Co-culture of rat brain cells as a tool for studying cell-cell interactions

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Brain capillary endothelial cells (BCECs) form the blood-brain barrier (BBB) in response to interaction with other brain cells (astrocytes, pericytes and neurons). BCECs are characterized by tight junctions (TJ), maturation and stabilization of which require different proteins, such as occludin.
When co-cultured with astrocytes and neurons, BCECs were found to form a monolayer resembling the natural BBB: paracellular flux of dopamine and sucrose (i.e. compounds which are unable to cross the BBB *in vivo*) significantly decreased (1), while the transendothelial electrical resistance (TEER) increased. In these conditions, BCECs produced a larger amount of occludin and tended to localize it at the cell periphery, thus suggesting formation of TJs (1). Since we also discovered that oligodendroglioma cells shed extracellular membrane vesicles (MVs; 2), we investigated whether also neurons and/or astrocytes can release MVs and whether these vesicles contained angiogenic factors. The results of these analyses demonstrated that all kinds of brain cells actually shed MVs containing FGF-2 and VEGF (3-4). On the basis of these findings, we investigated the possibility that the BBB model could be used to study the molecular events that result in BBB damage, in some pathological conditions, such as, for example, multiple sclerosis (5). We are now investigating whether cultured astrocytes shed vesicles containing aquaporin 4 (AQP4), a protein which has been involved in brain edema. Our results suggest that production of AQP4 increases in stressed astrocytes.

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Pharmacophore modelling as useful tool in the lead compounds identification and optimization

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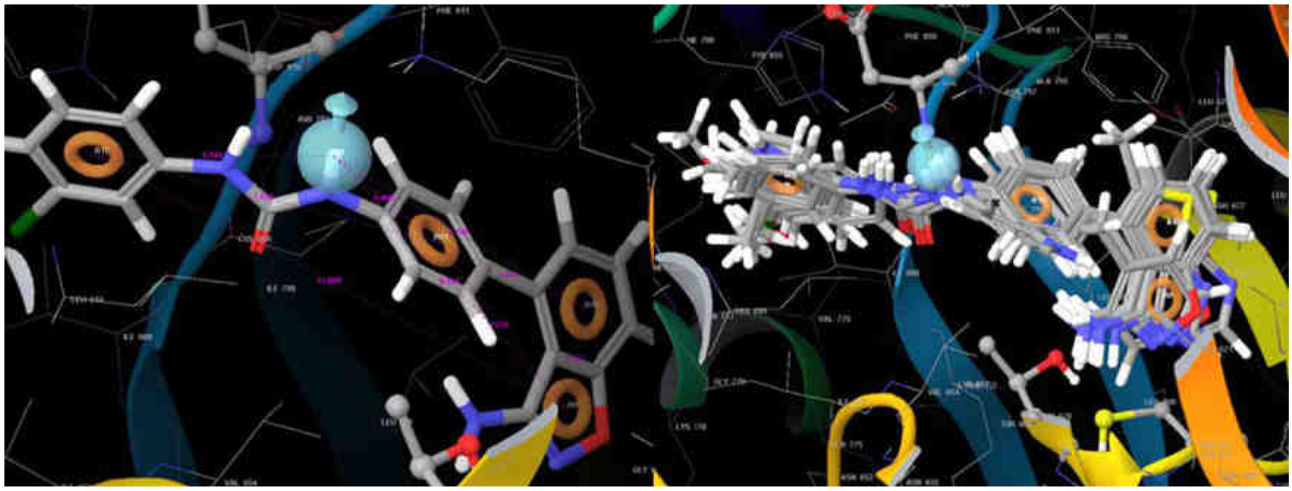
The goal of computer-aided molecular design methods in modern medicinal chemistry is to reduce the overall cost and time associated to the discovery and development of a new drug by identifying the most promising candidates to focus the experimental efforts on. Very often, many drug discovery projects have reached already a well-advanced stage before detailed structural data on the protein target have become available. A possible consequence is that often, medicinal chemists develop novel compounds for a target using preliminary structure–activity information, together with the theoretical models of interactions. Only responses that are consistent with the working hypothesis contribute to an evolution of the used models. Within this framework, the pharmacophore approach has proven to be successful, allowing the perception and understanding of key interactions between a receptor and a ligand[1]. In recent years, our research group exploited this useful modeling tool with the aim to identify new chemical entities and/or optimizing known lead compounds to obtain more active drugs in the field of antitumor, antiviral, and antibacterial drugs. In this communication, we present an overview of our recent works in which we used the pharmacophore modelling approach combined with induced fit docking, 3D-QSAR approach, and HTVS for the analysis of drug-receptor interactions and the discovery of new inhibitors of IKKβ, Bcl-xl, and c-kit tyrosine kinase, all targets involved into the initiation and the development of different types of cancer[2-5].



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Synthesis and evaluation of new LINEZOLID-like compounds[1]

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The alarming rates of emerging multidrug resistance, among Gram-positive bacterial pathogens, represents one of the major challenges for researchers. Linezolid (Zyvox) (see figure) is the lead compound of oxazolidinones, a new class of synthetic compounds with antibacterial activity.[2] Linezolid was approved in 2000 by FDA for the treatment of community-acquired and nosocomial pneumonia, complicated and uncomplicated skin and soft-tissue infections, and infections caused by MRSA and VRE. Nevertheless, recently, the wide and indiscriminate use of this drug promoted the development of bacterial resistance. In the present communication will be discussed the development of new Linezolid-like molecules by substitution of the oxadiazolinone heterocyclic moiety (A-ring) or the morpholine moiety (C-ring) with an 1,2,4-oxadiazole ring. These two series of 1,2,4-oxadiazoles, with different side-chain and various fluorine content, were synthesized and tested "in vitro" for activity against multidrug-resistant bacteria.

