MOLECULAR CHAPERONES, STAT5, AND HEPARANASE INHIBITORS AS NEW ANTITUMOR AGENTS

Molecular chaperones (heat shock proteins, Hsp) are involved in many mechanisms that regulate cell functions. They assist other proteins to fold properly in the cytoplasm, and stabilize mitochondria, thus containing the release of pro-apoptotic factors. These mechanisms are often overexpressed in cancer cell lines causing chemotherapy resistance, so inhibitors of molecular chaperones may find application as therapeutic agents.
The STAT5 proteins regulate cell cycle, apoptosis and proliferation of different cells through the influence on gene transcription. STAT5 proteins are suggested to play an important role in leukaemogenesis, as they are constitutively activated in some haematooncologic diseases. For this reason, inhibition of STAT5 proteins may represent a valuable treatment option for this type of cancers. It has been demonstrated that structural analogs of the neuroleptic pimozide are able to interfere with cell growth in some myelocytic leukemias lines, which is typical of Jak/STAT system overexpression.
Cancer cell migration to generate invasion and metastasis is promoted by heparanase, through remodeling of the connective tissue which contains the primary tumor. Inhibition in connective tissue remodeling is crucial for uncovering novel therapeutic targets and treatment strategies.

GOALS

- Design and synthesis of new derivatives as potential inhibitors of Hsp family, with particular regard to mitochondrial Hsp75.
- Synthesis and biological evaluation of a potential class of antitumor drugs acting through inhibition of activated STAT5, with the aim of finding alternative therapeutic options to bypass imatinib resistance in the treatment of myelocytic leukemias.
- Search for new molecules able to interfere with heparanase activity (in cooperation with Sigma-Tau)

INSTRUMENTS AND METHODS

Common techniques and equipment of a synthetic organic laboratory. Use of chromatographic (preparative HPLC, flash chromatography) and analytic techniques (mass spectrometry, NMR, IR) for the purification, identification and characterization of the synthesized compounds. Structural optimization based on literature data and preliminary biological test results of the new compounds.

MAIN SUBJECTS

Medicinal chemistry

RESEARCH GROUP

Prof. D. Simoni, Dr. R. Rondanin

COLLABORATIONS

Prof. M. Landriscina (University of Foggia), Dr. M. Tolomeo (Palermo General Hospital), Sigma-Tau SpA.

DESIGN AND SYTHESIS OF NEW ANTICANCER AGENTS

DNA minor groove binders constitute an important class of derivatives in anticancer therapy. The ongoing project intends to explore the possibility to target specific sequences in DNA with synthetic ligands. The minor groove of double helical B-DNA is becoming a site of great interest for developing new drugs since it is the site of non-covalent high sequence specific interactions for a large number of small molecules. The research activities also include the discovery and development of novel small molecules able to affect tubulin polymerization. It has been established that some tubulin-binding agents selectively target tumor vasculature and thus can also be considered vascular disrupting agents. The potential of vascular targeting agents as cancer therapeutics has been firmly established in experimental studies.

GOALS

- Development of new small molecules as DNA minor groove binders.
- Rational design, synthesis and pre-clinical evaluation of novel combretastatin analogues with potent cytotoxic and vasculature targeting properties which have potential as cancer therapeutic agents.

INSTRUMENTS AND METHODS

The compounds will be synthesized with the standard equipment technology for traditional liquid phase synthesis. The chemical structures and purity of the synthesized compounds will be determined by NMR, electrospray mass, UV and IR spectrometers.

MAIN SUBJECTS

Medicinal chemistry, organic chemistry, pharmacology

RESEARCH GROUP

Prof. P. G. Baraldi, Prof. R. Romagnoli

COLLABORATIONS

Prof. P. A. Borea (University of Ferrara), Prof. P. Geppetti (University of Florence)

DESIGN AND SYNTHESIS OF ADENOSINE RECEPTOR LIGANDS

Adenosine is a nucleoside produced following tissue injury involving ischemia and hypoxia. The production of extracellular adenosine and its subsequent signalling through adenosine receptors (ARs: A1, A2A, A2B and A3) play an important role in orchestrating injury responses in multiple organs. ARs have long been considered promising therapeutic targets in a wide range of conditions, ranging from cerebral diseases to cancer, including inflammatory disorders. Thus, the ongoing research project encompasses the design and the synthesis of new ligands for each AR subtype, primarily for their therapeutic potential but also as pharmacological tools in receptor studies.

