12th Conference in Advanced Medicinal Chemistry

ORGANIZATION

Department of Pharmaceutical Chemistry, School of Pharmacy, A.U.Th.

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All members of the Department of Pharmaceutical Chemistry, School of Pharmacy, A.U.Th.

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PROGRAMME

12th International Conference in Advanced Medicinal Chemistry

Thessaloniki, May 20-21, 2011

"Rational Drug Design and Development"

SCIENTIFIC PROGRAMME

FRIDAY, May 20, 2011

CHAIR:	***Associate Professor A. Geronikaki, University of Thessaloniki, Greece***
9:15 - 10:15	**Welcome Addresses** **Rector of the Aristotelian University of Thessaloniki** **President of the National Drug Organisation** **President of the Panhellenic Pharmaceutical Association** **Professor Emeritus P.N. KOUROUNAKIS, University of Thessaloniki, Greece** **"Introduction"** ***Professor D. Galaris, University of Ioannina, Greece***
CHAIR:	**Professor A. STEPHENSON, School of Pharmacy, University of London, UK**
10:15 - 11:00	**"Understanding NMDA receptors; key targets for therapeutic intervention in disorders of the Central Nervous System"**
11:00 - 11:45	***COFFEE BREAK - POSTERS***
11:45 - 12:30	**Professor G. GOLDSTEINS, University of Eastern Finland** **"Mitochondrial targets for neuroprotection"**
12:30 - 16:00	***LUNCH***
CHAIR:	***Professor A. Tselepis, University of Ioannina, Greece***
16:00 - 16:45	**Professor G. F. ECKER, Department of Medicinal Chemistry,** **University of Vienna, Austria** **"The molecular basis of drug/transporter interaction - Knowledge driven ligand design"**

16:45 - 17:45	***COFFEE - POSTERS***
17:45 - 18:30	**Professor G. CIRRINCIONE, Università degli Studi di Palermo, Italy** **"Potent antineoplastic activity of isoindolo-quinoxalines"**
18:30 - 19:15	**Professor Y. TACHE, Center for Neurobiology of Stress and Digestive Diseases Research Center, Los Angeles, CA, USA** **"Corticotropin releasing factor receptors and stress-related gut diseases: physiology and therapeutic potential"**

SATURDAY, May 21, 2011

CHAIR:	***Professor P. Cordopatis, University of Patras, Greece***
9:30 - 10:15	**Professor J. C. DEARDEN, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK** **"The art of developing valid QSARs"**
10:15 - 11:15	***COFFEE - POSTERS***
11:15 - 12:00	**Professor H. STARK, Institut fuer Pharmazeutische Chemie, Johann Wolfgang Goethe-Universitaet, Frankfurt am Main, Germany** **"From target discovery to clinical trial: the example of the histamine H_3 receptor and Pitolisant"**
12:00 - 15:45	***LUNCH***
CHAIR:	***Professor T. Theophanides, Technical University of Athens, Greece***
15:45 - 16:30	**Professor F. CORELLI, Faculty of Pharmacy. University of Siena, Italy** **"Targeting the endocannabinoid system: Synthesis, SAR and pharmacological evaluation of quinolone-3-carboxamides as potent and selective CB2 ligands"**
16:30 - 17:30	***COFFEE - POSTERS***
17:30 - 18:15	**Professor H. E. POULSEN, Clinical Pharmacological Laboratorium, Copenhagen, Denmark** **"Oxidative damage to nucleic acids: clinical relevance"**
18:15 - 18:30	**Professor P.N. KOUROUNAKIS, University of Thessaloniki, Greece** **Closing the 12th Conference in Advanced Medicinal Chemistry**

CONTENTS

CONTENTS……..8

Introduction

P.N. Kourounakis

Department of Medicinal Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Greece

Dear Invited Speakers and Colleagues,

Since I joined this department, in 1976, coming from the University of London (A.H Beckett) and University of Montreal (H. Selye), my main preoccupation was to modernise our department. Thus, I tried to update the material taught to the undergraduate students. It was then the first time students were taught about the role of physicochemical properties in drug action, subjects like structure/activity relationships, drug metabolism and the role of chemical bonds in the development of drug action, at the undergraduate level in Greece. The second objective was the postgraduate level, I am not certain the department had any PhD produced by 1977. Since then, more than 30 PhDs have been granted. Our research has gradually developed to work involving the majority of the expressions of Medicinal Chemistry. Most of us in this department try to apply the rational drug design in our research, as you can realise from the posters exhibited in this conference. We base our research on the pathobiochemistry of the disease, the molecular mechanism of action and/or drug metabolism, for lead discovery and optimisation. Oxidative stress, being implicated in many pathologic conditions, is also among our interests. The third effort made by myself and the other colleagues was to invite renowned scientists from abroad, to lecture in our department. Among them were A.H. Becket, W.H. Hunter, University of London, H. Timmerman, University of Amsterdam, B.B. Breimer, University of Leyden, E. Mutschler, University of Frankfurt, H. Oelshlager, University of Frankfurt. Those lectures were liked by everybody, therefore we decided to organise the Conferences in Advanced Medicinal Chemistry every second year, with prominent invited speakers from Europe and America. In this way, our students and young colleagues would have the opportunity, here in Thessaloniki, to meet researchers working at the frontiers of science and creating knowledge. Twenty four years later, we have a long and impressive list of speakers in 12 Conferences in Advanced Medicinal Chemistry. Unfortunately, some of them are not among us any more. Arnold Beckett passed away some months ago, and Corwin Hansch only few days before this conference.

According to the IUPAC definition prepared for publication by C.G. Wermuth, C.R. Ganellin, P. Lindberg and L.A. Mitscher in 1998, **Medicinal Chemistry** is a chemistry-based discipline, also involving aspects of biological, medical and pharmaceutical sciences. It is concerned with the invention, discovery, design, identification and preparation of biologically active compounds, the study of their metabolism, the interpretation of their mode of action at the molecular level and the construction of structure-activity

relationships. I am proud that our undergraduate teaching, as well as our research work were along the ideas presented in the above definition twenty years before its publication.

As I said two years ago, the ultimate recipient of the scientific achievements in the field of drug discovery is the patient. This humanitarian character increases the pride of all scientists involved in the development of better drugs.

With these thoughts, I feel strongly the need to thank very sincerely all invited speakers and the audience, colleagues and students, for your presence, which honours our efforts.

Our invited speakers of the 11 previous Conferences (1989, 1991, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2007 and 2009) were the following Professors:

Gh. Atassi, Suresnes, France; A. Bast, Maastricht, The Netherlands; J. Bauer, Freiburg, Germany; P. Beaume, Paris, France; A.H. Beckett, London, UK; Ach. Benakis, Geneva, Switzerland; I. Björkhem, Huddinge, Sweden; M. Botta, Siena, Italy; N. Bodor, Gainesville, FL USA; K.-P. Bøgesø, Valby, Denmark; B.B. Breimer, Leiden, The Netherlands; J. Caldwell, London, UK; A. Carlsson, Gothenburg, Sweden; E. De Clercq, Leuven, Belgium; A. Ebringer, London, UK; B. Faller, Basel, Switzerland; R. Franke, Berlin, Germany; B. Frølund, Copenhagenm Denmark; D. Galaris, Ioannina, Greece; C.R. Ganellin, London, UK; G.G. Gibson, Surrey, UK; J. Gorrod, London, UK; R.J. Gryglewski, Cracow, Poland; C. Halldin, Stockholm, Sweden; C. Hansch, Pomona, CA USA; R.W. Hartmann, Saarbrüken, Germany; R.C. Hider, London, UK; U. Holtzgrabe, Würzburg, Germany; M. Ingelman-Sundberg, Stockholm, Sweden; H. Ischiropoulos, Philadelphia, USA; D.E. Jane, Bristol, UK; P. Jenner, London, UK; T.M. Jones, London, UK; H. Kappus, Berlin, Germany; A. Katritzky, Gainesville, FL, USA; P. Krogsgaard-Larsen, Copenhagen, Denmark; H. Kubinyi, Ludwigschafen, Germany; D.M. Lambert, Brussels, Belgium; A. Makriyiannis, Storrs, CT, USA; E. Maser, Marburg, Germany; C. Monneret, Paris, France; H. Moereels, Gent, Belgium; H.M. Moutsopoulos, Athens, Greece; S. Neidle, London, UK; K.J. Netter, Marburg, Germany; K. Nicolaou, La Jolla, CA , USA; C.R Noe, Vienna, Austria; H. Oelschlager, Jena, Germany; F. Oesch, Mainz, Germany; R. Paolleti, Milan, Italy; O. Pelkonen, Oulu, Finland; R. Pellicciari, Perugia, Italy; J.H. Poupaert, Brussels, Belgium; J. Rogers, Sun City AZ, USA; M. Rowland, Manchester, UK; W. Schunack, Berlin, Germany; J.K. Seydel, Borstel, Germany; H. Sies, Düsseldorf, Germany; D. Spinelli, Bologna, Italy; S. Stolc, Bratislava, Slovakia; S. Szabo, Long Beach, CA, USA; Y. Taché, Los Angeles, CA, USA; A. Terzis, Athens, Greece; Th. Theoharides, Boston, USA; A. Tselepis, Ioannina, Greece; G. Tucker, Sheffield, UK; N.P.E. Vermeulen, Amsterdam, The Netherlands; C.G. Wermuth, Strasburg, France; J.P Tollenaere, Beerse, Belgium; J. Wengel, Odense, Denmark; W. Wiegrebe, Regensbourg, Germany; E. Wülfert, Brain-l'-Alleud, Belgium.

12th Conference in Advanced Medicinal Chemistry

SPEAKERS

Prof. Anne STEPHENSON, School of Pharmacy, University of London, UK

Professor Anne Stephenson's graduated from the University of Cambridge with an MA degree in Natural Sciences (Chemistry). An MSc in Neurochemistry at the Institute of Psychiatry, University of London followed which led to a PhD in Biochemistry at the University of Bath where her thesis work was on the nicotinic acetylcholine receptor and its role in myasthenia gravis. Following post-doctoral work at the University of California with Professor Richard Olsen where she began her study of the $GABA_A$ receptors, she joined Professor Eric Barnard FRS in the Department of Biochemistry Imperial College, London as an MRC Training Fellow. In 1983, Professor Stephenson was one of the first recipients of a Royal Society University Research Fellowship. She moved with Professor Barnard to the MRC Molecular Neurobiology Unit in Cambridge where she was a member of the Department of Pharmacology, University of Cambridge. In 1989, she joined The School of Pharmacy, University of London. She was appointed Professor of Molecular Neuroscience in 1995. Professor Stephenson has served on the British Neuroscience Association Committee, the Neurochemical Group of the Biochemical Society, the Editorial Board of the Biochemical Journal and Journal of Biological Chemistry, the BBSRC Biochemistry and Cell Biology Board and the MRC College of Experts. She is currently a member of the MRC College of Experts and also, the Molecular and Cellular Neurosciences Committee of The Wellcome Trust.

The focus of Professor Stephenson's research group is to elucidate fundamental mechanisms which contribute to the molecular organization of synapses, the major points of communication between neurones. Current projects include the assembly, trafficking and targeting of NMDA receptors to synapses including the investigation of the role of amyloid precursor protein in NMDA receptor biogenesis; mechanisms of mitochondrial transport in neurones and determining the molecular basis of specificity with respect to inhibitory synapse formation testing the hypothesis that transynaptic $GABA_A$ receptor protein-protein interactions are important in establishing inhibitory neuronal circuitry.

Prof. Gundars GOLDSTEINS, Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland.

