Review of Clinical Pharmacology and Pharmacokinetics

EPITHEORESE KLINIKES FARMAKOLOGIAS KAI FARMAKOKINETIKES
ΕΠΙΘΕΩΡΗΣΗ ΚΛΙΝΙΚΗΣ ΦΑΡΜΑΚΟΛΟΓΙΑΣ ΚΑΙ ΦΑΡΜΑΚΟΚΙΝΗΤΙΚΗΣ
INTERNATIONAL EDITION

VOLUME 25, 2011 ❁ No 1

Issue Devoted to Papers Presented at the
11th Conference with International Participation

Medicinal Chemistry:
Drug Discovery and Design

Organized by the

Departments of Chemistry and Pharmacy
of the University of Patras, Hellas

April 26-28, 2010
Patras, Hellas

ISSN 1011-6583

Review of Clinical Pharmacology and Pharmacokinetics

EPITHEORESE KLINIKES FARMAKOLOGIAS KAI FARMAKOKINETIKES
ΕΠΙΘΕΩΡΗΣΗ ΚΛΙΝΙΚΗΣ ΦΑΡΜΑΚΟΛΟΓΙΑΣ ΚΑΙ ΦΑΡΜΑΚΟΚΙΝΗΤΙΚΗΣ
INTERNATIONAL EDITION

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:

• ***Chemical Abstracts*** • ***Elsevier' Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE, Compendex, GEOBASE, FLUIDEX, TEXTILES***
• ***SCImago Journal and Country Rank Factor***

Review of Clinical Pharmacology and Pharmacokinetics
Επιθεώρηση Κλινικής Φαρμακολογίας και Φαρμακοκινητικής
Epitheorese Klinikes Farmakologias kai Farmakokinetikes
INTERNATIONAL EDITION

☸

Published three times a year by PHARMAKON-Press
Publisher Responsible According to the Law
Maria Plessa and Company, Limited Partnership
145 Michalakopoulou str., GR-115 27 Athens, Hellas
Tel.- Fax 00302107784700
Email: splessas@otenet.gr & stplessas@hotmail.com

Τετραμηνιαία Έκδοση από την ΦΑΡΚΑΚΟΝ-Τύπος
Ιδιοκτήτης-Υπεύθυνος κατά το Νόμο Μαρία Πλέσσα και ΕΕ
Μιχαλακοπούλου 145, 11527 Αθήνα, Ελλάς
Τηλ.- Fax 00302107784700
Email: splessas@otenet.gr & stplessas@hotmail.com

☸

☸

Original Papers, Review Articles, as well as short preliminary communications will be considered for publication and should be send to the Editors-in Chief
Πρωτότυπες Ερευνητικές Εργασίες, Άρθρα Επισκόπησης και Ενημέρωσης, καθώς και Κλινικές Μελέτες με άμεση σχέση το Φάρμακο και τη Θεραπευτική πρέπει να αποστέλλονται στους Εκδότες

☸

Online ISSN 1011-6583

Articles published in this Journal are ***Indexed*** or ***Abstracted*** in:
• ***Chemical Abstracts • Elsevier's Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE • SCImago Journal and Country Rank Factor***

GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS

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 provides a detailed focus on different properties, i.e. dosage, toxicology, drugs interactions and 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 *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*, and
- 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 *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.

VOLUME 25 ❁ 2011 No 1

CONTENTS

THIRTEEN YEARS GRADUATE PROGRAM
MEDICINAL CHEMISTRY

UNIVERSITY OF PATRAS
DEPARTMENTS OF CHEMISTRY AND PHARMACY

11th CONFERENCE WITH INTERNATIONAL PARTICIPATION

MEDICINAL CHEMISTRY:
Drug Discovery and Design

April 26-28, 2010
Conference and Cultural Center
University of Patras, Patras, Hellas

Letter from Guest Editor

This issue contains Abstracts of research work presented by specialists at the 10th Medicinal Chemistry Conference with International Participation held at the University of Patras, April 26-28, 2010. 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, high ranked in evaluations, is offered by the Departments of Chemistry and Pharmacy of the University of Patras, to selected graduate students from Departments of Chemistry, Pharmacy, Biology and Medicine. In particular, this issue contains articles, which are the results of novel work carried out by the researchers of the program and their graduate students, who take the post graduate program leading to Master of Science and PhD degrees. Abstracts cite in summary research findings from a broad area of Biomedical Fields, including Organic Synthesis and Drug Design Methods. The articles of the book are written by specialists in their field, who participated at the Conference and provide a global understanding of the recent activities in the field of Drug Discovery and Design in Greece and Abroad.

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

The Guest Editor

John Matsoukas

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

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 25: 11-13 (2010)

Forewords by Distinguished Scientists

Andrew V. Schally, Ph.D., MDhc (Multi), DSc hc

Miller School of Medicine, University of Miami, FL

Nobel Prize in Physiology or Medicine, 1977

For more than 50 years, I tried to the best of my ability to uncover some of the secrets of the nature hidden in the hypothalamus and help clinicians to convert these findings into diagnostic and therapeutic tools. At the inception of my scientific career, the concept of hypothalamic control of anterior pituitary function was in its formative stage. It was my good fortune to have arrived on the scene at such a crucial time and to have helped place it on the solid foundation on which it now rests. At present, the validity of this concept stands proven by the isolation, structural identifications, and synthesis of at least five hypothalamic regulatory hormones.

It will be my pleasure and honor to present at the 11th annual Medicine Conference in Patras the story of the hypothalamic luteinizing hormone releasing hormone (LHRH) also known as GnRH (gonadotropin releasing hormone). LHRH is the main link between the brain, the pituitary and the gonadal (reproductive) functions. The isolation and structural determination of LHRH, synthesis of LHRH and its analogs, the development of depot preparations, and first basic studies on prostate cancer with agonists of LH-RH were all carried out in my laboratory between 1970 and 1981. Later I participated in the first clinical study with LH-RH agonists in men with prostate cancer. The analogs of LHRH have many clinical applications in endocrinology, gynecology, urology, pediatrics and oncology.

In connection with this, I want to stress the importance not only of collaboration between various disciplines like chemistry, biology, medicine and pharmacology for progress in research but also of international cooperation in science and medicine. During many past years, we have been able to witness the significant impact of international collaboration between my laboratory, hospitals and academic institutions in various countries including Greece, the land of Alcmaeon, *Hippocrates* and *Aristotle*. Much valuable basic and clinical work was carried out and subsequently published in top journals.

It has been my good fortune to collaborate with outstanding Greek Clinicians and Scientists such as Dr George Tolis, Dr Sotos Raptis, Dr Hippokratis Kiaris, Dr Michael Koutsilieris, Dr Nektarios Barabutis and many other Greek investigators. In addition to performing research, we also formed strong bonds of friendship.

I would like to congratulate Professor John Matsoukas for organizing the conference and the students who will be presenting their research achievements.

Zeeto I Hellas!

K.C. Nicolaou, Ph.D.

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

Dear Professor Matsoukas and Faculty Members of the Departments of Chemistry and Pharmacy!

With this message I wanted to congratulate you for yet another year of success as you gather together with your students and guests to cele-

brate the 11th Conference on Medicinal Chemistry. I also congratulate your distinguished guests of honor, Nobel Laureate Andrew V. Schally and Composer and Chemist Dr Mimis Plessas, for their extraordinary contributions to science and culture that so profoundly benefted society.

Finally, but not least, my congratulations go to the students for their academic achievements. I wish them good luck in their future professional activities, and all the best to you at the University of Patras, as we all move forward to face the global challenges of the future through science and technology.

Warmly,
K.C. Nicolaou

Aristides Patrinos

President Synthetic Genomics Inc.

I am honored to be asked to prepare a brief foreword for this very important conference. I have many fond memories from the 2006 conference during which I was honored for my work with the Human Genome Project. Since that time I have followed with great interest the work of subsequent conferences and even though I have not yet succeeded in attending any of them I look forward to the day when I can indeed do just that. I applaud the choice of focus for this conference (cancer) and of the honoree (Dr. Andrew Schally). I am certain that the conference will see many lively and constructive discussions that will greatly benefit the next generation of scientists in whose minds and hands the future of the scientific enterprise will lay. We were perhaps a bit naïve when the human genome was finally sequenced because we expected a much faster route to the understanding and elucidation of biological processes, including cancer. Now we know that the road is longer and the difficulties still plentiful. However, we are still optimistic because we know that our tools are better and our young scientists are more creative than we ever were.

I congratulate the organizers of the conference and especially the tireless and inspired Dr John Matsoukas.

All the best,

Aristides A. N. Patrinos

Chemistry *vs* Biology: Neither Exists Independently of the Other

Vasso Apostolopoulos

Professor, Burnet Institute, Immunology and Vaccine Laboratory, Melbourne VIC Australia

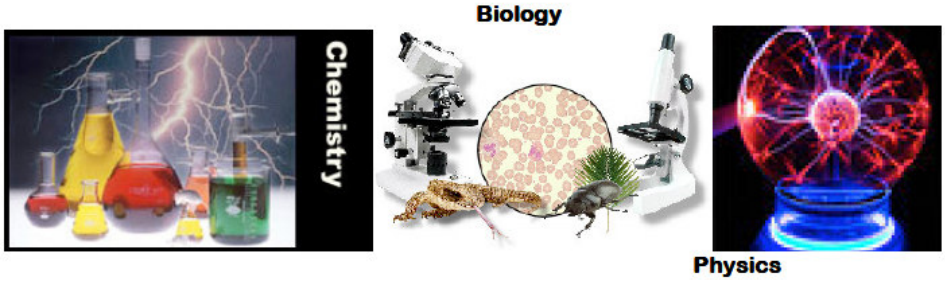
It was 2005, a nice sunny day, at Deakin University Australia. Dr Maria Katsara, a visiting PhD student in my lab, from the University of Patras, Department of Chemistry, Assoc Prof Fred Pfeffer, from Deakin University, Department of Biochemistry and myself, were sitting at the University restaurant having a nice lunch after an excellent seminar given by Dr Katsara on her immunological findings with cyclic peptides in MS. Out of the blue, a debate started between Dr Katsara and myself, on which was more important – Chemistry or Biology. After an hour, we had not come to a conclusion of which was better, even though Dr Katsara insisted chemistry was more important and I insisted that Biology (Immunology) was more important. Assoc Prof Fred Pfeffer did not want to take sides.

In April 2010, the 11th Medicinal Chemistry conference will be taking place at the University of Patras for the 11th consecutive year. There will be a range of excellent speakers once again, ranging from Biology to Chemistry to Medicinal Chemistry to Immunology. It is clear that neither chemistry or biology exists independently of the other. Each discipline is important and each discipline requires each other. In this years program, I happily see another great Scientist Professor Andrew Schally to participate presenting a hot topic on LHRH and the Applications in Medicine and Society.

I would like to congratulate the PhD students who will be presenting their research conducted

at collaborative laboratories around the world from the Medicinal Chemistry program. In addition, congratulations to the graduating Masters Students and congratulations on your poster or oral presentations.

Congratulations to the organising committee for a successful 11th Medicinal Chemistry Conference.



❁

Biomedical Research in Greece and Advances

Chris D. Platsoucas, Ph.D.

Dean, College of Sciences, Director, Center for Molecular Medicine, Professor of Biological Sciences, Old Dominion University, Norfolk, VA 23529, USA

The Graduate Program in Medicinal Chemistry at the University of Patras has completed its 12th year. This is a great achievement and will be celebrated with the 11th Medicinal Chemistry Conference. I am sure that the 11th Conference will be very interesting and exciting, like all the previous conferences organized by the Graduate Program in Medicinal Chemistry.

I would like to congratulate Professor Matsoukas, the other faculty members of the Program and the University of Patras for initiating and maintaining such a vibrant and productive graduate program. I am delighted for this success having graduated myself from the Department of Chemistry of the University of Patras more than thirty years ago. Also, I am very pleased that I have been associated with the Program as a member of the Advisory Committee from the time it was founded.

Warmest congratulations to the over 200 Ph.D. and Masters Students, who have graduated from the Program over the last 13 years. It is indeed remarkable the work that has been accomplished. The impact of this Program is very extensive for the continuing development of biomedical research in Greece. Also it provides higly trained scientists for the further development of the Greek economy. Many of these graduates have continued/are continuing their studies or are pursuing scientific careers in Greece, other European countries or in the U.S. Many are continuing to do research.

A major strength of this Program is that it is truly interdisciplinary in nature and extents across Chemistry, Pharmacology, Biology and Medicine. Major research advances have been realized recently in these areas. These advances have substantially increased our understanding of the molecular and cellular basis of human disease and are being translated to significant improvements in the diagnosis and treatment of these diseases. For these reasons interdisciplinary programs merging chemistry with biology and pharmacology are very timely and likely to be very fruitful.

With all best wishes for continuing success,
Chris D. Platsoucas, Ph.D.

❁

The Dream for Post-graduate Education

Michael Maragoudakis

Department of Pharmacology, Medical School, University of Patras, GR 26500 Patras, Hellas

Η σημερινή εικόνα των Πανεπιστημίων της χώρας μας θα ήταν τελείως διαφορετική, αν υπήρχαν πολλά Μεταπτυχιακά Προγράμματα, όπως αυτό της Ιατρικής Χημείας που λειτουργεί με το

ίδιο ενθουσιασμό διδασκόντων και διδασκομένων τα τελευταία 10 χρόνια.