GOALS

- Development of new A1 AR allosteric enhancers as potential cardioprotective agents.
- Development of new A2A AR agonists or antagonists (potentially useful for the treatment of Parkinson's disease and inflammation).
- Development of new A2B AR agonists or antagonists (potentially useful for the treatment of cardiac disease and asthma).
- Development of new A3 AR agonists or antagonists (potentially useful for the treatment of cancer and eye disorders).

INSTRUMENTS AND METHODS

The compounds will be designed and synthesized with the standard equipment technology for traditional liquid phase synthesis. The chemical structures and purity of the synthesized compounds will be determined with NMR, electrospray mass, UV and IR spectrometers

MAIN SUBJECTS

Medicinal chemistry, organic chemistry, pharmacology

RESEARCH GROUP

Prof. P. G. Baraldi, Prof. R. Romagnoli

COLLABORATIONS

Prof. P. A. Borea (University of Ferrara), Prof. P. Geppetti (University of Florence)

DESIGN AND SYNTHESIS OF CB2 RECEPTOR MODULATORS AS EMERGING OPPORTUNITY TO TREAT PAIN AND INFLAMMATION

While acute pain is a physiological sensation triggered in the nervous system to alert the organism against a possible injury, the condition of chronic pain represents a major challenge to healthcare providers because of its complex nature and unclear etiology. Existing therapies for chronic pain are far from effective. This underscores the importance of considering, validating, and pursuing alternative targets to treat refractory pain. The ongoing project is aimed to validate emerging opportunities in this therapeutic area offered by the activation of the cannabinoid CB2 receptor, both involved in the generation and/or transmission of pain signals.

GOALS

- Rational design, synthesis and biological evaluation of potent and selective TRPA1 receptor antagonists.
- Rational design and synthesis of potent CB2 cannabinoid receptor agonists with high selectivity versus the CB1 cannabinoid receptor subtype that could be in future investigated and evaluated as novel analgesic medicines especially for their emerging capability to improve pain control by increasing clinical efficacy of opioids.

INSTRUMENTS AND METHODS

The compounds will be designed and synthesized with the standard equipment technology for traditional liquid phase synthesis. The chemical structures and purity of the synthesized compounds will be determined with NMR, electrospray mass, UV and IR spectrometers.

MAIN SUBJECTS

Medicinal chemistry, organic chemistry, pharmacology

RESEARCH GROUP

Prof. P. G. Baraldi, Prof. R. Romagnoli

COLLABORATIONS

Prof. P. A. Borea (University of Ferrara), Prof. P. Geppetti (University of Florence)

DESIGN AND SYNTHESIS OF TRPA1 RECEPTOR MODULATORS AS EMRGING OPPORTUNITIES TO TREAT PAIN AND INFLAMMATION-RELATED DISORDERS

Transient receptor potential (TRP) channels constitute a large family of diverse ion channel proteins with impact on sensory signalling pathways. TRPA1 is the only known member of the ankyrin subfamily that, behaving as a chemosensor of by-products of oxidative/nitrative stress resulting from tissue damage and/or inflammation, plays a fundamental role in pain signalling. Furthermore, TRPA1 activation promotes the release of inflammatory neuropeptides. This dual function as a detector and instigator of inflammatory agents makes TRPA1 a gatekeeper of chronic inflammatory disorders of the skin, airways, and gastrointestinal tract. The ongoing project is aimed to validate emerging opportunities in these therapeutic areas mainly offered by the blockade of TRPA1 receptor.

