STRATAGEM
CA17104

ABSTRACT BOOK

COST Action 17104 (STRATAGEM)
WG2 Meeting and International Online Symposium on "Synthesis and nanodelivery strategies for new therapeutic tools against Multidrug Resistant Tumours"

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COST is supported by the EU Framework Programme
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STRATAGEM Action Summary

This Action will build the first multidisciplinary network, including academic laboratories, research institutes, small and medium enterprises (SMEs), with a wide range of excellent and non-overlapping expertise, aiming at improving at the same time the diagnosis and therapy of multidrug resistant (MDR) solid tumors. Until now, there are fragmented knowledge on biomarkers and therapeutic tools used against MDR tumors; there are not algorithms predictive/diagnostic of MDR tumors ex ante; all the past therapies against MDR tumors failed. The key challenge of this Action is to fill these gaps, by producing a comprehensive, open and user-friendly platform of knowledge on MDR tumors, identifying new diagnostic/predictive biomarkers, producing new and safe compounds applicable to personalized treatments of MDR tumors. Up to 70% of solid tumors are resistant at the diagnosis: this means poor life quality and poor prognosis for patients, high management costs for the European healthcare systems. This Action is working to improve diagnosis and treatment of patients with MDR tumors and reduce the costs for their management. Second, by creating fruitful collaborations between basic and industrial research, we will give impulse to the creation of new Start-up and SMEs in Europe. Finally, the Action aims at raising the level of European research on MDR, reducing the disparity in the research quality between EU countries and ITC, providing the necessary training for European early stage researchers (ESRs) to grow as future independent research leaders, regardless of location, age or gender.

Action website: https://stratagem-cost.eu/
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Dear Friends,

Welcome to the third WG2 online Meeting.

As initially announced at our annual meeting in Belgrade in February 2020, this meeting should have been held in Angers (France) and be followed by a congress of the French Society of Nanomedicine (SFNano). Given the current pandemic restrictions, the congress has been cancelled and we have decided to propose this meeting online, and to transform it into a small International Symposium to allow us to better know each other and exchange scientific ideas. As you know, the WG2 aims as identifying, via design and synthesis, new bio-active compounds that target multidrug resistance (MDR) tumours, and formulating them using nanocarriers to improve their anticancer action. However, this event is open to members from all WGs of STRATAGEM, with complementary scientific aims.

This one-day online Symposium includes two plenary lectures given by Prof. Romano Silvestri and Prof. Fabiana Quaglia, on topics that are highly relevant to WG2 objectives. Eight selected oral presentations will be given by scientists/students from different members' laboratories, from eight different countries.

Twenty-two posters are available at our webpage (following login) and will be shortly presented during the Symposium, in the form of 5-minute "Speed-talks".

At the end of the Meeting/Symposium, we will award prizes for the best presentations. These will be selected by the Prizes Selection Committee, consisting of members of the Scientific Committee who will attend sessions and are not co-authors of more than one communication.

The WG2 member Meeting itself, will take place at the end of the morning session, during which an update on relevant topics will be made.

We wish that this online Meeting/Symposium enhances the scientific knowledge of all participating scientists and students, providing a valuable experience and new opportunities for future collaborations.

Finally, and most importantly, we hope you enjoy this scientific day!

PROGRAM

9h30 Welcome

9h40 - 13h – MORNING SESSION

9h40 **Keynote Lecture I – Prof. Romano Silvestri (Sapienza University of Rome, Italy):** *New anti-Cancer Agents through an Interaction with Tubulin.*

10h30 **Anne Vessières (Sorbonne Université, France)**: *Biological activity of ferrocifens on human PDCLs of glioblastoma (GBMs). A step toward personalized medicine.*

10h50 **Kateřina Valentová (Czech Academy of Sciences, Czech Republic):** *Selectively halogenated flavonoids: Preparation, biophysical properties, and multidrug resistance modulation.*

11h10 **Arasu Ganesan (University of East Anglia, United Kingdom)**: *Multitargeting epi-epi drugs for multidrug resistance.*

11h30 **Alfonso T. Garcia-Sosa (University of Tartu, Estonia):** *Predicted Protein Kinase C isoform interactions and experimental inhibition of breast cancer stem cell-inducing spheres.*

11h50 Coffee break

12h - 13h WG2 member meeting (for WG2 members)

13h - 14h – INTERVAL (LUNCH BREAK)

14h - 18h – AFTERNOON SESSION

14h Keynote Lecture II – Prof. Fabiana Quaglia (Naples University, Italy): *Biodegradable nanoparticles delivering therapeutic combinations in MDR cancer.*

14h50 **Cristina Del Plato (Istituto Italiano di Tecnologia, Italy)**: *Design and synthesis of piperazine-based compounds conjugated to Humanized ferritin as delivery system of siRNA in cancer cells.*

15h10 **Veronica Bastos (University of Aveiro, Portugal)**: *UCNPs nanocapsules for doxorubicin targeting delivery in melanoma cell lines.*

15h30 **Denitsa Aluani (Medical University of Sofia, Bulgaria)**: *Micellar Encapsulation of Doxorubicin with CAPE Enhance its Cytotoxicity Against Lymphoma L5178 MDR1 cells*.

15h50 **Christina N. Banti (University of Loannina, Greece)**: *Novel silver glycinate metallodrug; A non toxic antiproliferative agent induces apoptosis on human breast cancer cells.*

16h10 Coffee break

16h20 Speed-talks of poster presentations

16h20 **Silvia Cammarone (Sapienza University of Rome, Italy)**: *Chalcones and Chalcone-mimetic Derivatives as Notch blocking agents in T-cell acute lymphoblastic leukemia.*

16h25 **Pierre Idlas (University of Angers, France)**: *Formulation of ferrocifen loaded lipid nanocapsules against multidrug resistant ovarian adenocarcinoma.*

16h30 **Florence O. McCarthy (University College Cork, Ireland)**: *Novel 11-substituted ellipticines as potent anticancer agents with divergent activity against cancer cells.*

16h35 **Francesca Picarazzi (University of Siena, Italy)**: *Structural elucidation of novel Imidazo[1,2-a]pyridine inhibitors of Aldehyde Dehydrogenase 1A Family.*

16h40 **Vera M. S. Isca (Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal)**: *Innovative nanosystems with natural cytotoxic royleanone diterpenes from Plectranthus spp.*

16h45 **Oscar Briz (University of Salamanca, Spain)**: *Transportome manipulation by gene therapy to sensitize liver and gastrointestinal tumors to chemotherapy.*

16h50 **Valeria Vergine (Sapienza University of Rome, Italy)**: *$mPEG_{5kDa}$-cholane/Glabrescione B delivery system as promising tool for the treatment of Hh-dependent tumors.*

16h55 **Nicolas Clere (University of Angers, France)**: *p722 ferrocifen loaded lipid nanocapsules improve survival of murine xenografted-melanoma via a potentiation of apoptosis and an activation of $CD8^+$ T lymphocytes.*

17h00 **Bruno M. F. Gonçalves (University of Lisbon, Portugal)**: *Exploring the efflux and modulation mechanisms of Human ABCG2 through Molecular Dynamics Simulations.*

17h05 **Arif Kivrak (Van Yüzüncü Yil University, Turkey)**: *Synthesis of Novel Artemisinin-Benzothiophene Hybrid Molecules.*

17h10 **Wolfgang Link (Instituto de Investigaciones Biomédicas "Alberto Sols", Spain)**: *Harmine and Piperlongumine revert TRIB2-mediated drug resistance.*

17h15 **Mariacristina Failla (University of Turin, Italy)**: *NO release regulated by doxorubicin as the green light-harvesting antenna.*

17h20 **Sundus Erbas-Cakmak (Konya Food and Agriculture University, Turkey)**: *Activatable Photodynamic Therapy Agents for Use in Multi-Drug Resistant Tumors.*

