Review of Clinical Pharmacology and Pharmacokinetics

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

VOLUME 28, 2014 ❁ No 1

Issue Devoted to Papers Presented at the
15th Conference with International Participation

Euromaster Gradate Program
Medicinal Chemistry
Drug Discovery and Design

Organized by the

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

April 9-11, 2014
Patras, Hellas

ISSN 1011-6583

Review of Clinical Pharmacology and Pharmacokinetics

INTERNATIONAL EDITION

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

• Chemical Abstracts • Elsevier' Bibliographic Databases: Scopus, EMBASE, EMBiology, Elsevier BIOBASE • SCImago Journal and Country Rank Factor

Further informations regarding the Journal can be seen in the web address: http://pharmakonpress.gr

Review of Clinical Pharmacology and Pharmacokinetics
INTERNATIONAL EDITION

☸

Published three times a year by PHARMAKON-Press
Publisher Responsible According to the Law
Helen S. Plessa
9A Kanari str., GR-1532, Nea Penteli, Athens, Hellas
Tel.- Fax 00302109756332
Email: splessas@otenet.gr & stplessas@hotmail.com

Τετραμηνιαία Έκδοση από την ΦΑΡΜΑΚΟΝ-Τύπος
Ιδιοκτήτης-Υπεύθυνος κατά το Νόμο Ελένη Πλέσσα του Σταύρου
Κανάρη 9Α, GR-15232, Νέα Πεντέλη, Αθήνα, Ελλάς
Τηλ.- Fax 00302109756332
Email: splessas@otenet.gr & stplessas@hotmail.com

☸

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

VOLUME 28 ❁ 2014 No 1

CONTENTS

Forewords

Abstracts Lectures

Abstracts Posters

GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS INTERNATIONAL EDITION

The Journal aims to promote optimum drug therapy by providing original papers and review articles covering important aspects of clinical and applied Pharmacology and Therapeutics. The focus of the Journal comprises drug evaluation reviews, which 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 *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry and Pharmacy of the University of Patras, Hellas*
- the *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*

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

INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

FIFTEEN YEARS GRADUATE PROGRAM
MEDICINAL CHEMISTRY

UNIVERSITY OF PATRAS
DEPARTMENTS OF CHEMISTRY AND PHARMACY

15th CONFERENCE
MEDICINAL CHEMISTRY

MEDICINAL CHEMISTRY:
Drug Discovery and Design

April 9-11, 2014
Conference and Cultural Center
University of Patras, Patras, Hellas

Letter from Guest Editor
Medicinal Chemistry Graduate Program
Drug Discovery and Design
A Program of Excellence

The *Medicinal Chemistry Graduate Program "Drug Discovery and Design* of the University of Patras has completed its 17th year of operation. The Program, a joint collaboration of the Departments of Chemistry, Medicine and Pharmacy, has been successful with outstanding research and academic activities in the field of Medicinal Chemistry. The Program has attracted the interest of world leading scientists for participation and research collaboration. Each year a distinguished scientist is honored for his/her contribution to Biomedical Research and Science.

So far the Program has honored outstanding scientists in the field: *Harald zur Hausen*, Nobel in Medicine and Physiology (2014); *Ada Yonath*, Nobel in Chemistry (2013); *Kleomenis Barlos* (2012); *James D. Watson*, Nobel in Medicine and Physiology (2011); *Andrew V. Schally*, Nobel in Medicine and Physiology (2010); *Dimitrios Nanopoulos* (2009); *Jean-Marie Lehn*, Nobel in Chemistry (2008); *Kyriakos Nikolaou* (2007); *Aristidis Patrinos* (2006); *Charalambos Gavras* (2005); *Konstantinos Sekeris* (2004); *Michael Maragoudakis* (2003); *Chris Platsoukas* (2002); *Athanasios Giannis* (2001); *Vasso Apostolopoulou* (2000).

The 15th Medicinal Chemistry Conference was held in the Cultural and Conference Center of the University of Patras on April 9-11, 2014. The Guest of Honor was *Harald zur Hausen*, Nobel in Medicine and Physiology. The title of Professor Harald zur Hausen Lecture was *Contributions of Infections to Human Carcinogenesis – Mechanisms and Perspectives***.** Professor Hausen was honored by the Department of Medicine of the University of Patras with the title of Doctor Honoris Causa.

The main research interests of the Program are focused on the Organic and Peptide Synthesis of Biomolecules, Rational Design with Aided Computer and Modeling Methods, Biological Evaluation *in vivo* and *in vitro*, Molecular Biology, Molecular Medicine, Toxi-cology, Biochemical Analysis, Pharmacognocy, Pharmacokinetics, Research Methods. The Program has organized, since 2000, fifteen Conferences with International Partici-pation.

Over three hundred students have graduated so far from the Program with MSc and PhD Degrees. The Program provides highly trained students to the benefit of the European Economy and Society. Emphasis is given to applied pharmaceutical research. Innovative Products and methods from the graduate students are published annually in high stand Journals. Graduate Research is the Key for Progress and Development in European and Worldwide dimension. The Program has been evaluated as a Centre of Excellence in Greece for its outstanding academic activities. The Program is an example of Excellence in the country, relating basic research with applied. It is the first graduate program in Greece, labeled by the title *Euromaster*, after evaluation by the ECTN Association.

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

The Guest Editor
John M. Matsoukas
Professor of Chemistry, University of Patras, Greece
Medicinal Chemistry: Drug Discovery and Design

15th Medicinal Chemistry Conference

Forewords

Official Opening: Introduction

John M. Matsoukas
Professor of Chemistry, University of Patras, Greece

It is a great Honor and privilege for all of us, to welcome Professor Harald zur Hausen in our Medicinal Chemistry Conference and in our University.

Professor Hausen has promptly accepted our invitation to participate in our 15th Conference, honoring our Program, our Students and our University. We are delighted, grateful and honored that he is today with us.

Professor Hausen won the 2008 Nobel Prize for his Discovery on the Role of the *Human Papillomavirus* in the cause of *cervical cancer*. This discovery is a scientific breakthrough in the cancer rerearch.

Today, with joy and pride we welcome Professor Hausen in our University and we celebrate his participation in our Conference.

Professor Hausen, from the deepest of our hearts, we all thank you for your contribution to Science and Society.

