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INTERNATIONAL EDITION

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Original Papers, Review Articles, as well as short preliminary communications will be considered for publication and should be send to the Editors-in Chief

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GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS INTERNATIONAL EDITION

The Journal aims to promote optimum drug therapy by providing original papers and review articles covering important aspects of clinical and applied Pharmacology and Therapeutics. The focus of the Journal comprises drug evaluation reviews, which provide a detailed focus on different properties, i.e. dosage, toxicology, drugs interactions and a place in therapy of both newer and established drugs. Other Review Articles offer state-of-the-art literature surveys covering broader topics. Practical Therapeutics Articles and Leading Articles provide recommendations for specific situations of connections or emerging areas, respectively.

The Journal publishes, in special issues, papers presented at:
- the *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry and Pharmacy of the University of Patras, Hellas*
- the *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*

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

INSTRUCTIONS TO AUTHORS

English is the preferred language for all papers. However, papers in French, German or other European languages can also be submitted, provided they are accompanied by an English summary.

FORMAT: Summary, Introduction, Materials and Methods, Results, Discussion Acknowledgements and References

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

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

Key words: A list of key words should be submitted, after summary

References: These should be numbered in the paper and listed under *References* in order of their appearance in the text. The author(s) surname followed by the initials should be given first, then the complete title of the article, the name of the Journal or Magazine (abbreviated according to the Index Medicus), the volume number, page numbers and year of publication in parenthesis.

Letter from Guest Editor

Post Graduate Program “Medicinal Chemistry”: A Program of Excellence

When we began the Post Graduate Program "Medicinal Chemistry", 17 years ago in our minds we had a vision and a goal. The aim was to establish a research program which would provide: Specialized executives in the vital field of Health, Innovation in the Development of Methods and Products, Innovative Products (active drugs). We also wanted the innovation and the products to be for the benefit of society. Moreover, we would like this program to contribute to the Advancement of Education and the Development of the Country.

Main research interests of the Program are focused on the Organic and Pep- tide Synthesis of Biomolecules, Rational Design with Aided Computer and
Modeling Methods, Biological Evaluations in vivo and in vitro, Molecular Biology, Molecular Medicine, Toxicology, Biomedical Analysis, Pharmacognocy, Pharmacokinetics, Research Methods. The program has organized fifteen (15) Medicinal Chemistry Conferences with International participation. The Program honors each year a distinguished scientist for the important contribution to Bio- medical Research and Science. So far the Program has honored outstanding scientists in the field: Harald zur Hausen, Nobel of Medicine, German Cancer Research Center, University of Heidelberg, Germany (2014), Ada Yonath, Nobel of Chemistry, Weizmann Institute of Science, Israel (2013), Kleomenis Barlos, University of Patras, Greece (2012); James D. Watson, Nobel of Medicine and Physiology, Cold Spring Harbor Laboratory, USA (2011); Andrew V. Schally, Nobel of Medicine and Physiology, University of Miami, USA (2010); Dimitrios Nanopoulos, University of Texas, USA (2009); Jean-Marie Lehn, Nobel of Chemistry, Louis Pasteur University, College de France, France (2008); Kyriakos Nikolaou, Scripps Research Institute, USA (2007); Aristidis Patrinos President Synthetic Geonomics Inc., USA (2006), Charalam- bos Gavras, Boston University, USA (2005); Konstantinos Sekeris, University of Athens, Greece (2004); Michael Maragoudakis, University of Patras, Greece (2003), Chris Plat- soukas, Temple University, USA (2002); Athanasios Giannis, University of Leip- zig, Germany (2001); Vasso Apostolopoulou, Austin- Burnet Research Institute, Australia (2000).

After 17 years, the Program with a brilliant path in Greek University, in research, in education, in innovation, and in Connecting with Society, with 250 graduates (with a brilliant professional and academic career), with leading collaborations with outstanding research results, with openness, with large investments in the Program's research, is an Island of Excellence and an Island of Development for the country.

The success was the result of collective efforts and was based on the cooperation, dedication, hard work, vision and objectives and furthermore on the right choice of associates and graduate students. The credibility of the research team relied on the excellent research work, published in leading peer-reviewed scientific journals. Behind all was love for the University, the Students, Research and a Vision. Biosciences in Greece are a priceless treasure. Innovation and Excellence of Greek Universities is one way for the Development and Prosperity of the country.

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 Prof. S. T. Plessas and Dr C. T. 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 M. Matsoukas
Professor of Chemistry, University of Patras, Greece
President of the Organizing Committee
Medicinal Chemistry: Drug Discovery and Design

UNIVERSITY OF PATRAS
DEPARTMENTS OF CHEMISTRY, MEDICINE AND PHARMACY

16th Medicinal Chemistry Conference

MEDICINAL CHEMISTRY: Drug Discovery and Design

November 11, 2015

Conference and Cultural Center
University of Patras, Patras, Hellas

Greetings for 16th Med Chem Conference

John Matsoukas
President of the Organizing Committee
Professor of Chemistry, University of Patras

I welcome all of you in our Conference! It is a great honor and I am delighted that today we have with us, among other distinguished scientists, Professor of Chemistry Graham Moore, from the Universities of Calgary and British Columbia, in Canada, and Professor of Medicine George Kolias, from the University of Athens, member of the Academy of Athens. They are the Guests of Honor, in our Conference.

