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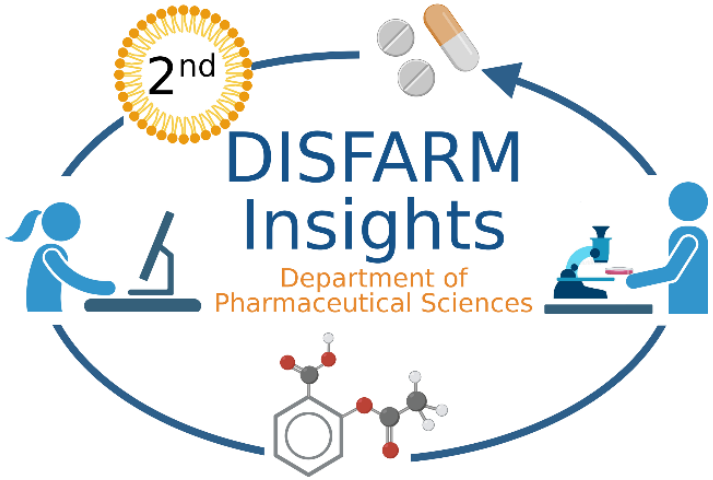

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Synthesis of MMP-responsive constructs and modified phospholipids suitable for incorporation into liposomes for diagnosis and treatment of neurological disorders

A.I. Antoniou[1,2], S. Giofrè[1], A. Amenta[1], D. Passarella[1], P. Seneci[1], S. Pellegrino[2]

[1]*Dipartimento di Chimica, Università degli Studi di Milano, Milano 20133, Italy*.
[2]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Milano 20133, Italy*.

antonia.antoniou@unimi.it

Keywords: enzyme-responsive liposomes, lipopeptides, hybrids, PET-imaging, cancer therapy

Due to limited access of biologically active compounds to the brain, current treatments for Alzheimer's disease and glioblastoma are suboptimal. Searching for more efficient therapies, we focused onto the rational design of innovative constructs to be included in suitable nanovectors for drug delivery to the brain [1], as well as to the construction of imaging-friendly liposomes suitable for diagnostics [2].
More specifically, dual ligand-modified liposomes were developed to cross the blood-brain barrier (BBB) and to release their cargo in a pathological matrix metalloproteinase (MMP)-rich microenvironment. Thus, the surface of the liposomes was functionalized with a mApoE modified peptide, known to promote cargo delivery to the BBB (*in vitro* and *in vivo* models), whereas the introduction of a MMP-sensitive moiety should cause the triggered release of the payload [3]. Therefore, MMP-sensitive peptides were functionalized either at both ends with hydrophobic stearate tails to yield MMP-sensitive lipopeptides, or the *N*-terminus was functionalized with a hydrophobic tail and the *C*-terminus was coupled with a natural phospholipid resulting to hybrid constructs (HYBs). Both lipopeptides and HYBs were later assembled into mApoE liposomes, and the latter were loaded with neuroactive drugs and tested for their bioavailability and disassembly in absence and presence of MMPs.
Furthermore, the insertion of a triple bond on the polar head of phospholipids led, after the characterization and assembly of the resulting modified phosphopeptide into liposomes, to nanoobjects suitable for diagnostics via a click chemistry approach – liposome functionalization with PET reagents, fluorescent molecules, and so on [4].
This study was implemented within the framework and with the financial support of the research project 'New frontiers of engineered nanovectors to improve treatment efficacy and safety in neurological disorders – NEVERMIND Project', CP2_16/2018 – Collaborative project II edition FRRB (Fondazione Regionale per la ricerca biomedica).

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In vitro and *in vivo* evaluation of an oral colon delivery system based on pectin and chitosan as the microbially degradable components

S. Moutaharrik[1], M. Cerea[1], M. Cirilli[1], I. Filippin[1], A. Foppoli[1], A. Maroni[1], A. Melocchi[1], L. Palugan[1], M. Uboldi[1], L. Zema[1], A. Gazzaniga[1]

[1]*Sezione di Tecnologia e Legislazione Farmaceutiche "M. E. Sangalli", Department of Pharmaceutical Sciences, University of Milan, 20133 Milano.*

saliha.moutaharrik@unimi.it

Keywords: Oral Colon delivery; Hydrophilic cellulose derivatives; Enteric polymers; Pectin; Chitosan.