Πιστεύω ότι για να αλλάξει η ζοφερή εικόνα που παρουσιάζουν τα Ελληνικά Πανεπιστήμια είναι απαραίτητο οι εμπνευσμένοι Πανεπιστημιακοί δάσκαλοι, ιδιαίτερα αυτοί που έχουν παραστάσεις και διεθνή αναγνώριση, να συμμετέχουν στη προσπάθεια για σοβαρή μεταπτυχιακή εκπαίδευση και στη δημιουργία νησίδων Επιστημονικής Αριστείας.

Ο άλλος παράγοντας που θα βοηθούσε καθοριστικά στη πραγματοποίηση αυτού του στόχου είναι η γεναιόδωρη και συνεχής χρηματοδότηση της μεταπτυχιακής έρευνας. Αυτό είναι απαραίτητο για την ανάπτυξη σύγχρονης ερευνητικής υποδομής και αύξηση του ερευνητικού δυναμικού της χώρας.

Είναι χρέος της πολιτείας και των ανθρώπων του πνεύματος προς τη νέα γενιά να ανοίξουν σε αυτή το δρόμο της επιστήμης. Η γενιά αυτή δικαιολογημένα αγανακτεί για την αβεβαιότητα της επαγγελματικής τους αποκατάσταση στην πρωτοφανή οικονομική κρίση και την κρίση αξιών σε όλα τα επίπεδα.

Ο δρόμος της επιστήμης πρέπει και μπορεί να προταθεί σαν καινούργιο μοντέλο ζωής, επειδή η επιστήμη συνδέεται ενδογενώς με την αξιοπρέπεια, την ανιδιοτέλεια, την ταπεινοφροσύνη και την αναζήτηση της αλήθειας.

Επιπλέον το Πανεπιστήμιο δεν έχει μόνο υποχρέωση να εκπαιδεύει ικανούς επιστήμονες και ώριμους πολίτες με αξίες και ιδανικά, αλλά και να συμβάλει στην ανάπτυξη και στην ευημερία της κοινωνίας. Η συνεισφορά των Πανεπιστημίων στην ανάπτυξη καινοτόμων προϊόντων και υπηρεσιών πληροφορικής, τηλεπικοινωνιών, Βιοϊατρικής κ.λπ. είναι γνωστή. Είναι βέβαιο ότι η υπέροχη κληρονομιά της Ελλάδος, το ταλέντο και η εργατικότητα των νέων ανθρώπων, όταν τους δοθούν τα κίνητρα και οι ευκαιρίες, θα συνεισφέρουν στην πρόοδο της οικονομίας και στην απομόνωση της βίας και της καταστροφής.

Ο Οικονομικός Δυναμισμός, η Παραγωγικότητα, η Ποιότητα Μόρφωσης και Αισθητικής, η Ποιότητα των ανθρωπίνων σχέσεων θα εξασφαλίσουν την καλύτερη ποιότητα ζωής και θα συμβάλλει στο Διεθνές Κύρος, την Ασφάλεια και την Αξιοπρεπεια της Χώρας.

PHARMACOKINETICS INTERNATIONAL
EDITION 25: 15-25 (2011)

Abstracts Lectures

Therapy of Various Cancers Expressing the Receptors for Luteinizing Hormone-Releasing Hormone (LHRH) with Targeted Cytotoxic Analogs of LHRH

Andrew V. Schally, Ph.D., MDhc (Multi), DSc hc

Miami Veterans Affairs Medical Center, University of Miami Miller School of Medicine, Miami, FL, USA

Targeted chemotherapy is a modern approach aimed at increasing the efficacy of systemic chemotherapy and reducing its side effects. The peptide receptors expressed mainly on cancerous cells can serve as targets for a selective destruction of malignant tumors. Binding sites for luteinizing hormone-releasing hormone (LHRH), also known as gonadotropin releasing hormone (GnRH), were found on human breast cancers, ovarian and endometrial cancers, prostatic carcinomas, bladder cancers, renal cell carcinomas (RCC), lymphomas, melanomas and pancreatic cancers. As LHRH receptors are not expressed on most normal tissues, these tumors represent a specific target for cancer chemotherapy with antineoplastic agents linked to an LHRH vector molecule. To test the efficacy of targeted chemotherapy based on LHRH analogs, we developed a cytotoxic analog of LHRH, designated AN-152, which consists of agonist [D-Lys^6]LHRH covalently linked to a widely used chemotherapeutic agent, doxorubicin (DOX). In addition, we synthesized a highly active derivative of DOX, 2-pyrrolino-DOX (AN-201), which is much potent than DOX. AN-201 is active against tumors resistant to DOX, and non-cardiotoxic. As in the case of DOX, AN-201 was coupled to carrier peptide [D-Lys^6]LHRH to form superactive targeted cytotoxic LHRH analog AN-207. AN-152 and AN-207 can effectively inhibit the growth of LHRH receptor-positive human breast, ovarian, endometrial, prostatic, urothelial (bladder) and pancreatic cancers, as well as lymphomas, melanomas and renal cell carcinomas xenografted into nude mice. DOX-containing cytotoxic LHRH analog AN-152 is in clinical phase II trials in patients with ovarian, endometrial and breast cancers. Phase I/II trials with AN-152 are in process for patients with prostatic, bladder, pancreatic and renal cancers. Targeted chemotherapy based on cytotoxic analogs of LHRH can improve the management of cancers expressing receptors for LHRH.

Overexpression & NMR Studies of Arkadia Ring Finger Engineered Mutants

A. Loutsidou[1], C.T. Chasapis[1], N.G. Kandias[1], D. Bentrop[2], V. Episkopou[3], G.A. Spyroulias[1]

[1]Department of Pharmacy, University of Patras, Rio, Patras, Hellas
[2]Institute of Physiology II, University of Freiburg, Germany
[3]Mammalian Neurogenesis, MRC Clinical Sciences Centre, Imperial School of Medicine, Hammersmith Hospital, London, United Kingdom

E3 ubiquitin ligases play a key role in the recognition of target proteins by catalyzing the covalent attachment of the ubiquitin and degradation by 26S proteasome (1). Arkadia is a RING finger protein and the first example of an E3 ligase that positively regulates TGF-β family signaling (2). The 68 a.a. of the Arkadia C-terminal, including the RING finger, was cloned, expressed in its zinc-loaded form, and studied through multinuclear and multi-dimensional NMR Spectros-

copy (3).

Additionally, mutations identified in the RING domain were studied in the light of the 3D NMR structure and new non-native RING finger forms were produced and isolated with the aim to study the structural integrity of the RING and its ability to interact with E2 partner enzyme. The new 68-a.a. RINGs were overexpressed in uniformly labeled ^{15}N form suitable for NMR studies. Among four mutated constructs three of them found to preserve their Zn-binding capacity while one abolish any metal binding ability, according to atomic absorption measurements & NMR data. The ^{1}H-^{15}N HSQC exhibit dramatic changes in respect with the native RING, but preserves the signal dispersion that is typical for a structured polypeptide, suggesting along with atomic absorption measurements that the mutated polypeptide although binds two Zn(II) ion per molecule, the RING architecture have been significantly modified.

References

1. Hershko A., Ciechanover A.: *Annu. Rev. Biochem.* 67: 425-479 (1998)
2. Mavrakis K.J., Andrew R.L., Lee K.L., Petropoulou C., Dixon J.E., Navaratnam N., Norris D.P., Episkopou V.: *PLoS. Biol. 5*: e67 (2007)
3. Kandias N.G., Chasapis C.T., Bentrop D., Episkopou V., Spyroulias G.A.: *BBRC* 378: 498-502 (2009)

Of Anthrax Lethal Factor Catalytic Site

P.V. Gkazonis[1], G.A. Dalkas[1], C.T. Chasapis[1], D. Bentrop[2], A. Vlamis Gardikas[3], G.A. Spyroulias[1]

[1]Department of Pharmacy, University of Patras, Rio, Patras, Hellas
[2]Institute of Physiology II, University of Freiburg, Germany
[3]Biomedical Research Foundation (BRFAA), Athens, Hellas

The most prominent virulence factor of the disease anthrax is the bacterium's lethal toxin (LeTx) and in particular a 90 kDa Zn-dependent highly specific metalloprotease called Anthrax Lethal Factor (LF) (1). LF exhibits high proteolytic specificity towards vital cellular signal transducers, the family of mitogen-activated protein kinase kinases (MAPKKs) cleaving them close to their N-termini, thus disrupting their ability to interact with and phosphorylate downstream substrates. The overall effect is alteration of signalling pathways and ultimately apoptosis (2). Moreover, the high cleavage specification of LF against these kinases, often found overexpressed in tumor cells and thus associated with cancer tumorgenesis (3), might spearhead to the development of an innovatory therapeutic treatment of nascent tumour (4) contrasting its potential use as a biological weapon.

Aiming to understand the structural-functional activity of the catalytic site of LF towards its kinase substrates, we studied the interaction *in silico*, performing Molecular Dynamics Simulations in eight LF-MEK/MKKs complexes (5). Likewise, NMR spectroscopy can provide experimental evidence for atomic level insights of the structure and the dynamics of proteins and enzymes, hence two polypeptide constructs of the LF catalytic domain (106a.a. and 170a.a.) were overxpessed as recombinant proteins for NMR structural studies. The recombinant LF catalytic domains were purified and reconstituted with the Zn^{2+} ion in order to be subjected to biochemical, biophysical and structural characterization *in vitro*.

References

1. Pannifer A.D., Wong T.Y., Schwarzenbacher R., Renatus M., Petosa C., Bienkowska J., Lacy D.B., Collier R.J., Park S. Leppla S.H., Hanna P., Liddington R.C.: *Nature 414*: 229 (2001)
2. Duesbery N.S., Webb C.P., Leppla S.H., Gordon V.M., Klimpel K.R., Copeland T.D., Ahn N.G., Oskarsson M.K., Fukasawa K., Paull K.D., Vande Woude G.F.: *Science 280*: 734-737 (1998)
3. Davies H., Bignell G.R., Cox C.: *Nature 417*: 949 (2002)
4. Huang D. Ding Y., Luo W.M., Bender S., Qian C.N., Kort E., Zhang Z.F., VandenBeldt K., Duesbery N.S., Resau J.H., Teh B.T.*: Cancer Res. 68*: 81-88 (2008)
5. Dalkas G.A., Papakyriakou A., Vlamis-Gardikas A., Spyroulias G.A. : *Protein Sci. 18*: 1774-1785 (2009)

The Discovery of Novel Targets for Antibiotics from *Old Style Science*: Thioredoxin Reductase

Alexios Vlamis

Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

Resistance to antibiotics is a growing problem requiring the continuous invention of novel antibacterial compounds. The wealth of knowledge acquired from classical biochemistry over the years and the description of the genomes of microbial and eukaryotic organisms acquired by novel technologies has made possible to pinpoint fine differences in the metabolisms of parasites and hosts. One such metabolic pathway is the reduction of disulfides that in the cytosol of *Escherichia coli* is performed by the thioredoxin and glutaredoxin systems. A pivotal component of the thioredoxin system is the enzyme thioredoxin reductase (TrxR) that delivers electrons to thioredoxin from NADPH. Due to the presence of selenocysteine at their active site, mammalian TrxRs have very broad specificity compared to the very narrow specificity to their prokaryotic counterparts that contain cysteine instead. The prokaryotic enzyme is thus a potential target for inhibitors that could be substrates for human TrxR. The situation is even more promising because the complementary glutaredoxin system, present in mammalian cells, is absent from anaerobic and gram positive bacteria, many of whom are known pathogens (e.g. *Mycobacteria*). These organisms are grately if not solely based on the thioredoxin system for their survival and are thus ideal targets for inhibitors of their prokaryotic TrxRs. An example of an inhibitor of prokaryotic TrxRs being at the same time a substrate for the mammalian enzyme is the antibacterial compound ebselen.

Recognition Pliability is coupled to Structural Heterogeneity: The Importance in the Disease

G. Deraos[1], J. Matsoukas[1], I. Gerothanassis[2], A. Tzakos[2]

[1]Department of Chemistry, University of Patras, Rio, Patras, Hellas
[2]Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, Ioannina, Hellas

Protein interactions and their complexes within regulatory networks should adapt in a spatiotemporal dependent dynamic environment in order to process and respond to diverse and versatile cellular signals. However, the principles governing recognition pliability in protein complexes are not yet well understood. We focused on a regulatory complex (between MBP-CaM) and found that is characterized by discrete structural heterogeneity. Our findings suggest that structural heterogeneity in protein complexes could potentially explain the way that transient and promiscuous protein interactions are optimized and tuned in complex regulatory networks.