I am also delighted that we have with us Mimis Plessas, a great composer and a legend in music and culture. Mimis is a member of our " University family". He is a graduate of the Cornell University with a Phd degree in Chemistry. He has been Honored with the title "Doctor Honoris Causa", from our University. It is a great pleasure and honor having him every year, since 2008, in our Conferences.

I would also like to mention the participation of Professor Avner Yayon, from Israel, University of Hadassah. Professor Yayon has a collaboration with our University and especially with medical people. We are happy to have him with us.

I am glad that our Medicinal Chemistry Program continuous the tradition of our annual Conferences. This is the 16th Medicinal Chemistry Meeting since 2000 and I wish that we will go on with this successful tradition, for many many years.

Introduction
16th Med Chem Conference
November 11, 2015

Dimitris Gatos
Professor of Chemistry, University of Patras, Greece
Head of Medicinal Chemistry Graduate Programm

I welcome all of you in our Conference and wish you will have a nice and productive time!

The Medicinal Chemistry Graduate Program “Drug Discovery and Design” of the University of Patras has completed its 18th year of operation. The Program, a joint collaboration of the Departments of Chemistry, Medicine and Pharmacy, has been successful with outstanding research and academic activities in the field of Medicinal Chemistry. The Program has attracted the interest of world leading scientists for participation and research collaboration. Each year a distinguished scientist is honored for his/her contribution to Biomedical Research and Science.

It is a great honor and we are delighted that today we have with us, among other distinguished scientists, Professor of Chemistry Graham Moore, from the Universities of Calgary and British Columbia, in Canada, and Professor of Medicine George Kolias, from the University of Athens, member of the Academy of Athens. They are the Guests of Honor, in our Conference.

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REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 30: 39 (2016)

Peptide Mimetics: A New Generation of Drugs, the Dream, the Perspectives

Prof. Graham J. Moore Ph.D., D.Sc. Principal

Pepmetics Inc.

Core interactions of biological molecules often involve peptide sequences contained within protein structures (hormones, receptors, enzymes, antibodies, etc.).
These core interactions can form the basis for therapeutic intervention using appropriately designed drug molecules.
There are two ways to discover new drug leads:

1) rational drug design based on detailed information from structure-activity relationships,
2) bioassay screening of combinatorial peptide libraries.

Finally, since peptides are too metablically unstable to be used as drugs, target peptides discovered using the above methods have to be converted into peptide mimetics [which contain no peptide bonds].

REVIEW CLINICAL PHARMACOLOGY AND
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An Integrated Approach for Drug Development: From Cheminformatics to Precision Medicine

George Kollias

Medical School, National and Kapodistrian University of Athens; Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece

Chronic inflammatory diseases include a wide range of pathologies such as rheumatoid arthritis, Crohn's disease, psoriasis, multiple sclerosis, etc. These are very frequent, as they affect 0.5 to 1% of the population. However to date, treatments remain inadequate, and generate significant side effects thus reducing the quality of life. Moreover, these treatments target for the most part the symptoms of these inflammatory diseases and not their causes, and do not provide a long-term cure, thus warranting further research for the development of improved therapeutics.

To that end, we have established a comprehensive, integrated drug development pipeline for the discovery of therapeutic lead compounds for clinical evaluation. Specifically, an effective in silico drug discovery workflow has been developed and applied for the virtual screening of plant origin natural products using the crystal structure of the TNF dimer with SPD304 (currently the most active published inhibitor of TNF). As a result of the in silico procedure, a priority list of 15 natural products potent direct inhibitors of TNF have been screened in several cellular and in vitro assays including L929 cytototoxicity, osteoclastogenesis, ELISA and cross-linking assays. The result was the identification of two potent direct inhibitors of TNF trimerisation and function with IC50 values comparable to those of SPD304 and minimal toxicity even at high concentrations. Most importantly, one of them proved to be a dual inhibitor able to inhibit both TNF and RANKL function, at low micromolar ranges. To our knowledge, these compounds constitute the first natural product TNF inhibitor and the first dual small molecule inhibitor of TNF and RANKL, and could serve as lead compounds for the development of novel treatments for chronic inflammatory and autoimmune diseases.

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Myelin and Multiple Sclerosis – The Dream, the Clinical Trials

John Matsoukas

Department of Chemistry, University of Patras, Rion-Patras, Greece 26504

In this short lecture, I would like to highlight milestones of research work towards peptide and non-peptide mimetics of important Key peptides. This work is carried out in the Department of Chemistry of the University of Patras and in the Science Park of Patras in collaboration with research groups in Greece and abroad. Also, the methods and steps we follow towards peptide mimetics for their Development as potential drugs will be mentioned. Methods include modeling, synthesis and Pharmacology.

The target of this work is to convert peptides to non-peptide mimetics, as being more stable.
Peptides suffer from disadvantages as they are easily hydrolyzed because of the amide bond.
As examples of tool peptides, I will refer to four important peptides or protein peptides, in particular, to Angiotensin (implicated in Cardiovascular Diseases), Myelin and Myelin Epitopes (implicated in Multiple Sclerosis), TRAP (implicated in Angiogenesis and Cancer) and GnRH (implicated in Fertility and Cancer).