Over the last decades, several strategies have been proposed for achieving site-selective drug delivery to the colon, particularly for successful treatment of inflammatory bowel disease (IBD). Such strategies are based on the exploitation of physiological features of the gastrointestinal tract (GIT) such as pH, microbiota and transit time [1]. The pH-dependent formulation approach, leveraging pH changes along the GIT, covers the vast majority of commercially available products indicated for the treatment of IBD. Indeed, typical gastroresistant polymers soluble above a 5 to 7 pH threshold are applied as coating agents. Nevertheless, these pH values are exceeded in the small intestine, while a pH below the threshold is typical of the proximal colon, especially in the inflamed organ. Thus, there is a need for addressing both issues of premature release or release failure [2]. Starting from these premises, a novel oral colon delivery platform based on a combined strategy was developed. The system consists of: (i) a drug core, (ii) an inner swellable low-viscosity hydroxypropyl methylcellulose (HPMC) layer, and (iii) an outer film coating based on a Eudragit® S: high-methoxyl pectin blend, optionally containing chitosan. Immediate-release convex tablets containing paracetamol as an analytical tracer or 5-aminosalicylic acid (5-ASA), were coated by aqueous spray-coating technique. The double-coated tablets exhibited pulsatile release profiles when tested according to the compendial two-stage procedure. Drug release from tablets with outer Eudragit® S: pectin : chitosan coatings was affected by the presence of bacteria from IBD patients in simulated colonic fluid as compared to control culture medium. Moreover, in a preliminary rat study, double-coated 5-ASA systems containing pectin and chitosan in the outer film were proved to control the progression of colitis and alteration of the microbiota more effectively than Pentasa®, marketed product for the therapy of IBD.

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Breakable organosilica nanoparticles for targeted drug delivery: the challenging case of S-adenosyl methionine

Simone Potenti[1], Luisa De Cola[1,2]

[1]*Dipartimento di Biochimica Molecolare e Farmacologia, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, 20156 Milano, Italy.*
[2]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, via Camillo Golgi 19, 20133 Milano, Italy.*

simone.potenti@marionegri.it

Keywords: Organosilica, nanoparticle, drug, loading, S-adenosyl methionine

Organosilica nanoparticles have been extensively investigated as valuable vehicles for targeted drug delivery, showing several advantages, such as high biocompatibility, degradability, large surface area for drug loading, stability and low costs [1]. Noteworthily, depending on the solubility and permeability properties of the drug [2], some nanoparticles and loading strategies are more suited than others, since they can guarantee higher loading factors and they can prevent drug leakage before the desired target is reached [3]. Besides, the outer surface of the nanoparticles can be conveniently functionalized, greatly expanding the potential of such vehicles. In particular, the charge of the nanoparticles can be exploited to load oppositely charged drugs, taking advantage of electrostatic interactions. The morphology of such nanoparticles includes disulfide groups, allowing their breakability in the intracellular medium [4]. Indeed, the glutathione present in the target cells is able to reduce the S-S bonds to thiols groups [5]. As far as the characterization is concerned, FTIR spectroscopy, TGA, DLS, and electron microscopy represent the most useful techniques to determine the physico-chemical properties of the nanoparticles. Moreover, UV-Vis and fluorescence spectroscopy are extensively used to determine the drug loading factor, through either direct or indirect approaches (spectroscopic analysis of the nanoparticle suspension after breakability test, or spectroscopic analysis of the surnatant solutions, respectively). Here, the challenging case of S-adenosyl methionine (namely SAM) will be described. SAM is a common co-substrate of a wide plethora of enzyme-catalyzed methylation reactions, but its biochemical use goes far beyond its most prominent role as a methyl donor [6]. Nevertheless, its solubility properties and its limited stability (strongly dependent on the physico-chemical properties of the dissolving medium [7]) are indeed delicate issues, incompatible with several common loading strategies. Eventually, careful optimization studies led to satisfactory protocols for the encapsulation of SAM into organosilica nanoparticles.

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Organ-retentive drug delivery systems based on shape memory polymers

M. Uboldi[1], M. Cerea[1], M. Cirilli[1], I. Filippin[1], A. Foppoli[1], A. Maroni[1], A. Melocchi[1], S. Moutaharrik[1], L. Palugan[1], L. Zema[1] and A. Gazzaniga[1]

[1]*Sezione di Tecnologia e Legislazione Farmaceutiche "M. E. Sangalli", Department of Pharmaceutical Sciences, University of Milan, 20133 Milano, Italy.*

marco.uboldi@unimi.it

Keywords: fused deposition modeling, hot melt extrusion, film coating, drug delivery systems, bladder, stomach.

Shape memory polymers (SMPs), also known as smart materials, are able to dynamically respond to specific external *stimuli* of non-mechanical nature (*e.g.* variations in temperature or light, and contact with water) by changing their shape over time. Due to this peculiar behavior, they currently represent one of the topics at the forefront of the research in many sectors. Within the pharmaceutical field, their use was demonstrated able to provide innovative performances to drug delivery systems (DDSs) and to overcome possible limitations entailed by current therapeutic approaches. These include poor patient compliance, ability to ensure effective drug levels at the target area for a prolonged period of time, fine tuning and customization of administration and release performance.
Based on these premises, the possibility of using SMPs of pharmaceutical-grade in the development of DDSs intended for long-lasting retention and release of active molecules into hollow-muscular organs, such as bladder and stomach, was approached [1,2]. Indeed, the shape shifting these materials are provided with would concomitantly allow safe administration.
A comprehensive overview of the scientific literature available was first undertaken, with a focus on the objective for which the shape changes of SMP-based DDSs were pursued [3]. From an experimental point of view, having identified a SMP of pharmaceutical grade (*i.e.* poly(vinyl alcohol), PVA), feasibility of matrix- and reservoir-like prototypes was preliminarily investigated, mainly taking advantage of hot melt extrusion and fused deposition modeling 3D printing, the latter resulting in 4D printing. The specimens obtained were characterized for dimensional variation and swelling behaviour upon contact with aqueous fluids, release and recovery performance as well as mechanical properties [4]. Film-coating of PVA-based prototypes was also shown an effective strategy to prolong the release without affecting the shape memory response [5]. Moreover, a comprehensive experimental campaign was coupled with computer-aided simulation modelling to accelerate the R&D stages and to enhance the overall performance of the retentive DDSs proposed [6]. Indeed, this approach would allow to predict the shape memory behavior of complex prototypes, reducing the number of intermediate samples to be manufactured and tested.

