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Epitheorese Klinikes Farmakologias kai Farmakokinetikes
INTERNATIONAL EDITION

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Journal of Applied Pharmacology and Current Therapeutics Reviews, Review Articles on Drugs and Drugs Therapy, Original Papers and Practical Therapeutics Articles

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Τετραμηνιαία Ιατροφαρμακευτική Έκδοση
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VOLUME 23, ❁ 2009 No 1

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GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS

publishes Applied Pharmacology and Current Therapeutics Reviews, Review Articles on Drugs and Drug Therapy, Original Papers and Practical Therapeutics Articles.

The *scientific standard* of the papers, which are accepted for publication, is controlled by the Editorial Board or by other Experts in the various fields of Pharmacology and Pharmacokinetics.

Manuscripts: These should mention, on the first page, the *Title*, *Author(s)* and the *Name of the Institution* at which the work was done. The complete *address* of the author, including Postal area code number, should be given under the rubric *Send reprint requests to*. Papers should follow the general form: *Introduction, Materials and Methods, Results, Discussion* and *References*. Drugs must be referred to by their generic or chemical name, but may be identified by trade name in parenthesis or o footnote. All papers should be submitted in duplicate.

Summary: A summary in English (maximum length 200 words) must accompany all manuscripts.

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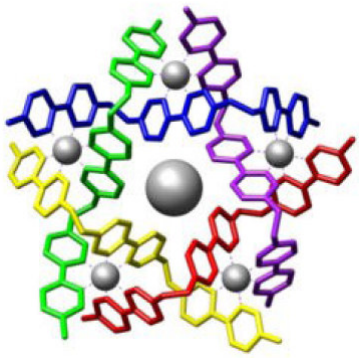
Papers should be submitted to the Editors-in-Chief: Prof. S.T. Plessas or Dr C.T. Plessas.

Ten Years Graduate Program Drug Discovery and Design



Guest Editor

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Department of Chemistry, University of Patras
26500 Patras, Greece
Head of Medicinal Chemistry Postgraduate Program
Medicinal Chemistry: Drug Discovery and Design

Letter from Guest Editor

This issue contains Abstracts of research work presented by specialists at the Ninth Medicinal Chemistry Conference held at the University of Patras, 27-20, March 2008. This Conference was organized by the Postgraduate EPEAEK Program *Medicinal Chemistry: Drug Discovery and Design* initiated and sponsored by the Ministry of National Education and Religion.

Distinguished scientists from Greece and Abroad participated in the Conference, among them Nobel' Laureate Jean Marie Lehn from the University of Louis Pasteur (Strasbourg) and Collège de France (Paris), with a plenary Lecture on Supramolecules.

This Program, high ranked in evaluations, is offered by the Departments of Chemistry and Pharmacy of the University of Patras, to selected graduate students from Departments of Chemistry, Pharmacy, Biology and Medicine. In particular, this issue contains articles, which are the results of novel work carried out by the researchers of the program and their graduate students, who take the post graduate program leading to Master of Science and PhD degrees. Abstracts cite in summary research findings from a broad area of Biomedical Fields, including Organic Synthesis and Drug Design Methods. The articles of the book are written by specialists in their field, who participated at the Conference and provide a global understanding of the recent activities in the field of Drug Discovery and Design in Greece and Abroad.

The Guest Editor, on behalf of the Postgratuate Program Committee, wishes to express his deep appreciation to all contributors in this book. We also thank the Editorial Board of *Review of Clinical Pharmacology and Pharmacokinetics* in particular Journal Editors S. Plessas and C. Plessas for invitation and for providing the suitable and high-standard forum through which important findings of this research will become available to the scientific community.

The Guest Editor

John Matsoukas

Professor in Chemistry
University of Patras, Greece
Head of Medicinal Chemistry Postgratuate Program
Medicinal Chemistry: Drug Discovery and Design

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
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Abstracts Lectures

Constitutional Dynamic Chemistry for Bioorganic and Medicinal Purposes

Jean-Marie Lehn

ISIS, Université Louis Pasteur (Strasbourg) and Collège de France (Paris), France

Key words: Chemistry, constitutional dynamic, bioorganic, medicinal purposes

Supramolecular chemistry is intrinsically a dynamic chemistry in view of the lability of the interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their constituents. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibility, so as to allow a continuous change in constitution by reorganization and exchange of building blocks. These features define a Constitutional Dynamic Chemistry (CDC) on both the molecular and supramolecular levels. When applied to mixtures of reagents CDC acquires combinatorial features. Thus, dynamic combinatorial chemistry (DCC) has developed recently as an approach that uses molecular and supramolecular self-assembly processes to generate libraries of chemical compounds. In contrast to the classical combinatorial chemistry which is based on vast collections of prefabricated molecules, DCC allows for the generation of libraries *via* the continuous interconversion between the library constituents by recombination of their building blocks. Spontaneous assembly/deassembly of the building blocks through reversible chemical reactions virtually encompasses all possible combinations, and allows the establishment of adaptive processes owing the dynamic interchange of the library constituents. Addition of a biological target molecule creates a driving force that favours the formation of the best-binding constituent through molecular recognition, a self-screening process that allows the identification of bioactive molecules and of lead compounds for drug discovery. Several implementations of this approach will be described.

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From Academic Research to its Exploitation for Medicinal Uses: Sartans, a new Generation of Drugs for Treating Hypertension and Cardiovascular Diseases (the Elsartan Case) – The Contribution of Greece

John Matsoukas

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Key words: Sartans, hypertension, cardiovascular diseases, exploitation

The discovery of Losartan a non peptide Angiotensin II Receptor antagonist was announced in 1989 during the Gordon Research Conference on Angiotensin and the Renin-Angiotensin-System (RAS). The drug was discovered in the Laboratories of Dupont and the announcement at the Conference was the approval for Clinical trials which led to the first Angiotensin II nonpeptide Receptor antagonist. Previous Angiotensin II peptide antagonists such as Sarilesin and Saralasin failed to become drugs due to its peptide nature rendering them susceptible to proteolytic enzymes which hydrolyze them. The announcement was the result of many years work on Angiotensin and the RAS System, since it was discovered 80 years ago. Breakthroughs in this evolution were the discovery of Captopril by Miguel Ondetti in 1975 and Losartan by Timmermans in 1989. In this lecture the main steps followed in our laboratories in Patras are mentioned which led to our Sartan, named Elsartan. Briefly the main steps are: (i) Peptide (The tool), (ii) Peptide Model (The ligand-receptor interaction), (iii) Cyclic Peptide (The drug lead), (iv) Non-peptide mimetic (The Drug). Also, the strategic steps are described, in the investment of this research and the incorporation of ELDRUG in order to develop Elsartan for Medical uses as antihypertensive.

Novel Fluoroketone Inhibitors of Phospholipase A_2: From Basic Research to Preclinical Studies

George Kokotos

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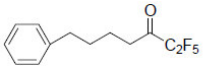
Key words: Fluoroketone inhibitors, novel, phospholipase A_2, preclinical studies

Phospholipase A_2 (PLA_2) plays a normal physiological role in phospholipids metabolism and signal transduction. However, under pathological conditions PLA_2 has been implicated in inflammation in a variety of tissues and organs. PLA_2 hydrolyzes the ester bond at the sn-2 position of phospholipids generating free fatty acids, such as arachidonic acid (AA), and lysophospholids, such as lysophosphatidylcholine (LPC). AA, *via* the cycloxygenase and lipoxygenase enzymes, gives rise to a variety of pro-inflammatory eicosanoids, while LPC is a potent demyelinating agent, as well as induces expression of pro-inflammatory chemokines and cytokines. Very recently, we have developed a variety of new fluoroketones presenting various selectivities against the various PLA_2 forms. This presentation will focus on the opportunities provided by the inhibition of PLA_2 for the development of new medications for multiple sclerosis and other neurological disorders. New methodology was developed allowing the synthesis of various pentafluoro, heptafluoro, tetrafluoro and trifluoro derivatives. To understand the role of PLA_2 in multiple sclerosis, we focused our study on their in vitro activity on two PLA_2 isoforms: the cytosolic GIVA $cPLA_2$ and the calcium-independent GVIA $iPLA_2$. Among the fluoroketones we synthesized and studied, compound 1 proved a potent and selective inhibitor of GVIA $iPLA_2$, whereas compound 2 strongly inhibited both GIVA $cPLA_2$ and GVIA $iPLA_2$. Both inhibitors *1* and *2* showed strong reduction in the clinical severity and progression of the experimental autoimmune encephalomyelitis (EAE) model, the widely used animal model for multiple sclerosis, indicating that PLA_2 inhibitors are likely to be excellent candidates for drug development.



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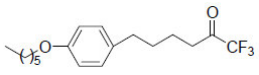

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Steroid and Thyroid Hormone Receptors and other Nuclear Transcription Factors in Mitochondria and their Role in Energy Metabolism and Apoptosis

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Key words: Receptors, steroid hormone, thyroid hormone, nuclear transcription factors, mitochondria, energy metabolism, apoptosis

Steroid and thyroid hormones are major regulators of metabolic, growth, immune and differentiation processes, exerting many of their biological effects by way of nuclear receptors and modulation of gene expression. In these processes cell replication and apoptosis are involved, the energy yield being a major factor in the cell's fate towards life or death. The mitochondrion is the major energy generating organelle of the cell and the site of many basic processes, including apoptosis. These functions are performed in concert with other cell compartments, importantly the nucleus, and are regulated, in addition to the hormone receptors, by other nuclear transcription factors. The demonstration of the presence of steroid and thyroid receptors and other nuclear transcription factors, such as NF-kB, AP-1, CREB and p53 in mitochondria raises the question of the role of these regulatory molecules in their *new environment*. In addition to the action of the nuclearly localized receptors on nuclear OXPHOS (enzymes of oxidative phosphorylation) gene transcription, a direct role of the mitochondrially localized hormone receptors on mitochondrial transcription, biosynthesis of OXPHOS and apoptosis is now being revealed. The coordination of transcription modulation in nuclei and mitochondria by the respective receptors is in part realized by their binding to common trans-acting elements in the two genomes. This principle of coordination of OXPHOS transcription in the two cell compartments by way of interaction of the regulatory agent with common binding sites in the nuclear and mitochondrial genome is expanding to encompass many of the other nuclear transcription factors. Their presence in the mitochondrion increases its arsenal of regulatory molecules and underlines the central role of this organelle in the integration of growth, metabolic and cell survival signals.

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Role of Thrombin/Thrombosis in Angiogenesis and Cancer Progression

Michael Maragoudakis

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Key words: Angiogenesis, thrombin, thrombosis, cancer progression

The frequency of blood coagulation in cancer patients was observed more than 150 years ago. Since then, many investigations have confirmed this finding and provided clinical laboratory and pharmacological data. More recently it was shown that exposure of tumor cells to thrombin, increases their metastic potential more than 150 fold. Furthermore, the metastatic potential of breast cancer cells is related to the number of activated thrombin receptor (PAR1). Recent epidemiological studies show an increase of cancer diagnosis by 6 fold after primary thromboembolism. A plausible explanation for those findings is our results that thrombin is a potent promoting

factor of angiogenesis, a process essential for tumor growth and metastasis. We have identified many cellular actions of thrombin, independent of its coagulation activity, which are involved in the activation of angiogenesis: Thrombin stimulates the release of VEGF from cancer cells, smooth muscle cells and platelets and at the same time upregulates the expression of VEGF receptors in endothelial cells. This leads to synergism of thrombin with the key angiogenic factor VEGF. In addition, thrombin up regulates the expression of integrin $\alpha_v\beta_3$, which is characteristic of the angiogenic phenotype of endothelial cells. Thrombin also up regulates the activation of MMP_2 and decreases the ability of endothelial cells to adhere to basement membrane proteins. All the aforementioned effects are specific and depend on the activation of thrombin receptor PAR1. On the other hand through its RGD sequence thrombin acts as chemotactic and aptotactic agent and provides antiapoptotic signals to endothelial cells. Since all these effects of thrombin on angiogenesis are unrelated to its blood coagulation activity (fibrin formation), these findings may serve as a basis for developing agents that antagonize the angiogenic activity of thrombin, which have therapeutic potential in cancer and other diseases without interfering with blood coagulation.