17h25 **Enrique Domínguez-Álvarez (Consejo Superior de Investigaciones Científicas, Spain)**: *Selenocompounds: a novel approach to fight cancer resistance.*

17h30 **Isabella Romeo (Istituto Italiano di Tecnologia, Italy)**: *Synergistic inhibition of the Hedgehog pathway by newly designed Smo and Gli antagonists bearing the isoflavone scaffold.*

17h35 **David S. P. Cardoso (University of Lisbon, Portugal)**: *Generation of a library of indole alkaloid derivatives as ABCB1 inhibitors in resistant cancer cells.*

17h40 **Philippe Bertrand (University of Poitiers, France)**: *Simplified tetraethylene oxide-mediated synthesis of gold nanoparticles and their internalization by cancer and neuronal cells.*

17h45 **Michela Puxeddu (Sapienza University of Rome, Italy)**: *New 1,1'-Biphenyl-4-sulfonamides as Potent and Selective Human Carbonic Anhydrase inhibitors.*

17h50 **Sarah Le Saux (University of Montpellier, France)**: *Stability, cellular interactions and post production modification of murine mesenchymal stem cells (mMSC) derived Extracellular Vesicles.*

17h55 **Florence O. McCarthy (University College Cork, Ireland)**: *Isoquinolinequinone N-oxides as anticancer agents effective against drug resistant cell lines.*

18h00 **Niamh M. O'Boyle (Trinity College Dublin, Ireland)**: *Combretazets: Enantiomeric β-Lactams for the Treatment of Breast Cancer.*

18h05 **Hulya Ayar Kayali (Izmir Biomedicine and Genome Center, Turkey)**: *Synthesis and Characterization of Therapeutic Antibody-drug Conjugates against Multidrug Resistant Ovarian Cancer Therapy.*

18h10 Prizes for best presentations and closing remarks

Abstracts - Oral presentations

The abstracts presented herein are organised as per the event programme

New anti-Cancer Agents through an Interaction with Tubulin

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Microtubules (MTs) are involved in a number of essential cellular functions, such as maintenance of cell shape, cell motility, intracellular transport and cell division. Interfering with the MT dynamic equilibrium, by either inhibiting tubulin polymerization or blocking MT disassembly, has resulted in a productive strategy for the development of efficient anticancer agents. On the other hand, MT stabilizing drugs show potential to treat Alzheimer's disease (AD) and related tauopathies. However, drug resistance, toxicity and unwanted side effects still prompt the search for new MT inhibitors as components of improved treatments.

A new series of tubulin targeting agents have shown to inhibit at nanomolar concentration the cancer cells including P-glycoprotein (Pgp) overexpressing lines NCI/ADR-RES and Messa/Dx5. Besides the inhibition of tubulin polymerization, the new agents stimulate the cytotoxic activity of natural killer cells at doses which do not severely affect cell viability, increasing NKG2D and DNAM-1 ligand up-regulation on HeLa cells. At higher concentrations, these compounds stably arrest mitotic progression, prevent mitotic slippage and the ensuing formation of aneuploid cells and induce cell death, with effectiveness comparable or superior to that obtained with VBL.

Besides the ability to inhibit tubulin polymerization, these scaffolds have shown to interfere with the Hedgehog signaling pathway and medulloblastoma D283 cells, and the Na^+/H^+ exchanger 3 regulating factor 1 (NHERF1) membrane adaptor protein. The results prompt further development to obtain agents with enhanced anticancer activity.

Biological activity of ferrocifens on human PDCLs of glioblastoma (GBMs). A step toward personalized medicine.

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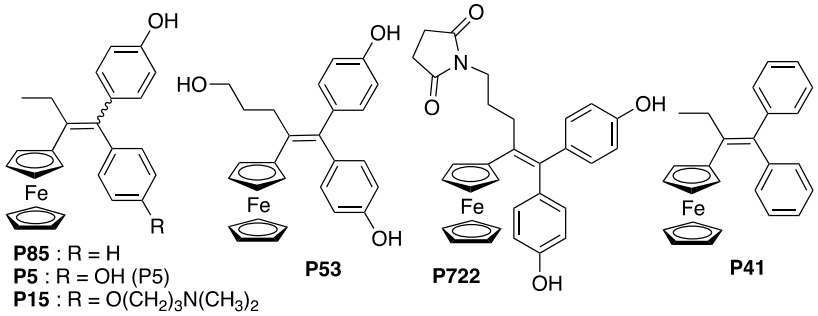
We have developed a family of iron metallo-drugs, called ferrocifens, that exhibited strong *in vitro* antiproliferative activities on multi-drug resistant (MDR) cancer cells including 9L cells (rat gliobastoma).[1,2] In collaboration with Pr C. Passirani, we found that intraveneous injections of **P5**, formulated in lipid nano capsules (LNCs) induced, *in vivo* in rats, strong regression of 9L ectopic and orthotopic tumors.[3,4]
We will present the evaluation of the antiproliferative activity of a selection of 6 ferrocifens on 15 human GBM patient-derived cell lines (PDCLs) available in Gliotex team of ICM.[5] These results showed that, three complexes, **P5, P85**, **P722** are very cytotoxic on some cell lines (IC_{50} as low as 0.01 µM), while they are only slightly cytotoxic on other cell lines (IC_{50} as high as 29.8 µM). For complex **P53**, the range of IC_{50} is smaller (0.2 - 6.9 µM) while **P15**, the complex with an amino side chain, displays high cytotoxicity on all cell lines. Regarding PDCLs, we found that the ratio highest/lowest IC_{50} values cover a very broad range (3.7-1041) evidencing large differences of sensitivity to the complexes between cell lines. In 12 out of 15 cell lines this ratio is higher than 10. These interesting results point out the wide variety of response of cancer cells belonging to the same type of cancer (GBMs) to these metallo-drugs and open the way to personalized treatment taking into account the molecular context of each patient's tumors.



P85 : R = H
P5 : R = OH (P5)
P15 : R = $O(CH_2)_3N(CH_3)_2$

P53 **P722** **P41**

A bioinformatics study is underway to understand the heterogeneity of response observed on the various cell lines, with the objective of identifying active signaling routes in sensitive cell lines.

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Selectively halogenated flavonoids: Preparation, biophysical properties, and multidrug resistance modulation

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Flavonoids represent a vast group of polyphenolic secondary metabolites found in most plants and fungi, and they are a common part of the human diet. Flavonoids typically have low toxicity for humans and beneficial biological effects such as anti-oxidative, anti-inflammatory, anti-mutagenic, and cytotoxic properties.[1] Besides it some flavonoids were recently described to inhibit transporters associated with multidrug resistance.[1] Selective modification, such as halogenation can provide new compounds and enlarge the biological potential of flavonolignans. Mono-, di- and trihalogenated derivatives of flavonoids were prepared and characterized. Selectively monobrominated derivatives were prepared using α,β-dibromohydrocinnamic acid in presence of a base.[2] Di- and trihalogenated derivatives were prepared with the use of corresponding *N*-halogensuccinimide. All prepared derivatives were tested for anti-inflammatory, antioxidant, reducing and anticancer activity in comparison to the parent compounds. Prepared compounds were tested for inhibition of transporters associated with multidrug resistance. The highest antioxidant and reducing potential was observed for the flavonoids and 2,3-dehydroflavonolignans and their respective halogenated derivatives. In the case of the flavonolignans, silybins and their derivatives showed very poor acitivity, while silychristin and its derivatives exhibit better antioxidant and reducing potential. Halogenated derivatives and parent compounds were compared for their ability to modulate the doxorubicin resistant phenotype in human ovarian carcinoma.