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Cardio-protective effects of montelukast, a cysteinyl-leukotriene receptor antagonist: an example of drug repurposing

M. Muluhie[1], L. Castiglioni[1], P. Gelosa[1], J. Rzemieniec[1], B. Mercuriali[1], L. Sironi[1]

[1]*Department of Pharmaceutical Sciences, University of Milan, Italy*.

majeda.muluhie@unimi.it

Keywords: Cysteinyl leukotrienes, myocardial infarct, montelukast, drug repurposing

Cysteinyl leukotrienes (CysLTs), a class of inflammatory mediator produced by arachidonic acid metabolism via the 5-lipoxygenase pathway, are involved in cerebro and cardio-vascular diseases. CysLT modifiers, which are generally safe and well tolerated, show a significant vascular protection in the experimental settings. Although data indicate a role of CysLTs in cardiac diseases, no data are available about the benefit of CysLTs receptor (CysLTR) antagonists in myocardial infarct (MI). This proposal aims to investigate the effects of montelukast, a CysLTR-1 antagonist, in mouse model of MI by investigating the effects on ventricular function, hypertrophic remodelling and inflammatory patterns.
MI was induced in C57BL/6N female mice by left anterior descending (LAD) coronary artery ligation. 24 hours after LAD ligation mice were subjected to cardiac magnetic resonance imaging (cMRI) and randomized to receive montelukast 10 mg/kg/die (MI+MTK, n=9) or vehicle (MI, n=9). Sham-operated mice were used as controls (SHAM, n=8). Four weeks after surgery mice were subjected to cMRI, sacrificed, and hearts collected. The expression of the inflammatory genes was measured by the use of qPCR 48 h after MI.
Our results demonstrated that compared to SHAM, 24 hours after LAD ligation, the infarcted groups (MI and MI+MTK) exhibited a reduction of ejection fraction (EF%) and regional contractility of remote non-infarcted region. At the end of experimental protocol, compared to MI group, MI+MTK mice showed a reduction in left ventricle (LV) volume, and a significant increase in EF% indicating a protective effect of montelukast in preserving LV contractility. Moreover, montelukast treatment was able to reduce LV mass and increase wall thickening.
MTK in MI animals prevented cardiomyocytes hypertrophy significantly counteracting the increase in pGSK3ß/GSK3ß, a regulator of hypertrophic pathway, and decreased mRNA expression of *Il1ß*, *Tgfß* and *CCl2* confirming its anti-inflammatory properties.
Our data show the ability of montelukast to protect from maladaptive remodelling following MI thus preserving LV contractility. The beneficial effects of MTK could be partially related to its anti-inflammatory properties exerted during the acute post-ischemic phase. These results providing the opportunity for montelukast “repurposing” in cardiovascular diseases and in particular in myocardial infarction.

Unravelling the role of estrogen signaling in macrophages in female reproductive functions

G.Pepe[1], C. Sfogliarini[1], A. Dolce[1], M. Locati[2], A. Maggi[1], E. Vegeto[1]

[1] *Center of Excellence on Neurodegenerative Diseases and Department of Pharmaceutical Sciences*.
[2] *IRCCS Humanitas Research Hospital and Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy*.

giovanna.pepe@unimi.it

Keywords: macrophage; estrogen; endometrium; reproduction

Macrophages are immune cells that play a key role in host defense against infections as well as in tissue remodeling and homeostasis, through the adoption of specific immune phenotypes. Alteration in macrophage activities contribute to the progression of reproductive disorders, such as gynecological cancers and endometriosis.
Recently, we demonstrated that 17β-estradiol (E2) induces the proliferation of peritoneal macrophages and their acquisition of an immune-tolerant phenotype. These responses are in line with the permissive propensity of the immune system in the estrogenic phases of the menstrual cycle, thus suggesting a physiological role for the estrogen-peritoneal macrophages signaling in female reproduction.
The aim of this study was to test the hypothesis that the macrophage response to estrogen directly participates in female reproductive organs and functions.
The proliferation and differentiation of ERα-negative epithelial cancer cells were analyzed after incubation with molecules secreted by primary peritoneal macrophages treated with E2. Moreover, the adoptive transfer of reporter macrophages was employed to study the specific involvement of peritoneal macrophages in reproductive functions and in response to estrogen.
In vitro data show that, after E2 treatment, macrophages produce signals that promote epithelial cells proliferation and expression of genes involved in endometrial protection against infections. Preliminary results from the animal model also demonstrated that peritoneal macrophages migrate in the ovaries and endometrium of adult female mice in stationary conditions but to a greater extent in response to estrogens and ovulation.
Altogether, our results suggest that peritoneal macrophages are directly involved in the physiology of ovarian and endometrial tissue by physically and functionally interacting with these tissues under the estrogenic control, with relevant implications for the etiology and therapeutic perspectives of reproductive inflammatory pathologies.