GOALS

- Design, synthesis and in vitro biological evaluation of new TRPA1 antagonists with high potency, selectivity, improved metabolic properties and water solubility.
- Design and synthesis of the first TRPA1 selective covalent/fluorescent antagonist probes.
- Design of fluorinated ligands eliciting a particular interest if considering that ^{18}F has been recognized as a useful positron emitting isotope with impact for in vivo imaging technology and wide application in drug discovery and development.
- Validation of new multi-targeting approaches involving the TRPA1 receptor.

INSTRUMENTS AND METHODS

The compounds will be synthesized with the standard equipment for traditional liquid phase synthesis. Flash chromatography and preparative HPLC, mass spectrometry, analytical HPLC, NMR, IR techniques will be used to isolate and characterize the synthesized compounds.

MAIN SUBJECTS

Medicinal chemistry, organic chemistry, pharmacology

RESEARCH GROUP

Dr. D. Preti

COLLABORATIONS

Prof. P. Geppetti (University of Florence), Prof. G. Calò (University of Ferrara), Dr. S. Cosconati (Second University of Naples)

SYNTHESIS OF AGONISTS AND ANTAGONISTS OF THE PURIGENIC RECEPTORS P1 AND P2

The purinergic receptors are widely distributed in body. There are two big families: P1 and P2. The P1 family includes four receptor subtypes named A1, A2A, A2B and A3. They are G-protein coupled receptors and the physiological ligand is adenosine. A2A receptors, in particular, are coupled to the dopamine D2 receptors: A2A adenosine receptors antagonists induce a better interaction of dopamine with its D2 receptors improving the dopaminergic cerebral functions, which are highly compromised in the Parkinson's disease due to the dopamine depletion. *Drug development* is limited by the presence of the *blood-brain barrier (BBB), so* there is need to obtain new compounds that can penetrate the BBB through chemical modifications of known scaffolds or the synthesis of molecular hybrids.
The big P2 family is divided in two subfamilies named P2Y and P2X, whose physiological ligands are ATP or ADP. The P2Y receptors are G protein coupled receptors, whereas the P2X ones are ion channel receptors. These subfamilies include several subtypes usually ubiquitarious in the body. Among all of the P2Y receptors subfamily, P2Y12 receptors are G protein coupled receptors involved in the process of platelet aggregation, and they are relatively present only on the platelets. Their physiological agonist is ADP, while ATP is the antagonist. The development of P2Y12 antagonists may represent a good biological target in the field of cardiovascular diseases in which the platelets aggregation is one of the major problems.

GOALS

Synthesis and biological evaluation of new purinic or pyrimidinic derivatives as ligands for the purinergic receptors.

INSTRUMENTS AND METHODS

Common equipment of a synthetic organic laboratory. Use of chromatographic (preparative HPLC, flash chromatography) and analytic (NMR, HPLC-mass spectrometry, IR) techniques for the purification, identification and characterization of the synthesized compounds.

MAIN SUBJECTS

Medicinal chemistry

RESEARCH GROUP

Dr. Barbara Cacciari

COLLABORATIONS

Prof. G. Spalluto (University of Trieste), Prof. S. Moro (University of Padova), Prof. C. Cattaneo (University of Milan), Prof. A. Dalpiaz e Prof. K. Varani (University of Ferrara)

SYNTHESIS AND ACTIVITY OF PROTEASOME INHIBITORS

The proteasome is a multicatalytic protease complex which takes an active part in the ubiquitin-proteasome (UPP) metabolic pathway of proteolysis in eukaryote cells. Fundamental cell functions are related to the degradation of proteins which are involved in processes such as cell cycle and differentiation, apoptosis and generation, and transcriptional regulation. Many natural and synthetic compounds have been evaluated as inhibitors of proteasome. In vitro and in vivo studies have demonstrated that proteasome inhibitors show anti-proliferative and pro-apoptotic activities towards solid and hematologic tumors. Other proteasome inhibitors have been tested against inflammation- and immune-associated disorders.