Professor Goldsteins has obtained his MSc in Chemistry, Department of Enzyme Chemistry, Faculty of Chemistry, Latvia State University, Riga, Latvia and PhD in Biochemistry, A.N. Bach Institute of Biochemistry, Moscow, Russia. He has been Senior Research Assistant at the Institute of Applied Biochemistry, Laboratory of Molecular Biology, BIOLAR, Olaine, Latvia and Research Assistant at Department of Applied Cell and Molecular biology, University of Umeå, Umeå, Sweden. Since 2003 he is Assistant Professor in Neurochemistry, A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland.

He is also: Supervising PhD and MSc theses, A.I.Virtanen Institute for Molecular Sciences, University of Eastern Finland. Member of the Society for Neuroscience. Research grant reviewer for MND Association. Referee for scientific journals such as Journal of Biological Chemistry, FEBS Letters, Apoptosis, and Journal of Neuroscience Research

Professor Goldsteins's research interests include: Mechanisms of neurodegeneration, focusing on models of acute and chronic neurodegenerative diseases. Contribution of mitochondrial dysfunction to the neurodegenerative diseases. Studies on the role of key enzymes and other targets that are involved in inflammation, oxidative stress or cell death and verification their significance in pathogenesis of the animal models of the brain diseases. Discovery of approaches to pharmacological treatment of the neurodegenerative diseases.

Prof. Gerhard F. ECKER, Department of Medicinal Chemistry, University of Vienna, Austria.

Gerhard Ecker is Professor for Pharmacoinformatics and Head of the Pharmacoinformatics Research Group at the Department of Medicinal Chemistry, University of Vienna. He also coordinates the research focus "Computational Life Sciences" of the Faculty of Life Sciences. Professor Ecker received his doctorate in natural sciences from the University of Vienna and performed his post-doctoral training at the group of J. Seydel in Borstel (Germany). He has published more than 100 articles mainly related to SAR and QSAR studies on P-glycoprotein (P-gp), edited 3 books and gave around 100 invited lectures. His research focuses on systematic drug design which not only led to the identification of highly active propafenone-type inhibitors of P-gp, but also paved the way for development of new descriptors and virtual screening approaches for identification of new scaffolds active at P-gp. With the increasing knowledge on the importance of P-gp for ADME, his interest moved towards the prediction of P-gp substrate properties. Recently he extended the studies also on other antitargets, such as the hERG potassium channel, as well as on the serotonin transporter, the GABA receptor and the insulin receptor. Gerhard is member of Editorial Advisory Boards and Editorial Boards of several journals and Editor of Molecular Informatics. He regularly organizes a summer school on drug design in Vienna and coordinates the EUROPIN PhD program in Pharmacoinformatics and the IMI project Open PHACTS. Currently he is also President of the European Federation for Medicinal Chemistry.

Professor Girolamo CIRRINCIONE, Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari, Università degli Studi di Palermo, Italy

Professor Girolamo Cirrincione graduated in chemistry from the University of Palermo, in March 1974. After completing one year of military service, he did his postdoctoral fellowship at the Medicinal

Chemistry Department of the University of Palermo from May 1975 to March 1976; from April 1976 to October 1981, he was a research fellow and from November 1981 to October 1994 an associate professor of medicinal chemistry at the same institution. Since November 1994, he has been a full professor of medicinal chemistry at the University of Palermo. He has obtained CNR-NATO Fellowships (September 1982–May 1983, July–August 1986, July– September 1989) and British Council Fellowship (August 1984), from the School of Chemical Sciences of the University of East Anglia (Norwich, UK). He has served as the director of the Istituto Farmacochimico (March 1995–June 1999) and director of the Dipartimento Farmacochimico Toss. Biol. (July 1999–December 2004 and July 2005–Dec. 2010). He is responsible for ERASMUS exchanges of the Faculty of Pharmacy of the University of Palermo; for the research sector 'Synthetic Analogues of Natural Structure of Biological Interest' of the ICTPN-CNR in a scientific capacity (January 1994–December 1998). He is Coordinator of the PhD course in Medicinal Chemistry from 2009 to date. He is member of the Drug Discovery Committee of the European Organization for Research and Treatment of Cancer, the Societa` Chimica Italiana, and the International Society of Heterocyclic Chemistry (which he has also served in the capacity of vice-president for the period 2004–05). He is a scientific editor of the journal ARKIVOC. The research activity is devoted to the design, synthesis, reactivity studies, biological evaluation and SAR studies of nitrogen heterocyclic systems of pharmaceutical interest.

Prof. Yvette TACHE, Center for Neurobiology of Stress and Digestive Diseases Research Center, Digestive Diseases Division, UCLA and VA Greater Los Angeles Health Care System, Los Angeles, CA, USA.

Yvette Taché is Professor of Medicine at the UCLA, Department of Medicine and co-Director of the NIH Digestive Diseases Research Center and NIH Women's Health and Functional Visceral Disorders Center, at the Digestive Diseases Division, Los Angeles, California, USA. She obtained her undergraduate degree in Human Biology and Animal Physiology from the Claude Bernard University in Lyon in 1969, France and in 1974 her Ph.D. in Experimental Medicine and Surgery from the University of Montreal, Canada, under the leadership of Professor Hans Selye, who pioneered the concept of stress and performed postdoctoral training at the Salk Institute, La Jolla, California, USA, with Dr. Vale's group who characterized the stress hormone, corticotropin releasing factor (CRF), and related family members and their receptors. Her field of expertise is brain-gut interactions as it relates to brain and autonomic pathways and transmitters influencing gastrointestinal function and gut hormones regulating food intake. Dr. Taché's work contributed to the current understanding of the brain peptidergic network involved in the gastrointestinal motor response to stress which may have implications to irritable bowel syndrome as published in 320 original articles and 150 chapters. She obtained NIHDDK MERIT Award, the

Distinguished Research Award in Gastrointestinal Physiology from the American Physiological Society, the Janssen Award for Basic Research in Gastrointestinal Motility, and in 2008, the Outstanding American Gastroenterology Association Women in Science

Prof. John C. DEARDEN, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, U.K.

Professor John Dearden is Emeritus Professor of Medicinal Chemistry at the School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, U.K. He has worked in the area of QSAR (quantitative structure-activity relationships) for over 40 years, using the technique to model and predict drug potency, drug toxicity, ADMET properties, ecotoxicity and adverse health effects, and physico-chemical properties of chemicals. He has published over 250 scientific papers and book chapters, and has edited two books on QSAR. He is an honorary member of the Royal Pharmaceutical Society of Great Britain for his pharmaceutical research work. In 2004 he received the International QSAR Award for significant contributions to QSAR in environmental sciences.

Prof. Holger STARK, Goethe University, Institute of Pharmaceutical Chemistry, Frankfurt, Germany

Professor Stark has studied Pharmacy at the Free University Berlin, Germany. Ph.D. "Drug Development of Prodrugs with Agonistic Active Compounds and of Antagonists of the Histamine H_3 Receptor", Free University Berlin, Germany (Supervisor W. Schunack); co-operation J.-C. Schwartz, Paris, and C.R. Ganellin, London; (summa cum laude). He has served as Scientific Worker and then as Assistant Professor (Hochschulassistent) at the Institute of Pharmacy, Free University Berlin. Since 2000 he is Professor for Pharmaceutical/Medicinal Chemistry at the Johann Wolfgang Goethe-University, Institute for Pharmaceutical Chemistry, Frankfurt am Main, Germany. He has been supervising Ph.D. and Master students on different topics in Medicinal Chemistry.

He has more than 170 original papers, review articles and book contributions, more than 380 national and international lectures and poster presentations, nine international patents with more than 60 national and divisional filings.

His research interests are in Medicinal Chemistry: Drug research for CNS drugs/neurotransmitters, dopamine receptor subtypes, histamine receptor subtypes, NMDA receptors, lipid signalling, sphingolipid modulators, biomarkers (fluorescence ligands), radioligands (PET, SPECT), prodrugs, partial agonists, inverse agonists, allosteric modulators, multiple target compounds, molecular modelling, metabolic enzymes, Parkinson's disease, Alzheimer's disease, drug addiction, schizophrenia, inflammation, immunology etc.

Prof. Federico CORELLI, Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Italy.

Professor Federico Corelli graduated in Medicinal Chemistry at the University of Rome La Sapienza in 1977 under the supervision of Prof. R. Giuliano. He joined Artico's group at the same University in 1980 and was appointed Assistant Professor in 1983. In 1987 he moved as Associate Professor of Medicinal Chemistry to the University of Siena, where he was appointed Full Professor since July 1st, 2000 and Dean of the Faculty of Pharmacy since November 1st, 2003 to October 31, 2009.

He served as the Chairman for two cycles of the Ph.D. School in Pharmaceutical Sciences of the University of Siena and in 2007 was appointed in the Scientific Committee of the Ph.D. School in Science of Drugs and Bioactive Substances of the University of Pisa. He was in the scientific/organizing board of twenty national and international scientific conferences. Since 2008, he is the Chairman of the Workshop on Synthetic Methodologies in Medicinal Chemistry of the Division of Medicinal Chemistry of the Italian Chemical Society.

His research interests include the synthesis of biologically active compounds, especially antiviral, antibacterial and antifungal compounds, CNS and antitumor agents. His current research activity mainly deals with the synthesis and biological evaluation of new compounds able to interact with enzymes (thymidine phosphorylase, purine nucleoside phosphorylase) and proteins (heat shock protein 90) involved in the origin and progression of cancer, with enzymes (reverse transcriptase, integrase) controlling HIV replication, with cannabinoid receptors. He is the author of 6 patents, 180 scientific papers, 8 chapters in books, more than 90 oral/poster communications in international and national meetings. He has also been editor of a book.

Prof. Henrik E. POULSEN, Clinical Pharmacolocal Laboratorium Q7642, Rigshospitalet,. Copenhagen, Denmark.

Medical doctor, University of Copenhagen 1976, doctoral thesis 1986.

1976-1978 Medical Training, Lund and Helsingborg University Hospital, Sweden

1978-1985 Research Fellow, Rigshospitalet, University Hospital Copenhagen

1985-1986 Intern, Department of Medicine, Rigshospitalet, Copenhagen

1986-1994 Associate Professor, Dept Pharmacology, University Copenhagen

1994-1996 Professor, Dept Pharmacology, University Copenhagen

1996-present Professor, Dept Clin Pharmacology, Rigshospitalet, Copenhagen.

Editorial Board Member: 5 international journals.

President Society for Free Radical Research Europe 2009-11.

Minimum 5 invited lectures at international conferences/meetings per year the last 5 years.

Main interests are oxidative stress and relation to disease development and complications. Pioneering work within non-invasive estimation of oxidative stress in humans with particular reference to nucleic acid oxidation, development of highly sophisticated mass spectrometry methods and a large number of clinical explanatory and intervention trials. Present research is centered about hemachromatosis and diabetes.

Other research interests include pharmacogenetics and pharmacoepidemiology.

PubMed count 266 publications, 7018 citations (4927 without self citations), Hirsch 41, Average citations 31.05, two top citations 478, 387.

12th Conference in Advanced Medicinal Chemistry

PLENARY LECTURES

Understanding NMDA receptors; key targets for therapeutic intervention in disorders of the Central Nervous System

F Anne Stephenson

School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, United Kingdom

The fidelity of synaptic function in the brain is dependent on the expression of the appropriate neurotransmitter receptor subtype, the targeting and trafficking of these receptors to synapses as well as the regulation of the actual number of receptors at synapses. The family of N-methyl-D-aspartate (NMDA) receptors are a subclass of the excitatory, ionotropic L-glutamate neurotransmitter receptors. NMDA receptors are important for normal brain function being both primary candidates for the molecular basis of learning and memory and in the establishment of synaptic connections during the development of the central nervous system. NMDA receptors are also implicated in neurological, neurodegenerative and psychiatric disorders. Their dysfunction which is primarily due to either hypo- or hyper-activity is pivotal to these pathological conditions. There is thus a fine balance between NMDA receptor-mediated mechanisms in normal brain and in diseased states where receptor homeostasis is perturbed.