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DNA Sensors

Theodore K. Christopoulos

Department of Chemistry, University of Patras, Rion-Patras, Greece 26504

This presentation focuses on the architecture and functional aspects of disposable, dipstick-type DNA biosensors that enable visual detection of single nucleotide polymorphisms. Their advantages are: simplicity, low cost, portability, no need for specialized equipment as well as elimination of multiple pipetting, incubation and washing steps. For genotyping, the interrogated sequence is subjected to amplification and allele-discrimination reactions. The product is applied to the sample area of the biosensor, which is then immersed in the appropriate buffer. The appearance of a characteristic colored line at the test zone indicates the presence of the 'normal' or 'mutant' allele in the sample.

REVIEW CLINICAL PHARMACOLOGY AND
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The Necessity or New Antibiotics

Dimosthenis Lykouras

Department of Pulmonary Medicine, Department of Medicine, School of Health Sciences, University of Patras, Patras 26500, Greece

Antibiotics are probably one of the most successful forms of chemotherapy in the history of medicine. Their development has totally changed human lives, as they have contributed to the control of infectious diseases that were once major causes of morbidity and mortality.

The modern antibiotic era is largely associated with the names of Paul Ehrlich and Alexander Fleming. The systematic screening approach introduced by Paul Ehrlich became the cornerstone of drug research strategies in the pharmaceutical industry; thus resulting in the innovation of several anti-infectives as well as other types of drugs. But it was for the somewhat unforeseen event on the September 3, 1928 that the penicillin discovery was made by Fleming. The penicillin mass production and distribution was achieved in 1945.

Since that very first antimicrobial drug a lot of work has been done, leading to the development and clinical use of various classes of both bacteriostatic and bactericidal drugs. The typical targets of antibiotics include: inhibition of cell wall synthesis (β-lactams, cephalosporins), inhibition of tetrahydrofolate synthesis (sulfonamides), inhibitors of DNA function (quinolones, nitroimidazole derivatives), inhibitors of protein synthesis (tetracyclines, aminoglycosides, macrolides, oxazolidinones).

Even before the extensive use of penicillin, there were some observations suggestive of its possible enzymatic degradation by bacteria. These were the first observations of bacterial resistance to drugs. Today, the mortality rates due to multidrug-resistant bacterial infections are quite high and each year about 25,000 patients in the EU die from an infection caused by multidrug-resistant bacteria. The estimated cost includes both prolonged hospitalizations and productivity losses of more than 1.5 billion EUR each year.

The last effective and widely used antibiotics were introduced in mid 00's; thus there has been no development of any anti-infective drugs for more than a decade. This suggests that the known antibacterial drug targets should be expanded either by testing new molecules on known targets or by using the new information that has been available in the genomic era for the identification of new targets.

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Genomic Medicine in the post-genomic era

George P. Patrinos

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The central aim of genomic medicine is to utilize the individual's genomic information to support the clinical decision-making process. In the post-genomic era, significant genomic research advances have been made in understanding the molecular etiology of a wide range of human genetic diseases. These advances have the potential to improve disease prognosis and treatment. In parallel, genomic technology has progressed rapidly, prompting the replacement of low-throughput genetic screening methods by new high-throughput genome-wide screening and massively parallel sequencing approaches. As a result, genomics research has the potential to aid clinicians in their task of estimating disease risk, as well as individualizing treatment modalities. This constitutes the basis of Genomic Medicine, a new specialty that promises to enhance opportunities for the customization of patient care including the personalization of conventional therapeutic interventions. Genomic Medicine relies on the transition from genomics and pharmacogenomics research from the bench to the bedside through a plethora of research activities in the fields of Public Health Genomics, Ethics in Genomics (or 'genethics'), Genome Informatics, the genetics education of healthcare professionals, genetics awareness of general public and health economic evaluation in relation to genomic medicine. In other words, genomic and pharmacogenomics research represents the bedrock of genomic medicine, supporting pillars, such as the disciplines described above, will still need to be erected for the genomic medicine superstructure to hold. At present, although the foundations of genomic medicine are becoming stronger and being attributed ever-increasing hopes and expectations, the pillars themselves are still largely under construction. At present, several international organizations and research consortia exist, aiming to support the translation of genomic research into clinical practice so that genomic medicine can ultimately be used to benefit the global community.

REVIEW CLINICAL PHARMACOLOGY AND
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Biological Targeting With Active Molecules

Thomas Mavromoustakos

Chemistry Department, National Kapodistrian University of Athens, Panepistimiopolis Zografou 11571, Greece

Medicine is the benefactor of our body when suffers from a disease. Its beneficial effects are attributed to the active substance or drug molecule that is included. Many times the medicine fails to exert the beneficial effects as the drug molecule does not reach the target organ. Then, it is necessary to be delivered through vehicles.
An example is given with the hepatoprotectnt lipophilic drug silybinin (Figure 1). Cyclodextrins (CDs) are a well-known class of supermolecules that have been widely used to protect drugs against conjugation and metabolic inactivation as well as to enhance the aqueous solubility and hence to ameliorate the oral bioavailability of sparingly soluble drug molecules.

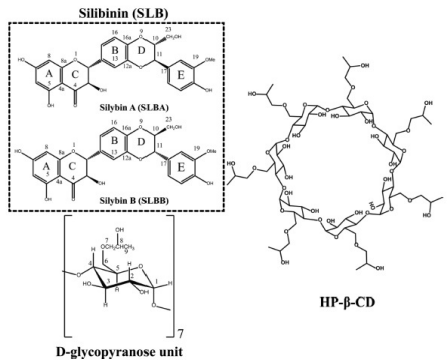

Figure 1: Structure of SLB (top, left) and the cyclic HP-β-CD (right) consisting of seven D-glycopyran segments.