References:
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Multitargeting epi-epi drugs for multidrug reistance

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Epigenetic therapy is now a clinical reality with eight approved drugs that target DNA methyltransferases, histone deacetylases (HDACs) and lysine methyltransferases. A further recent development is the concept of epigenetic multitargeting through the rational design of novel agents that combine the inhibition of an epigenetic pathway with a second non-epigenetic target and five such compounds have advanced to clinical development.
We are investigating the even newer concept of 'epi-epi' drugs that inhibit two separate epigenetic pathways. Such dual targeting agents have the potential to achieve higher efficacy against proliferating cancer cells while reducing tunor resistance. In this presentation, we report a selective dual histone deacetylase and demethylase inhibitor with an IC_{50} of of 0.55 and 0.14 □M against HDAC6 and LSD1 respectively. The compound was biologically profiled together with control compounds that were either single inhibitors or inactive against either enzyme. The dua inhibitor was active against a panel of leukemia cell lines at a micromolar level and induced apoptosis. Target engagement asays such as CETSA were employed to confirm the inhibition of HDAC6 and LSD1 in cells. Further experiments were carried out to identify synergistic effects with clinically approved agents and promising results were observed with doxorubicin.

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Predicted Protein Kinase C isoform interactions and experimental inhibition of breast cancer stem cell-inducing spheres

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The abietane diterpene royleanones are interesting compounds for cancer research [1]. Protein kinase C isoforms have been implicated in different cancers, and their modulation by small molecules is an avenue for development of possible therapeutics. Subtle changes in the binding site of each PKC isoform studied (δ/ι/α/β/θ/ζ/λ) can change the modeling [2] predicted interaction profiles of the ligands. Subtle changes in royleanone substitution patterns, such as a double substitution only with non-substituted phenyls, or hydroxybenzoate at position four that flips the binding mode of ParvD, can increase the predicted interactions in certain PKC subtypes. Inhibition of PKCζ may find use against tumorigenesis in breast [3] and pancreatic [4] cancer and as a tumor suppressor in prostate cancer [5].

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Biodegradable nanoparticles delivering therapeutic combinations in MDR cancer

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Cancer treatments using a single therapeutic agent often result in limited clinical outcomes. By tackling multiple targets, a combination of therapeutics aims either to improve the therapeutic index by increasing efficacy and overcoming resistance or to attain similar efficacy with reduced systemic toxicity.
The co-delivery of more than one therapeutic agent is pharmaceutically challenging and can benefit from a nanotechnological approach. Multicargo nanocarriers can unify the pharmacokinetics of the single drugs and ensure that programmed drug ratios are delivered at target. In the vast arena of possible nanocarriers achieving the “mission” of multimodal therapy, biodegradable polymeric nanoparticles (NPs) offer the advantages to be tailored through selection of the appropriate building blocks. Core-shell NPs can be logically designed to load drugs independently of their lipophilicity/size, release bioactive cargos at pre-programmed rates, incorporate targeting moieties, and evade clearance mechanisms.
In this contribution, our recent achievements in designing biodegradable NPs delivering combinations of cytotoxic drugs with nitric oxide photodonors, photosensitizers or nucleic acids will be illustrated, providing advanced proof-of-principles of therapeutic concepts.

Design and synthesis of piperazine-based compounds conjugated to Humanized ferritin as delivery system of siRNA in cancer cells

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Small interfering RNA (siRNA) represents revolutionary tool for gene therapy with a wide array of potential applications in the regulation of gene expression. However, a successful clinical application of nucleic acid-based therapy requires novel delivery options, because of the extremely labile nature of siRNA under physiological conditions, which hamper its efficient and sustained delivery. [1] With the aim to physically entrap siRNA duplexes in the inner cavity of an engineered Humanized ferritin from *Archaeoglobus fulgidus (*Hum*AfFt)*, piperazine-based compounds featuring one or two piperidine rings (**PAs**) were rationally designed and synthesized to promote electrostatic interactions with negative small nucleic acids. [2] In addition, these rigid-rod-like amines were functionalized with thiol-reactive crosslinkers (*i.e.* maleimide and fluorobenzene sulfonamide) for chemoselective conjugation of cysteine residues located inside the HumAfFt cavity. These systems allowed siRNA delivery into HeLa, HepG2 and MCF-7 cancer cells with improved silencing effect on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression with respect to traditional transfection methodologies and provided a promising TfR1-targeting system for multifunctional siRNA delivery to therapeutic applications. It is envisioned that the reported nanodelivery systems might be employed to multiple siRNA-based silencing for a wide range of biotechnological applications.

References:
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UCNPs nanocapsules for doxorubicin targeting delivery in melanoma cell lines

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Melanoma is the most aggressive skin cancer with limited and non-curative options in conventional clinical treatments [1]. Upconverting nanoparticles (UCNPs) have emerged as promising systems for cancer theranostics and multimodal therapy, due to their unique NIR-driven applications [2, 3]. The aim of this work was the production of a nano system for anti-tumor drugs encapsulation and delivery. Thus, UCNPs coated with mesoporous silica shells ($NaYF_4$:Yb,Er@$mSiO_2$) were functionalized with folic acid (FA) for tumor targeting and loaded with the antitumor drug doxorubicin hydrochloride (DOX) for chemical treatment. The functionalization with FA enhanced the drug release. The release of DOX was measured at different pHs, and the cytotoxicity of UCNPs@$mSiO_2$-FA_DOX was evaluated and compared with free DOX in melanoma cell lines. In conclusion, our results demonstrated that the developed UCNPs nanocapsules could serve as drug delivery systems for cancer therapy.

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Micellar Encapsulation of Doxorubicin with CAPE Enhance its Cytotoxicity Against Lymphoma L5178 MDR1 cells

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Besides the broad therapeutic potential of doxorubicin (Dox) in the treatment of breast, ovarian, lung, bladder cancer, its application is strongly related to clinical problems, such as multiple drug resistance (MDR) and dose-dependent cardiotoxicity. Among the approaches for overcoming MDR in doxorubicin therapy, double encapsulation with hydrophobic natural substances in nanosized drug-delivery systems could provide advantages, regarding its safety and efficacy.
The present study evaluated the impact of double encapsulation of doxorubicin and caffeic acid phenethyl ester (CAPE, a hydrophobic natural compound with antioxidant and antiproliferative properties) in mixed polymer micelles on its cytotoxic potential on L5178Y and multidrug resistant L5178 MDR1 lymphoma cells. The antiproliferative effects of micellar DOX/CAPE were compared with those of free DOX and CAPE, and the free combination of both active compounds.
Both, Dox and CAPE were loaded in mixed micelles based on triblock copolymers of poly(ε-caprolactone) with poly(acrylic acid) and poly(ethylene oxide). The mixed coloaded micelles were characterized with a small diameter (41 nm) and 5.8 and 5.2 % loading degree for Dox and CAPE, respectively. A non-loaded Dox caused statistically significant cytotoxic effects in L5178Y cells at 0.56 μg/ml, while in resistant L5178Y MDR1 cells cytotoxicity occurs in significantly higher concentrations (5.6 μg/ml). A solution of both non-loaded Dox and CAPE caused more pronounced concentration-dependent cytotoxicity in both cell lines compared to the effects of Dox or CAPE, administered separately. Interestingly, double loading of doxorubicin and CAPE leads to significantly higher cytotoxic effects to L5178 MDR1 cells compared to the combination of both non-loaded active compounds.
The co-delivery of DOX and CAPE encapsulated in mixed copolymeric micelles) is a promising approach to improve the synergistic effects of doxorubicin and hydrophobic natural substances as CAPE in resistant lymphoma L5178 MDR1 cells.

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Novel silver glycinate metallodrug; A non toxic antiproliferative agent induces apoptosis on human breast cancer cells.

