Review of Clinical Pharmacology and Pharmacokinetics

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ΕΠΙΘΕΩΡΗΣΗ ΚΛΙΝΙΚΗΣ ΦΑΡΜΑΚΟΛΟΓΙΑΣ ΚΑΙ ΦΑΡΜΑΚΟΚΙΝΗΤΙΚΗΣ
INTERNATIONAL EDITION

VOLUME 21, 2007 ❁ No 2

Medicinal Chemistry: Drug Discovery and Design

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MEDICINAL CHEMISTRY

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of the University of Patras, Hellas

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POSTERS

Drug Discovery and Design

Guest Editor
Prof. Jonh M. Matsoukas

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 Seventh Medicinal Chemistry Conference held at the University of Patras, 8-11, March 2006. 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.

This Program 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, Biochemistry, Biomedical Analysis, Molecular Modeling, Pharmacology 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

ORAL PRESENTATIONS

Science at the Interface of Disciplines and at the Service of Society

Aristides A.N. Patrinos

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Over the centuries science has served many masters. Science has given us both an improved quality of life as well as deadlier instruments of war. Scientists have always enjoyed a more privileged position in society even if we frequently complain that we are not well understood and are rarely rewarded at the level we believe we are worth. There is a greater urgency for scientists to engage more actively in society and to more responsibly address some of the problems that humanity faces today and in the future including the challenges of global environmental degradation and sustainable development.

Toward the end of the last century the scientific enterprise has been strengthened by the expanding emphasis on multidisciplinary research. Two vivid examples of multidisciplinary research have been the study of global environmental change and the sequencing of the human genome. Biology has been a major beneficiary of this transformation because of the availability of tools borrowed from the physical and computational sciences. Biological research is also poised to help better understand the processes of environmental degradation as well as provide innovative solutions to the challenges of sustainable development.

Two examples of the scientific revolution in biotechnology are the new field of environmental genomics and the use of microbial science in the production of renewable energy sources. Environmental genomics has given us a powerful new tool to peer into the workings of the Earth system at the microscopic level and to scale up our enhanced understanding to the ecosystem level. The discovery of new and novel genes in Nature's *toolbox* provide us with the knowledge to tackle serious environmental problems such as global climate change.

An exciting new prospect for the biotechnology revolution is the potential to produce significant amounts of renewable fuels such as ethanol. Although there is no general agreement of when the world will run out of petroleum there are both national security and environmental reasons to develop suitable alternatives to petroleum, particularly for the transportation sector. With strategic investments in biotechnology research it may be possible to create a world-wide *cellulose to ethanol* industry that can provide a renewable fuel for transportation needs.

Research and Technology in Greece

Dimitrios V. Nanopoulos

Professor of Texas A&M University and Academician, Chairman of Greek National Council of Research and Technology

Σύμφωνα με τα τελευταία στοιχεία από την EU (European Innovation Scoreboard 2005), η Ελλάδα κατετάγη 23^{η}, μόλις πριν από τη Λετονία και τη Μάλτα στην Έρευνα και την Καινοτομία. Αυτή η αντικειμενική άποψη είναι 180^{ο} αντίθετη από τη *ρόδινη εικόνα* που προβαλλόταν στην Ελλάδα μέχρι πρότινος. Μετά από πολλά χρόνια και μετά από πολλά εκατομμύρια ευρώ (€), είμαστε σχεδόν τελευταίοι. Τι συμβαίνει; Γιατί, αφού έχουμε ξεκάθαρα καλό πνευματικό υλικό, καταλήγουμε έτσι; Πού πάνε τα λεφτά; Θα προσπαθήσω να δώσω απαντήσεις σε αυτά τα ερωτήματα και πάνω σ' αυτές να στηρίξω τις θεσμικές αλλαγές που επιχειρούνται αυτή τη

στιγμή στη χώρα μας στον τομέα της Έρευνας και της Τεχνολογίας, όπου πραγματικά γίνεται μια μεγάλη προσπάθεια, καθολικού επιστημονικού χαρακτήρα και διεθνούς εμβελείας, ώστε να ξεφύγουμε από τις συντεχνίες, τη μιζέρια και την κακεντρέχεια που βασανίζουν αυτόν τον τόπο.

❁

From Weeping Willow to Modern Drugs

Athanassios Giannis

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Nature is still an important source of new drugs or, more commonly, of precursors to drugs. Natural compounds and their analogues are important tools for investigation of biological processes like for example cell adhesion, cell migration, signal transduction and cell proliferation. These investigations opened the way for discovery of innovative anti-cancer drugs, immune suppressants, as well as agents for treatment of central nervous diseases etc. Almost 40% of the 520 new drugs approved for the drug market between 1983 and 1994 were natural products or derived from natural products. Greater than 60% of the anticancer and anti-infective agents that are on the market or in clinical trials are of natural product origin or derived from natural Products. In my lecture I will discuss the impact of nature in drug discovery and development.

❁

Spesific Polymers in the Production of Fuzeon

Kleomenis Barlos

Department of Chemistry, University of Patras, GR- Rio, Hellas

Peptide production utilizing the method of the sequential condensation of suitably protected peptide fragments has been proved very effective during the multiton production of the anti-HIV peptide Fuzeon. This production has renewed the interest in the development of peptide and peptidomimetic pharmaceuticals. The example of the Fuzeon production has proved, that in many cases chemical methods are superior of the corresponding biological methods.

❁

Synthesis of the Bis-Spirocyclic Core of the Prunolides, Pinnatoxins and Pteriatoxins Using a Singlet Oxygen-Mediated Cascade Sequence

Georgios Vassilikogiannakis, Maria Tofi, Thomas Georgiou and Tamsyn Montagnon

Department of Chemistry, University of Crete, GR-71409 Iraklion, Hellas

In 1999, the first information regarding the isolation of a novel class of natural products, the prunolides (1–3), was reported in the literature (1). These secondary metabolites were shown to prevent the proliferation of cancer cells. In addition to their exciting biological activity, they possess an unusual architecture, particularly in their central bis-spiroketal ring system, which makes

them interesting and challenging targets for chemical synthesis. Marine toxin pinnatoxin A (4) was isolated in 1995 (1) from the shellfish *Pinna muricata*. Its unique molecular architecture, accompanied by its pronounced biological activity as a Ca^{2+}-channel activator, makes pinnatoxin A an intriguing synthetic target. The other members of the pinnatoxin family (5-7) were isolated from the same team. (1) Pteriatoxins A, B and C (8-10), extremely potent toxins (LD_{99S} from 100 to 8 μg/kg), were isolated from the Okinawan bivalve *Pteria penguin. (3).*

The synthesis of the intricate prunolide core was achieved in just four steps starting from furan itself and with a high overall yield of 30% (1) The key step of this short synthesis was a singlet oxygen-orchestrated cascade sequence in which a 1,2-difuryl alkene was oxidised and then dehydrated to furnish the prunolide core structure.

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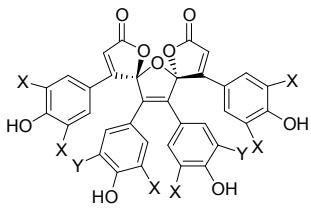
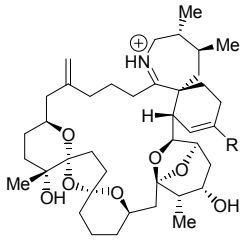
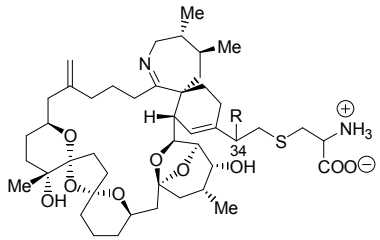


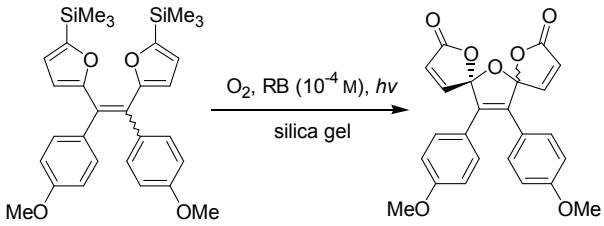
1: X=Br; Y = Br (prunolide A)
2: X=Br; Y = H (prunolide B)
3: X=H; Y = H (prunolide C)



4: Pinnatoxin A (R=-COO^-)
5: Pinnatoxin B (R=*(R)*-$CH(NH_3)COO^-$)
6: Pinnatoxin C (R=*(S)*-$CH(NH_3)COO^-$)
7: Pinnatoxin D (R=-$COCH_2CH_2COO^-$)



8: Pteriatoxin A (R=-OH)
9: Pteriatoxin B (R=-CH_2OH) } epimers
10: Pteriatoxin C (R=-CH_2OH) } at C34



Phospholipase A_2 as a Molecular Target for the Treatment of Pain, Inflammation and Multiple Sclerosis

George kokotos

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis, Zografou, Athens, Hellas

The interest in new treatments for pain and inflammation has been recently increased, because current therapies have been associated with undesirable side effects and in various

cases have been proved unsuccessful. In addition, the currently available treatments for neuroinflammation and in particular multiple sclerosis, which is characterized by inflammatory, demyelinating episodes, offer only partial relief or delay the disease progression. Therefore there is an urgent need to identify new molecular targets for multiple sclerosis therapy. Phospholipase A_2 (PLA_2) play a normal physiological role in phospholipids metabolism, host defence and signal transduction. However, under pathological conditions PLA_2 has been implicated in inflammation in a variety of tissues and organs. PLA_2 hydrolyze 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. This presentation will focus on the opportunities provided by the inhibition of PLA_2 for the development of new medications for pain, inflammation and multiple sclerosis. We have demonstrated that novel synthetic 2-oxoamide inhibitors of human GIVA PLA_2 regulate the production of arachidonic acid and PGE_2 in cells and demonstrate potent *in vivo* anti-inflammatory and analgesic activities.

Sofikiti N., Tofi M., Montagnon T., Vassilikogiannakis G., Stratakis M.: *Org. Lett. 7*: 2357-2359 (2005)

❁

Design and Synthesis of Antimicrobial Peptides: Conformational and Biological Studies

C. Sakarellos, S. Zikou, P. Mastora, A.I. Koukkou, C. Drainas, E. Panou-Pomonis, M. Sakarellos-Daitsiotis

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The widespread use of antibiotics in recent years led to the development of antibiotic-resistant microbial strains. Most of the natural antimicrobial peptides possess positive charge, a substantial portion of hydrophobic residues and amphipathic conformation. On the basis of these characteristics modified peptide analogues have been designed and studied for the development of new antibiotics. Our approach is based on the synthesis and study of polypeptides (Arg-X-Gly)n, where X=Ala, Val, Leu and cationic peptide models, Ac-(Aib-Arg-Aib-Leu)n-NH_2, where n=1-4. The peptides were tested for their antimicrobial activity, as well as for their proteolytic stability and toxicity. Conformational studies of the peptides by CD spectroscopy are in progress in order to study the peptide structure-bioligical activity relationship.

❁

Novel Methods of Antigen Delivery for Vaccines

Geoffrey A. Pietersz, Dodie S. Pouniotis and Vasso Apostolopoulos

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Delivery of antigens into the cytosol of antigen presenting cells (APC) and subsequent presentation of peptides by class I molecules to CD8 T cells is crucial for the induction of cellular responses. In addition, simultaneous presentation of peptide to CD4 T cells is advantageous to sustain immune responses and for long term memory responses. We have developed two delivery systems based on oxidised mannan (OxMan) and a membrane translocating peptide (penetratin, Int). The OxMan conjugates are internalised via mannose binding receptors and subsequently escape from the endosome into the cytoplasm and access the class I presentation pathway. Int linked in tandem to CD4 and/or CD8 cytotoxic T cell (CTL) epitopes or chemically conjugated to whole proteins internalise via endocytosis and similarly to OxMan, escapes

the endosome to access the class I pathway. In addition, these conjugates can also deliver antigen to the class II presentation pathway. We have utilised Int conjugates of ovalbumin as a model antigen and the tumour associated antigen mucin 1 (MUC1) to demonstrate mechanism of antigen presentation and induce protective immune responses in mice.

❁

Adjuvant Therapy with or without Fusion Protein Mannan-muc1 in Stage II Breast Cancer-Phase III

S. Vassilaros

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The pilot phase III study for evaluation of immunotherapy using Fusion Protein Mannan-MUC1 in stage II breast cancer with anti-oistrogenic therapy started on December 1997 and concluded after 8 years of follow-up. The results of that study were very interesting regardless the small number of participants (N=31). Four out of 15 patients that were vaccinated with Placebo developed metastases and none of the 16 patients that were vaccinated with the true vaccine (p=0.083).

Following the above results this new Phase III protocol *Adjuvant therapy with or without Fusion Protein Mannan-MUC1 in stage II breast cancer*, was introduced and approved by EOF on December 20th 2004. For statistical reasons 450 patients should be enrolled in this new porotocol. These women can be pre- or post-menopausal with histologically proved stage II carcinoma of the breast. The size of the primary tumor to be up to 3cm, with 1 to 4 metastatic axillary lymphnodes and no distant metastases.

The surgical procedure could be total mastectomy with axillay dissection or partial mastectomy with axillary dissection followd by postoperative radiation. They should have good haemopoitic, urinary and hepatic fuction and a life expectancy of more than 2 years. Mandatory is the patient's signed written consent.

The adjuvant postoperative treatment for all patients will be scheduled by their doctors. Within one month following the surgery they should start the vaccination program which is one subcutaneous vaccination every two weeks for 6 times and then once a month for 9 months. In parallel blood samples are collected in order to measure the levels of interferone and study the anti-bodies.

The vaccination vials are prepared in Austin Research Institute of the University of Melbourne, Australia and half of them contain the Mannan-MUC1 and the rest Placebo. So the patients by signing the written consent they know that by enrolling to this double blind study they have the possibility to be injected with the real vaccine or the placebo.

The interesting point and this is crucial in order to enroll all these patients, is that this time the University of Crete and Patra, as well as several Breast Centers in Athens, showed interest to participate in this study.

The aim is (a) to demonstrate the immune response to the Fusion Protein Mannan-MUC1 and (b) to demonstrate that by using this protein, improves the disease free survival.

❁

Newer Data and Mechanisms of Action of Monoclonal Antibodies against Compact Tumours

Haralampos Kalofonos

Oncology Department, Internal Medicine Clinic, University of Patras, Patra, Greece

Τα μονοκλωνικά αντισώματα (ΜΑ) αντιδρούν ανοσολογικά με συγκεκριμένα αντιγόνα στόχους και χρησιμοποιούνται από ετών για τη διάγνωση και τη θεραπεία κακοήθων νεοπλασιών. Οι μηχανισμοί δράσης είναι άμεσοι και έμμεσοι και διαφέρουν ανάλογα με το αντίσωμα και τη μέθοδο, περιλαμβάνουν δε ανοσολογική αντίδραση με υποδοχείς ανάπτυξης (EGFR, HER2), παρά-

γοντες ανάπτυξης της νεοαγγείωσης (VEGF), συνοδά αντιγόνα των όγκων (CD20, CEA) κ.α. Οι ραγδαίες εξελίξεις στο χώρο της βιοτεχνολογίας έχουν συντελέσει στην απομόνωση, ταυτοποίηση και μέτρηση και άλλων μοριακών στόχων οδηγώντας στην ανάπτυξη αντίστοιχων ΜΑ που είναι υπό διερεύνηση. Η επιλεκτική στόχευση με ΜΑ συγκεκριμένων σηματοδοτικών μορίων καθώς και ειδικών μεταγραφικών παραγόντων προσφέρει τη δυνατότητα βελτίωσης της ειδικότητας της θεραπευτικής προσέγγισης. Αξιόλογος αριθμός κλινικών μελετών έδειξε ότι τα ΜΑ είναι μερικά από τα σημαντικότερα φάρμακα μοριακής στόχευσης για τη θεραπευτική αντιμετώπιση ασθενών με καρκίνο. Κακοήθεις νεοπλασματικές παθήσεις που αντιμετωπίζονται με επιτυχία με ΜΑ είναι τα μη Hodgkin's λεμφώματα, λευχαιμίες, τα καρκινώματα μαστού, παχέος εντέρου, κεφαλής και τραχήλου, νεφρού, κ.α. Η χρησιμοποίηση ΜΑ σε συνδυασμό με χημειοθεραπεία ή/και ακτινοθεραπεία αυξάνει την κυτταροτοξική δράση στα νεοπλασματικά κύτταρα και πρόσφατες κλινικές μελέτες επιβεβαιώνουν τα παραπάνω δεδομένα. Συμπληρωματικές ερευνητικές δραστηριότητες είναι απαραίτητες αφ' ενός για να διερευνηθεί ο ρόλος των νεώτερων ΜΑ καθώς επίσης και των άλλων ανοσομορίων όπως τα $F(ab')_2$, Fab και Fv ανοσοτμήματα αλλά και για την ανεύρεση των άμεσων και απώτερων ανεπιθύμητων ενεργειών τους. Τα ΜΑ είναι νέοι αλλά καθιερωμένοι πλέον αντινεοπλασματικοί παράγοντες για την αντιμετώπιση ασθενών με κακοήθεις νεοπλασματικές παθήσεις και στο μέλλον θα έχουν ευρύτατη χρήση.

