed a relevant MOR affinity with Ki values in the range of 1.5-5.9 nM. Competition binding assays on σ1 and σ2 receptors were also performed using [^{3}H]-(+)-Pentazocine and [^{3}H]DTG as radioligands, respectively. Mouse vas deferens (MVD) assay is in progress for evaluating biological activity on opioid receptors. Moreover, the HDAC fluorimetric assay is programmed to study the inhibition properties of our hybrid compounds. Finally, in vivo evaluation in tail flick test will be programmed.

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Usage of the Universal Immune System Simulator (UISS) to predict the effects of immunotoxic substances on the immune system

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Computational modelling and simulation platforms can be used in pharmaceutical domain as well as in the chemicals one, for example, to simulate the adverse events that some immunotoxic compounds, such as perfluoroalkyl substances (PFASs), may have. The aim of this work is to investigate the adverse effects that PFASs may have on the immune system, through a computational modelling infrastructure called Universal Immune System Simulator (UISS). PFASs, especially perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are global environmental contaminants; the exposure to elevate concentration of PFASs is associated with lower antibody responses to vaccinations as well as with lower resistance to some diseases [1]. UISS is an In Silico Trial platform based on agent-based methodologies. It simulates the human immune system considering its main entities, interactions, and dynamics [2]. Firstly, the main information necessary to design a conceptual model are retrieved from the literature and then the implementation phase comes. In the first experiment, we generated two in silico cohorts of 100 virtual patients with a well-functioning immune system and age ranging from 18 to 60 years old. The first cohort was exposed to PFOA at time 0 and reached a serum concentration of 10 ng/ml, while the second one was unexposed to PFOA challenge. Each digital twin received two challenges to appreciate the effects of PFOA on the immune system response. In the second experiment we evaluated the effects of PFOA elicited in anti-Haemophilus Influenza Type B, anti-tetanus, anti-diphtheria antibodies, IL-10, and IFN-gamma levels in a specific children population, mirroring the ones observed in the study conducted by Abraham et al. [3]. A cohort of in silico pediatric patients is generated, simulating a commonly administered two-vaccination schedule. We predicted an inverse correlation between PFOA serum concentration levels and vaccine antibodies against Hib, tetanus and diphtheria, as well as IFN-gamma production. We also evaluated the effect of PFASs on the anti-H1N1influenza antibodies titers measured in the work by Looker et al [4]. We generated 411 digital twins who received influenza vaccination. Then, depending on the PFOA concentrations, the virtual patients were divided into 4 cohorts corresponding to specific quartiles. Finally, we compared the predicted anti-H1N1 antibodies titers against the data presented in the paper by Looker et al., obtaining in silico results in good agreement with the in vivo data. Thus, UISS-TOX is able to correctly represent the immune response and may offer the opportunity to estimate the immunotoxicity risk posed by compounds such as PFASs. To make the model strategy more robust, we will test in due course the applicability of UISS-TOX in a follow-up project in which we will evaluate the effects that skin sensitizing chemicals may have on the immune system.

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PACAP and NAP: effect of two functionally related peptides in diabetic retinopathy

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Diabetic retinopathy (DR) is a microvascular complication of diabetes leading to vision loss. The retinal impairments during DR can be ascribable to metabolic changes caused by hyperglycemia leading to microvascular alteration, retinal hypoxia, inflammation, impairments of retinal architecture resulting in blood retinal barrier (BRB) damage, and consequent general tissue dysfunction.

The protective effect of pituitary adenylate cyclase-activating peptide (PACAP) in DR it has been previously demonstrated [1]. It is well known that its effect in central nervous system is mediated through the activation of activity-dependent neuroprotective protein (ADNP) [2]. To study the role of ADNP, it is frequently used its smallest active element, known as NAP that includes eight amino acid sequence of ADNP. In the present investigation we have characterized the modulatory role of PACAP-ADNP axis on hypoxic and inflammatory event characterizing early stage of DR.

Results have demonstrated that intravitreal injection of 100µM NAP counteracts the reduction of STZ-induced retinal thickness. The immunolocalization analysis, conducted by using confocal microscopy, have revealed that a single intraocular administration of the octapeptide reduces retinal expression of hypoxic inducible factors HIF-1α and HIF-2α that are more intense in the inner nuclear layer (INL), in the outer plexiform layer (OPL) and in the photoreceptor layer (RCL) of STZ-injected rat. Moreover, NAP treatment decreases the signal intensity of vascular endothelial growth factor (VEGF) in RCL and in ganglion cell layer (GCL) as compared to STZ group. Furthermore, this peptide significantly decreases expression of inflammatory cytokine IL-1β and its related receptors IL1-RI and IL1-RII in OPL, inner plexiform layer (IPL) and RCL of diabetic retinas.
A further characterization of PACAP-ADNP axis involvement in DR could lead to identification of new therapeutic targets counteracting efficiently retinal damages.

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Glioblastoma multiforme: multimodal role exerted by pituitary adenylate cyclase-activating polypeptide

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Glioblastoma multiforme (GBM) is a lethal form of brain cancer affecting adults, characterized poor prognosis due to high rate of cells migration and invasion. The uncontrolled proliferation of cancer cells produces hypoxic niches in tumor mass. By inducing activation of hypoxia inducible factors (HIFs), hypoxia triggers many signaling cascades responsible to uncontrolled cell proliferation as well as release of vascular endothelial growth factor (VEGF), directly responsible to neoangiogenesis [1]. All these factors conduce to cancer development by promoting malignant progression and recurrence. Assumed the heterogeneity of tumoral mass, the actual therapeutic approach consists in a multimodal treatment comprising in surgery, radiation and chemotherapy with different molecules.

It has previously demonstrated that pituitary adenylate cyclase-activating polypeptide (PACAP) is involved in GBM since it interferes with the hypoxic microenvironment through the modulation of HIFs via PI3K/AKT and MAPK/ERK pathways inhibition [2]. Considering that hypoxic tumor microenvironment is strictly linked to epithelial-mesenchymal transition (EMT), in the present study, we have investigated the PACAP ability to regulate this process by using GBM frozen sample and human U87MG glioblastoma cells exposed to hypoxic mimetic agent, DFX. The immunolocalization analysis, conducted by using confocal microscopy, have revealed that PACAP and its related receptor PAC1R are expressed in cells with different phenotypes in GBM tissue. The peptide treatment decreases the expression of mesenchymal markers such as vimentin, matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) as well as simultaneously increases ZO-1 expression that represents a marker of epithelial cells. Moreover, PACAP exogenous administration significantly reduces the migratory ability of mesenchymal cells exposed to DFX-induced hypoxia, as demonstrated by the reduced expression of CD44 and vimentin.

Although additional investigations are warranted to determine PACAP role in GBM malignancy, in this study we have point out new insight on modulatory action exerted by PACAP in GBM.

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Green choice in antimicrobial therapy: potential of extracts from Sicilian plants.

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Introduction: In the last decades, the improper use of antimicrobial agents has led to a significant increase in the phenomenon of antimicrobial resistance. The growing emergence of resistant strains strongly limits the available therapeutic options to treat infections, causing an increased risk for complications and mortality. A further aspect that compromises the efficacy of chemotherapy is the expression of specific virulence factors, such as the biofilm, which allow microorganisms to elude the antibiotic and immune system's attack. To overcome such obstacles, scientific research has focused on the study of "green" antimicrobials, or rather products of natural origin able to counteract the growth and the invasion of the microorganisms.

In the oncologic field, the severe adverse effects of chemotherapy strongly impact on quality of life of oncologic patients. Furthermore, it is worth noting that one of the most common side effects of chemotherapy consists in the reduction of immune defenses. This condition favors the onset of opportunistic infections which can contribute to the worsening prognosis of oncologic patients. In this scenario, an integrative treatment with natural adjuvants, endowed with both antimicrobial and anticancer activity, could be considered a good strategy to limit the adverse effects induced by the chemotherapy, improving the life expectancy of patients.

Besides infectious and cancer diseases, many pathologies are caused by oxidative stress. The phytoextracts are a rich source of phenols and flavonoids, which are endowed with antioxidant properties. This characteristic highlight a further possible application of the natural extracts in all pathological conditions in which the oxidative stress plays a crucial role.

Therefore, in the present study, the antimicrobial, antioxidant, and antitumoral activity of extracts from plants grown in the eastern Sicily was explored. Specifically, the biological activities of the extracts of Juglans regia (L.), Orobanche crenata Forssk., Teucrium siculum Rafin, Castanea sativa Mill. and Opuntia indica L. Mill. were tested.

Materials and Methods: The antimicrobial activity on clinically relevant bacterial and fungal strains was evaluated by the microdilution method, according to the standard procedures of the CLSI. The anti-biofilm activity was tested through the crystal violet (CV) and MTT assays. The DPPH and the (SOD) s-like activity test were used to determine the antioxidant activity of the extracts. The cytotoxic and anti-invasive effects on human glioblastoma (A172) and colorectal adenocarcinoma (Caco2) cells were evaluated by the MTT assay and the cell migration assay, respectively.

Results: All the tested extracts demonstrated an effective antibacterial activity, particularly against Gram-positive bacteria. The extract of O. crenata was also able to inhibit the growth of yeasts belonging to the Candida genus. J. regia extract showed anti-biofilm activity against coagulase-negative Staphylococci. The extracts of T. siculum and O. crenata inhibited superoxide anion formation and the radical DPPH, in a dose-dependent manner. J. regia extracts significantly reduced A172 and Caco2 cell viability, whereas they did not produce any effect on primary human fibroblast cells (HFF-1). Furthermore, J. regia extract also inhibited the migration of A172 cells, showing an antimetastatic action.