We also thank Professor Hausen for his great support to Greece and the Greek Universities with his letter *Support for Greece*, to the European Leaders. His letter, which was published in Science, was co-signed by other 23 Nobel Laureates. The letter had a great impact in Greece and Europe. Greece is not alone. Greece will overcome the problem and will be again, as in the past, in the fronts of Science, Innovation and Technology.

Professor Hausen thank you very much for your great contribution to Science and Society!

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

Miller School of Medicine, University of Miami, FL

Nobel Prize in Physiology or Medicine, 1977

It was my honor and pleasure in 2010 to participate in the 11th annual Medicinal Chemistry Conference in Patras and present 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.

An interesting international collaboration on LHRH analogs and medicinal chemistry in general was started with the group of Prof. John Matsoukas. In connection with this, I want again 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.

This year, the program committee has organized another conference in medicinal chemistry at the University of Patras. Prof. Harald zur Hausen, the winner of the 2008 Nobel Prize in Physiology or Medicine was invited as the guest of honor and plenary speaker for this 15th Medicinal Chemistry Conference. I want to congratulate the program committee for following in the splendid tradition of previous conferences. I also congratulate the students who will be presenting their research achievements. I applaud the international collaboration for the progress of science and humanity. *I wish all participants the best of success.*

May I also convey my warm congratulations for outstanding achievements to the University of Patras on its 50th Anniversary together with my best wishes. It has been a privilege for me to take part in the academic program of the University of Patras.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 28: 11-20 (2014)

Abstracts Lectures

Contributions of Infections to Human Carcinogenesis - Mechanisms and Perspectives

Harald zur Hausen and Ethel-Michele de Villiers

Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Key words: Human carcinogenesis, mechanisms, perspectives, role of Infections

At present slightly more than 20% of the global cancer incidence can be linked to infections. Although approximately two thirds of infection-linked cancers are linked to virus infections, Helicobacter pylori, as a bacterium, also plays a significant role. In addition, in Africa and South East Asia parasitic infections are of local importance for carcinogenesis. The mechanism by which infectious factors contribute to carcinogenesis is increasingly understood. Some agents act as direct carcinogens, where persistence and expression of specific genes is a necessary precondition for the resulting malignant phenotype. Others seem preferentially to act as indirect carcinogens, commonly by inducing inflammatory reactions with the production of mutagenic by-products. Immune suppression induced by human immunodeficiency viruses frequently activates other latently persisting potential tumorviruses (e.g. Epstein-Barr virus, human Herpes virus type 8 and others) and could result in cancer induction after about 3 to 15 years.

None of the known infections induces cancer as a direct consequence of infection. The long latency periods between initial infection and cancer development, ranging in part up to 60 years, result from the requirement for additional modifications in host cell genes or within the persisting viral genome. Without modifications, the affected genes apparently play a protective role in preventing cancer and developed during a long co-evolution between the human host and potentially carcinogenic agents.

Epidemiological data suggest the involvement of additional infections in widespread human cancers. This accounts in particular for cancers of the colon, possibly also for breast cancer and childhood leukemias and lymphomas. For colon and breast cancer nutritional factors have been incriminated, in particular the consumption of *red meat*. The available data suggest a pivotal role of beef (*Bos taurus*) meat and possibly dairy product consumption. The isolation of novel viral DNAs from cattle serum and successful transfection of this DNA into human cells will be reported.

Development of a Breast and an Ovarian Cancer Vaccine: What have we achieved in 20 Years?

Vasso Apostolopoulos

College of Health and Biomedicine, Victoria University, Melbourne VIC Australia.
E-mail: vasso@scientist.com

Key words: Breast, ovarian cancer, vaccine

Targeting antigens to dendritic cell receptors has recently become a popular approach to inducing effective immune responses against cancer antigens. Almost 20 years ago, however, we had

demonstrated that targeting the mannose receptor on dendritic cells lead to strong cellular immune responses. We conducted over 16 human clinical trials (Phase I, II, IIb) demonstrating the effectiveness of oxidized mannan-MUC1 (M-FP) in MUC1+ adenocarcinoma patients.

Breast Cancer Vaccine: In one trial, in a small pilot Phase III double blind placebo trial (conducted by Prof Vassilaros, Prolipsis Medical Centre, Athens Greece), of direct M-FP injection, in early stage breast cancer patients, after 5-8 years the recurrence rate in patients receiving placebo was 27 % (4/15) compared to no recurrences in those receiving M-FP (0/16). We recently published the 12-15 years follow-up of these patients which showed that the recurrence rate in patients receiving placebo was 60% (9/15). In those receiving immunotherapy (M-FP), the rate was 12.5% (2/16). We will now present the clinical data follow up, 16.5 years later. The preliminary evidence indicates that M-FP is beneficial in the overall survival of early stage breast cancer patients. This long term clinical follow-up of patients strongly supports the necessity for a large phase III study of direct M-FP injection in early stage breast cancer patients, to evaluate immunotherapy as an adjuvant treatment for breast cancer.

Ovarian Cancer Vaccine: Using an ex-vivo approach, rather than direct injection of M-FP, a Phase I trial demonstrated that generation of patients cells ex vivo into dendritic cells, pulsed with M-FP and re-injected back into the patients led to strong cellular responses which were sustained at 1 year in monitored patients. Two patients with clearly progressive disease (ovarian and kidney) at entry became stable after initial therapy and underwent further treatment for over 3 years. This data supported further clinical evaluation of dendritic cell-vaccine immunotherapy approach. Indeed, the vaccine has been licenced as CVac™, has completed numerous trials, has been approved by regulatory agencies and is being commercialised.