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Background: Silver(I) metallodrugs exhibit significant *in vitro* antiproliferative activity against many cancerous cell lines, including adenocarcinoma cells [1]. The antiproliferative activity of silver ion is due to its binding to the DNA bases, on its binding to thiol groups of the protein and on its interaction with mitochondrion, which activates the mitochondrial apoptotic pathway of the cells [1]. Amino acids, on the other hand, as biocompatible ligands can deliver the metal ion to its biological target preventing its reduction under physiological conditions [2-3]. Metal complexes with amino acids can be more selective toward the abnormal cells in respect to the normal ones [3]. This is due to the over-expression of the amino acids receptors of the abnormal cells [1].
Aims: The combination of a biocompatible agent with a metal ion enhances its activity because of the easy penetration of the agent into cytoplasm and its delivery to intracellular biological targets, selectively [1].
Methodology and results: The new covalent polymeric silver(I) complex with glycine (GlyH), $[Ag_3(Gly)_2NO_3]_n$ (AGGLY) was synthesized and was characterized by spectroscopic techniques and single crystal X-ray crystallography. The *in vitro* cytotoxic activity of AGGLY was tested against human breast adenocarcinoma cancer cell lines: MCF-7 and MDA-MB-231. The *in vitro* and *in vivo* genotoxicity was evaluated by micronucleus assay and Allium cepa model. The mechanism of action was studied by cell morphology, cell cycle arrest, AO/EB Staining, and permeabilization of the mitochondrial membrane test. The molecular mechanism of it was studied by the binding affinity towards the calf thymus DNA
Conclusion: AGGLY is a non toxic antiproliferative agent which induces apoptosis on human breast cancer cells though mitochondrion pathway

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Abstracts - Poster presentations

The abstracts presented herein are organised as per the event programme

Chalcones and Chalcone-mimetic Derivatives as Notch blocking agents in T-cell acute lymphoblastic leukemia

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The Notch signaling pathway is an inter-cellular communication system driving many biological processes in different tissues and in a wide spectrum of organisms. Indeed, it is considered a rationale target in the therapy of cancers, particularly those harbouring Notch gain of function mutations, including T-cell acute lymphoblastic leukemia (T-ALL).[1] Although the currently available Notch-blocking agents are showing anti-tumor activity in preclinical studies, they are not effective in all the patients and often cause severe side effects, limiting their widespread therapeutic use. Since natural products have long been used as medicines for human diseases and are considered a relevant resource of lead compounds for drug discovery, an *in house* library of natural products and their derivatives was used as a potential source of inhibitors of the Notch signaling in T-ALL.[2] Eight representative molecules of the library were selected through a cheminformatics approach and tested *in vitro*. The chalcone scaffold emerged as a promising tool to inhibit Notch signalling; indeed, the synthesis of several chalcones combined with their biological evaluation highlighted the 2′,4-dihydroxy-4′-methoxychalcone (named chalcone **8**) as the most potent Notch inhibitor of the series, suggesting the synergistic activity of 2′- and 4-hydroxyl groups.[2] Based on hit-likeness and chemical diversity, a number of chalcones and chalcone-mimetic compounds were further designed and synthesized and their antiproliferative activity in KOPTK1 cells was evaluated.[3] Among them, 2',4-dihydroxy-3-methyl-4'-methoxychalcone (compound **1**) and 2,4-dimethoxyphenil-7-methoxy-1,2,3,4-tetrahydronaphtalen-2-yl-methanone (compound **18**) proved to be new promising Notch-blocking agents, exhibiting cell growth reduction and inhibitory effects comparable to that of compound **8**.

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Formulation of ferrocifen loaded lipid nanocapsules against multidrug resistant ovarian adenocarcinoma

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Keywords: nanomedicine, organometallic complex, ovarian cancer, SKOV3 cells, TLS peptide, multidrug resistance

Considering that ovarian adenocarcinoma is one of the deadliest epithelial malignancies in women because of resistance to the current treatments, new strategies are needed [1]. Ferrocifens, innovative organometallic complexes, could represent an alternative. Indeed, these molecules, composed of a tamoxifen scaffold covalently bond to a ferrocene moiety, have shown very interesting anti-proliferative and cytotoxic effects on several cancer cell lines [2]. However, as for many therapeutic molecules, one major problem is the high lipophilicity of ferrocifens. That is why, in order to be administered *in vivo,* there is a need to use a nanocarrier as, for example, the lipid nanocapsules (LNC). This nanoparticle is obtained by a free organic solvent process of phase inversion and composed of an oily core surrounded by amphiphilic surfactants [3]. The aim of this project is to formulate the corresponding ferrocifen loaded LNC in order to reach specifically the ovarian adenocarcinoma. For that, a cell-penetrating peptide specifically targeting the SKOV3 ovarian cancer cell lines, the TLS [4], will be added to the LNC surface either by adsorption or by covalent grafting.
Concretely, two kinds of ferrocifens, named P722 and P769, have been encapsulated in LNC. The maximal drug loading has been determined by UPLC. Stability studies of the corresponding nanocarriers have been performed by controlling the physicochemical characteristics of the nanosystems. No significant variation in terms of size and zeta potential has been found over one month. However, interestingly, the encapsulation of P769 has resulted in the formulation of a gel which seemed dependent on the concentration in P769. It is the first time that this kind of formulation has been obtained with ferrocifens.

In the future, further studies will be performed on these ferrocifen loaded LNC. *In vitro* and *in vivo* experiments will be undertaken to determine the efficiency of this innovative treatment on ovarian adenocarcinoma.

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Novel 11-substituted ellipticines as potent anticancer agents with divergent activity against cancer cells.

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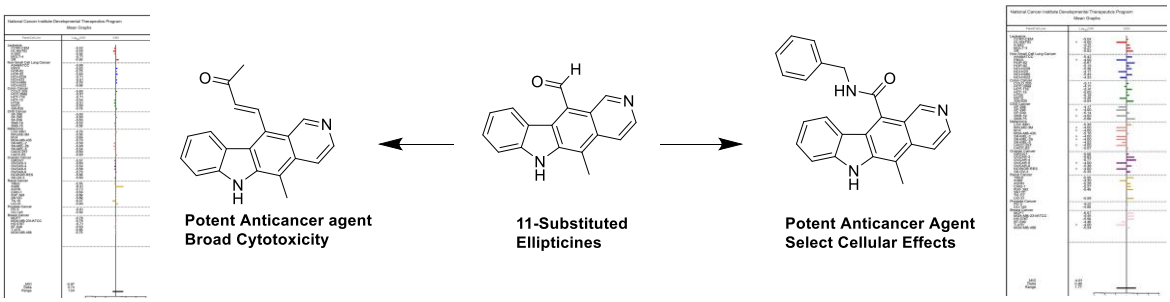
Ellipticine **1** (5,11-dimethyl-6H-pyrido [4,3-*b*]carbazole) is a natural product which was isolated in 1959 from a small tropical evergreen tree (Ochrosia elliptica). Over the past 60 years, the planar tetracyclic structure of ellipticine has been the focus of extensive chemical and pharmacological research with a number of ellipticines having well documented anticancer activity, in particular with substitution at the 1-, 2-, 6- and 9-positions. However, due to limitations in synthesis and coherent screening methodology, the full profile of this anticancer class has not yet been achieved and some positions on the tetracycle have received little attention.
In order to address this shortfall, we set out to explore the anticancer activity of this potent natural product by substitution. Herein, we describe the synthesis of novel 11-substituted ellipticines with two specific derivatives showing potency and diverging cellular growth effects. Screening of anticancer activity via topoisomerase II inhibition and NCI 60 cell screening methodology identifies significant potency and potential molecular targets.[1]



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Structural elucidation of novel Imidazo[1,2-*a*]pyridine inhibitors of Aldehyde Dehydrogenase 1A Family