❁

Angiotensin II: A Role in Inflammation and Cancer

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Angiotensin II (AII), is a pleiotropic hormone functioning as a major regulator of blood pressure and cardiovascular homeostasis. However, there is increasing evidence that AII is capable of serving as a neurotransmitter, pro-thrombotic agent and growth factor and thus is involved in the regulation of cell proliferation, angiogenesis and tissue remodelling. During the last years studies have shown that AII is in part able to trigger the pre-inflammatory reaction a mechanism that underlies many types of diseases including cancer. This is mainly due to the induction of nuclear factor-kB activity and release of pro-inflammatory cytokines and free radicals that are responsible for a cascade of events leading to DNA mutations and carcinogenesis. The angiotensin I-converting enzyme (ACE; conversion of angiotensin I to II) is expressed by a broad spectrum of normal tissue such as the uterus, placenta, vascular endothelium, heart, nervous system, adrenals, white blood cells and lung. Genomic epidemiologic data, increasingly supported by clinical outcomes results, strongly suggest that overactivity of ACE may underlie most inflammatory diseases such as atherosclerosis, heart failure, dementia, psoriasis, and many types of cancer. Studies on the expression and function of ACE and AII receptors in various aspects of cancer suggest that ACE inhibitors and AII receptor antagonists have beneficial effects on tumour progression, vascularization and metastasis, and indicate a potential role in cancer treatment. An overview of the major intracellular signalling pathways associated with AII activation in cancer cells, as well as in endothelial and inflammatory cells, is presented.

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On the Role of Reactive Oxygen Species in Angiogenesis and Cancer Cell Growth

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Reactive oxygen species (ROS) are produced by several intracellular systems and are generated under various physiological and pathological conditions. Although under oxidative stress ROS have cytotoxic effects, evidence also exists concerning their implication in cell signaling. Angiogenesis, the formation of new blood vessels from pre-existing ones, is a complex, multi-step process that characterizes a variety of physiological and malignant conditions. ROS seem to be implicated in the regulation of angiogenesis *in* vivo and activation of endothelial cells *in vitro*. Hydrogen peroxide (HP), among all ROS, is an ideal candidate for a signaling molecule. Small quantities of HP are produced by all types of cells, and activate several signal transduction pathways in mammalian cells. Under non-stress conditions, HP stimulates angiogenesis *in vivo*, as well as endothelial cell proliferation and migration *in vitro*. The angiogenic effect of HP seems to include activation of the transcription factor activator protein-1 (AP-1) and heparin affin regulatory peptide (HARP) up-regulation. HP added exogenously or produced endogenously after stimulation by fibroblast growth factor-2 (FGF-2), stimulates prostate cancer cell growth and migration via the same mechanism.

Identification of Genetic Alterations in Hereditary Breast and Colorectal Cancer in Greece

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Approximately 30-50% of the FAP patients harbor truncating germ-line mutations in the APC tumor suppressor gene (Adenomatous Polyposis Coli). Germ line mutations in genes encoding proteins involved in DNA mismatch repair are responsible for the autosomal dominantly inherited cancer predisposition syndrome HNPCC in colorectal cancer. Mutations in the *BRCA1/2* genes predispose individuals to breast and ovarian cancer. The lifetime risk of breast cancer in female carriers of a *BRCA1* mutation is 60-80% while that of ovarian cancer is 20-40%. The median age of diagnosis of breast cancer is 42 years.

Results and discussion

FAP: To date we have tested 90 members from 18 families. Pathogenic mutations were identified in 14 families (32 out of 37 patients 86,5%) including three novel truncating mutations - 2601delGA, A2767T, 1577insT, as well as three families (17%) with *de-novo* mutations and one family with a large deletion (*APC*del exons 6-15)

HNPCC: Here we describe the combination of different molecular biology techniques for the detection of mutations and large genomic rearrangements of the APC and MMR genes in familial CRC in Greek patients. A unique disease-causing mutation has been identified in 7/9 (78%) families. The types of mutations identified are nonsense (5/7) (*hMLH1*: E557X, R226X; *hMSH2*: Q158X, R359X and R711X), a 2 bp deletion (*hMSH2* 1704-1705delAG) and a 2.2 kb *Alu*-mediated deletion encompassing exon 3 of the *hMSH2* gene.

BRCA: We describe analysis of *BRCA1/2* in families from a Greek cohort. A pathogenic mutation in *BRCA1* was identified in 27.7 % of the families, where four distinct mutations have been observed. In one family MLPA revealed deletion of exon 20 of the *BRCA1* gene. Finally in one family the *BRCA2* gene was mutated. Here we describe the combination of different molecular biology techniques for the detection of mutations.

In conclusion, our results document and extent previous work, suggesting that genomic rearrangements account for a large proportion of identified mutations. This therefore warrants use of a combination of techniques capable of identifying both single base mutations in addition to large genomic rearrangements. In this respect, we have found that use of dHPLC for single base mutations and MLPA for large genomic rearrangements is a reliable and cost-effective combination for use as an initial screening step followed by sequencing for characterization of the mutations identified.

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Diagnostic and Prognostic Tools in Multiple Sclerosis

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Multiple Sclerosis is an autoimmune, demeylinating disease of the central nervous system in which the role of antibodies remains unclear. Based on its varied clinical presentation, natural history and response to therapeutic interventions, Multiple Sclerosis can be considered as a group of diseases with distinct pathogenetic mechanisms leading to the formation of myelin lesions. Four distinct patterns of Multiple Sclerosis pathology are proposed: cell-mediated demyelination; antibody-mediated demyelination; active myelin destruction; oligodendrogliopathy or oligodendrocyte dystrophy. Despite the concordance of clinical features, there is a poor definition of pathogenetic mechanisms in individual patients. We demonstrated that a β-hairpin peptide structure plays a fundamental role in exposing at the best the minimal epitope Asn(Glc) responsible for Ab recognition in Multiple Sclerosis patients. A rational design, by molecular modeling, lead to the selective Ab ligand CSF114(Glc), synthetic marker of the disease activity. Therefore, we developed the prototype of the first diagnostic test based on the use of CSF114(Glc) in ELISA, able to detect pathogenic auto-Abs in Multiple Sclerosis patients sera [1]. We demonstrated that CSF114(Glc) is a reliable and specific marker in a longitudinal study of Multiple Sclerosis patients followed for up to 3 years. Development of anti-CSF114(Glc) antibodies paralleled clinical activity and brain lesions positive to MRI. Therefore, a CSF114(Glc)-based immunoassay may have an important diagnostic/prognostic value in Multiple Sclerosis to monitor disease progression. This means that the changes in auto-Ab level could follow the instauration of different therapies. If a reduction of auto-Abs accompanies a good clinical response to the drugs, CSF114(Glc) can be proposed as a marker to help the patients to achieve a better quality of life. CSF114(Glc) is commercially available since October 2003.

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Safety and Efficacy of Myelin Cyclic Peptide Analogues Administration in Autoimmune Demyelination

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Myelin peptide analogues have been and still are tested regarding their treatment value in multiple sclerosis (MS) and the correspondent animal model, the experimental alergic ence-

phalomyelitis (EAE). Altered peptide ligand or analogue (APL) is a native peptide, which is modified by aminoacid substitutions at essential contact residues for the T-cell receptor (TCR). Several APLs were have been designed and tested both in EAE and MS. One of the major disadvantages of peptide therapy is the activation of proteolytic enzymes, leading to peptide degradation. To address this problem, an attempt to create more stable molecules by synthesizing cyclic peptide analogues has been reported. Previous reports indicated the amelioration of the clinical severity and underlying inflammation in animals suffering from EAE induced by myelin basic protein (MBP), following tretment with cyclic MBP-analogues. There are no reports however whether these potential therapeutic agents may have encephalitinogenic properties by themselves, a factor that might limit their use. Proteolytic protein (PLP: peptides 43-64, 103-116, 139-15l), is among the candidate autoantigens in MS and has been used in the induction of EAE. A cyclic PLP analogue has been synthesized and its possible encephalitinogenic properties, as well as its efficasy in chronic EAE model, were studied. Chronic EAE was induced with MOG in C57/B according to a standard protocol. Animals were were divided into two groups and vaccinated on the day of EAE induction either with cyclic PLP analogue or vehicle. In another group of animals, PLP was administered without previous induction of EAE. The clinical profiles as well as the underlying pathology were studied both at the acute and the chronic stages following the peptide immunization. During the acute stage of the disease both groups of animals exhibited severe relapse with no significantly different clinical scores, followed by a chronic progressive residual neurologic deficit. However, in cyclic PLP treated animals the deficit was significantly less compared to controls. Inflammation was equally severe at the acute stage of EAE for both groups of animals. However, the relative subpopulation of brain derived neurotrophic factor (BDNF) - positive inflammatory cells was higher and the axonal injury and loss was significantly less in the cyclic PLP - treated group. Naive animals vaccinated with cyclic PLP exhibited little, if any, paralytic signs with no concomitant inflammatory lesions or axonal injury. Our results indicate that cyclic PLP analogues have little, if any, encephalotinogenic properties and may benefit EAE due to their increased capacity to induce BDNF expression by the inflammatory cells. It may therefore be speculated that immunomodulatory rather than immunosuppressive effects of cyclic PLP analogues may have a neuroprotective effect in EAE, contributing to a better clinical recovery, thereby. It is of interest the fact that such an effect came out from a cyclic analogue that does not correspond to the peptide used for the EAE induction. It is of great importance to test the possibility whether similar effects may exist following the administration of cyclic MBP and MOG analogues in the same chronic EAE model.

Stress Neuropeptides

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The hypothalamic neuropeptides corticotropin-releasing factor (CRF) and the urocortins (Ucn) Ucn1, Ucn2, and Ucn3 represent ancient molecules that first appeared in teleost fish and in amphibians and later on in mammals. In mammals, they play a central role in the homeostatic response to stress. They also participate in the physiology of endocrine, cardio-vascular, gastrointestinal and immune system. Their role in the regulation of the inflammatory response appears to be central. They regulate, via the central nervous system, the production of adrenal glucocorticoids, the major endogenous suppressors of the immune system. In addition, and in a direct paracrine manner, they control a multitude of immune cells including macrophages, the dendritic antigen presenting cells, mast cells and lymphocytes. The precise role of the CRF neuropeptides in the pathophysiology of the adaptation to stress and its diseases, in obesity, insulin resistance and generally in the patho-physiology of the Metabolic Syndrome, as well as in a several other diseases is under intense investigation worldwide.

Effect of Myelin Basic Protein Peptides on T-Cell Activation Parameters of the Same Multiple Sclerosis Patient Tested at Presentation and One Year Later

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Introduction: In multiple sclerosis (MS) patients, the majority of myelin antigen (Ag)-specific T-cells isolated during active disease, secrete pro-inflammatory cytokines (type-1), whereas during remission, the balance shifts to anti-inflammatory-cytokine-producing T-cells (type-2). The effect of an experimental drug on type-1 and type-2 cytokine secretion profiles is one of the ways to estimate its potential to treat MS (1).

Patient, Materials and Methods: In the present study, we tested the effect of myelin basic protein (MBP) peptides (1) on T-cells derived from an MS patient at presentation and one year later. The patient was a 20 year old female, with remitting-relapsing (RR) MS and Expanded Disability Status Score (EDSS)=2 at presentation and EDSS=3 one year later. Between the 1st and 2nd visits, the patient was under continuous prophylactic therapy with IFN-β(Avonex, 30 mg in once/week). At both time points, the patient was in the acute phase of the disease, and blood samples were drawn before the administration of treatment (methylprednisolone, 1 g/day iv for 3d). The peptides tested were analogs of the guinea pig MBP epitope 72-85, and included the agonists Glu-Lys-Ser-Glu-Arg-Ser-Glu-*Asp*-Glu-Asn-Pro-Val (linear) and Glu-Lys-Ser-Glu-Arg-Ser-Glu-*Asp*-Glu-Asn-Pro-Val-NH_2 (cyclic), and the antagonists Glu-Lys-Ser-Glu-Arg-Ser-Glu-*Ala*-Glu-Asn-Pro-Val (linear) and Glu-Lys-Ser-Glu-Arg-Ser-Glu-*Ala*-Glu-Asn-Pro-Val-NH_2 (cyclic). In addition, an irrelevant control peptide was tested (Cyc[(D-F)LLRεKDap]) as negative control, and a peptide mapping to the human wild type (wt) MBP epitope 87-99 (Val^{87}-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro^{99}) as a positive control. Peripheral blood mononuclear cells (PBMC) were isolated from the patient and cultured with various concentrations of the peptides ± the T-cell-specific mitogen phytohaemaglutinin (PHA). Cell proliferation rates were measured using a BrdU proliferation assay. Cytokine concentrations in culture supernatants were determined by ELISA.

Results and Conclusions: Addition of the MBP peptides to unstimulated cultures of PBMC of the patient at both presentation and 1y later, reduced cell proliferation rates; the effective dose (ED) of all peptides was found to be 10 ng/ml. MBP peptides over-induced cell proliferation in PHA-stimulated cells, at the same ED. Maximum inhibition of proliferation was observed with the MBP(72-85) cyclic antagonist, and maximum induction with the MBP(72-85) linear agonist. The control peptide had no effect on cell proliferation.

Regarding cytokine secretion, the constitutive cytokine production by the cells of the patient *at presentation*, was dominated by IL-10 (type-2 profile). Culture with the MBP(72-85) linear or cyclic agonist resulted in a net increase of IFN-γ production or a net decrease of IL-10 secretion (switch to type-1). Culture with MBP(72-85) linear or cyclic antagonist resulted and a net increase of IL-4 or IL-10 (reinforcement of the type-2 profile). The wt MBP or control peptides had no effect on cytokine secretion patterns. *One year later*, the constitutive cytokine production by the cells of the patient was dominated by IL-2 and IFN-γ (type-1 profile). All peptides, regardless of their agonist or antagonist activity or conformation, resulted in a net reduction of IL-2 and IFN-γ secretion (weakening of the type-1 profile).

To our knowledge, this is the first time that it was demonstrated that (i) an RR-MS patient can present with a type-2 profile in the acute phase, (ii) refractoriness to IFN-β treatment can implicate a change of a type-2 profile to type-1 and (iii) the effectiveness of an immunomodulatory experimental drug should be tested not only on a per patient basis, but also on the precise time it is to be administered to the patient.

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Identification of Epitopes of the Sjogren's Syndrome (SS) La/SSB Autoantigen that Affect SS T-cell Proliferation

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INTRODUCTION: In autoimmune diseases such as Sjogren's syndrome (SS), systemic lupus erythematosus (SLE) and scleroderma, a section of the secondary immune response is directed toward autoantigens that are often large cellular complexes composed of proteins, DNA and small RNAs. At least three immunologically distinct proteins, La/SSB, Ro60kD and Ro52kD, complexed with human cytoplasmic RNAs, participate in the autoimmune response in SS and SLE patients (1,2). Although the pathogenetic mechanisms of La/SSB autoimmunity are largely unknown, considerable evidence suggests that autoantibody responses to La/SSB are antigen-driven, involving a number of B- and T-cell determinants, and/or that autoreactive B-cells specific for La/SSB exist in healthy individuals and their activation depends upon T-cell assistance provided by autoreactive T helper cells [3]. T-cells recognize antigen only after it is processed and presented on antigen presenting cells in association with histocompatibility glycoproteins. The histocompatibility proteins, encoded by highly polymorphic genes in the major histocompatibility complex (MHC), have the capacity to stably bind short peptides and display them to the T-cell-specific receptor resulting in the initiation of the immune response (4).

PATIENTS, MATERIALS AND METHODS: In this study, two predictive methods, based on the MHC class II peptide binding approach [5,6] were applied to identify epitopes of the La/SSB autoantigen recognised by T-cells. The resulting epitopes found by both methods were synthesized and tested for T-cell proliferation: WIDFVRGAG, IILFKEKAK, AELMEISEDKTKIR, KKIIEDQQESLNKW, FNVIVEALS and DLLILFKDDYFAKK. Peripheral blood mononuclear cells (PBMC) were isolated from 10 SS patients and 10 age- and sex-matched controls, and cultured with various concentrations of the peptides in the presence or absence of the T-cell-specific mitogen phytohaemaglutinin (PHA). Cell proliferation rates were measured using the BrdU proliferation assay.