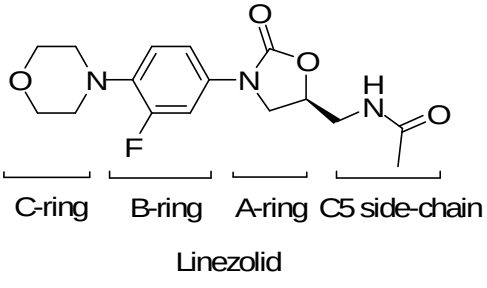

Figure 1. Structure and portions nomenclature of Linezolid

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Superparamagnetic Hydrophobic Polyaspartamide Nanoparticles For Anticancer Drug Delivery

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Superparamagnetic Fe_3O_4 nanoparticles (SPION) have been recently used experimentally for numerous in vivo application such as magnetic resonance imaging, hyperthermia, drug delivery, etc.[1-4]. In this study, a novel approach to prepare magnetic polymeric nanoparticles (MNPs) containing superparamagnetic domains and polymeric shell is reported.
PHEA-IB-poly(ButMA) were used as biocompatible coating copolymer to obtain MNPs with specific shape and size by emulsifying a clorophorm polymer solution in the simultaneous presence of SPION and the anticancer drug flutamide (FLU), in aqueous dispersion.
The results obtained from TEM (fig.1) and DLS analysis showed that the MNPs are spherical with average size of about 250 nm. The magnetic measurement studies revealed the superparamagnetic behavior of the MNPs and the saturation magnetization values confirmed the presence of superparamagnetic domains inside nanoparticles. Cytotoxicity profile of the MNPs on human prostate carcinoma cells (LNCaP) showed that the empty MNPs are nontoxic but the FLU loaded MNPs, compared to free drug, caused a significant reduction of LNCaP cells proliferation induced by dihydrotestosterone, and above all, at higher concentrations, it is able to induce cell death. In vivo biodistribution of drug loaded into MNPs in rats subjected to an external magnetic field is different in comparison with that obtained in the control group. FLU was concentrated most conspicuously in kidney, and less in the other organs (fig.2).
Obtained data shows that MNPs may be useful for in vivo applications in treatment of tumors.

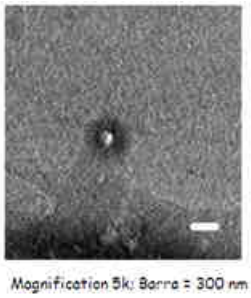

Figure 1

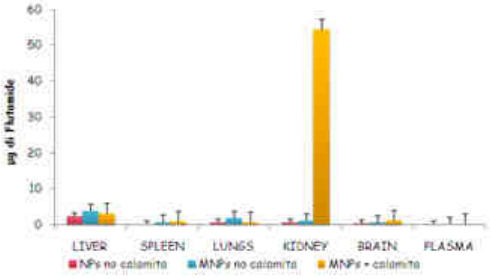

Figure 2

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Aminoacidic derivatives as novel CNS-targeted neurotherapeutics

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Drug delivery to the CNS is subject to the permeability limitations imposed by the BBB that regulates movements of actives in and out of the brain. During the drug discovery phase a key aspect could be the selection of the compounds properties crucial for brain penetration. Novel CNS-targeted neurotherapeutics should possess the optimal characteristics that allow passive diffusion through the BBB *via* the transcellular route, or have the structural features necessary to serve as a substrate for one of the endogenous transport systems of the BBB.
An attractive and rewarding chemistry-based strategy, employed to increase the CNS transport of poorly penetrating therapeutic agents, is the transient chemical modification. This approach could improve physicochemical, biopharmaceutical, pharmacokinetic and drug delivery properties of active agents that overcome barriers to a drug's usefulness.
With the aim of mask functional groups and modify the physicochemical properties relevant to bioavailability, in this work CNS-actives were covalently linked to different aminoacidic moieties. The chemical structure of the derivatives and Log D were determined. Chemical stability was evaluated in simulated biological fluids. Enzymatic stability was assessed *ex vivo*. The effects of derivatives were observed *in vitro* and their availability was measured in rat brain tissue. Finally, cellular toxicity was established.
Our data support that aminoacids could be considered as good masking agents for CNS-targeted drugs.

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Genome Wide Analysis Of Chromatin Poly-Adp-Ribosylation In Drosophila

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Poly-ADP-Ribosylation is a post-translational modification of proteins mediated by poly-ADP-ribose polymerases (PARPs). Using NAD+ as a substrate, PARPs catalyze the covalent attachment of ADP-ribose units to a wide variety of target proteins to generate long linear and branched poly-ADP-ribose (PAR) chains. PARPs are involved in the regulation of critical cellular functions, including transcriptional regulation. While it is widely demonstrated the accumulation of PAR at decondensed and transcriptionally active loci of highly inducible genes, very little is known about basal PARylation function in transcription in the absence of induced stimuli.

Unlike mammals, which have several PARP encoding genes, the model organism *D.melanogaster* has only one PARP gene, highly related to mammalian PARP-1, making flies a great model system to study PARP biology. In order to study the role of PARylation on chromatin in non-induced conditions we conducted a genome−wide analysis of PAR distribution on Drosophila chromosomes.

Our analisys revealed that PAR has ~5600 high affinity chromatin binding sites, both in genic and intergenic regions. In order to check if PAR binds a particular group of genes involved in specific biological processes, we conducted an analysis of PAR-bound genes based on their Gene Ontology (GO) classification. This GO analysis revealed that PAR binds genes encoding for factors involved

in a variety of essential biological functions and that PAR-bound genes were over represented in genes encoding factors involved in “Signal Transduction”.