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Aldehyde dehydrogenases (ALDHs) are oxidizing enzymes, whose main function is to convert exogenous and endogenous aldehydes into their corresponding carboxylic acids [1]. Members of the ALDH1A family are commonly acknowledged as hallmarks of cancer stem cells (CSCs). Overexpression of the ALDH1A3 isoform is significantly associated with tumorigenesis and chemotherapeutic resistance [2]. Accordingly, small molecule inhibitors of ALDH1A3 stand as a desirable option to fight cancer and related multi drug resistance (MDR).
Starting from the reference ALDH inhibitor GA11 previously developed by our group, a novel series of imidazo[1,2-*a*]pyridines was developed and optimized by means of a structure-based approach. These novel compounds were evaluated *in vitro* for their activity and selectivity against the ALDH1A family, and investigated through X-ray crystallography and modeling studies for their ability to interact with the catalytic site of the 1A3 isoform.
Among the most promising ALDH1A3 inhibitors, NH51 did not show a sufficient electron density in crystallographic studies. Molecular docking, molecular dynamics (MD) simulations and Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) [3] calculations helped to fill this structural gap. Furthermore, theoretical affinity of compound NR6 was evaluated by MD simulations, while alanine scanning calculations highlighted the thermodynamic role of Y472, specific for the ALDH1A3 isoform, which emerged as a fundamental residue in ALDH1A3/inhibitor interaction.
Overall, computational approach provided structural and thermodynamics information that are essential for the design and optimization of small molecules ALDH1A3 inhibitors. *In vitro* testing of some imidazo[1,2-a]pyridines on different populations of CSCs obtained from glioma, colorectal and prostate tissue specimens, exhibited a relevant anti-proliferative efficacy, thus paving the way for treating MDR cancers.

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Innovative nanosystems with natural cytotoxic royleanone diterpenes from *Plectranthus* spp.

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Natural products are important sources of new drug leads for cancer therapy. Several well-known anticancer agents including examples as paclitaxel and vinblastine, were obtained from plants [1]. *Plectranthus* genus (Lamiaceae) is an important source of bioactive compounds, namely, royleanone diterpenes [2]. Cytotoxic diterpenoids are frequently very low water-soluble compounds and nanotechnology can be a possible solution to improve drug solubility and targeted delivery without undesirable side effects. One example of this concept is related to the assembly of 6,7-dehydroroyleanone (DHR) with hybrid nanoparticles to potentiate DHR cytotoxicity in cancer cells [3].
In this study, we describe the isolation and derivatization of 7α-acetoxy-6β-hydroxyroyleanone (Roy) from *P. grandidentatus*. The main propose was to target to tumor area and to improve the royleanones' cytotoxicity by using nanotechnology. So far, several derivatives were obtained with over all good yields. Furthermore, two benzoylated derivatives exhibited promising cytotoxic potential to be further used in nano-formulations. Overall, we expect that nanoparticles delivery systems besides improving solubility and stability, they may extend a better therapeutic action of these royleanone derivatives.

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Transportome manipulation by gene therapy to sensitize liver and gastrointestinal tumors to chemotherapy

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Background: The response of liver and gastrointestinal cancer to pharmacological treatment is often limited by low intracellular levels of anticancer drugs due to reduced function/expression of drug uptake proteins of the Solute Carrier (SLC) superfamily and high activity of drug exporting ATP-binding cassette (ABC) pumps. **Aim:** To investigate the usefulness of gene therapy strategies to modify the transportome to enhance the intracellular amounts of anticancer agents and hence sensitize tumor cells. **Methods and results:** Transduction of cells derived from hepatocellular carcinoma, hepatoblastoma, and cholangiocarcinoma was carried out using lentiviral vectors containing the ORF of the organic cation transporter OCT1 (*SLC22A1*), whose expression is dramatically reduced in liver cancer. This maneuver increased their sensitivity to sorafenib, which was taken up through OCT1. Treatment with sorafenib markedly inhibited tumor growth in a xenograft mouse model but only if the tumor was formed by cells expressing OCT1. To increase the specificity of gene therapy towards tumor cells, the OCT1 ORF was placed under the transcriptional control of *BIRC5* promoter (*BIRC5pr*), whose activity is markedly high in gastrointestinal tumors. Adenoviruses carrying the *BIRC5pr-SLC22A1* (Ad-OCT1) construct were used to treat nude mice bearing intrahepatic tumor xenografts generated from liver cancer cells. This gene therapy resulted in a selective over-expression of OCT1 in the plasma membrane of the tumor cells, whereas no expression was detected in the adjacent peritumoral tissue. Co-administration of Ad-OCT1 and sorafenib resulted in the enhanced antitumor effect of this drug. Since the effectiveness of chemotherapy is often reduced by up-regulation of ABC export pumps, we used this characteristic to overcome tumor chemoresistance. We investigated the usefulness of inducing in tumor cells the expression of OATP1B1 (*SLCO1B1*), a drug carrier with a broad spectrum of substrates whose expression is also markedly decreased in liver tumors, under the control of the MRP2 promoter (*ABCC2pr*). LS174T/R colon cancer cells are highly chemoresistant, due in part to MRP2 overexpression. When these cells were transduced with *ABCC2pr-SLCO1B1,* enhanced OATP1B1 expression was observed, and this was accompanied by higher sensitivity to OATP1B1 substrates, such as paclitaxel and Bamet-UD2, a cisplatin bile-acid derivative synthesized by our group. In nude mice, xenografted tumors formed by LS174T/R cells transduced with *ABCC2pr-SLCO1B1* were markedly sensitized to Bamet-UD2. **Conclusion:** The induced expression of anticancer drug uptake transporters constitutes a promising approach to overcome the low response of liver and gastrointestinal tumors to chemotherapy due to reduced drug uptake and/or enhanced drug export.

$mPEG_{5kDa}$-cholane/Glabrescione B delivery system as promising tool for the treatment of Hh-dependent tumors

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Abnormal activation of Hedgehog (Hh) signaling is responsible for several tumors such as medulloblastoma (MB) [1]. Hh inhibitors acting on GLI1, the final effector of Hh signaling, represent a valuable opportunity to overcome the pitfalls of the existing therapies to treat Hh-driven cancers [2]. In a previous study we identified Glabrescione B (GlaB), a natural isoflavone that proved to inhibit Gli1/DNA interaction. [3] The physical availability of GlaB by isolation from plant is unfeasible due to important limitations, so the total synthesis of GlaB is proposed. [4] To overcome its poor water solubility, several formulation strategies will be investigated to encapsulate GlaB in polymeric micelles, to promote the delivery of the drug.[5] The most promising one, GlaB formulated with a self-assembling amphiphilic polymer forming micelles, called $mPEG_{5kDa}$-cholane, enhanced the solubility of the isoflavone. GlaB encapsulated in $mPEG_{5kDa}$-cholane micelles was tested both *in vitro* and *in vivo* Hh-dependent MB models, and the biodistribution in brain and cerebellum will be assessed by the High-Performance Liquid Chromatography (HPLC) combined with Mass Spectrometry (MS). Our findings reveal $mPEG_{5kDa}$-cholane/GlaB is a good candidate for preclinical practice in the treatment of Hh-dependent tumors.

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p722 ferrocifen loaded lipid nanocapsules improve survival of murine xenografted-melanoma via a potentiation of apoptosis and an activation of $CD8^+$ T lymphocytes

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Metastatic melanoma is a malignant tumor with a poor prognosis. Recent new therapeutics improved the survival of patients at a metastatic stage. However, the low response rate to immunotherapy, explained in part by resistance (MDR) to apoptosis, needs to develop new strategies. The ferrocifen family represents promising bioorganometallic molecules for melanoma treatment since they show potent anticancer properties. The aim of this study is (i) to evaluate the benefits of a strategy involving encapsulated p722 in lipid nanocapsules (LNC) in B16F10 melanoma mice models and (ii) to compare the beneficial effects with an existing therapy such as anti-CTLA4 mAb. Interestingly, LNC-p722 induces a significant decrease of melanoma cell viability. *In vivo* data shows a significant improvement in the survival rate and a slower tumor growth with p722-loaded LNC in comparison with anti-CTLA4 mAb. Western blots confirm that LNC-p722 potentiates intrinsic apoptotic pathway. Treatment with LNC-p722 significantly activates $CD8^+$ T lymphocytes compared to treatment with anti-CTLA4 mAb. This study uncovers a new therapeutic strategy with encapsulated p722 to prevent B16F10 melanoma growth and to improve survival of treated mice.