Sepsis Syndrome: From Research to Clinical Practice

C.A. Gogos and K. Akinosoglou

Department of Internal Medicine, University General Hospital of Patras, 26504, Rio, Greece

Key words: Sepsis syndrome

Sepsis represents a systemic inflammatory syndrome in response to infection, and is characterized by hemodynamic instability, hematologic parameter disturbance and multiple organ dysfunction. It currently remains a leading cause of mortality following cardiovascular events, posing significant financial burden on health care systems worldwide. Following trigger - usually consisting of host cell damage or pathogen associated molecular patterns - by an infectious pathogen, an inflammatory cascade is initiated, leading to pro-inflammatory agent secretion and immune cell activation, local and systemic inflammation and distant organ involvement. As time progresses, a compensatory anti-inflammatory response characterized by exhaustion and suppression of immune response takes place. The tight balance between these two stages determines outcome. Currently, prompt management with available antibiotics and intensive care represent the milestones of sepsis therapy. Even though, a trend of decreasing mortality has been observed during the last decades, incidence of severe sepsis cases is increasing, due to growing population of immune-compromised patients and drug resistant pathogens. At the time, research has focused upon attractive concepts of timely identification of septic patients through study of gene polymorphisms and product differential expression, therapeutic modulation of immune response in different stages, as well as biomarker identification for early diagnosis, prognosis and follow up of septic patients; all with encouraging pre-clinical results. Forward and reverse genetics have provided some useful insight in potential targets of future therapies, as well as, molecules that could serve as future biomarkers of sepsis time course, from early diagnosis to follow-up. However, results have been diverse and threshold values ambiguous in many of applied settings. In terms of therapy, approximately thirty six novel compounds, including pro- or anti-inflammatory mediators (i.e adhesion molecules, coagulation pathway mediators, cytokine/chemokines, NO, lipid derivatives etc) have been tested in clinical trials. Of those, 32 showed no clear

benefit, 3 worsened outcomes, while very much promising rhAPC has now been withdrawn. Lack in clinical translation of experimental data so far can be attributed in a variety of reasons, including redundancy of immune system molecules and overlapping pathways, need for *multimodal treatment* approach strategy, as well as, over-simplistic experimental designs that fail to simulate complex clinical settings, individual or pathogen variation. Interestingly, it appears that at the moment our rich armamentarium of bench resources is a long way from reaching its bedside target

❁

Single Chain Insulins: Novel High Tech Products

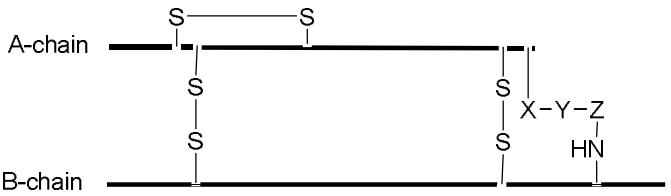
K. Barlos and D. Gatos

Department of Chemistry, University of Patras, 26504, Rio, Patras, Greece

Key words: Insulins single chain: Novel products

Η προϊνσουλίνη εμφανίζει το 1% της αντιδιαβητικής δράσης της ινσουλίνης. Παρ' όλο ότι είναι χημικά και βιολογικά σταθερότερη απ' αυτήν, δεν ήταν δυνατό μέχρι σήμερα να παραχθούν παράγωγα της προϊνσουλίνης με μεγαλύτερη δράση από αυτήν του 45% της ινσουλίνης. Ο λόγος είναι ότι για την βιολογική δράση είναι απαραίτητες η C-τελική καρβόξυλομάδα της Β-αλυσίδας και η Ν-τελική αμινομάδα της Α-αλυσσίδας που στην προϊνσουλίνη είναι δεσμευμένες.

Παράταση της δράσης των ινσουλινών επιτυγχάνεται με δύο βασικούς τρόπους. Στον πρώτο ρυθμίζεται το ισοηλεκτρικό σημείο της ινσουλίνης με τρόπο τέτοιο που αυτή να γίνεται δυσδιάλυτη και να καταβυθίζεται στο κύτταρο. Διαλυόμενη σταδιακά παρέχει παρατεταμένη προστασία σταθεροποιώντας τα επίπεδα ινσουλίνης στον οργανισμό. Στον δεύτερο τρόπο προστίθεται μία λυπόφιλη ομάδα στην πλευρική αμινομάδα της C-τελικής λυσίνης της Β-αλυσίδας. Η λυπόφιλη ομάδα αυξάνει την αυτοσυνένωση των μορίων της ινσουλίνης και την μεγαλύτερη διασύνδεσή της με την αλβουμίνη. Με αυτό τον τρόπο επιτυγχάνεται βραδεία απορρόφηση από το σημείο της ένεσης και παρατεταμένη παρουσία της ορμόνης στους ιστούς, με αποτέλεσμα παρατεταμένη δράση. Συνδυασμός του πλεονεκτήματος της χημικής και ενζυμικής σταθερότητας της προινσουλίνης και της τροποποίησης των ιδιοτήτων της πιστεύουμε ότι μπορούν να επιτευχθούν στα νέα μας παράγωγα της ινσουλίνης, στα οποία οι δύο αλυσίδες Α και Β συνδέονται μεταξύ τους μέσω μίας πλευρικής καρβοξυλομάδας της Α-αλυσίδας και της πλευρικής αμινομάδας της λυσίνης που ευρίσκεται στο τελικό άκρο της Β-αλυσίδας. Στα παράγωγα αυτά που παρουσιάζονται στο πάρα κάτω σχήμα, εισάγεται μία επί πλέον γέφυρα μεταξύ των Α και Β-αλυσίδων της ινσουλίνης. Με αυτό τον τρόπο αναμένεται σημαντική αύξηση της χημικής και ενζυμικής σταθερότητα των νέων παραγώγων συγκρινόμενων με την φυσική ινσουλίνη. Περαιτέρω βελτίωση των ιδιοτήτων τους αναμένουμε με την εισαγωγή μη φυσικών αμινοξέων στις αλυσίδες. Με τον τρόπο αυτό αποτρέπεται η ενζυμική αποικοδόμησή τους σε συγκεκριμένα ευπαθή σημεία. Επί πλέον η τρίτη γέφυρα X-Y-Z μεταξύ των αλυσίδων μπορεί να σχεδιασθεί έτσι, ώστε να προσδίδεται στα νέα παράγωγα η βέλτιστη διαλυτότητα, λυποφιλικότητα και δυνατότητα πρόσδεσης στον υποδοχέα.



Πλευρική διασύνδεση αλυσίδων ινσουλίνης. Ινσουλίνη μίας αλυσίδας.