NMDA receptors are activated by the co-agonists, L-glutamate and glycine, the alleviation of a voltage-dependent magnesium blockade by activation of adjacent non-NMDA glutamate receptors resulting in the opening of an integral ion channel with a high permeability for calcium ions. There are seven genes encoding NMDA receptor subunits. Functional NMDA receptors are hetero-tetrameric integral membrane proteins being composed of the obligatory NR1 glycine binding subunit assembled with different combinations of NR2 and NR3 NMDA receptor subunits to yield receptors with distinct biophysical and pharmacological properties. Receptor activity is due in part to the number of surface expressed receptors. NMDA receptors are localized at excitatory synapses. These synapses are highly structured but dynamic with interplay between NMDA receptors and NMDA receptor associated scaffolding proteins regulating the expression of functional cell surface synaptic and extra-synaptic receptors. Understanding the assembly and trafficking of this complex, heteromeric, neurotransmitter receptor family may therefore be pivotal to understanding diseases in which their altered activity is evident.

This lecture will describe studies from my research group that have generated highly specific antibody research reagents that have led to the elucidation of the subunit compositions of the major NMDA receptor subtypes, the contribution of the post-synaptic density-95 (PSD-95) membrane associated guanylate kinase (MAGUK) family of scaffold proteins to the organization and lateral mobility of NMDA receptors at

synapses and, key regulatory steps in the assembly and cell surface trafficking of receptors. Finally, I will describe our most recent work in which we have discovered a novel protein-protein interaction between NMDA receptors and amyloid precursor protein (APP), mutations of which have been strongly linked to familial forms of Alzheimer's disease. Despite intense study, the physiological function of APP remains unknown. One proposed role for APP is the regulation of neuronal trafficking. We have shown that APP may contribute to postsynaptic mechanisms via the regulation of the surface trafficking of key subtypes of NMDA receptors thus yielding new insights into the function of APP and possibly, new avenues to explore for the generation of novel therapeutic agents for the treatment of dementia.

The research in my laboratory is supported by the BBSRC (UK); The Alzheimer's Research Trust; the Medical Research Council (UK) and The Wellcome Trust.

Mitochondrial targets for neuroprotection

***Gundars Goldsteins* and Jari Koistinaho**

Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, PO Box 1627, 70211 Kuopio, Finland

Mitochondrial pathology has been described in a number of acute and degenerative brain diseases such as stroke, Parkinson's disease, Alzheimer's disease and ALS. Dysfunction of mitochondria leads to reduced ATP production, impaired calcium buffering, and generation of reactive oxygen species (ROS). Increase in mitochondrial ROS, combined with rise of calcium concentration, may cause opening of the mitochondrial permeability transition pore, resulting in mitochondria swelling, release of cytochrome c (CytC) to the cytoplasm and induction of apoptosis. Identification of mitochondrial targets and protective strategies represents a promising approach for the development of drug candidates fighting acute and age related neurodegeneration.

Mitochondria are the major intracellular source of superoxide, generated by one-electron reduction of oxygen. Instead of reverse oxidation, the detoxifying mechanism for superoxide includes dismutation to hydroperoxide and oxygen, exercised by superoxide dismutases. In parallel to manganese superoxide dismutase (SOD2), which is found in the mitochondrial matrix, a fraction of cytosolic copper-zinc superoxide dismutase (SOD1) is present in mitochondrial intermembrane space (IMS). We have established that, upon mitochondrial stress, SOD1 may compete with CytC for superoxide in the IMS and generate hydroperoxide, which then could react with CytC and form the peroxidase compound I-type intermediate, eventually leading to an increase in ROS production and cell injury. The results obtained demonstrate a novel mechanism, how the presence of SOD1 activity in the IMS causes paradoxically augmented ROS production upon mitochondrial stress.

Mitochondria also contain a unique phospholipid cardiolipin, which is almost exclusively located in the inner mitochondrial membrane. Cardiolipin peroxidation has a deleterious effect on the biochemical function of the mitochondrial membranes, altering membrane fluidity, ion permeability, structure and function of components of the mitochondrial electron transport chain, resulting in impaired oxidative phosphorylation and leading to apoptosis. Specifically, cytochrome c oxidase (COX) multi-protein complex requires binding to cardiolipin, and cardiolipin peroxidation results in decreased enzymatic activity. Recently discovered role of cardiolipin *in vitro* models of apoptosis suggests that it may be a trigger of apoptotic cell death and may serve as a therapeutic target.

Oxytosis, caused by glutamate induced glutathione depletion, results in an early cardiolipin peroxidation and COX inactivation. Our studies have demonstrated that tetracycline antibiotic minocycline efficiently protects cultured cells against the death caused by oxytosis. Minocycline prevents oxytosis through both, direct antioxidant effect and the effects targeted to the mitochondria. We have demonstrated, that minocycline inhibited increase in intracellular ROS and suppressed related oxidative injury markers. It also specifically prevented cardiolipin peroxidation, which is one of the most upstream events in vicious cell death cascade following oxidative stress. And finally minocycline prevented loss of COX activity, which is a keystone for proper mitochondrial function.

These studies shed light on the deleterious role of SOD1 in the mitochondrial IMS and its contribution to the cell death mechanisms of diseases in which mitochondrial pathology is implicated, including neurodegeneneration. The results obtained elucidate a novel mechanism of minocycline protection against oxytosis, preventing cardiolipin peroxidation, inhibiting COX inactivation and provide a basis for the development of neuroprotective therapies.

The molecular basis of drug/transporter interaction - Knowledge driven ligand design

Gerhard F. Ecker

Department of Medicinal Chemistry, University of Vienna, Althanstrasse 14, 1090 Wien, Austria.

Drug transporters play a major role in the uptake, disposition, efficacy and safety of drugs and drug candidates. Up to now more than 400 membrane transporters have been identified in the human genome. P-glycoprotein (P-gp), the paradigm transporter in the field, has been discovered more than 30 years ago as being responsible for multiple drug resistance in tumor cells. Although hundreds of compounds have been identified as inhibitors of P-gp and more than 20 entered clinical studies, none of them has been approved so far. This questions the druggability of P-gp and related ABC-transporters.

With an increasing understanding of the function and physiological role of ABC-transporters, their major contribution to bioavailability, brain permeation and clearance of drug candidates became evident. Thus, designing in and designing out substrate properties of potential drug candidates comprises a hot topic and – due to the polyspecificity of the transporters – also a major challenge. Within the talk an overview on our ligand- and structure-based approaches for modulating drug/transporter interaction will be presented. Special emphasis will be given on knowledge-driven concepts such as experimental data guided ligand docking and on the application of machine learning for classification of substrates and non-substrates. Finally, the expansion to other targets and antitargets, such as the serotonin transporter and the hERG potassium channel, will be presented.

Potent antineoplastic activity of isoindolo-quinoxalines

Girolamo Cirrincione

Università degli Studi di Palermo - Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari – Via Archirafi 32, 90123 Palermo, Italy

Polycyclic nitrogen heterocycles with planar structure can be good pharmacophores for classes of antitumor drugs since they can intercalate between the base pairs of double-stranded DNA. Well known cancer chemotherapeutic agents, such as Anthracyclines, Captothecin and Amsacrine, characterized by planar polycyclic systems are able to interfere with DNA-processing enzymes (Topoisomerases I and II) by forming a ternary complex involving the drug, the DNA and the enzyme.[1] Quinoxalines and the structurally related compounds represent an important class of heterocycles which showed a wide range of anticancer properties.[2] Recently some of this type of molecules have been reported as candidates for the treatment of cancer and disorders associated with angiogenesis functions.[3]

Angiogenesis is a critical determinant of tumour growth and the development of metastases. Tubulin inhibitors have been shown to be effective inhibitors of angiogenesis. Microtubules generated by polymerization of tubulin α,β-dimers are the main constituents of the mitotic spindle, whose formation and activity are required for chromosome segregation during mitosis and for other cell processes.

As part of our continuing work to search for novel antitumor compounds, we synthesized isoindolo[1,2-*a*]quinoxalin-4(5*H*)ones which showed remarkable antineoplastic activity. At the molecular level, such compounds act through inhibition of tubulin polymerization and topoisomerase I activity.[4] The synthesis of more water soluble compounds of this series, toxicity and *in vivo* studies will be discussed.

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Corticotropin releasing factor receptors and stress-related gut diseases: physiology and therapeutic potential

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Gastrointestinal (GI) motility disorders such as irritable bowel syndrome (IBS), functional dyspepsia, and gastroesophageal reflux diseases represent conditions commonly seen by gastroenterologists. Research efforts are needed on the numerous systems and processes that may be impaired in functional and motility disorders, including the brain-gut interactions, the enteric nervous system (ENS), and sensory mechanisms. In animal models and human diseases, gut inflammation, altered gut mucosal immunity, psychiatric conditions or psychosocial factors all may modulate the reciprocal pathways between the brain and the gut, inducing state of hypersensitivity. Advances have been made in the degree to which emotional processes participate in the generation and/or exacerbation of symptoms in chronic functional disorders. In particular dysregulation of stress circuit can affect sensory and motor function of the gut. Clinical investigations also support the notion that stress contributes to visceral hypersensitivity of the gut observed in patients with irritable bowel syndrome (IBS) as established by their lowered threshold of pain to colorectal distension (CRD). Prospective studies also established that there is a 4%-31% incidence of post infectious IBS following bacterial gastroenteritis and stressful life events increase the risk to develop such post-infection IBS.

The characterization and distribution of corticotropin-releasing factor (CRF) family of peptides, CRF, urocortin 1 (ucn 1), urocortin 2 (ucn 2) and urocortin 3 (ucn 3), and the two G-protein coupled receptors, CRF_1 and CRF_2, as well as the development of selective CRF_1 and CRF_2 receptor antagonists provided novel means to understand mechanisms involve in the stress response. CRF has preferential affinity toward the CRF_1 receptor while Ucn 1 has equal affinity to both receptors and Ucn 2 and Ucn 3 are selective ligands for CRF_2. The activation of brain CRF_1 signaling pathway plays a primary role in the endocrine (activation of pituitary adrenal axis), behavioral (anxiety, depression), autonomic (sympathetic and sacral parasympathetic activation, vagal inhibition), and decrease immune responses to stress. Combined anatomical, pharmacologic and molecular approaches support a role of CRF receptor activation in the brain as part of key mechanisms involved in stress-related alterations of gut propulsive function. Inhibition of gastric emptying and stimulation of colonic motor function are the commonly encountered patterns resulting from exposure to various stressors in animals and humans. Activation of brain CRF_2 receptors mediates stress-related inhibition gastric motor function while that of CRF_1 receptors are involved in the stimulation colonic secretory and motor functions.

The brain and the gut overlap in their peptidergic content and recent studies provide anatomical support for the existence that the CRF signaling system is also part of the brain-gut peptidergic axis. The CRF system in the gut is regulated under stress conditions particularly in response to immune challenge. In our studies we established that

CRF_{2b} receptor is a predominant subtype isoform expressed in the gastric corpus with a70-fold higher mRNA level than CRF_1 receptors. Lipopolysaccharide injected intraperitoneally at a low dose induces a time dependent increases in mRNA levels of ucn 1, ucn 2 or ucn 3 in both mucosa and submucosa plus muscle layers at 2, 6, 9 h, with peak levels at 6h, and returned to the control level at 24h while there was a downregulation of $CRF_{2b}R$ mRNA in the gastric mucosa and submucosa/muscle layers 2, 6, 9 h respectively after LPS injection, and recovery at 24h. LPS injection inhibits gastric emptying that was prevented by the peripheral injection of CRF_2 receptor selective antagonist, astressin$_2$-B while the CRF_1 antagonist, CP-154,526 had no effect. We also established that the exogenous administration of Ucn 2 decreased dose-dependently gastric phasic activity and peptide action was blocked astressin$_2$-B. These data indicate that CRF_2 signaling pathway is prominently expressed in the rat stomach and regulated by LPS and plays a role in the associated delayed gastric emptying.