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Exploring the efflux and modulation mechanisms of Human ABCG2 through Molecular Dynamics Simulations.

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ABCG2 is an ATP-binding cassette (ABC) protein transporter closely associated with the development of multidrug-resistance (MDR) when overexpressed in cancer cells. The efficient modulation of the ABCG2 function has been pointed as a powerful therapeutic strategy to overcome MDR by improving the pharmacokinetics and efficacy of chemotherapeutic agents. However, the development of clinically useful modulators of ABCG2 has been hampered by the lack of knowledge about the structural and functional understanding of this efflux pump [1]. The publication of the first Cryo-EM structure of human ABCG2 (PDB ID: 5NJ3) in 2017 [3] brought new information and demanded new independent assays to get new insights on the ABCG2-efflux mechanism.
In this communication, we will present our contributions in clarifying key points of the mechanism of ABCG2-substrate recognition and ATP-driven transport as well as on the structural basis of small-molecule inhibition of ABCG2 efflux. A refined model of full-length ABCG2 was built based on the incomplete cryo-EM structure and our previous homology model [4] and refined through molecular dynamics (MD) simulations at physiological conditions. To understand how ABCG2 conformational changes lead to substrate efflux, the global motion patterns displayed by the apo ABCG2, and ABCG2 in the presence of specific subtracts or inhibitors during 500 ns MD simulations were comprehensively analyzed. Relevant differences were observed between the Apo and the substrate-bound structures, suggesting that substrate binding can induce specific conformational changes that could be part of the mechanism used by ABCG2 to change from inward-open to outward-open conformation. Distinct interactions and binding energies were also observed when comparing protein-inhibitor and protein-substrate simulations. The analysis of these interactions gives valuable information to identify a possible explanation for the mechanism of ABCG2.

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Synthesis of Novel Artemisinin-Benzothiophene Hybrid Molecules

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Design and synthesis of novel organic molecules or isolation of natural products from plants have been gained big importance for the treatment of cancer and other diseases.[1] Artemisinin and its semi-synthetic derivatives are a group of drugs that possess the most rapid action of all current drugs against malaria.[2] Last decades, there have been many research including biological properties of artemisinin and their derivatives. However, there are a few studies for the design and synthesis of novel artemisinin derivatives. Artemisinin structure has been displayed anti-cancer activity for different kind of cancer cells.[3] Benzothiophenes are well known biologically important organic molecules.[4] They have been used as anti-bacterial, anti-parasitic, anti-cancer, anti-inflammatory and anti-tumor agents. In the present study, we design and synthesis novel artemisinin-benzothiophene hybrid molecules as potential candidates for the treatment of cancer.

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Harmine and Piperlongumine revert TRIB2-mediated drug resistance

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Main text of the abstract: Therapy resistance is responsible for most relapses in patients with cancer and the major challenge to improving the clinical outcome. The pseudokinase Tribbles homologue 2 (TRIB2) has been characterized as an important driver of resistance to several anti-cancer drugs including the dual PI3K/mTOR inhibitor dactolisib (BEZ235) [1]. TRIB2 promotes AKT activity leading to the inactivation of FOXO transcription factors which are known to mediate the cell response to antitumor drugs [2]. To characterize the downstream events of TRIB2 activity, we analysed the gene expression profiles of isogenic cell lines with different TRIB2 status by RNA sequencing. Using a connectivity map-based computational approach, we identified drug-induced gene-expression profiles that invert the TRIB2-associated expression profile. In particular, the natural alkaloids harmine and piperlongumine not only produced inverse gene expression profiles but also synergistically increased BEZ235-induced cell toxicity. Importantly, both agents promote FOXO nuclear translocation without interfering with the nuclear export machinery and induce the transcription of FOXO target genes. Our results highlight the great potential of this approach for drug repurposing and suggest that harmine and piperlongumine or similar compounds might be useful in the clinic to overcome TRIB2-mediated therapy resistance in cancer patients.

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NO release regulated by doxorubicin as the green light-harvesting antenna

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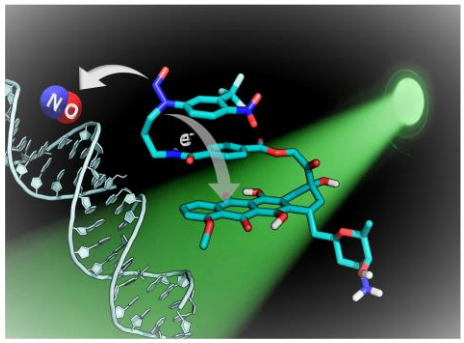
The development of multidrug resistance (MDR) to chemotherapy remains a major challenge in treating cancer. Doxorubicin (DOX) is a chemotherapeutic agent that is widely used in treating a variety of tumors, including solid tumors, soft tissues sarcomas, and many malignancies of the blood [1]. The clinical use of this important antibiotic is hampered by the development of resistance and by its cardiotoxicity. One of the most studied mechanisms of resistance is the increased efflux of antineoplastic drugs from tumor cells associated to the overexpression of ATP binding cassette (ABC) transporters [2].
Nitric oxide represents a multitarget cytotoxic agent, generally not affected by MDR issues, because of its ability of inactivate efflux pumps. However, concentration, location and dose of NO strictly influence its biological effects [3]. The photogeneration of NO achieved using compounds able to release NO under the action of the visible light, namely NO photodonors (NOPDs), has received great attention as potential new anticancer therapy. NOPDs allow the action of NO to be confined to the irradiated area with high spatial precision, and its dosage to be controlled with accuracy by tuning the duration and intensity of the irradiation [4].
We report for the first time a NO photodonor operating using DOX as the light-harvesting antenna. This permits NO uncaging from an N-nitroso appendage upon selective excitation of DOX with highly biocompatible green light, without precluding its typical red emission. This NOPD effectively binds DNA and photodelivers NO nearby, representing an intriguing candidate for potential multimodal therapeutic applications based on the combination of DOX and NO.



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Activatable Photodynamic Therapy Agents for Use in Multi-Drug Resistant Tumors

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Activatable photodynamic therapy agents allow selective oxidative destruction of tumor either by remote control or by the markers of the disease [1,2]. Most of the multi-drug resistant tumors have elevated reduced glutathione which enables drug detoxification or efflux [3]. Role of esterase enzymes in passivation of the drug via chemical transformation is another common pathway adopted by resistant tumors [4]. A novel photodynamic agent responsive to both esterase and glutathione is investigated. These two multi-drug resistant tumor markers, together with light, is expected to activate the photodynamic agent by leading to conversion of a pyridinium based agent into a pyridine bearing agent. This conversion leads to a large shift in the extinction coefficient of the agent at the wavelength of interest, enabling activation. Molecular encryption requires application of each input, that unlocks the key, or in other words activates the agent, in a pre-determined order [5]. Photodynamic therapy agent developed by the project is a molecular keypad lock, a molecular logic gate which, up to date, has no defined biological application in literature. In the project, for the first time such molecular systems will be developed for the treatment of multi-drug resistant tumors. Stimuli responsiveness of the photodynamic agent will be discussed and progress in the project will be presented.