Development of a picomolar potency corrector of F508del CFTR for the treatment of cystic fibrosis: an overview on discovery and backup strategies

A. Giraudo[1,2], F. Berti[2], A. Rodríguez-Gimeno[2], S.M. Bertozzi[2], F. Sorana[2], G. Ottonello[3], A. Armirotti[3], R. Spanò[3], R. Bertorelli[3], I. Penna[2], D. Russo[2], E. Caci[4], L. Ferrera[4], V. Tomati[4], E. Sondo[4], A. Gianotti[4], E. Pesce[4], P. Scudieri[5], N. Pedemonte[4], L.J.V. Galietta[5,6], F. Bertozzi[2], T. Bandiera[2]

[1] *DISFARM, Università degli Studi di Milano, Italy*.
[2] *D3-PharmaChemistry, Istituto Italiano di Tecnologia (IIT), Genova, Italy*.
[3] *Analytical Chemistry and Translational Pharmacology, Istituto Italiano di Tecnologia (IIT), Genova*.
[4] *U.O.C. Genetica Medica, Istituto Giannina Gaslini (IGG), Genova, Italy*.
[5] *Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (Napoli), Italy*.
[6] *Department of Translational Medical Sciences (DISMET), University of Naples, Italy*.

alessandro.giraudo@unimi.it

Keywords: cystic fibrosis, F508del CFTR, corrector, drug discovery

Cystic fibrosis (CF) is the most frequent autosomal recessive disease in Caucasians caused by mutation of the *CF Transmembrane conductance Regulator (CFTR)* gene, encoding for the CFTR chloride channel. Several mutations have been identified impairing CFTR function by different mechanisms causing disfunction in its synthesis, folding, trafficking, gating and conductance.[1] The deletion of phenylalanine in position 508 (F508del) is the most frequent CFTR mutation, inducing trafficking defects, premature degradation due to impaired folding and stability, and also gating defects. Effective treatments for F508del CF patients require at least a *corrector*, to increase CFTR levels at the cell surface, and a *potentiator*, to increase the opening frequency of the mutant CFTR channel.[2] A recently approved triple combination of two correctors, and a potentiator, represents an important step forward in the treatment of CF. However, there is still the need to continue developing new CF therapies. In this context, a drug discovery program supported by Italian CF Foundation was established to identify novel correctors for the treatment of CF caused by F508del mutation. Using a high-throughput functional phenotypic assay, based on the Halide-Sensitive Yellow Fluorescent Protein (HS-YFP),[3] a set of about 15,000 maximally diverse commercial small-molecules was screened in two different cell types (FRT and CFBE41o-) stably expressing F508del-CFTR. This activity led to the identification of a hit compound that was optimized through rounds of chemical synthesis, biological testing, and evaluation of drug-like properties. Based on its potency and efficacy in cell-based assays, a lead compound was then identified and used as starting point for further structure-activity relationships' evolution. This work allowed the discovery of ARN23765 [4] as a potent CFTR corrector, that is currently under preclinical development investigation. A medicinal chemistry activity to identify a backup compound of ARN23765 was also conducted, leading to the analysis of more than 100 analogues. Furthermore, the synthesis and the biological evaluation of known CFTR correctors [5] allowed assessing the therapeutic relevance of the newly discovered corrector and possibly delineate its future development. This work was supported by the Italian Cystic Fibrosis Research Foundation as part of the "Task Force for Cystic Fibrosis" project.

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Preventing Rac1 inhibition to hamper intellectual disability

C. Coppa[1], A. Bazzoli[1], C. Liaci[2], S. Festa[1], I. T. Norten[1], S. Pellegrino[1], G. R. Merlo[2] and A. Contini[1]

[1]*Dipartimento di Scienze Farmaceutiche − Sezione di Chimica Generale e Organica "Alessandro Marchesini", Università degli Studi di Milano, Via Venezian, 21 20133 Milano, Italy.*
[2] *Department of Molecular Biotechnologies and Health Sciences, University of Torino, Italy.*