By contrast, in the colon CRF_1 receptor signaling is a key component of the local arm of the colonic response to stress. We showed that CRF mRNA level, detected by reverse transcription-polymerase chain reaction (RT-PCR) was 1.3-fold higher in the distal than proximal colon and 3.4-fold higher in the proximal colonic submucosa plus muscle layers than in mucosa. CRF immunoreactivity is located in the epithelia, lamina propria and crypts, and co-localized with tryptophan hydroxylase, a marker for enterochromaffin (EC) cells, and in enteric neurons. Lipopolysaccharide increased defecation by 2.9-fold and upregulated CRF mRNA by 2.5-fold in the proximal and 1.1-fold in the distal colon and increase circulating levels of CRF as detected using a novel RAPID method of blood processing. LPS-induced increased CRF mRNA expression occurred in the submucosa plus muscle layers (1.5-fold) and the mucosa of proximal colon (0.9-fold) and at the protein levels CRF immunoreactivity was elevated in the submucosal and myenteric plexuses of proximal and distal colon compared to saline groups. Laser capture microdissection combined with RT-PCR and immunohistochemistry in longitudinal muscle myenteric plexus whole-mount colonic preparations revealed CRF_1 receptor expression in myenteric neurons. When CRF is injected peripherally, the peptide activate selectively myenteric plexus of proximal and distal colon as shown by Fos immunoreactivity, a marker of neuronal activation while no Fos was found in gastric corpus, antrum, duodenum, jejunum and ileum myenteric neurons. Fos immmunoreaticity induced by CRF was located in 55 +/- 1.9% and 53 +/- 5.1% of CRF_1 receptor-IR myenteric neurons and in 44 +/- 2.8% and 40 +/- 3.9% of cholinergic neurons with Dogiel type I morphology, and in 20 +/- 1.6% and 80 +/- 3.3% of nitrergic neurons in proximal and distal colon respectively. Further functional studies established that intraperitoneal (ip) injection of the selective CRF_1 agonist cortagine (10 ug/kg ip) results in a significant decreased of distal colonic transit time by 45% without affecting gastric transit, increased distal and transverse colonic contractility by 35.6 and 66.2%, respectively, and induced a 7.1-fold increase in defecation and watery diarrhea in 50% of rats during the 1st hour postinjection. Cortagine ip also increased colonic permeability, activated proximal and distal colonic myenteric neurons, and induced visceral hypersensitivity to a second set of phasic colorectal distention (CRD). The CRF antagonist astressin abolished ip cortagine-induced hyperalgesia whereas injected intracerebroventricularly, it had no effect indicative of receptor interaction occurring in the periphery In addition, CRF_1 antagonists studies showed that peripheral CRF_1 receptor activation contributes to the development and maintenance of hyperalgesia to CRD under conditions of acute or chronic stress. Likewise in mice, cortagine stimulated defecation by 7.8-fold, induced 60% incidence of diarrhea, and increased visceral pain to CRD. The peptide also induces a pro-inflammatory profile selectively in terminal

ileum unlike the proximal colon as shown by the up-regulation of TNFα and interleukin (IL)-1ß and down-regulation IL-10 and IFN gamma mRNA expression 2 h after injection. Reduced mRNA levels of the tight junction molecules claudin-1 and -8 in the ileum, were associated with increased bacterial load in mesenteric lymph node and liver and apoptosis of ileal epithelial and lamina propria cells 8 h after cortagine. Therefore stress-like ileal and colonic alterations are induced by ip cortagine in rats and mice through restricted activation of peripheral CRF_1 receptors. These pre-clinical studies support a role for peripheral CRF_1 signaling as peripheral effectors of the stress response in the gut. In addition of clinical phase I data support that targeting of CRF_1 receptors may open new therapeutic venues for stress-related functional gastrointestinal disorders

In summary, CRF and related peptide, ucn 1 acting on CRF_1 receptor signaling pathways have been identified and characterized to coordinate the various facets of the endocrine and behavioral responses to stress. The role of CRF signaling system at both central and peripheral levels is gaining also recognition as part of the neurobiological common denominator of IBS symptoms susceptible to stress and anxiety/depression. There is evidence for elevated levels of CRF in patients with major depression, anxiety and vulnerability to stress as well as those suffering from obsessive compulsive disorders, posttraumatic stress disorders or childhood trauma. Investigations in IBS patients indicate also that there is an overactivity of the hypothalamic pituitary axis and enhanced plasma CRF response to mental stress. In experimental animals, CRF receptors are expressed in hypothalamic, limbic nuclei and pontine circuitries regulating anxiety and autonomic outflow to the gut as well as in colonic myenteric neurons, mast cells and enterochromaffin cells. Recently we developed an experimental model that recapture cardinal features of IBS-diarrhea predominant patients with regard to stress-related hyperalgesia to CRD, increased colonic permeability, mast cell degranulation, activation of the enteric nervous system, propulsive motor function (motility, transit, defecation and development of diarrhea using the administration of selective CRF_1 receptor agonists, cortagine or $stressin_1$ in rodents. CRF_1 receptor antagonists blunt acute and chronic stress-induced visceral hyperalgesia and colonic propulsive events. By contrast, activation of CRF_2 receptors attenuated the CRF_1 mediated colonic and hyperalgesic responses to either administration of CRF_1 agonists or stress and contributed to gastric stasis induced by immune stressors. These data suggest that dysregulation of CRF signaling pathways including an overactivity of CRF_1 signaling and/or defective mounting of the CRF_2 inhibitory mechanisms may play a role in the manifestations of symptoms in diarrhea predominant IBS.

The art of developing valid QSARs

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A QSAR (quantitative structure-activity relationship) is a mathematical correlation between a property (biological, chemical or physical) of interest and one or more indicators of molecular structure. There are four broad stages in the development of a QSAR model. Firstly, values for the property of interest have to be obtained, either by experiment or from the literature, for a number of representative compounds. Secondly, indicators of molecular structure (commonly termed descriptors) have to be obtained, by experiment, from the literature, or by using appropriate software. Thirdly, a statistical technique has to be used to select those descriptors that best model the property of interest, and to derive the mathematical expression that is the QSAR. Fourthly, the QSAR has to be validated; that is, the model has to be tested to check that it has predictive ability, so that it can be used with confidence to predict the value of the property of interest for compounds not used in the development of the model.

Most descriptors fall into three broad classes – hydrophobic, electronic, and steric. Hydrophobicity generally models transport of a xenobiotic within an organism, although it can also reflect hydrophobic binding to a receptor. It is usually represented by the octanol-water partition coefficient (P). Electronic descriptors are the broadest class, and include polarity, polarisability, hydrogen bonding, atomic charge, molecular energy levels and many others. Steric descriptors model molecular size and shape; the former is quite simple to model, and includes such descriptors as molecular weight and molar volume, but molecular shape is more difficult, and there is as yet no good universal way of doing so.

Some years ago, the OECD published guidelines for QSAR development, as follows: a valid QSAR should have:

1. a defined endpoint;
2. an unambiguous algorithm;
3. a defined domain of applicability;
4. appropriate measures of goodness of fit, robustness and predictivity;
5. a mechanistic interpretation, if possible.

Within each of those requirements, there are a number of more detailed requirements. However, sadly it is still the case that many published QSAR studies do not meet all those requirements. A recent publication from our laboratory (Dearden et al, *SAR & QSAR in Environmental Research* **20** (2009) 241-266) reported

21 different types of error that occur in published QSAR studies. These include failure to take account of data heterogeneity, use of inappropriate endpoint data, use of collinear descriptors, use of incomprehensible descriptors, error in descriptor values, poor transferability of QSARs, inadequate and/or undefined applicability domain, unacknowledged omission of data points, use of inadequate data, replication of compounds in datasets, too narrow a range of endpoint values, over-fitting of data, use of excessive numbers of descriptors, lack of and/or inadequate statistics, incorrect calculation, lack of descriptor auto-scaling, misuse and/or misinterpretation of statistics, lack of consideration of residuals, inadequate training set and/or test set selection, failure to validate a QSAR correctly, and lack of mechanistic interpretation.

This presentation will consider each of these errors, and will offer guidance on the correct development of QSARs.

From target discovery to clinical trials: The example of the histamine H_3 receptor and Pitolisant

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Ligands for histamine H_1 and H_2 receptor subtypes have advanced to blockbuster drugs in the last century [1], and H_3 and H_4 receptor ligands may follow in the near future. One of the recent members of the histamine GPCR family, the histamine H_3 receptor (H3R), has been recognized as a a promising target in the development of new small molecule therapeutics for the potential treatment of mainly centrally occurring diseases e.g. sleep/wake state, cognitive impairment, epilepsy, schizophrenia, migraine, pain but also allergic rhinitis.

Based on derivatisation and modification of the endogenous ligand we have developed different classes of highly potent and selective imidazole-containing antagonists/inverse agonists (e.g. ciproxifan [2]) which show important species variances on affinity and efficacy mainly acid caused by a single amino acid change [3]. Shifting to non-imidazole antagonists made it possible to develop a more robust general H3R pharmacophore which can be applied on heterogeneous drug design strategies. Pharmacokinetic and toxicological optimization can be addressed as well as the design of multiple targeting ligands. Drug optimization leading to pitolisant as promising H3R antagonist in late stage of clinical development for narcolepsy and excessive daytime sleepiness in Parkinson patients shows a good example of translational academic/industrial research from basic to applied sciences [4].

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Targeting the Endocannabinoid System: Synthesis, SAR and pharmacological evaluation of quinolone-3-carboxamides as potent and selective CB2 ligands

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Research in medicinal chemistry during the last years has led to the identification of a spectrum of compounds with different affinities and selectivities for cannabinoid type 1 (CB1) and type 2 (CB2) receptors, compounds that have been essential in characterizing the role of cannabinoid receptors in the body. Differences in receptor distribution and signal transduction mechanisms are likely to account for the relative absence of CNS side effects induced by CB2 ligands. These considerations suggest that novel pharmacotherapies selectively targeting CB2 receptors may have considerable therapeutic potential. Accordingly, significant medicinal chemistry efforts have been directed to the characterization of selective CB2 agonists, leading to the identification of compounds eliciting antinociceptive effects in models of acute pain, persistent inflammatory pain, post-operative pain, cancer pain, and neuropathic pain.

In the last years a number of reports have suggested that CB2 inverse agonists/antagonists may possess antiinflammatory activity, being able to inhibit carrageenan-induced paw oedema in mice, to reduce leukocytes trafficking and to impair the migration of cells towards cannabinoid agonists. CB2 receptor is also an emerging target in oncology research (mainly gliomas) as well as in osteoporosis disease modification, as it has been reported to regulate bone mass, but conflicting results have been reported with regard to its effects on bone resorption and osteoclast function.

This presentation will show our contribution to this field, focussing in particular on

- the preparation of a large set of quinolone-3-carboxamides bearing at position 5, 6, 7, or 8 diverse substituents, such as halides, alkyl, aryl, alkoxy, and aryloxy groups, differing for their steric/electronic properties
- in vitro pharmacological evaluation of the new compounds
- SAR development
- investigation of the functional profile of the most interesting compounds in vitro and/or in vivo
- structural optimization aimed at improving water solubility through a bioisosteric approach.

Oxidative damage to nucleic acids: clinical relevance

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Oxidative stress and 8-hydroxylation of guanine in DNA (e.g. 8-oxodG) can result in base mispairing and GT:TA transversion mutations, and can induce proliferation, apoptosis or necrosis. Several conditions include increased oxidative stress, i.e. smoking, extreme exercise, high iron status and several diseases. There is growing evidence that oxidative stress, and in particular modifications to DNA induced by oxidative stress, is of importance in the pathogenesis of several diseases and/or their complications.