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Multilayered Supported Ionic Liquid As Catalysts for High-Throughput Study Of Co2 Cycloaddition Reaction

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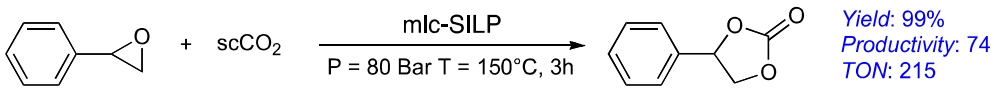
The development of green processes based on chemical fixation of carbon dioxide has drawn a great deal of attention in industrial chemistry from the perspective of the protection of environment and resource utilization.[1] One of the few commercial routes using CO_2 as a raw material is the insertion of CO_2 into epoxides to produce cyclic carbonates. In terms of green chemistry and atom economy, the process is promising because CO_2 can be incorporated into epoxides with no formation of side products.

Recently several supported ionic liquid materials, mainly based on imidazolium cations, have been employed as catalysts for the cycloaddition of CO_2 to various epoxides.[2]

Herein the synthesis of a new class of multilayered covalently supported ionic liquid phase (mlc-SILP) is presented.[3]

These materials were prepared using a new approach based on the grafting of different bis-vinylimidazolium salts on amorphous silica. The materials, which contain a highly cross-linked polymeric network, were characterized and tested as catalysts in the reaction of supercritical carbon dioxide with various epoxides to produce cyclic carbonates.



The material prepared by supporting an iodide bis-imidazolium salt was identified as the most active catalyst for the synthesis of cyclic carbonates and displayed improved productivity compared to known supported ionic liquid catalyst. The catalyst can be successfully reused in consecutive catalytic runs. The rapid and parallel screening of the catalysts was efficiently carried out by means of high-throughput (HT) experimentation under supercritical carbon dioxide conditions.

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Development of cattle babesiosis diagnostic tools and research of Babesia bigemina vaccine candidates

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Cattle babesiosis is a tick-borne disease transmitted by haemoparasites belonging to the phylum of Apicomplexa, such as *Babesia bovis* and *B.bigemina*. The pathology affects cattle mainly in tropical and subtropical areas, but also in Europe, reducing meat and milk production. This study was addressed to the molecular characterization of Italian *B.bigemina* strains isolated from infected animals, with attention to the genes codifying for surface antigens, putative candidates for vaccine and diagnostic tools development.
B.bigemina Apical Membrane Antigen-1 (AMA-1) is an apical protein involved in the host red blood cells invasion. The AMA-1 sequences from many Apicomplexa were compared and useful information on this gene from Italian strains of *B.bigemina* is provided. Data about the immunogenicity of the purified protein obtained by synthesis in *E.coli* are reported.
We further report the sequences of the rap-1b and gp45 genes from Italian strains of *B.bigemina*. The rhoptry associated protein-1 (rap-1) is a vaccine candidate against bovine babesiosis. gp45, a surface antigen bound to the membrane by a Glycosylphosphatidylinositol (GPI-anchor), has an important role in the bovine erythrocyte invasion by the parasite. Sequence analyses revealed that *B.bigemina* Gp45 is highly polymorphic. In conclusion, the research provides useful information about the parasite and contribute to the improvement of disease control, in concert with the study of potential vaccine and diagnostic tools.
The research was funded by Italian Ministry of Health projects IZSSI 02/07 and IZSSI 11/10.

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Synthesis of the new ring system pyrrolocyclohepta[1,2-*b*] pyridin-9(1H)-one as photochemotherapeutic agents.

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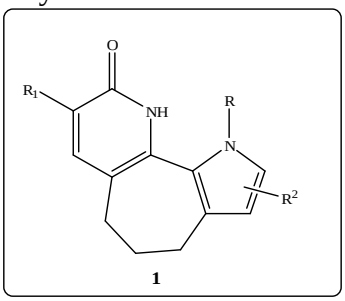
Psoralen and Angelicin, respectively linear and angular furocoumarins, are photoactivable drugs which, upon UVA light irradiation, intercalate into DNA and photobind to it.[1] With the aim of studying new photoreactive agents with enhanced antiproliferative activity and decreased side effects we reported the synthesis of the new ring systems pyrrolo[2,3-*h*]quinolinone, pyrrolo[3,4-*h*]quinolinone and pyrrolo[3,2-*h*]quinolinone which differ for the condensation of the pyrrole ring to the quinolinone moiety.[2-5] Although the three classes of compounds revealed a remarkable antiproliferative activity reaching the submicromolar range, the pyrrolo[3,2-*h*]quinolinones were the

most promising. In fact, studies on the mechanism of action demonstrated that they induce apoptotic cell death mediated by mitochondria without any DNA damage which is the main origin of the side effects of the PUVA therapy. On continuing our studies on photochemotherapeutic drugs, we focused our attention on the modification of the lead structure of pyrrolo[3,2-*h*]quinolinone replacing the central six membered ring with a seven membered one generating the new ring system *tetrahydropyrrolo[3',2':6,7]cyclohepta[1,2-b]pyridin-9(1H)-one* **1**. Our synthetic approach consisted on the annelation of the pyridone ring on the cycloheptapyrrole moiety using, (dimethylamino)methylene-4,5,6,7-tetrahydrocyclohepta[b]pyrrol-8(1H)-one as building blocks. All derivatives of the new ring system will be subjected to photobiological studies in order to investigate their antiproliferative activity both in the dark and under UVA light.