Thus, silibinin is complexed with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and is characterized by differential scanning calorimetry, mass spectrometry, solid and liquid high-resolution NMR spectroscopy. The chemical shift changes using 13C CP/MAS on the complexing of the guest with the host provided significant information on the molecular interactions, and they were in agreement with the 2D NOESY results. These results point out that in both solid and liquid forms, the drug is engulfed and interacts with HP-β-CD in identical manner. Molecular dynamics calculations have been performed to examine the thermodynamic characteristics associated with the silibinin–HP-β-CD interactions and to study the stability of the complex (Figure 2)

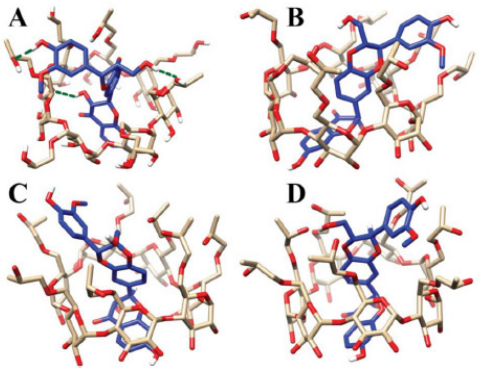

Figure 2: SLBA-HP-β-CD complex: (A) as predicted initially by GlideXP (hydrogen bonds are shown in green), (B) after 100 ns of MD simulation, (C) after 200 ns, and (D) at the end of the simulation.

The physiological conditions, the aqueous solubility and dissolution characteristics of the complex at pH states simulating those of the upper gastrointestinal tract have been applied and showed better properties. The antiproliferative activity of silibinin–HP-β-CD complex comparatively to silibinin in MCF-7 human cancer cells, MTT assays has been increased.

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Secretomics in Precision Medicine

T. Katsila and G. P. Patrinos

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Key words: precision medicine, "secretomics-informed pharmacogenomics" approach

Today, great individual variability is a major hurdle in terms of disease management and health care. Precision medicine holds the promise of tailored-made therapeutic strategies via patient stratification and biomarker discovery. We feel that "omics" strategies can greatly enhance this initiative. Herein, the paradigm of secreted phosphorylated-EGFR (pEGFR) is presented as a candidate biomarker of response to cetuximab in colorectal cancer (CRC). Secretomics, a rather complementary discipline, focuses on cell-cell communication and cell microenvironment. For this, a "secretomics-informed pharmacogenomics" approach that will merge the two "omics" strategies is expected to both accelerate and broaden the scope of the analysis of pharmacogenomic candidate genes and pathways, unraveling disease mechanisms and guiding drug research.

REFERENCE

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REVIEW CLINICAL PHARMACOLOGY AND
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Mannan-conjugated myelin peptides induce dendritic cell (DC)-driven tolerogenic response

Maria Rodi[1], Anne-Lise de Lastic[1], Stefanos Prekatsakis[2], Anthi Tapinou[3], Theodoros Tselios[3], Ioannis Matsoukas[3], Panagiotis Papathanasopoulos[2] & Athanasia Mouzaki[1]

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Key words: Aldose reductase, inhibitors, non-anionic, efficient

Introduction: The induction of immune tolerance using dendritic cells (tDCs) is a therapeutic strategy for multiple sclerosis (MS). We explored the potential of tDCs loaded with mannan-conjugated myelin peptides for MS immunotherapy.
Materials and Methods: Peripheral blood monocytes and T-cells were isolated from 2 patients with remitting-relapsing MS and 2 age/sex-matched controls. tDCs were generated from monocytes cultured with IL-4/GM-CSF/vitD3 for 6d. The resulted tDCs were loaded with myelin peptides conjugated with mannan (or peptide alone) and co-cultured with T-cells±IL-2 for 3 rounds of peptide stimulation (total of 25d). Cells were analyzed by flow cytometry to determine the phenotype of tDCs and the resulting T-cell populations. The cytokine profiles of DCs and T-cells consisting of IL-1β, IL-2, IL-4 IL-6, IL-8, IL-10, IL-12p70, IL-17A, TNF-a and IFN-γ, were measured by CBA.
Results: tDCs showed a semi-mature phenotype and secreted low to zero levels of proinflammatory cytokines. After d3 of co-culture the lymphocytes were >90% CD3+CD4+, and after the 1st antigen presentation all were differentiated into memory (CD3+CD4+CD45RO+) cells. At the end of the culture period, in the point with the mannan-myelin-peptide-loaded-tDCs (i) Tregs were higher than in cultures with tDCs+peptide alone, (ii) T-cells displayed the least activation potential and (iii) the cytokine profile was antinflammatory.
Concusions: Our results display that the generated DCs are both phenotypically and functionally optimized to induce tolerance in vitro, in healthy subjects and patients. In the context of myelin antigen presentation, the conjugation of myelin peptides with mannan is clearly superior in tolerogenic effect compared to the unconjugated peptide.
This study indicates that mannan-myelin peptide loaded tDCs can be eventually used as immunotherapy in MS patients.

This work was supported by GGSR “Cooperation” grant 09SYN-21-609.