Interestingly, RNA is more prone to oxidation than DNA because it is predominantly single stranded and do not have bound protective proteins, but has attracted very little attention. In the last decade, however, there have emerged several reports that emphasize the importance of RNA oxidation, although it still is very little researched. In certain areas of the brain there are report of oxidation of guanines in more than 50% of RNA molecules in degenerative brain disorders compared to very little in controls, and furthermore it the location in the brain is not random, and occur very early and before disease symptoms are evident. The consequences of RNA oxidation are only emerging, and mutated proteins, lower concentrations of proteins, cell deterioration and even cell death.

Measurement of the guanine 8-hydroxylation can be done in DNA, or excreted repair products can be measured in urine. In DNA the levels of unmodified guanines are 6 orders of magnitudes higher than the 8-hydroxylated guanine, and the repair products are excreted into urine together with a large number of products of similar size/structure in considerable concentrations. Multicenter initiatives such as ESCODD and the present ESCULA projects have established methods providing much more reliable and validated analytical methods for use not only in chemistry and cells, but also in whole organisms including man, and also have demonstrated the proper interpretation of the measurements. The most reliable, specific and sensitive methodology for measurement of DNA modification by oxidation in tissues, and for measurement of repair products into urine seems to be liquid chromatography coupled with tandem mass spectrometry. This method also includes the possibility of measuring several products at in the same run.

While there is no doubt that oxidation of DNA, exemplified by the prototype lesion 8-oxodG, occurs in vivo and can lead to changes with severe disturbances in cells, the relative contribution of oxidation of DNA to the variety of other possible insults is less clear. Regarding RNA oxidation there is emerging evidence that it is of similar importance and might even be more sensitive for measuring very early envents

in the assumed pathogenesis.

Still, data than create links from prospective trials in humans between oxidative DNA lesions and diseases, are sparse, but the development of sufficient methodology is in place and such evidence is emerging. One interesting example of such a link between iron status, nucleic acid oxidation and diabetes.

There is a dire need for application of the developed and validated biomarkers to prospective studies between nucleic acid oxidation and disease development and disease complications, and there is great hope that such biomarkers can be used to find and monitor treatment modalities.

12th Conference in Advanced Medicinal Chemistry

POSTERS

Novel thiazolimino–5–aryliden–4-thiazolidinones as potent antibacterial agents. Estimation of structure–activity relationship

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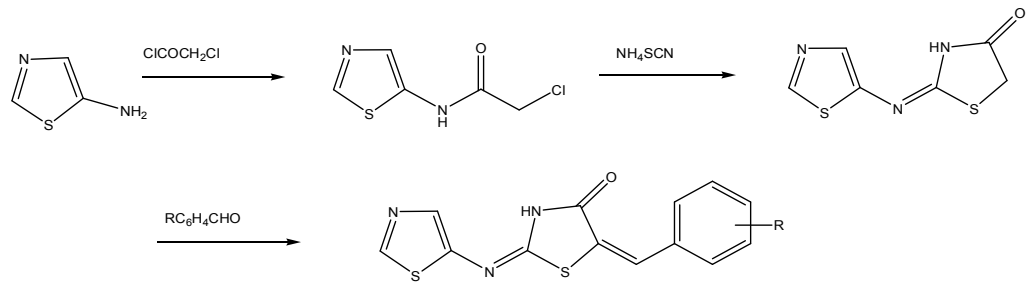
Despite the rapid progress of science, the treatment of infectious diseases still remains a serious problem mainly because of the different factors leading to the emergence of these diseases and also the increased number of pathogenic microorganisms with multiple resistance to drugs. Therefore, countering the emerging resistance of microorganisms requires the proper and balanced use of antimicrobial drugs.

Heterocycles represent "privileged structures" capable of binding to receptors with high affinity. During our outgoing project on the synthesis of compounds with potent biological action, was testified that many thiazole derivatives act as antibacterial agents. This led us to proceed to the synthesis of the title compounds and evaluation their possible antibacterial activity. The synthesis and structures of the compounds are shown in Scheme 1.

In order to determine their antibacterial activity, they were tested against human pathogenic bacteria by using the microdilution method. The following Gram-negative bacteria were used: Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Enterobacter cloacae (human isolate) and the following Gram-positive bacteria: Listeria monocytogenes, Bacillus cereus (clinical isolate), Micrococcus flavus, and Staphylococcus aureus. Some of the title compounds have shown desirable action against specific kinds of bacteria.



R= 1) 3-F, 2) 4-F, 3) 3-Br, 4) 2,6-diCl, 5) 2,4-diCl,6) 4-CH_3,7)2,3diCl

Scheme 1

TOWARDS MORE EFFECTIVE ALDOSE REDUCTASE INHIBITORS: DESIGN SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SELECTIVE PYRROLYL-DIFLUOROPHENOLS WITH ADDITIONAL ANTIOXIDANT CAPACITY

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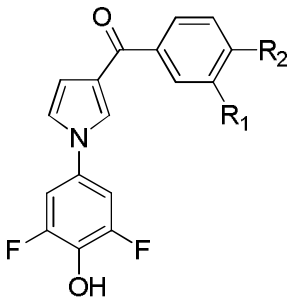
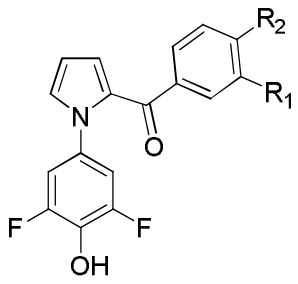
Diabetes mellitus is a complex metabolic disorder that affects westernalized societies all the more in the last decades[1]. According to the National Center for Health Statistics of the USA[2], diabetes consists the 6th leading cause of death in the USA, an attribute highly related to the emergence of chronic diabetic complications. Macroangiopathies, such as coronary artery disease, peripheral vascular disease and cerebrovascular disease are some of the reasons of high mortality, accompanied by nephropathy, neuropathy and retinopathy that minimize the quality of the diabetic's life[3].

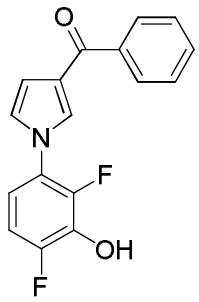
During the last decades the polyol pathway has been widely recognized as one of the main routes that give rise to diabetes chronic implications, along with oxidative stress and the formation of advanced glycation end products (AGEs)[4,5]. Aldose reductase (ALR2), as the first and rate limiting enzyme of the polyol pathway, has therefore emerged as an intriguing molecular target for the prevention of chronic implications of diabetes. Even though many aldose reductase inhibitors (ARIs) with high potency have been synthesized, some of which even reached clinical trials, there is only one commercially available in Japan (Epalrestat, Ono Pharmaceuticals, Japan) but still none approved by the FDA. The most studied ARIs are derivatives of the carboxylic acid or the hydantoin moiety and these scaffolds have been correlated to either poor biodistribution or acute adverse effects. The high ionization potential of carboxylic acids seems to cause the defects on pharmacokinetics, and the parallel inhibition of ALR2 homologous enzymes (such as aldehyde reductase, ALR1) has been related to the adverse effects observed. On that regard, there is an effort to discover novel chemotypes that can maintain ALR2 inhibitory activity, exhibit improved pharmacokinetics and selectivity towards ALR2. Additionally, the potent antioxidant activity of new ARIs could amplify the therapeutic effect via inhibition of oxidative stress and AGE formation, since all these routes are responsible for the onset and progression of diabetes chronic implications.



1 R_1, R_2 = H
2 R_1 = H, R_2 = OCH_3
3 R_1 = OCH_3, R_2 = H



4 R_1, R_2 = H
5 R_1 = H, R_2 = OCH_3
6 R_1 = OCH_3, R_2 = H



7

2,6-Difluorophenol is considered as a lipophilic bioisoster of the carboxylic acid moiety[6]. Based on this, compound **1** was synthesized and found to be a potent ARI[7] that should also exhibit improved pharmacokinetics compared to the bioisosteric carboxylic acids. In order to determine the effect of substitution on ALR2 inhibitory activity, compounds **2-6** were synthesized through the synthetic strategy of electrophilic aromatic substitution of chemically protected 2,6-difluoro-pyrrol-1-ylphenols. Also, we studied how the position of the difluorophenol ring affects activity. On this regard, compound **7** was synthesized by an Ullmann type conjugation reaction. The synthesized compounds were evaluated for their inhibitory activity on ALR2 and ALR1. ALR1, a member of the oxidoreductase superfamily, exhibits the highest similarity towards ALR2 (65%)[8], and therefore is used as a selectivity determinant. Additionally, the antioxidative activity of the most potent compound (**5**) was determined on the homogenous system of DPPH and the heterogeneous system of DOPC liposomes.

2,6-Difluoro-4-(1H-pyrrol-1-yl)phenol proved more potent than its isoster 2,6-difluoro-3-(1H-pyrrol-1-yl)phenol as a scaffold and some of its substituted derivatives exhibited submicromolar ALR2 inhibitory activity along with high selectivity index and important antioxidant activity.

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IN VITRO & IN VIVO EVALUATION OF A NEW ANTIDYSLIPIDEMIC MOLECULE. A PRELIMINARY PHARMACOKINETIC STUDY

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The majority of cardiovascular diseases and thereby mortality are caused by atherosclerosis which is the result of hyperlipidemia, oxidative stress and inflammation. We have previously developed substituted morpholine derivatives that reduce cholesterol and triglyceride levels, acting as squalene synthase inhibitors, as well as antioxidants.[1] Based on these data and rational drug design, we developed a new lipophilic morpholine derivative (**1**). The new derivative was synthesized employing modifications of known methods and its structure was confirmed by ^{1}H-NMR and elemental analysis. It was found to be a very potent antioxidant with an IC_{50} value of 5.9 μM against lipid peroxidation of **rat** microsomal membranes, while at a concentration of 1 μM it fully inhibited $CuSO_4$-induced oxidation of **human** LDL. *In vivo* (rat) antidyslipidemic evaluation of the new molecule revealed its significant lipid-lowering effects, reducing cholesterol and triglycerides by 90% and 76%, respectively. We further developed an HPLC method for the analysis of *in vivo* samples containing this derivative in order to determine its concentration at its sites of action i.e. blood (prevention of LDL oxidation) and liver (squalene synthase inhibition) after *i.p.*/*p.o.* administration to **rats**/**mice**. The new derivative **1** could be fully extracted from liver tissue and plasma, and after respective validation studies, the estimated LOD was found to be 0.06 μg/mL. The analysis of blood samples at predetermined time points showed detectable amounts of compound **1** in plasma within 10 and 30 minutes after *i.p.*/*p.o.* administration, respectively, as well as at 24 hours after administration, while it is present in liver at 4 hours after administration.

In conclusion, the combination of *in vitro*/*in vivo* activity assays and the developed HPLC method for monitoring the *in vivo* fate of the new morpholine derivative will assist in the correlation of its various therapeutic (i.e. potent antioxidant and antidyslipidemic) effects with its concentration *in vivo* and contribute to the optimization of dose-response relationships for further *in vivo* pharmacological investigations.

[1]Kourounakis A. et al. *Bioorg. Med. Chem.* 2010; 18(21):7402-12.

NEW HETEROCYCLIC ARYLIDENE DERIVATIVES WITH ANTIOXIDANT & ANTI-INFLAMMATORY ACTIVITY

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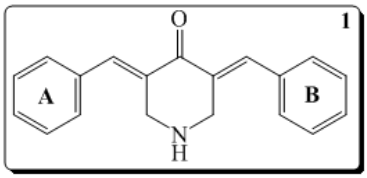
There are many evidences, which led to a close association between chronic inflammation and cancer. It is generally known that chronic inflammation increases the risk of cancer and treatment with NSAIDs reduces the risk of certain cancer. Also the presence of a variety of inflammatory mediators, including cytokines, chemokines, tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2), matrix metalloproteases (MMP) in tumor tissues, angiogenesis are similar to that in chronic inflammation responses [1].