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Effects Of P14^{arf} Ectopic Expression After Mad2 Depletion In Hct116 Cells

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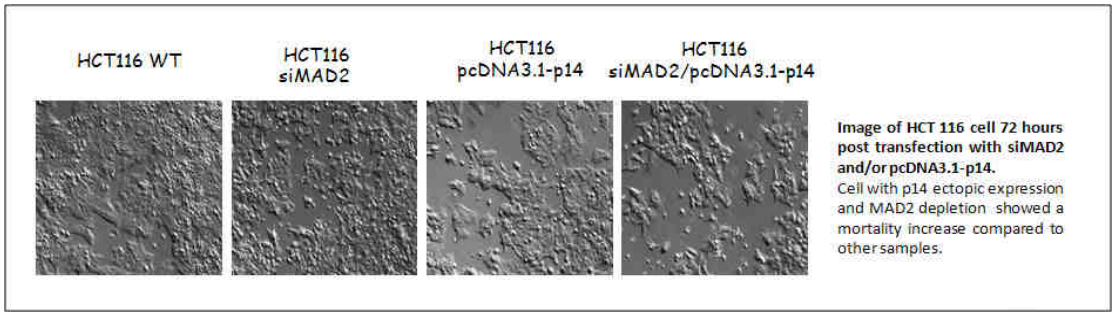
Reduced expression of MAD2, an important component of the Spindle Assembly Checkpoint, induces chromosome instability and aneuploidy, a hallmark of malignant cells (1).
p14^{ARF} is a tumor suppressor encoded by INK4a/ARF locus frequently altered in human cancers. P14^{ARF} is up-regulated by oncogenic stimuli and it increases p53 stability through interaction with MDM2 (E3-ubiquitin ligase) (2).
Previous data suggest that p14^{ARF} could function to block the development of aneuploid cells (3). To this aim, p14^{ARF} was ectopically expressed in HCT116 cells, a stable near diploid cell line, after MAD2 depletion that induced aneuploidy. We observed increased cell death associated to aneuploid cell numbers reduction, accompanied by the increase of p53 and p21 protein levels. In addition by immunofluorescence analysis, we detected a decrease of spindle alterations and mitotic abnormalities, in comparison to MAD2 post transcriptional depleted cells. Altogether these results suggest that p14^{ARF} could prevent proliferation of aneuploid HCT116 cells.



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Novel antimicrobial peptides (AMPs) against biofilms
The treasure of sea-cucumber

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With the aim to face the threat of pathogen biofilms intrinsically resistant to conventional antibiotics, we focused on coelomocytes, the immune mediators in echinoderms, as source of novel antimicrobial peptides (AMPs). In this study the antimicrobial and antibiofilm activity of the sea-cucumber *Holothuria tubulosa* coelomocytes were evaluated. The peptide fraction <5kDa from coelomocytes cytosol (5-HCC) was tested against a group of Gram positive and Gram negative reference strains. The 5-HCC resulted active against all tested strains at concentrations ranging from 11.2 to 44.8 μg/mL and showed a MIC of 10 μg/mL against staphylococcal isolates of animal origin. The ability to prevent biofilm formation of staphylococcal isolates was also observed at 5 μg/mL.
In order to detect the presence of antimicrobial peptides and determine their sequences, the 5-HCC was characterized by capillary RP-HPLC/nESI-MSMS. The MS/MS data were used to investigate protein database of *Echinodermata* and to perform *De Novo peptide sequencing*.
By this approach, nine principal peptides whose molecular masses ranging from 805.5 to 2215.7 Da, were identified. Seven of nine peptides, for their chemical-physical characteristics and their similarity with naturally occurring cationic peptides produced by a variety of organisms, have good chance to be novel AMPs.
Some of these novel AMPs could have a high potential to act on slow-growing or even non-growing bacteria that exhibit a reduced susceptibility to conventional antibiotics and represent a reservoir for recurrent biofilm associated infections [1].

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Trans-epithelial transport of the betalain pigments indicaxanthin and betanin across Caco-2 cell monolayers and influence of food matrix.

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Trans-epithelial transport of the phytochemicals indicaxanthin and betanin in Caco-2 cell monolayers seeded on Transwell^{R} inserts was measured in apical to basolateral (AP-BL) and basolateral to apical (BL-AP) direction, under an inwardly directed pH gradient (pH 6.0/7.4, AP/BL) mimicking luminal and serosal sides of human intestinal epithelium. AP-to-BL apparent permeability coefficients (P_{app}) were $(4.4\pm0.4) \times 10^{-6}$ and $(3.2\pm0.3) \times 10^{-6}$ cm s^{-1} for indicaxanthin and betanin, respectively. Transport of indicaxanthin was non-polarized, linear as a function of time and unaffected by inhibitors of membrane transporters. Betanin exhibited significantly different bidirectional P_{app} values and non-linear efflux kinetics, which appeared related to a multidrug resistance associated protein 2 (MRP2)-mediated apical efflux. Neither indicaxanthin nor betanin underwent metabolic transformation. Permeation of both betalains increased remarkably after opening tight junctions by EDTA treatment of the cell monolayer. Betalainic food matrix did not affect trans-epithelial transfer of indicaxanthin, but reduced the absorption rate of betanin, red beet more than cactus pear.

Our data indicate that dietary indicaxanthin and betanin can substantially be absorbed through paracellular junctions of intestinal epithelial cells. Additional trans-membrane permeation can be considered for betanin, whose absorption is limited by a MRP2-mediated efflux and is negatively affected by its food matrix. Present findings are consistent with the quite higher bioavailability of indicaxanthin over betanin established in humans (1).

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[1,2]Oxazolo[5,4-*e*]isoindoles, new potent antitumor agents.

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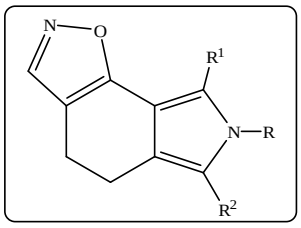
Antimitotic agents, one of the major classes of cytotoxic drugs for the treatment of cancer, have gained in recent years great attention. They cause the mitotic arrest in eukaryotic cells by interfering with the normal microtubule polymerization/depolymerization process. Taxol, vincristine, colchicine and combretastatins, are leading examples of this class of compounds.[1] Combretastatin A4 phosfate (CA4p),[2] a water soluble prodrug of CA4, is currently in phase III clinical trials for the treatment of cancer and early results are very promising. To date many efforts have been done to

discover new combretastatin analogues replacing the alkenyl bridge of the natural CA4. Among these a number of analogues of CA4 were prepared using five-membered heterocycles such as imidazole, pyrazole, oxazole and [1,2]oxazole as linkers.[3,4] Considering our interest on heterocycles containing the pyrrole moiety with antitumor activity, we planned the synthesis of the new ring system *[1,2]oxazole[5,4-e]isoindole*. A first series of thirteen derivatives was synthetized and evaluated for antitumor activity at the NCI of Bethesda, and two of them (mean pGI_{50} 5.80 and 6.68) were selected for Hollow Fiber Assay. Considering the good results, new compounds were prepared properly modificated from their original structure. Fourteen out of twenty four compounds were evaluated against the full panel of 60 tumor cell lines at the NCI and four of them showed in some cases higher activity than the lead compounds reaching the nanomolar level (mean pGI_{50} 6.61-7.19). Results will be discussed.