REVIEW CLINICAL PHARMACOLOGY AND
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Interactions of AT1 antagonists in the crystallized AT1 receptor with the view to finding innovative molecules with improved pharmacological profile

Maria-Dionysia Mania[2], John Matsoukas[1], Thomas Mauromoustakos[2]

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[2]Department of Pharmacognosy & Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Zografou, 15771, Greece

The structure of the human angiotensin II receptor type1 (AT1R) was determined by serial femtosecond crystallography at an X-ray free-electron laser (SFX at XFEL) in a complex with the selective antagonist ZD7155 which is the precursor of the antihypertensive molecule of candesartan.AT1R has a transmembrane α-helix(7TM) with an extracellular N-terminus, three intracellular loops (ICL 1-3), three extracellular loops (ECL 1-3), one amphoterix helix VIII and an intracellular C- terminus.

Because of the different chemical structure of the commercially available AT1 antagonists, the nature of the interactions of each inhibitor with the active side of the AT1R is different. However, most of them bound in a similar way and are involved in interactions with the three critical aminoacids Arg167, Trp84, και Tyr35 that are also involved at the binding of ZD7155.

As part of my PhD thesis, I have initiated the study of the molecular binding of AT1 blockers (losartan, irbesartan, olmesartan, valsartan, candesartan, telmisartan, azilsartan, eprosartan,c1-c14, EXP3174 και V8001-V8004). The obtained results will be discussed during the presentation.

REVIEW CLINICAL PHARMACOLOGY AND
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In vitro evaluation of natural compounds for their estrogenic and osteoprotective activity and their contribution to breast carcinogenesis

Glava M.[1], Meligova AK.[1], Boulaka A.[1], Vontzalidou A.[2], Pilalis E.[1], Makropoulou M.[1,2], Chatziioannou A.[1], Magafa V.[3], Mitsiou DJ.[1], Aligiannis N.[2], Mitakou S.[2], Alexis MN.[1]

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Osteoporosis is the most common metabolic bone disorder affecting post-menopausal women and it results from an imbalance between the processes of bone formation and resorption. In the present project we tested in vitro natural compounds found in edible plants for their potential to promote osteoblast differentiation and inhibit osteoclast differentiation, for their estrogenic and/or antioxidant activity and their interference with the differentiation of normal breast epithelial cells. The combinatorial assessment of the above activities highlighted Kaempferol and Hydroxytyrosol as potentially osteoprotective compounds. Kaempferol, because of its estrogenic potential, is suitable only for low breast cancer risk women, while Hydroxytyrosol, which lacks estrogenic potential, is suitable for high risk women as well.

REVIEW CLINICAL PHARMACOLOGY AND
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Hypomethylating agents in vitro induce HLA-G expressing regulatory T cells

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DNA methylation has a well-established role in the generation, maintenance and regulation of the function of T-regulatory cells. Recently, it was shown that administration of hypomethylating agents (HAs) in mice prevented the development of Graft-versus-Host-Disease (GVHD) through induction of FoxP3^{+} immunoregulatory cells. In-vitro azacitidine (aza) induced suppressor function, was shown to be FoxP3 independent, suggesting that aza-induced suppressor function depends on the modification of other hypomethylated genes. Human leukocyte antigen-G (HLA-G) is a non-classical HLA-I immunoregulatory molecule, the expression of which is epigenetically regulated. In this study, we addressed whether in-vitro HAs can induce HLA-G^{+} immunoregulatory T-cells.

Negative selected peripheral T-cells of healthy individuals were stimulated and subsequently treated with HAs for 72hours. Phenotypical characterization was performed with FACS, mRNA expression was quantitated by Real-time PCR. The effect of HAs on the upstream regulatory region of HLA-G gene was assessed with Bisulfite Pyrosequencing. The suppressive function of induced HLA-G^{+} T-cells was assessed by in-vitro suppression assays, ± blocking anti-HLA-G or ILTR2 antibodies.

We report that in-vitro treatment of HLA-G$^{neg.}$CD4T-cells with HAs induces de-novo HLA-G expression (HLA-G+ T-cells: 6,88±3,9%, p=0,0022, fold-increase in HLA-G mRNA expression: 3.8±0.8). Methylation analysis revealed that treatment with HAs caused hypomethylation of the HLA-G proximal-promoter. Stability assays revealed that HA-induced HLA-G gene hypomethylation and protein expression is evident 3 days after the removal of decitabine. In-vitro HA-induced HLA-G^{+}CD4 T-cells are FoxP3^{negative} and exert potent immunosuppressive function, which is largely but not exclusively HLA-G dependent.

Conclusively, in-vitro HAs, induce HLA-G gene expression through hypomethylation, on activated T-cells. HAs-induced HLA-G surface expression, not only characterizes a potent regulatory CD4^{+}T-cell population, therefore can be used as a marker for isolation of cells with suppressor activity, but also is involved in regulatory function. These findings may provide a promising methodology to generate ex-vivo HLA-G^{+} immunoregulatory T-cells for adoptive immunotherapy.