100 Years from the Discovery of the Renin-Angiotensin-Aldosterone System

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Key words: Renin-angiotensin-aldosterone system, 100 years from the discovery

The RAAS (renin-angiotensin-aldosterone system) is present in the majority of animal species and is committed to the regulation of circulatory homeostasis. Renin was described more than 100 years ago by Tigerstedt and Bergman, is secreted by the kidney and represents the first and rate regulating enzyme for this complicated cascade. The second enzyme of RAAS cascade is a metalloproteinase called angiotensin converting enzyme (ACE) and is responsible for the generation of the active octapeptide angiotensin II, both into the systemic circulation, and also locally into the heart, the vascular wall and other tissues. The first step in the development of RAAS inhibitors was the discovery of ACE by Skeggs and colleagues in 1956. The X-ray crystal structure of ACE has been reported recently for both the human testicular and drosophila enzymes. In 2000, another enzyme, called ACE-2, was identified. This enzyme does not generate angiotensin II, but increases the formation of angiotensin 1–7, a vasodilatory and antiproliferative hormone. The effects of all angiotensin peptides are mediated through specific cell surface receptors. The AT1 receptor mediates most of the effects usually associated with angiotensin II, such as vasoconstriction and growth. The AT2 receptor antagonises many effects of the AT1 receptor, for example, cell proliferation. The AT4 receptor for angiotensin IV affects kidney tubular function and improves the memory of rodents. Captopril was the first orally-active ACE inhibitor discovered in 1975 and approved by FDA for clinical use in 1981. Since then, at least twelve other ACE inhibitors have been marketed. Of note, ACE inhibitors do not completely prevent the formation of angiotensin II, as there are other conversion enzymes i.e. the chymase, which is especially expressed in diseased or injured vascular tissue. The octapeptide saralasin, discovered in mid 1970's, was the first angiotensin II receptor blocker to work in man, but it was not suitable for clinical use. However, it facilitated the development of sartanes, another major class of drugs, which over the last 15 years have been widely used to treat hypertension, heart failure, renal diseases etc. Currently, there is only one renin blocker available, called Aliskiren, which in 2007 was approved by the FDA for high blood pressure treatment. New aspects of the RAAS continue to emerge and could become targets for novel therapeutic strategies. For example, the ACE molecule itself could act as a cell surface receptor, mediating outside–in signalling in endothelial cells *in vitro*. Finally, a receptor, which mediates the uptake of renin (and prorenin, or

both) was cloned by Nguyen and colleagues in 2002 and found to have profibrotic effects not mediated through angiotensin II.

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Physiologic and Genomic Effects of Angiotensin in the Heart

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Key words: Angiotensin II, heart, physiologic effects, genomic effects

Angiotensin II (Ang II) has long been known as a potent vasoconstrictor and stimulator of adrenal steroid synthesis. The coronary, renal and cerebral vasculatures are far more sensitive to the pressor effect of Ang II than vasculature of musculocutaneous tissues. Ang II excess is cardiotoxic, causing widespread areas of myocardial necrosis. Ang II receptor blockade or angiotensin-converting enzyme (ACE) inhibition increase regional blood flow to vital organs and exert cardioprotective and renoprotective effects. Even at non-hypertensive doses, exogenous Ang II was shown to have vasculotoxic and tissue damaging effects. More recently Ang II has been recognized to have trophic, mitogenic, thrombogenic and inflammatory properties, thus causing hypertrophy and/or proliferation of cardiomyocytes and vascular smooth muscle cells and promoting atherothrombotic processes. While the physiologic and pathologic results of Ang II excess have been demonstrated in experimental animal models and their human counterparts, the underlying molecular events that precede and trigger these pathologic responses remain poorly understood. We recently conducted a series of experiments in mice involving infusion of Ang II at 0.9 ng/h for either 24h or 2 weeks, in order to evaluate alterations in expression or genes relevant to cellular integrity, proliferation, metabolic and tropic functions triggered by acute or chronic Ang II excess. Using microarray technology we scanned the whole mouse genome and found downregulated expression of over 2,000 genes, including genes for mitochondrial metabolism, electron transport and fatty acid oxidation; unregulated expression of 1,300 genes, including genes whose products are involved in growth regulation, oxidative stress, vasoactive hormone receptors and glucose metabolism; and another 1,456 genes which were upregulated by acute and downregulated by chronic Ang II excess. Of particular interest was the finding of excessive upregulation of a hitherto unknown transcript that eventually led to the discovery of a novel gene, the cardiomyopathyassociated Cmya3 gene, whose product remains unknown, but seems to be involved in the development of ischemic cardiomyopathy induced by Ang II excess.

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Research at the Cutting Edge and Spin-Offs

E. A. Couladouros

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Key words: Cutting edge, spin-offs, proACTINA

Chemical synthesis is one out of the most important steps both for the discovery of a new pharmaceutical and for its exploitation to become a drug. Bioactive natural products of complicated structure are the outmost challenge in this field. Apart from the purely academic interest, such endeavours may lead to the development of general and convergent routes for the preparation of

designed and diversified libraries, thus facilitating their further exploitation. In parallel, researchers remain updated with the most recent knowledge, while future expertises are well trained for spin-off and high-tech companies. A short presentation of our SME proACTINA and the most recent achievements of our laboratory will be presented.

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Molecules that Changed the World

K.C. Nicolaou, PhD

Department of Chemistry and The Skaggs Institute for Chemical Biology - The Scripps Research The Department of Chemistry and Biochemistry, University of California, San Diego, CA, USA

This lecture, based on the recent book by Nicolaou and Montagnon (1), will expound on our learned knowledge of some of Nature's most intriguing molecules and the ability of Man to discover, synthesize, modify and use them to our advantage in what was not formerly envisioned. Through the development of the theme, it is hoped that one will also discover just how profound the impact of chemistry is in our lives. The lecture will also explore some of the most exciting frontiers in modern science and medicine, and the opportunities they present to young students for future careers. Illustrated in a colourful style, this presentation will aim to provide insights about the role of chemistry in society in general and how chemical synthesis, the art and science of constructing natural and designed molecules, in particular, shaped and continues to shape our world. Indeed, the lectures will touch upon fascinating tales about molecules and their presence in, among many items, foods, perfumes, dyes, high tech materials, textiles, vitamins, nutritional supplements, pesticides, insecticides, and above all, medicines. The history of total synthesis, the flagship of chemical synthesis, as unraveled within the scope of this lecture will hopefully serve to underscore how admirably chemical synthesis enabled and facilitated world-shaping innovations since its inception in 1828 by Friedrich Wohler. The molecules to be discussed include the following.

REFERENCE

1. K.C. Nicolaou and T.Montagnon (eds): Molecules That Changed The World. Wiley-VCH, 2008

Urea & Acetic Acid	Penicillin	Gingkolide	Brevetoxin B
Glucose	Longifolene	Cyclosporin	Ecteinascidin 743
Aspirin®	Prostaglandins	FK506 & Rapamycin	Epothilones
Camphor	Vitamin B12	Calicheamicin γ1	Resiniferatoxin
Tropinone	Erythronolide B and	Palytoxin	Vancomycin
Haemin	erythronolide A	Taxol®	Quinine
Morphine	Monensin	Mevacor®	Thiostrepton
Steroids & The Pill	Avermectin	Zaragozic Acids & The	Small molecule drugs
Strychnine	Amphotericin B	CP molecules	Biologics

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Peptide Based Vaccines: A Structural and Immunological Perspective

Vasso Apostolopoulos

Burnet Institute (Austin campus), Studley Road, Heidelberg, Australia

Key words: Vaccines, peptide-based, structural and immunological perspectives

Peptides usually bind MHC class I or II molecules by anchoring specific side chains into specific pockets in the peptide binding groove. Peptides that do not contain these canonical anchor resi-

dues are believed to bind with low affinity that result in loss of stable complex formation and loss of immunogenicity. Our results indicate using conventional computer algorithms to predict MHC binding peptides that a range of potentially effector T cell epitopes are missed. In this regard, we have determined immunologically and by x-ray crystallography at least ten novel families of peptide MHC binding modalities. These include, peptides using alternative anchor pockets, peptides devoid of central anchor amino acids, low affinity peptides, mutations to make high affinity peptides, short peptides, long peptides, looping out peptides, mimic peptides and glycopeptides for anchoring to MHC. We suggest that limitations in the ability to predict MHC binding peptides have hampered the detection of the whole spectrum of immunogenic peptides. The molecular interactions and immunological information elucidated in these non-canonical peptide MHC complexes should uncover additional immunogenic peptides from primary protein sequences and aid in the design of alternative peptide-based vaccines.

Endocannabinoids and Erythropoietin: Exploiting the Therapeutic Potentials of two Endogenous Molecules on Experimental Autoimmune Encephalomyelitis

Athanasios Lourbopoulos, Roza Lagoudaki, Olga Touloumi, Nikolaos Grigoriadis

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Key words: Experimental Autoimmune Encephalomyelitis, endogenous biological molecules, 2-arachidonylglycerol, erythropoietin, therapeutic potential

Experimental Autoimmune Encephalomyelitis (EAE) is widely used as a model of inflammatory disease of the Central Nervous System (CNS), with numerous applications of experimental treatments. Our laboratory has recently addressed the possible preventive effect of 2 endogenous biological molecules in the clinical course and pathology of EAE. The first of these two molecules is the endogenous cannabinoid receptor ligand 2-arachidonylglycerol (2-AG). Acute monophasic EAE was induced in SJL mice and animals were treated with intraperitoneously (IP) injections of 2-AG for 14 days from day of disease induction. Our clinical data indicated that 2-AG treatment significantly ($p<0.05$) ameliorated the disease in the treated group, whichhad lower Mean Maximal Scores (MMS), lower disease burden and a delayed disease onset. Pathological data of the subchronic phase of the disease revealed modified infiltrating processes, reduced axonopathy and a surprisingly increased microglial reaction within the CNS of treated animals. The second molecule was erythropoietin (Epo), which has recently been attributed with a wide range of neuroprotective and anti-neuroinflammatory activities. The chronic model of myelin oligodendrocyte glycoprotein (MOG peptide 35-55) EAE was used and Epo was daily administered IP, from day 1 to day 15 post-disease-induction. Pathology was studied at acute and chronic phase of the disease and revealed that Epo administration was highly efficient in almost completely blocking the onset of the disease, for as long as it was administered, but not efficient in preventing the long term disability. Pathology agreed with the clinical data and inflammations, axonopathy and demyelination were significantly lower at acute phase but did not differ at chronic phase. Additionally, we found that Epo seemed to retain BBB integrity during acute phase but not in the long term (chronic phase). In conclusion, our results show that both molecules exert a beneficial effect on EAE clinical course and pathology through different mechanisms (immune modification and BBB altering) both of which are promising for further exploration.

Linear and Cyclic Mutant Analogues of self Antigens (MBP and PLP) Manipulate Immune Responses in SJL/J Mice

Maria Katsara[1,2], Elizabeth Yuriev[3], Paul Ramsland[1], George Deraos[2], Theodore Tselios[2], Spyros Deraos[2], John Matsoukas[2] and Vasso Apostolopoulos[1]

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Key words: Self antigens (MBP and PLP), linear and cyclic mutant analogues, manipulate immune responses, SJL/J mice

Multiple sclerosis (MS) is a commonly occurring chronic, inflammatory and disabling disorder of the central nervous system (CNS). It is widely considered that $CD4^+$ T helper type 1 (Th1) cells play a pivotal role in mediating an autoimmune attack against components of myelin sheath. Additional cells, such as $CD8^+$ T cells, macrophages and complement are also involved in axonal damage and neurodegeneration. Several autoantigens, such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) have been proposed as candidate antigens in the induction of MS based on auto-T cells and auto-antibodies which are present in patients with MS. The design of peptide mutants of disease-associated myelin epitopes to alter immune responses from Th1 to Th2 offers a promising avenue for the treatment of MS. Hence, a number of peptides were designed and synthesised by mutating principal TCR contact residues based on MBP_{87-99}, MBP_{83-99} and $PLP_{139-151}$ peptide epitopes in their linear or cyclic forms. Peptides were either emulsified in equal volumes of complete Freund's adjuvant (CFA) and phosphate buffer saline (PBS) or conjugated to reduced mannan via a KLH linker, in order to examine their cytokine and antibody profile. Mannan was previously found to generate either a Th1 response (IL-2, IFN-γ, IL-12, TNF-α and IgG2a antibodies) or Th2 response (not IFN-γ or IL-12, but significant amounts of IL-4, IL-10 and TGF-β and IgG1 antibodies) depending on the mode of conjugation, oxidised or reduced mannan, respectively. IFN-γ, IL-4 and IL-10 or antagonism experiments were conducted using a capture ELISpot method, proliferation assays were performed to evaluate the regulation of the peptides in vitro and ELISA was performed to assess antibody responses *in vivo*.