References

1. Tzakos et al.: *Mol. Biosyst.,* 2009
2. Tzakos et al.: Recognition pliability is coupled to structural heterogeneity, *submitted*

Molecular Determinants in the Corticotropin Releasing Factor Receptor Type 1 for Peptide Binding

K. Gkountelias[1] T. Tselios[2] and G. Liapakis[1]

[1]Department of Pharmacology, Faculty of Medicine, University of Crete, Herakleion, Hellas
[2]Department of Chemistry, University of Patras, Rio, Patras, Hellas

The corticotropin releasing factor (CRF) is a 41 amino acid peptide that plays an important role in many physiological and pathophysiological processes. The amino-terminal portion of CRF and its related peptides, such as sauvagine, has been shown to be essential for peptide function, most likely by interacting with CRF receptors, which belong to subfamily B of G-protein coupled receptors (GPCRs). The CRF receptors like the other GPCRs, consists of 7 membrane-spanning segments (TMs), connected by 3 intracellular and 3 extracellular loops. Previous studies have shown that the extracellular loops of type 1 CRF receptor (CRF_1) play an important role in peptide binding most likely by interacting with the amino-terminal portion of peptides. However the precise interactions have not been determined so far. For first time we determined the interactions between the amino-terminal portion of CRF and sauvagine with CRF_1, by mutating to Ala the residues in the second extracellular loop (EL2) of receptor, modifying the structure of CRF and sauvagine and determining the binding affinities and potencies of the natural and modified peptides for wild type CRF_1 and Ala mutants. Our findings suggest that Trp259 and Phe260 in the EL2 of CRF_1 interact with a region between the amino-terminal residues 8-10 of sauvagine and the corresponding region in CRF. These results in conjunction with those from studies in progress will ultimately elucidate the mode of interaction of the ELs of CRF_1 with CRF family peptides, thus putting the basis for the design of CRF_1-selective peptides.

Novel GnRH Peptide Analogues Using Leuprolide as a Model for the Treatment of Prostate Cancer

T. Katsila[1], E. Balafas[2], N. Kostomitsopoulos[2], D. Laimou[3], T.Tselios[3] and C.Tamvakopoulos[1]

[1]Laboratory of Pharmacology-Pharmacotechnology, [2]Center for Experimental Surgery, BRFAA, Athens, Hellas
[3]Department of Chemistry, University of Patras, Rio, Patras, Hellas

Currently, Gonadotropin-releasing hormone (GnRH) agonists, such as Leuprolide, are widely used for the treatment of prostate cancer and other hormone related disorders. However, GnRH agonists, due to their peptidic nature, exhibit poor pharmacokinetics and are accompanied by side effects (flare phenomenon). In an effort to provide alternative treatments, novel GnRH analogues were evaluated *in vitro* and *in vivo*. A variety of *in vitro* and *in vivo* bioassays were coupled to liquid chromatography mass spectrometry (LC-MS/MS), a technique that provides qualitative and quantitative features of the studied peptides and corresponding metabolites. *In vitro* peptide metabolism and stability was determined using mouse kidney membranes in the presence and absence of inhibitors. Such studies potentially provide insight in the mechanism of degradation. Additionally, a mouse model was developed for the pharmacokinetic evaluation of GnRH and analogues following intraperitoneal administration. *In vitro* and i*n vivo* data showed good correlation. Finally, *in vivo* pharmacodynamic studies were conducted upon administration of selected analogues and action via the GnRH receptor type I was demonstrated.

The rational design of GnRH analogue peptides but also other peptide therapeutics with improved pharmacokinetics/pharmacodynamics is facilitated with the above approaches.

From Angiotensin II to Losartan and Elsartan: Towards Clinical Trial

John Matsoukas

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

The discovery of Losartan a non peptide Angiotensin II Receptor antagonist was announced in 1989 during the Gordon Research Conference on Angiotensin and the Renin – Angiotensin – Sys-

tem (RAS). The drug was discovered in the Laboratories of Dupont and the announcement at the Conference was the approval for Clinical trials which led to the first Angiotensin II nonpeptide Receptor antagonist. Previous Angiotensin II peptide antagonists such as Sarilesin and Saralasin failed to become drugs due to its peptide nature rendering them susceptible to proteolytic enzymes which hydrolyze them. The announcement was the result of many years work on Angiotensin and the RAS System, since it was discovered 80 years ago. Breakthroughs in this evolution was the discovery of Captopril by Miguel Ondetti in 1975 and Losartan by Timmermans in 1989. In this lecture the main steps followed in our laboratories in Patras are mentioned which led to our Sartan, named Elsartan. Briefly the main steps are: 1. Peptide (The tool), 2. Peptide Model (The ligand-receptor interaction), 3. Cyclic Peptide (The drug lead), 4. Non-peptide mimetic (The Drug). Also, the strategic steps are described, in the investment of this research and the incorporation of ELDRUG in order to develop Elsartan for Medical uses as antihypertensive.

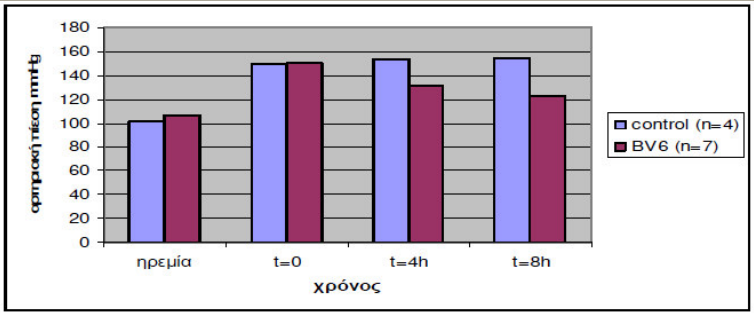
Elsartan (BV6): The New Lipid-Soluble Sartan for Trasdermal Use

P. Keppa[2], D. Kalavrizioti[2], A. Resvani[2], I. Matsoukas[2], G. Aggelis[2], A. Drakou[1], D. Vlachakos[1]

[1]Nephrology Department, Attikon University Hospital, Athens, Hellas
[2]Chemistry Department, University of Patras, Rio, Patras, Hellas

INTRODUCTION AND AIMS: Previous experimental in vitro and in vivo studies in mices and rabbits showed that BV6 succesfully inhibits the hypertensive reaction from angiotensin II (AII) per. os., iv, sc and transdermal administration. This study was designed in order to evaluate the effectiveness of transdermal use of BV6. METHODS: Mices of 300 gr were used. Their blood pressure calculated with CODA II device of Kent Scientific. Hypertensive responce of 50mmHg was steadily reproduced with sc administration of AII at a dose of 50 μg/kg. The previous day the back from the experimental animals were shaved and pealed. In the clean skin of 7 of them (group BV6) a mixture of 50 μg/kg BV6 in an excipient of 30% azone, 30% ethylic alcohol, 30% propyloglycol and 10% of water was placed. In another group of 4 (*placebo* group) only excipients (without BV6) was placed. The hypertensive reaction after the administration of 50 μg/kg AII was measured at the beginning and after 4 and 8 hours, respectively. RESULTS: The following table includes the results of the arterial blood pressure of the experimental animals in mmHg. The values are as a mean value ± standart deviation. CONCLUSIONS: The new lipid soluble sartan BV6 can succesfully penetrate the dermis to the systematic circulation of the experimental animals and inhibit the hypertensive responce of AII. Further experiments are required in order to procede to clinical trials.

RESULTS				
	Blood pressure baseline	1st bolus AII (t=0)	2nd bolus AII (t=4h)	3rd bolus AII (t=8h)
placebo (n=4)	101± 22	148,5± 28	154±32	155±26
BV6 (n=7)	107±12	150±14	131±19	122±20



Novel Therapeutic Approaches on Multiple Sclerosis

Nikolaos Dimisianos

Resident in Neurology, Postgraduate Student, Neurology Clinic, Patras University Hospital, Rio, Patras, Hellas

Multiple Sclerosis (MS) is considered an autoimmune, inflammatory, demyelinating disease of the central nervous system, with destruction of the myelin sheath, neurodegeneration and accumulation of neurological deficit. Until 1993 there were no approved therapies for MS. Since then several therapeutic agents have been tested and used, which function by modulating the immune response (immunomodulatory treatments or disease modifying therapies - DMTs), including interferon β, glatiramer acetate and the monoclonal antibody natalizumab. As our understanding of the immunopathophysiological mechanisms that underlie the disease expand and modify, novel approaches have emerged that target different paths of these mechanisms, many of which have been tested in phase 2 and 3 clinical trials with promising results. In this presentation we will make a brief review of the existing MS treatments and we will focus on the novel therapeutic approaches that will change the way we treat this devastating disease.

❁

Regulatory T-Cells (Tregs) in Remitting-Relapsing Multiple Sclerosis (RR-MS) Patients: Effects of Disease Activity and Treatment Regimens

A. Mouzaki[1], N. Dimisianos[2], K. Chatzantoni[1], P. Sakellaraki[1], Z. Dervilli[1], G. Deraos[3], J. Matsoukas[3] and P. Papathanasopoulos[2]

[1]Division of Hematology, Department of Internal Medicine, Medical School; [2]Neurology Clinic, University Hospital; [3]Department of Chemistry, University of Patras, Rio, Patras, Hellas

The main culprits of multiple sclerosis (MS) pathogenesis are thought to be CNS-specific effector Th cells, and defective T regulatory cells (Tregs). In this work, PBMC from 49 remitting-relapsing (RR)-MS patients and 25 controls were analyzed by FACS, to determine the sizes of their Treg populations and the effects of disease activity and treatment regimens (methylprednizolone or natalizumab) thereof. The groups of Th cells analyzed were $CD4+CD25^{high}$, $CD4+CD25^{high}Foxp3+$ and $CD4+CD25^{-/low}Foxp3+$. PBMC were also cultured with an antigenic peptide to determine the responsiveness of Tregs of the various study groups. No significant difference in the % of $CD4+CD25^{high}$ T-cells was observed between patients and controls. The % of $CD4+CD25^{high}Foxp3+$ T-cells was significantly lower in patients than controls, with relatively higher levels in the stable and natalizumab-treated patients. The % of $CD4+CD25^{-/low}Foxp3+$ T-cells was significantly higher in natalizumab-treated patients than controls and patients in the acute phase±methyl-prednizolone, and marginally higher than patients in remission. $CD4+CD25^{high}Foxp3+$ Tregs of natalizumab-treated patients responded well in culture to the peptide (40% increase) compared to Tregs of patients in remission (28% increase) or controls (15% increase) whereas Tregs of patients in the acute phase±methylprednizolone responded adversely to the peptide (18-20% decrease). Our results demonstrate control levels of $CD4+CD25^{high}$ T-cells in RR-MS patients but lower levels of their $CD4+CD25^{high}Foxp3+$ T-cells. Natalizumab-treated patients had higher levels of Tregs than the remaining study groups and their $CD4+CD25^{high}Foxp3+$ T-cells responded better to an antigenic peptide, indicating that this drug may help restore immunological tolerance through Treg induction.

❁

Agonists, Inverse Agonists, and Allosteric Modulators of G Protein-Coupled Receptors

Leonardo Pardo

Laboratori de Medicina Computacional, Unitat de Bioestadística, Facultat de Medicina Universitat Autònoma de Barcelona, Barcelona, Spain

In the ligand-free basal state, G protein-coupled receptors (GPCRs) exist in equilibrium of conformations, each stabilized by a network of intramolecular interactions. Agonists binding at the native (or orthosteric) site stabilize conformational changes in cytoplasmic domains that increase receptor signaling. Conversely, inverse agonists decrease the basal, agonist-independent level of signaling by stabilizing different conformational changes. In addition, ligand binding to an allosteric site (that is topographically distinct from the orthosteric site) might enhance (positive allosteric modulators) or decrease (negative allosteric modulators) the response of orthosteric agonists. We will show, combining the latest information about GPCR structure with chemical synthesis, site-directed mutagenesis, biophysical experiments and computational modeling, how the binding of the ligand to the allosteric or orthosteric site influences this equilibrium of conformations.

The Role of GnRH Analogues in Cancer

T. Makatsoris, H. Kalofonos

Department of Medicine, Univercity of Patras, Rio, Patras, Hellas

GnRH (gonadotropin-releasing hormone), a decapeptide produced by the hypothalamus, plays an important role in the reproduction by regulating the pituitary-gonadal axis. Continuous high doses of GnRH or its agonists result in desensitization of the pituitary gonadotropes and a suppression of sex steroid production by the gonads (chemical castration). Based on these effects, the treatment with GnRH agonists has become a widely used hormonal therapy of the sex-steroid dependent tumors.

GnRH analogues can cause tumor regressions in hormonally responsive breast cancers and have received approval for the treatment of metastatic breast cancer in premenopausal women.

Ovarian suppression/ablation, alone or combined with tamoxifen, are also effective strategies for the adjuvant treatment of hormone-receptor positive breast cancer in premenopausal women. Clinical trials have demonstrated that ovarian suppression/ablation (OS/OA) is effective when used as a sole method of adjuvant systemic therapy, and is as effective as certain chemotherapy regimens, such as cyclophosphamide, methotrexate, and fluorouracil (CMF).

Gonadotropin-releasing hormone analogues result in a medical orchiectomy in men and are used as a means of providing androgen ablation for metastatic prostate cancer. Furthermore, randomized trials demonstrated that adjuvant androgen deprivation therapy (ADT) with gonadotropin-releasing hormone (GnRH) agonists decreases cancer-specific and, in some cases, all-cause mortality for men with locally advanced or high-grade localized prostate cancer.

Cerebrosides, Myelin, Hormone LHRH, BRAIN

Thomas Mavromoustakos

Laboratory of Organic Chemistry, Chemistry Department, Capodistrian University, Athens, Hellas

This short speech is dedicated to prolific and honored composer and graduated Chemist from Cornell University of New York, USA, Mimis Plessas. Dr M. Plessas is not only a great musician well known nationally and internationally, but also he is a chemist studied the cerebroside biomolecules existing in the brain which can serve as a treasure storage of energy. Structurally similar to cerebrosides, are sphingomyelins, present in the myelin. Myelin forms a sheath to a neuron axon and its reduction leads to Multiple Sclerosis.