Discussion and Conclusion: All the tested extracts showed antimicrobial, antitumoral and antioxidant action. The promising results promote further studies aimed at elucidating the mechanism of action through which the "green" antimicrobials act and at evaluating new possible applications in the pharmacological field.

A Dynamic molecular investigation of doxorubicin and curcumin thermal release from Poly(N-isopropylacrylamide) nanocomposites.

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A large number of molecules with a wide range of therapeutic applications need, for various reasons such as poor stability, water solubility, bioavailability, pH sensitivity, tissue irritation, to be conveyed through the use of appropriate release systems. To overcome this issue intense research activities have been focused on the development of new nanomaterials as nanocarriers and delivery systems. Among these, the pH- and thermal-responsive Poly(N-isopropylacrylamide) (PNIPAM), and its nanocomposite formed with graphene oxide, SiO_2 surfaces and carbon dots, Fig.1, have been extensively studied for drug delivery [1-5].

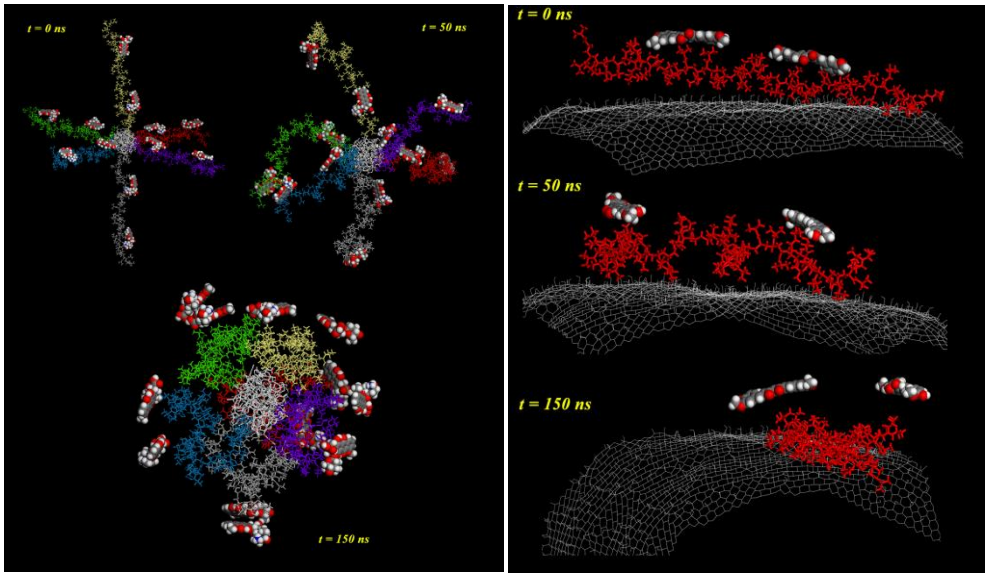

Fig.1 Snapshots of nanocomposites at 310 K taken at 0 ns, 50 ns and 150 ns

Curcumin and doxorubicin possess the characteristics listed above and were used as test molecules to investigate, by means of computational approach, the potential of PNIPAM nanocomposite for delivery and temperature-tunable drug release.

The structural and dynamical properties studied indicate that the substrate modulates the Lower Critical Solution Temperature (LCST) of the polymer affecting the release of the physisorpted molecules, in particular hydrophilic surfaces hinder the coil-to-globule transition increasing the LCST up to 315 K by means of H-bond interactions between PNIPAM acceptor groups and hydroxyl groups on the surfaces [6]. As to carbonized polymer dots (CPDs) is concerned results demonstrate that a higher number of polymer chain anchored to the carbon core decreases the coil-to-globule transition time due to the more intense inter-chain interactions which destabilize the highly cooperative water cluster that is at the base of the coil form stability. Finally, kinetics study of charged vs neutral species of curcumin and doxorubicin confirm a more marked release of the charged species highlighting the crucial role of the environment.

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Lipid Nanoparticles as Tools for the Administration of Active Natural Products Aimed to the Treatment of Nervous System Disorders

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Nowadays, the incidence of central nervous system (CNS) disorders has greatly increased and they represent a serious problem, not only concerning the high costs of treatment, but in addition, the quality of life of the patient. Among these, Alzheimer's disease (AD) is a neurodegenerative disorder of which the main cause is a marked oxidative stress at the level of the encephalic cells. Recent studies indicate that the normalization of antioxidant capacity could represent a very promising therapeutic for the treatment of AD [1,2]. Moreover, research has made a significant effort to develop innovative therapies based on natural compounds, as old synthetic drugs cause numerous side effects.
Curcumin (CUR) and astaxanthin (AST) are two natural antioxidants that possess therapeutic properties for the treatment of AD, although they show poor bioavailability due to their high lipophilicity. In order to overcome these limits that compromise their therapeutic use [3-5], the researchers' attention is focused on the development of innovative and efficient stealth carriers (sSLN) loaded with these antioxidants, capable of avoiding the defense line represented by the macrophages and improving the drug stability, in order to achieve good bioavailability in the brain. The best strategies to realize sSLN, suitable for parenteral administration, were to coat the CUR-SLN surface with hydrophilic polymer PEG (CUR-pSLN), while the AST-SLN with surfactant polysorbate 80 (AST-p80SLN).
CUR-pSLNs and AST-p80SLNs showed a good mean particle size suitable for parenteral administration (<200 nm), as confirmed by TEM [6,7]. Moreover, both formulations showed greater antioxidant activity over time than free CUR and AST, confirming the key role of encapsulation in preserving and therefore increasing the antioxidant activity of powerful active compounds [8].
In conclusion, the pharmacological activity of the formulations has been evaluated by *in vivo* assay using transgenic mice TgCRND8. The obtained results showed that the cognitive deficit was completely recovered. Therefore, CUR-pSLNs and AST-p80SLNs could be regarded as promising carriers for the treatment of CNS disorders, through systemic administration.

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New benzylpiperazine derivatives with high affinity for the sigma -1 receptor and antinociceptive and anti-allodynic effects *in vivo*

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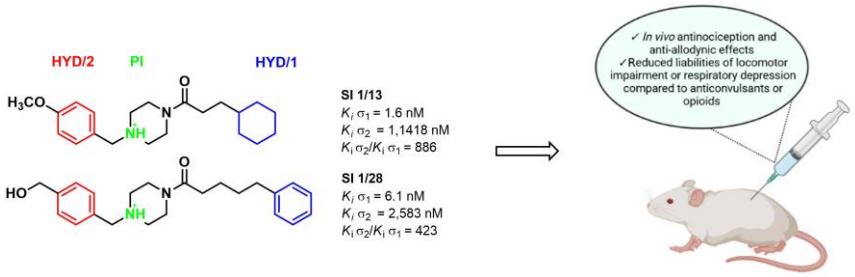
The sigma-1 receptor (σ_1R) is a unique endoplasmic reticulum chaperone protein involved in the regulation of the trafficking of a variety of proteins [1]. For instance, due to its ability to modulate nociception without opioids' liabilities of use, the σ_1R has been established as a druggable target to safely treat pain and other medical conditions including cancer [2]. In particular, σ_1R antagonists have shown promise in pre-clinical and clinical studies as a novel non-opioid analgesic [3]. Based on these premises, the Pharmaceutical Chemistry section of the Department of Drug and Health Sciences, for many years, has been engaged in the design, synthesis, and pre-clinical evaluation of selective σ_1R ligands [4]. Here, we reported the development of a series of benzylpiperazine derivatives as selective σ_1R ligands. The two most potent analogs, 3-cyclohexyl-1-{4-[(4-methoxyphenyl)methyl]piperazin-1-yl}-propan-1-one (**SI 1/13**) and 1-(4-{[4-(hydroxymethyl)phenyl]methyl}piperazin-1-yl)-5-phenylpentan-1-one (**SI 1/28**), showing a suitable σRs binding profile (K_i σ_1 < 10 nM e K_i σ_2/K_i σ_1 > 500), were tested for their antinociceptive and anti-allodynic effects *in vivo*. Inhibition of inflammatory (formalin) and chemical (acetic-acid) pain, chronic nerve constriction injury (CCI) induced mechanical allodynia, and potentially confounding adverse effects of sedation in a rotarod assay or respiration and locomotion using the Comprehensive Lab Animal Monitoring System (CLAMS) were assessed after i.p. administration to male mice. Both **SI 1/13** and **SI 1/28** produced dose-dependent antinociception in the formalin test. Likewise, **SI 1/28** produced dose-dependent antinociception against visceral nociception and anti-allodynia against CCI-induced neuropathic pain. Also, while opioid analgesic morphine produced psychostimulation and respiratory depression, even high doses (60 mg/kg, i.p.) of **SI1/28** demonstrated no significant effect on locomotor activity, and did not affect respiration rate. In summary, the putative σ_1R antagonist **SI 1/28** proved efficacious in the treatment of chronic pain without producing confounding locomotor or respiratory effects.