We noted that the use of CFA with either MBP_{87-99} or MBP_{83-99} mutant peptide analogues, in general, for immunisation, induced higher levels of IFN-γ and lower levels of IL-4, when mutant analogues were used. However, very high levels of IL-4 and IL-10 and no IFN-γ were secreted by T cells, when mice were immunised with reduced mannan peptide conjugates. Antibody responses to native peptide, mutant peptides, linear and cyclic peptides and to whole MBP protein, in addition to, T cell proliferation and EAE experiments were also been assessed.

Overall, the linear $[Y91]MBP_{83-99}$ peptide conjugated to reduced mannan showed the best cytokine and antibody profile and could antagonise T cell responses in vitro, thus, gives promise for the immunotherapy of MS and needs to be pursued for further testing in human studies. For the first time, structural alignment of existing crystal structures revealed the peptide binding motif of H2 I-As (MHC class II). Molecular modelling was used to identify novel H-bonds and van der Waals interactions between peptides and MHC (I-As). Finally, mannosylation of $PLP_{139-151}$ peptide could protect mice from EAE in a prophylactic vaccination setting.

Individual Molecular Profile Influences Treatment Decision in Metastatic Breast Cancer

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Key words: Metastatic breast cancer, treatment decision, individual molecular profile

Breast cancer is the most common cancer in women in the U.S. and Europe. Six to ten of these patients present with metastatic breast cancer (MBC) disease achiving a median survival of 2–3 years. Therefore, for the majority of patients with MBC, *cure* is not the goal of treatment; instead, more conservative treatments are preferred to obtain maximum control of symptoms, avoid treatment complications while preserving a good quality of life (QoL). With the growing understanding of the molecular biology mechanisms underling MBC exciting new tumour targets eligible for anticancer therapy have been identified. These targets such as HER2 are implicated in several pathways relevant to the survival of the cancerous cell such as the signal transduction pathway, the cell cycle, the apoptotic pathway, and the angiogenesis/metastasis pathway. Amplification and overexpression of the her-2/neu gene occurs in approximately 25% of invasive ductal carcinomas and is an adverse prognostic factor. Herceptin is a humanized monoclonal antibody approved for the treatment of patients overexpressing HER-2. The combination of Herceptin with chemotherapy has significantly prolonged the survival of MBC patients. Pooled results from randomized trials of herceptin in breast cancer showed a significant reduction of mortality ($p < 0.05$) and recurrence ($p < 0.05$). Despite the impressive results obtained with herceptin in a percentage of MBC patients, about 50% of patients with HER-2–positive disease will not benefit from this agent, and the median duration of response is between 9 and 12 months. It is likely that acquired resistance to herceptin occurs and other molecular factors are involved in the resistance to this agent. Another interesting finding linked to the molecular profile of these tumours is that HER-2 overexpression is associated with resistance to the endocrine treatment with tamoxifen. Significant preclinical evidence suggests that intensive crosstalk between HER-2 and Estrogen Receptor occurs in MBC cells and there is a rationale to combine herceptin with other antiestrogens such as aromatase inhibitors that act at the level of the ligand and not the estrogen receptor. With the development of genomics and proteomics we increasingly understand that breast cancer is not just a single entity but a complex disease with considerable molecular diversity that often translates into different clinical phenotypes and thus responses to treatment. Further definition of these subtypes will probably change our treatment approach, moving from the era of empirically based treatment to the era of tailored therapies for each individual patient. Nevertheless, this disease requires a multidisciplinary team approach, with the early involvement of psychosocial support and palliative interventions as part of routine patient care. Patients must be encouraged to be actively involved in the treatment decisionmaking process.

Structural Insight into EIF3 Recruitment to the 40S Subunit: Molecular Recognition of EIF3J by EIF3B-RRM Domain

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Key words: EIF3 recruitment, recognition of EIF3J, EIF3B-RRM domain

Mammalian eIF3 is a ~800 kDa multiprotein complex essential for initiation of protein synthesis in eukaryotic cells. eIF3 stimulates eIF2-GTP-tRNAiMet ternary complex binding to 40S subunits, aids correct positioning of mRNAs within 48S initiation complexes and serves as an anti-association factor preventing 60S subunit binding to 40S subunits prior to the subsequent 48S initiation complex formation. eIF3 consists of 13 subunits (eIF3a-m), among which eIF3b serves as a major scaffolding protein. To gain insights into the role of this conserved domain, we investigated its structure and interactions with other eIF3 subunits and the 40S subunit. The structure of human eIF3b-RRM determined by NMR spectroscopy reveals a non-canonical RRM with a negatively charged surface in the b-sheet area contradictory with potential RNA binding activity. Instead, eIF3j, which is required for stable 40S ribosome binding of the eIF3 complex, specifically binds to the rear b-helices of the eIF3b-RRM, opposite to its b-sheet surface. Moreover, we identified that an N-terminal amino acid peptide of eIF3j is sufficient for binding to eIF3b-RRM and that this interaction is essential for eIF3b-RRM recruitment to the 40S ribosomal subunit. To identify the critical residues mediating this interaction, we determined the structure of the complex of eIF3b-RRM bound to the N-terminus of eIF3j. Our results provide the first detailed structural insights into the interaction between two eIF3 subunits essential for stable eIF3 recruitment to the 40S subunit.

Designing Coumarin Derivatives as Serine Protease Inhibitors

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Key words: Coumarin derivatives, serine protease inhibitors, designing

Many biological functions rely on proteases, including food digestion, lysosomal degradation, and signaling cascades. Precise spatial and temporal regulation of proteolytic activity is essential to human physiology. Modulation of protease activity with synthetic peptidomimetic inhibitors has proven to be clinically useful for treating human immunodeficiency virus (HIV) and hypertension and shows potential for medicinal application in cancer, obesity, cardiovascular, inflammatory, neurodegenerative diseases, and various infectious and parasitic diseases. A large number of natural or synthetic inhibitors have been presented over the last years. Inhibitors can be generally classified into 2 large groups based on their structural dichotomy: low molecular weight peptidomimetic inhibitors and protein protease inhibitors composed of one or more peptide chains. Some protease inhibitors inhibit non- specifically more than one type of proteases. Despite this impressive progress, there is much to learn about the cross talk between signal transduction pathways and protease activation cascades. Additionally, development of successful protease inhibitors for clinical use is reliant on maximizing bioavailability, specificity, and potency of inhibition of the target enzyme. However, only few serine protease inhibitors have managed to reach market. Numerous inhibitors of serine proteases have been reported during the past three decades. Among them, coumarintype molecules displayed a high inhibitory potency towards carious serine proteases. A number of coumarin derivatives have been designed, synthesized and evaluated as serine protease inhibitors. A series of coumarin derivatives have been studied from our research group as serine protease inhibitors (trypsin, chymotrypsin and thrombin). A molecular modelling study has been performed. Docking experiments have been used to rationalize the SAR in this series of compounds. 2D and 3D QSAR studies have been performed to further evaluate the biological dataas to design more potent and effective compounds. Discovery of novel selective inhibitors can proceed only through combination of screening of chemical libraries, rational design, computational technology, and exploration of natural compounds. Furthermore, future research into the synergistic capabilities of inhibitors will help elucidate the

most effective combination therapies. Proteases inhibitor research should be viewed as a promising field in which medical advances are likely to be realized.

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A Novel Asymmetric Synthesis of the NK-1 Receptor Antagonist CP-122,721 Based on the Sulfur Ylide-mediated Epoxidation

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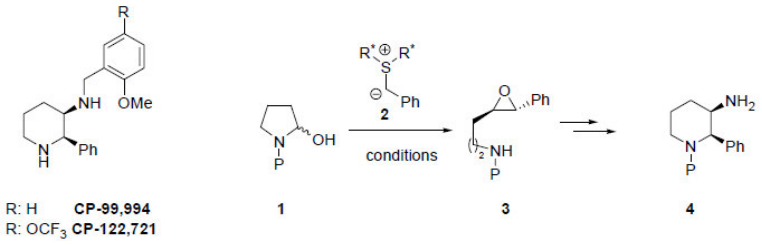
Key words: Nonpeptide NK-1 receptor antagonists, CP-99,994, CP-122,721

The neuropeptide substance P (SP) is involved in various biological activities exerting its action through binding to the neurokinin-1 (NK-1) receptor. The search for nonpeptide NK-1 receptor antagonists led initially to the discovery of CP-99,994 and then to CP-122,721, which was developed as a second–generation NK-1 receptor antagonist showing superior in vivo blockade of NK-1 receptor mediator responses, together with a potent anti-emetic activity. Herein, we present a novel methodology for the synthesis of CP-122,721 based on a new sulfur ylide-mediated epoxidation. N-Protected hemiaminals *1* were converted to epoxides *3* by treatment with sulfur ylide *2* in good yields and high enantioselectivities. Epoxide *3* was readily transformed to either five-membered or six-membered nitrogen heterocyclic products as well as the desired piperidine *4*, which is the key intermediate for the synthesis of the target NK-1 receptor antagonists.



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Induction of Antigen-specific Regulatory T Cells in Wild-type Mice: Visualization and Targets of Suppression

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Key words: Y chromosome-encoded transplantation antigens, immune responses, T cells

Immune responses to Y chromosome-encoded transplantation antigens (HY) can have life-threatening consequences in clinical settings and antigen-specific transplantation tolerance in the absence of immunosuppressive drugs is a rarely achieved goal. This can be achieved, however, by adopting a procedure developed in TCR transgenic mice to convert naive T cells into male-specific Foxp3+ regulatory T cells (Tregs) in wild-type (WT) female mice. For this purpose, female mice were infused by osmotic mini-pumps with a single class II MHC-presented HY peptide and Tregs visualized by tetramer staining. As a result, animals developed Treg-mediated long term tolerance to all HY transplantation antigens, irrespective of whether they were recognized by CD4 or CD8 T cells, on skin or hematopoietic grafts from male donors.

Adrenal beta-Arrestin 1 Mediates Physiological Production of Aldosterone *in vivo*

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Key words: Aldosterone, physiological production, adrenal beta-arrestin 1

Aldosterone is one of the various hormones with detrimental functions for the failing heart, whose circulating levels are elevated in chronic heart failure (HF). Aldosterone is produced and secreted by the adrenal cortex in response to angiotensin II (AngII) acting through AngII type 1A receptors (AT_{1A}Rs), which are expressed in the adrenocortical zona glomerulosa cells. The AT_{1A}Rs are G-protein coupled receptors (GPCRs) that have recently been shown to also signal through G-protein-independent pathways. The scaffolding actions of b-arrestin-1 and -2 (barr1 and -2), originally discovered as terminators of GPCR signaling following phosphorylation of these receptors by GPCR kinases (GRKs), have a central role in mediating G-protein-independent signal transduction by these receptors. *Hypothesis*: We hypothesized that adrenal barr1 mediates, at least in part, AT_{1A}R signaling to aldosterone production/secretion in vivo. *Methods*: To test this hypothesis, we used in vivo adenoviral-mediated gene transfer to the adrenal gland of normal young male Sprague-Dawley rats to induce overexpression of barr1, GRK2, or the GRK2 inhibitor peptide bARKct. One week post-infection, the animals were sacrificed, in vivo transgene overexpression was assessed by Western blotting and plasma aldosterone levels were determined by ELISA. *Results*: All three transgene proteins were robustly overexpressed specifically in the adrenal glands of these rats. Adrenal-targeted barr1 overexpression (Adbarr1) led to a significant increase in plasma aldosterone levels in the infected rats (536±50 pg/ml) compared to control rats that received adenovirus encoding for Green Fluorescent Protein (AdGFP) in their adrenals (235±40 pg/ml, $p<0.01$, $n=5$). Adrenal GRK2 overexpression caused a small but significant increase in plasma aldosterone levels, whereas bARKct overexpression was without effect compared to control AdGFP rats. Studies are currently being done in the HF model trying to determine if aldosterone levels in HF can be manipulated through alterations of barr function. *Conclusions*: Adrenal barr1 appears to mediate physiological and pathological (increased in HF) aldosterone turnover in vivo, and appears to do so independently of the actions of its co-factor in receptor desensitization, GRK2.