Altered Peptide Ligants of Myelin Basic Protein Mutants Conjugated to Reduced Mannan Modulate Immune Responses in Mice

M. Katsara[1,2], E. Yuriev[3], P. Ramsland[1], T. Tselios[2], G. Deraos[2], A. Lourbopoulos[4], N. Grigoriadis[4], J. Matsoukas[2] and V. Apostolopoulos[1]

[1]Immunology and Vaccine, and Structural Immunology Laboratories, Burnet Institute, Australia
[2]Department of Chemistry, Section of Organic Chemistry, Biochemistry, and Natural Products, University of Patras, Rio, Patras, Hellas
[3]Medicinal Chemistry and Drug Action, Monash University, Australia
[4]B' Department of Neurology, University Hospital, Aristotle University, Thessaloniki, Hellas

Mutations of peptides to generate altered peptide ligands, capable of switching immune responses from T helper 1 (Th1) to T helper 2 (Th2), are promising candidates for the immunotherapy of autoimmune diseases such as multiple sclerosis (MS). We designed and synthesised two mutant peptides from MBP_{87-99}, an immunodominant peptide epitope identified in MS. Mutations of residues K^{91} and P^{96}, known to be critical TCR contact sites, resulted in the mutant peptides [R^{91}, A^{96}]MBP_{87-99} and [A^{91}, A^{96}]MBP_{87-99}. Immunisation of mice with these altered peptide ligands emulsified in complete Freund's adjuvant induced both interferon-γ (IFN-γ) and interleukin-4 (IL-4) responses compared with only IFN-γ responses induced to the native MBP_{87-99} peptide. It was of interest that [R^{91}, A^{96}]MBP_{87-99} conjugated to reduced mannan induced 70% lower IFN-γ responses compared to the native MBP_{87-99} peptide. However, [A^{91}, A^{96}]MBP_{87-99} conjugated to reduced mannan did not induce IFN-γ secreting T cells, but elicited very high levels of IL-4. Furthermore, antibodies generated to [A^{91}, A^{96}]MBP_{87-99} peptide conjugated to reduced mannan did not cross-react with the native MBP_{87-99} peptide. By molecular modelling of the mutant peptides in complex with major histocompatibility complex (MHC) class II, I-A^s, novel interactions were noted. It is clear that the double mutant peptide analogue [A^{91}, A^{96}]MBP_{87-99} conjugated to reduced mannan is able to divert immune responses from Th1 to Th2 and is a promising mutant peptide analogue investigating potential treatment of MS.

❁

Cellular Grafts in Experimental Focal Ischemia

A. Lourbopoulos, N. Grigoriadis

Laboratory of Experimental Neurology and Neuroimmunology, University Hospital, Thessaloniki, Hellas

Adult Bone Marrow Stromal Cells (BMSCs) and neonatal Neural Precursor Cells (NPCs) are both candidate cellular grafts for treatment of ischemic stroke. Intravenous, intraarterial or intraparenchymal routes of administration for these cells have been widely studied. However, the intraventricular (ICV) administration of both grafts 6 hours after severe stroke has not been studied so far.

Adult male Wistar rats were subjected to 2-hours transient occlusion of the middle cerebral artery (MCAO model), using a model recently developed in our Laboratory. BMSCs and NPCs were cultured, phenotypically characterized and transplanted into the lateral ventricles. Animals were clinically monitored at specific time-intervals and were studied for neuropathology at 3 (acute) and 60 (chronic phase) days post-infarction.

The 2 different grafts exhibited significantly different effects and behavior in the host brain. Both cellular grafts were widely dispersed throughout the ventricular system but display significantly different intraparenchymal migratory capability (BMSCs at a medium distance and for short period of time; NPCs at a long distance and continious through time). BMSCs, but not NPCs, rendered a significant clinical benefit with reduced post-infarction mortality and improved clinical scores at 2 months. Possible mechanisms for these effects included significant reduction of a) post-ischemic edema, b) neuronopathy and axonopathy and c) astrocytic activation and scar during acute and chronic phase after stroke. In addition, BMSCs expressed trophic factors (VEGF and HGF). No cellular graft reduced the infarction volume. However, BMSCs developed masses attached to the ventricular walls and possibly constituting a graft-rejection reaction.

Conclusively, both cellular grafts do not seem

to behave as *cellular replacement therapy* after stroke. On the contrary, BMSCs but not NPCs seem to function as supportive *cellular trophic reactors*, which desire further pharmaceutical exploitation.

❁

Growth Factor Activities Are Dependent On Multiple Receptor Complexes: The Example of Pleiotrophin

M. Koutsioumpa, C. Mikelis and E. Papadimitriou

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Rio, Patras, Hellas

Growth factor activities depend on their binding and activation of specific receptors. Pleiotrophin (PTN) is a heparin binding growth factor with diverse biological activities, among which a direct role on tumor growth and angiogenesis. It is well known that PTN acts on endothelial and tumor cells through its receptors ALK (anaplastic lymphoma kinase) and RPTPβ/ζ (receptor protein tyrosine phosphatase β/ζ) (1). We have recently found that integrin $\alpha_v\beta_3$ forms a functional complex with RPTPβ/ζ on the cell surface and is required for PTN-induced migration of endothelial and tumor cells. In the absence of $\alpha_v\beta_3$, PTN inhibits cell migration (2). It seems that another component of the cell surface functional complex that is required for PTN-induced cell migration is nucleolin, which is found only on the surface of cells that also express $\alpha_v\beta_3$ (3). These data suggest that PTN-induced cell migration requires the presence of at least three different cell surface receptors, each of which may participate in a different way in the diverse biological activities of PTN.

References

1. Papadimitriou E. et al.: *Eur. Cytokine Netw. 20*: 180-190 (2009)
2. Mikelis C., et al.: *FASEB J. 23*: 1459-1469 (2009)
3. Koutsioumpa M., et al.: submitted.

❁

Saffron: Study of Chemopreventive Potential and Identification of Bioactive Components

Fotini N. Lamari

Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Rio, Patras, Hellas

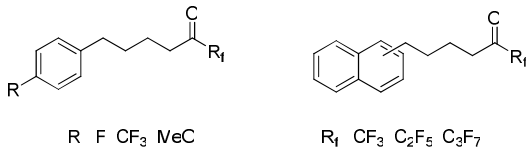
Saffron is a prevalent spice and important component of folk medicine around the Mediterranean and Middle East. Its phytochemical composition is unique due to the presence of water-soluble carotenoids, crocins, which are glycosidic esteres of crocetin. In order to investigate the chemopreventive/anticancer properties of saffron and identify the bioactive components, we carried out a series of experiments *in vitro* and *in vivo* with the crude extract, the major crocin (*trans*-crocin-4), crocetin and safranal (major essential oil component). The antioxidant properties were studied in cell lines treated with H_2O_2, in neonatal rats treated with sodium selenite (20 mg/kg body weight) and in normal adult mice. Both the extract and crocetin offered significant antioxidant protection. Crocetin properties gain particular importance when taking into account that in healthy adult volunteers, two hours after consumption of saffron tea, crocetin is detected in blood plasma (1-4 μM). Crocetin was also identified as the main ingredient causing significant inhibition of proliferation and invasive capacity of MDA-MB-231 cancer cells. This effect was accompanied by a significant reduction of expression and secretion of membrane type 1 and 2 matrix metalloproteinases.

❁

New Fluoroketones as Inhibitors of Human Ca^{2+}-Independent Phospholipase A_2

A. Smyrniotou, V. Magrioti, K. Nikolaou, S. Kyriakos and G. Kokotos

Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis GR-15771, Athens, Hellas



R F CF_3 MeC R_1 CF_3 C_2F_5 C_3F_7

Ca^{2+}-Independent phospholipase A_2 (GVIA $iPLA_2$) has recently emerged as a novel pharmaceutical target. The discovery of potent and selective $iPLA_2$ inhibitors is of great importance for the development of new therapies for neurological disorders, like multiple sclerosis (1). Our group has recently disclosed a series of polyfluoroketones (trifluoromethyl, pentafluoroethyl and heptafluoropropyl ketones) which include an aromatic ring and a linker of four carbon atoms, between the aromatic ring and the polyfluoroalkyl ketone group (2). In the present work, we describe the synthesis of new polyfluoroketones which contain a substituted aromatic ring or an extended aromatic ring such as the naphthyl moiety.

For the synthesis of the desired polyfluoroketones, commercially available substituted benzaldehydes or 1- or 2-naphthaldehyde were used as starting materials. At first, each aldehyde underwent a Horner-Wadsworth-Emmons reaction with triethyl 4-phosphonocrotonate to yield the corresponding unsaturated ester. After catalytic hydrogenation and saponification, the carboxylic acid was converted to acyl chloride and after treatment with pyridine and trifluoromethylacetic or pentafluoroethylpropionic or heptafluoropropylbutyric anhydride, the desired polyfluoroketones were obtained. The study of the in vitro activity and selectivity for $iPLA_2$ is in progress.

References

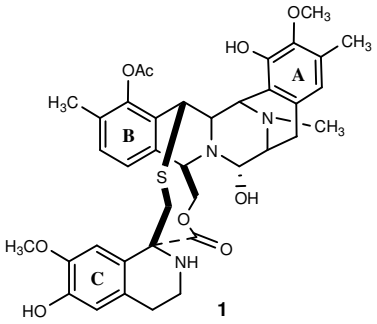
1. Kalyvas A., Baskakis C., Magrioti V., Constantinou-Kokotou V., Stephens D., Lopez-Vales R., Lu J.-Q., Yong V.W., Dennis E.A., Kokotos G., David S.: *Brain 132*: 1221-1235 (2009)

2. Baskakis C., Magrioti V., Cotton N., Stephens D., Constantinou-Kokotou V., Dennis E.A., Kokotos G.: *J. Med. Chem. 51*: 8027-8037 (2008)

Studies toward the Total Synthesis of Ecteinascidin-743: New Synthetic Technologies in Medicinal Chemistry

V. Psarra, P. Magriotis

Department of Pharmacy, University of Patras, Rio, Patras, Hellas



1

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

1. (a) Rinehart K.L., Holt T.G., Fregeou N.L., Stroh J.G., Keifer P.A., Sun F., Li L.H., Martin D.G.: *J. Org. Chem. 55*: 4512-4515 (1990). (b) Rinehart K.L.: *Med. Drug Rev.*: 1-27 (2000)
2. (a) Zewail-Foote M., Hurley L.H.: *J. Med. Chem. 42*: 2493-2497 (1999). (b) Hedge S., Schmidt M.: *Ann. Rep. Med. Chem. 43*: 492-493 (2008)
3. Molinski T.F., Dalisay D.S., Lievens S.L., Saludes J.P.: *Nat. Rev. Drug Discov. 8*: 69-85 (2009)

Adress: see email: fakis@upatras.gr

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 25: 26-41 (2010)

Abstracts Posters

Human Studies of Linear and Cyclic Double Mutant Peptides [A^{91},A^{96}] MBP_{87-99}: Implications in the Immunotherapy of Multiple Sclerosis.

G. Deraos[1], K. Xatzantoni[3], L. Synodinos, M. Matsoukas[1], S. Deraos[1], A. Papalois[5], H. Kaldbacher[4], A. Mouzaki[3], V. Apostolopoulos[2*], J. Matsoukas[1*]

[1]Department of Chemistry, [2]Department of Medicine, University of Patras, Rio, Patras, Hellas
[3]Department of Biochemistry, University of Tubingen, Germany
[4]Experimental-Research Center Elpen,
[5]Burnet Institute, Centre for Immunology, Immunology & Vaccine Lab, Prahran, VIC, Australia

Multiple Sclerosis is a demyelinating autoimmune disease mediated primary by $CD4^+$ T cells of the Th1 subset. We have shown that mutated MBP 87-99 constrained peptide analogs with modifications at the principal TCR contact sites 91 (Lys) and 96 (Pro), are able to divert immune responses from Th1 to therapeutic Th2 subset. In particular in this study we have evaluated wild type MBP_{87-99} (P2), linear mutant $MBP_{87-99}[A^{91}, A^{96}]$ (P3) and cyclo(87-99) $MBP_{87-99}[A^{91}, A^{96}]$ (P4) with substitution at positions 91, 96 critical for TCR contact, for their effects on the cytokine secretion by PBMC culture derived from 28 MS patients and their ability to induce TH1/TH2 pathways.