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Selenocompounds: a novel approach to fight cancer resistance

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Selenium is a trace element that plays a crucial role in cell biology and human health due to its antioxidant and prooxidant properties [1]. In line to this, our group has synthesized selenoesters and selenoanhydrides as novel cytotoxic and antiproliferative agents in cancer cell lines. The most active compounds were able to scavenge free radicals, exerted a potent antiproliferative activity at nanomolar concentrations against a prostate cancer cell line, as well as a cytotoxic activity in several cancer cell lines at a concentration below 5 μM [2].
Recent studies confirmed this activity in additional cancer cell lines, finding that these derivatives are strong apoptotic inducers. In addition, these selenoesters and selenoanhydride could reverse multidrug resistance (MDR) in cancer by inhibiting the MDR transporter P-glycoprotein (ABCB1), being stronger inhibitors than the reference verapamil [3]. An ADMETox evaluation of the compounds was performed, revealing that none of the compounds was mutagenic, and relevant physico-chemical properties (solubility, permeability, stability) were also investigated with positive results.
These promising results led us to evaluate the selenoesters in combination with a wide selection of chemotherapy agents; finding that they showed a potent synergistic interaction with vincristine, doxorubicin, methotrexate, topotecan, cyclophosphamide and 5-fluorouracil [4]. Additionally, a similar synergistic enhancement of the anticancer activity of phenothiazines was observed [5].
Concluding, selenoesters and selenoanhydrides have shown in different studies and cell lines a potent anticancer and antioxidant activity, a strong inhibition of P-glycoprotein and a significant synergistic enhancement of the activity of known chemotherapeutic drugs. All these facts show that the application of these selenocompounds could be a promising novel approach to fight resistant cancers.

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Synergistic inhibition of the Hedgehog pathway by newly designed Smo and Gli antagonists bearing the isoflavone scaffold.

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Hedgehog (Hh) signaling pathway inhibition has emerged in recent years as a druggable target for anticancer therapy. [1] We previously identified Glabrescione B (GlaB), an isoflavone naturally found in the seeds of *Derris glabrescens* (*Leguminosae*), as a novel small molecule that proved to interfere with Gli1/DNA interaction. [2] We provided the total synthesis of GlaB based on the deoxybenzoin route, allowing the structure activity relationship elucidation through the preparation of a small-size focused library of isoflavones. Target preference has been achieved by the introduction of specific bulky substitutions at *meta* position (targeting Gli1) or *para* position (targeting Smo) of the isoflavone's ring B that is able to block the pathway respectively at the downstream effector Gli1 or the upstream receptor Smo. [3] Interestingly, the combined administration of two different isoflavones behaving as Smo and Gli1 antagonists in primary medulloblastoma cells has shown synergistic Hh inhibition at doses that are around 20-*fold* lower than single administration, thus leading the way to further and innovative combination therapy for the treatment of Hh-dipendent tumors. This approach seems to effectively overcome the drug resistance, particularly at the level of Smo. Thus, we combined the pharmacophores targeting Smo and Gli1 into a single and individual isoflavone, compound 22, which inhibits the Hh pathway at both upstream and downstream level and suppresses medulloblastoma growth *in vitro* and *in vivo* through antagonism of Smo and Gli1 providing a novel mechanism of action in Hh inhibition. Our study encourages the use of a multitargeting approach for the treatment of Hh-driven tumors and provides significant support in oncology research for the development of new clinically relevant Hh inhibitors.

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Generation of a library of indole alkaloid derivatives as ABCB1 inhibitors in resistant cancer cells

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Multidrug resistance (MDR) remains the main hindrance for a successful chemotherapy in cancer treatment. One of the most important factors behind MDR is the overexpression of ATP-binding cassette (ABC) transporters, by pumping out of the cells chemotherapeutic agents. P-glycoprotein (P-gp/ABCB1) is one main ABC transporters associated with MDR.
Aiming at finding effective MDR reversers, in the present work two major indole alkaloids, from the African medicinal plant *Tabernaemontana elegans* plant, were derivatized and afterwards tested as P-gp inhibitors. Twenty-six new derivatives were obtained by alkylation of the indole nitrogen, and thirty by condensation reactions at the carbonyl group. Their MDR reversal activity was evaluated on multidrug resistant human colon adenocarcinoma and human *ABCB1*-gene transfected L5178Y mouse lymphoma cells, overexpressing ABCB1. A noteworthy increase of activity was found for most of the derivatives, displaying outstanding ABCB1 inhibitory results those containing *N*-phenethyl moieties, for the alkylated series [1], and naphthyl moieties among azine derivatives. Moreover, regarding *in vitro* combination chemotherapy assays, most of the compounds displayed strong synergistic interactions with the antineoplastic drug doxorubicin, highlighting their potential as MDR reversers. Addicional insights for an efficient drug-receptor interaction were evaluated using 3D-QSAR models.

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Simplified tetraethylene oxide-mediated synthesis of gold nanoparticles and their internalization by cancer and neuronal cells

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For biological applications, gold nanoparticles synthesis requires non-toxic and biocompatible reagents, and excludes current techniques with toxic reagents like CTAB. Inspired by nanoparticle synthesis using polyethyleneoxide (PEO) in alkaline conditions[1,2] or natural polysaccharide[3,4] we developed a simplified and safe gold nanoparticles synthesis in aqueous tetraethylene oxide (PEO_4). This synthesis was performed at 80°C and particles were obtained within 25 minutes. Moreover we observed that alkaline conditions are not necessary. Spherical and homogenous Nanoparticles were obtained with a mean diameter of 13 nm (Figure 1) and their absorbance was maximal at 538 nm. Based on zeta potential and XPS analysis, we proposed an updated mechanism of nanoparticles formation correlated with current bibliographic knowledge.

As nanoparticles are tools for cellular imaging and therapeutic delivery, we investigated the uptake of such nanoparticles in two different adherent cell lines: A549 non-small cell lung cancer cell line and PC-12 cell line derived from a transplantable rat pheochromocytoma and induced to differentiate into dopaminergic neurons. Indeed, as observed by electron microscopy these particles were able to enter these cells and localized in the cytoplasm.

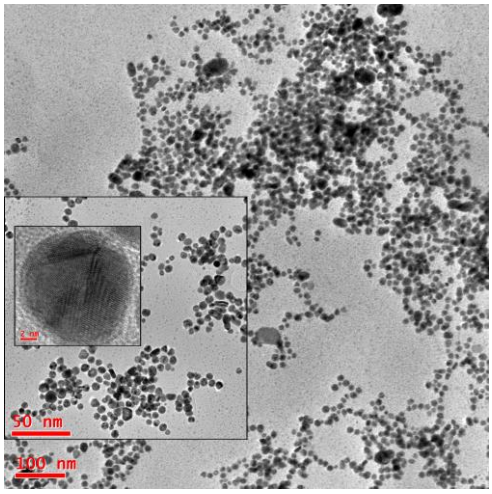

Figure 1. Transmission electron microscopy of gold nanoparticles in solution

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New 1,1'-Biphenyl-4-sulfonamides as Potent and Selective Human Carbonic Anhydrase inhibitors.

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Carbonic anhydrases (CAs) are an attractive and versatile target for the development of new agents active in different diseases. The different isoforms of this metalloenzyme are involved in key physiological processes, so that there is growing interest in the design of new CA inhibitors. We have developed a first series 1,1'-biphenylsulfonamides as human CA (hCA) inhibitors [1], evaluating their activity in hCA isoforms I, II, IX and XIV, using acetazolamide (AAZ) as reference compound. These compounds are characterized by a sulfonamide group, a substituent which, in its anionic form, can coordinate the zinc atom in the catalytic site. Most of the derivatives inhibited all the isoform, with K_i values in the nanomolar range of concentration, outperforming AAZ. In particular, the major activity is on the XIV isoform, involved in epilepsy and retinopathies. X-ray crystallography and molecular modelling studies on the adduct of these derivatives with the different hCAs provided insights into the molecular determinants responsible for its high affinity toward the target enzyme. Pursuing our studies on hCA inhibitors, we designed new 4'-substituted 1,1'-biphenyl-4-sulfonamides [2], moving the amino or carboxyphenyl unit from 4" to 3" or 2" position. This shift results in a substantial change in the inhibitory activity: it moves selectivity towards hCAII with K_i values in the subnanonmolar range, which is involved in the onset of glaucoma, and it causes a drop of activity against hCAIX. The reported new compounds have potential as novel therapeutic agents. In fact, a small change in the structure leads to a selectivity towards different isoforms, each one involved in specific pathologies such as glaucoma, epilepsy, cerebral edema and some types of tumors.