RESULTS AND CONCLUSIONS: None of the peptides tested on control PBMC cultures ±PHA affected their proliferation rates. In addition, none of the peptides tested affected cell proliferation of SS PBMC in the absence of mitogenic stimulation. In SS PBMC cultured with PHA, the WIDFVRGAG and IILFKEKAK peptides increased proliferation rates by at least 40%, whereas the peptides AELMEISEDKTKIR and KKIIEDQQESLNKW decreased proliferation rates by 30%. The effective concentrations of the peptides ranged between 50-150 ng/ml. Peptides FNVIVEALS or DLLILFKDDYFAKK had no effect on the proliferation of SS PBMC cultured with PHA. Future experiments will delineate the mechanisms by which the peptides WIDFVRGAG, IILFKEKAK, AELMEISEDKTKIR and KKIIEDQQESLNKW increase or inhibit T-cell proliferation in SS, to assess whether they may be of pharmacological significance.

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Protein Profiling *vs*. Peptide Mass Fingerprinting in Proteomic Applications in Life Sciences

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Desorption (DI) and spray (ESI) ionization methods are mass spectrometric (MS) tools which have favoured the tremendous explosion of proteomic based research. The comprehension of the physiopathologic mechanisms of many diseases, including cancer, depends on the proper use of mass spectrometry in protein profiling and/or in peptide mass fingerprinting. An essential aspect of this type of research is represented by the availability of data banks for a rapid identification of the protein source and a fast sequence assignment to the related peptides. Protein Profiling has been applied with success in the search for markers of ageing. Chemical fractionation of plasma proteins followed by MALDI-TOF/TOF analysis allowed the identification of a protein at *m/z* 8861.96, in one of the fraction taken from the plasma of a centennial woman, which might be used as marker of ageing in population screening. This protein, however, is too heavy to be directly sequenced in the gas phase by MS/MS methods and its identification would require a quite difficult isolation by chromatographic techniques. This is probably the main drawback of the *profiling* methodology which relies on the presence of objects which may not be easily identifiable. A bottom-up approach was used in the proteomics of the peripheral nerves. A low-molecular weight P0 related protein was isolated by SDS-PAGE from bovine sciatic nerve. In this case the unique opportunity offered by the TOF/TOF configuration of the instrument used was successfully exploited in the structure elucidation of the molecule by MALDI-MS/MS. In this approach the second MS dimension allows to overcome drawbacks which may arise from protein overlapping in the 1-D gel electrophoretic separation. The same approach can be used to trace typical cattle diseases and to provide an early warning to dairy factories operators. Milk protein profiling integrated with peptide mass fingerprinting has been exploited to check the development of mastitis in cows. Profiling and peptide fingerprinting could be successfully applied to the detection of conjugates between drugs and plasma proteins and to the identification of the distribution of drugs, or their metabolites, in a target organ. This approach might prove useful in drug designing.

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NMR Spectroscopy: A Valuable Tool in the Weaponry of Medicinal Chemistry

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Medicinal Chemistry constitutes a branch of Chemistry aiming in the design and synthesis of novel drugs to treat major diseases. 2D NMR Spectroscopy has especially become a valuable tool in the hands of Medicinal Chemists since the majority of drugs are organic compounds of low molecular weight (<1000) or peptides (<5000).

The first step after the synthesis of potential pharmaceutical products is their structure elucidation. To fulfill this aim 2D NMR spectroscopy has offered a tremendous help. It is not an exaggeration to say that 2D NMR spectroscopy aids not only the Medicinal Chemists to characterize the new products but also Pharmaceutical technologists providing information about polymorphism of drug powders and of drugs in tablets. The synthetic drugs must have pharmacokinetics that prohibit their toxicity. NMR spectroscopy has its contribution on this aspect by studying drug metabolism through analysis of biological fluids. Its coupling with liquid chromatography offers the separation and characterization of metabolic products (LC-NMR).

NMR has many applications in the Medicinal Chemistry. NOE effect allows to study the conformations of drug molecules in many environments that simulate the biological ones. The conformation of the molecule is related to its bioactivity. NMR permits to comprehend on the stereoelectronic parameters that govern the bioactivity of drug molecules and therefore to design and synthesize novel drugs with optimized pharmacological profile. This rationale design minimizes the synthetic compounds to be prepared and the man power to be involved.

In vitro NMR spectroscopy has been developed and HSQC (Heteronuclear Single Quantum Coherence) experiment offers the possibility to study the binding of a drug in the active site. Thousands of molecules can be tested and new molecules can be developed through this approach. Recently *in vivo* NMR has been applied using the same experiment but in a real biological environment.

In conclusion, NMR spectroscopy constitutes a valuable tool in Medicinal Chemistry and offers new avenues in the adventurous research trip towards the discovery of novel drugs that will ameliorate the suffering of humanity from undesired diseases.

Hydration Degree Study of Amino Acids and their Derivatives in Aqueous Solution as a Function of pH Using Nuclear Magnetic Resonance ^{14}N Methods

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The ^{14}N NMR line widths of the α-amino groups of several protein amino acids and their acetyl and methyl derivatives were measured in aqueous solution, with composite proton decoupling, to estimate the effect of the molecular weight on the line widths. The ^{14}N line widths, under composite proton decoupling, increase with the bulk of the amino acid, and increase at low pH. Statistical treatment of the experimental ^{14}N and literature ^{17}O NMR data was performed assuming two models: (i) an isotropic molecular reorientation of a rigid sphere in a medium of viscosity η, and, (ii) a stochastic diffusion of the amino and carboxyl groups comprising contributions from internal (τ_{int}) and overall (τ_{mol}) motions. Assuming a single correlation time from overall molecular reorientation (τ_{mol}), then, a linear correlation was found between the line widths and the molecular weights of the protein amino acids at the pH values 0.5 and 6.0, which are characteristic of the cationic and zwitterionic forms respectively (1). The slopes of the straight lines were found to be dependent of pH for ^{14}N, contrary to the ^{17}O linear correlations whose slopes were found to be independent of pH (2). Assuming effective correlation times of the amino and carboxyl groups which comprise contributions from the internal (τ_{int}) and overall (τ_{mol}) motions, then, a significant improvement of the statistics of the regression analysis was observed. The ^{14}N relaxation data, in conjunction with ^{17}O NMR line widths, can be interpreted by assuming that the ^{14}N quadrupole coupling constants (NQCCs) are influenced by the protonation state of the carboxyl group, the ^{17}O NQCCs remain constant, and the cationic form of the amino acids is hydrated by an excess of one to two molecules of water relative to the zwitterionic state.

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Structural Studies of Large Biologicaly Important RNAS

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Three-dimensional structure determination of small proteins and oligonucleotides by solution NMR is established. With the development of novel NMR and labeling techniques, structure determination is now feasible for proteins with a molecular mass of up to ~100 kDa and RNAs with size up to 35 kDa. Beyond these molecular weights special techniques and approaches are required for applying NMR as a multiprobe method for structural investigations of proteins and RNAs. It is our aim of to identify new approaches to advance the molecular mass limit of NMR of large RNAs (up to 100 kDa).

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Structure and Dynamics of E3 Ligases Hdm2 & Hdmx Tumor-Suppressor Protein p53 Regulators

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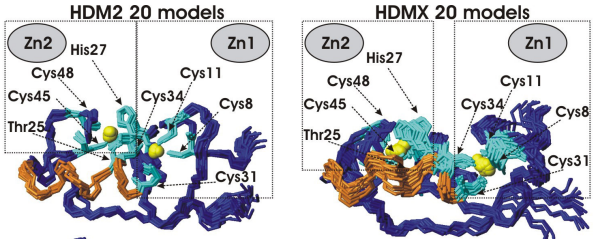

p53 protein is one of the most effective defensive weapons of human body against carcinogenesis, due to its tumor suppression properties (1). It has been noticed, in many types of cancer, that the functions of p53 are being downgraded or even suppressed and this fact is ought to the presence of mutated forms of p53 or to the complete absence of the protein (2). The suppression of p53 levels is being indirectly regulated by the protein itself, which activates the expression of a gene, the oncogene mdm2 *(murine double minute 2),* which expresses the MDM2 protein, known as human-MDM2 or just HDM2 (3,4). HDMX protein (5) is a homologue protein to HDM2 and is being implicated, through various biological processes, in the suppression of p53. The C-terminal regions of HDM2 and HDMX are being characterized by their function as E3 ubiquitin ligases. These domains catalyze the latter stage of protein signaling for proteolysis by the 26S proteasome, through the ubiquitin pathway. The C terminal regions of HDM2 and HDMX are two E3, *Ring finger* type, ligases, which are characterized by their high content in cysteines and the binding of two Zn(II) ions. The models of HDM2 and HDMX *Ring fingers* (the crystal structure of c-Cbl used as template) are studied using molecular dynamics simulations, and are analyzed in a Structure-Function correlation basis.

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Antidepressant Strategies and the Putative Role of Somatostatin

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The understanding that antidepressant drugs (ADs) MAOI & TCAs, discovered in the 1950s, act through monoaminergic mechanisms led to the formulation of the monoamine hypothesis of depression. This hypothesis was further supported by the development, in the early 1980s, of a new class of ADs, the selective serotonin reuptake inhibitors (SRIs). Their development was the result of sophisticated research on presynaptic aminergic transporters. The need to explain the delay of antidepressant treatment efficacy in humans and the limitations of the monoamine hypothesis triggered research, over the past 30 years, on the long term effects of the ADs, and the implication of other neurochemical systems. The neuropeptide somatostatin has been reported to be implicated in the pathophysiology of depression. Our experimental results on brain somatostatin function following acute and chronic ADs support the importance of somatostatin not only in the pathophysiology but also in the therapeutics of depression. It is hoped that the shift in the antidepressant paradigm from the biochemistry of monoamines to the identification of other targets will lead to new psychiatric drug development. [Supported by Ministry of Education, EPEAEK Neuroscience and an EC contract (QLG3-CT-1999-00908) to K.T.]

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Polyunsaturated Fatty Acids: NMR Study of Fish Oils and their Enrichment in ω-3 Fatty Acids

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Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have specific physiological functions, are necessary for proper growth and development and are therefore of nutritional importance. Several sources of information suggest that human originally consumed a diet with a ratio ω-6 to ω-3 fatty acids of about 1:1, whereas today this ratio ranges from 10:1 to 20-25:1 in Western societies. Fish are excellent sources of ω-3 fatty acids, especially 20:5 (EPA) and 22:6 (DHA). This presentation will focus on the NMR study of lipids extracted from sardines, as well as their lipase-assisted hydrolysis products. NMR technique was chosen since is a not destructive and time consuming method. Ttriglycerides are the main lipidic components of sardines. DHA is preferentially esterified at the β-position of triacylglycerols, while EPA is nearly equal distributed in the α- and β-position of glycerol backbone, as suggested by ^{13}C-NMR spectra. Lipases of microbial origin as *Candida rugosa* and *Aspergillus niger* lipases were used for the hydrolysis study. *Candida rugosa* lipase is a non-specific enzyme hydrolyzing PUFAs leading to a mixture containing half amount of the ω-3 fatty acids. *Aspergillus niger* lipase is a 1- and 3-positional specific enzyme leading to the enrichment of PUFAs in DHA.

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A General Strategy in the Design of Fluorescent Probes for the Detection of Ions in Biological Systems

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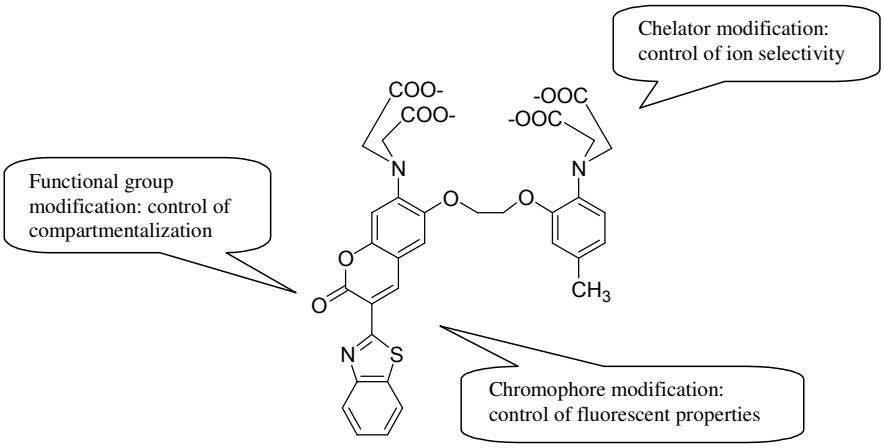
The interpretation of phenomena that take place at the cellular level is of vast importance for the understanding of the etiology of a plethora of diseases. Medicinal research, in an impressive overlap with chemistry and biology, is employing a number of tools provided by the latter sciences. Synthetic probes that undergo distinct changes upon binding ions with high selectivity have enabled researchers to investigate changes in the intracellular ion concentration levels. Fluorescence has been long viewed as a powerful tool for the selective study of single components in a complex biological system. There is a rapidly growing interest in the development of fluorescent probes with high selectivity for their cellular component-targets. The polycarboxylate fluorescent ion probe method was first envisioned by R. Y. Tsien who, in an elegant approach to the problem, introduced a number of intracellular calcium probes. The need of property optimization in the dyes initially introduced by Tsien prompted scientists to synthesize a series of probe analogs. Studies on the impact of structural changes in the parent molecules to the properties of the new probes led to the conception of a general synthetic strategy depicted in the following Scheme.



Mass Spectrometry for the Quantification of Bioactive Peptides

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The study of pharmacologically active peptides is central to the understanding of disease and development of novel therapies. It would be advantageous to monitor the fate of bioactive peptides in biological fluids following their *in vivo* administration or the modulation of endogenous factors (e.g., peptide hormones) affected by the administration of a pharmacological agent. Measurement of administered compounds (small molecules) in plasma is a mature field. However, measurement of pharmacologically active peptides presents particular problems for quantitative mass spectrometry, including challenges from selectivity and sensitivity perspec-

tives. Current approaches towards peptide quantification in biological fluids include immunoassays and mass spectrometric techniques. Immunoassays, although sensitive, lack the necessary selectivity for distinction between peptide and metabolites. Modified molecules induced by metabolic transformations (e.g., N- or C-terminal truncation of the peptide) might not be differentiated by the antibody used in the assay, leading to cross-reactivity. However, although it is generally accepted that mass spectrometry is an ideal technique for the quantification of trace levels of analytes in biological fluids, immunological techniques are still characterized by better limits of detection. In this presentation, novel mass spectrometric approaches and strategies on peptide quantification will be reviewed and the current capabilities and prospects for advances in this critical area of research will be examined.

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❁

Evaluation of Immunosuppressive Agents in an Experimental Model of Hepatocyte Xeno-Transplantation in Rats with Acute Liver Failure

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Objective: Acute liver failure (ALF) is a demanding clinical entity carrying a dismal prognosis in 80% of cases if left untreated. Although orthotopic liver transplantation (Tx) is considered as treatment of choice. The existing donor shortage restricts the opportunity of transplantation in only one third of potential recipients. During the last decade cellular liver replacement methods have been developed in order to support detoxification and liver regeneration as bridging therapy to Tx. The aim of our study was to evaluate the efficacy of mycophenolate mofetil (MMF), Sirolimus (S) and Daclizumab (Dmab) in a rabbit to rat model of hepatocyte (Hc) Xeno-Tx after induction of ALF.

Materials-Methods: White New Zealand rabbits were used as donors of fresh isolated Hc (collagenase method) with a viability > 90%. Rats were injected with N-Dimethylnitrosamine 24h before Tx (dose 18mg/kg). Hc were transplanted to the lower splenic lobe (>5.000 clusters of Hc). Recipients (male Wistar rats) were randomized to the following study groups (12 animals in each group) : 1- ALF without any treatment. 2- ALF and Tx of Hc without immunosuppression (Im). 3- ALF and Tx of Hc with Cyclosporine (Cyc) as Im. 4- ALF and Tx of Hc with MMF as Im. 5- ALF and Tx of Hc with S as Im. 6- ALF and Tx of Hc with S and Cyc as Im. 7- ALF and Tx of Hc with Dmab as Im. 8- ALF and Tx of Hc with Dmab and Cyc as Im. All surviving animals were sacrificed on day 15.

Results : Survival rate in the treated groups with MMF or Dmab and Cyc was statistically significant higher than in groups treated with S or S and Cyc. Biochemical data showed significant differences among groups regarding Creatinine, K^+, Na^+, γ-GT, Glucose, SGOT, SGPT and Total Bilirubin levels. Histopathology confirmed the engraftment of Hc in the treated groups.

Conclusions: We conclude that the Hc Tx in a rat experimental xeno-Tx model improved the survival rate and the biochemical data in cases of toxic ALF especially in the MMF and Dmab treated groups.