GOALS

The project aims at the synthesis of new potent and selective proteasome inhibitors, with satisfactory *pharmacokinetic properties,* to be used in new therapeutic protocols. It follows previous development of many classes of peptide-based proteasome inhibitors bearing *C*-terminal suitable pharmacophoric units that are able to interact with the catalytic threonine through a Michael-type addition. At present, we are studying the design and synthesis of new peptide-based proteasome inhibitors lacking the *C*-terminal electrophilic function. We expect to obtain molecules that could give non-covalent interactions with the proteasome catalytic sites in order to achieve a reversible inhibition of the enzymatic complex. Potential therapeutic applications of such analogs should not suffer from the undesired side effects that are displayed by irreversible inhibitors, that instead covalently bind to the enzymatic complex active subsites.

INSTRUMENTS AND METHODS

Solution- and solid-phase synthesis. Combinatorial chemistry techniques. Purification techniques, such as flash chromatography and preparative HPLC. Mass spectrometry, analytical HPLC, NMR, IR techniques to determine either structure or purity of final compounds.

MAIN SUBJECTS

Pharmaceutical chemistry, organic chemistry, biochemistry

RESEARCH GROUP

Prof. M. Marastoni

COLLABORATIONS

Prof. R. Gavioli, Prof. C. Trapella, Dr. V. Ferretti (University of Ferrara); Dr. M. Bazzaro (Masonic Cancer Center and Department of Obstetrics, Gynecology and Women's Heath, University of Minnesota, Minneapolis, Minnesota, USA).

STRUCTURE-ACTIVITY RELATIONSHIP OF BIOLOGICALLY ACTIVE PEPTIDES

Despite their low bioavailability, peptides have recently been enjoying a resurgence of interest as potential drug candidates, due to their wide range of specific functions as hormones, neurotransmitters, or neuromodulators. Amino acid replacement, peptide-bond modification as well as peptidomimetic design are fundamental in identifying new biologically active compounds. Chemically-modified peptides can be used as pharmacological tools in preclinical studies, but also to develop innovative drugs.

GOALS

- Identification of the main structural requirements that determine either the potency or the efficacy of peptides.
- Peptide-sequence stabilization toward peptidases and synthesis of compounds showing in vivo prolonged activities.
- Identification of the chemical moieties responsible for peptide binding to receptors, typically the G protein-coupled (GPCRs) ones.
- Development of pharmacophoric models to be used for rational design of new peptidic and non-peptidic ligands.

INSTRUMENTS AND METHODS

Solution- and solid-phase peptide synthesis. Purification techniques, such as flash chromatography and preparative HPLC. Mass spectrometry, analytical HPLC, NMR, IR techniques to determine either structure or purity of final compounds.

MAIN SUBJECTS

Pharmacology, molecular biology, conformational analysis.

RESEARCH GROUP

Prof. S. Salvadori, Prof. R. Guerrini, Dr. E. Marzola

COLLABORATIONS

Prof. G. Calo' (University of Ferrara); Prof. D. Picone (University of Naples); Prof. M. P. Costi (Universiy of Modena and Reggio Emilia); Prof. T. Costa (ISS, Roma); Prof. D. Lambert (University of Leicester, UK); Prof. Mei-Chuan Ko (Wake Forest University, NC, USA).

INNOVATIVE DELIVERY SYSTEMS AND TARGETING OF DRUGS TO THEIR ACTION SITES

The aim of the project is the formulation of innovative systems able to target neuroactive and anticancer drugs to their action sites. In general, neuro-active drugs are not able to reach the brain at therapeutic concentrations and several anticancer agents show the multidrug resistance phenomenon. These effects are mainly due the active efflux transport (AET) systems, that recognize and efflux in the bloodstream the drugs that have reached the brain or the cancer cells. The design of appropriate prodrugs, pharmaceutical co-crystals, or micro- and nano-particulate systems can allow to obtain formulations that, upon non-invasive administrations, are able to target the drugs to their action site. In particular we are designing innovative formulations constituted by prodrugs or pharmaceutical co-crystals able to elude the AET systems as micro- and micro- or nano-particulate systems. Microparticles are formulated for nasal administration and brain targeting of neuroactive drugs; nanoparticles are formulated for intravenous administration of anticancer and anti-HIV agents, respectively.