REVIEW CLINICAL PHARMACOLOGY AND
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Morphological and preliminary phytochemical analysis of the styles of Crocus nivalis

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Crocus nivalis is a member of the *Crocus* genus of Iridaceae family; *C. sativus* (better known as saffron) belongs also to this family. It appears in the Balkan area and in Greece, specifically, in most phytogeographical areas except from KK, EC and NAe. It grows in the stony slopes, forest clearings and mountain tops at an altitude ranging between 400 and 2400 meters. The structure of the *C. nivalis* flower resembles to that of *C. sativus* since both contain distinctive colored purple petals as well as a style ending in three stigmas and three yellow-orange stamens. Aim of our research was the fingerprinting of the qualitative and quantitative composition of the essential oil and the methanol extract of its styles. The plant material was acquired from mountains of Peloponnese; two areas of Mountain Panachaico and one area (Xirokambos) of Mountain Chelmos. The styles of *C. nivalis* flowers from the Panachaico and Chelmos is characterized by mean length of 2.5cm and 2.2 cm, mean-weight of 3.4mg and 2.6 mg, and three branches of 0,6 cm and 0,4 cm mean-length, respectively. For the isolation of the essential oil, styles were extracted with diethyl ether in ultrasonic bath and followed with a wash of ether's extract with saturated sodium chloride and finally, concentration with nitrogen gas. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the absence of the essential oil's characteristics components such as those identified in the relevant extract of *C. sativus*. The analysis of styles methanolic extract by High Performance Liquid Chromatography (HPLC) shows the presence of new components in the extracts of *C. nivalis*, which are not contained in the relevant extract of *C. sativus*. These components absorb in a higher rate at the wavelength of 440 nm and have elution times between 30 and 40min. This preliminary investigation shows the presence of new crocins, the identity of which has to be established.

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Benefit and Cost in Medical Imaging

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Medical imaging has a special offer in the field of medicine. It has a history of more than a hundred years and is constantly evolving, as it has taken advantage of the latest developments in physics and technology. Today, it has in its disposal a wide range of analog or digital imaging modalities, making use of ionizing or non-ionizing radiation. Indicatively, such techniques are the projection imaging, computed tomography (CT), ultrasonography (U/S), magnetic resonance imaging (MRI), positron emission tomography (PET) and hybrid techniques such as CT /MRI. The great benefit of medical imaging is based on the rich range of valuable information offered regarding the patient's anatomical structure and physiology data. This benefit has been enriched by the contribution of the processing and analysis of medical image. In particular, the greatest contribution of medical imaging is the early diagnosis, which is especially important for the increased chance of cure. Therefore, appropriate screening programs have been established. Medical imaging has significant contribution in the field of radiotherapy, as some of the stages of the radiotherapy process are based on medical imaging, such as the planning and the follow up. Equally important is the contribution of medical imaging in the field of surgery, as many modern surgical techniques are performed under fluoroscopic guidance, resulting in a reduction of invasive surgery and improve of surgical outcome. Such interventions are particularly common today in the field of cardiology, orthopedics, urology, gastroenterology, etc. Unfortunately, the medical imaging in addition to the important benefits offered is accompanied by significant cost. This cost is first of all economic. The imaging systems are high cost systems, resulting in high cost per examination. Obviously, the modern societies have limited financial resources and have many financial and social needs, a factor that limits the full exploitation of modern imaging technology to the patient. Second, important is the labor cost, due to the development of imaging technology, as the radiographers and radiologists should familiarize themselves with these new technologies and the issue having a special role in this is lifelong learning. Finally, the biggest cost of medical imaging is the “cost” due to the use of radiation, as all radiations, particularly the ionizing radiation (x, γ), when expose patients and staff result to significant risks including increase of the probability of carcinogenesis and death, depending on the level of exposure to radiation. That is why there are very strict international and national guidelines, such as the principle of justification, the principle of optimization and the diagnostic reference levels, which contribute to reducing the dose to the patient and staff. The medical radiation physicist has a significant role in this area. In conclusion, each imaging examination is accompanied by an expected benefit and a possible cost and in any case the benefit must be greater than cost.

REVIEW CLINICAL PHARMACOLOGY AND
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Investigation of the use and misuse of antibiotics formulations of University of Patras' students

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Key words: antimicrobial resistance to antibiotics, sensitivity of bacteria in antibiotics, self – medication, use and misuse of antibiotics, students of University.

Introduction: The misuse of antibiotics leads to development of antimicrobial resistance to antibiotics. This resistance is considered to be a major problem of public health that puts in risk the use of medicines. Avoiding the non-rational use of antibiotics is the best way to face the durability.
Purpose: The aim of this study is to investigate the knowledge, behavior and use of antibiotics of University of Patras' students.
Materials and Methods: We made a questionnaire that has 32 questions, open and close type. Subsequently, a pilot test of the questionnaire in the area of the University of Patras accomplished where 10 students answered and commented the questions for checking the validity of its content. At the beginning of October 2015 were asked 814 students from each department of University of Patras. The participation in this study was voluntarily and anonymous. Finally, we did statistical analysis of answers by using the statistical package IBM SPSS, edition for Windows.
Results: The questionnaire was answered totally by 814 students, where 71.4% were women and 28.6% were men. The 91.8% of students said that they know the definition of antibiotics. Most students (97.2%) received antibiotics at least once as therapy. In the question how they react/receive antibiotics in influenza symptoms/cold the 29.3% answer with doctor's prescription. The 28.7% of students declare that they have purchased antibiotics from a pharmacy without doctor's prescription. The 37.4% of students obtain preventive antibiotics from the pharmacy or home before traveling. The 49.5% of students declare that they used antibiotics in the last 12 months. The 63.4% holds residues of antibiotics at home. The 47.3% of the students has interrupted the therapy when the symptoms disappeared. The antibiotics with the most often use are Augmentin with 67.3% and Amoxil with 48.4%. The 66.8% of students know that antimicrobial resistance to antibiotics is a public health problem and the 60.7% of them are informed about health issues mainly from the Internet in percentage 90.4%.
Conclusions: Although the students are informed about health issues and know that resistance of bacteria in antibiotics is a public health problem, they present abusive behavior to antibiotics and they don't receive their medication rationally.
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Design, Synthesis and Biological Evaluation of Citrullinated Analogues of the Epitope 82-98 of Myelin Basic Protein (MBP)