❁

Role of the A Chain of Thrombin

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Key words: Thrombin, A chain, B chain stabilization

Thrombin is a Na^+ activated, allosteric serine protease involved in blood clotting and is composed of two polypeptide chains of 36 (A chain) and 259 (B chain) residues that are covalently linked through a disulfide bond between residues Cys1 and Cys122 (chymotrypsin numbering). The A chain has received little attention in thrombin studies and is considered an appendage of the activation process from prothrombin. Previous studies have focused on the B chain which carries the catalytic properties of the enzyme and suggested that the A chain may be dispensable for function. However, several naturally occurring mutations of prothrombin involve residues of the A chain and are associated with severe bleeding. The A chain contributes to stabilization of the B chain through numerous ionic interactions. Disruption of any such interactions may produce

effects that ultimately influence the interaction with the B chain and its properties. In this study, human α-thrombin was subject for the first time to a systematic mutagenesis strategy leading to the construction of thrombin molecules with mutations in residues of the A chain that are critical in terms of charge and evolutionary conservation. The functional study of these molecules provided insight to the importance of the A chain on catalysis of thrombin. The alanine substitution of most of the residues of the A chain that were studied did not significantly affect the function of the enzyme. However, mutations that perturbed the ion cluster Arg4-Glu8-Arg14-Glu14c in the A chain elicited noticeable perturbations in the interaction of thrombin with chromogenic and physiological substrates. These findings demonstrate that there is a mechanistic linkage between the A-chain, at the back of the molecule, and the B chain, which hosts the active site, the primary specificity pocket and the Na^+ binding site.

Targeting of Inositol 1,4,5-Trisphosphate Receptors

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Key words: Inositol 1,4,5-trisphosphate receptors, targeting

Inositol 1,4,5-trisphosphate receptors (IP3Rs) are intracellular C^{a2+} channels, responsible for mediating the release of Ca^{2+} from internal stores. IP3Rs form tetramers. Each subunit has an N-terminal IP3-binding site and towards the Cterminal there are six transmembrane domains (TMDs), the last pair of which, with their linking luminal loop, form the pore of the channel. The precise targeting of IP3Rs to specific subcellular compartments underlies their ability to generate spatially organized Ca^{2+} signals, which subsequently regulate a plethora of cellular responses. Whatever the final destination of an IP3R, it is first directed to the endoplasmic reticulum (ER). We previously demonstrated that a fragment of IP3R1 comprising only TMD1-2 and the intervening luminal loop was targeted to the ER, while TMD1 or TMD2 alone were expressed in mitochondria (Parker et al., 2004). We address aspects of IP3R targeting by identifying the minimal requirements for the targeting of IP3R1 in the ER, examining the differential distribution of IP3R subtypes (IP3R1-3) and by evaluating the mobility of these subtypes. For this purpose, N-terminal fragments of IP3R1 tagged with enhanced green fluorescent protein (EGFP) and full length IP3R subtypes tagged with different fluorophores were prepared and expressed in COS-7 cells. Their localization was examined using biochemical analyses and confocal microscopy and their mobility studied using fluorescence recovery after photobleaching (FRAP).

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Generation of a Recombinant apoE Form with Improved Biological Functions

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Key words: Apolipoprotein E, recombinant, biological functions

Apolipoprotein E (apoE) is probably one of the most important proteins of the lipoprotein transport system and is responsible for maintaining normal plasma cholesterol and triglyceride levels.

ApoE-deficient mice or apoE-deficient humans have plasma cholesterol levels higher than 400 mg/dl and develop premature atherosclerosis. ApoE has three natural isoforms in humans: apoE2, apoE3, and apoE4. Despite the beneficial effects of wild-type apoE, it has a reduced therapeutic value for the correction of dyslipidemias, because it acts as a double-edged sword: at physiological concentrations, it maintains lipid homeostasis and is atheroprotective; at high concentrations, apoE causes high triglyceride levels (hypertriglyceridemia) and fails to clear cholesterol from the circulation. We have used transient gene transfer methodologies in apoE-deficient mice to correct the apoE deficiency. These studies provided the following new insights on the role of apoE in cholesterol and triglyceride homeostasis in the circulation and the molecular etiology of type III hyperlipoproteinemia. a) Truncated apoE forms can clear efficiently cholesterol from the plasma of apoE-deficient mice; full-length apoE induces hypertriglyceridemia. b) Residues L261, W264, F265, L268 and V269 of apoE can account for the apoE-induced hypertriglyceridemia in vivo, and they also affect formation of HDL. Substitution of these residues by Ala provided a recombinant apoE form with improved biological functions which may have therapeutic applications in the future. c) The amino terminal domain 1-185 of apoE is sufficient to mediate efficient clearance of the apoE-containing lipoprotein remnants *in vivo via* the LDL receptor. d) ApoE-induced hypertriglyceridemia is due to increased hepatic VLDL triglyceride secretion and reduced activity of lipoprotein lipase in vivo. e) The LDL receptor deficiency or apoE mutations increase the sensitivity to apoE-induced hypertriglyceridemia. Based on these findings we were able to generate a recombinant full-length apoE form (apoE4[L261A/W264A/F265A/L268A/V269A] designated as apoE4mut1) with improved biological functions. Specifically, using adenovirus-mediated gene transfer and bolus injection of pure apoE, we found that apoE4mut1can efficiently correct the high cholesterol levels of apoE-deficient mice and promote the formation of HDL, even when expressed at high plasma concentrations. The ability of this protein to prevent atherosclerosis and stabilize unstable atherosclerotic plaques is currently under investigation. We believe that this recombinant apoE form is a potential new lead compound for the cure of dyslipidemias and possibly atherosclerosis in the future.

Human Autoimmune Diseases are Specific Antigen-Driven T-Cell Diseases: Identification of the Antigens

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Key words: Specific antigen-driven T-cell, human autoimmune diseases, identification

We are investigating the hypothesis that most human autoimmune diseases are specific antigen-driven T-cell diseases. Tcell clones responding to specific antigenic epitopes are responsible for the initiation and/or the propagation of these diseases. Similarly, specific antigen-driven T-cell responses are responsible for the rejection of organ allografts and the immune response to tumors. Activated T cells provide the *engine* for the chronic inflammation that is associated with autoimmune diseases, organ graft rejection and tumor immunity. The best way to identify whether specific antigen-driven T cell responses are involved in the initiation and/or propagation of these disorders is to investigate whether T cells that infiltrate relevant tissues from these diseases contain monoclonal or oligoclonal, that is to say clonally expanded, populations of T cells. Identification of the T-cell antigen receptor (TCR) transcripts employed by the clonally expanded T cells in these patients permits the identification of the specific antigens that elicit these T-cell responses. These antigens may be responsible for the pathogenesis of these diseases. We have developed an approach to identify the antigens recognized by the alpha/beta TCR or gamma/delta TCR transcripts of these *in vivo* clonally expanded T cells. We constructed by

PCR-mediated ligation full-length copies of the clonally expanded TCR transcripts. All combinations of these full-length TCR transcripts were expressed in a mutant T-cell line, using a retroviral vector. These T-cell lines were screened for recognition of target cells (such as tumor cells) or peptides presented to the T cells by an autologous EBVtransformed B-cell line using appropriate assays (calcium influx, cytotoxic or apoptotic assays, cytokine production, others). Using this approach we have identify a functional molecular mimicry between myelin oligodendrocyte glycoprotein (MOG) peptides and Hepatitis A virus (HAV) polyprotein peptides in a patient with pediatric MS (Zhang et al.). It is likely that HAV infection resulted in activation of virus specific T cells in this patient, the activated T cells crossrecognized self-antigen, MOG peptides, through molecular mimicry, and resulted in the demyelinating disease observed in this patient. In other experiments we expressed in Jurkat T cells, using the same approach, clonally expanded gamma- and delta-chain TCR transcripts in solid tumors and/or peripheral blood of patients with epithelial ovarian carcinoma (Whitfield et al.). These transduced Jurkat T cells expressing the clonally expanded gamma- and delta-chain TCR transcripts were able to lyse cells of ovarian tumor cell lines, providing the basis for the development of new approaches for gamma/delta TCR+ T-cell immunotherapy.

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Abstracts Posters

Design and Synthesis of Analogues of the Sequence 558-565 Loop of A2 Subunit of the Factor VIIIa Blood Coagulation

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Key words: Blood coagulation, factor VIIIa, A2 subunit loop, sequence 558-565, design and synthesis of analogues

The coagulation of blood is important for the precaution of an organism from bleedings and takes place through a process where thrombin is produced. However, the activation process of coagulation in physiological conditions is limited in local level at the lesion of the bleeding vessel. The irregular and increased production of thrombin further from the natural procedure is undesirable. Consequently, any effort that prevents the causing venous or arterial thrombosis and protects from potentially lethal complications it obviously improves and the conditions of survival. The present research reports in the synthesis of biologically active peptides, which are expected to inhibit selectively the maximisation of thrombin production depended on factor IX (FIX) and accordingly the additional activation of platelets. These peptides are based on the regions in which the factor VIIIa interacts with the factor IX. Both FVIII and FIX are essential for normal coagulation and deficiency of either is associated with the bleeding diathesis. The FVIIIa includes the subunits A1-A2-B-A3-C1-C2 with a molecular mass ~300 kDa. The sequence 558-565 of A2 subunit is the following:

Ser^{558}-Val^{559}-Asp^{560}-Gln^{561}-Arg^{562}-Gly^{563}-Asn^{564}-Gln^{565}

The present research work covers the synthesis and biological evaluation of linear and cyclic peptides, analogues of the A2 subunit, aiming at the inhibition of interaction of FVIIIa with FIXa. Thus the process of activation of coagulation is suspended and the aggregation of platelets is avoided. Substitutions have been taken place at Asn564, which are related with the existence of side-chain pharmaceutical groups, e.g. side-chain of Asp564. All the synthesized analogues are purified (RP-HPLC), identified (ESI-MS) and are under investigation for their biological activity against the FVIIIa factor of blood coagulation.

Acknowledgements: This Research Project is co-financed: 75% by European Union – European Social Fund and 25% by General Secretary Research & Technology (PENED 03ED569)

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Synthesis of PAR 1 Thrombin Receptor Mimetics, Using Piperidine as Template

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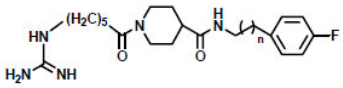
Key words: PAR1 thrombin receptors, novel non-peptide analogues, synthesis

Thrombin receptors are attractive drug discovery targets because of their mediation to a variety of cellular actions of thrombin, such as platelet aggregation. The cellular actions induced by thrombin trigger the activation of PAR receptors. PAR1 receptor is cleaved and activated by the serine protease a-thrombin and is involved in many cellular responses associated with hemostasis, proliferation and tissue injury. In the present study, novel non-peptide analogues have been synthesized based on conformational analysis of SFLLR of thrombin receptor in order to inhibit or prevent fibrin development without affecting thrombin actions. This work led to compounds 1 and 2 containing the rigid heterocyclic template of piperidine bearing spatially the key pharmacophoric groups. These two analogues differ in the side chain, in order to augment the lipophilicity of the molecule as well as to induce their ability to penetrate cell walls and cross biological membranes.



1: n=0; *2*: n=1

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Naphtho[de]1,2-oxazines: Synthesis and Application

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Key words: Naphtho[de]1,2-oxazines, synthesis, application

1,2-Oxazines (*1*), either as structure entities in their own merit or as core units/intermediates of carbo(hetero)cyclic frameworks are of considerable significance and potential. This is attributed to the facile scission of the N-O bond of the oxazine ring, hence their use, eventually, as precursors to regioselectively substituted naphthols/naphthalenes. Although 1,2-oxazines, partly or fully saturated, are abundant in the literature, there is only a dearth of reports on their aromatic congeners. The present work is targeted on: a) development of synthetic methodology towards substituted naphtha [de] 1,2-oxazines (*2*), and b) synthesis of suitably substituted naphthols and amino acids. These unnatural amino acids can be used as surrogates of amino acid residues of selected peptide sequences, in the hope that the, thus, generated semimetics will exhibit improved biological activity.

Acknowledgments: We thank the European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II), and particularly the Program PYTHAGORAS I, for funding the above work.

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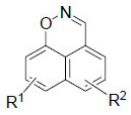

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Synthesis of the Kazal-type Inhibitor LEKTI Domain 6

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Key words: Kazal-type inhibitor, LEKTI domain 6, synthesis

Proteinase inhibitors are important negative regulators of proteinase action and are thus involved in several pathophysiological processes. LEKTI (lympho-epithilial Kazal-type related inhibitor) is a novel multidomain proteinase inhibitor consisting of 15 potential serine proteinase in-

hibitory domains. Defects within the gene encoding LEKTI, SPINK5 (serine protease inhibitor Kazal-type5), have been associated with several skin diseases and atopic disorders, including Netherton syndrome and atopic dermatitis. Hence, LEKTI represents a potential drug for treating these disorders. Here we report the synthesis of the 68-residue LEKTI domain 6 using Fmoc-based convergent and native chemical ligation techniques. For the protection of the four cysteines an orthogonal scheme was selected in order to achieve regioselective formation of the two disulphide bridges.