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More resistant cells in stem cell population.

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Dipartimento STEMBIO

Most of the stem cells used in damaged tissue repair, when injected or engrafted, die as they are subjected to cellular stress caused by ROS release in the inflamed area. Some of them survive and the question arises whether the stem cell population is composed of cells arranged differently to withstand oxidative stress. To find more resistant cells inside a stem cell population we treated them with the highest H_2O_2 dose (400μM 24h) that it might be found in inflamed tissues. We applied this severe treatment to three different cell types: mesoangioblast isolated from both embryonic and adult mice and mouse fibroblasts, fully differentiated cells. Our results show that these treatments are better tolerated by differentiated cells, such as fibroblasts or adult stem cells than by embryonic ones, which seem to be less resistant to a severe treatment. In embryonic mesoangioblasts we analyzed: cell cycle phases, proliferation activity/doubling time, cell death during the treatment and during the recovery in the following 8 days. Our results showed total growth arrest and 96% of cell death. We then isolated cell clones after 8 days of recovery from cells which have restart

proliferation and have re-established cell cycle phase distribution. These cell clones are more resistant to a second H_2O_2 treatment than wild type cells and they retain all stemness features. A treatment with H_2O_2 in embryonic stem cells induces an autophagic process possibly to remove damaged parts of cells. In a second step starts the apoptotic process of cell death as the damaged parts were not deleted.

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The study of gelling ability of some germinal imidazolium salts

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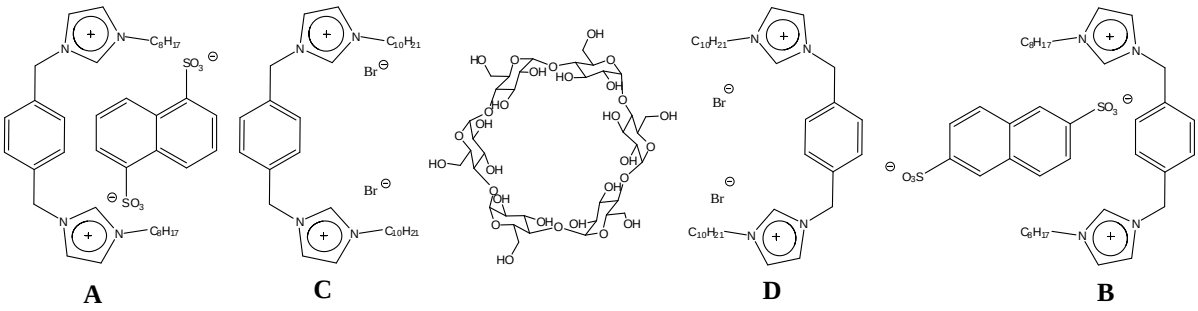
Self-organization is a process in which systems capable of spontaneously generating well-defined, organized and functional supramolecular architectures from their components, behave as programmed systems.[1] In this research area, a growing interest derives from gelation processes, in particular those in which supramolecular gels are hierarchically built-up of fiber from the molecular into supramolecular level.[2]

In the framework of our interest, we synthesized some diimidazolium organic salts to study their properties as Ionic Liquids.[3] In particular, the 3,3'-di-*n*-octyl-1,1'-(1,4-phenylenedimethylene)diimidazolium 1,5-naphthalendisulfonate (**A**), the 3,3'-di-*n*-octyl-1,1'-(1,4-phenylenedimethylene)diimidazolium 2,6-naphthalendisulfonate (**B**), the 3,3'-di-*n*-decyl-1,1'-(1,4-phenylenedimethylene)diimidazolium bromide (**C**) and the 3,3'-di-*n*-dodecyl-1,1'-(1,4-phenylenedimethylene)diimidazolium bromide (**D**) were prepared.



A **C** **D** **B**

Unfortunately, they are salts with high melting point, higher than 200 °C. But surprisingly, they were able to behave as Low Molecular Weight Organogelators when dissolved in some alcohols with alkyl chain from C2 to C6 and as Hydrogelators, in presence of well defined amount of α-cyclodextrin.

We characterized the obtained gels determining their T*gel* and Δ*H* values and we analyzed their thixotropic behavior. The gelation process and gel properties were investigated by means of Resonance Light Scattering, UV-vis measurements and Scanning Electron Microscopy.