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On the size, shape and energetics of the hydration shell around alkanes and the side chain of some aliphatic amino acids

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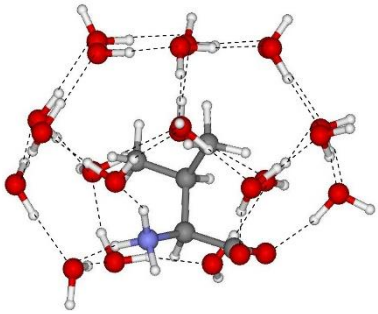
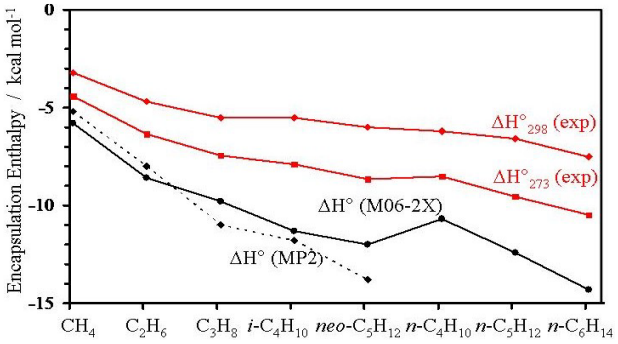
The water distribution around hydrophobes and related interactions are one of the most important and discussed topics in chemistry and structural biology since they are at the origin of the hydrophobic effect.[1] Very important issues, such as molecular aggregation, protein folding, molecular recognition, and biological membrane formation, intimately correlate with the self-organization processes arising due to the low solubility of molecules (or parts of them) in water.

Thermodynamic data on the solubility of simple hydrocarbons in water show a specific signature: i) negative enthalpy of solution ($\Delta H_s<0$); ii) large unfavourable entropy of solution ($\Delta S_s>0$); iii) positive heat capacity changes ($\Delta C_{Ps}>0$); and iv) decrease in volume ($\Delta V_s<0$). Analysis of these data have suggested that water molecules, forming the very first shell, are not totally ‘‘random’’ oriented, like in bulk water; rather they are constrained in configurational spaces and adopt structures in which O–H bonds, in contact with the hydrophobe surface, are tangentially oriented.[2,3]

High level quantum chemical calculations, in combination with a bottom-up approach, have been used to construct the very first hydration shell around alkanes and the side chain of some hydrophobic amino acids in their zwitterionic form.[4,5] It emerges a light iceberg model, in which the water molecules in direct contact with the hydrophobe should satisfy some geometrical constraints to get energetically suitable configurations: (i) minimize the cavity size; (ii) tetrahedral 4-fold coordination, and (iii) no O─H bond or O-lone pair protrusions inside the cavity. With these restrictions, the most stable and hence statistically significant structures resemble those found in clathrate hydrates with spheroidal shapes for both alkanes and amino acids side chains (Figure on the left for valine).

The computed encaging enthalpy for various hydrocarbons is similar to the enthalpy of solution measured at a temperature just above the melting point of aqueous hydrocarbon solutions (Figure on the right), thus indicating that water molecules should not deviate too much from the configuration with O–H bonds tangentially oriented with respect to the solute surface. The computed trend differs from the enthalpy of solution measured at room temperature, thus the very first hydration shell departs, up to a certain degree, from the clathrate-like structures.





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CHIM PO15

FAR PO16

Nano-platforms for light-controlled nitric oxide release

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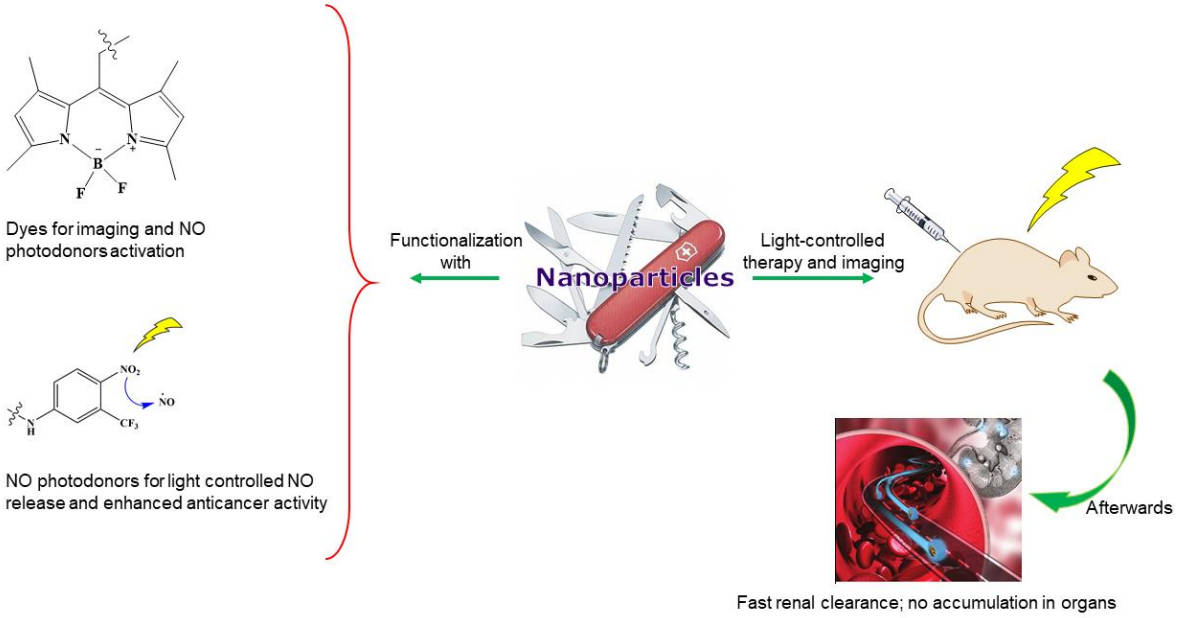
Non-invasive early diagnosis and therapy are key in the fight against diseases such as cancer or neurological illnesses. Nanotechnology plays a crucial role in this scenario since nanoparticles can not only anchor different kinds of molecules on their surface allowing for theranostic properties, but, if properly engineered, also show high biocompatibility and low accumulation in vital organs.[1] At the same time, many recent studies have highlighted the importance of nitric oxide (NO) in cancer therapy, especially concerning its ability to inhibit several efflux transporters mostly responsible for multidrug resistance in cancer cells. In this frame, nano-systems that can release NO in a controlled way are of outermost importance.[2-3] Here, we report about silica nanoparticles modified with dyes and nitric oxide photodonors for the light-controlled release of NO. Both nanoparticles and the functional molecules have been synthesized and characterized. After functionalization, the nano-objects have been studied for their NO photo-releasing properties under light irradiation.



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In silico design of recombinant multi-epitope vaccine against influenza A virus

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Influenza A virus is one of the diffused causes of mortality. The emergence of novel escape variants of the influenza A virus is still a substantial challenge in annual vaccine production [1]. Recently, multi-epitope vaccines have been increasingly investigated. In this view, an immunoinformatic approach to design a recombinant multi-epitope vaccine could be very useful as the one we proposed here based on a highly conserved epitope of hemagglutinin, neuraminidase, and membrane matrix proteins. The starting point consisted on the collection of the potential B cells, cytotoxic T lymphocytes (CTL), and CD4 T cell epitopes [2]. Furthermore, some bioinformatics online servers and datasets were used to evaluate the immunogenicity and chemical properties of selected epitopes [3]. In addition, the Universal Immune System Simulator (UISS), an *in silico* trial computational framework based on Agent-Based methodologies, was run after an influenza challenge exposure and recombinant multi-epitope vaccine administration, showing a suitable immune response in terms of immunoglobulins of class G (IgG), and interferon-gamma (IFN-g) levels. *In silico* dynamics of IFN-g are shown in Fig. 1 [4]. In the first scenario, the peak level of IFN-g is about 1×10^6 molecules at day 50 (Fig.1 panel A), while in the second one, the IFN-g level is considerably higher than after influenza exposure at day 25 (Fig. 1, panel B). Fig 1, panel C shows a higher second peak, highlighting the effect of the vaccination in response to the influenza challenge. High levels of IgG characterize the recombinant multi-epitope vaccine response (approximately 130,000 titers) (Fig. 2, panel B), while after influenza exposure, the IgG level is fewer (24,000 titers) compared to the one after vaccine simulation (Fig. 2, panels A–C). The suggested vaccine formulation was found to have an acceptable immunogenicity score. Therefore, a multi-step bioinformatics approach with the concomitant UISS usage, could enhance vaccine development and boosts the probability of maintaining good results in pre-clinical and clinical settings.

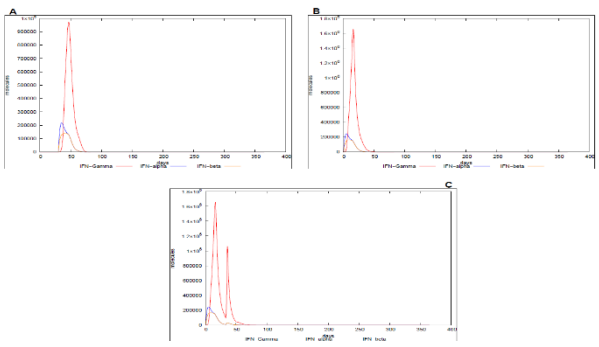

Fig.1. In silico dynamics of IFN-g.

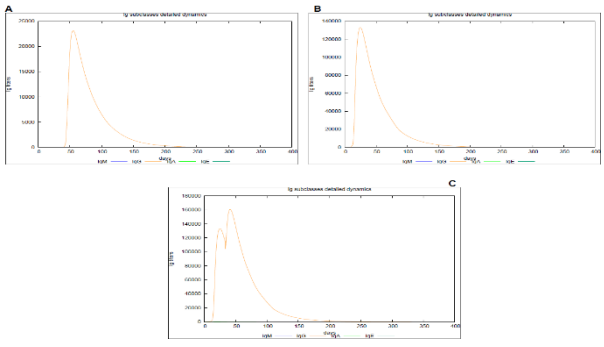

Fig.2. In silico dynamics of IgG.