Chalcones is a series of compounds, in which the two aromatic rings are joined by a three carbon, α,β-unsaturated enone system These compounds seem to have cytotoxicity, antitumor, anti-inflammatory, antiplasmodial, immunesuppression, antioxidant and many other biological properties. [2, 3]. Also they posses marked affinity for thiol but not for amino or hydroxyl groups, found in nucleic acids. Since thiols are absent in nucleic acids mutagenic and carcinogenic affects should be absent. Conversion of certain conjugated enones into the corresponding Mannich bases led to significant increases in both the rates of thiol alkylation and cytotoxicity [4, 5]. In our laboratory we tried to synthesize a series of 3, 5- bis (arylidene)-4-piperidones for future development as anti-inflammatory, antioxidant and anticancer agents. These compounds considered as Mannich bases of dienones. We followed two different ways of chemical synthesis. Following a Claisen-Schmidt condensation between 4-piperidone hydrochloride and the appropriate hetero-aryl aldehyde led to the formation of novel derivatives [6, 7]. The compounds have been identified using IR, ^{1}H-NMR, ^{13}C-NMR, elemental analyses and mass spectroscopy. The role of lipophilicity is detrimental for the biological response, thus lipophilicity was determined experimentally as R_m values using RPTLC.



We present the preliminary results from antioxidant and anti-inflammatory activity tests *in vitro* and *in vivo.* The results are discussed in terms of structural characteristics. Further investigation is in progress for their anticancer activity.

SYNTHESIS OF 3′-*C*-CYANO & 3′-*C*-CYANO-3′-DEOXY PYRIMIDINE PYRANONUCLEOSIDES AS NOVEL CYTOTOXIC AGENTS

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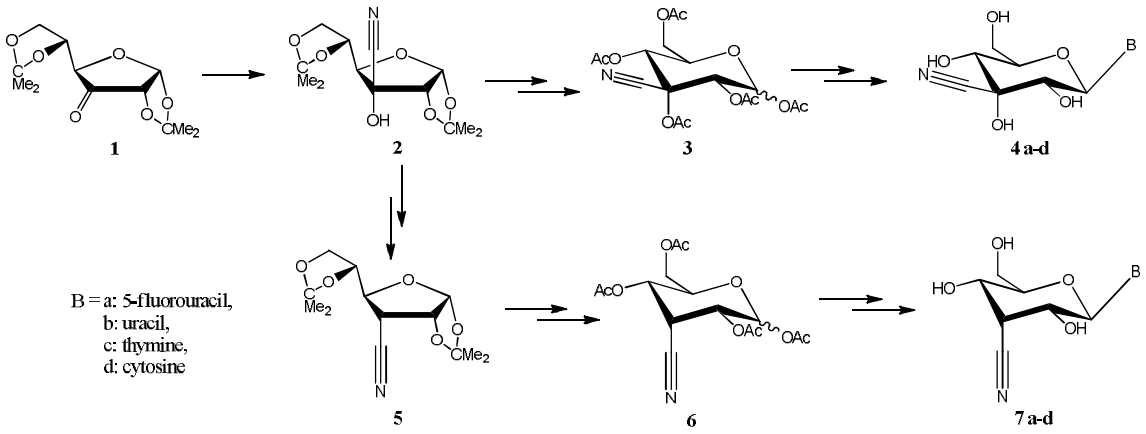
A novel series of 3′-*C*-cyano & 3′-*C*-cyano-3′-deoxy pyrimidine pyranonucleosides has been designed and synthesized. Reaction of 3-keto glucoside **1** with sodium cyanide gave the desired precursor 3-*C*-cyano-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**2**). Hydrolysis followed by acetylation led to the 1,2,3,4,6-penta-*O*-acetyl-3-*C*-cyano-D-allopyranose (**3**). Compound **3** was condensed with silylated 5-fluorouracil, uracil, thymine and N^4-benzoyl cytosine, respectively and deacetylated to afford the target 1-(3′-*C*-cyano-*β*-D-allopyranosyl)nucleosides **4a-d**. Routine deoxygenation at position 3′ of cyanohydrine **2**, led to 3′-*C*-cyano-3′-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**5**). Hydrolysis of **5** followed by acetylation led to the 1,2,4,6-tetra-*O*-acetyl-3-*C*-cyano-3-deoxy-D-allopyranose (**6**). Coupling of sugar **6** with silylated pyrimidines and subsequent deacetylation yielded the target 1-(3′-*C*-cyano-3′-deoxy-*β*-D-allopyranosyl)nucleosides **7a-d**. The novel analogues were found to exhibit promising antitumor activity against murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa). In particular, *C*-cyano nucleoside of 5-fluorouracil **4a** was highly cytostatic at an IC_{50} (50% inhibitory concentration) ranging between 0.6 and 10.0 μM.



DESIGN OF NOVEL BIOACTIVE COMPOUNDS BASED ON OXIDATIVE STRESS.

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The increasing interest in better drugs, the high safety standards, the concern about the economic burden for drug discovery and pharmacotherapy lead to the Rational Drug Design. In Rational Drug Design there are three main steps: 1) Lead compound discovery; 2) Pharmacophore finding, and 3) Optimisation. Rational Drug Design may be based on the pathobiochemistry of the disease and the molecular mechanism of drug action. Herein, examples of designing and producing molecules effective in few pathologic conditions implicating free radicals and oxidative stress are presented.

1) Inflammation: The actions of lipoxygenase and cycloxygenase on arachidonic acid involve the formation of peroxides and other reactive oxygen species. Furthermore, activated leukocytes produce superoxide anion radical. We present the design and synthesis of efficient non steroidal anti-inflammatory compounds, free of gastrointestinal side effects.

E.g.: Amide of ibuprofen with cystein ethyl ester.

2) Atheromatosis: It involves increased levels of LDL-cholesterol, oxidative stress at the site of the vascular damage and increased leukocyte activity. The tissue suffers from inflammation. We report compounds with efficient antioxidant, anti-inflammatory and antidyslipidemic activities.

E.g.: Amide of diclofenac with cystein ethyl ester.

3) Biologic stress and implications: Biologic stress induces oxidative stress, uropepsinogen increase and thymus involution. We describe a compound which can act as an antioxidant, reduces uropepsinogen and normalizes thymus weight in stressed rats.

E.g.: Conjugation of lorazepam - GABA - butylated hydroxybenzoic acid.

4) Senile dementia Alzheimer's type: It is characterised, among others, by oxidative stress, high levels of LDL-cholesterol and brain inflammation. We present a compound that can reduce inflammation act as an antioxidant and lower blood LDL-cholesterol.

It is almost certain that all the above mentioned conditions are multicausal. We, therefore, believe that diseases of this character can be more effectively treated by polyfunctional compounds.

E.g.: Conjugation of ibuprofen - proline- cystein ethyl ester.

SECONDARY METABOLITES OF *STAEHELINA UNIFLOSCULOSA* SIBTH. & SM.

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The genus *Staehelina* (Asteraceae, tribe Cardueae) is represented only by 7 species worldwide. *S. unifloscu*losa Sibth. & Sm is a Balkan endemic species and had not been studied previously. This study is a continuation of the ongoing phytochemical analysis of plants from Asteraceae family. The crude extract of the aerial parts of *S. unifloscu*losa was fractionated by using several chromatographic methods. So far, five flavonoids (eriodictyol, nepetin, hispidulin, jaseocidin and eriodictyol 3´-O-glucopyranoside), one phenolic acid (protocatechuic acid), one phenolic glucoside (arbutin), one lignan (pinoresinol) and four sesquiterpene lactones (artemorin, tamirin, tanachin and reynosin) were isolated and identified. Structure elucidation of the pure compounds was achieved by using spectroscopic methods (1D and 2DNMR).

Acknowledgements: The authors are grateful to Dr. N. Krigas (Scientific collaborator of Department of Biology, Aristotle University of Thessaloniki) for the identification of the plant material.

COMPARISON OF IN VIVO ANTI-INFLAMMATORY ACTIVITY BETWEEN NOVEL SYNTHESIZED SUBSTITUTED (E)-1-(4-METHYL-2-(METHYLAMINO)THIAZOL-5-YL)-3-PHENYLPROP-2-EN-1-ONES AND (E)-1-(2-(ETHYLAMINO)-4-METHYLTHIAZOL-5-YL)-3-PHENYLPROP-2-EN-1-ONES

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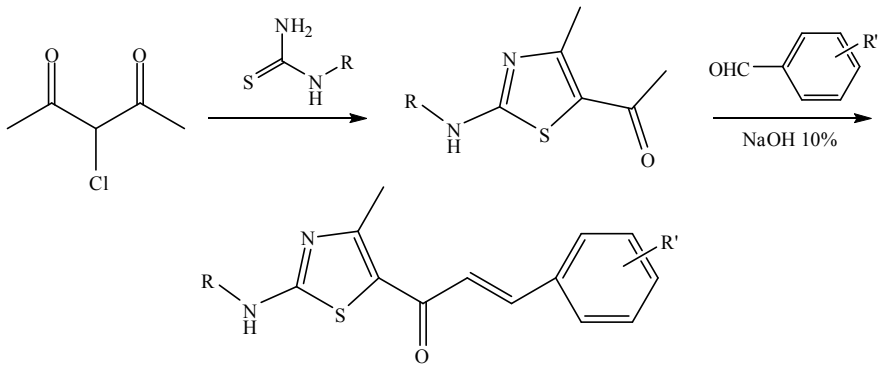
During our outgoing research project on the synthesis of chalcones with 2-(alkylamino)thiazol-5-yl moiety as potent anti-inflammatory agents, was noticed that small changes on the length of the alkylamino chain have significant effect on the % in vivo anti-inflammatory activity on mice.

A series of the title compounds were synthesized according to the procedure given in Scheme 1. Their anti-inflammatory activity was evaluated *in vivo* on mice, using the carrageenin induced paw edema method.

The preliminary results have shown that elongation of the alkylamino chain from methylamino to ethylamino had as a result the improvement of the in vivo anti-inflammatory activity of the 3 out of 4 chalcones. Especially the m-Cl compound exhibited a significant (+12.4 %) improvement of its anti-inflammatory activity.

These promising results will be studied in order to be correlated with the lipophilicity, stereochemistry and COX/LOX *in vitro* inhibitory activity of the title compounds.



R	R'
CH_3 or CH_2CH_3	p-NO_2
CH_3 or CH_2CH_3	m-NO_2
CH_3 or CH_2CH_3	p-Cl
CH_3 or CH_2CH_3	m-Cl

Scheme 1

SYNTHESIS OF NON-SUBSTITUTED/SUBSTITUTED (E)-5-BENZYLIDENE-2-(THIAZOL-2-YLAMINO)THIAZOL-4(5H)-ONES AS POTENT ANTIBACTERIAL AGENTS

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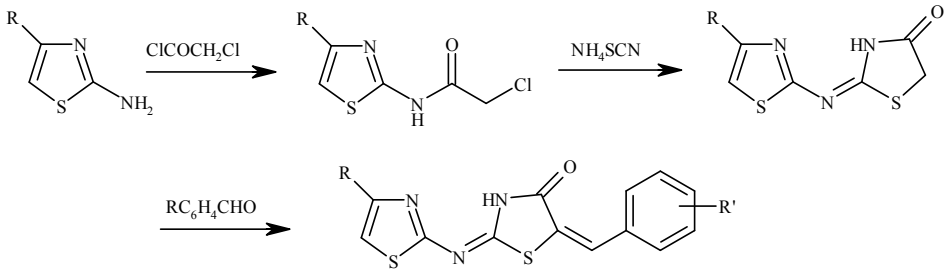
A potential approach to overcome the urgent need for antimicrobials is to design innovative agents with original modes of action that could target both sensitive and resistant strains. Heterocycles represent "privileged structures" capable of binding to receptors with high affinity. During our outgoing project on the synthesis of compounds with potent biological action, was testified that many thiazole derivatives act as antibacterial agents. This lead us to proceed to the synthesis of the title compounds and evaluated their possible antibacterial activity. The synthesis and structures of the compounds are shown in Scheme 1.