❁

Synthesis of New Analogues of C-Terminal Hexapeptide of Neurotensin

R. Exarchakou[1], V. Magafa[1], N. L. Assimomytis[2], F. N. Lamari[1], F. Bazoti[3], G. Varvounis[4] and P. Cordopatis[1]

[1]Department of Pharmacy, University of Patras, Rio, Patras, Hellas
[2]Department of Mechanical Engineering, TEI of Patras, Patras, Hellas
[3]*GAIA* Research Center, The *Goulandris* Natural History Museum, Athens, Hellas
[4]Department of Chemistry, University of Ioannina, Ioannina, Hellas

Neurotensin (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) [NT] is a tridecapeptide originally isolated from bovine hypothalamus and later from intestines. NT displays a wide spectrum of biological actions both in the central and peripheral nervous systems of different mammalian species. These actions have led to the proposal that this peptide fulfills a dual function as a neurotransmitter/neuromodulator in the brain and as a hormone/cellular mediator in peripheral tissues. The physiological and biochemical actions of NT are mediated through binding to NT receptors (NTRs). Up to now, three subtypes of neurotensin receptors (NTS1, NTS2 & NTS3) have been cloned. All three receptors recognize the same C-terminal hexapeptide fragment of NT [NT(8-13)], which corresponds to the shorter fragment of NT that maintains full biological activities. Although NT(8-13) possesses high receptor binding affinity, it is rapidly degraded by peptidase action. Therefore, it is important to synthesize analogues with stabilized bonds against metabolic deactivation which do not lose binding affinity. Based on these findings, we herein report the synthesis of new analogues of NT(8-13) with modifications in the basic structure needed for high affinity binding in order to improve the metabolic stability. The analogues were synthesized by the Fmoc/Bu^t solid phase methodology utilizing 2-chlorotrityl chloride resin. Electrospray MS was in agreement with the expected results.

Synthesis of N^{α}-Hydrazino-Peptoides Corresponded to Substance P C-Terminal Fragments and their Trypsin Inhibitory Effect

M. Ziovas[1], R. Papi[2], D. Kyriakidis[2,3], C. Poulos[1] and G. Stavropoulos[1]

[1]Dept. of Chemistry, University of Patras, Rio, Patras, Hellas
[2]Dept. of Chemistry, Aristotle University, Thessaloniki, Hellas
[3]National Hellenic Research Foundation, Athens, Hellas

A number of small synthetic peptides, analogs to Substance P C-terminal fragment, were designed based on the structure of inhibitors active against trypsin or chymotrypsin. These serine protease inhibitors are widespread in nature and play an important role in the regulation of proteolytic enzymes. Trypsin-like serine proteases are important in various biological processes and the upregulation of the members of this group is an interesting research target for the development of potential therapeutic agents.

In the present work we have studied a series of N^{α}-hydrazino-peptoides for their trypsin inhibitory effect. The stability of these peptoides is increased against the action of peptidases due to the incorporation in their structures D-amino or N-amino acids and hydrazine-derivatives.

All the syntheses were carried out stepwise by SPPS, using the Fmoc/Bu^t methodology on the solid support 2-chlorotrityl chloride resin and DIC/HOBt as coupling reagent. The products were purified (HPLC) and identified (ESI-MS). They are studied for their inhibitory effect against trypsin.

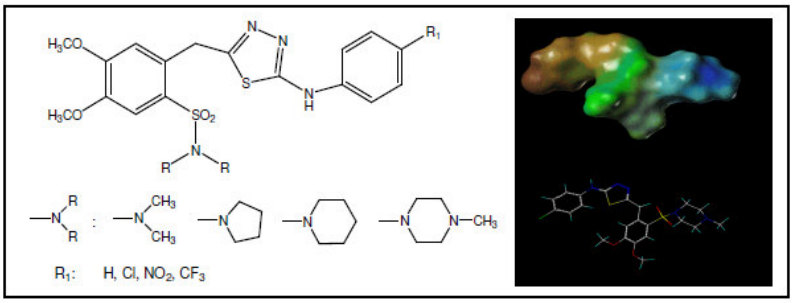
1. Glp[1]-Glu(OBzl)[2]-NPhe[3]-Gly[4]-[NH-N(Bzl)-CH_2-CO][5]-D-Trp[6]-Leu[7]-OH
2. Glp[1]-Glu(OBzl)[2]-NAla[3]-Gly[4]-[NH-N(Bzl)-CH_2-CO][5]-D-Trp[6]-Leu[7]-OH
3. Glp[1]-Glu(OBzl)[2]-Tic[3]-Gly[4]-[NH-N(Bzl)-CH_2-CO][5]-D-Trp[6]-Leu[7]-OH

❁

Sulfonamide-1,2,4-Thiadiazole Derivatives as Antifungal and Antibacterial Agents: Synthesis, Biological Evaluation, Lipophilicity, and Conformational Studies

C. Camoutsis[1], A. Geronikaki[2], P. Zoumpoulakis[3], M. Zervou[3], A. Ciric[4], M. Soković[4]

[1]Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, School of Health Sciences, University of Patras, Rio, Patras, Hellas
[2]Department of Pharmaceutical Chemistry, School of Pharmacy, *Aristotelian* University, Thessaloniki, Hellas
[3]Laboratory of Molecular Analysis, Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Athens, Hellas
[4]Mycological Laboratory, Department of Plant Physiology, Institute of Biological Research, Belgrade, Serbia



A series of thirteen new thiadiazole compounds were synthesized and evaluated for *in vitro* antifungal and antibacterial activity. All compound tested showed significant antifungal activity

against all the micromycetes, compared to the commercial fungicide bifonazole. Differences in their activity depend on the substitution of different reactive groups. More specifically, best antifungal activity was shown for the synthetic analogue with methylpiperazine reactive group. Furthermore, it is apparent that different compounds reacted on different ways against bacteria with pyrrolidine derivatives showing higher activity with respect to piperidine, methylpiperazine and dimethylamino derivatives. An effort was made to correlate the above mentioned differences in activity with lipophilicity profiles showing that the increase in activity goes in parallel with their lipophilicity expressed as the calculated ClogP values. NMR and molecular modelling were used to obtain the main conformational features of the most potent analogues in contrast to the less potent, for *in silico* future studies.

Nogo-A Expression in the Central Nervous System of Mice Induced with Experimental Autoimmune Encephalomyelitis

P. Theotokis, A. Lourbopoulos, E. Salta, R. Lagoudaki, E. Polyzoidou, N. Grigoriadis

Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Thessaloniki, Hellas

Nogo-A, a neurite outgrowth inhibitor, has been found to play a significant role in controlling axonal regrowth after injury. The mRNA expres-sion patterns of Nogo-A have been studied in the adult rat central nervous system (CNS) both in naive animals and in animal models, such as traumatic brain and spinal cord injury. Nogo-A mRNA was abundantly expressed in most brain nuclei of naive animals, including neurons of the hippocampus, habenular nuclei, piriform cortex, red nucleus, oculomotor nucleus and pontine trigeminal nucleus. Given that no similar pattern expression has ever been investigated in demyelinating diseases, we performed MOG-induced experimental allergic encephalomyelitis (EAE) in adult C57BL/6 mice, an animal model of multiple sclerosis (MS).

We studied the NOGO-A mRNA expression at various time points following the induction of the disease. Naive (non-EAE) animals were used as controls. *In situ* Hybridization (ISH) was performed in order to detect and localize the specific Nogo-A mRNA's in 26μm brain and spinal cord sections using digoxigenin-labelled riboprobes. Real-time PCR was used in order to quantify the expression among all groups of animals and data were normalized and analysed for statistical significance.

Histochemical analysis with ISH revealed an mRNA expression pattern similar to that previously reported in naive adult rats. Real-time PCR exhibited a time-independent change in mRNA levels in brain homogenates. On the other hand spinal cords exhibited a time-progressive upregulation of the transcripts culminating in acute phase of the disease followed by a mere fall in chronic. Evidently, these changes were related to the inflammatory reaction within CNS at the correspondent time-point.

Current results indicate that the expression of NOGO-A mRNA and the concomitant axonal regeneration is dependent on the kinetics of the underlying immune-mediated demyelination.

Elsartan-A Selective Antagonist of AT1 Receptor of Angiotensin II

D. Kalavrizioti[1], M.-E. Androutsou[2], A. Resvani[2], G. Agelis[2] and J. Matsoukas[2]

[1]Department of Medicine, [2]Department of Chemistry, University of Patras, Rio, Patras, Hellas

Angiotensin II (AII) receptor antagonists play a significant role in the regulation of blood pressure and the treatment of hypertension. The AII receptor antagonists block the AT_1 receptor in a competitive or insurmountable way and prevent AII from binding to the AT_1 receptor. In the present study, we demonstrate that Elsartan is a selective antagonist for AT_1 receptor and represses the action of AII in a dose dependent manner. *In vitro* displacement binding assay was carried out in

order to determine the binding affinity of Elsartan compared to Losartan (control). The IC_{50} values of Losartan and Elsartan were 347.6 nM (K_i= 207.4 nM) and 208.4 nM (K_i= 124.3 nM), respectively, for the inhibition obtained in human AT_1 receptor produced in sf9 cells.

Furthermore in the intracellular Ca^{2+} mobilization assay, Elsartan (10^{-5} M) caused a total inhibition of the action of AII. The IC_{50} values of Elsartan and Losartan were 68.7 nM and 149.5 nM, respectively. These studies indicate that Elsartan is a potentially promising antihypertensive agent.

References

Fierens F.L.P., Vanderheyden P.M.L., De Backer J.P., Vauquelin G.: *Eur. J. Pharmacol. 372*: 199-206 (1999)

An Update on Hypertension Management and the Role of Angiotensin Receptors Antagonists (Sartans)

V. Karantalis, P. Davlouros, D. Alexopoulos

Cardiology Department, Patras University Hospital, Rio, Patras, Hellas

Blood pressure has a unimodal distribution in the population as well as a continuous relationship with cardiovascular risk down to systolic and diastolic levels of 115-110 mmHg and 75-70 mmHg, respectively. In Greece, according to the Hellenic Society for the Study of Hypertension, 25% of the population is affected by it, only 25% of who are diagnosed, treated and have controlled their blood pressure sufficiently. What is even more alarming is that 50% of the hyper-tensive population remains either undiagnosed (40%) or untreated even when diagnosis is established (10%). For every 10 mm and 20 mm increase in diastolic and systolic pressure respectively from the optimal levels of 75/115mm there is a documented two-fold increase for both a mortal stroke and a myocardial infarction.

In hypertensive patients, the primary goal of treatment is to achieve maximum reduction in the long term total risk of cardiovascular disease. This requires treatment of the raised BP per se as well as of all associated reversible risk factors. BP should be reduced to at least below 140/90 mmHg (systolic/diastolic), and to lower values, if tolerated, in all hypertensive patients. Target BP should be at least 130/80 mmHg in diabetics and in high or very high risk patients. In order to more easily achieve goal BP, antihypertensive treatment should be initiated before significant cardiovascular damage. Lifestyle measures should be instituted, whenever appropriate, in all patients, including those who require drug treatment.

In ESC/ESH 2007 Guidelines, a combination of two drugs was considered superior over monotherapy. More specifically a combination of a diuretic and an Angiotensin Converting Enzyme (ACE) inhibitor or an Angiotensin Receptors Blocker (ARB) or a Calcium Channel Blocker (CCB) seems to be slightly more favored than other combinations. An ARB and a CCB seem also to be a logical and effective combination.

So the role of ARBs in hypertension management remains crucial and is even increased by the fact that ARBs are drugs that can be used in various other combined diseases such as diabetic nephropathy and congestive heart failure. Many trials in the past years have shown and continue to show ARBs participation in antihypertensive combinations to be one of the first choices of the clinician dealing with a hypertensive patient.

A Facile Synthesis of 1-Biphenylmethyl Tetrazole Substituted Imidazole AT_1 Angiotensin II Receptor Antagonists Based on Urocanic Acid

K. Kelaidonis, A. Resvani, J. Matsoukas and G. Agelis

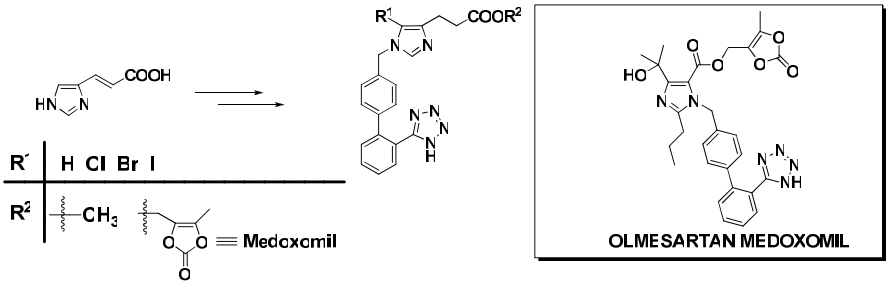
Department of Chemistry, Section of Organic Chemistry, Biochemistry and Natural Products, University of Patras, Rio, Patras, Hellas

The hormone Angiotensin II (Ang II) is the main factor of the Renin-Angiotensin System (RAS) and plays a significant role in the regulation of arterial blood pressure. Non-peptide AT_1 Angio-

tensin II receptor antagonists have been successfully used for the treatment of hypertension. In the present study, a series of *E*-urocanic acid derivatives was designed and synthesized, as potent AT_1 Ang II receptor antagonists. The synthesized imidazole analogues bear a biphenylmethyl tetrazole moiety at the 1-position and a bulky lipophilic and electron-withdrawing group such as halogen atom at the 5-position of the imidazole ring. Additionally, the saturated acid side chain of the urocanic acid was lengthened by esterification, resulting in the methyl ester or the bulky ester group (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl of Olmesartan Medoxomil, which *in vivo* is metabolized to the carboxyl moiety and may proved an effective structural element, emerging to compounds with improved activity.