❁

A_2 Adrenergic System: From Neurotransmitans to Neuroprotection

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α_2-Adrenergic receptors (α_2-ARs) have a widespread distribution in the central nervous system and affect a number of biochemical and behavioral functions, including stimulation of prefrontal cortex and cognitive function. Until recently the functional significance of the a_2-AR system was mostly understood in the regulation of cardiovascular function. However, in addition to its role as a classical neurotransmitter and the regulation of sympathetic system outflow, norepinephrine has been recently shown to exert an important influence on the plasticity in areas of the brain where neurogenesis persists in the adult, notably subgranular zone within dentate gyrus of hippocampus and olfactory bulb. In regulating adult neurogenesis, the noradrenergic system is functionally integrated with chronic stress and depression. Chronic stress, depression or depletion of norepinephrine *in vivo* suppress and antidepressant treatments induce hippocampal neurogenesis by down- or up-regulating, respectively, cell proliferation. Using clones of PC12 cells stably transfected with individual a_2-AR subtypes we have demonstrated that a_2-ARs induce subtype-specific differentiation rather than cell proliferation. Recently we have shown that a_2-ARs generated signalsare relayed via transactivation of growth factor receptors with tyrosine kinase activity. Together these data make it conceivable that α_2-ARs might contribute neurotrophic actions *in vivo* synergistically or in permutation with other neurotrophic factors.

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The Molecular Basis of Hypertension by the Use of Biophysical Methods

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The rennin angiotensin system (RAS) is characterized by a sequence of enzymatic reactions leading to the release of angiotensin II. Over activity of RAS has been implicated in the development of cardiovascular diseases such as hypertension, congestive heart failure, coronary ischemia and renal insufficiency (1). In order to shed light to the discrete paths of this system, in an atomic level, we utilized the following aproaches. (i) Structural investigation of polypeptide models of the active site of angiotensin converting enzyme, ACE (2-4). (ii) Detailed modeling studies and investigation of the origin of the different chloride activation of the two catalytic domains of the enzyme. (iii) Investigation of the domain specificity of inhibitors for the two catalytic sites of ACE through flexible docking calculations (4). (iv) Detailed NMR and molecular dynamics study of the solution struc-tures of AII, its agonist and antagonists and AI in aqueous solution (5,6). (v) Investigation of the molecular path of recognition of the bioactive hormone angiotensin II from its GPCR AT1 and also construct, analyze the structure and design new inhibitors for the receptor site.

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Selective Phosphinic Inhibitors of Angiotensin Converting Enzyme (ACE)

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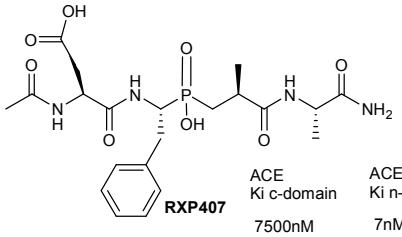

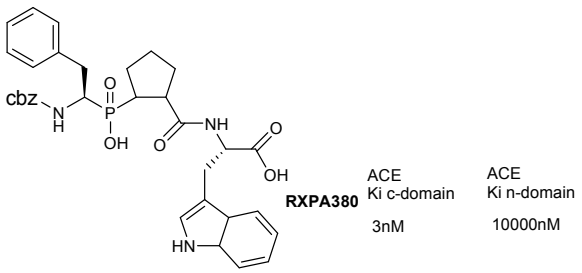

Somatic angiotensin – converting enzyme (ACE) contains two homologous domains, each bearing a functional active site with different substrate specificity and different activation requirements (1). The *in vivo* contribution of each active site to the release of angiotensin II (Ang II) and the inactivation of bradykinin (BK) - two peptide hormones that play a key role in the regulation of blood pressure - is still unknown. To gain insights into the functional roles of these two active sites, two phosphinic inhibitors RXP407 (2) and RXPA380 (3), were designed and synthesized. These two phosphinic compounds are able to selectively inhibit only one active site of ACE. We will present here: a) the synthetic strategy of the two selective phoshinic inhibitors using combinatorial chemistry and b) the *in vitro* and *in vivo* results.

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From Angiotensin to Losartan and Sartans: A new Generation of Anti-hypertensives

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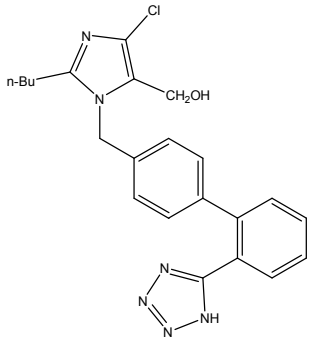
Losartan was the first drug of the Sartan series to be marketed. Using molecular models of Angiotensin II and mimetic technology developed early in 1990's researchers from Patras and Calgary were able to design and synthesize Losartan analogues, which were found to be strong inhibitors. One of them was equally potent to Losartan with longer period of action. However analogue, named Vivartan, was not further developed as synthesis was not cost effective. Latest efforts with structural modi-fications have reduced steps to achieve a cost effective

synthesis of related compounds with strong potency. This presentation will describe methods to design potent ANG II receptor anta-gonists.



Losartan

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Renin-Angiotensin System and the Regulation of Erythropoiesis

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Several clinical and experimental observations suggest that an intact and activated renin-angiotensin system (RAS) may be an important determinant of erythropoiesis in a variety of clinical conditions, including hypertension, chronic renal insufficiency or failure, chronic obstructive pulmonary disease, and congestive heart failure. Accordingly, RAS inactivation may confer susceptibility to the hematocrit-lowering effects of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Indeed, a dose-dependent decrease in hematocrit is observed within the first month of such therapy. In the majority of patients with hypertension decrements in hematocrit values after RAS inactivation are small and not clinically important. In extreme conditions, however, such as erythrocytosis after successful renal transplantation, secondary polycythemia of chronically hypoxemic COPD patients, erythrocytosis associated with renovascular hypertension, severe cardiac or renal failure, the hematocrit-lowering effect of angiotensing-converting enzyme inhibitors and angiotensin receptor blocker may be profound and even lead to or worsen anemia. Hematocrit reachs its nadir value within three months, and then it remains stable during long-term observations. After discontinuation of RAS blockade, hematocrit values rise gradually over the next three to four months towards the pretreatment levels. The mechanism(s) related to this phenomenon is not yet fully understood, but angiotensin II seems to be responsible for inappropriately sustaining secretion of erythropoietin despite hematocrit elevation and capable to directly stimulate the erythroid progenitors to produce erythrocytes.

❁

Genes and Coronary diseases

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Ο ρόλος των γονιδίων στην εμφάνιση των καρδιοπαθειών δεν μπορεί πλέον να αμφισβητηθεί. Έχουν αναγνωρισθεί αρκετές γονιδιακές μεταλλάξεις που είναι υπεύθυνες για την εμφάνιση πρώιμης αθηρωμάτωσης. Οι πιο γνωστές είναι οι μεταλλάξεις στο γονίδιο του LDL υποδοχέα

(προκαλούν την οικογενή υπερχοληστερολαιμία), η οικογενής έλλειψη της apoB–100 (η μετάλλαξη αυτή προκαλεί μειωμένη πρόσδεση της LDL με τον LDL-υποδοχέα), οι ισομορφές της apoE (ελέγχονται γονιδιακά και επηρεάζουν τον κίνδυνο για στεφανιαία νόσο). Επιπρόσθετα έχει αποδειχθεί ότι τα ετερόζυγα άτομα για το γονίδιο του ABCA1 εμφανίζουν πάχυνση του ενδοθηλίου και του μυϊκού χιτώνα των περιφερειακών αρτηριών. Επίσης ο πολυμορφισμός του ACE και του αγγειοτασινογόνου έχει βρεθεί ότι συσχετίζεται με την στεφανιαία νόσο. Οι διαταραχές στη φυσιολογική λειτουργία των αιμοπεταλίων και των ενδοθηλιακών κυττάρων συμβάλλουν στην ανάπτυξη καρδιαγγειακών επεισοδίων. Ενεργοποιημένα αιμοπετάλια και ενδοθηλιακά κύτταρα εκφράζουν στην επιφάνειά τους τις P και E-σελεκτίνες. Έχει παρατηρηθεί ότι η έκφραση των P και E-σελεκτινών αυξάνεται στην ύπαρξη αθηρωματικής πλάκας και αυξημένα επίπεδα των μορίων στο πλάσμα έχουν βρεθεί σε ασθενείς με έμφραγμα του μυοκαρδίου και ασταθή στηθάγχη. Χρειάζονται και άλλες μελέτες που θα περιλαμβάνουν όλα όσα έχουν αναφερθεί, όπως επίσης και την αλληλεπίδραση και με άλλα γονίδια.

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Gene Therapy of Hypertension

Helen Triantafyllidi, Haralambos Gavras

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Cardiovascular diseases represent the first cause of death in developed countries. Hypertension as a leading predisposing factor of cardiovascular disease consists main clinical and research target. Although, management of hypertension is based on proper drug treatment as well as dietary counseling we have not achieved to reduce the incidence of hypertensive patients. Failure is due to patient incompliance according daily treatment against an asymptomatic disease with detrimental consequences. Gene therapy of hypertension, using plasmid or viruses as genetic carriers, has advantages over conventional treatment since we use a biologic therapy against a biologic problem. We still need to solve problems ac-cording patients' safety, increased production with low cost and improvement of gene delivery. Although we are still far away of the clinical application of gene therapy in hypertension it seems that we are in the right direction.

❁

Dilated Cardiomyopathy in Clinical Practice

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Η Διατατική Μυοκαρδιοπάθεια αποτελεί το συνηθέστερο τύπο μυοκαρδιοπάθειας και τη συνηθέστερη εκδήλωση της Συμφορητικής Καρδιακής Ανεπάρκειας. Παρά το γεγονός ότι η διάγνωση της μυοκαρδιοπάθειας απαιτεί τον αποκλεισμό κάθε αιτιολογικού παράγοντα που οδηγεί σε καρδιακή ανεπάρκεια, αναμφισβήτητα τα κλινικά, σε συνδυασμό με τα επεμβατικά και μη επεμβατικά ευρήματα, επιτρέπουν συνήθως επαρκώς την ασφαλή διάγνωση της νόσου. Οι ειδικού τύπου μυοκαρδιοπάθειες που οφείλονται σε συγκεκριμένη αιτιολογία δεν περιλαμβάνονται στον όρο *ιδιοπαθής διατατική μυοκαρδιοπάθεια*. Ωστόσο, όλοι οι τύποι των διατατικών μυοκαρδιοπαθειών έχουν τον ίδιο φαινότυπο, αλλά διαφορετικό γονότυπο. Γονιδιακές διαταραχές που οδηγούν συνήθως στον ίδιο φαινότυπο έχουν σχέση με γονιδιακές διαταραχές του σαρκομεριδίου, της τιτίνης ή συναφών πρωτεϊνών του δίσκου Z, του σαρκειλήμματος και γενικότερα του κυτταροσκελετού, καθώς και των ενδιαμέσων ινιδίων. Οι γονιδιακές μεταλλάξεις, σε συνδυασμό με τις μεταβολές των περιβαλλοντολογικών συνθηκών φαίνεται ότι είναι υπεύθυνες για το χρόνο ενάρξεως και τη σοβαρότητα της Διατατικής Μυοκαρδιοπάθειας.

Crystal Structure of the Anchor Modified Non-Canonical Tumor Associated MUC1-8 Peptide, Showing Enhanced MHC Class I Binding and Immune Responses, in Complex with H-2K^b

Eliada Lazoura[1], Jodie Lodding[1], William Farrugia[2], Paul A. Ramsland[2], James Stevens[3], Ian A Wilson, [3,4] Geoffrey A. Pietersz,[5] and Vasso Apostolopoulos[1]

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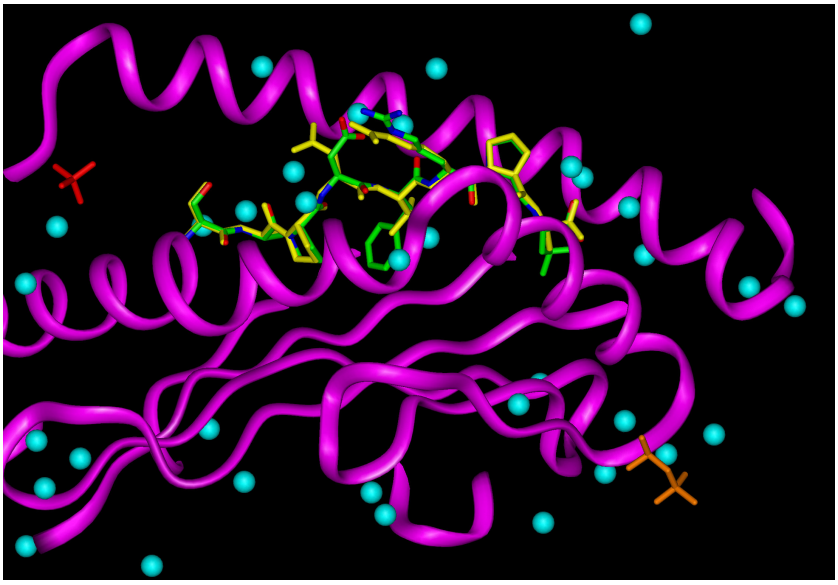
Peptide-based vaccine design for therapeutic applications in cancer immunotherapy has in the past involved immunization with high affinity tumor-associated antigenic peptides. To-date, however, low immunogenicity has been observed for such peptides which may be attributed to the lack of T cells to these peptides, having been deleted during thymic development. More recently, low-to-medium affinity peptides, which represent more suitable candidates, have yielded promising results. In addition to the difficulty in identifying such antigens, peptide binding to MHC and hence their ability to induce a strong immune response is limited. Therefore, in order to enhance binding to MHC and improve immune responses, anchor modifications of non-canonical tumor-associated peptides would be advantageous. In this study, modification of the non-canonical tumor associated peptide from MUC1, MUC1-8 (SAPDTRPA), at the MHC anchor residues (SAPD**F**RP**L**; MUC1-8-5F8L) resulted in enhanced binding to H-2K^b and improved immune responses. Furthermore, crystallographic studies revealed that binding of MUC1-8-5F8L to H-2K^b MHC was similar to that of the canonical OVA8 (SIINFEKL).



Crystal structure of the H-2K^b-MUC1-8-5F8L complex. The H-2K^b molecule is shown in pink, and the MUC1-8-5F8L peptide coloured according to the atoms (C in green, N in blue and O in red). The MUC1-8 parent peptide (yellow) is also shown for comparison. PO_4^{3-} molecules are in red and MPD molecules in orange. Water molecules are represented by cyan spheres

Immunomodulation in EAE through Regulation of T Helper Cell Subsets

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The possibility to modulate the outcome of a disease by manipulations that induce the selective maturation of T helper cell subsets is a recurrent theme in biomedical research. It has been established, by using different experimental approaches, that favoring the maturation of type 2 helper cells inhibits the progression of CNS inflammation in experimental autoimmune encephalomyelitis (EAE), the most widely used animal model of the disease multiple sclerosis (MS). Recent developments show that the protective effect might result from alterations on the different T cell pools of the organism such as effector, memory or regulatory T cells. An overview of the current approaches for the possible therapeutic utilization of such knowledge will be presented.

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Synthesis of Polyamine Conjugates with the Antimalarial Drug Artemisinin

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Artemisinin (**I**) and some of its analogs which are used for the treatment of malaria are presently considered as the only potent drugs against the very serious form of this illness (1). A series of artemisinin analogs have been already synthesized, a number of which show considerable biological activity. In this work, the synthesis of novel conjugates of artemisinin (**III-IV)** with linear and cyclic polyamines (**PA**) will be presented using a suitable linker (**II**) and as cross-linking reaction the well-established selective acylation of primary amino functions by α,β-unsaturated succinimidyl esters (2).