In order to determine their antibacterial activity, they were tested against human pathogenic bacteria by using the microdilution method. The following Gram-negative bacteria were used: Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Enterobacter cloacae (human isolate) and the following Gram-positive bacteria: Listeria monocytogenes, Bacillus cereus (clinical isolate), Micrococcus flavus, and Staphylococcus aureus. Some of the title compounds have shown desirable action against specific kinds of bacteria.



R = H, R_1 =2-OMe, 2,5-OMe, 4-Br, $N(CH_3)_2$, 2-OH,5-Br

R = Ph, R_1 = 4-Cl, 3-Cl; R= Me, R_1 = 4-=Cl, 2-Cl.

Scheme 1

NOVEL [Re/^{99m}Tc(L)(CO)$_3$] COMPLEXES WITH NSO AND NSN TRIDENTATE BIFUNCTIONAL CHELATORS. SYNTHESIS, CHARACTERIZATION AND *IN VITRO* STABILITY.

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Organometallic *fac*-[Tc/ReL(CO)$_3$] complexes have gained significant attention in radiopharmaceutical chemistry, since they exhibit kinetic inertness and they are conveniently synthesized at the tracer level. The successful development of targeted diagnostic and therapeutic radiopharmaceuticals involves the bifunctional chelating agent (BFCA) strategy for suitable radiolabeling of biomolecules. [1,2].

In the present work, we describe the synthesis of two novel rhenium complexes of the general formula, *fac*-[Re(NSO)(CO)$_3$] and *fac*-[Re(NSN)(CO)$_3$]$^+$ with novel tridentate BFCAs. NSO is 2-(carboxyethylthio)-3-(1H-imidazol-4-yl)-propanoic acid and was synthesized in a two-step reaction from L-histidine. NSN is 3-(2-aminoethylthio)-3-(1H-imidazol-4-yl)propanoic acid, and was synthesized in an one-step reaction from *trans*-urocanic acid.

The neutral *fac*-[Re(NSO)(CO)$_3$] complex was synthesized by reacting the precursor [NEt$_4$]$_2$[Re(CO)$_3$Br$_3$] with an equimolar amount of the NSO in water. The cationic *fac*-[Re(NSN)(CO)$_3$]$^+$ complex was synthesized by reacting the precursor *fac*-[Re(CO)$_3$(OH$_2$)$_3$]$^+$ with an equimolar amount of the NSN ligand in water. The characterization of the complexes was accomplished by ^{1}H- ^{13}C NMR and FT-IR spectroscopies. RP-HPLC analysis of the reaction mixture of *fac*-[Re(NSO)(CO)$_3$] revealed one major peak, while that of *fac*-[Re(NSN)(CO)$_3$]$^+$ revealed two peaks in a 1:1 ratio; the latter was consequently analyzed by NMR, where the presence of two isomers was established. Both complexes contain a free carboxylate group and can be used for conjugation of a suitable biomolecule.

The tracer *fac*-[^{99m}Tc(L)(CO)$_3$] complexes were prepared by reacting the suitable precursor *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ with mmolar concentration of the ligands. The identity of technetium-99m complexes was established by comparative RP-HPLC using the well characterized rhenium complexes as reference. Studies of the *in vitro* stability in 1mM L-histidine and 1mM L-cysteine over 24 hours will be reported.

In our previous studies [3], we have shown that similar tridentate ligands coordinate potently at the tracer level (^{99m}Tc and ^{188}Re) and show promise for the development of diagnostic and therapeutic radiopharmaceuticals, respectively.

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SYNTHESIS OF 5-SUBSTITUTED PYRIMIDINE GLUCOPYRANONUCLEOSIDES AS NOVEL ANTITUMOR AGENTS

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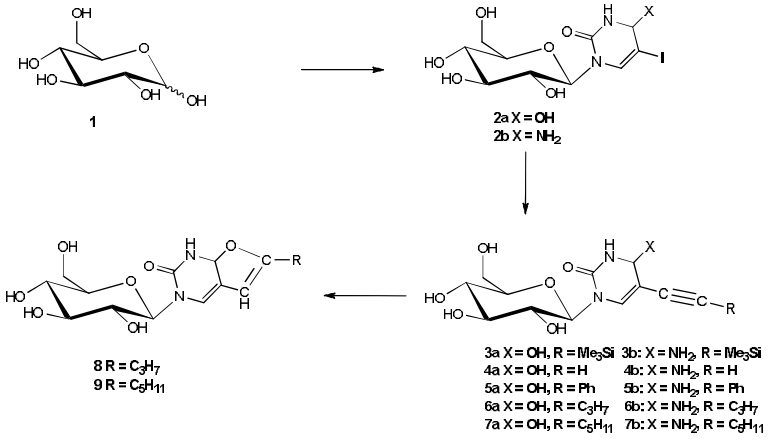
The synthesis of the desired 5-substituted pyrimidine glucopyranonucleosides **4-9**, is described. Acetylation of D-glucose (**1**), followed by coupling with silylated 5-iodouracil and 5-iodocytosine, respectively and subsequent deacetylation of the *β*-nucleosides formed, led to 1-(*β*-D-glucopyranosyl)nucleosides of 5-iodouracil **2a** and of 5-iodocytosine **2b**. Different substituents were then introduced by reaction of terminal alkynes with deprotected nucleosides **2a**,**b** under optimized Sonogashira Pd(0) catalyzed reactions. Thus, reaction of the nucleosides **2a,b** with trimethylsilylacetylene (TMS) in the presence of triethylamine (base), CuI (cocatalyst) and $Pd(PPh_3)_4$ (catalyst) in anhydrous *N*,*N*-dimethylformamide afforded nucleosides **3a,b**, which by the removal of the trimethylsilyl group with *n*-tetrabutylammonium fluoride (*n*-Bu_4NF) yielded 5-ethynyl derivatives of uracil **4a** and of cytosine **4b**. By the same manner, coupling of **2a,b** with phenyl acetylene, pentyne and heptyne respectively, gave the corresponding 5-phenylethynyl-glucopyranonucleosides of uracil **5a** and of cytosine **5b**, 5-pentynyl glucopyranonucleosides of uracil **6a** and of cytosine **6b** and 5-heptynyl glucopyranonucleosides of uracil **7a** and of cytosine **7b**. Finally, treatment of **6a** and **7a** with $AgNO_3$ led to the furanopyrimidine nucleosides **8** and **9**. The novel *C*-5-alkynyl pyrimidine *β*-D-glucopyranonucleosides **4-7** were found to exhibit promising antitumor activity against murine leukemia cells (L1210), human T-lymphocyte cells (CEM), and human cervix carcinoma cells (HeLa). In particular, *C*-5 phenyl ethynyl nucleoside analogue **5a** was highly cytostatic at an IC_{50} (50% inhibitory concentration) ranging between 5.2 and 6.2 μM.



NOVEL BENZOXAZINE AND BENZOTHIAZINE DERIVATIVES AS MULTIFUNCTIONAL ANTIHYPERLIPIDEMIC AGENTS

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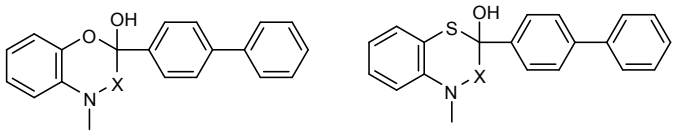
Atherosclerotic cardiovascular disease remains the leading cause of death and/or disability in the developed world. Over the past years, evidence has accumulated suggesting that free radicals, and the lipid peroxidation they induce, could trigger most of the factors involved in atherosclerotic vascular injuries (such as cytotoxicity, inflammation, formation of atheromatic plaques). Oxidative modification of LDL can lead to its increased uptake by macrophages triggering a cascade of cellular processes that lead to the formation of fatty streaks and eventually atherosclerotic lesions at the arterial wall. Since atherosclerosis is a complex disease resulting from multiple molecular abnormalities, we applied a strategy that involves the design of a single chemical compound able to simultaneously modulate more than one targets.

Based on our previous studies[1,2] we hereby present the design and synthesis of novel benzoxazine and benzothiazine derivatives (Figure 1) that demonstrate a significant antioxidant activity inhibiting *in vitro* microsomal lipid peroxidation induced by Fe^{2+}/ascorbate with IC_{50} values between 9 and 422 μM while the most active compound inhibits the oxidation of human LDL, induced by $CuSO_4$, with an IC_{50} of 7.8 μM. Moreover, they have considerable antioxidant activity *in vivo*, reducing plasma MDA levels of hyperlipidaemic rats by 31-63%. The new derivatives combine squalene synthase inhibitory activity *in vitro* (IC_{50} values between 5 and 16 μM) and a significant antihyperlipidemic activity *in vivo*. Plasma levels of total cholesterol, LDL-cholesterol and triglycerides were reduced by 26-74% increasing the ratio HDL/LDL up to 97%.



1 X = CH_2
2 X = CO

3 X = CH_2
4 X = CO

Figure 1.

The increased antioxidant and antihyperlipidaemic activity of the novel derivatives render them interesting leads for establishing a new pharmacophore. Together with 2-hydroxy substituted thiomorpholine

derivatives that have been synthesized and evaluated, these molecules show promise as potential antiatherosclerotic agents.

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[2]Kourounakis, A.P.; Matralis, M.N., Nikitakis, A. Design of more potent squalene synthase inhibitors with multiple activities. *Bioorg. Med. Chem.* **2010**, *51*, 7402-7412.

DESIGN, SYNTHESIS AND BIOLOGICAL PROPERTIES OF NEW ALKYLATING STEROIDAL ESTERS OF 2-[*p*-*N*,*N*-BIS(2-CHLOROETHYL)AMINOPHENYL]BUTYRIC ACID

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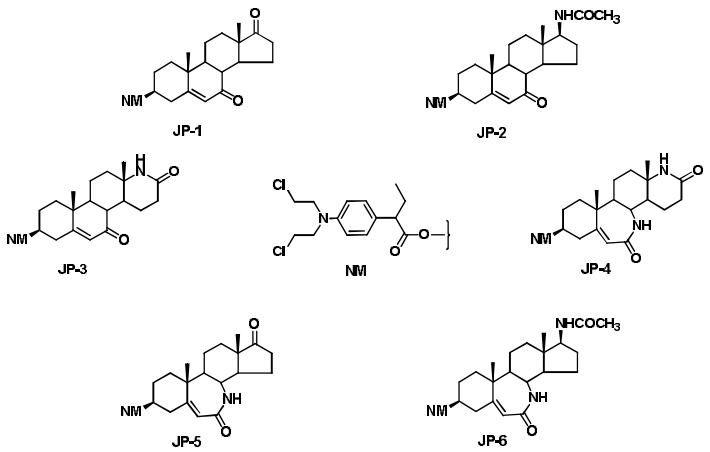
Nitrogen mustards (NMs) such as chlorambucil and melphalan are among the alkylating agents currently used in the treatment of a variety of cancers. Recently, bendamustine, a novel NM with unique structural and mechanistic features, was approved by FDA for the treatment of chronic lymphocytic leukemia.[1] Despite their therapeutic value, NMs produce severe unwanted side effects due to their high inherent chemical reactivity. Different approaches have been used to minimize these undesirable side effects and improve drug efficacy. Among them, the conjugation of aromatic NMs to steroidal hormones has produced steroidal esters with potent *in vitro* and *in vivo* antileukemic activity and reduced toxicity compared to the parent NMs.[2] Additionally, structure-activity relationship (SAR) and 3D quantitative SAR (QSAR) studies have revealed crucial structural features of both the steroidal part and aromatic NM for enhanced antileukemic activity.[3]



Based on these observations as well as on the predictions of *in silico* design, we designed and synthesized six new steroidal esters (**JP1-6**) bearing the 2-[*p*-*N*,*N*-bis(2-chloroethyl)aminophenyl]butyric acid (**NM**) as alkylating agent. The synthesis of the intermediate and target molecules as well as preliminary results related with biological properties of the compounds will be presented.