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Multiple Sclerosis (MS) is an autoimmune, inflammatory disease of the Central Nervous System (CNS). It is thought to be caused by the coordinated attack of the immune system against the myelin, resulting in the inflammation of the axons and the appearance of neurological dysfunction. Several epitopes are involved in the pathogenesis of MS, including the Myelin Basic Protein (MBP). A feature of MBP is the large number of post-translational modifications, such as deimination, an enzymatic reaction named citrullination and consists of the conversion of a residue of arginine to citrulline. Due to this modification, the protein loses its positive charge and the subsequent conformational changes may generate new antigenic epitopes that could trigger an immune response [1,2]. In this work, we synthesized a linear and a cyclic citrullinated analogue of MBP_{82-98} (Dirucotide), an investigational drug for MS, which failed to delay the disease progression in phase III clinical trials. The synthesized analogues MBP_{82-98} (Cit^{97}) and cyclone (91,98) MBP_{82-98} (Cit^{97}) were further studied for their activity in Peripheral Blood Mononulear Cells (PBMCs) of healthy individuals and a MS patient. Their biological evaluation showed that they did not have any toxic activity. Moreover, the analogue MBP_{82-98} (Cit^{97}) resulted in the reduction of the ratio type 1/type 2 cytokine secretion in comparison with MBP_{82-98}, while cyclo (91,98) MBP_{82-98} (Cit^{97}) had no influence on this ratio. However, further investigation is needed in order to confirm their positive action.

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PEGylated Analogues of the Epitope 82-98 of Myelin Basic Protein (MBP) in Research related to Immunotherapy of Multiple Sclerosis

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Chemically, the term of pegylation refers to the modification of a protein, peptide or non-peptide molecule by the linking of one or more polyethylene glycol (PEG) chains [1]. PEG is frequently used to improve the clinical properties of the compounds, with which it is combined. Recently, PEG is applied in an already administrated drug for Multiple Sclerosis (MS), the INF-β-1α, leading to positive results, including a decrease of relapses rate and disability progression in patients with relapsing-remitting MS, reduction of side-effects and increase of bioavailability [2]. In this work, PEG is combined with the epitope MBP_{82-98} (Dirucotide), as well as a modified analogue of it. The synthesised analogues PEG_2-MBP_{82-98} and PEG_2-MBP_{82-98} (Cit^{97}) were studied for their activity in Peripheral Blood Mononuclear Cells (PBMCs) of healthy individuals and a MS patient. The results showed that these analogues did not have any toxic activity and they resulted in a reduced ratio of type1/type 2 cytokine secretion, as compared to MBP_{82-98} analogue. However, further studies are needed in order to confirm their positive action.

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REVIEW CLINICAL PHARMACOLOGY AND
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Application of Wireless Electric stimulation and Pulsed Elecromagnetic Radiation in Protein Crystallization

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X-ray diffraction is the most powerful tool to obtain the three-dimensional structure of proteins. The first step in this procedure is to grow protein crystals from solution suitable for X-ray diffraction studies. Crystallization is trial and error method and the problems which crystallographers encounter are many, such as the amount of protein and the control of crystal size. Among the efforts that have been achieved, in order to improve crystallization, are the use of magnetic fields and interior or exterior electric fields. The electric fields consist a significant methodological advancement and have been used in order to enhance nucleation and crystal growth.

In this work two technologies are investigated upon crystallization: the influence of non-contact current transfer using a prototype ion-generator (O_2) device (NCCT device) and the influence of electro-impulses using an electronic patch (RX patch). The use of these two devices is much simpler and easier than other devices with classic electrodes. Also the risk of contamination is minimized.

The influence of these two devices is investigated upon three pattern proteins: Lysozyme, Insulin, RNase-A. The vapor diffusion method was used in both hanging and sitting drop setups. All three proteins were in powder form and diluted in crystallization buffers in several concentrations.

Crystals appeared earlier in samples exposed to NCCT than in non-exposed samples under identical condition. Crystallization trials with RX patch have shown similar results, indicating that both techniques are influencing protein nucleation and crystal formation.

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Structure-Based Design of Non-Peptide Mimetics Based on the Trimolecular Complex Involved in Multiple Sclerosis

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Key words: Aldose reductase, inhibitors, non-anionic, efficient

Multiple Sclerosis (MS) is an autoimmune, inflammatory, demyelinating disease, characterized by the destruction of the myelin of the Central Nervous System (CNS), leading to serious medical conditions. It is believed that the T-cells that are stimulated by the immunodominant epitopes of the myelin protein are responsible for the induction of the disease [1]. T-cell activation is triggered by the formation of the trimolecular complex between the Human Leukocyte Antigen (HLA), the immunodominant myelin protein epitopes and the T-cell receptor (TCR) [2,3]. In this study, a detailed mapping of the interactions during the creation of the trimolecular complex HLA-MBP83-96-TCR, was carried out (pdb file: 1YMM) [4,5].The 83-96 epitope was chosen for the rational design since it is considered as the main immunodominant epitope involved in MS. The aim of this study was the structure-based design of non-peptide mimetic molecules that bind to the TCR, avoiding the interaction with the HLA. The non-peptide mimetics were designed to prevent the formation of the trimolecular complex and the further stimulation and proliferation of the encephalitogenic T-cells. A pharmacophore model was developed and the search for new candidates was performed using the ZINC database. The structure-based design was achieved using the MOE and LigandScout softwares in a LINUX operating system. Semi-empirical (SE) and DFT methods were used to predict the binding energy between the proposed non-peptide mimetics and the TCR.