Crescenzo.coppa@unimi.it

Keywords: GAP, Mental disorder, computational modelling, drug discovery

The Rho GTPase Rac1 plays a key role in many cellular processes. For example, it is essential for reorganization of the actin cytoskeleton, an activity at the basis of neuronal migration, neurogenesis and spine formation.[1] Due to its important role in cellular development, Rac1 iper-activation is connected to different cancers.[2] Conversely, its downregulation is connected to some types of intellectual disabilities (ID),[3] and this last aspect is of our interest. Rac1 is brought to an inactivated state by GTPase activating proteins (GAP). Among GAPs, arhGAP15 is of interest for ID since it is disruption leads to Rac1 hyperactivation.[4] Aim of this work is to mitigate Rac1-related IDs by targeting ArhGAP15, thus impairing its interaction with Rac1 and restoring its physiological activity. First, arhGAP15::Rac1 interaction was evaluated by molecular dynamics (MD) simulations. By computational alanine scanning we identified the hotspot residues of both Rac1 and arhGAP15 involved in the protein-protein binding. Thus, we selected a sequence of amino acids to use as a template for the development of peptides inhibiting arhGAP15::Rac1 interaction. To improve affinity to the target, and stability, this sequence was further modified using computational residue scanning. The obtained peptides were evaluated by MD simulations. The most promising were synthesized and evaluated experimentally by microscale thermophoresis (MST) and on specific cell models. The two most interesting mutations of the wild type sequence were then combined to generate a new peptide. This peptide was synthesised and tested *in silico* together with the original one via classical MD and enhanced sampling simulations. To assess selectivity for arhGAP15, the peptides were also evaluated *in silico* on homology models of two alternative GAPs (arhGAP33 and Bcr). Computational results suggest that this new peptide is more selective than the original one, even though binding to Bcr is also possible. In conclusion we were able to identify three peptides that can bind arhGAP15 while modulating Rac1 activity.

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Development of a vaping machine for the determination of THC yielded by a dry herb Cannabis vaporizer

N. Mangano[1], A. Cilona[1], E. Casagni[1], S. Arnoldi[1], V. Gambaro[1], G. Roda[1]

[1]*Department of Pharmaceutical Sciences, Università degli Studi di Milano, Via L. Mangiagalli 25, 20133, Milan, Italy.*

nicoletta.mangano@unimi.it

Keywords: Cannabis, THC, Vaporizer, Cannabinoids, Terpenes

Cannabis Sativa L., known for its psychoactive and therapeutic effects, is widely used in Italy in the form of Cannabis oils: galenic preparations orally submitted for the treatment of various diseases such as multiple sclerosis, Tourette syndrome and in easing pain, nausea and vomiting associated to cancer. Oral submission, though providing good compliance and easy adjustment of dose, has slow onset of action (ill-suited for acute exacerbation of symptoms), variable bioavailability and extensive first pass metabolism; therefore more and more patients resolve to Cannabis vaporization or "vaping". This method of administration provides the advantages of the pulmonary route, while avoiding the respiratory hazards of smoking, mainly the production of toxic pyrolytic compounds or byproducts that occurs during combustion.
In this study we evaluated a dry herb Vaporizer (Ryah Smart Inhaler®) in order to determine vaporization yield of delta-9-tetrahydrocannabinol (THC) at three different heating temperatures (335°F,370°F,400°F) and volumes inhaled (500mL, 2000mL and 5000mL). A homemade vaping machine was developed for the purpose and equipped with a solvent trap system for the recovery of cannabinoids from the vapor, providing a semi-quantitative measure of its composition. The experimental setup was optimised by testing different sampling bottles, filter types and solvents to improve recovery efficiency and assessed in its reproducibility. The Cannabis material was tested for cannabinoid and terpenes content, respectively through LC/UV and HS-GC/MS: it was found that the vast majority of terpenes are vaporized within the first 500mL inhaled regardless of the temperature, while the decarboxylation process of THCA and the degradation of THC to CBN was characterized at different temperatures and an extrapolation of the estimated THC dose was proposed.

Deep Eutectic Solvents as sustainable media for organic transformations

E. Brambilla[1], V. Pirovano[1], M. Tiecco[2], G. Abbiati[1]

[1]*Department of Pharmaceutical Sciences, Sezione di Chimica Generale e Organica "A. Marchesini", University of Milan, 20133 Milan, Italy.*
[2]*Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi di Perugia, Perugia, Italy*.

elisa.brambilla@unimi.it

Keywords: Deep Eutectic Solvent, sustainable chemistry, A3 coupling, cyclization

In the last few years, the development of new sustainable synthetic methods was oriented toward the development of strategies that promoted the employment of environmentally friendly solvents, such as the Deep Eutectic Solvents (DESs).[1] Deep Eutectic Solvents represent an innovative class of solvents related to the category of ionic liquids and they are a eutectic mixture of a hydrogen bond donor and a hydrogen bond acceptor. DESs present low toxicity and biodegradable characteristics with the possibility of recycling use. Thanks to their composition and features, DESs are employed in a variety of chemical transformations not only as "innocent" solvents but also as "active" participants in the reaction.[2]

Herein, we report our recent results in the development of multicomponent reactions and intra- and intermolecular cyclization approaches promoted by the presence of DESs (fig 1). In particular, we will disclose the A^3 coupling reaction[3] and a couple of cyclization reactions involving arylalkynes with a reactive group in the ortho position promoted by carefully selected DES as "active" solvents.[4]