References

1. Carini D.J., Duncia J.V., Aldrich P.E., Chiu A.T., Johnson A.L., Price W.A., Santella J.B., III; Wells J., Wexler R., Wang C., Yoo S.E., Timmermans P.B.M.M.: *J. Med. Chem. 34*: 2525 (1991)
2. Yanagisawa H., Amemiya Y., Kanazaki T., Shimoji Y., Fujimoto K., Kitahara Y., Sada T., Mizuno M., Ikeda M., Miyamoto S., Furukawa Y., Koike H.: *J. Med. Chem. 39:* 323 (1996)



Tumor Antigen Vaccines in the Treatment of Lung Cancer

A. Koumarianou[1], S. Loukides[2], C. Kotoulas[3]

[1]Second Department of Internal Medicine, Medical Oncology Unit, [2]Department of Respiratory Medicine, Attiko General Hospital, Athens University, Athens, Hellas
[3]Department of Cardiothoracic Surgery, 401 General Military Hospital, Athens, Hellas

Tumor cells express membrane antigens that can serve as targets for vaccine treatment. One well studied antigen is MUC1 mucin, a self-antigen that is over-expressed in many adenocarcinomas such as lung and breast cancer. Several MUC1 mucin vaccine protocols have been developed during the past 15 years but with limited results. Limitations of cancer immunotherapy with vaccines are mainly due to a) immune suppression caused by the tumor itself, b) insufficient expression of co-stimulatory molecules on tumor, c) the presence of T regulatory cells suppressing the tumor specific immune response, d) trial errors such as the inclusion of patients with advanced disease or patients with insufficient expression of the antigen and e) the difficulty with the available vaccine approaches to induce a strong CD8 and CD4 cell response that drive strong cytolytic reactions.

There are many vaccine constructs (allogeneic tumor cells, protein and DNA preparations) currently under evaluation. It is not known which antigens are the most potent rejection stimulants nor which chemical construct is the most effective.

Similarly to immunization protocols in infectious diseases, it looks that tumor immunizations are needed to be conducted repeatedly to maintain a strong long-lasting immune response. The concomitant use of adjuvants and GM-CSF facilitate the break of tolerance and amplify the response. Cytokines such as IL-12 may skew the response towards a type 1 T-cell response where as IL-2 might not be useful as it expands T regulatory subpopulation. Induction of T regulatory cells might be abolished by the concomitant treatment with a low dose of cyclophosphamide.

Regardless the use of new generation drugs NSCLC remains an untreatable disease with a 5-year survival of approximately 15%. However, some encouraging clinical results in patients immunized with tumour vaccines have recently been published. In stage IIIB and IV NSCLC patients following chemotherapy a long peptide of

MUC-1 vaccine (Stumuvax) showed to prolong survival, 17.2 months for vaccine treated patients as compared to 13.0 months for non treated control group. Median survival for patients with stage III B and logo-regional disease was 30.6 months for the vaccine group as compared to 13.3 months for the control group. A world-wide phase III trial (START) is ongoing. There are also some other promising vaccine candidates that target EGF and TGF-b in NSCLC.

In conclusion, if therapeutic vaccines are planned to be studied alone, they should be applied in the adjuvant setting whereas in advanced disease they have to be combined with chemotherapy. It is expected that in the future therapeutic vaccines will be part of the trials for NSCLC.

❁

Comparative Conformational Analysis Study of Candesartan CV with Eprosartan and Losartan

E. Kritsi[1,2], S. Chatziaggeloglou[1,2], P. Zoumpoulakis[1], C. Potamitis[1], N. Papakonstantopoulos[2], T. Mavromoustakos[1,2]

[1] Laboratory of Molecular Analysis, Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Athens, Hellas
[2]Organic Chemistry Section, Chemistry Department, Kapodistrian University, Athens, Hellas

Our laboratory has been engaged, for long term, in the study of the conformational analysis of commercially available AT1 receptor antagonists (sartans) in a membrane simulated environments. Although sartans exert their activity through the same mechanism on the AT1 transmembrane receptor, they exert different pharmacological profiles because: (a) they adopt different location and orientation in the core of the membrane bilayers; (b) they exert different molecular interactions with the amino acids which are crucial for the antagonism in the active site. In order to comprehend the key interactions of pharmacophore segments with the amino acids that constitute the active site, we have already studied the conformational features of losartan, valsartan and eprosartan. Here, we are continuing our effort with conformational analysis of candesartan CV, the active metabolite of candesartan cilexetil. A conformational comparative analysis study will be made with the other already studied AT1 antagonists.

❁

Effects of 23S RRNA Mutations on the Interaction of Telithromycin with *Escherichia Coli* Ribosomes

O. Kostopoulou, A. Petropoulos, E. Kouvela, G. Dinos and D. Kalpaxis

Department of Biochemistry, School of Medicine, University of Patras

The rapid and sudden increase in erythromycin resistance led to the development of improved compounds, termed ketolides. Telithromycin represents one of the most distinguished member of ketolides, with improved activity against erythromycin-resistant bacteria. Kinetic and chemical footprinting analysis shows that telithromycin (I) interacts with *Escherichia coli* ribosomes (R) in a two-step process: $R+I \rightleftarrows RI \rightleftarrows R^*I$. The first step is rapidly equilibrated and involves a relatively low-affinity binding site, placed at the entrance of a tunnel for the exit of the nascent polypeptide chain. The second step represents a slow conformational change with an isomerization constant, K_{is}, equal to 58.9, which pushes the drug deeper into the tunnel, in a high-affinity site. Mutation of nucleoside U754 in 23S rRNA to adenosine moderately reduces the translocation of telithromycin to its final position (K_{is}=25.7), through minor effects on the forward and reverse rate constants. In contrast, mutation U2609C reduces 5-fold the shift of the drug to the high-affinity site and also destabilizes the final complex R^*I, leading to a value for K_{is} equal to 2.6. Taking into account that both ribosomal nucleosides have been crystallographically localized at the same region of the exit tunnel, our results emphasize the notion that some ribosomal residues, although placed at neighboring positions, may have entirely different contribution on the accommodation

of a drug into the ribosome.

Acknowledgments: We thank Sanofi-Aventis, Inc. for providing telithromycin.

❁

Design, Synthesis and Conformational Studu of Potent Cyclic LHRH Analogues

D. Laimou, M. Agelidakis, A. Vitoratou, T. Tselios*

Department of Chemistry, Section of Organic Chemistry, Biochemistry and Natural Products, University of Patras, Rio, Patras, Hellas

Luteinizing-hormone-releasing hormone (LHRH) plays a central role in the biology of reproduction. Synthetic LHRH analogues have proven valuable in the treatment of a wide variety of endocrinological and non-endocrinological disorders. LHRH was first isolated from mammalian hypothalami as the decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly.NH_2) (1). Following the elucidation of the amino acid sequence of LHRH native hormone, modifications to the sequence were developed in the expectation of greater potency and improved receptor-binding. LHRH analogues (agonists or antagonists) have achieved widespread clinical use for the control of reproduction. In this report, modified cyclic LHRH analogues (2,3) were designed and synthesized using the Fmoc/tBu methodology (4). Moreover, the conformational analysis of synthesized analogues was performed in order to explore the stereochemical requirements for agonistic activity. Computer calculations were performed on a Pentium IV workstation using MOE2008.10 as simulation software. The conformational study is of great value through pharmacophore analysis in order to rationally design and synthesize new potential non-peptide mimetics of LHRH with antitumor activity.

References

1. Schally A.V., Arimura A., Baba Y., et al.: *Biochem. Biophys. Res. Commun. 43*: 393-399 (1971)
2. Samant M.P., Hong D.J., Croston G., Rivier C., Rivier J.: *J. Med. Chem. 49*: 3536-3543 (2006)
3. Koerber S.C., Rizo R., Struthers S.R., Rivier J.: *J. Med. Chem. 43*: 819-828 (2000)
4. Tselios T., Daliani I., Probert L., Deraos S., Matsoukas E., Roy S., Pires J., Moore G, Matsoukas J.: *Bioorg. Med. Chem. 8*:1903-1909 (2000)

❁

New European Directives for the Treatment of Multiple Sclerosis: Immunomodifying Therapies and Symptomatic Treatments

E. Balogianni[1], G. Thomaidis[1], I. Daglis[1], E. Tsanaki[1], A. Kalpatsanidis[1], A. Orologas[1,2]

[1]Hellenic Society for Multiple Sclerosis
[2]Neurologic Clinics, B' Dept. of Neurology, *AHEPA* University Hospital, Thessaloniki, Hellas

Η Multiple Sclerosis Therapy Consensus Group (MSTCG), ομάδα ιατρικών συμβουλευτικών επιτροπών των ευρωπαϊκών εταιρειών Σκλήρυνσης κατά πλάκας (ΣΚΠ) δημοσίευσε το 2008 τις τελευταίες κατευθυντήριες οδηγίες της σχετικά με τις ανοσοτροποποιητικές θεραπείες και αναθεώρησε τις οδηγίες του 2006 για τη συμπτωματική αγωγή της νόσου.

Στην ανοσοτροποποιητική θεραπεία περιλαμβάνεται (α) η αντιμετώπιση της υποτροπής (ώσης) και (β) η τροποποίηση της πορείας της νόσου (ανοσοσοτροποποιητική θεραπεία). Στην πρώτη περίπτωση χορηγούμε *i.v.* υψηλή δόση μεθυλπρεδνιζολόνης-MP για 3+2 ημέρες. Εναλλακτικά χορηγείται MP per os. Προϋπόθεση χορήγησης αποτελούν το πλήρες ιστορικό, η επιβεβαίωση πως πρόκειται για ώση και η απουσία αντενδείξεων χορήγησης κορτικοστεροειδών. Η ανοσοτροποποιητική θεραπεία (Disease Modifying Treatment - DMT) με συνεχή *προληπτική* αγωγή μπορεί να εφαρμοστεί μετά από πιστοποίηση πως πρόκειται για ΣΚΠ και εκπλήρωση ορισμένων κριτηρίων όπως αυτά ορίζονται από την MSTCG. Στην κατηγορία αυτή περιλαμβάνονται

σκευάσματα Ιντερφερόνης-β (IFN-β), Οξικής γλατιραμέρης (Copaxone®), Αζαθειοπρίνης (Imuran®), Μιτοξανδρόνης (Novantrone) και Κυκλοφωσφαμίδης (Endoxane®) καθώς και το μονοκλωνικό αντίσωμα Natalizumab (Tysabri®). Καμία IVIG δεν έχει εγκριθεί προς το παρόν για τη θεραπεία της ΣΚΠ.

Με τη Συμπτωματική θεραπεία της Πολλαπλής Σκλήρυνσης γίνεται προσπάθεια να αντιμετωπιστούν τα συμπτώματα της νόσου χωρίς όμως να υπάρχει καμιά επίδραση στην εξέλιξη της ΣΚΠ. Αναλυτικότερα ο θεράπων Νευρολόγος, σε συνεργασία με άλλες ιατρικές ειδικότητες καθώς επίσης και με συμβολή του Φυσιοθεραπευτή, αντιμετωπίζει τα συμπτώματα συστηματικά με σκοπό τη βελτίωση της ποιότητας ζωής του ασθενούς και την παροχή της καλύτερης φροντίδας, ολοκληρωμένα και αποτελεσματικά. Στα συμπτώματα αυτά περιλαμβάνονται η κόπωση, διάφορα σύνδρομα Πόνου και γνωσιακές δυσλειτουργίες, η κατάθλιψη, οι επιληπτικές κρίσεις και τα οφθαλμοκινητικά συμπτώματα (Υπερπυρηνική Οφθαλμοπληγία και Νυσταγμός), Δυσφαγία, Δυσαρθρία και Δυσφωνία, Σπαστικότητα, Τρόμος και Αταξία, Νευρογενής Δυσλειτουργία του Εντέρου και Νευρογενής κύστη καθώς τέλος και Σεξουαλικές διαταραχές.

Σκοπός των παραπάνω οδηγιών είναι η καλύτερη κατάρτιση των ιατρών που ασχολούνται με την Σκλήρυνση κατά πλάκας με στόχο την ολοκληρωμένη και μεθοδευμένη αντιμετώπιση της νόσου με όλα τα εφόδια που προσφέρει ως τώρα η επιστημονική έρευνα.

Structure Elucidation of Aliskiren, a Potent Renin Inhibitor, Using Nuclear Magnetic Resonance (NMR) and Molecular Modeling Techniques

M.-T. Matsoukas[1], P. Zoumpoulakis[2], T. Tselios[1]

[1]Department of Chemistry, University of Patras, Rio, Patras, Hellas
[2]Laboratory of Molecular Analysis, Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, Athens, Hellas

Aliskiren is the first successful non-peptidic renin inhibitor in the pharmaceutical market which specifically and potently inhibits the human renin enzyme. The first synthetic renin inhibitors (i.e., remikiren and zanikiren) suffered from their poor oral bioavailability, rapid elimination, low efficacy and high cost of synthesis. X-ray crystallography of the recombinant glycosylated human renin receptor with aliskiren has already provided structural data of aliskiren's docking conformation. Here we present the conformational behavior of aliskiren in water solution, using high resolution 600MHz NMR spectroscopy, and molecular modeling techniques. Data on the interatomic distances between distant protons of the molecule's functional groups are combined with molecular dynamics simulations for inspection and evaluation of the drug's molecular properties. The obtained conformations of aliskiren in solution, are compared to those obtained from X-ray crystallography. Results will give insights for the structural characteristics of this antihypertensive agent and will be further used for *in silico* docking studies.