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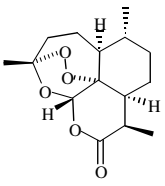

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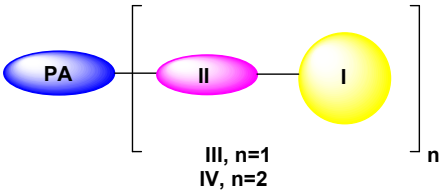

III, n=1
IV, n=2

❁

Computational Approaches in the Design and Analysis of Bioactive Compounds as Inhibitors Towards the Proteolytic Enzyme *Lethal Factor* of *Bacillus Anthracis*

Georgios A. Dalkas[1], Athanasios Papakyriakou[1], Georgios F. Vlachopoulos[1], Georgios A. Spyroulias[1] and Paul Cordopatis[1]

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Anthrax is a disease of animals and humans, caused by the bacterium *Bacillus anthracis*. Anthrax toxin (AT) consists of three proteins, one of which is anthrax lethal factor (ALF), a Zn-dependent metalloprotease (~90.000 kDa) with an important role in the pathogenesis of the disease (1). LF is a highly specific protease that cleaves members of the mitogen-activated protein kinase kinase (MAPKK) family near to their amino termini, leading to the inhibition of one or more signaling pathways (2). It is envisaged that, inhibition of the proteolytic activity of LF is the most promising approach for treating the disease. Computational procedure has been proved to be an effective tool in the design, analysis and optimization of bioactive compounds. Using this approach we have created libraries of dipeptides and tripeptides using the natural aminoacids. Initially, we carried out docking simulations to the biomolecular target with all the dipeptides that can be set up by using all the natural aminoacids (that is 20^2 dipeptides). The docking simulation results indicate that dipeptides which contains as structural units the aminoacids Arg, Trp and Lys form stable ALF-dipeptide complexes (low binding energy). The above analysis leads to the design and development of tripeptide libraries. These libraries contain as building blocks the aminoacids Arg, Trp and Lys in combination with other natural and not aminoacids, organic and chelating groups, in order to increase the binding affinity of the ligand-ALF complexes and to increase their stability.

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❁

Synthesis of new Pyrroloazepinoindole Skeleton Analogues with Potential Protein Kinase Inhibitory Activity

Efthymia Koutsandrea, Manolis Fousteris, Stella Kokoli, David O. Onyango, Ioannis Stamos, Anna Koutsourea and Sotiris Nikolaropoulos

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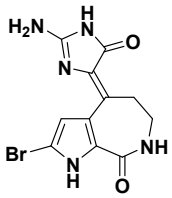
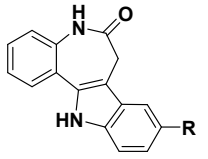
Cell cycle progression is being controlled by a variety of protein kinases, among of which cyclin dependent kinases (CDKs) play a pivotal role. Deregulation of their activity is associated with various diseases, including cancer, diabetes and neurodegenerative diseases. As a consequence, considerable efforts have been directed towards the development of new compounds with potential CDK inhibitory. Pyrrolo- and indolo-heterocycles with fused seven membered lactamic ring, like Hymenialdisine **I** and Paullones **II**, have exhibited inhibitory activity against several protein kinases, CDKs included. Aiming to the synthesis of new lead-compounds with potential CDK inhibitory activity, we designed and synthesized new analogues of the basic scaffold **4**, which maintain structural features of already known inhibitors. Pyrrolo[2,3-*c*]azepino-4,8-dione **3** is a key-intermediate of the synthetic plan, while the final products are obtained after Fischer indolization in high yields.

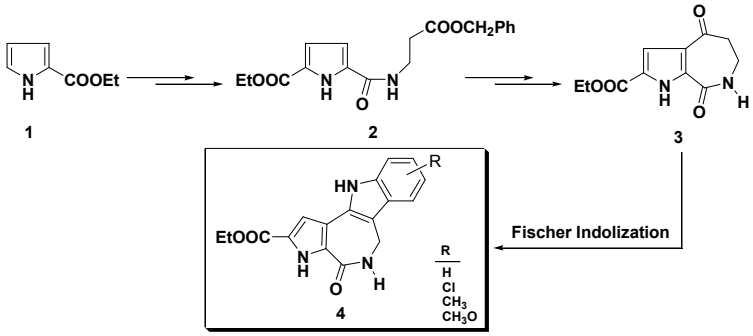


I



Kenpaullone, R = Br
Alsterpaullone, R = NO_2

II



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Design and Synthesis of Heme Peptides and Bioremediation

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The purpose of this work is to produce a peptide able to degrade dyes which are extremely pollutants to the environment. At this point, we are interested in a class of enzymes, the *peroxidases,* which are able to efficiently degrade azo and anthraquinonic dyes. In particular, we are working on the *versatile peroxidase (VP) of Pleurotus Eryngii*, [a] a fungus, which can perform this degradation. So, our goal is to try to synthesize a peptide based on this peroxidase and emulate its biological function. For the design of the sequence of the peptide which can sustain this action, an analysis was performed on the theoretical model of VP and the important amino acids for the desired function of the active site were identified. In particular, the important residues for the coordination of the heme, the Mn binding site and the Long Range Electron Transfer (LRET) pathway, were pin-pointed. Furthermore the radius of the heme cavity was calculated. We proceeded in the design of linear and cyclic peptides. The next step was the synthesis of these peptides in order to verify the coordination of the heme and optimize the sequence of these peptides. The synthesis of these peptides was performed by solid phase synthesis following the Fmoc/tBu strategy in a semi-automatic instrumentation. The next step will be to test whether these products are able to coordinate the heme and as soon as we verify this, we can move on to the production of the best peptide, maintaining all the functions of *Pleuritus Eryngii* enzyme, and hopefully performing its functions at the best.

REFERENCE

[a] *PDB ID:* **1A20** Molecular Model for a Pleurotus Eryngii Peroxidase Oxidizingmnii as well as Different Phenolic and Non-Phenolic Aromatic Compounds and Dyes, Theoretical Model'

❁

Design and Synthesis of Nitroxy Esters of Aromatic Heterocyclic Compounds: A new Class of Ischemic Preconditioning Agents?

Theano Fotopoulou, Maria Koufaki[1], Andrew Tsotinis[2], Ioanna Andreadou[2], Efstathios Iliodromitis[3], Dimitrios Kremastinos[3]

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Ischemic preconditioning is a well-established endogenous mechanism of protection of the ischemic heart, which reduces the infarct size when brief periods of ischemia and reperfusion proceed to a more sustained episode of ischemia. The pharmaceutical interventions that trigger this mechanism are nicorandil and adenosine. The activity of nicorandil has been linked to a concurrent stimulation and opening of the mitochondrial K_{ATP} channels, the release of NO, and the activation of PKC. Conversely, adenosine exerts its action by activating the appropriate kinases. In this communication we report on the design and synthesis of a series of new aromatic heterocycles, which bear the pharmacophoric nitroxy ester group. The new analogues are currently evaluated as to their ability to trigger ischemic preconditioning.

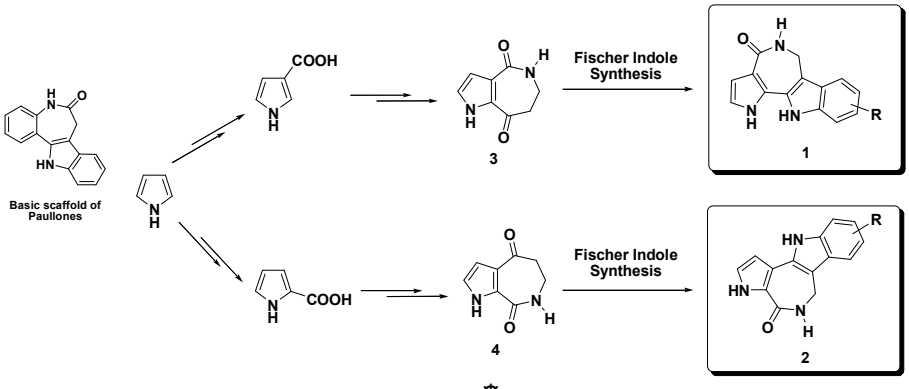
❁

Synthesis of new Pyrroloazepinoindole Analogues, with Potential CDK Inhibitory Activity

Stella Kokoli, Manolis Fousteris, Efthymia Koutsandrea, Andromaxi Papageorgopoulou, Ioannis Stamos, Anna Koutsourea and Sotiris Nikolaropoulos.

Laboratory of Medicinal Chemistry, Department of Pharmacy, School of Health Sciences, University of Patras, Rio, Greece

The transition of a cell from one phase of the cell cycle to another, is controlled from different cell proteins, among of which cyclin-dependent kinases (CDKs) play a crucial role. Deregulation of CDKs contributes to human cancers and neudegeneratine disorders. The investigation of novel compounds as candidate inhibitors of CDKs, offers a modern therapeutic approach for the treatment of the above diseases. Among other already known CDK inhibitors, Paullones, a group of 7,12-dihydro-indolo[3,2-*d*][1]benzazepin-6(5*H*)-ones, are well established CDK inhibitors with *in vitro* antiproliferative activity. Aiming towards the development of new lead compounds with potential CDK inhibitory activity, we designed and synthesized analogues, which bear the basic pyrroloazepinoindole skeletons **1** and **2**. According to the following synthetic plan, the pyrroloazepinodiones **3** and **4** constitute key-intermediate molecules. The latters were employed as building blocks in Fischer indolization conditions for the construction of final products core.



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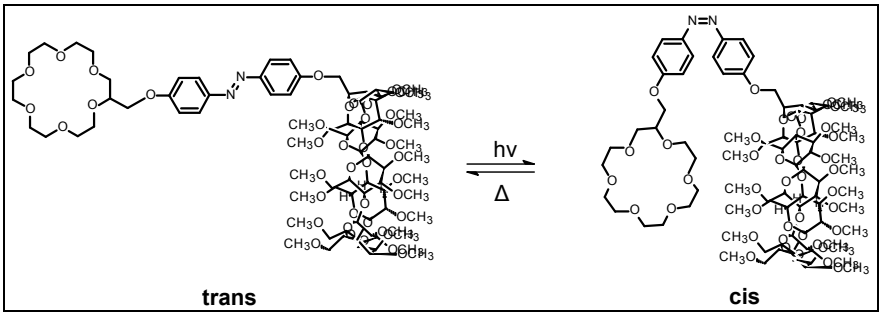
Photochromic Biomimetic Systems

S. Zachariadou, N. Kyritsis and G. Tsivgoulis

Laboratory of Organic Chemistry, Department of Chemistry, University of Patras, Rio, Greece



Scheme 1

Cyclodextrins (CD) are toroidal shaped molecules that show excellent complexation properties as a result of their unique structure. Thus, cyclodextrins have been used extensively in the construction of supramolecular systems and many examples where cyclodextrin derivatives have served as biomimetic systems in catalysis and/or transport have been already described. Crown ethers, also, represent an important category of supramolecular compounds because of their ability to complex metal ions and cations in general. Literature data show that molecules combining cyclodextrins with crown ethers can act as supramolecular multireceptors of charged organic molecules like aminoacids and benzoic acid salts. The aim of the present study is to synthesize and study the multifunctional molecule shown in Scheme 1. In this molecule, a permethylated β-cyclodextrin is connected to a 18-crown-6 moiety through an azobenzene linkage. Azobenzenes are well-known photochromic molecules capable to interconvert between a trans and cis conformation. Thus, the target molecule will exist in two different conformations (Scheme 1), while only the cis form will bind strongly substrates as amino acids. Therefore, molecule 1 is expected to act as a carrier for such compounds.

The Development of Surgery: A short Chronicle

N. Papadakis

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Κατά την εξέλιξή της η χειρουργική, από ένα αρχικό μείγμα θρησκείας και μαγείας σε ένα πεδίο επιστήμης και τεχνολογίας, έχει προκαλέσει ισχυρά συναισθήματα, ελπίδας και θαυμασμού, φόβου και απαγορεύσεως. Ποτέ δεν έχει προκαλέσει αδιαφορία. Η σημερινή πολύπλοκη χειρουργική μπορεί να ανιχνευθεί μέσα στις χιλιετίες και τα έθνη, από τους προϊστορικούς χρόνους, όπου ένας τυχερός ασθενής θα μπορούσε να έχει επιζήσει ενός τρυπανισμού του κρανίου. Επίσης, η σημερινή πολύπλοκη χειρουργική μπορεί να ανιχνευθεί στους κλασσικούς πολιτισμούς με τις εν πολλοίς ορθές ιατρικές μεθόδους, στις φοβερές επιδημίες και τις χονδροειδείς εγχειρήσεις στα πεδία των μαχών του Μεσαίωνος. Επίσης, στους σκαπανείς ιατρούς του 19ου αιώνος, που έκαναν δυνατή την εξέλιξη της Χειρουργικής, μέχρι την σύγχρονη εποχή μας με τη μεταμόσχευση οργάνων, πλαστική Χειρουργική και εγχειρίσεις εγκεφάλου.

POSTERS

Isolation and Characterization of Carbohydrates in Mastic Gum

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Departments of [1]Pharmacy and [3]Chemistry, University of Patras, GR-26500 Patras, Rio, Greece; [2]Department of Chemistry, Aristotle University, Thessaloniki, Greece

Mastic gum var. *Chia* is a resinous, aromatic substance that comes from the trunk and the thickest branches of the gum mastic tree *Pistacia lentiscus* species. Characterization of the gum constituents is prerequisite for understanding its bactericidal activity against Gram (+) and Gram (-) bacteria and especially *Helicoba-cter pylori*. Aim of this study is the isolation and characterization of carbohydrate macromolecules of mastic gum. Extraction was performed with 20 mM Tris/HCl buffer, pH 7.5, containing 0.1 M NaCl and the removal of molecules of low molecular weight and salts was accomplished with dialysis against water. Extract characterization was performed with colorimetric methods for the determination of neutral sugars, hexosamines, uronic acids, sialic acids, and protein. Separation and isolation of carbohydrate macromolecules was performed with anion-exchange chromatography on a Mono Q column. With low salt concentration, two main glycoconjugates of high molecular weight were eluted. Their contribution to the biologic properties of mastic gum is under investigation.

❁

Efficient Synthesis of Polyamines Bearing Tetrazolyl Units on their Amino Functions

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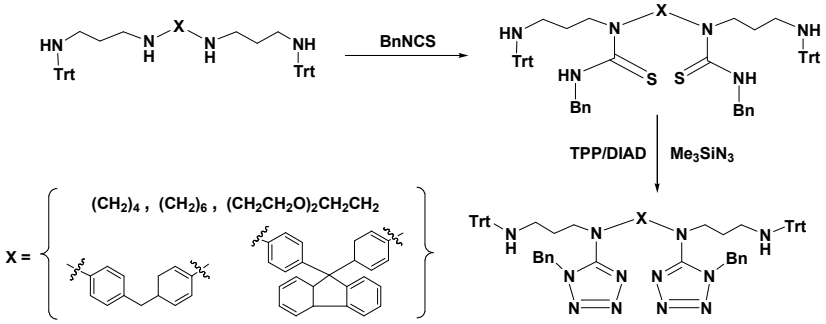

Linear N^{α}-trityl-α,ω-diamines and N^{α},N^{ω}-ditrityl-polyamines were efficiently converted to the corresponding *N*-substituted, with the 1-benzyltetrazolyl unit, derivatives upon treatment with benzylisothiocyanate (1) followed by the reaction of the thus obtained thioureas with azidotrimethylsilane under Mitsunobu reaction conditions (2). Deprotection was effected by TFA-mediated acidolysis or catalytic hydrogenolysis.

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Synthesis of Peptoid-Peptide Analogs of Substance P Fragments Incorporating D-Amino Acids

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It is well known that synthetic peptides are currently under investigation as possible antitumors agents. Specifically for SP, it has been proved that either the hormone or its C-terminal fragments stimulates the secretion of tumor-necrosis factor–α (TNF-a) and cytokines (IL-6, IL-10) from peripheral blood monocytes and cord blood monocytes-derived macrophages in synergetic fashion with liposacharides *in vivo* and *in vitro*. Continuing this research project on SP analogs, we have synthesized C-terminal peptide analogs of SP in liquid phase having the sequences Glp^1-D-Trp^2-$MePhe^3$-D-Trp^4-$Glu(OBzl)^5$-NH_2 and Glp^1-D-Trp^2-$MePhe^3$-D-Trp^4-Leu^5-$Glu(OBzl)^6$-NH_2. In SPPS we have synthesized the C-terminal hexapeptide peptoid-peptide analogs of SP, which contain the [-N(Bzl)-CH_2-CO-] monomer in their sequence. The incorporation of N-substituted glycine in the peptide chains has been proved to improve their stability against proteases and give biologically active peptides. The corresponding analogs which have been synthesized are Glp^1-D-Trp^2-[N(Bzl)-CH_2-CO]^3-D-Trp^4-Leu^5-$Glu(OBzl)^6$-NH_2 and Glp^1-D-Trp^2-[N(Bzl)-CH_2-CO]^3-D-Trp^4-Leu^5-$Glu(OBzl)^6$-OH. All the synthesized analogs are purified (RP-HPLC), identified (ESI-MS) and set about for study their biological properties and activity against the cancer cells proliferation.