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The identification of irradiated Crustaceans and evaluation of the dose by thermoluminescence: Intercomparison between two methods for extracting minerals

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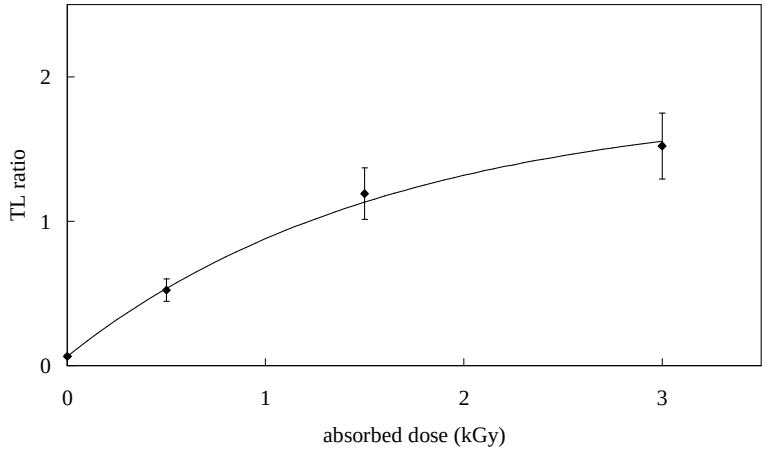
The thermoluminescence (TL) is one of the physical methods recommended by the European Committee for Standardization for the identification of irradiated food from which silicate minerals can be extracted. The efficacy of the method strongly depends on the quantity and purity of the extracted minerals, and therefore on the extraction procedure. In this work we applied the TL for the identification of crustacean *Nephrops norvegicus* irradiated at 0.5 – 1.5 – 3.0 kGy, comparing two different procedures for extracting minerals: by means of a density gradient or with acid hydrolysis. The identification of the irradiation treatment was always achieved with both procedures, without any false positive. An innovative dose reconstruction method was also set up and tested, based on the experimental determination of a calibration function (TL Ratio vs, dose). A little preference should be given to the acid hydrolysis extraction procedure, that requires less quantity of food sample and reagents, as well as less time to be carried out, and gives a satisfactory reconstruction of dose.



TL Ratio vs. dose for the minerals extracted with acid hydrolysis. The continuous line is the fitting function.

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Regulation of antibiotic biosynthesis in Actinomycetes

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Filamentous actinomycetes are the main producers of secondary metabolites endowed with potent biological activities, such as antibiotics, currently used for human therapy (1). Most drugs in commercial use are obtained at industrial scale by fermentative production. To increase production yield, genetic manipulation of the producer could be necessary and a thorough knowledge of antibiotic biosynthetic pathways is necessary.

The actinomycete *Nonomuraea* sp. ATCC 39727 produces the glycopeptide A40926, precursor of dalbavancin, a promising candidate for treating infections caused by multi-drug resistant Gram-positive bacteria. The *dbv* gene cluster for A40926 biosynthesis includes 37 *orfs* participating in antibiotic biosynthesis, regulation, resistance, and export. Specifically, the cluster encodes the putative regulators Dbv3 (LuxR-like) and Dbv4 (StrR-like), as well as the putative response regulator Dbv6 and the sensor-kinase Dbv22 that may be part of a two-component regulatory system (2, 3).

In order to investigate the role of these regulators, mutants in all the four gene were generated. Bioassay revealed that *dbv3* and *dbv4* mutants did not produce antibiotic. On the other hand, the absence of *dbv6* and *dbv22* did not seem to influence A40926 production. To understand their involvement in antibiotic biosynthesis, transcriptional and proteomic analyses of the mutants in respect to wild type strain are ongoing.
These studies will provide new insights in regulation of glycopeptide biosynthesis and could be used to specifically manipulate the strain and increase the productivity.

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New benzamido derivatives: synthesis, cytotoxycity, and inhibitory effects on tubulin polimerization.

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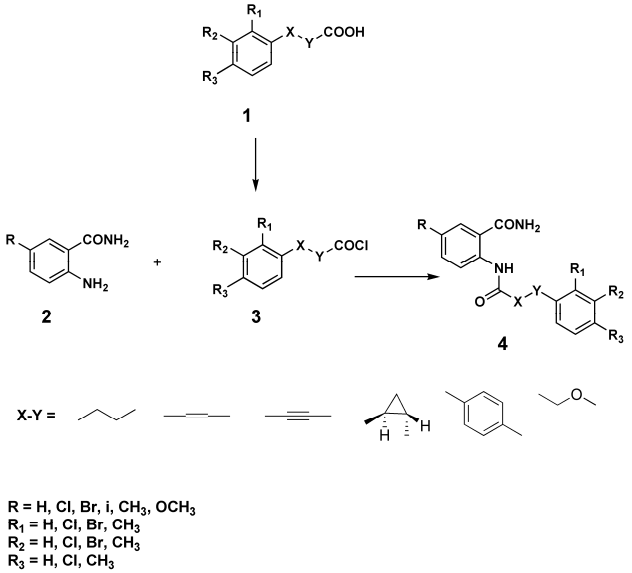
The cinnamammidobenzamides represent a class of biological active substances of great importance in medicinal chemistry [1,2]. Moreover, despite their wide range of biological activities, a review of the literature revealed that no anticancer activity is described for this kind of substances. Considering that the 2-cinnamamido-5-iodobenzamide, a precursors keep in our laboratory, resulted able to inhibit the leukemic cell line K-562 proliferation with a percent of inhibition of 74% at 10μM concentration, we undertake the following structural modifications on cinnamamidobenzamide skeleton: the introduction of various substituents both on the benzamido and the cinnamamido moieties [3], the substitution of vinilidene moiety with the ethylidene, ethynylidene, cyclopropylidene p-phenylene and oxymethylene ones as reported in the scheme. Compounds **4** caused growth inhibition against many tumor cell lines at low micromolar and

submicromolar concentrations against every tumor cell line investigated. COMPARE analysis, effects on tubulin polymerization and cell cycle distribution (G2-M phase block), including induction of apoptosis, indicate that these new antiproliferative compounds act as antitubulin agents [3].