❁

Immunotherapy of Experimental Autoimmune Encephalomyelitis Using APC-targeted Myelin Peptides as Prophylactic and Therapeutic Vaccines

Vivian Tseveleki[1], Theodore Tselios[2], Ioannis Kanistras[1], Olga Koutsoni[3], Irene Friligou[2], Vasso Apostolopoulos[4], Eleni Dotsika[3], John Matsoukas[2], Hans Lassmann[5] and *Lesley Probert*[1]

[1]Laboratory of Molecular Genetics, [3]Laboratory of Cellular Immunology, Hellenic Pasteur Institute, Athens, Greece; [2]Department of Chemistry, University of Patras, Rio, Patras, Greece; [4]Immunology and Vaccine Laboratory, Burnet Institute, Melbourne, Australia, [5]Division of Neuroimmunology, Brain Research Institute, Vienna, Austria

Key words: Encephalomyelitis autoimmune, myelin peptides APC-targeted

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS that shows autoimmune features and causes demyelination, early axonal injury and progressive neurological impairment. Current treatments induce non-specific immune or T cell suppression but are only partially effective and are often associated with adverse effects. Selective treatments that target the immune cells involved in the pathological processes are needed. One approach is to identify disease-associated auto-antigens and to induce antigen-specific T cell tolerance. Antigen-presenting cells, including dendritic cells, are important for priming T cell responses to antigen and for maintaining peripheral tolerance. We tested mannan-peptide conjugation as a method for targeting myelin peptides to antigen-presenting cells to induce T cell tolerance and resistance to central nervous system (CNS) inflammation in a mouse model for multiple sclerosis, named experimental autoimmune encephalomyelitis (EAE), that can be induced in mice by immunization with the 35-55 peptide of myelin oligodendrocyte glycoprotein (MOG). MOG peptide that was synthesized with a (Lys-Gly)5 linker and conjugated to mannan polysaccharide in its oxidized (OM) or reduced (RM) forms, but not unconjugated MOG or mannan, protected mice against EAE when administered in prophylactic or therapeutic protocols, with OM-conjugated peptides giving best results. Protection was peptide-specific, resistant to immune adjuvants and associated with normal T cell proliferation and priming of Th1 and Th17 cell responses to antigen *in vitro* and *in vivo* but failure of activated T cells to infiltrate the CNS and induce demyelination. The mechanism of tolerance appeared different to those previously described for DC-induced tolerance because populations of Th1 and Th17 cells, which are responsible for inducing EAE, and $CD4^{+}Foxp3^{+}$ regulatory T cells expand efficiently in the periphery of OM-MOG-treated animals. However, autoreactive T cells isolated from the tolerized mice displayed functional defects and the precise mechanism of OM-MOG-induced tolerance is currently under investigation. Overall, our results show that APC-targeting of myelin peptides by mannan conjugation is an efficient method for inducing peripheral T cell tolerance and reducing sensitivity to CNS autoimmune disease through the differentiation of antigen-specific Th1 and Th17 T cells with compromised effector functions. This approach shows promise as a therapeutic approach for the treatment of multiple sclerosis.

Elmyelin, a Novel Potential Vaccine in the treatment of Multiple Sclerosis: Preparation of IND File towards Phase I Clinical Investigation

John Matsoukas, Maria-Eleni Androutsou

Department of Chemistry, University of Patras; Eldrug S.A, Patras Science Park, Patras, Greece

Key words: Elmyelin, potential vaccine, multiple sclerosis, phase I clinical investigation

Multiple sclerosis (MS) is an autoimmune disease in which the body's immune system mistakenly attacks nerve fibres and their protective insulation, the myelin sheath, in the brain and spinal cord. Drug development for multiple sclerosis (MS) faces numerous challenges, with many drugs failing at various stages of development. Therefore safety and efficacy should be the two main topics to be considered in MS therapies.
This study includes the pre-clinical evaluation of Elmyelin in several experimental models of MS. Elmyelin is a mutated myelin epitope of MOG protein conjugated to mannan and has been evaluated in a number of *in vivo* and *in vitro* assays. This study focuses on the activities related to the manufacture of the drug substance such as the synthesis, highlighting the physicochemical parameters and analytical methodologies. Toxicity and stability studies are in progress with promising results so far. The final results will be included in the Investigator Brochure, part of the IND file to be submitted to the authorities for approval of Phase I clinical trials.

Hepcidin: A new Peptide Modulator of Erythropoiesis

Demetrios D. Vlahakos M.D.[1], Athena Drakou, M.D.[2]

[1]Professor of Medicine and Nephrology, [2]Research Fellow, Renal Unit, Attikon University Hospital, Athens University School of Medicine, Athens, Greece

Key words: Hepcidin, erythropoiesis

Iron is essential for cell life and especially for erythropoiesis, which is the major body consumer of iron for red cell production. Ferroportin is the major iron export protein located on the cell surface of enterocytes, macrophages and hepatocytes, the main cells capable of releasing iron into plasma for transport by transferrin.

Anemia is a common complication of chronic kidney disease (CKD) that develops early in the course of CKD, and becomes increasingly severe as the disease progresses. The management of anemia in CKD patients requires an appropriate balance between stimulating the generation of erythroblasts with erythropoetin and maintaining sufficient iron levels for optimum hemoglobin (Hb) production. Thus, assessing iron status is integral to both iron and anemia management in CKD patients, as iron is essential for Hb formation. Several factors may contribute to the development of iron deficiency in patients with CKD, including poor iron intake due to dietary restrictions, depression or cachexia, occult gastrointestinal bleeding due to uremic gastritis, chronic use of aspirin or anticoagulant agents, repeated phlebotomies for laboratory evaluation, and increased proinflammatory cytokines attending the uremic syndrome.

Hepcidin, a 25-amino acid peptide exclusively synthesized by the liver, is the main iron regulatory hormone via its ability to directly bind to ferroportin and decrease its functional activity by causing it to be internalized from the cell surface and degraded. The hepatocyte is thought to react to either an increase in iron saturation of transferrin or to increased iron stores in hepatocytes themselves, by inducing the synthesis of hepcidin by an as yet unknown mechanism. Thus the physiological response to iron overload under normal circumstances would be the hepcidin mediated shut down of iron absorption (enterocyte), recycling (macrophage) and storage (hepatocyte).