❁

Design, Synthesis and Molecular Modeling Studies of a Non-peptide Antagonist of Par 1 Thrombin Receptor, Using Piperazine as Template

Maria–Eleni Androutsou[1], Konstantinos Alexopoulos[2], Amalia Resvani[1], Konstantinos Kelaidonis[1], André Aubry[3], Theodore Tselios[1] and John Matsoukas[1]

[1]Department of Chemistry, University of Patras, Patras 26500, Greece; [2]Blood Department, *Agios Andreas* Hospital, Patras 26335, Greece;[3]LCM3B, Biocrystallography Group, UHP Nancy I, BP 239, 54506 Vandoeuvre les Nancy Cedex, France

Thrombin is a serine protease that plays a central role in thrombosis and hemostasis (1,2). Inhibition of Thrombin Receptor (PAR-1) is a promising therapeutic approach for the treatment of various cardiovascular disorders such as unstable angina, acute myocardial infarction and restenosis. This research is focused on the development of PAR-1 inhibitors that do not interfere with the coagulation cascade (3,4). 1-(6-guanidohexanoyl)-4-(3-phenylalyl) piperazine (*CIN3*) has been designed based on Nuclear Magnetic Resonance (NMR) Constraints and Molecular Dynamics (MD) studies of the bioactive SFLLR and SFLLR-NH_2 peptides (5). Piperazine was chosen as template because of its ability to take two low energy conformations, in order to bring closer in space or remove far away the Phenyl and Guanidine group, which play major role in the bioactive pentapeptide. *CIN3* was found to inhibit platelet aggregation induced by thrombin and stimulate the endothelial PAR 1 receptor in rat aorta relaxation assay in a concentration dependent manner.

This work was supported by NATO Collaborative Linkage Grant No 97 9856

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Study of Thioester Ligation on Solid Phase

I.Vassilopoulos, D. Gatos and K. Barlos
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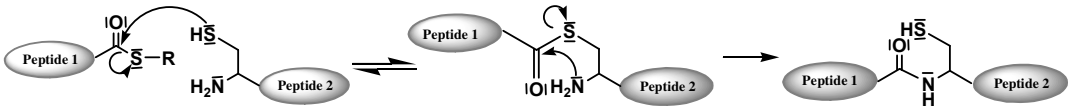
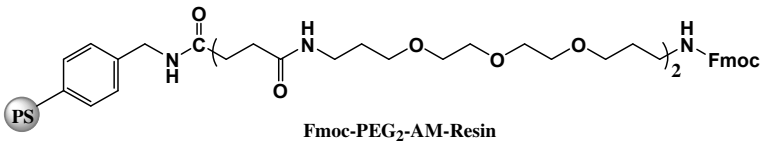


Figure 1



Fmoc-PEG$_2$-AM-Resin

Figure 2

Despite the numerous methodological advances made over the last two decades, chemical synthesis of large peptides and proteins still remains problematic. The technique of native chemical ligation has enabled the total chemical synthesis of proteins with molecular weights far in excess of those achievable by conventional SPPS and convergent peptide synthesis. The method involves the condensation of two unprotected peptide segments, one bearing a C-terminal thioester and the other an N-terminal cysteine residue, to afford a protein with a native amide linkage at the site of ligation (Fig.1). Our aim was to synthesize C-terminal peptide thioesters and study the ligation of these fragments on solid and liquid phase. For this purpose we used a new hydrophilic solid support, PEG (polyethyleneglycol) aminomethyl resin (Fig.2).

❁

Protonless NMR for the Study of Protein-Protein Interactions

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Interactions between proteins are at the basis of many biological processes. The mechanisms of molecular recognition and interaction of macromolecules can only be studied through the elucidation of the three dimensional structure of the formed complexes and NMR spectroscopy is a powerful tool to determine it in solution. Even if the complex is transient, it is possible to map the intermolecular contacts by analyzing the chemical shift variations in simple 2D NMR spectra. This is generally achieved by monitoring the spectral changes in a 1H-15N correlation map. However, this approach often lacks of selectivity, as the exchangeable NHs are very sensitive to any variation in their chemical environment, and so their chemical shift. Furthermore, in some cases the NH signals are lost due to exchange broadening. The chemical shift of aliphatic nuclei is less prone to such variations and the chemical shift mapping of the side-chain nuclei offers a probe of meaningful changes in the structure. Copper, like other metal ions, needs the so-called metallochaperones in its pathways within the cytoplasm. In *Saccharomyces cerevisiae* the copper chaperone Atx1 delivers Cu(I) to the soluble copper domains of Ccc2, an ATPase. Solution structures of the native Cu(I)-bound and the reduced apo-forms of both yeast Atx1 and the first soluble domain of Ccc2 have been solved in our laboratory and models of their in-

teraction have been refined. We have applied the novel set of experiments that we have developed for correlating N, CO, Cα, Cβ carbon nuclei, as well as new *protonless* sequences specifically designed to assign side-chains nuclei, on the Atx1-Ccc2 complex with and without copper(I). The ensemble of information obtained demonstrates the capability of heteronuclear direct-detected experiments in unrevealing details of the interaction between proteins and metal ion uptake.

❁

Synthesis and Pharmacochemical Study of New Pyrrolyl-Derivatives with Possible Antiinflammatory Activity

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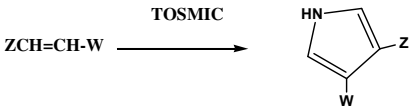
Chalcones constitute a class of compounds of the general structure: Z—CH=CH—W. They are known for a number of biological activities (1) among which, anti-inflammatory activity, antagonism, disposal and captivation of free radicals. Furthermore they act as inhibitors of several enzymes such as the ones that interfere in the inflammatory process, lipoxygenase and cycloxygenase, lysozymes and β-glucuronidase. Chalcones also are precursors for the synthesis of a series of pyrrole derivatives (2,3), inhibitors of COX-1/COX-2. Within the framework of our tries to synthesize dual inhibitors COX/LOX and using the computer-aided design, we have synthesized some new chalcones and their correspondig pyrolyl-derivatives. The synthesized compounds have been studied for: (a) the *in vitro* inhibition of lipoxygenase, (b) the *in vitro* inhibition of β-glucuronidase, (c) the *in vitro* inhibition of lipid peroxidation, (d) the interaction with the stable, N-centered, free radical DPPH and (e) *in vivo* their anti-inflammatory activity by the inhibition of the carrageenan induced rat paw edema.



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❁

Design, Synthesis and Biological Evaluation of the MBP83-99 Epitope Conjugated with Oxidised or Reduced Mannan: A Pilot Study of the *in vitro* Effects of the Mannan-peptides on T-Cells Isolated from Multiple Sclerosis (MS) Patients And Controls

G. Deraos[1], K. Chatzantoni[2], P. Papathanassopoulos[2], E. Friligou[1], S. Deraos[1], T. Tselios[1], J. Matsoukas[1] and A. Mouzaki[2]

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MS is a chronic demyelinating disease of the central nervous system characterized by local T-cell (mainly CD4+) and macrophage infiltrates, demyelination and loss of neurologic function. A novel strategy in the immunotherapy of MS is the use of carriers/adjuvants to *bias* the immune response from the predominant type-1 (pro-inflammatory) to type-2 (anti-inflammatory) phenotype. Mannan targets the mannose receptors on anigen presenting cells, enhancing their antigen-presenting capacity. In the present report, the immunodominant MS epitope MBP83-99 was congugated with oxidised (OxMannan) or reduced mannan (RMannan) and the resulting

peptidic constructs were tested for their ability to switch the cytokine production pattern of T-cells derived from MS patients from type-1 (IL-2 was tested) to type-2 (IL-10 was tested). To this end, T-cells were isolated from 3 MS patients and 3 controls and all the types of peptides tested in this study were presented to them by homologous dendritic cells prepared *in vitro*. The best results, so far, were achieved with the OxMannanMBP83-99 peptide, that reduced the ammount of IL-2 secreted by the MS T-cells, but had no effect on the control T-cells.

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❁

ATRA Inhibits Prostate Cancer Cell Growth through Regulation of Heparin Affin Regulatory Peptide Expression

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It is becoming increasingly recognized that all-trans retinoic acid (ATRA) plays a role in cancer cell growth arrest through regulation of the expression of several genes (1). Heparin Affin Regulatory Peptide (HARP) is an 18 kDa secreted polypeptide growth factor with high affinity to heparin. HARP is mitogenic for endothelial cells, stimulates angiogenesis in vitro and in vivo and plays a key role in the progression of several types of tumors of diverse origin (2). In the present study we found that exogenous ATRA significantly decreased human prostate cancer LNCaP cell proliferation. Heparin affin regulatory peptide (HARP) seems to be involved in the inhibitory effect of ATRA, because the latter had no effect on stably transfected LNCaP cells that did not express HARP. Moreover, ATRA significantly decreased HARP mRNA and protein amounts in a concentration- and time-dependent manner. These data suggest that ATRA affects prostate cancer LNCaP cell growth through an effect on the expression of HARP and further studies are in progress to elucidate mechanisms involved.

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❁

New Synthetic Analogues of LHRH Modified in Positions 3 and 6

A. Thomopoulou[1], E. Athanasopoulou[1], D. G. Chryssanthi[1], A. A Zompra[1], V. Magafa[1], F. Lamari[1], N. K. Karamanos[2] and P. Cordopatis[1]

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Reproduction in mammals is controlled by interactions between the hypothalamus, anterior pituitary and gonads. The hypothalamus secretes pulses of Luteinizing Hormone–Releasing Hormone (LHRH), a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) that is the primary regulator of gametogenesis and steroidogenesis. The hormone acts by binding to a sin-

gle class of G protein coupled receptors (GPCR) leading to synthesis and release of gonadotropins Luteinizing Hormone (LH) and Follicle-stimulating-Hormone (FSH). Although hypothalamus and pituitary are the principal source and target sites for LHRH, several reports have recently suggested extrahypothalamic LHRH and LHRH receptors in various reproductive tissues such as ovaries, placenta, endometrium, prostate and mammary glands. LHRH and its analogues have been proven valuable in the treatment of a wide variety of endocrinological and nonendocrinological disorders. In this study, we report an improved synthesis of new analogues of LHRH in order to study the effect of modifications in positions 3 and 6 on breast cancer cell proliferation. The structural criteria for the synthesis of the new analogues of LHRH were on the basis that the most of the superagonists usually incorporate a D-amino acid substituting for Gly in position 6 and an Pro-ethylamide residue instead of the terminal Gly-NH_2 in position 10 (Fujino modification). These chemical modifications decrease the susceptibility of the peptide to proteolytic degradation and lead to peptides with high binding affinity to the receptor of LHRH. The synthesized analogues bear the Fujino modification , while Gly^6 was substituted by the hydrophobic amino acid D- and L- cyclohexyl-alanine (Cha) and Trp^3 by D-Trp, D- 1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid (D-Tic), D-tetrahydro-isoquinoline-1-carboxylic acid (D-Tiq) and D-2-naphtylalanine [D-Nal(2)]. Peptide synthesis has been performed through solid phase chemistry on a [3-((Ethyl-Fmoc-amino)-methyl)-1-indol-1-yl]-acetyl AM resin *via* Fmoc/t-Bu methodology. The analogues were tested for the inhibition of proliferation on human breast cancer cells (MCF-7).

❁

Synthesis of Fragments of the Diuretic Hormones Dippu-DH_{46} and Dippu-DH_{31}

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Insect Diuretic Hormones are crucial for control of water balance. Two diuretic hormones have been identified from the cockroach, *Diploptera Punctata*, the CRF (corticotropin-releasing factor) -related diuretic peptide Dippu-DH_{46} and the calcitonin-like peptide Dippu-DH_{31}, which increase cAMP production and fluid secretion in Malpighian tubules of several insect species. Our aim was to synthesize fragments analogues of the Dippu-DH_{46} and Dippu-DH_{31}, the C-terminal Dippu-$DH_{46(38-46)}$, Dippu-$DH_{46(32-46)}$, Dippu-$DH_{31(23-31)}$, Dippu-$DH_{31(17-31)}$ for structure-activity studies and to relate their activity with Locmi-DH_{32-46} (fragment from CRF-related diuretic peptide Locmi-DH) which presented antifeeding activity in locusts. The synthesis of above analogues was performed in solid phase on the 2-Chlorotrityl chloride Resin using the Fmoc/tBu method.

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❁

Immune Responses of Myelin Basic Protein (MBP_{83-99}) Analogues (Alone or Conjugated with Mannan) in SJL/J Mice

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Multiple Sclerosis (MS) is an autoimmune demyelinating disease, characterized by inflammatory foci in the brain white matter with variable axonal damage. It is believed that MS is an au-

toimmune disease mediated by CD4+ T cells of Th1 subset. Candidate self-antigens include constituents of the myelin sheath - Myelin Basic Protein (MBP), Proteolipid Protein (PLP) and Myelin Oligodendrocyte Glycoprotein (MOG). Modern approaches toward the therapeutic management of MS involve the design and use of peptide analogues of disease-associated myelin epitopes to induce peripheral T-cell tolerance. We designed a number of antagonist peptides by mutating principal TCR contact residues. The synthesis of the linear peptide agonist MBP_{83-99}, as well as of the cyclic analogue was carried out by the Fmoc/tBu methodology, utilizing the 2-chlorotrityl chloride resin. Cyclization was achieved using O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxy-7-azabenzotriazole, 2,4,6 collidine allowing fast reaction and high yield cyclization product. The purification was achieved using HPLC reversed-phase chromatography and the peptide purity was assessed by analytical HPLC and by mass spectrometry (ESI-MS).

MBP_{83-99} linear and cyclic peptide analogues synthesized in this study, were either, (i) emulsified in equal volume of Complete Freund's Adjuvant (CFA) and PBS and injected once or (ii) conjugated to reduced mannan via a KLH linker and injected twice on days 0 and 14; all mice were injected intradermally (i.d) at the base of tail. Previous studies demonstrated that reduced mannan had the ability to generate a Th2 response with high IL-4/IL-10 production and low INF-□. T cells were isolated from spleen and were examined *in vitro* for their cytokine production profile (Th1/Th2). IFN-γ and IL-4 were measured using a capture ELISpot method and antibody responses were measured by ELISA. We note that the use of CFA for immunization induced low levels of IFN-γ and IL-4 when the antagonist peptides were used. In contrast, high levels of IL-4 were secreted by T cells when mice were immunized with reduced mannan-KLH conjugated to antagonist peptides. Cytokine and antibody responses to all agonist, antagonist, linear and cyclic peptides will be discussed. We are currently determining the effectiveness of the peptides in EAE models.

❁

Synthesis, Structural Characterization and Biological Activity of Sn(IV) and Zn(II) Complexes with Peptides which Contain α-Aminoisobutyric Acid (Aib)

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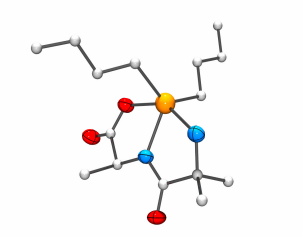

Structure of complexe $[(n\text{-}Bu)_2Sn(Aib\text{-}L\text{-}Ala)_2]\cdot MeOH$

The interactions of peptides with metal ions have attracted the interest of bioinorganic chemists in order to investigate, among others, the influence on their reactivity upon coordination, the possibility that these metal complexes exhibit biological/pharmacological activity and the potential for the formation of supramolecular structures. We describe here the synthesis, the structural characterization and spectroscopic properties of Sn(IV) and Zn(II) complexes with the synthetic peptides H-Aib-L-Ala-OH, H-Aib-L-Leu-OH and H-Aib-Aib-Aib-OH. The Sn(IV) complexes exhibit satisfactory antiproliferative activity, while the structures of the Zn(II) complexes are of particular interest.

Quantitative Structure Activity Relationships on Thromboxane A_2 Inhibitors and on Thromboxane A_2 Receptor Antagonists

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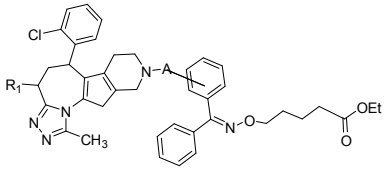

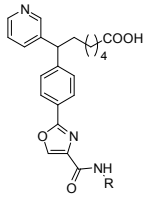

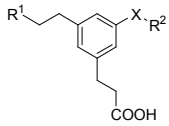

Thromboxane A_2 (TXA_2) is an unstable endogenous metabolite of arachidonic acid. It is a potent vasoconstrictor, bronchoconstrictor and platelet-aggregating agent. It has been also implicated as a causative factor in a number various diseases, such as circulatory disorders, unstable angina and stroke (1). Therefore, TXA_2 receptor antagonists (TRAs) are expected to be effective for the treatment of these diseases, and a number of TXA_2 receptor antagonists have been clinically investigated (2). Considerable effort has been also devoted to the design of TXA_2 synthase inhibitors (TSIs), which may have utility in the therapy of these diseases (1). Because of the lack of clinical efficacy with these agents, theoretical arguments have been made to support potentially superior antithrombotic efficacy of using a combined TRA/TSI over either class of agent alone or aspirin (3). A number of compounds (1,3,4) have been synthesized as to act either as thromboxane A_2 receptor antagonists or as thromboxane A_2 synthase inhibitors. They have been studied here in terms of QSARs and it has been reported that lipophilicity and steric parameters seem to play a crucial role for their activity.