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Biocompatible hydrogels based on hyaluronic acid cross-linked with a polyaspartamide derivative as delivery systems for epithelial limbal cells

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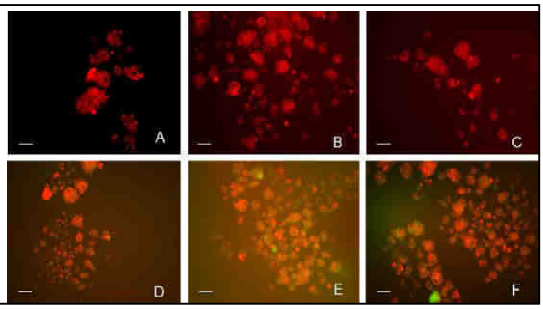
Human amniotic membrane is used as a carrier for delivering cultured limbal stem cells to the cornea after its breaking down on the eye thus leaving limbal cells in place (1).
However, the biodegradation time is not constant as it depends on the processing of the amniotic membrane and on the particular storage regimes used in the tissue banks (2)
Also in spite of extensive screening of the maternal donors before the membrane is used, there is still some risk of viral disease transmission that cannot be completely eliminated.
Therefore, there is a clinical need to develop a synthetic, biocompatible and slowly biodegradable material which could be used as substitute for the amniotic membrane allowing both the attachment of the cells and their subsequent delivery onto the cornea.
The aim of this work was to evaluate the potential use of hydrogels based on hyaluronic acid (HA)

chemically cross-linked with α,β-poly(N-2-hydroxyethyl) (2-aminoethylcarbamate)-D,L-aspartamide (PHEA-EDA) (3) as substitutes for the amniotic membrane able to release limbal cells for corneal regeneration.
Contact lenses have been coated, in their inner surface, with HA/PHEA-EDA film and adhesion have been performed by using immortalized human corneal epithelial cells (HCEC), rabbit limbal epithelial cells (RLEC) and/or rabbit limbal fibroblasts (RLF).
We demonstrate that cell adhesion is only transitory, indeed after three days, viable cells are released in the culture medium thus suggesting a potential application of HA/PHEA-EDA hydrogels, for delivering limbal cells in the treatment of corneal damage.

Confocal images of RLEC (A, B, C) and co-culture of RLEC (red) + RLF (green) (D, E, F) after 3 days of culture on each HA/PHEA-EDA film coating the inner surface of a contact lens: film X = 0.5 (A, D), film X = 1.0 (B, E) and film X = 1.5 (C, F).



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The *trim-box* containing gene *strim1* is involved in the epithelial-mesenchymal network that regulates the skeletal morphogenesis of *Paracentrotus lividus*

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The sea urchin embryo is an excellent model to study morphogenetic processes such as skeletogenesis (1). Indeed, the complex transcriptional gene regulatory network that underlies the specification of embryonic skeletogenic cells, named PMCs, has been recently elucidated (2). Also, additional evidence confirm that biomineralization is induced by a signaling directed from the ectoderm to PMCs (3-4). The recently identified *strim1* gene encodes a tripartite motif-containing protein that regulates such a signaling (5). First, *strim1* transcripts are specifically localized in regions of oral ectoderm cells adjacent to the ventrolateral PMCs clusters that initiate skeletogenesis. By gain and loss of function experiments we demonstrated that *strim1* influences most of the PMC dynamics, including the directional migration, positioning, terminal differentiation and biomineralization activity. Furthermore, by blastomere transplantations, we established that these effects specifically depend upon *strim1* misexpression in ectoderm cells. We also implemented a rescue assay showing that a clonal source of *strim1*, superimposed on *strim1* knocked-down embryos, is able to fully restore the skeletogenic program.
To correctly place *strim1* into the hierarchy of the regulatory network, we identified some of its downstream indirect targets, such as *otp*, *pax2/5/8*, and *fgfA* in ectoderm cells, and *sm30* in PMCs. Remarkably, the functional link between *strim1* and the *otp* and *pax2/5/8* transcription factors has been further demonstrated by rescue assays.

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Autophagy is related to apoptosis in *Paracentrotus lividus* embryos cadmium exposed

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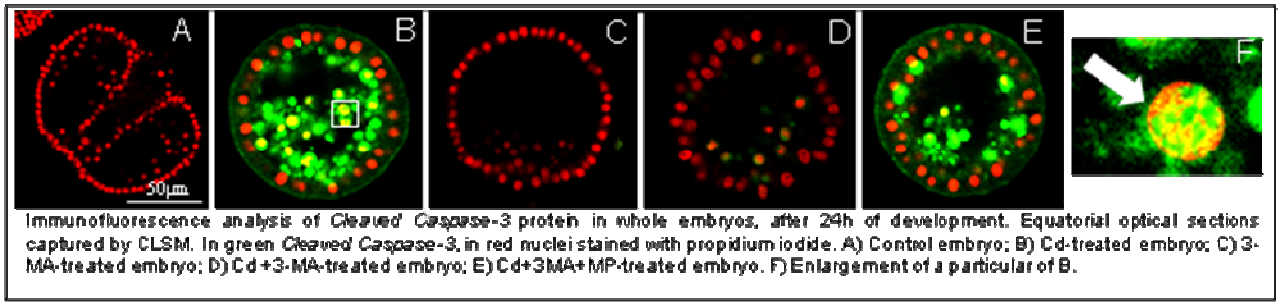
P. lividus embryo offers an excellent opportunity to investigate the adaptive response of cells exposed to different stress. We previously demonstrated that cadmium treatment triggers the accumulation of metal in embryonic cells and the activation of defense system depending on concentration and exposure time, through the synthesis of HSPs and/or the initiation of apoptosis.
Analysing autophagy, by neutral red, acridine orange and LC3-detection, we demonstrated that Cd-exposed embryos adopt this process as an additional stratagem to safeguard the developmental program. We observed that embryos treated at subletal Cd concentration activate a massive autophagic response after 18h, which decreases between 21 and 24h, in the opposite of apoptotic process.
In order to investigate a possible temporal relationship between autophagy and apoptosis, we tested apoptotic signals by TUNEL and immunofluorescence in situ assays of cleaved caspase-3. We showed that embryos activate a massive apoptosis after 24h of Cd-exposure. Therefore a functional relationship between autophagy and apoptosis was estimated evaluating apoptotic signals in Cd-exposed embryos, upon treatment with the autophagic inhibitor 3-methyladenine (3-MA). We found that the inhibition of autophagy produced a reduction of apoptotic signals, suggesting that the two phenomena are functionally related. In effect using methylpyruvate (MP), a substrate for ATP production, apoptosis was substantially restored.
This suggests that autophagy could energetically contribute to apoptotic execution through its catabolic role.