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Production and Properties of IgY Antibodies against the 11-24 Peptide of Biglycan

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Key words: IgY antibodies, 11-24 epitope of Biglycan, production, properties

Biglycan, a small leucine-rich proteoglycan, is believed to play a significant role in the mineralisation of bone. There is also evidence that biglycan binds and stimulates growth factor TGF-beta 1. For the production of IgY antibodies against 11-24 epitope of Biglycan (Big11-24), we synthesized a suitable peptide which is conjugated to polysaccharide mannan. With this conjugation, hens were immunized for the production of antibodies against 11-24 epitope of Biglycan. Moreover, in this report the design and synthesis of three tested peptide analogs (linear and cyclic), based on the immunodominant 11-24 epitope of Biglycan (Big11-24) using the Fmoc/tBu methology on the 2-chlorotrityl chloride resin, were described. The avidity and cross reactivity of the produced specific antibodies to peptide analogs of Big11-24 was measured. The aim of this work is to find the most suitable synthesized peptide for the purification of the produced specific IgY antibodies using affinity chromatography.

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Synthesis of Peptide Mimetics Based on C-Terminal Fragments of Substance P and their Biological Activity

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Key words: Substance P, C-terminal fragments, peptide mimetics, synthesis, biological activity

We have reported that small synthetic peptides, analogs of Substance P C-terminal fragments, increase the secretion of tumor necrosis factor α (TNF-α) and prevent the proliferation of several cancer cell lines1,2. We have also shown the antiproliferative activity in vitro of tri-and tetrapeptides, against the breast (T47D, SK-BR-3) and prostate (PC-3) cancer cell lines. N-MePhe and D-Trp were incorporated in the sequence of the synthesized peptides. In the present study we report the synthesis of a series of hydrazinopeptoide analogs of SP C-terminal hexa- and octapeptide, containing residues like NH-N(Bzl), D-Trp, Tic and the NPhe and NAla3, N-substituted glycine residues. The latter are residues having their side chains shifted from the chiral α-carbon atom of the corresponding amino acid onto the anchiral nitrogen. All the syntheses were carried out stepwise by SPPS, using the Fmoc/But methodology on the solid support 2chlorotrityl chloride resin and DIC/HOBt as coupling reagent4. All the above analogs were purified (HPLC) and identified (ESI-MS). The analogs No 5, 6 and 7 have been tested for their antipro-

liferative activity against the cancer cell lines MDA-MB-231 and HT-29 and also for their trypsin inhibitory activity. All the results will be discussed.

1: Glp[1]-NPhe[2]-Glu[3]-Phe[4]-Gly[5]-D-Trp[6]-NPhe[7]-D-Trp[8]-OH
2: Glp[1]-NPhe[2]-Glu[3]-Phe[4]-Pro[5]-D-Trp[6]-NPhe[7]-D-Trp[8]-OH
3: Glp[1]-NAla[2]-Glu[3]-Phe[4]-Gly[5]-D-Trp[6]-NPhe[7]-D-Trp[8]-OH
4: Glp[1]-NAla[2]-Glu[3]-Phe[4]-Pro[5]-D-Trp[6]-NPhe[7]-D-Trp[8]-OH
5: Glp[1]-NPhe[2]-Gly[3]-[NH-N(Bzl)-CH_2-CO][4]-D-Trp[5]-Leu[6]-OH
6: Glp[1]-NAla[2]-Gly[3]-[NH-N(Bzl)-CH_2-CO][4]-D-Trp[5]-Leu[6]-OH
7: Glp[1]-Tic[2]-Gly[3]-[NH-N(Bzl)-CH_2-CO][4]-D-Trp[5]-Leu[6]-OH

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Two Aromatic Residues of Type 1 Corticotropin Releasing Factor Receptor are Important for Peptide Binding

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Key words: Corticotropin releasing factor receptor, aromatic residues, peptide binding

The type 1 receptor (CRF1) for the corticotropin releasing factor (CRF) consists of seven membrane-spanning segments, connected by alternating three intracellular and three extracellular loops. To determine the functional role of the residues from Leu251 to Val266 in the second extracellular loop (EL2) of CRF1 we performed an alanine-scanning mutagenesis study. We found that mutation to alanine of the residues from Leu251 to Lys257 and from Gly261 to Val266, did not significantly reduce the binding affinity of the CRF-like peptide, sauvagine, as well as, its potency to stimulate the adenylyl cyclase. In contrast, substitution of Trp259 and Phe260 by alanine significantly reduced sauvagine affinity and potency. In addition, alanine mutation of Trp259 and Phe260 reduced the affinity of the agonist, CRF, without however affecting the affinities of the antagonists, astressin and antalarmin. These antagonists have been shown, in previous studies, to interact with different, than the EL2, regions of CRF1. Thus, mutation of Trp259 or Phe260 to alanine did not appear to significantly alter the overall conformation of CRF1, which is consistent with that Ala mutation, is a well-tolerated mutation that eliminates the side chain of a protein residue beyond the β-carbon, leaving a cavity, with little perturbation of the overall structure of protein. The above results suggest that Trp259 and Phe260 play a critical role in the binding of CRF and sauvagine to CRF1. Additional studies are now in progress, aiming to verify the interaction of sauvagine and CRF with the Trp259 and the Phe260 of CRF1, and to determine its nature. These studies will ultimately elucidate the mode of interaction of CRF1 with the peptides of CRF family, thus putting the basis for the design of CRF1-selective drugs.

❁

Synthesis of the Bicyclic Common Core of Polycyclic Polyprenylated Acylphloroglucinols; Synthetic Studies towards Hyperforin

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Key words: Polycyclic polyprenylated acylphloroglucinols, hyperforin, synthetic studies

Polycyclic Polyprenylated Acylphloroglucinols (PPAs) constitute a class of compounds with fascinating molecular architecture and a broad spectrum of bioactivity, including antioxidant, antiviral, and anticancer. Hyperforin, is a well known member and responsible for much of the antidepressant activity of Hypericum perforatum (1) which is used for the treatment of mild depression, anxiety and schizophrenia. Our efforts, targeting the development of a general method for the synthesis of the common bicyclic ring system of a plethora of PPAs as well as the total synthesis of Hyperforin will be presented.

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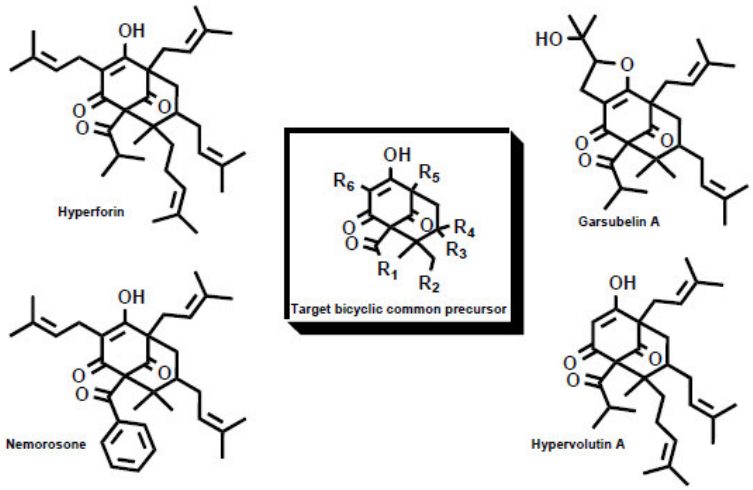

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Hypertension alters the Pattern of Adrenergic Receptors in Primate Prefrontal Cortices

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Key words: Hypertensive cerebrovascular disease, adrenergic receptors pattern, prefrontal cortices, primate

Using a primate model of hypertensive cerebrovascular disease, we investigated whether the prevalence of adrenoceptors in prefrontal cortices is altered as a consequence of chronic elevated blood pressure. Using quantitative in vitro receptor autoradiography we determined the high affinity binding sites for α_2 and β-adrenoceptors using [^{3}H]-clonidine and [^{3}H]-DHA, in prefrontal cortices in adult normotensive and surgically induced hypertensive rhesus monkeys. All areas investigated showed consistent pattern in the receptor laminar profile, with α_2 receptors showing the highest densities in cortical layer I, and β receptors in layers II, III and IV. Discriminate analysis revealed that hypertensive animals were characterized by higher density of α_2 receptors in comparison to controls in medial areas 14 and 9 (all layers), and areas 32, 24 (layer I), lateral area 46

(all layers), orbital areas OPro and 11, (superficial layers), and the gustatory area (layer I). Beta adrenoceptors showed a more complex profile in hypertensive cortices, up-regulation in areas 14 (all layers), 32 (layers I, II+III, VI), OPAll (layer I) and in the dysgranular insula (layer I), but down-regulation in lateral area 8 (all layers except VI), premotor area 6 (all layers), deep layers of area OPAll, and in subcortical structures (caudate, claustum). The modulation of α_2 and β adrenoceptors in prefrontal areas indicates that high-order association cortices are significantly affected in hypertension. The changes in noradrenergic neurotransmission in prefrontal cortices are in accord with evidence suggesting that the prefrontal cortex may be an important locus for impairment of executive function in hypertension.

Acknowledgements: Partly supported by EU QLRT-1999-31629 and NIH-NIA R37 AG17609

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Effect of Sodium Selenite Administration on Oxidative Stress Parameters in Crystalline Lens and Brain Areas of Neonatal Rats

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Key words: Oxidative stress, selenite, crystalline lens, brain areas, neonatal rats

Oxidative stress is implicated in the pathogenesis of neurodegenerative diseases, lens cataract and macular degeneration. Selenium is a trace element necessary for human organism. Selenium-dependent enzymes catalyze several important biologic functions, including antioxidant function. High concentrations of selenium are cytotoxic or possibly carcinogenic. Sodium selenite in large amounts induces the formation of superoxide anion and DNA damage. In the present study, the aim was to determine the effect of sodium selenite subcutaneous administration (20 και 25 μmol/kg body weight) to the oxidative stress parameters in the lens and the different brain regions of rats (cortex and midbrain). In particular, total antioxidant power was determined with a colorimetric assay (FRAP), malondialdehyde (MDA) was determined with a fluorimetric assay, and the lens proteins with polyacrylamide gel electrophoresis (SDS-PAGE). Sodium selenite administration on the 9th day after birth pups caused permanent cataract. Incidence of cataract was observed on the 16th postnatal day when the pups first opened their eyes. On day 20, animals were sacrificed and the tissues were isolated. Significant differences in the MDA levels of brain cortex were observed.

❁

Synergetic Use of 3D QSAR, Molecular Docking and Molecular Dynamics Simulations for the Conformational Analyses of Drugs

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Key words: Drugs conformational analyses, 3D QSAR, molecular docking, molecular dynamics simulations

In three-dimensional quantitative structure-activity relationship (3D QSAR) studies two most important steps are the selection of bioactive conformations of compounds being studied and their alignment based on the template compound. These steps not only affect significantly the results of the analysis, but they are also define the design of novel molecules. If the x-ray structure of a protein target and the experimental data concerning the ligand-receptor complex are available, the selection of the bioactive conformer is a simple procedure and the molecules can be aligned with greater credibility. However, when no experimental structural information is available, molecular modeling techniques can be used to explore the bioactive conformations of the drug. Molecular docking and molecular dynamics (MD) simulations of the selected bioactive conformations of the ligand at the binding site of the receptor contribute in the clarifications of its structural and dynamical properties at the receptor. Moreover, including the protein-membrane interactions in the drug design protocol one may get very reliable conformations of small molecules which help to avoid conformational artifacts of experimental measurements. An example of the usefulness of this approach is given by the recent combined 3D QSAR and molecular docking study of cannabinoids [Durdagi, S. et al., Bioorg. Med. Chem. Lett., 2007, 17, 6754]. In addition, MD simulations of analgesic, antihypertensive and antiviral bioactive ligands at the active site of the receptors including protein-membrane interactions are in progress

Chemical Biology of Small Molecules Interfering with Protein-Synthesis

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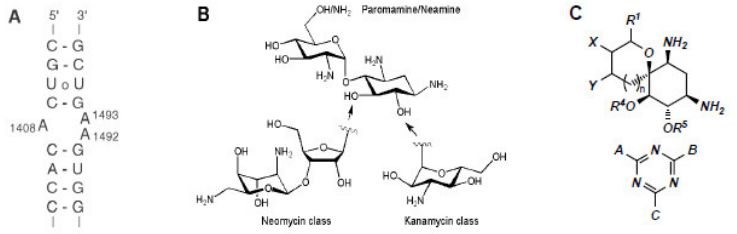
Key words: Small molecures, chemical biology, protein synthesis



The bacterial ribosome is a key target for many natural and semi-synthetic antibiotics, such as aminoglycosides, tetracyclins, and macrolides, which interact preferentially with its RNA components (rRNA). The decoding- or A-site, an internal loop within the 16S rRNA, is the molecular target for natural aminoglycoside antibiotics (Scheme), which interfere with the conformational flexibility of two adenines involved in mRNA decoding, thereby inducing an increased error rate in protein synthesis and ultimately leading to bacterial cell death. Recent crystallographic advances in the field of RNA along with our molecular modeling studies, assay development and synthetic chemistry efforts, will be presented. Our studies aim at improving the pharmacological and resistance profiles of natural aminoglycoside antibiotics. Furthermore, they provide the necessary tools for specifically screening small molecules against well defined and characterized RNA targets.