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Study of Coumarin Derivatives in Cardiovascular Diseases

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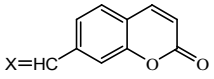
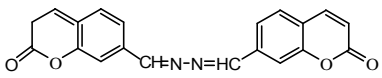
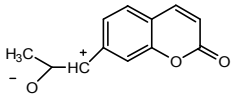


X=O, NHOH, $NHOCH_3$, NNHPh, $NNHCH_3$, NPh





Arterial thrombosis develops on disrupted atherosclerotic lesions and causes the clinical complications of myocardial infarction, sudden cardiovascular death, stroke and acute limb ische-

mia, the most common causes of mortality in the Western world today (1) Inflammation and coagulation play fundamental roles in the pathogenesis of atherothrombosis (2,3). Inflammation leads activation of coagulation. Coagulation also considerably affects inflammatory activity. It has been proved that inflammation plays an important role in post-thrombolytic complications whereas it is induced by thrombolytic therapy in patients with acute myocardial infarction (4). Coumarin derivatives have been found to act as anticoagulants, and to posses anti-inflammatory anticoagulant and antioxidant activities (5,6). In this work it is studied in vitro: inhibition of blood coagulation, (calculated as prothrombin time), inhibiton of lipid peroxidation and inhibition of troponine I, (a diagnostic marker of myocardial function). These compounds were studied for their possiblity to act as NO donors.

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❁

Synthesis and Biological Evaluation of Oxytocin Analogues Containing *β*-(2-Thienyl)-alanine in Positions 7 and 9

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Oxytocin (OT) is a hypothalamic cyclic nonapeptide that is released into the general circulation from the neural lobe of the pituitary, inducing uterine contractions during parturition and milk ejection during lactation. The widespread distribution of OT receptors in the brain and the specific behavioral effects of centrally applied OT have firmly established OT as a central neurotransmitter with roles in reproductive and social behaviors. Specifically, a role in mediating maternal behavior, sexual receptivity and partnership bonding has been proposed. The role of oxytocin in triggering preterm labor led the design of synthetic peptide and non-peptide OT antagonists as potential tocolytic agents for the prevention of preterm births. Of the many OT antagonists reported to date, only one, Atosiban, has been approved (in Europe) under the Trade name Tractocile for the treatment of preterm labor. The design of OT antagonists is based, on data from structure-activity studies. The *C*-terminal tripeptide and especially the proper orientation of the *C*-terminal glycine carboxamide is crucial for obtaining oxytocin analogues with high potency. Antagonistic activity also depends from the configuration and the hydrophobicity of the amino acid at position 2. Based on these findings we synthesized new oxytocin analogues containing *β*-(2-thienyl)-alanine (Tha) in positions 3, 7 or 9. Basic modification at positions 7 or 9 was combined with Mpa[1], D-Tyr(Et)[2], D-Nal(1)[2] modifications and their various combinations. For the synthesis we use the Fmoc/tBu solid phase methodology utilizing as solid support the Rink Amide MBHA resin, to provide the peptide amide. Electro-spray MS was in agreement with the expected results. The analogues were tested for rat uterotonic activity *in vitro*, in the rat pressor assay and for binding affinity to human OTR.

❁

Synthesis and Anti-Inflammatory Activity of 5-Nonsubstituted/ Substituted 2-[(4-Adamantine Thiazol–2–YL)imino]-4Thiazolidinones

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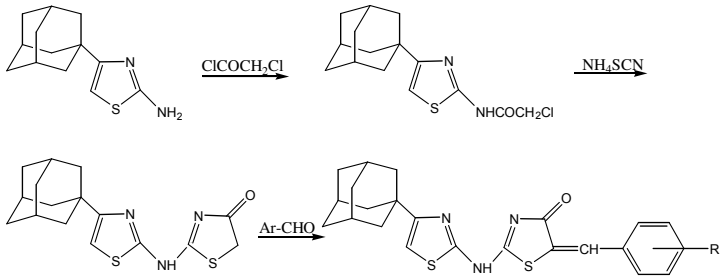

R = 1) p-NO_2, 2) m-NO_2, 3) o-NO_2, 4) p-Cl, 5) m-Cl, 6) o-Cl, 7) p-OCH_3, 8) p-OH, 9) p-OH-3-OCH_3, 10) H

SCHEME 1

Mammalian bodies respond to a variety of hostile agents such as parasites, pathogenic microorganisms, toxic chemical substances and physical damage with inflammation. A variety of non-steroidal anti-inflammatory drugs (NSAIDs) are widely used clinically for the treatment of inflammation diseases such as arthritis, lumbago and rheumatism, etc. The inhibitory action of these agents is exhibited on the cycloxygenase that catalyzes the biosynthesis of prostaglandins and troboxane from the arachidonic acid. Several substituted thiazolyl-derivatives are reported to possess anti-inflammatory activities. This research is an extension of our previous work on the synthesis of thiazole derivatives with prospective anti-inflammatory activity. The title compounds were synthesized according to scheme 1. The anti-inflammatory activity of the tested compounds was evaluated using carrageenin induced mouse paw edema. Lipophilicity of the title compounds was calculated using various programs.

❁

Synthesis and Pharmacochemical Study of Chalcones *and Relative Mannich Bases*

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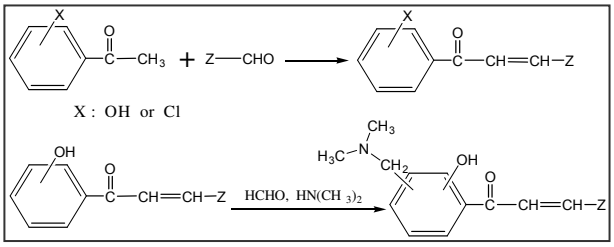

Employing the computer aided drug design an attempt has been made in order to design and synthesize a series of new chalcones and their corresponding Mannich bases with possible

inhibitory activity on lipoxygenase, antiviral and anticancer abilities as well as anti-inflammatory activity. Researches that have been recently published confirm that cancer is directly related to the complicated inflammation phenomenon. The synthesis method that has been used is the above indicated and the structure of the new compounds was verified by spectroscopic methods and their elemental analysis. Lipophilicity is an important property involved in biological activity. Thus lipophilicity was determined theoretically and experimentally. The new compounds were tested in vitro for their: a) interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, b) inhibition of lipid peroxidation, c) inhibition of trypsin proteolytic activity, d) inhibition of chymotrypsin proteolytic activity, e) interaction with glutathione (GSH) and in vivo f) inhibition of the carragenin induced rat paw edema.

❁

Photochromic Molecules in Biological Systems

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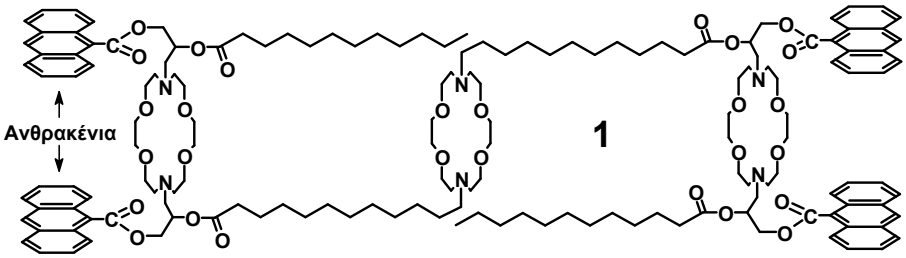

Figure 1

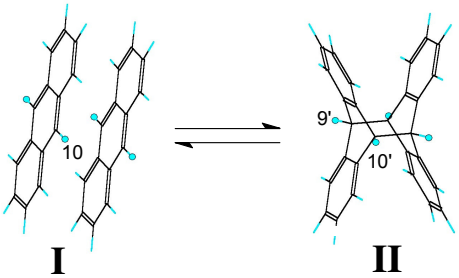

Figure 2

The synthesis of components capable to mimic natural systems, as transportation of ions and molecules through membranes, via channels or carriers, is very important. Some elegant examples of synthetic compounds capable to perform in that way have been reported in the literature. For example, Gokel's group has synthesized a tris(macrocyclic) ether that can transport sodium cations through membranes, via the channel type mechanism. In the present research, the introduction of anthracenyl groups in this tris(macrocyclic) ether is described (Figure 1), so that the opening and closing of the channel's entrance (gated channel) will be possible. Anthracene derivatives are well known photochromic compounds, as they can change between two structurally different forms (I, II Figure 2), by using light or temperature as triggers**.** As a result, the target molecule will also will exist in two different structures, one that will permit and one that will not permit the transportation of sodium cations through this channel.

Regulation of Heparin Affin Regulatory Peptide Expression by cAMP in Glioma Cells

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Glioblastoma is the most frequent and malignant human brain tumor. None of the therapeutic approaches used up to date have significantly improved the clinical outcome of this disease and most patients with GBM die in less than a year (1). Among the factors that may play a significant role in the progression of these tumors is cAMP, which seems to be decreased in high-grade tumors (2) and significantly decreases glioma cell growth (3). Heparin affin regulatory peptide (HARP), also known as pleiotrophin or heparin-binding growth-associated molecule, is an 18-kDa secreted growth factor that has high affinity for heparin. HARP is expressed in various cancer cell lines, among which glioblastomas (4, 5). The role of HARP in glioma cell growth has not been clearly defined, although preliminary results support an inhibitory effect on glioma cell growth and angiogenicity (6). In the present work, we investigated the possible regulation of HARP expression by cAMP in glioma cells. It seems that HARP expression is regulated by cAMP and plays a significant role in the inhibitory effect of cAMP on glioma cell proliferation.

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Adrenal GRK2 in Murine Heart Failure

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Heart failure (HF) represents one of the most significant health problems worldwide. One of its salient symptoms is elevated Sympathetic Nervous System (SNS) activity and outflow, reflected mainly by enhanced levels of circulating catecholamines (CAs) in HF subjects, a significant aggravating factor for the disease. CA secretion from the adrenal medulla, along with nor-epinephrine release from the Central Nervous System, is a major component of SNS outflow and activity. Various G-protein coupled receptors (GPCRs) have been shown to regulate it, some enhancing it (e.g. beta adrenoceptors, beta-ARs), while some others inhibit it, most importantly the alpha2-ARs. Regulation of these receptors in the adrenal medulla and, specifically in the chromaffin cells, remains largely elusive. On the other hand, since SNS activity is elevated in HF, adrenal CA secretion is expected to be enhanced and therefore the inhibitory alpha2-ARs (and possibly other GPCRs) might be down-regulated in HF adrenals. GPCR kinases (GRKs) play a prominent role in GPCR regulation. For this purpose, we investigated potential alterations in GRK expression and activity, in conjunction with levels of CA secretion and alpha2-AR function in adrenal medullae from a genetic mouse model of chronic HF. Interestingly, we found a significant up-regulation of adrenal GRK2 in these mice, which correlated with alpha2-AR desensitization and down-regulation, resulting in enhanced CA secretion. Therefore, adrenal GRK2 might be a novel therapeutic target for HF.

Efficient Total Syntheses of Novel Acitretin-Type Retinoids with Variable Electron Density in the Aromatic Ring

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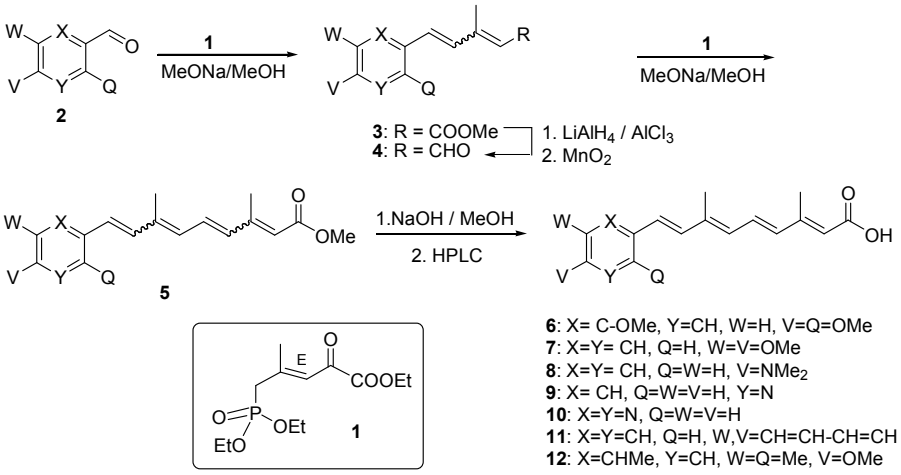

Synthesis of the novel acitretin(**12**)-type retinoids **6**-**11** was realized by initially coupling the phosphonate **1** with the commercially available aromatic aldehydes **2** through a Horner-Emmons (1) reaction to give esters **3**. Phosponate **1** was obtained exclusively as the E-isomer for the first time. Reduction of esters **3** followed by oxidation of the thus obtained alcohols afforded the corresponding dienals **4**. The latter were coupled again with phosphonate **1**, in order to form the required tetraene chain to give esters **5** as a mixture of geometric isomers. From this mixture, the projected *all-trans* acids were readily obtained through saponification, followed by either recrystallization or purification using RP-HPLC.

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A Putative Bioactive Conformation for the APL of Myelin Basic Protein and Inhibitor of Experimental Autoimmune Encephalomyelitis, [Ala^{96}] MBP$_{87-99}$

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[Ala^{96}] MBP$_{87-99}$ is an altered peptide ligand (APL) of myelin basic protein (MBP), shown to actively inhibit experimental autoimmune encephalomyelitis (EAE), which is studied as a model of multiple sclerosis (MS). The APL has been rationally designed by substituting two of the

critical residues for recognition by the T-cell receptor. A conformational analysis of the APL has been sought using a combination of 2D NOESY NMR experiments and detailed molcular dynamics calculations, in order to comprehend the stereoelectronic requirements for antagonistic activity, and to propose a putative bioactive conformation based on spatial proximities of the native peptide in the crystal structure. The proposed structure presents backbone similarity with the native peptide, especially at the important for MHC binding N-terminus. Primary (Val^{87}, Phe^{90}) and secondary (Asn^{92}, Thr^{95}) major histocompatibility complex (MHC) anchors occupy the same region in space, whereas T-cell receptor (TCR) contacts (His^{88}, Phe^{89}) have different orientation between the two structures. A possible explanation, thus, of the antagonistic activity of the APL is that it binds to MHC, preventing the binding of myelin epitopes, but it fails to activate the TCR and hence to trigger the immunologic response. NMR experiments coupled with theoretical calculations are found to be in agreement with X-ray crystallography data and open an avenue for the design and synthesis of novel peptide restricted analogues as well as peptide mimetics that rises as an ultimate goal.

Orthogonal Thioester Ligation of Selected Peptide Fragments on Solid and Liquid Phase

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The chemical synthesis of proteins by the traditional linear solid phase methodology is somewhat restricted by the difficulty of ensuring complete amino acid conjugation and N^{α}-deprotection throughout the elongation of peptide chain, especially when synthesis of long peptides is attempted. An alternative synthetic approach to large peptides is orthogonal thioester ligation. The method involves nucleophilic attack of the thiol side chain of a N^{α}-cysteine peptide on a thioester segment. The amide bond is formed spontaneously through intramolecular S→N acyl transfer. In our study we used selected unprotected peptide fragments of Mdm2 (Murine double minute 2) and Hir (Hirudin) to perform thioester ligation on solid and liquid phase. For the thioester ligation on solid phase, a fragment of Mdm2 was coupled to a novel hydrophilic resin, bearing a thiol group, leading to the desired thioester and reacted with a fragment of Hir. Correspondingly, in liquid phase ligation, the C-terminal peptide thioester of Mdm2 was prepared in solution and left to react as well in solution with a N^{α}-cysteine peptide fragment of Mdm2. All fragments were synthesized on 2-chlorotrityl chloride resin, using the Fmoc/ ^{t}Bu method.

Signal Transduction Differences between WT and Polymorphic α_{2B}-Adrenergic Receptor in LLC-PK1 Cells

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The aim of the present study was to characterise the signalling pathway triggered by activated human α_{2B}-adrenergic receptor (WT) in trasfected LLC-PK1, a renal tubular cell line. Moreover to investigate differences between the wild-type (WT) and a polymorphic (MUT) α_{2B}-AR, which has a deletion of three glutamic acids (Del301-303) in its third intracellular loop. Upon

agonist stimulation with UK 14304, both receptors (WT and MUT) induced ERK1/2 and Akt phosphorylation. Phosphorylation of Akt was abolished by prior treatment of the cells with PD 98059, a MEK specific inhibitor, and phosphorylation of ERK1/2 was abolished by prior treatment of the cells with LY 240092, a pharmacological inhibitor of PI3K. Activation of both ERK and Akt was prevented by PP1, the Src selective inhibitor. Treatment of the cells with UK 14304 induced IKK phosphorylation and NF-κB-driven luciferase activity and this activation was abolished by pretreatment of the cells with LY 240092. The participation of PKA and c-Src in UK 14304-induced IKK/NF-κB activation in both systems (WT and MUT) was demonstrated by employing specific pharmacological inhibitors. Contrary to WT α_{2B}-AR, the polymorphic receptor displayed impaired agonist-promoted phosphorylation and subsequent early desensitization, as assessed by GTP-γS binding and different kinetics of β-arrestin 1/2 recruitment to plasma membrane. Furthermore, the activation of IKK/NF-κB was absolutely dependent on prior ERK activation, in clear demarcation to WT-induced NF-κB activation, which is ERK independent. These data demonstrate basic differences in the signalling pathways employed by the WT α_{2B}-AR and the MUT α_{2B}-AR, which may entail functional and pharmacological consequences.