Immunofluorescence analysis of *Cleaved Caspase-3* protein in whole embryos, after 24h of development. Equatorial optical sections captured by CLSM. In green *Cleaved Caspase-3*, in red nuclei stained with propidium iodide. A) Control embryo; B) Cd-treated embryo; C) 3-MA-treated embryo; D) Cd+3-MA-treated embryo; E) Cd+3MA+MP-treated embryo. F) Enlargement of a particular of B.

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The study of the palladium(II)-catalyzed [3,3] aza-Claisen rearrangment of 3-allyloxy-5-aryl-1,2,4-oxadiazoles

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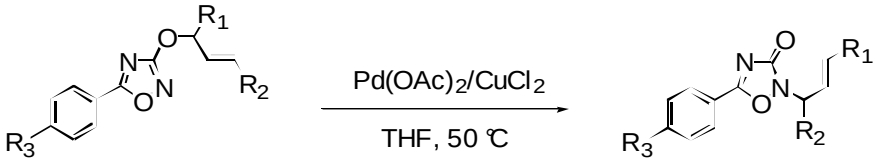
The [3,3] aza-Claisen rearrangement is a sigmatropic process that involves an allylic transfer in *N*-allyl, *N*-vinyl amines or related systems[1] that brings to the formation of ???-unsatured imines. It was efficiently employed in a number of useful transformations such as the stereospecific synthesis of 2',3'-dideoxynucleoside precursors[2]. Pd(II) salts are known to catalyze many synthetically useful [3,3]-sigmatropic rearrangements[3] in 1,5-dienes, allyl vinyl ethers and also in aza-Claisen-type processes. 1,2,4-Oxadiazoles present many applications in medicinal chemistry, such as peptidomimetics and enzyme inhibitors, apoptosis inducers or as unnatural bases in the extension of DNA strands. Among them, *N*(2)-substituted 1,2,4-oxadiazolones are considered an emerging class of bioactive molecules, mainly represented by the quisqualic acid, the sole natural 1,2,4-oxadiazole, responsible for the QUIS effect, i.e. the agonist effect toward a number of amino acids receptors in the SNC[4]. In this work we developed a new Pd(II)-catalyzed [3,3] aza-Claisen sigmatropic rearrangement involving 3-allyloxy-1,2,4-oxadiazoles (**1**) to give the related *N*(2)-substituted 1,2,4-oxadiazol-3-ones (**2**). The mechanism of the reaction was studied by analyzing the regiochemical and highly stereoselective course of this chemical transformation. The results obtained indicated the intervention of a cationic pallada-cycle similar to the one postulated for the



1 **2**

Cope rearrangement of 1,5-dienes.

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Synthesis and antitumor activity of 3-(2-phenyl-1,3-thiazol-4-yl)-1*h*-indoles and 3-(2-phenyl-1,3-thiazol-4-yl)-1*h*-7-azaindoles

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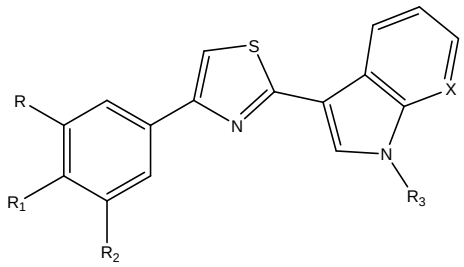
Marine indole alkaloids have emerged as an important structural class because of their great variety of biological activities including antimicrobial, antiviral and antitumor properties.[1] In particular nortopsentins A-C, having a characteristic 2,4-bis(3'-indolyl)imidazole skeleton, have been considered important lead compounds for the discovery of new biologically active derivatives.[2] We reported the synthesis and the antitumor activity of different bis-indolyl-5-membered heterocycles, in which the imidazole moiety of nortopsentin was replaced by thiophene, pyrazole, isoxazole, and furan rings. Some of these compounds showed antitumor activity from micromolar to sub-micromolar concentrations.[3] Many other analogues such as 2,4-bis(3'-indolyl)-thiazoles and 3,5-bis(2-indolyl)pyridines showed strong inhibitory activity against a wide range of human tumor cell lines. A marked cytotoxic effect was observed when one indolyl ring was replaced by a phenyl moiety.[4] In the attempt of looking for novel antitumor compounds, we thought it was interesting to synthesize 3-(2-phenyl-1,3-thiazol-4-yl)-1*H*-indoles **1** and 3-(2-phenyl-1,3-thiazol-4-yl)-1*H*-7-azaindoles **2**.



1 X=CH; **2** X=N

All compounds were tested on about 60 tumor cell lines and derivatives of 7-azaindole series were particularly active showing antitumor activity in a wide range of tumor cell lines from micromolar to nanomolar concentration. Two of them exhibited a high affinity for CDK1, with IC_{50} values of 0.41 and 0.85 µM. These promising results set the foundation for future investigations into the development of anticancer therapies.

[1] a) J. Shin, et al. *J. Nat. Prod*. **1999**, 62, 647 b) A. Casapullo, et al. *J. Nat. Prod*. **2000**, 63, 447;c) B .Bao, et al. *J. Nat. Prod*. **2005**, 68, 711; [2] Sakemi, S. et al. *J. Org. Chem.* **1991**, 56, 4304; [3] a) Diana, P. et al. *Bioorg. Med. Chem. Lett*. **2007**, 17, 2342; b) Diana, P. et al. *Bioorg. Med. Chem. Lett.* **2007**, 17, 6134; c) Diana, P. et al. *Bioorg. Med. Chem.* **2010**,*18*, 4524; [4] X-H.GU, et al. *Bioorg. Med. Chem. **2000**, 8, 363*

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