In addition, the synthesis and release of hepcidin is rapidly mediated by bacterial lipopolysaccaride and cytokine release, especially interleukin-6. Thus, hepcidin gene is an acute-phase responsive gene which is overexpressed in response to inflammation. Cytokine mediated induction of hepcidin caused by inflammation or infection is now thought to be responsible for the anemia of chronic disease, where iron is retained by the key cells that normally provide it, namely enterocytes, macrophages and hepatocytes. Retention of iron leads to the hallmark features of the anemia of chronic disease, with low transferrin saturation and high ferritin levels. The nature of the hepcidin receptor is presently unknown, however an exciting future prospect may be the development of agents to block the receptor in order to treat the anemia of chronic disease.

In a recent prospective observational study in iron-deficient adult nondialysis patients with stage 3, 4, or 5 CKD, we found that among various demographic, clinical, hematological and biochemical parameters, lower plasma hepsidin level prior to treatment emerged, as an inde-

pendent predictor of a clinically meaningful hemoglobin response to IV administration of ferric carboxymaltose (Ferinject). Similar, results have been reported in cancer patients, in whom those with the lower hepcidin levels were much more likely to have positive response to the IV iron plus darbepoetin combination. Therefore, we propose that serum hepcidin levels could help to distinguish a subset of patients who may have relative or absolute iron deficiency and may benefit from IV iron therapy.

❁

Biosensors: Progress and Prospective

Theodore Christopoulos

Professor, Department of Chemistry, University of Patras, Rio, Patras, Greece

Key words: Biosensors, progress, prospective

Η παρουσίαση περιλαμβάνει μια περιήγηση στις εξελίξεις στην επιστήμη και τεχνολογία των βιοαισθητήρων για τον προσδιορισμό μικρο- και μακρο-μορίων σε ποικιλία δειγμάτων. Η πρόοδος στην γονιδιωματική, πρωτεομική και μεταβολομική έδωσε ώθηση στην ανάπτυξη βιοαισθητήρων τόσο για τη διάγνωση και παρακολούθηση ασθενειών όσο και για την εφεύρεση νέων φαρμάκων. Η πρόοδος στην νανοτεχνολογία έχει προσφέρει πληθώρα νανοσωματιδίων και νανοδομών με καινούργιες οπτικές, ηλεκτρικές και μαγνητικές ιδιότητες, παρέχοντας νέες δυνατότητες για απλή και ταχεία ανίχνευση *in vitro* και *in vivo*.

❁

From the DNA Structure to Gene and to Genetic Medicine

G. Patrinos

Assoc. Professor of Pharmacogenomics, Department of Pharmacy, University of Patras, Patras, Rio, Greece (gpatrinos@upatras.gr)

Key words: Genetic medicine, DNA, gene

Κάθε υπέρβαση στην ιατρική επιστήμη, όπως τα αντιβιοτικά ή τα εμβόλια, είχε σαν αποτέλεσμα την βελτίωση των παρεχομένων ιατρικών υπηρεσιών επ' ωφελεία του ασθενούς. Η φαρμακογονιδιωματική εγκαινιάζει μια νέα εποχή στην εξατομικευμένη ιατρική. Η φαρμακογονιδιωματική χρησιμοποιεί γενετικούς δείκτες για την εξακρίβωση της ανταπόκρισης ενός ασθενούς σε συγκεκριμένη φαρμακευτική αγωγή με σκοπό την επίτευξη εξατομικευμένης θεραπείας. Αυτό κατέστη δυνατό όχι μόνο από την διαλεύκανση της αλληλουχίας του ανθρώπινου γονιδιώματος αλλά και από τις θεαματικές τεχνολογικές εξελίξεις της τελευταίας δεκαετίας στην μοριακή διαγνωστική. Η φαρμακογονιδιωματική όχι μόνο συμβάλλει στην εξεύρεση εκείνων των ασθενών στους οποίους ένα φάρμακο θα είναι οριακά ή και καθόλου επωφελές ή ακόμη και επικίνδυνο αλλά και στον περιορισμό της υπερκατανάλωσης φαρμάκων, μιας και τα φάρμακα δρουν αποτελεσματικά μόνο στο 30-50% των ασθενών. Επίσης, η φαρμακογονιδιωματική επιτρέπει την επιλογή της κατάλληλης, σε εξατομικευμένο επίπεδο, θεραπείας, μειώνει τις ανεπιθύμητες παρενέργειες και βελτιώνει την ανταπόκριση των ασθενών σε μια συγκεκριμένη θεραπεία. Επιπλέον, η φαρμακογονιδωματική βοηθά τις φαρμακευτικές εταιρείες να επιτύχουν καλύτερες κλινικές δοκιμές, να βελτιώσουν την παραγωγή φαρμάκων, να μειώσουν τον χρόνο, κόστος και ποσοστό αποτυχίας των κλινικών δοκιμών και να επαναφέρουν στο εμπόριο φάρμακα τα οποία είχαν αποτύχει σε προηγούμενες κλινικές δοκιμές. Γενικότερα, η φαρμακογονιδιωματική επιτρέπει τη μείωση του κόστους της ιατροφαρμακευτικής περίθαλψης. Παρολαυτά, θα πρέπει βαθμιαία να υπερπηδηθούν ηθικά, τεχνικά και κοινωνικά εμπόδια για την καθιέρωσή της φαρμακογονιδιωματικής στην κλινική πράξη.

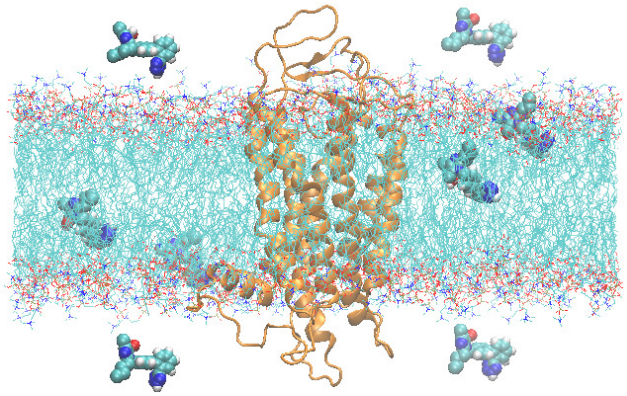
Drug: Membrane Interactions and Drug Discovery

Thomas Mavromoustakos

University of Athens, ,Department of Chemistry, Laboratory of Organic Chemistry, Zografou 15771, Athens, Greece, tmavrom@chem.uoa.gr

Key words: Membrane Interactions, drug discovery

The biological membranes were considered in the past as a barrier that allowed only necessary and useful molecules to enter the cell. Today, the knowledge at the molecular level of the role of biological membranes has increased considerably. The biological membranes constitute a complex molecular system that governs biological importance procedures. Different pharmaceutical molecules in order to exert their biological action must interact with their components. These drug-membrane interactions can determine their biological action. Therefore, these interactions are studied through biophysical methodologies in order to comprehend on their mode of action and design more efficient molecules.