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REVIEW CLINICAL PHARMACOLOGY AND
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A new and versatile approach for the directional cloning of PCR products for recombinant protein expression

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The expression of recombinant proteins has become the most common method for protein production, particularly for research purposes. The cloning of protein-coding genes into expression vectors is required to be directional for efficient expression, and versatile in order to make it easy for a gene to be inserted in to many different vectors for expression tests. TA-GC cloning method is a new, simple and efficient method for the directional cloning of protein-coding genes in expression vectors, which presents several advantages over existing methods, which tend to be relatively more labour intensive, inflexible or expensive. The proposed method relies on the complementarity between single A- and G-overhangs of the protein-coding gene, obtained after a short incubation with T4 DNA polymerase, and T and C overhangs of the novel vector pET-BccI, created after digestion with the restriction endonuclease BccI. The novel protein-expression vector pET-BccI also facilitates the screening of transformed colonies for recombinant transformants. Evaluation experiments of the proposed TA-GC cloning method showed that 61.7% of the transformed colonies contained recombinant pET-BccI plasmids, and 94.6% of the recombinant colonies expressed the desired protein. This demonstrates that TA-GC cloning could be a valuable method for cloning protein-coding genes in expression vectors.

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Conformational study of proteins by heteronuclear NMR spectroscopy

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Conformational study of proteins is of high importance in the field of Medicinal Chemistry. It provides the opportunity to map the target protein in order to rationally design bioactive molecules and learn more about their mechanism in molecular level. Conformational Analysis of a Protein with Heteronuclear Nuclear Magnetic Resonance Spectroscopy was conducted in this study, a technique that becomes increasingly popular in structural characterization of low molecular weight proteins and domains as well as kinetic parameters of the intermolecular interaction.

The protein studied was the R957C mutant of the RING domain E3 Ligase Arkadia. E3 Ligases, especially Arkadia play an important role in the specific degradation of proteins through the Ubiquitin-Proteasome pathway. These mutations may alter their conformation and lead to disastrous consequences for the Cell.

In the present study a conformational analysis with high resolution analysis was conducted, resulting in a very good compliance of NOE correlations and torsion angle restraints, suggesting that the mutated R957C RING preserve the native ββα topology. Conformational study was also conducted with methods that use chemical shifts for prediction of three dimensional structure of the protein. The results were compared with traditional approaches. Although these methods are still under evaluation, it seems that they perform quite accurate predictions during the early stages. Further study of the dynamic behavior of the protein based on NMR experiments revealed a well folded, rigid domain with disordered tails in both ends and the protein exists in solutions as a monomer.

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Mannan-conjugated Myelin Peptides Protect Mice Against Autoimmune Encephalomyelitis Without Altering T Cell Trafficking Into the Central Nervous System

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Recent studies in our lab showed that mannan-conjugated myelin peptide epitopes induce peripheral T cell tolerance and protect mice against experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (MS), when administered in prophylactic (vaccination) and therapeutic protocols. In vitro experiments further showed that tolerance was associated with reduced antigen-specific proliferation responses and the induction of peptide-specific T cell anergy, even though the production of Th1 and Th17 effector cytokines was not reduced [1]. The aim of this study was to understand whether mannan-conjugated peptides protect mice by altering the trafficking of activated T cells into the CNS during EAE. We generated chimeric mice, in which MOG35-55(MOG)-specific T cells isolated from 2D2 MOG-specific T cell receptor transgenic mice [2] that had previously been vaccinated with mannan-MOG or PBS control, were labeled with fluorescent markers (EGFP or CFSE) and adoptively transferred into recipient mice with ongoing MOG-EAE. Interestingly, mannan-conjugated peptide did not alter the trafficking of activated MOG-specific T cells into the CNS parenchyma compared to PBS-treated control cells, even though they showed significantly reduced antigen-specific proliferation and, as previously shown, were unable to induce clinical symptoms of EAE. Our results show that the protective effects of mannan-conjugated myelin peptides in mice are not associated with reduced homing and trafficking of activated T cells into the CNS tissues during the development of EAE.

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Effects of Wireless Electrostimulation in Cancer Cell Proliferation

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In recent years, the medical literature discloses the therapeutic effects of electrostimulation and the application of electromagnetic fields. On the occasion of the spectacular clinical results, in vitro experimental models began, in order to study the effect of the Wireless Electrostimulation (WE) in cell lines.

In this study, using a Wetling-200 DENMARK device, wireless electrostimulation was administered in cell lines PC-3 (prostate cancer cells) and LLC (lung cancer cells) at different times in a measurable current intensity range from 0.5 to 4 μA, having always a witness for each dish.

The purpose of the study is to examine the effect of WE on the morphology and cell proliferation in cell culture. The first results of our experiments gave us interesting information on the effects of wireless electrostimulation in cancer cell proliferation.