GOALS

- Nasal formulations based on polymeric or lipidic microparticles for the drug targeting in the central nervous system.
- Formulations based on polymeric nanoparticles for the encapsulation and the controlled release of neuroactive drugs.
- Development of cellular models for the *in vitro* studies of drug permeation across physiologic barriers.
- Bile acid prodrugs able to elude the active efflux systems: studies of drug targeting in the central nervous system or enhancement of drug action in tumors.
- Nanoparticulate formulations based on bile acids prodrugs for the targeting of anti-HIV drugs in macrophages.
- Development of cellular models for the *in vitro* studies of new strategies able to elude the multidrug resistance phenomenon of anticancer drugs .
- Hybrids obtained by anticancer drugs: studies of pharmacokinetics and multidrug resistance.
- Pharmaceutical co-crystals: studies of dissolution, permeability and bioavailability.
- Essential oils: studies of their potential pharmaceutical activity and bioavailability.

INSTRUMENTS AND METHODS

HPLC techniques for the in vitro and in vivo quantitative analysis of the prodrugs and their related prodrugs; emulsion or nanoprecipitation methods for the formulation of the particulate systems; cell culture of monolayers for permeation studies of the prodrugs and their parent drugs by HPLC; pharmacokinetic studies in physiologic fluids.

MAIN SUBJECTS

Pharmaceutical technology, medicinal chemistry, biology

RESEARCH GROUP

Prof. A. Dalpiaz

COLLABORATIONS

Prof. S. Scalia, Prof. M. Fogagnolo, Dr. P. Marchetti, Dr. C. Contado, Dr. V. Ferretti, Prof. V. Bertolasi (University of Ferrara); Sassari, Modena and Reggio Emilia, Catania Universities; Thomas Jefferson University, PA, USA; Department of Health Sciences, Luleå University of Technology, Sweden.

DEVELOPMENT OF MICRO AND NANOPARTICELLAR SYSTEMS FOR THE DELIVERY OF ACTIVE PRINCIPLES OF PHARAMACEUTICAL AND COSMETIC INTEREST

Micro and nanoparticellar systems are suitable tools for the delivery of pharmaceutical and cosmetic active principles, owing to the fact that they enable controlled release, localization at specific skin sites or the targeting to either the breathing apparatus or the nasal mucosa.

GOALS

- Development of innovative micro and nanoparticellar systems for pharmaceutical and cosmetic applications: we will synthesize and characterize polymeric/lipidic micro and nanoparticellar systems suitable for pulmonary and nasal drug delivery. These systems are expected to enhance stability and vehicolation in the treatment of respiratory diseases. These studies will be supported by suitable cell culture models.
- Development of lipid nanoparticle formulations, prepared with biodegradable and biocompatible materials, to be used for topical antioxidant application.

INSTRUMENTS AND METHODS

Chromatographic analysis, preparation of micro and nanoparticles *via* fusion-emulsion, in vitro pulmonary deposition studies, photostability testing, in vitro and in vivo transdermal absorption studies, skin analyses concerning elasticity, hydration, pH value.

MAIN SUBJECTS

Pharmaceutical technology, analytical chemistry, chemical physics, organic chemistry, cosmetic chemistry

RESEARCH GROUP

Prof. S. Scalia, Dr. A. Bianchi

COLLABORATIONS

Prof. D. Traini (Woolcock Institute of Medical Research - University of Sydney, Australia), Prof. M. Haghi (Graduate School of Health - University of Technology, Sydney, Australia), Prof. I. F. Almeida (Departamento de Ciências do Medicamento, Faculdade de Farmácia da Universidade do Porto, Porto, Portogallo), Dr. V. Iannuccelli (University of Modena e Reggio Emilia).