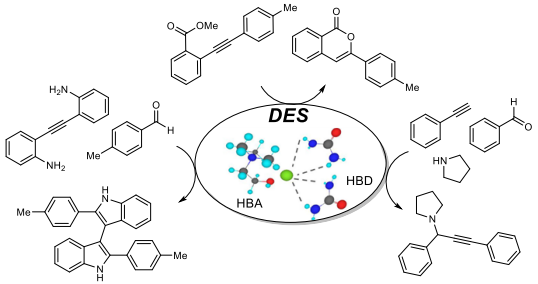

Figure 1

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Carnosine derivatives: the role of secondary amines in the quenching mechanism of reactive carbonyl species

A. Artasensi[1], S. Mazzotta[1], I. Sanz[1], G. Aldini[1], G. Vistoli[1], L. Regazzoni[1], and L. Fumagalli[1]

[1]*Department of Pharmaceutical Sciences, University of Milan, Via L. Mangiagalli 25, 20133, Milano, Italy.*

angelica.artasensi@unimi.it

Keywords: Carnosine, Antioxidants, Enamine catalysis, Aldol reaction

Carnosine (β-alanyl-L-histidine; CAR) is a natural dipeptide present in high concentrations in brain, muscles, and gastrointestinal tissues of humans[1]. Although the endogenous role is still not clarified, evidence demonstrate that CAR prevents several oxidative based diseases including lung disease, type 2 diabetes, cardiovascular disorders, neurodegenerative and kidney diseases[2]. More in detail, CAR protects cells from damage promoted by reactive species of oxygen (ROS), nitrogen (RNS) or carbonyl groups (RCS) by quenching these harmful species[3]. The RCS-sequestering ability of CAR is based on a multi-step mechanism that includes Michael addition, Schiff base formation, and/or Paal-Knorr reaction in which both the primary amine and the imidazole ring play important roles. Unfortunately, CAR bioavailability is limited due to the rapid degradation by carnosinase enzyme in tissues and serum[4]. Since the antioxidant properties of CAR had been documented, several efforts had been made to discover new compounds characterized by similar beneficial effects together with enhanced plasma stability. Thus, we synthesized a novel set of L-carnosine derivatives characterized by a secondary amine function instead of the primary one. This choice has been inspired by organocatalysis, which exploits secondary amine functions to promote the enamines' formation.
To assess the difference between a secondary and a primary amine the design has been focused on the effects of both steric hindrance and electronic properties of the secondary amine functional groups on the aldol condensation kinetics. All synthesized compounds have been tested for their quenching activity against 4-hydroxy-trans-2-nonenal (HNE), chosen as prototype of α,β-unsaturated RCS produced by lipid peroxidation. The assays comprise monitoring the kinetic by HPLC-UV analysis and the formation of corresponding adducts via mass spectrometry analyses. Finally, the metabolic stability of the tested compounds in human serum has been also investigated.

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Conditional Marketing Authorisation: a "lifeboat" for unmet medical needs

S. Manellari[1], P. Rocco[1], P. Minghetti[1]

[1]*Department of Pharmaceutical Sciences, University of Milan, G. Colombo 71, 20133, Milan.*

sara.manellari@studenti.unimi.it

Keywords: conditional marketing authorisation, early access

The traditional regulatory framework of medicinal products is built on the three "pillars" of Quality, Safety and Efficacy, which, for industrial products, are normally guaranteed through granting Manufacturing and Marketing authorisations (MA) by competent Authorities. The European Medicines Agency (EMA) evaluates and recommends to the European Commission the release of MAs for only those medicines eligible to the centralised procedure provided by Regulation (EU) No. 726/2004.
Effective and authorised medicines for life-threatening pathologies, rare diseases, cancer or intended to deal with emergency situations, such as the Covid-19 pandemic, may not be available on the market. The European legislation has foreseen the occurrence of these events and has set up regulatory pathways that facilitate the authorisation for placing on the market medicines of interest to both public health and individuals. One early access route is represented by the conditional marketing authorisation (CMA), ruled by Regulation (EU) No. 507/2006 and No. 726/2004, through which, under certain conditions and by fulfilling specific requirements, medicines can be authorised although some data are missing at the time of submission of the dossier (e.g. overall survival data for antineoplastic medicines or vaccine effectiveness data). At the same time, it is expected that the risk-benefit assessment of the medicinal product is positive and the applicant complies with all obligations within the times and in the ways established by the EMA, under penalty of withdrawal of the product from the market.
Till now, most CMAs have been granted for cancer treatment. Some of them have switched to full authorisations after few years and only one has been withdrawn for commercial reasons. During the pandemic, the CMA has proved, even more, to be a useful and successful tool in addressing unmet medical needs, as it has allowed a faster route to the MA for 6 different Covid-19 vaccines, although some quality and clinical data, such as manufacturing data and vaccination coverage, were missing due to the emergency situation. Moreover, the applicants fulfilled the obligations set down by EMA, so that most of vaccines' CMAs have been renewed for another year.