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Lipid nanoparticles as carriers for the development of sunscreen formulations containing bemotrizinol

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The efficacy and safety of UV-filters could benefit from their incorporation into lipid nanoparticles because of the ability of such colloidal carriers to reflect solar radiations, thus acting as physical sunscreens after their application on the skin surface [1]. In addition, lipid nanoparticles, namely solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC), show many advantages as topical delivery systems including high biocompatibility, controlled release of their payload and ability to modulate drug skin penetration/permeation [2]. These features mainly depend on the composition of their lipid matrix being both types of nanoparticles made up of a lipid core stabilized by surfactants in aqueous media.

Therefore, the aim of this work was to assess the effects of different lipid core compositions on the technological properties of SLN and NLC loading bemotrizinol (BMTZ), a widely used broad-spectrum sunscreen agent that absorbs UV radiation in the range 280-380 nm [3] to design novel sunscreen formulations with improved safety and efficacy.

Mixtures containing different ratios of solid lipid (cetyl palmitate) and liquid lipids (isopropyl myristate, decyl oleate and caprylic/capric triglyceride) were assessed to prepare BMTZ-loaded NLC containing oleth-20 (8.7% w/w) and glyceryl oleate (4.4% w/w) as surfactant and co-surfactant, respectively. The evaluation of mean particle size, polydispersity index and zeta potential pointed out that isopropyl myristate (IPM) provided NLC with better technological properties allowing BMTZ loading up to 8% w/w. Differential scanning calorimetry analyses confirmed IPM greater ability to favor BMTZ incorporation into the lipid core of NLC. Different concentrations (5, 10, 20% w/w) of the optimized BMTZ loaded NLC (IPM 3.5% w/w; BMTZ 8 % w/w) were incorporated into cosmetic vehicles (aqueous gel and O/W emulsion) and BMTZ in vitro release from these formulations was evaluated by means of Franz-type diffusion cells using the same vehicles containing different percentages of free BMTZ as control. BMTZ release from O/W emulsions was not affected by its incorporation into NLC while gel formulations provided lower BMTZ release from NLC compared to free BMTZ at the greatest concentration tested.

The results of this study pointed out the possibility of modulating BMTZ release from cosmetic formulations by loading this UV-filter into NLC incorporated in suitable vehicles, thus allowing the design of sunscreen formulations with improved efficacy and safety.

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CFA PO18

Antimicrobial activity of *Juglans regia* L. pellicle extract

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Introduction

The use of plant extracts for the treatment of a large variety of human diseases is well established since ancient time. Currently, there are growing interests on the natural extracts as a prominent source of chemical compounds with different biological activity. *Juglans regia* L. (common walnut) is a deciduous tree belonging to Juglandaceae family. Walnut was widely used in traditional medicine for its antidiabetic, antioxidant, antimicrobial, anti-atherogenic and anti-inflammatory effects. The aim of the present study was to evaluate the antibacterial and antiviral activities of walnuts pellicle extract.

Materials and Methods

Antibacterial activity of the extract was tested against thirty-two clinical isolates and eight ATCC strains by the microdilution method. Antiviral activity was evaluated against some DNA and RNA viruses, including *Herpes simplex* virus type 1 (HSV-1) and 2 (HSV-2) in VERO cells, *Echovirus* 9 (Hill strain) in LLCMK2 cells, *Poliovirus* 1 (Sabin strain), *Coxsackievirus* B1 and *Adenovirus* 2 in HEp2 cells. Total phenol content was determined spectrophotometrically, using the Folin-Ciocalteu method. Total flavonoid content was measured using a colorimetric assay. Ultra-Performance Liquid Chromatography coupled to Mass Spectrometry (UPLC-Ms/Ms) was performed to identify phytochemical compounds.

Results

Gram-positive strains were more sensitive, with MIC values ranging from 8.59 to >275.00 µg/ml. Gram-negative strains were less susceptible, with MIC values ranging from 275 to >275.00 µg/ml. Antibacterial activity of the extract was compared to that of reference antibiotic ciprofloxacin. *J. regia* L. pellicle extract inhibited HSV-1 and HSV-2 replication at doses below the cytotoxic dose. No virucidal effect was observed. The compound was ineffective against Polio 1, Adeno 2, ECHO 9, Coxsackie B1, viruses. Low flavonoid contents were found in the extract. These results are consistent with other studies showing that *J. regia* phenolics are mainly non-flavonoid type. UPLC-Ms/Ms confirmed that walnut pellicle extract is an important source of phenolic compounds.

Discussion and Conclusions

In antibacterial assay, Gram-negative strains were more resistant than Gram-positive. This could be explained by a different composition of their cell wall, formed by a thin layer of mucopeptides and a thick layer of lipoproteins and lipo-polysaccharides that make it not permeable to external agents. Interestingly, the extract showed also antiviral activity against HSV-1 and HSV-2. Taken together these data demonstrate a protective role of walnut pellicle extract against microbial infections. However, further studies will be need to investigate the possible mechanism of action of the biologically active chemical compound.

Olfactometric and sensorial properties of black pepper extracts loaded into lipid nanocarriers

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Black pepper (BP) shows several biological properties, including anti-inflammatory, analgesic, antioxidant, antidepressant, draining, and anti-cancer activities, in addition to boosting absorption of nutrients and improving blood sugar control [1]. However, the sharp smell could limit its use in pharmaceutical, cosmetic and nutraceutical formulations. Due to their ability to improve the perception of smell and taste, lipid nanoparticles could be a promising tool to design formulations containing black pepper with favorable organoleptic properties. Therefore, the aim of this work was to load black pepper (as dry extract) into lipid nanoparticles (LNPs) to modify its olfactometric profile and to assess the resulting hedonistic sensorial properties.

BP-loaded LNPs were prepared using GRAS (Generally Regarded As Safe) raw materials and characterized to assess their technological properties by determining their mean size, polydispersity index (PDI), zeta potential, and stability after storage for two months at room temperature. BP-loaded LNPs showed technological features (mean size < 100 nm, PDI < 0.300, zeta potential -17.5) suitable for their application in pharmaceutical, cosmetic and nutraceutical field.

Dynamic olfactometry was used to evaluate the odor concentration, expressed in European odor units per cubic meter (ouE/m3) [3], of BP-loaded LNPs samples, while the hedonistic sensorial evaluation was performed using a panel of six volunteers who gave their written informed consent to be enrolled in the present study.

The results of dynamic olfactometry revealed that loading BP dry extract into LNPs modified the olfactometric profile while maintaining a similar smell intensity compared to the control (1554 ouE/m^3 BP in ethanol, 1729 ouE/m^3 BP in LNPs). The hedonist sensorial evaluation pointed out a greater pleasantness of the investigated BP-loaded LNPs when compared to free BP.

Therefore, loading black pepper into lipid nanoparticles could provide a promising strategy to enlarge the field of application of this vegetable extract.

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Green approach in the development and optimization of colon-targeted delivery systems based on food-grade polymer matrices

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The development of systems for the targeted delivery of active substances to ileum and colon (cTDS) has attracted in recent years the attention of researchers and industries for their peculiar therapeutic profile. The release of actives can in fact be concentrated in the colon, ensuring a low systemic absorption and a localized effect on the target area, particularly useful in conditions such as inflammatory bowel disease (IBD) and colon cancer. In addition, this strategy allows to protect the carried compounds from the enzymatic action and the acidic environment of the first tracts of the digestive system. Analogously, the beneficial compounds that could damage the gastric mucosa become no longer a problem.

From the initial studies on medicinal drugs, the cTDS technology is progressively moving towards the applications in the nutraceutical field [1,2]. In recent researches from our group, the food-grade copolymers Eudraguard® Biotic (EUGB) and Control (EUGC) (made by Evonik Health Care, Germany) were proposed to produce microparticles loaded with natural nutraceutical ingredients [3,4]. These systems were formulated by an Emulsion Solvent Evaporation (ESE) technique. The present study has allowed to further optimize the process, obtaining microcarriers with appreciable technological performances for loading and delivery of both pure active compounds, such as resveratrol and ellagic acid, as well as of herbal extracts containing the same active ingredients, and whose use if largely more beneficial and costless for the interested companies. Among the used techniques, the Solvent Evaporation (SE) one has been developed to obtain systems with the required drug release profiles as a function of the pH values present along the gastro-intestinal tract. The method allows to produce totally ‘food-grade’ formulations; it does not require the purification of Eudraguard® commercial suspensions and allows using low-toxicity (ICH class 3) ‘green’ solvents.

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Novel mucoadhesive hybrid nanosystems for melatonin delivery to the posterior eye segment

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Melatonin is a neurohormone of promising interest as a neuroprotective agent in ocular pathologies [1]. Topically administered formulations for the treatment of diseases affecting the posterior eye segment are the most convenient but least absorbed. An approach to increase the bioavailability of eye drops could be to load drug into nanomedicine to modify their mucoadhesive properties [2,3]. Here, with the requirement to enhance melatonin delivery to the retina, minimize frequency of administration and provide controlled and sustained drug release, the design and optimization of melatonin-loaded lipid-polymer hybrid nanoparticles (mel-LPHNs) for topical administration were proposed. To improve mucoadhesion and mucopenetration to corneal tissues, mel-LPHNs were obtained from PLGA-PEG copolymer coated with a cationic lipid shell. The optimized formulation showed appropriate requirements for an eye drop, resulting in homogeneous particles populations (PDI 0.260), with suitable size for ocular administration (189.4 nm), a positive surface charge (+39.8 mV), high encapsulation efficiency (79.8%), adequate pH (6.3) and osmolarity (296 mOsm/Kg) values, good mucoadhesive properties and a controlled release profile. Infrared and thermal analyses confirmed the encapsulation of melatonin in the systems and the successful interaction of lipids with the polymer matrix. Stability studies conducted according to the ICH QA(R2) guidelines on the optimized formulation ensured a stability of 6 months under refrigerated storage conditions. In conformity with the European Pharmacopoeia, mel-LPHNs samples passed the sterility test after UV exposure for 30 minutes. Biological assay in an *in vitro* model of diabetic retinopathy revealed superior neuroprotective and antioxidant activity of mel-LPHNs compared to aqueous melatonin solution. The Draize test was conducted to evaluate the ocular tolerability of mel-LPHNs, which showed no signs of ocular irritation. These findings suggested the development of novel hybrid nanoparticles as a safe and promising platform suitable for the topical management of retinal diseases.