The Smallest on line Bioreactor: Drug Development in the NMR Tube

Alexandra Chatzikonstantinou[1], Maria Chatziathanassiadou[1], Nisar Sayyad[1], Alexandra Primikiri[1], Efstathia Giannopoulou[3], Haralambos Kalofonos[3], John Matsoukas[4], Haralambos Stamatis[2], Ioannis P. Gerothanassis[1], Andreas G. Tzakos[1]

[1]Department of Chemistry, [2]Department of Biological Applications and Technologies, University of Ioannina, Greece
[3]Patras Medical School, University Hospital of Patras, [4]Department of Chemistry, University of Patras, Patras, Rio, Greece

Key words: Bioreactor on line, NMR tube, drug development

Developing a new drug is a complex costly and time-consuming process which can take 12-15 years and cost in excess of $1 billion. Currently, innovative pharmaceutical companies are facing huge financial problems due to the high attrition rates in their drug discovery schemes and the reduced likelihood of being able to produce a *blockbuster* to the market. An ultimate key is thus to reduce this attrition rate by transforming drug discovery into a high-throughput, rational process.

Natural products cover a very interesting chemical space of biological relevance due to their vast chemical diversity, and fine-tuning for

optimal interactions with biological macromolecules through evolutionary selection. However, their inherent scaffold intricacy and the associated complexity in their synthetic chemistry have challenged their effective integration in the drug discovery pipeline. To overcome this, we exploit chemoenzymatic tools to regioselectively enable rapid installation of chemical functional groups to the natural product core. In order to successfully elaborate the *in situ* and on line rapid enzymatic product formation monitoring of the enzymatic reactions the NMR tube was used as an efficient small bioreactor and

Herein we will present the capability of utilizing the NMR tube both as an efficient small enzyme bioreactor to perform biologically orientated synthesis but in the same time conducting hit to lead optimization, two of the most crucial steps in the early drug discovery process.

Macromolecular Micro-crystallography: Perspectives in Drug Discovery

Irene Margiolaki

Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, GR-26500 Patras, Greece, E-mail: imargiola@upatras.gr

Key words: Macromolecular micro-crystallography, drug discovery

The study of biological macromolecules in the absence of good quality single crystals is a challenging field which attracts particular scientific interest in recent years. The development of the X-ray powder diffraction methods and algorithms, has already allowed for the structural characterization of a range of proteins and has been established by our group as a useful complementary technique to the traditional single crystal diffraction techniques (1,2).

Protein polycrystalline samples consist of a large number of randomly oriented microcrystals. They often form in a short period of time and under different conditions of physico-chemical environment. To date, our studies on Urate Oxidase (3) and complexes of human insulin with organic ligands (4,5), revealed a high degree of polymorphism, as well as crystalline polymorphs which were not previously identified probably due to the lack of adequate tools to characterize microcrystalline protein precipitates. Part of these results and the potential use of the newly identified polymorphs in future drugs to treat epidemics such as diabetes correspond to the main topic of this talk.

REFERENCES

1. Margiolaki I., Wright J.P.: Powder crystallography on macromolecules. Acta Crystallogr. A *64*: 169-180 (2008)
2. Margiolaki I.: *Macromolecular Powder Diffraction*. International Tables of Crystallography, Volume H: Powder Diffraction (Invited Book Chapter to be available in 2014)
3. Collings I., Watier Y., Giffard M., Dagogo S., Kahn R., Bonneté F., Wright J.P., Fitch A.N., Margiolaki I.: Polymorphism of microcrystalline urate oxidase from Aspergillus flavus. *Acta Crystallogr. D Biol Crystallogr. 66*: 539-548 (2010)
4. Margiolaki I., Giannopoulou A.E., Wright J.P., Knight L., Norrman M., Schluckebier G., Fitch A.N., Von Dreele R.B.: High-resolution powder X-ray data reveal the T(6) hexameric form of bovine insulin. *Acta Crystallogr. D Biol Crystallogr. D69*: 978-990 (2013)
5. Karavassili F., Giannopoulou A.E., Kotsiliti E., Knight L., Norrman M., Schluckebier G., Drube L., Fitch A.N., Wright J.P., Margiolaki I.: Structural studies of human insulin co-crystallized with phenol or resorcinol via powder diffraction. *Acta Crystallogr. D Biol Crystallogr. D68:* 1632-1641 (2012)

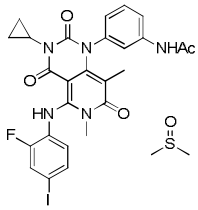
Trametinib: The first Approved MEK Inhibitor for the Treatment of Cancer

Gerasimos Rassias[*†], Richard A. Ward, Neil G. Stevenson, Katherine M. P. Wheelhouse, Kate L. Llewellyn, Augustine Ochen, Chris W. Jones, Elliot C. Hook

Global API Chemistry, GlaxoSmithKline Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY, UK

†Present address: Department of Chemistry, University of Patras, Rio 26504, Patras, Greece

Key words: Trametinib, MEK inhibitor, treatment of cancer



Trametinib dimethylsulfoxide (GSK1120212)

Dysregulation of the RAS/RAF/MEK/ERK signal cascade has been detected in over 30% of all human tumours and 70% of melanoma cases. MEK inhibition has been the subject of intense academic and industrial research and several MEK inhibitors have entered clinical trials, however, only trametinib has shown efficacy and acceptable pharmacokinetic and safety profile. It is the first drug of this type to receive regulatory approval from the FDA. This presentation focuses on the efforts in addressing the challenges involved with developing the commercial manufacturing process for trametinib capable of delivering the drug substance within specifications and on multi-kilogram scale. Central to this work has been the control of several key impurities based on extensive optimization experiments and an understanding of the mechanisms involved in the underlying processes spanning 10 synthetic steps. Several novel chemistry aspects are discussed with particular emphasis on a formamidine reduction with unusual selectivity and a novel rearrangement step.