Covalent Proximity Scanning of a Distal Cysteine to Target PI3Kα

C. Borsari[1,2], E. Keles[1], J. McPhail[3], A. Schäfer[4], R. Sriramaratnam[1], W. Goch[5], T. Schaefer[1], M. De Pascale[1], W. Bal[6], M. Gstaiger[4], J. Burke[3], MP. Wymann[1]

[1]*Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland.*
[2]*Department of Pharmaceutical Science, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy.*
[3]*Department of Biochemistry, University of Victoria, British Columbia V8W 2Y2, Canada.*
[4]*Institute of Molecular Systems Biology, ETH Zurich, Otto-Stern-Weg 3, 8093 Zürich, Switzerland.*
[5]*Department of Physical Chemistry, Medical University of Warsaw, 02-097 Warsaw, Poland.*
[6]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland.*

chiara.borsari@unibas.ch

Keywords: covalent probes, electrophiles, cysteine targeting, phosphoinositide 3-kinase

Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered as valuable assets in cancer therapy. A considerable effort has been dedicated to the development of drugs targeting class I PI3Ks, which are currently evaluated in preclinical and clinical studies.[1-4]
Herein we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib)[1,5], into a highly selective PI3Kα-covalent inhibitor aiming to minimize the on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting a PI3Kα non-conserved nucleophilic amino acid side chain, namely Cys862.
A combination of warhead activity design, proximity and orientation allows a tight control of reversible inhibitor binding and isoform selective covalent binding. To avoid off-target reactions, we have set up a method to quantitatively evaluate warheads' reactivity and optimize for selectivity and Cys862 modification. An extensive Structure Activity Relationship (SAR) study was performed and a wide range of linear and restricted rotation linkers introduced. A comprehensive understanding of the kinetics of irreversible inhibition exploiting biochemical assays allowed to interpret SAR and identify compounds with optimal kinact (maximum potential rate of inactivation). X-ray crystallography and bottom-up LC-MS/MS based proteomics validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351[6]. Moreover, our compounds are metabolically stable (rat liver microsomal assay) and outperform the rapidly metabolized CNX-1351.
Our strategy to investigate and tune warheads' reactivity represents a major step forward in the rational design of covalent chemical tools. We provide highly selective irreversible probes to dissect the role of PI3K isoforms in physiology and disease.[7]

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Epigenetic governs macrophage phenotypes during Mycobacteria infections

Anna Griego[1,2], Stefano Muzzioli[1,2], Edoardo Scarpa[1,2], Alessandra Fasciani[3], Chiara Cordiglieri[3], Maria Lucia Sarnicola[4], Mariacristina Crosti[4] and Loris Rizzello[1,2]

[1] *Infection Dynamic laboratory, Department of Pharmaceutical Science, University of Milan.*
[2] *Infection Dynamic laboratory, National Institute of Molecular Genetics (INGM), Milan.*
[3] *Microscopy facility, National Institute of Molecular Genetics (INGM), Milan.*
[4]*Flow Cytometry facility, National Institute of Molecular Genetics (INGM), Milan.*

anna.griego@unimi.it

Keywords: Epigenetics, single-cell, mycobacteria, phenotypic heterogeneity

Mycobacterial infections are very dynamic processes, where alveolar macrophages (AMs) are the first line of the host defense. Because AMs must finely tune the immune response to pathogens and particulates in the lung without disrupting gas exchange, AMs show a pattern of gene expression distinct from other macrophages. They simultaneously express indeed clusters of genes belonging to both classically (M1) and alternatively (M2) activated macrophages. Interestingly, it has been proposed that histone posttranslational modification (PTMs) might chiefly shape AMs transcriptional potential. As a result, histone PTMs, such as the acetylation of H3 lysin 14 (H3K14ac), represent a timely mechanism that provides AMs a temporary, yet long-standing, extra layer of transcriptional flexibility. This suggests that histone H3K14ac might be one of mechanisms driving AMs cell-to-cell phenotypic variation. We hypothesized that such PTMs might result in the coexistence of multiple macrophages subpopulations exhibiting different pro- and/or anti-inflammatory properties, and that epigenetics modification can thus tailor intracellular mycobacterial survival and clearance. Hence, we molecularly probed the effect of H3K14 acetylation in host phenotypic diversity and studied the role played during mycobacterial infections at single-cell level, by combining high-resolution confocal microscopy, image analysis and flow cytometry.
We started our explorative study by analyzing the cell-to-cell printing of H3K14ac within an AMs-like cell line (MPI-2) in steady-state condition. We demonstrated that AMs-like cells exhibit a bimodal distribution already in basal condition. Multi-parametric single-cell analyses identified two coexisting subpopulations having low ($H3K14ac_{low}$) and high ($H3K14ac_{high}$) levels of acetylation. We then further characterize $H3K14ac_{low}$ and $H3K14ac_{high}$ AMs subpopulations bacterial sensing and infection resolving properties, revealing the crucial role of epigenetics in controlling the progression of the infection.
In sum, this project aims to exploit the molecular dynamic governing histone PTMs to propose a novel and alternative host-directed therapy that might enhance the host's ability to resolve mycobacteria infection while avoiding the arising of antimicrobial drug resistance.