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Effect in prostate cancer cells of essential oils obtained from *Artemisia arborescens* L. growing on the island of Lipari, Sicily

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Prostate cancer is the second leading cause of cancer-associated mortality in Western countries. In spite of the attempts and extensive research for developing new anticancer therapies, this cancer is still one of the major human diseases with poor prognosis and high mortality (1). Therefore, finding effective anticancer drugs endowed with low toxicity to formulate new treatment strategies is important in patients with prostate cancer. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) exerts proapoptotic effects on malignant cells without any harmful effects to normal cells. However, some tumor cells including prostate cancer are resistant to TRAIL-induced apoptosis. Recently, research reports have shown that natural chemopreventive agents have the therapeutic potential to sensitize prostate cancer cells to TRAIL. *Artemisia arborescens* L. (Compositae) is an aromatic shrub widely used in traditional medicine whose essential oils are considered a potential source of molecules with pharmaceutical interest. In particular, our previous data reported that two essential oil samples from *Artemisia arborescens* L. growing on the island of Lipari, were able to induce apoptosis in melanoma cells (2). Therefore, in this study we evaluated the effect of these essential oils on androgen-sensitive (LNCaP) and androgen-insensitive (DU-145) human prostate cancer cells. In addition, we examined apoptotic and cytotoxic effects of TRAIL in combination with these natural products in TRAIL-resistant prostate cancer LNCaP cells. The essential oils were assayed for their anti-growth activity testing several parameters, such as cell viability and cell membrane integrity. The evaluation of DNA fragmentation and caspase-3 activity assay were employed for the detection of apoptosis. The expression of Bcl-2, Bax, cleaved caspase-3, cleaved caspase-9 proteins was evaluated by Western blot analysis. Generation of reactive oxygen species (ROS) were also measured. It was observed that our samples showed a dose-response relationship in the range of 3.12-12.5 μg/ml concentrations in LNCaP and DU-145 cells, activating an apoptotic process. But, the novel finding, in the present study, is that our tested essential oils sensitizes LNCaP prostate cancer cells to TRAIL-induced apoptosis. Our data reveal a potential way of chemoprevention of prostate cancer by enabling TRAIL-mediated apoptosis.

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CHIM PO23

Investigation of DASA properties in liposome

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Photochromes are molecules that can be reversibly switched between two forms by means of light. The variation of properties between the two isomers, especially when different polarities are observed, have been often used to trigger the behaviour of molecular compounds or materials.

Most of the time, photochromes require high-energy UV light for their transformation which reduces their use, especially for bio-medical applications. On the contrary, Donor–Acceptor Stenhouse Adduct (DASA) can switch from an open coloured form to a closed colourless form with low energy light stimulation [1]. The photoisomerization induces not only a change in the absorption properties but also a drastic evolution of the polarity with a hydrophobic to hydrophilic transition triggered by visible light. These changes open the way for multiple applications of these new photochromes such as membrane and phase transfer.

Some properties of DASA in organic solvents have already been described [2], their behaviour in more complex environment, such as liposome still need to be explored. In this contribution, we used FRET phenomenon between a fluorescent probe and DASA linear isomer in order to go deeper into the compression of DASA photo-induced isomerization in POPC liposome.

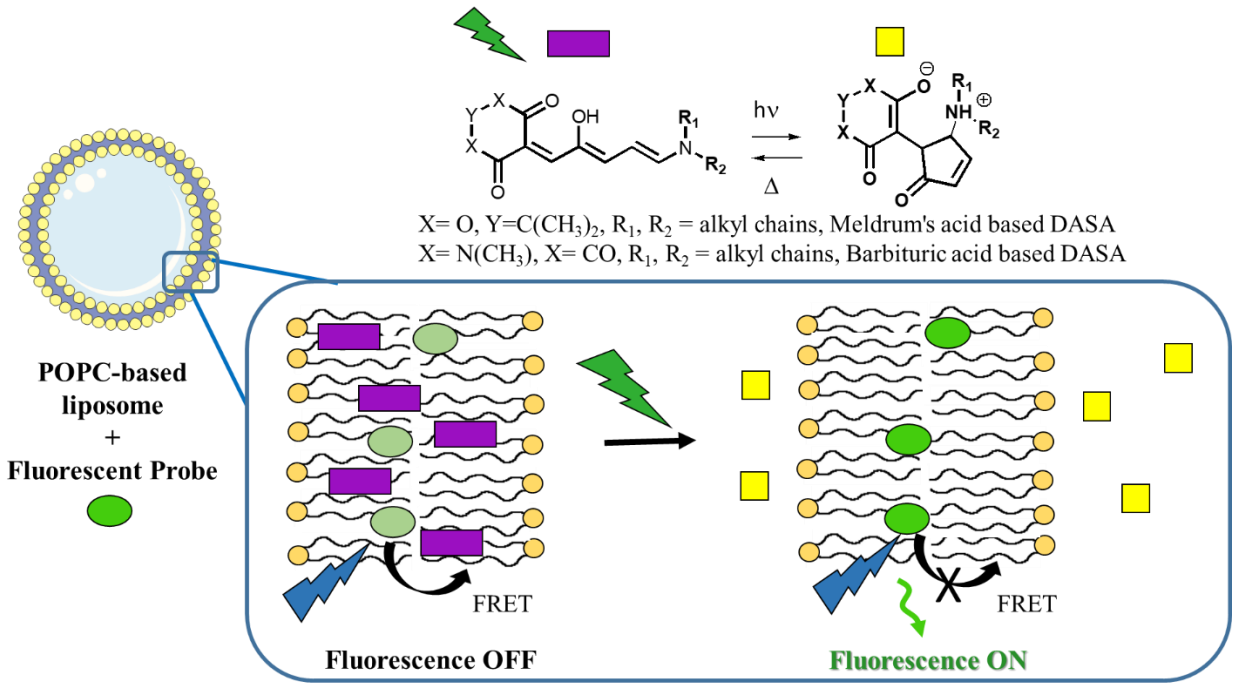

Figure 1: Schematic of DASA photo-isomerization in fluorescent-labelled liposome.

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Beneficial effects of extracts obtained from processing waste of the main agri-food products typical of the Mediterranean area

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The awareness of the large amount of waste produced along the food chain starting from the agricultural sector, from industrial transformation to the domestic context, has in recent years aroused strong concern also in public opinion, which wonders about the possible consequences that this could have on environmental sustainability, resource waste and human health. The *Waste Framework Directive* (2008/98 / EC) establishes the criteria for managing food waste and establishes that the "by-product" can still be fruitful for its use in the industrial sector. With a view to environmental sustainability and in a context of circular economy, the recovery of nutrients and bioactive molecules obtained from production chains derived waste is fundamental for the development of functional products with high added value to be used in various production sectors, from nutrition, nutraceuticals, cosmetics. In particular, this project program focuses its attention on the by-products and processing waste of the main agri-food productions typical of the Mediterranean area such as *pastazzo* (set of peels, pulps and seeds) and the *olive leaves*. The former derives from the processing of oranges and it is particularly rich in anthocyanins, flavanones and hydroxycinnamic acids, endowed with numerous nutraceutical properties (1, 2); the latter derive from olive tree pruning which contribute to the generation of a huge amount of waste rich in components such as oleuropein, elenolic acid, hydroxytyrosol, tyrosol and rutin molecules for which it has been demonstrated an ability to control blood pressure, blood sugar, triglyceride and cholesterol levels (3). The aim of the project is the recovery of substances with high added value from waste, by-products and production surpluses for the preparation of natural extracts which, thanks to the effect of antioxidants naturally present, can be useful for preventing, counteracting or delaying the onset of the complications of the Metabolic Syndrome. The extract obtained from the starting matrix consisting of peels and pulps deriving from the industrial process of pressing the oranges was concentrated and analytically characterized with respect to the anthocyanin and flavanone content. The anti-radical activity of this extract was evaluated with the DPPH assay. Human liver cells (HepG2) treated with free fatty acids were used as a model of hepatic steatosis. The extract obtained from the *pastazzo* has a good antioxidant capacity and is able to reduce the accumulation of lipid droplets. The preparation of a new formulation deriving from the combination of the extract obtained from citrus fruit pulp enriched with extracts obtained from olive leaves will be tested in order to create a synergic antioxidant effect.