REFERENCES

1. Gilmartin A.G.: Bleam M.R., Groy A., Moss K.G., Minthorn E.A., Kulkarni S.G., Rominger C.M., Erskine S., Fisher K.E., Yang J., Zappacosta F., Annan R., Sutton D., Laquerre S.G.: GSK1120212 (JTP-74057) is an inhibitor of MEK activity and activation with favorable pharmacokinetic properties for sustained *in vivo* pathway inhibition. *Clin. Cancer Res. 17*: 989-1000 (2011)

2. Infante J.R., Fecher L.A., Falchook G.S., Nallapareddy S., Gordon M.S., Becerra C., DeMarini D.J., Cox D.S., Xu Y., Morris S.R., Peddareddigari V.G., Le N.T., Hart L., Bendell J.C., Eckhardt G., Kurzrock R., Flaherty K., Burris H.A 3rd, Messersmith W.A.: Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: a phase 1 dose-escalation trial. *Lancet Oncol. 13*: 773-781 (2012).

3. Falchook G.S., Lewis K.D., Infante J.R., Gordon M.S., Vogelzang N.J., DeMarini D.J., Sun P., Moy C., Szabo S.A., Roadcap L.T., Peddareddigari V.G., Lebowitz P.F., Le N.T., Burris H.A. 3rd, Messersmith W.A., O'Dwyer P.J., Kim K.B., Flaherty K., Bendell J.C., Gonzalez R., Kurzrock R., Fecher L.A.: Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. *Lancet Oncol. 13*: 782-789 (2012)

4. Flaherty K.T., Robert C., Hersey P., Nathan P., Garbe C., Milhem M., Demidov L.V., Hassel J.C., Rutkowski P., Mohr P., Dummer R., Trefzer U., Larkin J.M., Utikal J., Dreno B., Nyakas M., Middleton M.R., Becker J.C., Casey M., Sherman L.J., Wu F.S., Ouellet D., Martin A.M., Patel K., Schadendorf D., METRIC Study Group. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med. 367*: 107-114 (2012)

5. Abe H., Kikuchi S., Hayakawa K., Iida T., Nagahashi N., Maeda K., Sakamoto J., Matsumoto N., Miura T., Matsumura K., Seki N., Inaba T., Kawasaki H., Yamaguchi T., Kakefuda R., Nanayama T., et al.: Discovery of a highly potent and selective MEK inhibitor: GSK1120212 (JTP-74057 DMSO solvate). *Med. Chem. Lett. 2*: 320-324 (2011)

Design and Development of New Silver, Organotin, and Antimony Metallo-drugs which Bind to Lipoxygenase and DNA, Modulating their Function and Inducing Apoptosis

Sotiris K. Hadjikakou

Section of Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece. e-mail: shadjika@uoi.gr

Key words:

The synthesis, characterization and biological activity of a series of silver(I), tin(IV) and antimony(III) complexes with thioamides or carboxylic acids is reported. Silver(I) complexes with arylphosphines (PR_3) and/or thioamides (HD) or carboxylic acids (HD or H_2D) of formulae $[AgX(HD)]_n$, $[AgX(PR_3)_n]$, $[AgX(HD)]_n$, $[AgX(HD)(PR_3)_n]$, or $[Ag(D)(PR_3)_n]$, organotin(IV) complexes of formulae $[[R_3Sn(D)]$, $[R_2Sn(D)_2]$, $\{[R_3Sn]_2(D)]$ or $[R_2SnCl(D)]$ {R is Phenyl, *n*-Butyl or Methyl-group}, and antimony(III) complexes of thioamides with formulae $\{[SbX_2(HD)_4]^+ X\}$, $[SbX_3(HD)_2]$, $\{[SbX_2(HD)_2]_2\}$,

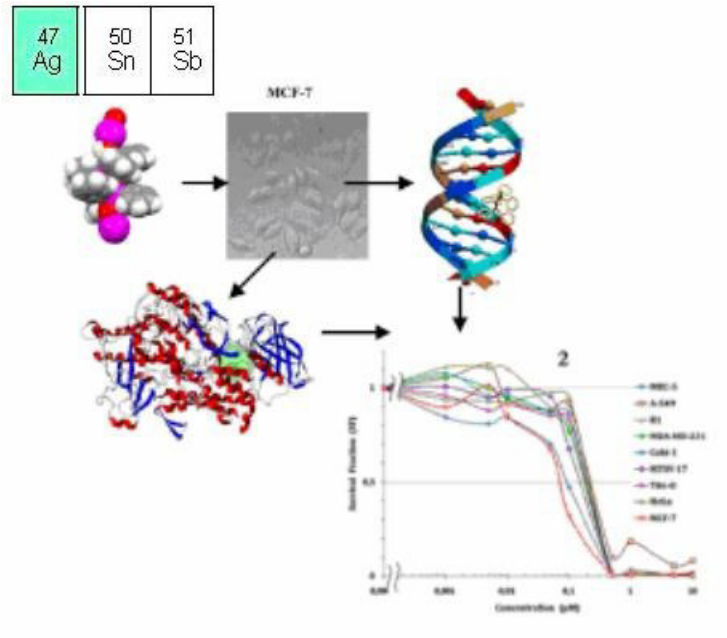
were synthesized and characterized by spectroscopic (NMR, IR, Raman, Mossbauer etc) and X-Ray diffraction techniques. Theses complexes cause *in vitro* cells death against leiomyosarcoma (LMS), cervical (HeLa), ovarian (OAW-42), breast, ER positive (MCF-7), breast, ER negative (MDA-MB-231), lung (A549), renal (Caki-1) cells and against the normal human lung cell line MRC-5 (normal human fetal lung fibroblast cells) and normal immortalized human mammary gland epithelial cell line (MTSV17) through apoptosis. Their activities are compared with that of cisplatin. Their mechanism of action is studied by the mean of their inhibitory activity towards the enzyme lipoxygenase (LOX) in relation to their binding properties to calf thymus (CT) DNA. Conclusions are drawn on structure activity relationship.