Cellular Parameters of a Remitting-Relapsing Multiple Sclerosis (RR-MS) Patient Refractory to Conventional Treatments and Bone Marrow Transplantation (BMT) who responded to Natalizumab in Comparison with other RR-MS Patients and Healthy Controls

A. Mouzaki[1], N. Dimisianos[2], P. Sakellaraki[1], M. Koutsokera[2], M. I. Garyfallou[1], Z. Dervilli[1], K. Chatzantoni[1] and P. Papathanasopoulos[2]

[1]Division of Hematology, Department of Internal Medicine, [2]Neurology Clinic, Medical School and University Hospital, University of Patras, Rio, Patras, Hellas

In severe, drug-resistant multiple sclerosis (MS), bone marrow transplantation (BMT) was intro-duced as a treatment option 15 years ago. Up to date, BMT has been applied to relatively

few patients worldwide with moderate success, and recent studies suggest that patients with early highly aggressive MS benefit most from this treatment approach. In this work we determined the peripheral blood lymphocyte populations of a patient (Patient A) with remitting-relapsing MS (RR-MS), refractory to conventional treatments, who underwent BMT, relapsed, and has being treated with natalizumab for the last 20 months. Eleven RR-MS patients in the acute phase of the disease untreated or treated with IFN-β and 20 healthy subjects were studied in parallel and served as controls. Natalizumab treatment of Patient A resulted in lymphocytosis, a relative increase in the percentage of CD20+ B-cells, especially the CD20+CD5+ subset, and CD4+CD25+FoxP3+ T regulatory cells (Tregs). The patient maintained relatively low CD3+ T-cells, especially the naive CD4+CDRA+ subset, and very low levels of CD3-CD56+ NK cells throughout. The Tregs of Patient A responded well in culture to a peptide mapping to a myelin basic protein antigenic epitope (mean 42% increase) than Tregs of healthy controls (mean 15% increase) whereas the Tregs of the MS controls did not respond to the peptide (mean 3% decrease). Since the beginning of natalizumab treatment, Patient A has had no relapses and his EDSS score has improved.

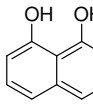
❁

Syntheses of Pharmaceutically Interesting Polyamine-Dithranol Conjugates

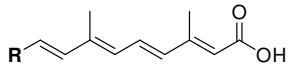
A. Boulnta, N. Tsiakopoulos, C.M. Athanassopoulos and D. Papaioannou

Laboratory of Synthetic Organic Chemistry, Department of Chemistry, University of Patras, Rio, Patras, Hellas

The present work refers to the synthesis of conjugates of the antipsoriatic drug dithranol with natural polyamines and acidic retinoids with potential antipsoriatic activity. Key-step in the synthesis is the efficient chemical modification of commercially available dithranol in a way which allows its conjugation to polyamine molecules and the preparation of symmetric and asymmetric (with retinoids) conjugates.



dithranol



acidic retinoid

❁

Solid Phase Peptide Synthesis of Cyclic(87-99) $MBP_{87-99}(Ala^{91,96})$ Using Microwave Irradiation on CLTR-Cl Resin

T. Neroutsos, I. Friligou, J. Matsoukas and T. Tselios

Department of Chemistry, Section of Organic Chemistry, Biochemistry and Natural Products, University of Patras, Rio, Patras, Hellas

We report the synthesis of an altered peptide ligand of Myelin Basic Protein [cyclic(87-99) $MBP_{87-99}(Ala^{91, 96})$] implicated in Multiple Sclerosis (MS). Microwave Enhanced Solid Phase Peptide Synthesis (MW-SPPS) has successfully been used for the fast synthesis of large peptide chains with high yields in terms of purity because of the enhancement of kinetic and thermodynamic of reactions (Fmoc deprotection and couplings), and with low racemization (1,2). The linear protected peptide was synthesized using the Liberty™ Microwave Peptide Synthesizer of CEM on 2-

chlorotrityl chloride resin (CLTR-Cl) (3). Fmoc deprotection was achieved with 20% Piperidine in DMF, while for the coupling reactions were used HOBt/DIC in DMF. After cleavage of the protected peptide from the resin, head to tail cyclization was carried out in solution utilizing TBTU/HOAT/2, 4, 6-collidine (4). The final crude product was purified by semi-prep HPLC and identified by ESI Mass Spectrometry.

References

1. Hayes B.L.: In: Microwave Synthesis: Chemistry at the Speed of Light. CEM Publishing, Matthews, NC., 2002
2. Rizzolo F., et al.: *Int. J. Pept. Res. Ther. 13*: 203 (2007)
3. Barlos K., et al.: *Angew. Chem. Int. Ed. 30*: 590 (1991)
4. Tselios T. et al.: *Bioorg. Med. Chem. Lett.*, **18**, 2713 (2000)

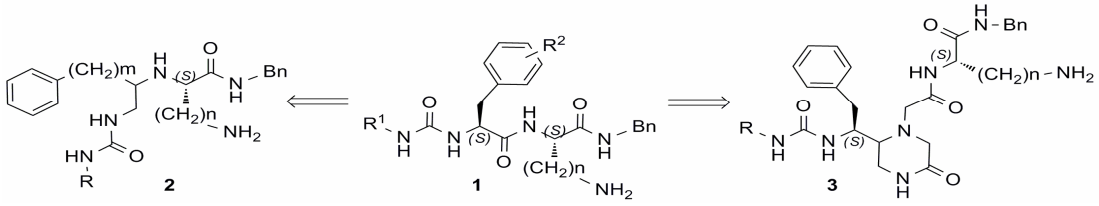
❁

Evaluation of New Peptidomimetic Antagonists of the Thrombin Receptor PAR1

I. Pappos[1], A.M. Valdivielso[2], V.-A. Pilar[2], M.T. Garcia-Lopez[2], M. Gutierrez-Rodriguez[2], R. Herranz[1], N.E. Tsopanoglou[1]

[1]Department of Pharmacology, Medical School, University of Patras, Rio, Patras, Hellas
[2]Instituto de Quimica Medica (CSIC), Juan de la Cievra 3, Madrid, Spain

In addition to the key role of thrombin in blood coagulation processes, this serine protease regulates multiple cellular responses, such as platelet aggregation and tumor cell proliferation. These cellular effects are mainly mediated by the activation of the protease-activated receptor PAR1. This receptor is activated by the thrombin-catalyzed cleavage of the N-terminal extracellular domain at the Arg^{41}/Ser^{42} peptide bond, which unveils the recognition sequence SFLLRN that acts as a tethered activation ligand. New peptidomimetic molecules have been composed and desinged as likely PAR1 antagonists. The first potent PAR1 antagonists were SFLLRN-based peptidomimetic ureas, represented by the general formula *1*. Taking into account these precedents, we have designed two series of urea derivatives of general formulas *2* and *3* as potential PAR1 antagonists. In this study, the new synthesized molecules were evaluated in SFLLRN-induced platelet aggregation assay and the first results are presented.



❁

Antiproliferative Activity On Prostate Cancer Cells, Enzymatic Stability and Conformational Studies of New GnRH Analogues

E.V. Pappa[1], A.A. Zompra[1], Z. Spyranti[1], Z. Diamantopoulou[2], F.N. Bazoti[3], F.N. Lamari[1], P. Katsoris[2], G.A. Spyroulias[1] and P. Cordopatis[1]

[1]Department of Pharmacy, [2]Department of Biology, University of Patras, Rio, Patras, Hellas
[3]*GAIA* Research Centre, The *Goulandris* Natural History Museum, Athens, Hellas

Analogues of GnRH, including [DLeu[6], *des*Gly[10]]-GnRH-NHEt (Leuprolide®, commercially available), have been widely used in oncology to induce reversible chemical castration. Several studies have provided evidence that besides their pituitary effects, GnRH analogues may exert direct anti-proliferative effects in tumor cells. In order to study the effect of modifications in position 4 and 6 of GnRH on prostate cancer cell proliferation we synthesized twelve new GnRH ana-

logues. To improve enzymatic stability, NMeSer was incorporated in position 4 and the rate of hydrolysis by a-chymotrypsin and subtilisin was investigated. Our results demonstrate that this incorporation increases enzymatic stability in all analogues of GnRH, while the antiproliferative effect on PC3 and LNCaP prostate cancer cells is similar to that of leuprolide. Conformational studies have been performed in an attempt to elucidate structural changes occurring upon substitution of native residues and to study structure-activity relationship for these analogues. The solution models of [DLeu^6, *des*Gly^{10}]-GnRH-NHEt (leuprolide), [NMeSer^4, DGlu^6, *des*Gly^{10}]-GnRH-NHEt, [Glu^6, *des*Gly^{10}]-GnRH-NHEt and [DGlu^6, *des*Gly^{10}]-GnRH-NHEt peptides have been determined through 2D NMR spectroscopy in DMSO-d_6. NMR data provide experimental evidence for the U-turn like structure preserved in all four analogues, which could be characterized as β-hairpin conformation.

Chemical Composition Mapping of Mandible Bones Using Spectroscopic Techniques

M. Pelardis[1], K. Lianos[2], S. Panteliou[2], C. G. Kontoyannis[1], M. G. Orkoula[1]

[1]Department of Pharmacy, [2]Department of Mechanical Engineering and Aeronautics, University of Patras, Rio, Hellas

Raman spectroscopy was used for the chemical composition mapping of human mandible bone throughout the external surface. The depth profile, by analyzing the cortical and trabecular part of the specimen, was also recorded. It was found that the chemical composition in the center of the mandible is different than the composition at the edges. At the center the bone density is higher than the respective density at the edges (higher hydroxyapatite and collagen Raman intensity). Also the collagen to apatite ratio is also larger than the respective ratio recorded from the edges of the mandible indicating that not only there is less cortical bone density but also the mineral part of the bone is less than the organic part. It was also found that the chemical composition of the trabecular bone located in the center of the mandible differs considerably to the chemical composition of the trabecular bone at the edges of the specimen.

Expression of Histone Acetyltransferase and Deacetylase Genes in Neural Precursor Cells in the Presense of Trichostatin-A and Cytokines, *in vitro*

K. Poulatsidou, R. Lagoudaki, E. Polyzoidou, S. Ravanidis, N. Tascos, M. Grigoriou, A. Chlichlia, N. Grigoriadis

Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Thessaloniki, Hellas

Introduction: Recent studies have proposed neural precursor cell (NPCs) transplantation for Multiple sclerosis (MS) therapy. Neuronal and oligodendrocyte precursor cell development and differentiation are modulated by histone acetyltransferases (HATs) and Histone deacetylases (HDACs), the enzymes that catalyze acetylation and deacetylation, respectively. In this study we investigated the mRNA expression of three HDACs (HDAC1, HDAC2, HDAC3) and two HATs (CBP, P300) in NPCs treated either with Trichostatin A (TSA) -an HDAC inhibitor- or cytokines, which simulate the inflammatory environment of MS.

Materials & Methods: NPCs isolated from newborn C57bl/6 mice were cultured as floating spheres for seven days. Groups of $5*10^5$ NPCs were treated a) with TSA in high or low concentration for 24 or 48 hours (TSA-h and TSA-l respectively), b) with cytokines for 48 hours (IFNg and TGFb groups), c) with vehicle as control. RNA extracted from each group was reversely

transcribed to cDNA and used for semi-quantitative Real time PCR analysis.

Results: HDAC1 (3.37 fold; P<0.0001) and HDAC2 (1.75 fold; P<0.0001) were significantly elevated in TSA-h 24h group. CBP (1.54 fold; P=0.0058) and P300 (3.36 fold; P<0.0001) showed increased expression in groups TSA-l 24h. In groups treated with cytokines no variance was observed in genes expression.

Conclusion: HDAC1, HDAC2, CBP and P300 genes seem to be up-regulated by TSA. In addition, either pro- or anti-inflammatory cytokines found in abundance in MS, were not adequate to cause changes in acetylation gene expression, *in vitro*. Further study of histone acetylation and differentiation of NPCs in inflammatory environment, could elucidate the mechanisms of action of inflammation at endogenous NPCs, in order to induce remyelination in MS.

Modulation of Expression of Chemokines and Their Receptors in Experimental Autoimmune Encephalomyelitis Model Following Transplantation of Neural Precursor Cells of Central Nervous System

S. Ravanidis, E. Polyzoidou, A. Lourbopoulos, R. Lagoudaki, K. Poulatsidou, N. Tascos, G. Mosialos, M. Arsenakis, N. Grigoriadis

Laboratory of Experimental Neurology and Neuroimmunology, AHEPA University Hospital, Thessaloniki, Hellas

Introduction: The transplantation of neural precursor cells (NPCs) from Central Nervous system (CNS), has been proposed as a therapy in Multiple sclerosis (MS) due to the immunomodulatory action which affects the host. Important molecules in MS pathogenesis are the chemokines, like CCL3 and CCL5 which contributes to the recruitment of macrophages and autoimmune CD4+ cells, respectively, inside the CNS. Their receptor is CCR1, which is expressed in activated macrophages during relapses in experimental autoimmune encephalomyelitis (EAE). In the present study, it was tested, if NPCs induce differences in chemokines' expression in CNS and in peripheral lymphocytes.