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STUDIES TOWARD A NOVEL TOTAL SYNTHESIS OF ECTEINASCIDIN-743: NEW REACTION OF TMS-IMINES WITH TMS-NUCLEOPHILES

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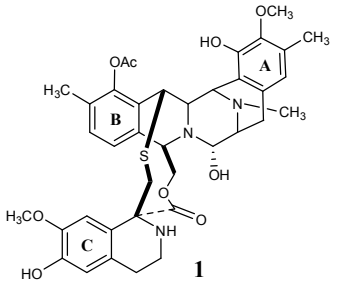
Ecteinascidin 743 (**1**) isolated from the Caribbean tunicate *Ecteinascidia turbinate*,[1] is arguably the most potent cytotoxin known as indicated by its evaluation against the National Cancer Institute's human *in vitro* cell line panel including melanoma, non-small-cell lung, ovarian, renal, prostate, and breast cancer, demonstrating ***potencies*** ranging from ***1 pM to 10 nM***.[2] In fact, the antiproliferative activity of Et 743 is greater than that of Taxol, camptothecin, adriamycin, mitomycin C, cisplatin, bleomycin, and etoposide by 1-3 orders of magnitude, propelling trabectidin (**1**) to become the first marine anticancer drug to be approved (October 2007) in the European Union (EU),[3] as a first-line treatment for soft tissue sarcomas. The complexity of molecular architecture, the remarkable biological activities, and the restricted natural availability (1.0 g from about 1.0 ton of tunicate) have made **1** an exceedingly attractive synthetic target for total synthesis.[4] Our studies toward the validation of key elements of our retrosynthetic analysis will be presented including the general and useful reaction of the title.



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SOME ASPECTS ON XENOBIOTIC METABOLISM, FROM HEPATIC FUNCTION TO PROSPECTIVE DRUGS:
EFFECT OF XENOBIOTICS ON CYTOCHROME P450 – DRUG DESIGN

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Drug metabolism, the molecular changes of bioactive compounds performed by the organism, is profoundly implicated in a number of pharmacochemical activities, e.g. drug design, toxicity, interactions, drug response. Xenobiotic biotranformations depend greatly on the metabolising enzymes, mainly cytochrome P450 (CYP). CYP functions are regulated intrinsically, e.g. sex, age, and by external conditions, like drugs, other xenobiotics, diet.

In view of the importance that CYPs play in health and disease, we present some of our research, may assist in the elucidation of the molecular mechanism of this phenomenon and may find useful applications in related medical interventions or help in finding more efficient drugs.

Therefore, in this investigation we present the interplay between drug metabolism and conditions like dyslipidemia, arthritis, biologic and oxidative stress. Furthermore, the importance of xenobiotic structural features for CYP induction or inhibition is studied, aiming to the development of structure - activity relationships.

Rat liver microsomal preparation is used for determining spectral binding constants and influence on the metabolic intermediate formation of tofenacine, for a series of potential histamine (H_1, H_2) receptor antagonists

Rats are treated with ethanol, CCl_4, $(CH_3)_2Hg$. Spironolactone, pregnenolone-16α-carbonitrile or triamcinolone are given liver function and drug metabolic activity are assessed.

Several 16-substituted pregnenolones (β-CN, α-CN, -H, $-CH_3$, $-CH_2CN$, $-CONH_2$, -COOH) and cyanopregnenolones (2α-, 6, 1, 17α-) are administered to rats, in vitro and in vivo drug metabolism is studied in all groups.

Two non steroidal anti-inflammatory drugs, ibuprofen and fenbufen) are given to rats at three dose levels, i.p. for 3 days. CYP and peroxisomal activities are determined.

The reductive metabolism of metyrapone analogues in human hepatic microsomes and epithelial Chang cells, as well as their toxicity are studied.

The natural, non toxic guaiazulene is administered to rats and the effect on CYP, as well as on paracetamol hepatotoxicity (GSH, GSH peroxidase and reductase) are examined.

A new derivative of the anxiolytic lorazepam, synthesised as antioxidant and anti-stress agent, is administered to stressed animals and its effect on the stress-induced elevation of CYP-mediated drug metabolism is examined.

From our results is can be realised that:

Numerous drugs influence xenobiotic metabolism. Proper molecular manipulations can separate their main pharmacologic action from CYP modulation (e.g. H_1, H_2 antagonists, insecticide metyrapone derivatives).

Stress can increase drug metabolism and induce oxidative insult. Antioxidant and/or anti-stress agents can offer protection.

Investigation of structural factors affecting CYP induction or inhibition would help in the development of selective, efficient and safe drugs.

DESIGN,SYNTHESIS AND PHARMACOCHEMICAL STUDY OF THIOMORPHOLINE DERIVATIVES WITH MULTIPLE TARGETING AGAINST HYPERLIPIDEMIA

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In recent years there is a growing view that multi-causal diseases are better dealt with multi-purpose compounds. Thus, we seek to combine in one molecule structural characteristics which are expected to address individual causes of disease. Atherosclerosis is a pathological condition in the progression of which dyslipidemia, oxidative stress and inflammation are important risk factors. Therefore, molecules with antidyslipidemic and antioxidant capacity could be useful means for the treatment of atherosclerosis. In our laboratory we have shown that appropriately substituted 2-diphenyl-morpholines reduces lipid levels and progression of atherosclerotic lesions. These compounds act as inhibitors of squalene synthase, SQS. In this study we present new compounds, which retain the structural characteristics of squalene synthase, but the basic ring is a thiomorpholine. Substituents of nitrogen are groups expected to offer antioxidant capacity. The hypolipidemic activity of the new compounds is estimated in vivo from the inhibition of hyperlipidemia, induced in rats by Triton WR 1339 (200mg, i.p). The compounds were administered i.p. at the dose of 56 μmol / kg. The biochemical parameters determined in plasma of rats were: triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol.

The antioxidant activity of the compounds is estimated in vitro by the inhibition of lipid peroxidation, which is induced by Fe^{2+}/ascorbate system, using rat hepatic microsomal membranes.

In addition, representative compounds are tested as inhibitors of squalene synthase, SQS, in a preliminary in vitro experiment using hepatic microsomal membranes and [^{3}H]farnesyl pyrophosphate as substrate.

In conclusion, the compounds developed in this study are novel structures that combine significant antidyslipidemic action, probably by acting as inhibitors of SQS, antioxidant capacity while they cause a large increase of HDL.

ANTIPLATELET EFFICACY OF LONG-TERM TREATMENT WITH CLOPIDOGREL BESYLATE IN PATIENTS WITH A HISTORY OF ACUTE CORONARY SYNDROME. COMPARISON WITH CLOPIDOGREL HYDROGEN SULFATE.

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Purpose: Clopidogrel besylate (CB) exhibits similar pharmacokinetic and pharmacodynamic properties compared with clopidogrel hydrogen sulfate (CHS) in healthy volunteers. However, it remains to be established whether the antiplatelet and clinical efficacy of CB resembles that of CHS in patients with cardiovascular disease.

Methods: Sixty-two patients aged 68.8±8.6 years, (47 men) with a history of acute coronary syndrome (ACS) participated in the study. At the time of inclusion to the study all patients were receiving 100mg/day aspirin, 75mg/day CHS (Plavix®) as well as 40mg/day atorvastatin. Patients were then randomized either to continue with CHS therapy (n=30) (CHS Group) or to switch to 75mg/day CB (Clovelen®) (n=32) (CB Group). Aspirin and atorvastatin therapy continued through all over the study. At 6 months after randomization, citrated blood samples were collected for platelet analysis. The platelet aggregatory response to ADP (10, 5, 2.5 μM) was studied by Light Transmission Aggregometry. Flow cytometric analysis of vasodilator-stimulated phosphoprotein (VASP) phosphorylation was performed and the platelet reactivity index (PRI) was calculated. We also studied the membrane expression of P-selectin and the circulating platelet-leucocytes conjugates by Flow Cytometry in whole-blood, before and after activation with 100 μM of ADP.

Results: From the VASP analysis, a wide variability in the PRI values (5.0%-87.3%) among participants was observed. Two patients (6.7% of total) of the CHS group and 3 patients (9.4% of total) of the CB group, were clopidogrel nonresponders (PRI values ≥50%). The mean±SD values of ADP-induced maximum platelet aggregation at 6 months of follow-up in CHS group were 23±8% for 2.5μM, 35±12% for 5μM and 50%±23% for 10μM. Similar results were obtained for the CB group, 25±10% for 2.5μM, 39±14% for 5μM and 46%±19% for 10μM. No difference between the 2 groups was observed in the P-selectin expression (% positive cells) (CHS group: 4.1±2.8% and 28.0±11.2% in unstimulated and ADP-activated cells, respectively. CB group: 4.2±2.5% and 23.1±12.4%, respectively). Similar results in the

platelet-monocyte and platelet-neutrophil conjugates (% positive particles) were obtained between the two groups either in unstimulated or in ADP-activated blood samples.

Conclusions: We show for the first time that the platelet aggregation to ADP and the platelet-mediated inflammatory response in patients with a history of an ACS receiving long-term therapy with CB is similar to that of patients treated with CHS.

THE "2+1" APPROACH FOR THE DEVELOPMENT OF NOVEL [$^{99m}Tc^{I}(NS)(P)(CO)_3$]-TYPE RADIOPHARMACEUTICALS: SYNTHESIS, STRUCTURAL STUDIES AND RADIOCHEMISTRY

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Technetium-99m radiopharmaceuticals are widely used in Nuclear Medicine. The organometallic [$^{99m}Tc^{I}(CO)_3$] core forms stable complexes with a variety of ligands in high yield, which are currently investigated as novel molecular imaging agents. Our interest in this work, lies in the design and synthesis of "2+1" mixed-ligand tricarbonyl $^{99m}Tc(I)$ complexes with potential radiopharmaceutical applications.

Herein, we present the synthesis and characterization of novel mixed-ligand complexes of the formula *fac*-[Re/$^{99m}Tc(Spy)(P)(CO)_3$] and [$Re(Spy)(P)_2(CO)_2$], where 2-mercaptopyridine (Spy) is the (NS) bidentate and triphenylphosphine (P) is the monodentate ligand. Complex *fac*-[$Re(Spy)(P)(CO)_3$] was synthesized by reacting the dimeric complex $Re_2(CO)_6(Spy)_2$, with triphenylphosphine and complex [$Re(Spy)(P)_2(CO)_2$] was synthesized by reacting Spy with the precursor *mer, trans*-[$Re(CO)_3(P)_2Cl$]. The complexes were characterized by IR, NMR spectroscopies and X-ray crystallography. The synthesis of the analogous *fac*-[$^{99m}Tc(Spy)(P)(CO)_3$] complex was conducted by reacting both the ligands (10^{-3}M) with the precursor *fac*-[$^{99m}Tc(OH_2)_3(CO)_3]^+$ at 80^{o}C for 30 min. Identification of the ^{99m}Tc complex was accomplished by HPLC chromatographic comparison with the analogous prototype rhenium complex.

Stability studies were conducted by incubation of the purified ^{99m}Tc complex in 1 mM cysteine and histidine at 37 ^{0}C and HPLC analysis showed that it remained intact over 24 hours. These results suggest that the complex *fac*-[^{99m}Tc (Spy)(P)$(CO)_3$] can be used for the development of ^{99m}Tc radiopharmaceuticals.

Author index