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Synthesis and Biological Evaluation of New Arginine Vasopressin (AVP) Analogues

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The pituitary hormone Arginine[8]-Vasopressin (AVP) is a cyclic nonapeptide (H-Cys[1]-Tyr[2]-Phe[3]-Gln[4]-Asn[5]-Cys[6]-Pro[7]-Arg[8]-Gly[9]-NH_2) that incorporate a disulfide bridge (between Cys[1] and Cys[6]). It is synthesized in neurones in the hypothalamus that project to the posterior pituitary from which AVP is released into the circulation. There are three distinct AVP receptor subtypes (V_{1a}, V_2, V_{1b}). All have seven transmembrane spanning domains and all are G protein coupled with significant structural homology to one another. The primary physiological role of AVP involves regulation of cardiovascular smooth muscle, via V_{1a} receptor, and antidiuretic actions on the kidney (blood osmolality regulation), via V_2 receptor. Binding of AVP on the V_{1a} receptor subtype also stimulates glycogenolysis in the liver and promotes platelet aggregation. In addition, activation of the V_{1b} (also known as V_3) receptor causes adrenocorticotropic hormone release from the anterior pituitary. Observations in bibliography suggest that the configuration and the hydrophobicity of the aromatic amino acid in position 2 are important for the antagonistic activity while, elimination of the N-terminal amino group plays an important role on the half-life of the AVP. On the basis of these findings we set out the synthesis of new AVP analogues containing mercapto propionic acid (Mpa) in position 1, D-Tyrosine(O-Ethyl) [D-Tyr(Et)], Tyrosine(O-Methyl) [Tyr(Me)] or 2-Naphtylalanine [Nal(2)] in position 2, Citrulline (Cit) or Arginine (Arg) in position 3 and L-a-*t*-butyglycine [Gly(Bu^t)] in positions 4 or 9. We also studied the impact on biological potency of the new AVP analogues modified the *C*-terminal amide. The analogues were synthesized by Fmoc/Bu^t solid phase methodology utilizing a Rink Amide MBHA, a [3-((Ethyl-Fmoc-amino)-methyl)-1-indol-1-yl]-acetyl AM resin and a 2-chlorotrityl-chloride resin to provide the *C*-terminal amide, ethylamide and carboxyl acid, respectively. The new synthesized analogues were tested for their pressor, antidiuretic and uterotonic potency.

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Synthesis of 3-[4-(4-Bromo-benzyloxy)-phenyl]-aryl-acetic and Aryl-hydroxamic Acids Inhibitors of Lipoxygenase with Anti-oxidant and Anti-inflammatory Activity

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Lipoxygenases are monomeric proteins, widely distributed in nature and they catalyze the incorporation of dioxygen into 1,4-*cis*,*cis*-pentadiene containing fatty acids (e.g., linoleic and arachidonic acids) to form hydroperoxide products. They contain a “non–heme” iron per molecule in the active site as high–spin Fe(II) in the native state, and high–spin Fe(III) in the activated state. The main product of LO are the leukotrienes, which have been implicated as important mediators in several diseases including asthma, arthritis, psoriasis and inflammatory bowel disease, thus inhibition of LO represents a potential new approach for their therapeutic intervention. Simple stable molecules containing the hydroxamic acid functionality have been shown to inhibit LO. In fact, several hydroxamates are orally active inhibitors of the enzyme as determined by their ability to block the biosynthesis of leukotrienes *in vivo*. In order to establish the inhibitory utility of simple hydroxamates several aryl-acetic and aryl-hydroxamic acids were synthesized[1]. In an attempt to expand and delineate these results we tried to synthesize some more with different subgroups for a further pharmacochemical study. 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) inhibition of lipid peroxidation, e) scavenging of superoxide anion radicals, f) interaction with GSH and g) *in vivo* for the inhibition of carrageenin induced rat paw edema. The compounds have shown important antioxidant activity, very good anti-inflammatory activity comparable to indomethacin and high inhibition of soybean lipoxygenase as a result of their physichochemical features.

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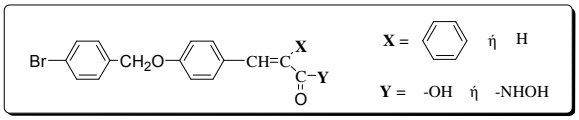

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Conformational Analysis of AT_1 Antagonist Valsartan using 2D NMR Spectroscopy and Computational Analysis. Determination of Thermodynamic Parameters through Dynamic NMR Spectroscopy and Semi-empirical Calculations.

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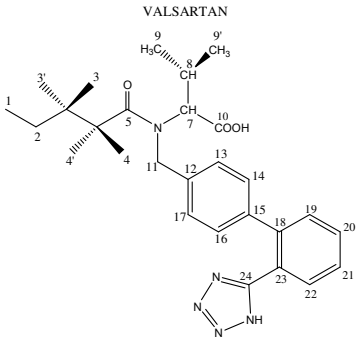
Valsartan is an antihypertensive drug acting as an angiotensin II type I receptor blocker. Two distinct conformational diastereoisomers were observed at the ^{1}H NMR spectrum caused by the hindered rotation of its amide bond. The conformational properties of Valsartan were studied using a combination of 2D NMR spectroscopy and Computational Analysis. More spe-

cifically, intramolecular distances from 2D ROESY experiments were set as constraints for the calculation of the low energy conformers with the application of Computational Analysis. In order to estimate the Gibbs free energy of activation ($\Delta G^{\#}$) for the interconversion between the two conformations, it is necessary to know the rate constants of the equilibrium. These con-stant rates can be determined by dynamic NMR spectroscopy using 2D EXSY NMR experiments at different temperatures and various mixing times. Comparative theoretical studies are under progress using semi-empirical calculations.



Rational Design and Synthesis AT1 Angiotensin II Receptor Antagonists Based on Imidazole

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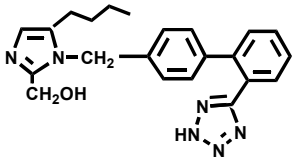

The Renin-Angiotensin System (RAS) plays a key role in the regulation of cardiovascular homeostasis and electrolite balance (1). The octapeptide Angiotensin II (Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]) which is the main factor of RAS, regulates the arterial blood pressure and induces hypertension. An approach for the treatment of hypertension is the design and synthesis of non peptidic antagonists (mimetics) (2) of AT1 Angiotensin II receptor. Our aim was to synthesize molecules based on Imidazole derivative on which the following pharmacophoric groups : the biphenyl group (Phe), the acidic group of tetrazol (-COOH C-terminal) and the butyl group (Ile) are bound, according to the Losartan structure (3). The Angiotensin AT1 receptor antagonist namely (5-butyl-1-{[(2´´-tetrazol-5´´´-yl)biphenyl-4´-yl]methyl}-2-hydroxymethylimidazole) has been tested for its ability to reduce blood pressure (4).

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Application of new Hydrophilic Polymers on the Organic Synthesis

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The development of combinatorial chemistry and automatic synthesis has leaded, since a few years, the interest of the scientist to the synthesis of novel polymeric substrates with ampholytic character. These new polymeric substrates exhibit high swelling properties in a great number of polar and non polar solvents and highly pure products. These substrates also demonstrate improvement of the reaction speed. The main structural characteristic of these polymers is polyethylene glycol (PEG). The polymers: AM-PS and MBHA-PS (of several substitutions) have been synthesized and then grafted with PEG molecules (w.v. =543), in order to produce the fmoc-PEG_n-AM-PS, $\{(fmoc\text{-}PEG)_2\}_n$-Lys-AM-PS, fmoc-$PEG_n$-MBHA and $\{(fmoc\text{-}PEG)_2\}_n$-Lys-MBHA, n=1-10, resins. These resins have been studied for their swelling properties and their substitution. The study has further been focused on the purity of the products (calcitonin exapeptide), that have been distracted from these resins. Kinetic measurements on their reactions have as well been applied.

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Synthesis of Linear and Cyclic non RGD Peptides Containing Tηιosalicylic Acid and their Antiplatelet Activity *in vitro*

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We have already demonstrated that small cyclic peptides containing the sequence RGD show extremely strong antiplate©let activity on human platelets (1,2). Continuing this research project we have synthesized a new series of RGD analogues, incorporating salicylic acid derivatives, by conventional solution techniques and/or by solid phase synthesis. In these non RGD peptides the residue Arg has been replaced by His whereas the Gly by β-Ala. The synthesized linear and cyclic analogues were tested for inhibitory activity on human platelet aggregation *in vitro*, by adding common aggregation reagents (collagen, ADP, ristocetin) to citrated platelet rich plasma.

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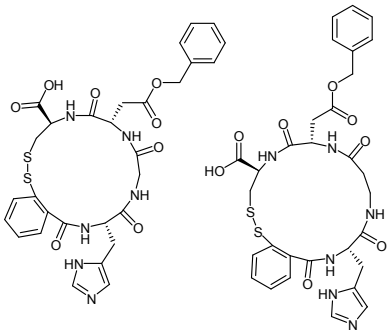

The Mitochondrion as Site of Action of Agents Involved in Regulation of Energy Production, Immunity and Apoptosis

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Mitochondria are the main energy providers of the cell by way of oxidative phosphorylation (OXPHOS) and are involved in apoptosis. Recent findings support a further role in neuroimmunomodulation. These processes are regulated by hormones, growth factors, cytokines and neurotransmitters, acting primarily on the nucleus and the extramitochondrial space. Experimental results have been presented for a direct mitochondrial action of these regulatory agents. As regards steroid and thyroid hormones, it is known that they activate transcription of nuclear OXPHOS genes by way of cognate receptor proteins. Receptors for these hormones have been also found in mitochondria and in the case of the mitochondrial thyroid hormone receptor, a direct effect on mitochondrial gene transcription has been shown. Additional nuclear transcription factors, some involved in neuroimmunomodulation, have been recently detected in mitochondria (NF-κB, AP-1, p53, CREB), translocating from the extramitochodrial space. Binding sites of these factors on the mitochondrial genome have been found,interaction with mitochondrial regulatory proteins, receptors and with the genome has been shown and, in some cases, modulation of mitochondrial transcription observed, with possible effects on energy yield. The intermembrane space of mitochondria store a host of critical apoptotic activators and inhibitors. The release of these factors could be another possible action of the mitochondrially translocated regulatory proteins.

Design and Synthesis of a Cyclic Analogue of Gonadotropin-Releasing Hormone (GnRH) for the Treatment of Cancer

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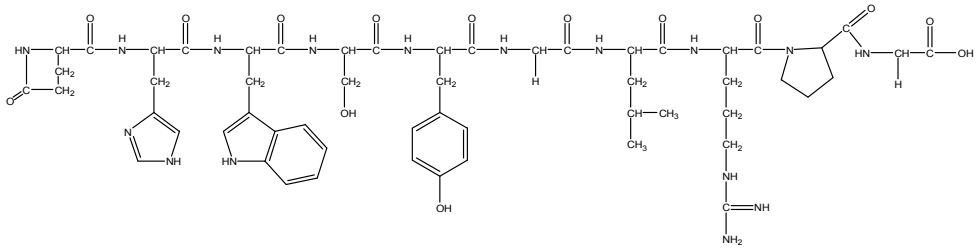

GnRH

Gonadotropin-releasing hormone (GnRH) stimulates production of gonadotropin hormones(FSH and LH) through interaction with specific receptors triggering important biological functions (1). Several linear analogues, Triptorelin, Leuprolide, Buserelin, Goserelin, Nafarelin, Histrelin, are known in clinical use for cancer treatment and reproductive disorders. In this report, the rational design and synthesis of a cyclic peptide analogue, namely cyclo(1-10)[Pro^{1}, Tyr^{5}(OMe), D-Leu^{6}, Aze^{9}, 3-aminobutyric acid^{10}] GnRH is described. D-amino acid (D-Leu) and Azetidine (Aze,) at positions 6 and 9 respectively were used in order to stabilize the important for biological activity β-turn between residues 5 to 8 (Tyr^{5}-Gly^{6}-Leu^{7}-Arg^{8}) of GnRH and for reducing the enzymatic degradation (2). Moreover, protected Tyr [Tyr(OMe)] was used to avoid, on a large scale, desensitization of GnRH receptors.

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Neuroproteomics: Challenges and Prospectives in Schizophrenia Treatment

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Schizophrenia affects about 1% of the general population. In neuroscience, *neuromics* (proteomics of the central nervous system) are in their infancy, with a paucity of studies in the context of the brain. An extensive, albeit inconclusive foray into protein profiling, involved attaining biomarkers in schizophrenia. The studies focused on the prefrontal cortex, limpic system striatum, and the hippocampus of the brain, because these are the areas in the brain where the positive and negative symptoms of the disease are thought to originate from. Many neuroanatomical investigations of patients with schizophrenia have been conducted and indicate gross disorganization of cortical microstructure, abnormalities within cortical neuronal circuits and ventricular volume changes. Many biochemical markers studied over the years failed to provide conclusive results, whereas more recent studies show that differences in the duration of treatment with various drugs induce distinct proteomic changes in the brain. High-throughput protein profiling has provided scientists with new tools that will help to delineate the mechanisms of disease pathogenesis and identify new therapeutic targets.

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Biotechnology and Culture: Research after Laboratory

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The term culture not only describes the total of technical achievements and artistic pursuits of a community, but the mesh of attitudes, notions and concepts based on which the individual orientates his social life and structures his everydayness. A scientific consideration confined to the presentation of technical advancements, remaining indifferent about the way that society welcomes them and the problematic that they arise, is only sketchy and arrogant. Aiming to eliminate this risk of scientific one-sidedness, the field of Bioethics attempts to establish a framework that shall facilitate, on the one hand, the apperception of the research status quo by a wide public and on the other hand protect it against potential extravagances or imprudence. However, pluralism of the contemporary societies and the variegation of positions that describes them, render unfavourable the formulation of a single regulatory framework and the identification of principles having features of global acceptance. Religious convictions, social prejudices or moral conceptions dictate to the entities a different comprehension about the scientific research applications (the scientific research itself is an entity subject to philosophical overdetermination), not only among different cultural groups but also among members of the same community. The establishment of absolute principles and their legal entrenchment, thus

claiming global power, suggest a coaction that waives sensitivities and underestimates particularities. The need to develop regulatory principles compatible with the multicultural character of postmodern societies has constituted the starting point for an extended dialogue already in progress, having wider consequences for the modern human than the occasion that stimulated it.

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Receptor Tyrosine Kinases Mediate α_2-Adrenergic Receptor Actions

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α_2-Adrenergic receptors (α_2-ARs) are members of the G-Protein-coupled receptor family of transmembrane receptors, which are present in the central and peripheral nervous system at both pre-and post-synaptic autonomic ganglia. They mediate physiological responses to the endogenous catecholamines, epinephrine and nor-epinephrine by modulating the activity of a large panel of effectors including adenylate cyclase, GIRK, phospholipase Cβ, MAPKs and PI3K (1). 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 (2). Besides their well established role in neurotransmission and the regulation of sympathetic outflow (3), α_2-ARs have been shown to exert neuromodulatory and neurotrophic actions (4). Although α_2-adrenoceptor agonists are widely used for analgesia, anxiolysis, sedation, sympatholysis and as anaesthetic-adjuncts for many years, their potential use as neuroprotectans has so far been confined to laboratory experiments. Despite the large body of evidence from both *in vivo* and *in vitro* studies, their exact neuroprotective mechanisms remain elusive. Using PC12 cells stably transfected with the human α_2-AR genes, we have previously shown that stimulation of all three subtypes causes subtype-specific morphological and molecular neuronal differentiation (5) similar to that by growth factors suggesting that α_2-ARs may also use similar signal transduction mechanisms. In the present study, we demonstrate that α_2-adrenergic receptor signaling in PC12 cells is mediated by the consecutive transactivation of two receptor tyrosine kinases, an early activation of EGFR and a late transactivation of TrkA, providing evidence for a putative mechanism that might underlie the neurotrophic actions of α_2-ARs.

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