Materials and methods: NPCs were isolated from neonatal C57/Bl6 mice brain and were cultured for 7 days. EAE was induced in 15 female mice. On day 7 post EAE induction, either NPCs (NL group, n=8) or vehicle (CP group, n=7) were subcutaneously administered. During the acute phase of EAE, RNA was extracted from brain (B), spinal cord (S.C.) and lymphnodes, for semi-quantitative Real Time PCR. Unpaired t-test was used to evaluate the statistical significance.

Results: CCL3 (B:4.7 fold, p= 0.0381, S.C. p=0.7873) and CCL5 (B:2.72 fold, p=0.0164, S.C.:1.71 fold, p=0.003) expression was reduced in CNS of transplanted animals. CCR1 expression as well was reduced at peripheral lymphocytes compared to control group (22 fold, p<0.0001).

Conclusion: Our findings show that one perspective of immunomodulation of NPCs in EAE, may be the down regulation of the expression of CNS chemokines and their receptors, making them very important molecules for pharmaceutical intervention.

Chalcone Derivatives Designed as Anti-Inflammatory and Antioxidant Agents

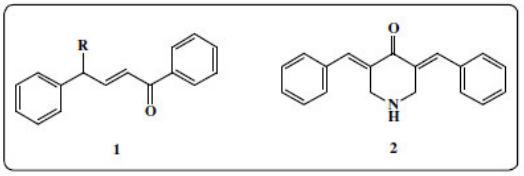
A. Repanas, A-M. Katsori, D. Hadjipavlou-Litina

Department of Pharmaceutical Chemistry, School of Pharmacy, *Aristotle* University, Thessaloniki, Hellas

Chalcones are open analogues of flavonoids, in which the two aromatic rings are joined by a three

carbon, α, β-unsaturated carbonyl system. These compounds seem to have cytotoxicity, antitumor, anti-inflammatory, antioxidant and many other biological properties. One of the main characteristic is that they interact with cellular thiols and less with hydroxyl and amino groups found in nucleic acids and thus, genotoxic side effects associated with many alkylating agents may be prevented (1,2). Dimmock and his coworkers attached styryl groups to the cyclic Mannich base 4-piperidone, formatting the rigid analogue *2*. This compound showed high activity toward the murine lympocytic leukemia P388/MRI cell line. Also the hydrochloride salt of compound *2* did not cause fatalities (3).

In our laboratory, we have synthesized several chalcones and Mannich bases as antioxidant/anti-inflammatory agents. Based on the fact that free radicals (4) and lipoxygenase (5) are implicated in cancer and the close relationship between cancer and chronic inflammation (6), we expanded our research synthesizing analogues of compound *1* and *2* by the base-catalyzed Claisen-Schmidt (7). We used suitable substituted aldehydes with acetophenone or 4-piperidone hydrochloride (8) under appropriate conditions. The compounds have been identified using IR, ^{1}H-NMR, ^{13}C-NMR, elemental analyses and mass spectroscopy. Lipophilicity as Rm values was determined using RPTLC. We present here the preliminary results for antioxidant and anti-inflammatory activity testing these new analogues *in vitro* and *in vivo*. The results are discussed in terms of structural characteristics. Further investigation is in progress for their anticancer activity.



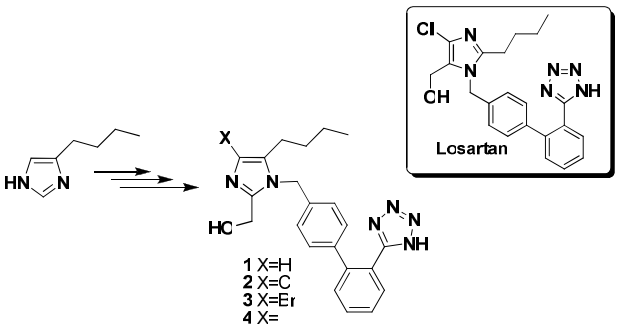
References
1. Dimmock J.R. et al.: *J.Med.Chem. 42:* 1358 (1999)
2. Rousseaux C.G. et al.: *Toxicol. Sci. 14*: 318, (1990)
3. Dimmock, J.R. *et al. Drug Des. Deliv.* 6, 183, **1990.**
4. Okada, F. *Redox Rep.* 7, 357-68. **2002**.
5. Romano, M.; Claria, J. *FASEB J.* 17, 1986, **2003**.
6. Mantovani, A. *et al. Nature***,** *454*, 436, **2008.**
7. Dannhardt G *et al. Arch. Pharm.* 326, 157-162, **1993**.
8. Dimmock, J. R., *et al. J. Med.Chem.* 44, 586-593, **2001**.

Acknowledges: A.-M. Katsori is thankful to *Bodossakis foundation* for PhD scholars

An Efficient Synthesis and Biological Evaluation of Imidazole AT1 ANG II Receptor Antagonists: Reorientation of Imidazole Pharmacophore Groups in Losartan Reserves Antihypertensive Activity

A. Resvani[1], K. Kelaidonis[1], J. Slaninová[2], J. Matsoukas[1] and G. Agelis[1]

[1]Department of Chemistry, University of Patras, Rio, Patras, Hellas
[2]Department of Biological Chemistry, University of Charles, Prague, Chech



The discovery of losartan has stimulated extensive research in the development of potent and selective antagonists. Herein, we describe a short, efficient and regioselective synthetic approach of AT1 Angiotensin II receptor antagonists based on 4(5)-butylimidazole in which the hydroxymethyl and butyl groups attached to the imidazole ring present different topographical positions compared to losartan. Furthermore, a halogen atom is introduced at the 5-position of

imidazole moiety as a lipophilic, electron-withdrawing substituent. The analogue *1* as well as the brominated analogue *3* showed high antihypertensive activity (pA_2 = 7.97, 7.58, respectively) similar to losartan (pA_2 = 8.33), indicating that reorientation of butyl and hydroxymethyl groups on the imidazole template retains antihypertensive activity.

References

1. Carini D.J., Duncia J.V., Aldrich P.E., Chiu A.T., Johnson A.L., Pierce M.E., Price W.A., Santella J.B., III., Wells G.J., Wexler R.R., Wong P.C., Yoo S.E., Timmermans P.B.M.W. M.: *J. Med. Chem. 34*: 2525-2547 (1991)
2. Wahhab A., Smith J.R., Ganter R.C., Moore D.M., Hondrelis J., Matsoukas J., Moore G.J.: *Arzn.-Forsch./Drug Res.* 43: 1157-1168 (1993)
3. Roumelioti P., Resvani A., Durdagi S., Androutsou M.-A., Kelaidonis K., Mavromoustakos T., Matsoukas J., Agelis G.: *Comput.-Aided Drug Des.* (Accepted for publication) 2010

Synthetic Studies on the Convergent Synthesis of ω-MVIIA Conotoxin

D. C. Tataraki, K. Barlos

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

A toxin is characterized as a poisonous substance produced by living cells or organisms. Among them omega-conotoxins are peptides of 24-30 amino acids in length with three disulfide bonds. A member of the family, omega conotoxin MVIIA is a potent and selective N type calcium channel blocker and is reported to be 100-1000 fold more potent than morphine as an analgesic but is not addictive. This conotoxin (also known as SNX-111) is under phase III clinical trials as an extremely potent analgesic, in patients who no longer respond to the administration of opioids. For the synthesis of ω-MVIIA we studied various approaches where fragments with a preformed disulfide bonds were used in the condensation reactions.

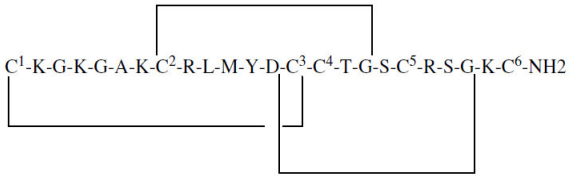
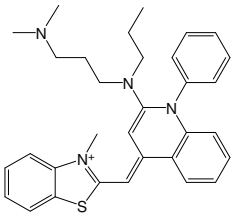


Figure 1: Primary structure of ω-MVIIA conotoxin

Photophysics of Sybr Green I Dye Intercalated in Single, Double and Triple Stranded DNA Complexes. A Femtosecond Time Resolved Study

M. Fakis, P. Persephonis, V. Giannetas

Department of Physics, University of Patras, Rio, Patras, Hellas



Sybr Green I (SG) is the most widely used dye for the fluorometric detection/determination of double-stranded DNA (dsDNA) with a high selectivity and sensitivity. It belongs to the family of asymmetric cyanine dyes consisting of two different heterocyclic ring systems (benzothiazolium and phenylquinolinium) linked via a single methine bridge. The fluorescence intensity of Sybr Green I increases when it is complexed with dsDNA due to restriction of the internal motion of SG as a result of the interaction with DNA. In this work, the photophysics of SG has been studied in free form and when bound in single, double and triple stranded DNA. It has been found that the fluorescence quantum yield (QY) of SG increases

dramatically when bound to DNA. The QY becomes highest when SG is bound in triple stranded DNA. In free form, SG exhibits ultrafast decay dynamics indicating the existence of large-amplitude internal motions. Upon binding to DNA, the dynamics becomes slower exhibiting four decay components. This is mainly due to the restriction of the internal motions of the dye caused by the relatively rigid environment of the dye complexed with DNA. The slowest decay dynamics are observed in triple stranded DNA indicating that this type of DNA provides the most rigid environment for the dye.

Corresponding author email: fakis@upatras.gr

❁

Solid-Phase Peptide Synthesis of a Multiple Antigen Peptide (MAP) Containing the Immunodominant Myelin Epitope MBP_{83-99} Using Microwave Irradiation

I. Friligou[1,2,3], F. Rizzolo[2,3], T. Tselios[1], J. Matsoukas[1], P. Rovero[3,4] and A. M. Papini[2]

[1]Department of Chemistry, University of Patras, Rio, Patras, Hellas
[2]International Laboratory of Peptide & Protein Chemistry & Biology, [3]Department of Chemistry *Ugo Schiff*, [4]Department of Pharmaceutical Sciences, University of Florence, Italy

Tam pioneered the synthesis and biological interest of multiple antigen peptide systems (MAPs), a distinct type of dendrimers. MAPs contain a lysine dendrimer scaffold bearing multiple copies of an antigenic peptide, which enhance its immunogenicity. MAPs with two, four, eight, or sixteen copies of synthetic peptide antigens can be produced by utilizing oligolysine cores with one to four sequential levels of lysine residues. Here we report the MW-SPPS of a MAP with two branches, containing the immunodominant epitope 83-99 of Myelin Basic Protein (MBP_{83-99}) implicated in Multiple Sclerosis. The C-terminus core matrix, consisting of an alternate Gly-Lys motif, was synthesized on Wang resin. Fmoc-Lys(Mtt)-OH was used in order to reach the desired branching level. On the N^{α} amino group of the resin-bound lysyl core matrix we synthesized stepwise the epitope MBP_{83-99}. After capping of the N-terminus and removal of Mtt group, the second copy of the antigen MBP_{83-99} was synthesized on the core. In order to prevent steric hindrance, β-Ala was used as a spacer between the core and the two antigens copies. The MAP synthesis was carried out on a Liberty^{TM} Microwave Peptide Synthesizer (CEM). We demonstrated that microwave energy can represent also in the case of MAPs solid-phase synthesis a fast and efficient way to enhance both Fmoc deprotections and coupling reactions, both critical steps in hindered dendrimers synthetic strategies.

❁

SPE-HPLC Method for Quantification of Crocetin in Human Plasma

D. G. Chryssanthi[1*], C. D. Georgakopoulos[2], P. Cordopatis[1], F. N. Lamari[1]

[1]Laboratory of Pharmacognosy & Chemistry of Natural Products, Department of Pharmacy,
[2]Department of Ophthalmology, School of Medicine, University of Patras, Rio, Patras, Hellas

Saffron (styles of *Crocus sativus* L.) is a well-known spice with many attributed therapeutic uses throughout centuries. Although studies have demonstrated that crocetin and crocins from saffron have various biological functions, issues concerning the route and way of saffron administration, the absorption and metabolism of saffron carotenoids in humans have not been answered yet. In the present study, an isocratic reversed-phase liquid chromatographic method was developed and validated for the determination of crocetin in plasma. Samples were pre-treated by solid phase extraction (recoveries >72%) and were chromatographed on a Luna C-18 column (4.6 mm x 250 mm, 5 μm) with a mobile phase consisting of methanol/water/trifluoroacetic acid (75.0/24.5/0.5, % v/v/v) at a flow rate of 1.0 ml min^{-1}. The HPLC method developed resulted in

sharp peaks at 10.7 (*trans*-crocetin) and 18.6 min (*cis*-crocetin), whereas the calibration curve of total crocetin in plasma displayed a good linearity for concentrations of 0.020 to 20 μM (R^2=0.999). Specificity, precision, accuracy and stability were also studied with spiked plasma samples and were acceptable. The developed method was applied to the determination of crocetin levels in plasma of four healthy human volunteers before and after consumption of one cup of saffron infusion (200 mg of saffron in water 80 °C for 5 min). Results showed that the concentration of crocetin was high after 2h (1.24 -3.67 μM) and still determined after 24 h (0.10-0.24). Interestingly, the percentage of the *cis*-isomer ranges from 25 to 50%, suggesting *in vivo* isomerization.

Acknowledgment: The authors acknowledge with thanks the Research Committee of University of Patras for financial support under the *K. Karatheodori* Grant C178.