The anti-asthmatic drug montelukast promotes oligodendrocyte maturation in a model of neuroinflammation in vitro

J. H. Castro e Silva[1], M. Boccazzi[1], C. Peri[2], V. Murtaj[2], D. Marangon[1], D. Lecca[1], P. Panina-Bordignon[2], G. Martino[2], M. P. Abbracchio[1]

[1] *Department of Pharmaceutical Sciences, University of Milan. Laboratory of Molecular and Cellular Pharmacology of Purinergic Transmission – Milan, Italy.*
[2] *Neuroimmunology Unit, Institute of Experimental Neurology (INSPE), Division of Neuroscience. IRCCS San Raffaele Hospital and Vita Salute San Raffaele University – Milan, Italy.*

juliana.castro@unimi.it

Keywords: multiple sclerosis, remyelination, GPR17, drug repurposing

Multiple Sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system characterized by demyelination. Although immunomodulatory drugs can significantly ameliorate the symptoms, no therapies are able to promote remyelination of the lesions, especially in the progressive forms characterized by neurodegeneration. GPR17 is a G protein-coupled receptor physiologically expressed by oligodendrocyte precursor cells (OPCs) and by pre-oligodendrocytes, and it has to be downregulated to allow oligodendrocyte terminal maturation. In demyelinating lesions, OPCs proliferate and migrate to the lesion sites. Nevertheless, they remain stuck in an intermediate differentiation stage in which GPR17 is overexpressed and do not become mature, thus not contributing to remyelination. Previous studies have shown that montelukast, an approved anti-asthmatic drug, was able to antagonize GPR17. Additionally, montelukast was demonstrated to promote neuroprotection in a mouse model of brain ischemia and to reduce cognitive decline in rats. However, its direct effect on brain OPCs is largely overlooked. Here, we evaluated the ability of montelukast to promote the differentiation of rat OPCs in vitro in both physiological and inflammatory conditions, after exposure to a cocktail of pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-1β). As expected, cytokine stimulation strongly impaired differentiation, whereas the treatment with montelukast restored the levels of typical mature markers after cytokine stimulation. Ongoing experiments aim at evaluating the effects of montelukast on human induced pluripotent cells-derived oligodendrocytes obtained from MS and control subjects. We have characterized the expression of GPR17 and other markers in this culture from days of differentiation 6, 12, 20, and 26 and showed that they express the receptor, with its peak expression on day 20. Transcriptomic analysis in this culture will also unveil the mechanisms underlying the protective effect of montelukast. Our data may open new pharmacological opportunities to repurpose montelukast as a remyelinating drug.

Predicting hERG-related cardiotoxicity of drugs by machine learning models based on molecular docking.

S. Vittorio[1]

[1]*Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli, 25, I-20133 Milano, Italy.*

vittorio.serena@unimi.it

Keywords: hERG channel, cardiotoxicity prediction, machine learning, molecular docking

The human ether-à-go-go-related potassium channel (hERG) is a voltage-gated potassium channel involved in the repolarization of the cardiac action potential. Off-target inhibition of hERG is the most frequent cause of drug-induced arrhythmias thus representing one of the major safety concerns in the early stages of drug development. Therefore, there is an increasing interest in the development of *in vitro* and *in silico* models to assess the hERG-related cardiotoxicity of new chemical entities in the early phases of drug discovery process. In particular, *in silico* approaches have emerged as attractive tools being less expensive and time-consuming. In this context, several ligand-based approaches have been used to predict hERG inhibition, spanning from pharmacophore mapping to machine learning models [1]. Recently, the 3D structure of hERG channel has been experimentally solved in its apo (PDB ID 5VA1) [2] and bound (PDB ID 7CN1) [3] states. This allows the development of structure-based predictive models which include information derived from the protein-ligand interactions. Herein, machine learning approaches have been employed to develop structure-based models for the prediction of hERG-related cardiotoxicity. Specifically, a dataset of 12789 hERG binders, collected from available databases, was submitted to docking simulation employing the 3D structure of the holo protein by means of three different software: Plants, LiGen and Gold. The resulting poses underwent to a rescoring procedure by using the Rescore+ tool as implemented in the VegaZZ suite of programs. The so obtained scoring functions were exploited to train random forest (RF) regression and classification models with satisfactory and comparable performances. Due to the high promiscuity of hERG ligands, the presence of allosteric sites has been postulated [1]. Basing on this, the GENEOnet method [4] was applied to detect additional binding sites on this protein channel. As result, two putative ligand binding pockets have been identified and employed for docking and rescoring analysis. RF models were trained on the obtained data and consensus models, incorporating the results yielded by the docking and the rescoring runs performed on the three pockets, were developed. Overall, the generated models showed a good ability in identifying hERG inhibitors offering new useful tools for cardiotoxicity assessment of novel drug candidates.

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