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New Contrast Agents for Magnetic Resonance Imaging Targeting Cancer Cells

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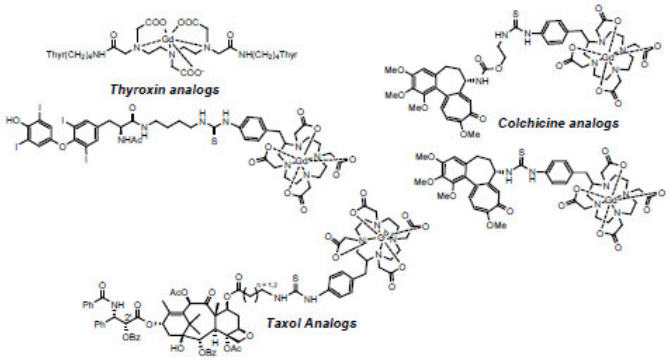
Key words: Cancer cells, magnetic resonance imaging, new contrast agents



Magnetic resonance imaging (MRI) is a non-invasive clinical imaging technique, which relies on thedetection of NMR signals emitted by hydrogen protons in the body placed in a magnetic field. MRI contrast agent (CA) is a unique class of pharmaceuticals that enhances the image contrast between normal and diseased tissue, and indicates the status of organ function or blood flow after administration, by increasing the relaxation rates of water protons in tissue, in which the agent accumulates. Contrast agents are being used in MRI since 1983 when the first injection of gadolinium (Gd) DTPA was performed in men. Since then CA have gained great acceptance. They function by shortening of T1-relaxation time, thereby increasing signal intensity (SI) (positive contrast enhancement). Conceptually, antibodies or other tissue-specific molecules may be combined with paramagnetic centers to provide disease-specific MRI agents. The challenge with regard to delivering sufficient quantity of paramagnetic label is substantial. On that front, we have synthesized a series of gadolinium conjugates with Colchicine, Taxol™ and Thyroxin, known for their tubulin- and hormone receptor-binding properties respectively, targeting the discovery of novel, cancer specific CA. Cytotoxicity and relaxation time measurements have been performed for all new complexes.

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Synthesis of 5-Amino-substituted 1,2,4-Oxadiazole Derivatives with Possible Antiinflammatory Activity

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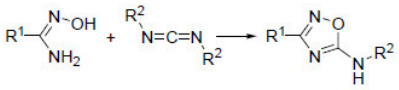
Key words: 5-Amino-substituted 1,2,4-oxadiazoles, derivatives, anti-inflammatory activity



1: R^1 = -C_6H_4-*p*-CH_3O, **1a**: R^2 = cyclohexyl
2: R^1 = -C_6H_4-*p*-CH_3, **2a**: R^2 = cyclohexyl
3: R^1 = -C_6H_4-*m*-NO_2, **3a**: R^2 = cyclohexyl
4: R^1 = -CH_2-C_6H_5, **4a**: R^2 = cyclohexyl
5: R^1 = -CH_2-C_6H_4-*p*-Cl, **5a**: R^2 = cyclohexyl
-CH_2-C_6H_4-*p*-Cl, **5b**: R^2 = isopropyl
6: R^1 = -CH_2-C_6H_4-*p*-NO_2, **6a**: R^2 = cyclohexyl
7: R^1 = -cyclohexyl
8: R^1 = -butyl

Various 5-amino substituted 1,2,4-oxadiazoles were synthesized in one step and in high yields via reactions of amidoximes with carbodiimides (1). Studies of the antiinflammatory activity of 1,2,4-oxadiazole derivatives are rather scarce in the literature (2,3). The prepartion in a highly efficient way of a number of aromatic and aliphatic derivatives gave us the opportunity to study their mechanism of action and to reach to important conclusions regarding their structure-activity relationships. All amidoximes *1-8* were synthesized under standard conditions, whereas the heterocyclic derivatives *1a-6a* were obtained via a novel methodology in good to excellent yields. The compounds were tested in vitro on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) action as NO donors in the presence of a thiol factor and d) in vivo for the inhibition of carrageenin induced rat paw edema. The compounds have shown significant antioxidant activity, satisfactory anti-inflammatory activity comparable to indomethacin and high inhibition on soybean lipoxygenase as a result of their physicochemical features.

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Synthesis of Sulfonamide-1,2,4-triazole-[3,4,b] [1,3,4]-Thiadiazoles and Sulfonamide-1,2,4-triazole-[3,4,b] [1,3,4]-Thiadiazines as possible Antibacterial and Antifugal Agents

Ch. Camoutsis[1], V. Tsinias[1], D. Natsiou[1], A. Stroumpini[1], E. Topouzidou[1] and P. Zoumpoulakis[2]

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Key words: 1,2,4-Triazole derivatives, fused heterocycles, antibacterial and antifugal agents

Various 1,2,4-triazole derivatives and fused heterocycles, as s-triazole[3,4-b] [1,3,4]-thiadiazole and s- triazole[3,4-b] [1,3,4]-thiadiazines, are found to be associated with diverse pharmacological activities (1,2). These compounds are obtained by fusing the bio-labile 1,2,4-triazole with 1,3,4-thiadiazole and 1,3,4-thiadiazine rings together, Prompted by these observations and in continuation of our search for bioactive molecules, we designed the synthesis of a series of novel compounds and in particular various sulfonamide-s-triazole[3,4-b] [1,3,4]-thiadiazole and sulfonamide-s-triazole[3,4-b] [1,3,4]-thiadiazines, in order to screen them for anticacterial and antifungal activity.

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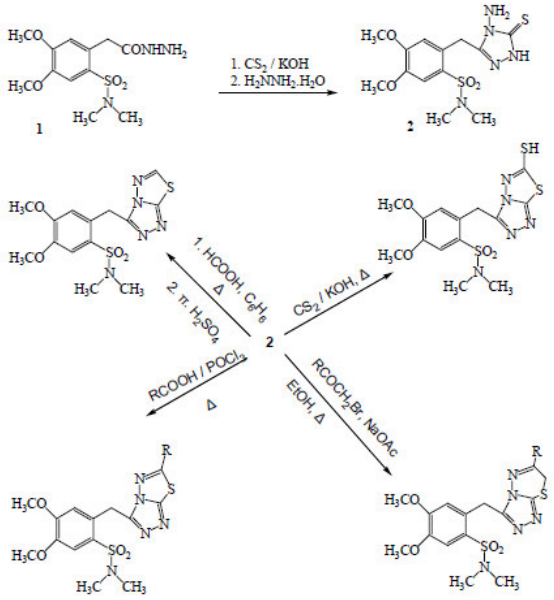

Sustained Activation of CREB Mediates Neuronal Differentiation in α_2-AR Transfected PC12 Cells

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Key words: α_2-Adrenergic receptors, transfected PC12 cells, cAMP-response element-binding protein, neuronal differentiation, activation

The α_2-adrenergic receptors (α_2-ARs) are members of the G-protein coupled receptor (GPCR) super family. They mediate physiological responses to the endogenous catecholamines, epinephrine and norepinephrine and have fundamental functions in neuronal physiology. Three different genes encode the human α_2-adrenergic subtypes (α_{2A}, α_{2B} and α_{2C}), that differ in their ligand binding properties, tissue distribution, chromosomal location and signaling pathways (1). Recent studies have demonstrated that epinephrine induces subtype-specific morphological and molecular neuronal differentiation of PC12 cells transfected with α_2-adrenergic receptors (2). Specifically both PC12α_{2B} and PC12α_{2C} cells displayed clear differentiation responses to epinephrine, whereas PC12α_{2A} displayed minimal differentiation response. The molecular basis of these differences has not been clearly understood. Neuronal functioning and many aspects of development, such as precursor proliferation, neuronal survival and differentiation and process outgrowth are regulated by the transcription factor cAMP-response element-binding protein (CREB) (3). In the present study we have investigated the role of CREB in α_2-AR induced differentiation (4). We employed a luciferase assay and western blotting analysis and we have found sustained activation of CREB by epinephrine during the differentiation process in a subtype-specific manner that closely resembles the differentiation properties of these subtypes. This prolonged signaling is mediated by the transactivation of the receptor tyrosine kinase TrkA (5), and is critically involved in α_2-AR induced differentiation as indicated by the effect of dominant nega-

tive mutants of CREB on the expression of Cyclin D1 and the neuronal marker peripherin as well as neurite outgrowth. Furthermore, overexpression of CREB in PC12α2A clone resulted in morphological (neurite outgrowth) and molecular (expression of peripherin) differentiation comparable to that of PC12α_{2B}/α_{2C}, by prolonging the time period of CREB activation.

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Studies towards the Development of Convergent Methodologies for the total Synthesis of Acitretin Analogs Incorporating Changes in the Lipophilic Part

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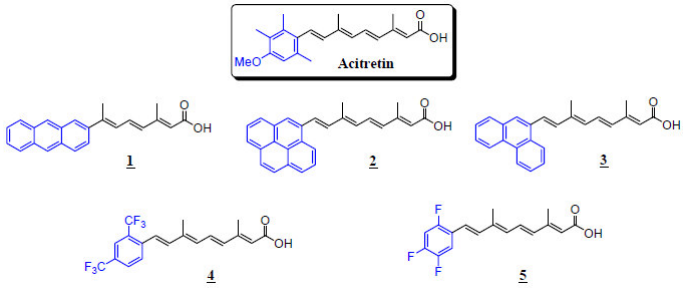
Key words: Acitretin analogs, lipophilic part, convergent methodologies, synthesis

Acitretin is a second generation monoaromatic acidic retinoid currently used in the treatment of severe psoriasis and other dermatoses. In the present work, we describe our efforts towards the development of convergent methodologies for the assembly of the retinoid skeleton using the well-established reactions Wittig and Horner-Emmons. These methodologies are expected to provide access to acitretin and a series of novel analogs suitable for structure-activity relationship studies, such as the retinoids *1-5* with condensed aromatic rings or electron-withdrawing fluoro/trifluoromethyl substituents on the aromatic ring.



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Pharmacokinetics and *in vitro* Metabolism of Leuprolide in Mice. Mass Spectrometry based Evaluation of Efficacy and Toxicity

T. Katsila[1], Z. Sofianos[1], N. Kostomitsopoulos[2], E. Balafas[2], J. Matsoukas[3], T. Tselios[3] and C. Tamvakopoulos[1]

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Key words: Leuprolide, pharmacokinetics, metabolism, spectrometry, efficacy, toxicity, mice

Prostate cancer is a leading cause of illness and death in the United States and Europe being the second most common malignancy among men in western societies after lung cancer. It is estimated that one in eight men will be diagnosed with the disease. Focusing on the molecular mechanisms of prostate cancer, the development and progression of the disease is mediated by androgens (testosterone, dehydrotestosterone) and growth factors. Based on the above, novel approaches were designed and performed having Leuprolide, a well known peptide drug for the treatment of prostate cancer, as the model compound: (i) an in-house *in vitro* protocol was established for metabolic studies using mouse kidney membranes, (ii) an *in vivo* protocol was developed for the determination of pharmacokinetic parameters in mice and (iii) a novel approach was developed for the quantification of testosterone (efficacy/toxicity biomarker) in mouse plasma following the administration of Leuprolide. Mass spectrometry based approaches that include the use of a *Hybrid Quadrupole Linear Ion Trap Mass Spectrometer System*, a platform that combines the potential of triple quadrupole instruments with that of a Linear Ion Trap, were a central part of this study. Considering the current need for novel improved therapies, this approach offers the potential for the in vitro and in vivo evaluation of Leuprolide peptide analogues (and other bioactive peptides) as well as the monitoring of efficacy/toxicity biomarkers.