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Rapha Myr® extract exhibits multifaced activity by inhibiting angiognesesis, tumour growth and migration of human astrocitoma cells

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Tumorigenesis is a multistep process which is driven by the sequential accumulation of genetic and epigenetic changes in oncogenes and tumour suppressor genes. Also, cells of the tumour microenvironment play a key role in all stages of tumorigenesis as facilitate abnormal cell proliferation directly and indirectly through stimulation of cancer-associated angiogenesis.
Compelling studies demonstrated that cruciferous vegetables, rich in isothiocyanates (ITCs) as sulforaphane, show potent cancer-prevention activities by modulating numerous cellular processes such as induction of apoptosis, inhibition of cell proliferation, migration ability, metastasis and neo-angiogenesis.
We treated human astrocytoma 1321N1 and human umbilical vein endothelial cells (HUVECs) with different concentrations of a blend of sulforaphane glucosinolate and myrosinase (Rapha Myr®) aqueous extract (10mg/mL), focusing on various aspects of carcinogenesis and their molecular and cellular mechanisms.
Rapha Myr® exhibited low antioxidant capability and exerted antiproliferative and genotoxic effects on 1321N1 cells by blocking the cell cycle, disarranging cytoskeleton structure and focal adhesions, decreasing the integrin 5 expression, renewing anoikis and modulating some important epigenetic pathways independently of the cellular p53 status. Moreover, Rapha Myr® inhibited endothelial cell migration and the capability of tube formation on matrigel by suppressing the expression of VEGF, its receptor 2 (VEGFR2) and ANG.
We proved the Rapha Myr® exerts anti-cancer effects on human astrocytoma cells by targeting multiple cancer survival mechanisms via epigenetic regulation. Beyond, Rapha Myr® aqueous extract modulates tumour microenvironment by inhibiting in-vitro neo-angiogenesis so affecting tumour growth indirectly.
Overall, our findings suggest a multiple therapeutic targeting of cancer by Rapha Myr® and support the need of clinical studies to test the effectiveness of Rapha Myr® in the prevention and treatment of cancer.

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TFA PO26

Nanotechnology for ocular drug delivery: from design to characterization

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The complex structure of the eye and the poor drug bioavailability are the main obstacles for conventional ocular dosage forms. To overcome these limitations, nanotechnology delivery systems have proven to be useful tools [1,2]. The biggest challenge associated with ocular nanotechnology is the development of safe, effective, and highly tolerated innovative carriers. Our research group was mainly focused on the delivery of active molecules in the treatment of pathologies affecting the anterior and posterior tract of the eye such as glaucoma, diabetic retinopathy, age-related macular degeneration, uveal melanoma, and retinitis pigmentosa. The search for a more patient-friendly topical route has been extensively investigated and remains the prospect of ongoing studies. The QbD application to nanocarriers has been the common thread, ensuring a rational design, which begins with the delineation of an appropriate design space and results in the achievement of the desired quality profile [3]. Supported by EMA and FDA, the ICH guidelines (Q8-Q11) suggest a systematic approach of Quality by Design (QbD) to ensure optimized high-quality products.

Thus, several approaches have been tested to optimize formulations with the goal of achieving controlled release profiles, prolonged residence time and increased bioavailability. In the last decade, our research group developed different platforms for the ocular delivery of active drugs. To reduce ocular drainage and clearance, nanogels and *in-situ* forming gels have been investigated. Polymers responsive to ions, pH and temperature variations have been used in the design of such systems [4,5]. To improve the diffusion to the back of the eye lipid nanosystems (i.e., SLN and NLC) have been studied [6,7] cationic compounds are used to enhance the interaction with negatively charged mucin on the ocular surface [8,9]. Biodegradable and biocompatible polymers (e.g., PLGA, PLA) were also investigated for the development of drug polymer-based nanoparticles to ensure controlled release and enhanced bioavailability [10].

Once the systems have been optimized, characterization is performed to assess their compliance with ophthalmic administration. Physico-chemical parameters such as size, polydispersity index, zeta potential, pH and osmolarity are evaluated. Other relevant aspects to consider in ocular delivery include purity and sterility of eyedrops; different processes have been compared to select the most suitable method for each type of system. Thermal (DSC) and spectroscopic (HPLC, UV-Vis, FT-IR, fluorescence) analytical techniques are applied for quantitative and qualitative determination of molecules. Simulated tear fluid was used to carry out *in vitro* experiments such as the determination of mucoadhesive properties and drug release profiles. Physical stability is monitored by thermoregulated optical analysis using a Turbiscan® Ageing Station [4]. Final batches of formulations can be subjected to stability studies according to ICH guidelines [QA(R2)].

In order to improve the stability during storage, freeze-drying process is investigated, along with cryoprotection studies to ensure the integrity of the colloidal formulation during the process. Experimental findings are finally confirmed by *in vivo* studies, demonstrating the efficacy and safety of designed systems for potential ophthalmic application.

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TFA PO27

Self-nanoemulsifying drug delivery systems (SNEDDS): a novel platform to enhance ocular bioavailability of hydrophobic drugs

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The difficulty of delivering molecules into the deeper ocular tissues makes the treatment of degenerative eye diseases in continuous development. The poor bioavailability of many molecules combined with the stability problems of formulated systems lead to the search for innovative technologies. In this respect, self-nanoemulsifying drug delivery systems (SNEDDS), due to the presence of biocompatible and biodegradable ingredients and the ease of large-scale production, combined with the opportunity for drug targeting, are of great interest. These systems allow for enhanced solubilisation, stability, and improved absorption, resulting in greater bioavailability of BCS Class II compounds. SNEDDS, consisting essentially of three components in an anhydrous mixture: oil, surfactant and co-surfactant, are emulsified directly at the site of action, avoiding the problem of drug loss during storage. These systems can represent an advancement of actual micro- and nanoemulsion technology [1]. The choice of starting materials and the ratio in which they are combined is the crucial point for obtaining a good bioavailability *in vivo*. Once in contact with an aqueous environment and exposed to gentle agitation, the system emulsifies spontaneously and quickly, forming a clear solution with a particle size within 100 nm. This is due to the use of surfactants with a high HLB in a minimum concentration of 40% (w/w) and the addition of a co-surfactant. Surfactants with an HLB above 10 facilitate the spontaneous emulsification even in sites, such as the ocular surface, where a very low amount of available aqueous phase is present. Achieving an optimised formulation is easier if done by computational methods. The preformulation phase is performed by ternary plot construction, to identify the emulsion zone. The optimization phase can also be done using statistical experimental design, such as the Box-Benkhen design, the central composite design, the simplex lattice design, the full-factorial design and the D-optimal design. The most popular applications are in the oral route, as the large capacity of the gastro-intestinal fluid certainly allows the formation of emulsions [2,3]. In the latest SNEDDS generation applications involving the pulmonary, parenteral and ocular routes are investigated. In the latter case, the contact with the tear fluid and slight agitation, due to the blinking phenomenon, can form an emulsion directly *in situ*. Voriconazole-loaded SNEDDS and econazole-loaded SNEDDS have been developed to improve transcorneal drug permeability [4,5]. Characterization involves the simulation of their behaviour in the ocular environment, as well as physico-chemical and technological analyses. Once produced, SNEDDS are evaluated for size, homogeneity, emulsion time, clarity by turbidimetry, drug content, mucoadhesion studies and *in vitro*/*in vivo* studies for biodistribution. Currently, they are evolving into mucus-permeable, supersaturated, solid and targeted SNEDDS to be shaped and optimized to different applications.

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FAR PO28

Antioxidant Activity of Fluoxetine and Vortioxetine in a Non-Transgenic Animal Model of Alzheimer's Disease

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Depression is a risk factor for the development of Alzheimer's disease (AD). Neurobiological and clinical links exist between AD and depression, with neuroinflammation and oxidative stress being involved in both diseases. Second generation antidepressants, in particular selective serotonin reuptake inhibitors (SSRIs), are currently investigated as neuroprotective drugs in AD. By employing a non-transgenic AD model, obtained by intracerebroventricular (i.c.v.) injection of amyloid-β (Aβ) oligomers in 2-month-old C57BL/6 mice, we recently demonstrated that the SSRI fluoxetine (FLX) and the multimodal antidepressant vortioxetine (VTX) reversed the depressive-like phenotype and memory deficits induced by Aβ oligomers rescuing the levels of transforming growth factor-β1 (TGF-β1). Aim of our study was to test FLX and VTX for their ability to prevent oxidative stress in the hippocampus of Aβ-injected mice, a brain area strongly affected in both depression and AD. The long-term intraperitoneal (i.p.) administration of FLX (10 mg/kg) or VTX (5 and 10 mg/kg) for 24 days, starting 7 days before Aβ injection, was able to prevent the over-expression of inducible nitric oxide synthase (iNOS) and NADPH oxidase 2 (Nox2) induced by Aβ oligomers. Antidepressant pre-treatment was also able to rescue the mRNA expression of glutathione peroxidase 1 (Gpx1) antioxidant enzyme. FLX and VTX also prevented Aβ-induced neurodegeneration in mixed neuronal cultures treated with Aβ oligomers. Our data represent the first evidence that the long-term treatment with the antidepressants FLX or VTX can prevent the oxidative stress phenomena related to the cognitive deficits and depressive-like phenotype observed in a non-transgenic animal model of AD.