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Stability, cellular interactions and post production modification of murine mesenchymal stem cells (mMSC) derived Extracellular Vesicles

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In regards to their key role in intercellular communication, extracellular vesicles (EVs) have a strong potential as bio-inspired drug-delivery systems. Yet, EVs still failed to convince users as a pertinent drug delivery system (DDS) mainly due to handling issues (low production and drug loading yields, heterogeneity and instability). With the aim of circumventing some of these well-known issues, we specifically focused on switching the biological vision of these entities to a more physico-chemical one, and to consider and fine-tune EVs as synthetic vectors. To allow a rational use, we first performed a full physico-chemical (size, concentration, surface charge, cryoTEM), biochemical (western blot, proteomics, lipidomics, transcriptomics) and biological (cell internalisation) characterisation of murine mesenchymal stem cell (mMSC)-derived EVs. A stability study based on evaluating the colloidal behaviour of obtained vesicles was performed in order to identify optimal storage conditions (freezing, freeze-drying). We evidenced the interest of using EVs instead of liposomes, in regards to target cell internalisation efficiency and identified a different endocytic route between EV and liposomes. Effect of physical methods scarcely investigated with EVs was evaluated (extrusion through 50 nm membranes, freeze-drying, sonication) on EV size, concentration, structure and cell internalisation properties [1].
Based on these results, we are currently evaluating sEV-mMSC for antibody fragments (single-chain variable fragment, scFv) transfer in cell cytoplasm, as no reference synthetic vectors were reported till now, especially for *in vivo* use. Our preliminary results confirm the interest of associating scFv with sEV-mMSC for intracellular delivery of a functional, biologically active protein.

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Isoquinolinequinone *N*-oxides as anticancer agents effective against drug resistant cell lines.

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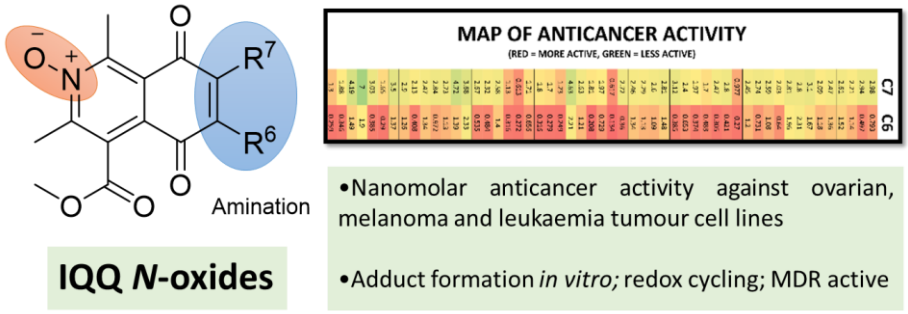
The isoquinolinequinone (or isoquinoline-5,8-dione) pharmacophore is a privileged framework in known cytotoxic natural product families, caulibugulones and mansouramycins with notable anticancer properties. Exploiting both families as seeds for drug discovery, we report for the first time on the structured development of an isoquinolinequinone *N*-oxide anticancer framework which exhibits growth inhibition of cancer cells in the nM range across melanoma, ovarian and leukaemia cancer cell lines.[1]



A new lead compound (**16**, R^6 = benzyl, R^7 = H) exhibits nM growth inhibition (GI50) values against 31/57 human tumour cell lines screened as part of the NCI60 panel and shows remarkable activity against doxorubicin resistant tumour cell lines. An electrochemical study highlights a correlation between electropositivity of the isoquinolinequinone *N*-oxide framework and cytotoxicity. Preliminary studies were conducted to identify adduct binding to sulfur based biological nucleophiles glutathione and cysteine observed *in vitro* pointing to a potential mechanism of action. This new framework possesses significant anticancer potential and future efforts will explore the mechanism by which this effect arises.

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Combretazets: Enantiomeric β-Lactams for the Treatment of Breast Cancer

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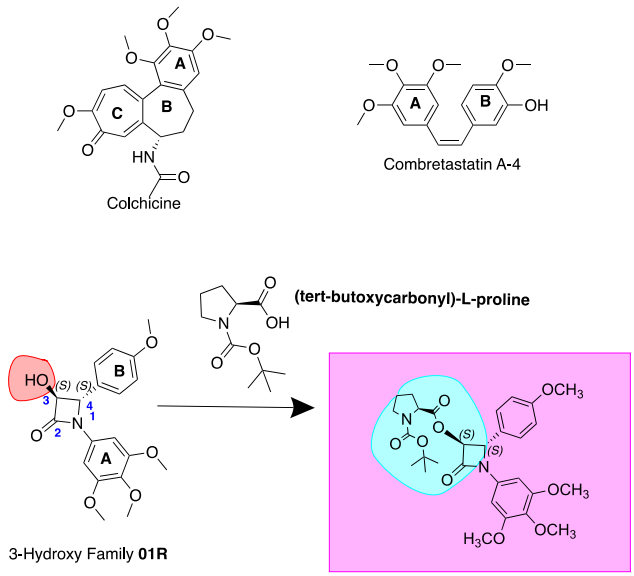
The combretastatins (CAs) are diaryl stilbenoid natural products isolated from the bark of the South African willow tree *Combretum caffrum*. CA-4 is a potent anticancer agent, which inhibits cancer cell proliferation and microtubule polymerisation by binding at the colchicine-binding site of tubulin [1]. Only the *cis* configuration of CA-4 possesses anticancer bioactivity. It readily isomerizes *in vivo* during metabolism and upon storage into the more thermodynamically stable but significantly less active trans isomer [2]. We are developing the 'Combretazets' with the aim of overcoming this undesirable isomerization to target multi-drug resistant cancers. Substituting the ethylene bridge with a 1,4-diaryl-2-azetidinone ring (compound **01**) allows similar structural arrangement between CA-4's two aromatic rings and overcomes *cis/trans* isomerization. It is essential to distinguish the eutomer from the distomer. Here, we describe the synthesis, resolution, characterization and biochemical activity of the enantiomers of **01**.



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Synthesis and Characterization of Therapeutic Antibody-drug Conjugates against Multidrug Resistant Ovarian Cancer Therapy

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The main treatment methods available for ovarian cancer include surgical intervention and the use of taxane and platinum-based chemotherapeutic drugs. However, these limit effective treatment because they have side effects that seriously affect the patient's quality of life and create multiple drug resistance (MDR) [1]. Biological drugs started to come to the fore in cancer treatment. In recent years, Antibody Drug Conjugate (ADC) have started to be investigated in cancer treatment, which minimizes the side effects and MDR of drugs and combines the strong effect of cytotoxic drug on target specific therapeutic power of mAbs alone. The monoclonal antibodies contained in ADCs are specific for the target cancer antigen and cytotoxic drug used in ADC are activated after it is taken into cancer cells. Therefore, ADCs are more effective and safer than chemical drugs [2,3]. Despite the many studies, only a few ADCs have been approved by the FDA. This shows the difficulty in optimizing key parameters of ADC, such as selecting a strong cytotoxic drug, determining a suitable target, a stable linker and conjugation method of the linker to the mAb [4]. In this study, a monoclonal antibody (mAb), which has been highly expressed by ovarian cancer cells, will be linked to DNA-targeted cytotoxic drug using click chemistry. Both the use of the target antibody and the use of a hydrophilic linker aim to overcome the MDR of the ADC to be synthesized. In addition, due to the enzymatic conjugation method to be used in the project, the conjugation of the cytotoxic drug to the antibody in a homogeneous manner is also aimed. Until now, endoglycosidase and its mutant enzyme have been produced and optimization work is carried out to find the growth medium that can provide the highest enzyme. These will be used to hydrolyze the glycans on the mAb and to transfer the glycans we will produce to the same place again, respectively. The synthesized ADC will allow to effectively overcome the ovarian cancer, which is one of the most lethal diseases among women.

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