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Biomimetic Chemistry: Zn(II) and Cd(II) Complexes as Structural Models for Substrate Activation in Hydrolytic Metalloenzymes

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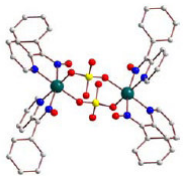
Key words: Biomimetic chemistry, hydrolytic metalloenzymes, complexes Zn(II) & Cd(II), structural models, substrate activation



One of the mechanisms for the substrate activation during hydrolytic processes in natural systems is the incorporation of metal ions in the active site of hydrolytic enzymes. The main natural choice is Zn(II), which has been identified in a great number of enzymes, while other choices are Ni(II), Cd(II), Mn(II) etc. In many of these metallohydrolases one metal ion per active site is available (carboxypeptidases, thermolysine), while in others two or three metal ions are implicated in the catalytic process (alkaline phosphatase, nuclease P1, sulfurylases). The elucidation of the role of the metal ions and the overall catalytic mechanism is a subject of great interest for bioinorganic chemists, not only from theoretical and mechanistic points of view, but also as a guide for the development of biomimetic catalysts. We report here the synthesis and study of the complexes $[Zn_2(PO_3F)_2(LH)_4]$ (*1*) (where LH: 2-pyridylaldoxime), $[Zn_2(SO_4)_2(LH)_4]$ (*2*) and $[Cd_2(SO_4)_2(LH)_4]$ (*3*) (where LH: phenyl-2-pyridyl ketonoxime), which are structural models of the transition state during the catalytic mechanism of some phosphohydrolases and sulfurylases.

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Conformational Analysis of Leuprolide Analogue in DMSO Solution Using Nuclear Magnetic Resonance (NMR) and Molecular Dynamics (MD)

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Key words: Leuprolide acetate analogue, conformation analysis, nuclear magnetic resonance, molecular dynamics

Leuprolide acetate (LPA) (pGlu-His-Trp-Ser-Tyr-(D)Leu-Leu-Arg-Pro-NHEt) is a potent gonadotropin-releasing hormone (GnRH or LHRH) agonist and is used to treat a wide range of sex hormone related disorders, including advanced prostatic cancer, endometriosis and precosious puberty. Despite its widespread use, only limited information based on spectroscopic evidence regarding the solution conformation of Leuprolide can be found in the literature. The aim of this study was to characterize the solution conformation of a modified linear analogue of Leuprolide (pGlu-His-Trp-Ser-Tyr(OMe)-(D)Leu-Leu-Arg-Aze NHEt) in DMSO using Nuclear Magnetic Resonance (NMR) and Molecular Modeling techniques. By using both NMR and Molecular Modeling we tried to characterize the secondary structural preferences of this Leuprolide analogue. Computer calculations were performed on a Silicon Graphics O2 workstation using Quanta 2005 and CHARMm force field.

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❁

Design, Synthesis and Conformational Analysis of Histamine Analogues for the Treatment of Hypertension

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Key words: Histamine analogues, design, synthesis, conformational analysis, hypertension

High blood pressure and its results cause hypertension. The octapeptide Ang II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) is the main factor or the Renin-Angiotensin System (RAS). It regulates arterial blood pressure and increases it. The main treatments of hypertension that are used today concern the suspension of Ang II activity with non peptide antagonists (analogues) of AT1 receptor of Ang II. The present work includes the synthesis and the molecular modeling analysis of (4) histamine analogues with possible antihypertensive activity through RAS system and adrenergic receptors. These analogues were syperimposed and compared with Losartan and specifically its active metabolite EXP 3174 so we can conclude to the analogues with the most possible bioactive conformation.

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❁

Synthesis of Cyclic RGD Analog Peptides Incorporating Thiosalicylic Acid and their Biological Activity

Fotios Ntellos[1], Yiannis Sarigiannis[1], George Stavropoulos[1]*, Maria Liakopoulou-Kyriakidou[2] and Pantelis Makris[3]

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Key words: RGD, cyclic RGD analogs, thiosalicylic acid, synthesis, biological activity

We have already demonstrated that small cyclic peptides containing the sequence Arg-Gly-Asp (RGD) show extremely strong antiplatelet activity on human platelets (1,2). Continuing this research project we have synthesized a new series of cyclic RGD analogs (I-V), incorporating salicylic acid derivatives, by solid phase peptide synthesis. The analogs were tested for inhibitory activity on human platelet aggregation in vitro, by adding common aggregation reagents (collagen, ADP) to citrated platelet rich plasma, following the method of Born and recording the increase of light transmission.

I. Cyclo-[C_6H_4(S)CO-Arg-Gly-Asp-Cys]-OH
II. Cyclo-[C_6H_4(S)CO-Arg-Gly-Asp(Bzl)-Cys]-OH
III. Cyclo-[C_6H_4(S)CO-Arg-Gly-Asp(Bzl)-β-Ala-Cys]-OH
IV. Cyclo-[C_6H_4(S)CO-β-Ala-Arg-Gly-Asp(Bzl)-Cys]-OH
V. Cyclo-[C_6H_4(S)CO-Arg-Gly-Asp(Bzl)-Cys]-NH_2

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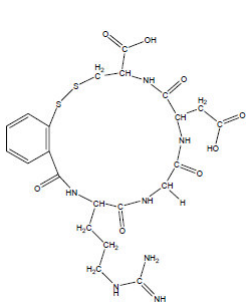

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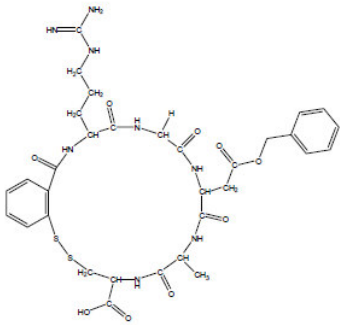

III

❁

Xanthone in Synthesis: Regioselective Derivatisation *via* Metalation and Electrofilic Substitution

M. Odrowaz-Sypniewski[1], P.G. Tsoungas[2] and P. Cordopatis[1]

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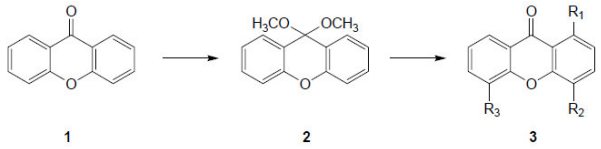
Key words: Xanthone system synthesis, regioselective derivatisation, metalation, electrophilic substitution

The xanthone core *1* is present in a large family of natural products with a broad spectrum of biological and pharmacological activities. During the past century, a variety of methods of differently

substituted xanthone system synthesis have been developed. In the overwhelming majority of cases they started from suitably substituted building blocks. Very few methods are based on the direct derivatisation of xanthone. In this work we would like to present the first general strategy of direct introduction of variety of functional groups to the xanthone. Depending on the conditions of reaction of 9,9-dimethoxyxanthene *2* with butyllithium, C4, C4 and C1 or C4 and C5 lithiation takes place. After quenching with an electrophile, corresponding derivatives are obtained. Thus alkyl R=CH_3, halogen R=I, formyl R=CHO, dialkylaminocarbonyl R=$CONEt_2$ and arylhydroxymethyl R=CHPh(OH) groups were introduced into xanthone system.

Acknowledgments: We thank the European Social Fund (ESF), Operational Program for Educational and Vocational Training II (EPEAEK II), and particularly the Program PYTHAGORAS I, for funding the above work.



❁

Effect of certain Parameters on the Physicochemical and Aerodynamic Properties of Formoterol Fumarate Mixtures Used for Dry Powder Inhalers

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Key words: Formoterol fumarate mixtures, physicochemical & aerodynamic properties, dry powder inhalers

The current study regards the effect of certain parameters (such as relative humidity and temperature) on the formulation of formoterol fumarate (a long acting $beta_2$-adrenergic agonist) and lactose mixtures and their filling into blisters, for inhalation via a dry powder inhaler (DPI). The ideal conditions for powder formulation and blisters' filling were examined, as to achieve the optimum therapeutic fraction FPD (Fine Particle Dose). Several batches were manufactured by blending micronized formoterol fumarate with lactose (carrier) at different room conditions. It was observed that the increased relative humidity (53%) led to a decrease of FPD because of the particles' cohesion and agglomeration. Decrease of relative humidity down to 45% gave more satisfactory FPD values, but the powder bore electrostatic charges that affected the uniformity of filling. When the RH was further lowered (< 30%) these charges were eliminated, thus giving acceptable weight and content uniformity results, as well as excellent drug deposition (in vitro control). Finally, the ideal conditions for satisfactory powder flowability, blisters' filling and chemical stability of formoterol fumarate (substance sensitive to temperature and humidity) were determined as follows: RH < 30% and T< 20 ^oC.

❁

Rational Design of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) Inhibitors Based on Molecular Dynamics Simulations

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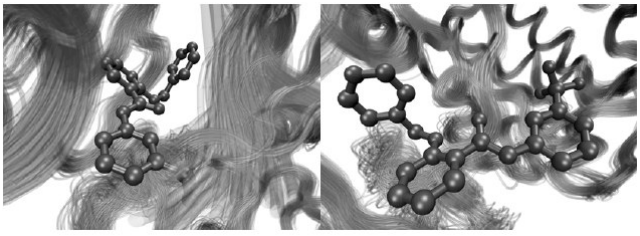
Key words: Vascular endothelial growth factor receptor-2 inhibitors, molecular dynamics simulations

It is widely accepted that inhibition of angiogenesis may constitute a revolutionary method for cancer treatment, exemplified by the clinical success of an anti-VEGF monoclonal antibody, Avastatin®, for the treatment of metastatic colorectal cancer. Known problems associated with the high cost of antibody preparation, along with its intravenous administration generates an immense interest towards small molecule inhibitors. The tyrosine kinase Flk-1/KDR (VEGFR2) high affinity receptor of VEGF represents the major target for the development of small molecule inhibitors of angiogenesis. The inherent flexibility of the kinase receptor VEGFR-2, especially in the area close to the catalytic site, complicates the rational design of novel inhibitors based on the available crystallographic information. For this reason we have employed docking calculations in combination with molecular dynamics (MD) simulations in explicit solvent. Data analysis of several enzyme–inhibitor complexes in the presence of water molecules, comprising thousands of MD snapshots, reveals interesting structural features, not evident from the x-ray crystal structures.



❁

Synthetic Studies towards Laurenditerpenol, a Novel HIF-1 Inhibitor

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Key words: Laurenditerpenol, HIF-1 inhibitors, synthetic studies

Laurenditerpenol (*1*) is a structurally novel bicyclic diterpene isolated by bioassay-guided fractionation of the lipid extract of the red alga Laurencia intricata. This natural product potently and selectively inhibits hypoxia-activated HIF-1 (IC50: 0.4 M) and hypoxia-induced VEGF (a potent angiogenic factor) in T47D cells (1). HIF-1 inhibitors represent potential novel anticancer drug leads (2). Laurenditerpenol features an unprecedented 7-oxabicyclo[2.2.1]heptane ring system and several contiguous stereogenic centers. Until recently, only the absolute configuration at C1 and the relative configuration of the bicyclic ring system have been established (3). The combination of structural novelty and complexity along with its important biological properties led us to design a synthetic strategy towards this natural product. Our exploratory synthetic studies that led to the preparation of two analogues of laurenditerpenol (*2, 3*) will be presented.

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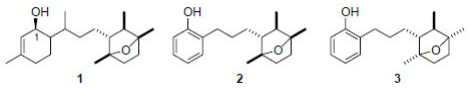

❁

Hypertension Study in Anaesthetized Rabbits: A New Protocol for AT_1 Antagonists Screening

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Key words: AT_1 antagonists, antihypertensive agents, losartan, active metabolite EXP 3174, protocol, anaesthetized rabbits

Hypertension is considered a major risk factor for cardiovascular diseases. Although many antihypertensive agents are available, blood pressure is poorly controlled in many patients. Thus, the necessity for the design and synthesis of new drugs is urgent and many animal models are used for the pharmacological screening of potential antihypertensive agents. In our study we used continuously infused with Ang II anaesthetized rabbits, as animal model. The Ang II infusion lasted for 20 min and the administered drugs were the AT_1 antagonist losartan or its active metabolite EXP 3174. The present study addressed the following issues: (i) the possible tachyphylaxis effect induced by the continuous infusion with Ang II, (ii) the produced blood pressure decrease after administering single bolus or sequential injection of 3 boluses of the studied drugs (post-treatment) and, (iii) the effectiveness of the pre-treatment injection with losartan or EXP3174 versus the posttreatment procedure. The results of the experiments may establish an optimized protocol for studying the *in vivo* antihypertensive effect of novel synthetic compounds in terms of the optimum duration of the study as well as the minimum injected dose of the tested molecule.