Food-grade Eudraguard® Control Microparticles loaded with Melatonin and L-Tryptophan for oral administration

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Although science has made great strides through the discovery of innovative routes of administration, to date 90% of the global market share of all pharmaceutical formulations for human is accounted for by those administered orally [1]. The orally administered drug requires protection from the external environment to ensure that it reaches the target site without being degraded or metabolised by biological fluids in order to exert its full pharmacological action.
In this project, the target site identified is the small intestine, as this is where most absorption occurs [2], and the carrier is a food-grade polymer and generally recognized as safe, namely Eudraguard® Control which has the characteristic of being gastro-resistant and of controlled release of the active ingredient at pH 6.8 [3].
A carrier system such as this is designed to transport two active ingredients, namely melatonin and L-tryptophan. Melatonin-loaded Eudraguard® Control microparticles (MEc-MPs) and L-tryptophan-loaded Eudraguard® Control microparticles (TEc-MPs) were prepared by the Solvent Evaporation method. The preparations were formulated with different ratios of active ingredient and polymer, i.e. 1:10 (MEc-MPs-10 and TEc-MPs-10) and 1:20 (MEc-MPs-20 and TEc-MPs-20). Subsequently, all systems were purified by centrifugation and lyophilised. The powders obtained were characterised at the physico-chemical level by sieving method to perform particle size analysis, drug loading (DL%) and encapsulation efficiency (EE%), FT-IR spectroscopy, DSC analysis, and preliminary *In vitro* mucoadhesion studies by studying electrostatic interactions between mucin and microparticles in artificial fluids such as stomach, small intestine and colonic fluid.
Particle size analysis showed particle sizes ranging from 90 to 500 μm, while DL% values are in the range 1.23-2.84 % and EE% values are between 46 to 71%. Both FT-IR and DSC analysis confirmed that the active ingredient is in dispersed form in the polymer matrix. Finally, from the results of preliminary *In vitro* mucoadhesion studies, both unloaded and loaded systems show strong interactions with mucin showing that the Eudraguard® Control polymer has potential mucoadhesive properties: its mucoadhesivity appears to increase as pH decreases.
Further physico-chemical characterisation and evaluation of the release of the active ingredients in various artificial fluids such as simulated gastric, intestinal and colonic fluids are in progress.

[1] Alqahtani, Mohammed S et al. Advances in Oral Drug Delivery. *Frontiers in pharmacology* 12,618411, 2021
[2] Kiela PR, Ghishan FK. Physiology of Intestinal Absorption and Secretion. *Best Pract Res Clin Gastroenterol*. 30(2):145-159, 2016.
[3] Curcio C, Greco AS, Rizzo S, et al. Development, Optimization and Characterization of Eudraguard® -based Microparticles for Colon Delivery. *Pharmaceuticals (Basel)* 13(6):131, 2020.

DSFS PHARMA DAY 2022

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2^ EDIZIONE, Catania, 1 giugno 2022

OTH PO30

Development of a technological platform for the functional testing of nutraceutical-based molecules

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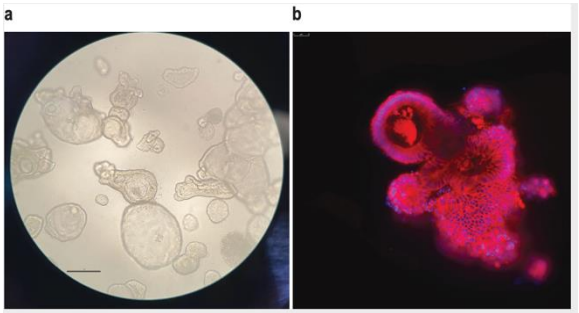
We have started the development and validation of a technological platform for the functional testing of nutraceutical-based molecules.
Recently, the trend to use natural molecules or extracts with nutraceutical activity has become increasingly widespread, both in the zootechnical field (feed) and in the human field (food supplements).
The in vitro test of nutraceutical molecules (usually, in relation to legislation and EFSA regulation, efficacy and safety tests, and limited to the evaluation of the cytotoxic or cytostatic potential) is carried out on cell lines, primary and / or immortalized. These tests obviously have a series of limitations, mainly due to the typology of the experimental model, since the models in use of two-dimensional (2D) cell cultures are not completely representative of the human physiology (or pathology) of the organ of interest or of the response of the organ. organism to molecules and other compounds. In fact, these models are unable to recapitulate the three-dimensional (3D) microenvironment typical of tissues in vivo, nor the cell-cell / cell-matrix interactions. Finally, the diffusion kinetics of the molecules under analysis vary considerably; effective doses in 2D are therefore often ineffective when re-proportioned on animals or humans.
The organoid model, produced both from ES (normal or iPSCs) or biopsies, is a major technological breakthrough that has already been established as an essential tool in many basic biology and clinical applications. This near-physiological 3D model facilitates an accurate in vitro investigation of a range of in vivo biological and biochemical processes including tissue renewal, stem cell/niche functions, transport and metabolism of nutrients and drugs, and tissue responses to drugs, mutation or damage. Moreover, human intestinal organoids represent a superior model of the intestinal epithelium and might help to implement the 3Rs (Reduction, Refinement and Replacement) principle in basic science as well as the preclinical and regulatory setup.
We have set up a robust platform for the production of intestinal and hepatic organoids from human biopsies or from different hiPSCs, in order to test nutraceuticals and natural extracts for toxicity and efficacy. To track specific cellular functions within organoids, using a 3D confocal time-lapse microscopy, we have started an approach using fluorescent vital-dyes specific for staining subcellular organelles, to follow changes in the fluorescent signal indicating specific actions within the cell. This approach will permit to monitor in time lapse the effect of molecules on organoids, and can be extended in the future to drug discovery approaches.



a. Intestinal patient-derived-organoids, day 20, bright field. Scale bar 250 mm, 10X; b. live confocal imaging with vital dyes. Scale bar 50 mm, 20X: nuclei in blue (Hoechst) and cell membranes in red (Cell-mask orange).

CERNUT - Centro Interdipartimentale di Ricerca in Nutraceutica e Prodotti Salutistici (Research Centre for Nutraceuticals and Health Products)

Il CERNUT, "Centro Interdipartimentale di Ricerca in Nutraceutica e Prodotti Salutistici", istituito il 23 Dicembre 2021, ha come scopo principale quello di promuovere ricerche multidisciplinari su integratori alimentari, "functional food", probiotici e sulle proprietà nutraceutiche degli alimenti e delle piante attraverso un approccio scientifico che ne dimostri i possibili benefici per la salute umana.

Afferiscono al Centro numerosi docenti e ricercatori da vari Dipartimenti dell'Ateneo di Catania, ognuno dei quali mette a disposizione le proprie skills ed expertise. Le principali linee di ricerca puntano alla caratterizzazione analitica degli ingredienti attivi e all'identificazione del ruolo e degli effetti dei composti health promoting a valenza nutraceutica nei contesti di wellness, aging, nutrigenomics e medicina preventiva, avvalendosi di modelli sperimentali in silico, in vitro ed in vivo e di studi mirati per il delivery & targeting di sostanze bioattive.

Tra le finalità del CERNUT rientrano:

• riunire numerosi esperti in ricerca di base e ricerca clinica, i cui risultati possono contribuire a migliorare la qualità della vita e lo stato di salute della popolazione
• collaborare con Aziende, Istituzioni ed Enti di Ricerca pubblici e privati nel settore della nutraceutica e della salute
• promuovere le attività di Terza Missione, organizzando eventi di educazione e divulgazione scientifica.

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Centro di ricerca per l'Imaging Molecolare, Preclinico e Traslazionale
Molecular Preclinical and Translational Imaging Research Centre
- IMPRonTe -

The Molecular Preclinical and Translational Imaging Research Centre IMPRonTe is an inter-departmental Research Centre of the University of Catania instituted in 2019, whose director has been elected Prof Massimo Gulisano (DSFS). Vice Director of the Centre is Prof Rosalba Parenti (BIOMETEC) and Scientific Director is Prof Gianluca Cicala (DICAR). The Centre has 103 regular members among the University of Catania researchers and 13 external experts.

The Centre promotes studies, research, documentation and scientific debate, with specific reference to interdisciplinary research, in the field of preclinical and molecular imaging, with particular reference to: (a) the identification of molecular targets to be used as targets for the development of imaging technologies also through the aid of computational modeling and subsequent validation; (b) identification of chemical and / or biological molecules to be used as contrast media or for detecting biological processes or phenomena; (c) validation of methodologies and protocols for the quantification of disease progression at the molecular level; (d) implementation of alternative experimental models to animal testing, including in silico systems, cell models, organoids and organ-on-chip, VR and AR systems; (e) application of convergent technologies to preclinical and molecular imaging (including microelectronics, photonics, bioengineering, robotics, sensors, additive manufacturing, ...) to the development of new instruments for diagnostic and / or therapeutic purposes guided by diagnostics (theranostics); (f) innovative applications in radiomics and radiogenomics.

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Centro di Ricerca in Nanomedicina e Nanotecnologia Farmaceutica – "NANOMED"

Il Centro intende promuovere gli studi, le ricerche, la documentazione ed il dibattito scientifico, con specifico riferimento alla ricerca interdisciplinare, nel settore delle Terapie Innovative (Drug Delivery & Targeting) e delle Nanotecnologie biomediche e farmaceutiche.

Principali campi di studio e di ricerca del Centro sono:

a. la caratterizzazione, l'analisi quali-quantitativa, la valutazione preclinica della biodisponibilità e degli effetti di sostanze attive farmaceutiche di potenziale applicazione nel campo della nanomedicina e del drug delivery & targeting;
b. le modifiche della struttura chimica di sostanze di interesse farmaceutico, al fine di migliorarne la farmacocinetica e la farmacodinamica nell'organismo;
c. lo studio preformulativo e formulativo di nuovi sistemi di veicolazione (delivery) e direzionamento (targeting) di farmaci;
d. lo studio degli effetti e della veicolazione carrier-mediata di sostanze a valenza diagnostica e teranostica, nei diversi campi della medicina preventiva e della terapia;
e. lo sviluppo formulativo e la valutazione di sistemi di veicolazione di farmaci in modelli consolidati di patologie neurologiche e neurodegenerative, per investigarne il potenziale terapeutico, ristorativo e/o neuroprotettivo;
f. lo sviluppo formulativo e pre-industriale di sistemi di veicolazione di farmaci in modelli consolidati di patologie oftalmiche, per investigarne il potenziale terapeutico e clinico;
g. la valutazione in modelli sperimentali in vitro, ex-vivo e in vivo, inclusi modelli bioinformatici e computazionali, delle attività biologiche e farmacologiche di sostanze bioattive;
h. le applicazioni della metabolomica mediante tecniche spettroscopiche e analitiche alla ricerca nel settore;
i. l'analisi critica della letteratura scientifica nel settore della nanomedicina e delle nanotecnologie per applicazione biomedica e farmaceutica;
j. il contributo, mediante il dialogo con gli organismi preposti, all'aggiornamento normativo nel settore delle nanotecnologie biomediche e farmaceutiche.

Website:
http://www.dsf.unict.it/it/content/nanomed-centro-di-ricerca-nanomedicina-e-nanotecnologia-farmaceutica

NACTUre srl

Nacture S.r.l. is a Spin-off of the University of Catania, established in 2017 on the proposal of some Professors with expertise in the Microbiological, Botanical-Nutraceutical and Chemical-Pharmaceutical fields.
The company is engaged in the research, development, production and marketing of biologically active substances which, if taken in adequate quantities, can help maintain and / or prolong the state of human health.
The project is distinguished by the possibility of carrying out research on numerous substances, especially of bacterial and plant origin, and of being able to test their activity in vitro.
The attention is also directed to the study of any synergistic actions between already known active compounds, not limiting to the simple mixing of substances with similar activity, as it often happens in other situations.
Nacture also offers consultancy on behalf of third parties for design and training.
In 2019 the first product was marketed: "URONACT". A food supplement, in which, thanks to the synergic action of Cranberry and D-mannose at the bladder level, with tamarind and probiotics at the intestinal level, we achieve the restoration of the physiological balance of the urinary tract, if compromised by bacterial infection uropathogens (*E. coli*).
Current research has shown interesting biological activities (antimicrobial, anti-infectious, antioxidant and anticancer) on other plant extracts, for which we hypothesize an industrial fallout.

Some publications of the members of Nacture srl:

1. Antimicrobial, antioxidant, and cytotoxic activities of *Juglans regia* L. pellicle extract. Antibiotics, 2021; 10(2):159.
2. The double effect of walnut septum extract (*Juglans regia* L.) counteracts A172 glioblastoma cell survival and bacterial growth. International Journal of Oncology, 2020, 57(5):1129
3. Phytochemical composition and biological activities of *Orobanche crenata* Forssk.: a review. Natural Product Research, 2020, 1-17.
4. Antioxidant, antimicrobial and anticancer activities of *Castanea sativa* (Fagaceae) extract: new therapeutic perspectives. Plant Biosystems, 2020, 1-9.
5. *Betula etnensis* Raf. (Betulaceae) extract induced ho-1 expression and ferroptosis cell death in human colon cancer cells. International Journal of Molecular Sciences, 2019, 20: 2723.
6. Antibacterial and anti-biofilm activities of walnut pellicle extract (*Juglans regia* L.) against coagulase-negative staphylococci. Natural product research, 2019, 1-6.
7. Effects of a new combination of plant extracts plus d-mannose for the management of uncomplicated recurrent urinary tract infections. Journal of Chemotherapy,2018, 30(2):107-114
8. Effects of an extract of *Celtis aetnensis* (Tornab.) strobl twigs on human colon cancer cell cultures. Oncology Reports, 2016, 36(4):2298-2304.
9. Effects of *Tithonia diversifolia* (Hemsl.) a gray extract on adipocyte differentiation of human mesenchymal stem cells. PLOS ONE, 2015, 10(4) -320
10. Anti-adhesion activity of a2-type cranberry proanthocyanidins on uropathogenic *E. coli* and *P. mirabilis* strains. Antibiotics, 2014, 3(2):143-154

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Mimesis
In Silico Medicine

MIMESIS srl

MIMESIS is an innovative startup and academic spin-off of the University of Catania that applies computational models to the pharmaceutical/nutraceutical sector, allowing the simulation of structural-chemical-biological phenomena. MIMESIS focuses on the design, development, implementation and marketing of modelling and simulation for the evaluation of the efficacy and safety of medicinal products.
MIMESIS offers the biomedical industry and pharmaceutical companies the first generation of In Silico solutions with the aim of reducing the time and costs of drug research and development.
The estimated cost for the approval of a new drug by regulatory agencies (EMA and FDA) varies between 2 and 3 billion dollars and it takes around 10 years for the drug to reach the market. Thanks to In Silico technologies, MIMESIS is capable to provide approximately a 60-80% reduction of the time required for drug discovery and development, with a significant decrease of the time to move from preclinical to clinical phase I. MIMESIS can also predict the outcome of phase II clinical trials thus increasing confidence in investing in a Phase III study.
Furthermore, the academic spin-off is focused also on the optimization and refinement of clinical tests, allowing a ~70% reduction in number of in vivo experiments and the maximization of the chances of success of in vivo and in vitro experiments, with a consequent extreme reduction (~60-80%) of the costs and relative time to the clinical validation.
MIMESIS flagship product is beyond any doubt the Universal Immune System Simulator general framework (UISS). UISS is a multi-scale (at cellular and molecular level), multi-compartment, polyclonal, agent-based simulator of the immune system dynamics. It is able to simulate each single entity of the immune system (and consequently its dynamics), along with the significant immune responses induced by a specific pathogen or stimulus.
MIMESIS addresses pharmaceutical and nutraceutical companies, doctors, and public and private health in general but also researchers, making available its innovative tools in the field of digitization, integration and analysis of multidisciplinary data to model and test new drugs on virtual patients, thus allowing the prediction of the best treatment regimen for the profile of the patient of interest.

Selected publications:

1. Russo G, Di Salvatore V, Sgroi G, Parasiliti Palumbo GA, Reche PA, Pappalardo F. A multi-step and multi-scale bioinformatic protocol to investigate potential SARS-CoV-2 vaccine targets. Brief Bioinform. 2021 Oct 5:bbab403. doi: 10.1093/bib/bbab403. Epub ahead of print. PMID: 34607353; PMCID: PMC8500048.

2. Curreli C, Pappalardo F, Russo G, Pennisi M, Kiagias D, Juarez M, Viceconti M. Verification of an agent-based disease model of human Mycobacterium tuberculosis infection. Int J Numer Method Biomed Eng. 2021 Jul;37(7):e3470. doi: 10.1002/cnm.3470. Epub 2021 May 12. PMID: 33899348; PMCID: PMC8365724.

3. Musuamba FT, Skottheim Rusten I, Lesage R, Russo G, Bursi R, Emili L, Wangorsch G, Manolis E, Karlsson KE, Kulesza A, Courcelles E, Boissel JP, Rousseau CF, Voisin EM, Alessandrello R, Curado N, Dall'ara E, Rodriguez B, Pappalardo F, Geris L. Scientific and regulatory evaluation of mechanistic in silico drug and disease models in drug development: Building model credibility. CPT Pharmacometrics Syst Pharmacol. 2021 Aug;10(8):804-825. doi: 10.1002/psp4.12669. Epub 2021 Jul 13. PMID: 34102034; PMCID: PMC8376137.

4. Pappalardo F, Russo G, Pennisi M, Parasiliti Palumbo GA, Sgroi G, Motta S, Maimone D. The Potential of Computational Modeling to Predict Disease Course and Treatment Response in Patients with Relapsing Multiple Sclerosis. Cells. 2020 Mar 1;9(3):586. doi: 10.3390/cells9030586. PMID: 32121606; PMCID: PMC7140535.

5. Pappalardo F, Fichera E, Paparone N, Lombardo A, Pennisi M, Russo G, Leotta M, Pappalardo F, Pedretti A, De Fiore F, Motta S. A computational model to predict the immune system activation by citrus-derived vaccine adjuvants. Bioinformatics. 2016 Sep 1;32(17):2672-80. doi: 10.1093/bioinformatics/btw293. Epub 2016 May 9. PMID: 27162187.

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been® THE NEW ERA OF DNA PREDICTION

BEEN srl

Been is a health and wellness SpinOff company of the University of Catania, not yet incorporated, dealing with molecular analysis and propose to provide on-site molecular solutions within just minutes.

"We will help people to improve their well-being
by providing access to their genetic heritage
when needed and wherever they want."

Been has developed an easy-to-use sampling and processing system to be used everywhere allowing safe rapid and multiple molecular test for DNA/RNA detection and analysis.
This device simplifies the way of doing tests and paradigmatically changes times and places to perform DNA/RNA analysis, allowing rapid analysis of any biological sample outside conventional lab centers, moving out in places of daily attendance (such as for example pharmacy or patient bed), with short times (30 minutes from swab to result) and low cost: a simple biological sample obtained by a swab permit an almost immediate diagnosis of a wide range of predispositions, pathologies and intolerances.

The first tests developed for the Been platform are able to identify in particular the predisposition to baldness, to skin imperfections such as premature wrinkles, stretch marks, sensitivity to sunlight, celiac disease and food intolerances (eg hypolactasia). Molecular tests for gynecology infections and COVID-19 are under development.
This system, which put together expertise in molecular biology, mechatronics and the development of a specific software for the management of control algorithms and the generation of diagnostic data, fits within the mega trend of personalization of medicine that increasingly proposes solutions designed for single patient thanks to the analysis of his/her genetic characteristics.
The Been platform stays at the intersection between the development lines of the POC (Point-Of-Care) and DIY (Do-It-Yourself) diagnostic tests.

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