❁

Designing Lipoxygenase Inhibitors with Antioxidant/Anti-Inflammatory Activity Based on QSAR Findings

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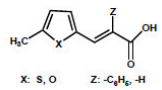
Key words: Lipoxygenase inhibitors, designing, antioxidant/antiinflammatory activity, QSAR study

Eicosanoids are a family of lipid mediators derived from the metabolism of arachidonic acid. The cascade involves two major pathways: a) the lipoxygenase (LO) is the first enzyme in a cascade which produces leukotrienes (LTs) while cycloxygenase (COX) initiates the cyclic pathway

leading to prostanoids. These eicosanoids have a wide range of biological actions including potent effects on inflammation and immunity. Inhibition of LO results inhibition of the synthesis of HPETE's, HETE's and leukotrienes, which have been implicated as important mediators in several diseases including asthma, arthritis, psoriasis, inflammatory bowel disease and others. According to the literature (1,2) and from the QSAR study some new phenyl-substituted or not 3-(5-methyl-thiophen/furan-2-yl)-acrylic acids have been synthesized in order to improve their biological activity. The compounds are tested in vitro on: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, c) the $HO^{\cdot}$ mediated oxidation of DMSO, d) antiradical activity as loss of $ABTS^{\cdot}$ radical, e) inhibition of lipid peroxidation using the AAPH protocol, f) scavenging of superoxide anion radicals, g) *in vivo* for the inhibition of carrageenin induced rat paw edema. The compounds have shown potent antioxidant activity, good anti-inflammatory activity and high inhibition of soybean lipoxygenase as a result of their physicochemical features.



Acknowledgements: Acknowledgements to the *Foundation for Education and European Culture* for financial support and to the Research Committee of the Aristotle University of Thessaloniki for scholarship.

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❁

Interactions of a Pair of Synthetic Peptide Analogues of Myelin Basic Protein with Lipid Bilayers Using DSC and High Resolution Solid State ^{13}C NMR

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Key words: Myelin basic protein, synthetic peptide analogues, DSC, ^{13}C NMR

MBP87-99 epitope peptide and its antagonist MBP87-99 (Arg91, Ala96) interactions with lipid bilayers of dipalmitoylphosphatidylcholine bilayers were investigated using Differential Scanning Calorimetry (DSC) and high resolution solid state ^{13}C NMR spectroscopy in an attempt to explore the role of the lipid bilayers in their physiological action. Increasing molar ratios of the synthetic peptides ranged between 1-10%, were used in order to examine their thermal and dynamic properties. DSC results showed that both molecules are incorporated in lipid bilayers and affect significantly their properties in a distinct and concentration dependent way. Preliminary results of 13C solid state NMR showed the incorporation of agonist and antagonist in lipid bilayers in agreement with DSC results.

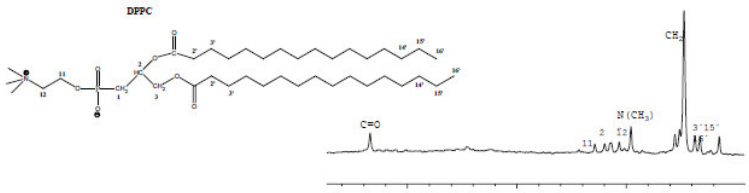

Figure 1: ^{13}C solid state MAS spectrum of DPPC bilayers containing 10% molar ratio of agonist MBP87-99. The spectrum was obtained with a 400 MHz Varian spectrometer at a spinning rate of 8 MHz

Synthesis and Conformational Analysis of a Non-peptide AT1 Receptor Angiotensin II

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Key words: Non-peptide AT1 receptor angiotensin II, synthesis, conformational analysis

The octapeptide Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) is the major factor of the Renin–Angiotensin System (RAS) and plays a significant role in the regulation of arterial blood pressure. In the present study, we have designed and synthesized a non-peptide Angiotensin II AT1 antagonist V8, based on Losartan. The synthetic strategy concentrated on changes in the orientation of the imidazole ring relative to the substituents, which were maintained in a similar pattern to that found in Losartan. From Structure Ativity Relationship and Conformational Analysis studies, the antagonist V8 was synthesized using 4(5)-butylimidazole as a template bearing the crucial pharmacophoric groups of Angiotensin II, thus imidazole, phenyl, tetrazole. Simultaneously, Conformational Analysis was performed using a Grid Scan Search in order to derive all the possible conformations from which six energy local minima (syn and anti) were extracted after a Cluster Analysis. Furthermore, these different conformations were superimposed with Losartan, where a spatial correlation among the pharmacophoric groups is observed.

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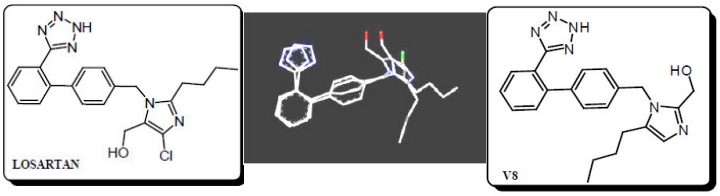

Effect of Altered Peptide Ligands of Myelin Basic Protein on the Subpopulation of T Regulatory Lymphocytes Isolated from Multiple Sclerosis Patients

Panagiota Sakellaraki[1], Panagiotis Papathanasopoulos[2], Kokona Chatzantoni[1], Odysseas Kariotis[2], Maria Koutsokera[2], Georgios Deraos[3], Spiros Deraos[3], Theodoros Tselios[3], John Matsoukas[3] and Athanasia Mouzaki[1]

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Key words: Multiple sclerosis, patients, myelin basic protein, altered peptide ligands, T regulatory lymphocytes

Multiple sclerosis (MS) is a chronic inflammatory degenerative disease of the central nervous system (CNS) characterized by macrophage and lymphocytic infiltrates, demyelination and progressive loss of neurological function. MS has a strong autoimmune basis, characterized by the

presence of CNS-specific CD4+ and CD8+ T lymphocytes. A major target of autoreactive T-cells in MS is myelin basic protein (MBP). In this study we used peptide analogs of MBP and assessed their effect on T regulatory cells (Tregs), a group of lymphocytes that play a crucial role in immune tolerance. The most common type of Tregs in humans express the surface markers CD4 and CD25 ± the transcription factor FoxP3. In the present study peripheral blood mononuclear cells (PBMC) were isolated from 13 MS patients and 8 controls and cultured ± MBP peptide analogs for 0 or 24 hours. The number of Tregs was determined by flow cytometry. The results show that 24h incubation with certain peptides changed dramatically the percentage of Tregs of the MS patients.

❁

Synthesis AT_1 Angiotensine II Receptor Antagonists Based on Imidazole

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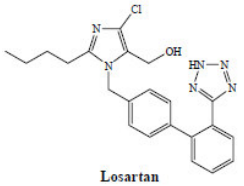
Key words: AT_1 Angiotensin II receptor, antagonists, imidazole, synthesis

The Renin-Angiotensin System (RAS) plays a significant role in the regular of cardiovascular homeostasis and electolite balance (1). The octapeptide angiotensin II

(H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH)

is the main factor of the RAS, regulates the arterial blood pressure and induses hypertension. An approach for the treatment of hypertension is the design and synthesis of non peptidic antagonist analogues (mimetics) (2) of AT_1 Angiotensin II receptor. In this study, we synthesized molecules based on 4-butyl-imidazole derivative on which the following pharmacophoric groups are incoporated: the (bi)phenyl group (Phe), the acedic group of tetrazol (-COOH C-terminal) and the hydroxymethyl group (-OH, Tyr) are bound according to the Losartan structure.



Losartan

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❁

Synthesis and Biological Evaluation of keto and Exomethylene D-Lyxopyranonucleoside Analogues

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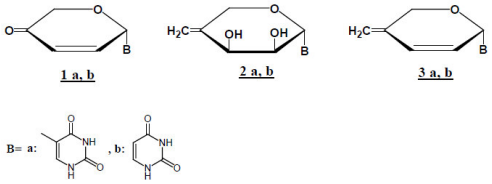
Key words: D-Lyxopyranonucleoside analogues, keto, exomethylene, synthesis, evaluation

Nucleoside analogues display a wide range of biological activity as anti-tumor, antiviral and chemotherapeutic agents. Lately, nucleosides with a six-membered carbohydrate moiety have

been evaluated for their antiviral, antibiotic properties and as building blocks in nucleic acid synthesis. Among them, unsaturated keto nucleosides are well established for their antineoplastic activity and immunosuppressive effects. Furthermore, modified nucleosides bearing an exocyclic methylene or fluoromethylene group in position 2´, 3´or 4´ exhibited potent anti-tumor and antiviral activities. Based on these findings we have designed, synthesized and pharmacologically evaluated a new series of unusual nucleosides, i.e., the unsaturated keto (1a,b), exomethylene (2a,b) and unsaturated exomethylene (3a,b) D-lyxopyranonucleosides as potential antiviral or anti-tumor agents.



Structure-activity Relationships Studies on Angiotensin II Antagonists and Aldosterone Production

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Key words: Angiotensin II antagonists, aldosterone production, structure-activity relationships

Angiotensin II is a peptide hormone composed by eight aminoacids (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH, AngII). Angiotensin-converting enzyme, which is bound to the plasma membrane of endothelial cells, cleaves two amino acids from AngI to form AngII. AngII has several important actions integral to maintaining circulatory homeostasis, including promoting the constriction of the arterioles within the renal and systemic circulations and the reabsorption of sodium in proximal segments of the nephron. It also stimulates the adrenal cortex to secrete aldosterone, which promotes the reabsorption of sodium (in exchange for potassium) in distal segments of the nephron, in the colon and the salivary and sweat glands. At present, there are three recognized angiotensin receptor subtypes AT_1, AT_2 and the AT_4. Classically, AT_1 and AT_2 relay signals by directly activating heterotrimeric guanine nucleotide-binding proteins (G proteins). Increasing evidence indicates that AT_1 may also signal through G protein-independent pathways in particular, GRKs/beta Arrestins. Consequently in order to inhibit aldosterone production new antagonists of beta Arrestin 1 are required, alternatively new Sartans, which will compete activation of beta Arrestin 1 by AT_1 receptor. Six Analogues of AngII were synthesized by Fmoc/But solid phase in high yields. The new analogues are:

[Sar^1,Ala^8]-AngII,
[Sar^1,Val^5,Ala^8]-AngII
[Sar^1,Ile^4,Ile^8]-AngII
[Sar^1,Aib^4,Ile^8]-AngII,
[Sar^1,Deg^4,Ile^8]-AngII
[Sar^1,Gly(But)^4,Ile^8]-AngII.

Information on their binding affinity to AT_1 and the signal transduction pathways elicited by them, will contribute to the structure-activity relationships and the development of more potent AngII analogues.

Microwave Enhanced Solid Phase Peptide Synthesis of Human Myelin Epitope MOG97-108, Utilizing 2-Chlorotrityl Chloride Resin

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Microwave energy represents a fast and efficient way to enhance both the Fmoc deprotection and coupling reactions in 9-fluorenylmethyloxycarbonyl (Fmoc) solid phase peptide synthesis (SPPS). Unlike conventional heating, microwave energy directly activates any molecule with a dipole moment and allows for rapid heating at the molecular level (1). A 12mer peptide, MOG_{97-108}, which is an immunodominant epitope of human myelin, was synthesized with Microwave Enhanced Solid Phase Peptide Synthesis utilizing 2-chlorotrityl chloride resin. The protected peptide was synthesized using the CEM Liberty automated microwave peptide synthesizer in 18 hours with a 3-minute Fmoc deprotection and 5-minute coupling reactions with microwave energy. The maximum temperature reached during both the deprotection and coupling reactions was 80 ^{o}C. Fmoc deprotection was achieved with 25% Piperidine in DMF, while coupling reactions were achieved with a 5-fold molar excess of 0.2 M Fmoc-protected amino acids dissolved in DMF and HOBt/DIC in DMF at a concentration of 0.5M as activating reagents. The final crude product was of high purity as shown in Scheme 1.

REFERENCES

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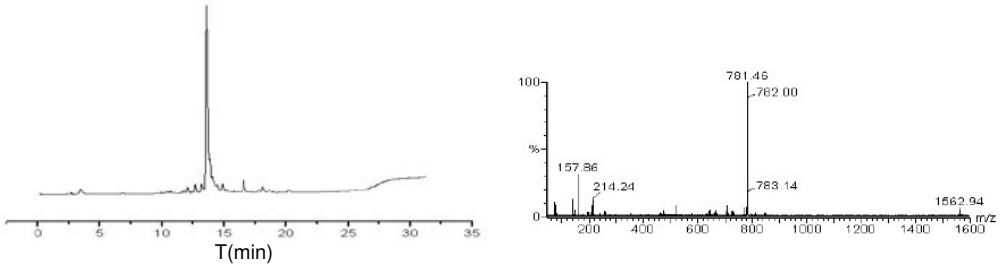

Scheme 1: Analytical profile, RP-HPLC and ESI-MS, of final crude product

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