REFERENCES

1. Balas V.I., Banti C.N., Kourkoumelis N., Hadjikakou S.K., Geromichalos G.D., Sahpazidou D., Male L., Hursthouse M.B., Bednarz B., Kubicki M., Charalabopoulos K. Nick HadjiliadisN: Structural and in vitro biological studies of organotin(IV) precursors; selective inhibitory activity against human breast cancer cells, positive to estrogen receptors. *Aust. J. Chem. 65*: 1625-1637 (2012)

2. Shpakovsky D.B., Banti C.N., Beaulieu-Houle G., Kourkoumelis N., Manoli M., Manos M.J., Tasiopoulos A.J., Hadjikakou S.K., Milaeva E.R., Charalabopoulos K., Bakas T., Butler I.S., Hadjiliadis N.: Synthesis, structural characterization and in vitro inhibitory studies against human breast cancer of the bis-(2,6-di-tert-butylphenol)tin(IV) dichloride and its complexes. *Dalton Trans. 41*: 14568-14582 (2012)

3. Banti C.N., Giannoulis A.D., Kourkoumelis N., Owczarzak A.M., Poyraz M., Kubicki M., Charalabopoulos K., Hadjikakou S.K.: Mixed ligand-silver(I) complexes with anti-inflammatory agents which can bind to lipoxygenase and calf-thymus DNA, modulating their function and inducing apoptosis. *Metallomics 4*: 545-560 (2012)



REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 28: 21-46 (2014)

Abstracts Posters

Docking Studies on Coumarin and Quinolinone Derivatives as $sPLA_2$ GIIA Inhibitors

S. Vasilakaki, T. Mavromoustakos, G. Kokotos

Department of Chemistry, Laboratory of Organic Chemistry, University of Athens, Zografou GR-15771, Athens, Greece

Key words: $sPLA_2$ GIIA Inhibitors, coumarin, quinolone derivatives

Secretory phospholipase A_2 is an enzyme related to inflammatory symptoms contributing to arachidonic acid metabolism. It has been proved that activity of $sPLA_2$ group IIA is associated with inflammatory diseases such as rheumatoid arthritis (1). We docked the substituted heterocyclic systems of coumarin and quinolinone on the active site of $sPLA_2$ GIIA (PDB:1KQU), using the docking program GOLD 5.2. The structures were sketched and optimized using Sybyl 8.0 molecular modeling package. The ring substitutions of the well known for their inhibitory activity Methyl Varespladib and Me-Indoxam (2,3) were applied on the heterocyclic systems of coumarin and quinolinone. We also docked 2-oxoamides derivatives of coumarin and quinolinone based on the fact that long chain α-ketoamide derivatives of α-amino acids have been proved a novel class of inhibitors of $sPLA_2$ GIIA (4).

Acknowledgmends: This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program *Education and Lifelong Learning* of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

REFERENCES

1. Dennis E.A., Cao J., Hsu Y.H., Magrioti V., Kokotos G.: Phospholipase A2 enzymes: Physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev. 111*: 6130-6185 (2011)
2. Tomita Y., Jyoyama H., Kobayashi M., Kuwabara K., Furue S., Ueno M., Yamada K., Ono T., et.al.: Role of Group IIA Phospholipase A2 in rat colitis induced by dextran sulfate sodium. *Eur. J. Pharmacol. 472*: 147-158 (2003)
3. Singer A.G., Ghomashchi F., Le Calvez C., Bollinger J., Bezzine S., Rouault M., Sadilek M., Nguyen E., et al.: Interfacial kinetic and binding properties of the complete set of human and mouse groups I, Ii, V, X, and XII secreted phospholipases A2. *J. Biol. Chem. 277*: 48535-48549
4. Mouchlis V.D., Magrioti V., Barbayianni E., Cermak N., Oslund R.C., Mavromoustakos T.M., Gelb M.H., Kokotos G.: Inhibition of Secreted Phospholipases A_2 by 2-Oxoamides Based on α-amino acids: Synthesis, in vitro evaluation and molecular docking calculations. *Bioorg. Med. Chem. 19*: 735-743 (2011)

❁

Study for the Effect of p68, RNA-Helicase on the Growth of different Types of Cancer *in vitro* and *in vivo*

Kleio Katsikani, Georgios Stathopoulos

Physiology Laboratory, Department of Medicine, University of Patras, Rio GR-26504, Patras, Greece

Key words: p68, RNA-helicase, cancer

An inheritable genomic function, which is not based on the sequence of the denuded DNA can be characterized as epigenetic. Such functions allow the same genotypes to give different phenotypes and there are a large number of chemical modifications that affect different epigenetic levels. Genomic methylation patterns are epigenetic changes that play a key role in the growth of mammals and differentiate in cancer. The enzymes responsible for the creation, main-

tenance and differentiation of these patterns are methyltransferases (Dnmts), which methylate, de novo, certain spots on the DNA. These enzymes show no sequence-specific affinity to DNA, so guidance to specific epitopes and modulation of the activity must be mediated by other molecules with which they interact. The helicase p68, also known as Ddx5, has been identified as directly interacting with members of the Dnmts family and is investigated as a potential tumor promoter. The non-protein coding RNAs are believed to be an additional level of epigenetic control due to their ability to establish epigenetic milestones and control gene expression. The effect of p68 on them is clear, since it is an RNA helicase and it collocates with RNA of all types, particularly in the nucleus but in the cytoplasm as well. The Ddx5 is a member of the DEAD-box protein family and is an RNA-dependent ATPase. It also effects genes and their transcription at the stage of the initiation of transcription as a transcriptional co-activator or a pro-transcriptional factor and promotes cell proliferation and cell survival.

Through transfections of cell lines, immunoblotting biochemical techniques and immunocytochemical and immunohistochemical techniques the different expression levels of p68 helicase were identified in a number of cell lines. Experimental protocols of cell culture were selected for the *in vitro* study of the effect of Ddx5 on growth of specific tumor cell lines. The study was completed with *in vivo* experiments designed for C_{57}Bl/6 mice, *via* intrapleural or subcutaneous infusion of tumor cells genetically modified to express different levels Ddx5.

In vitro Study of the Effects of Imiquimodin Isolated Peripheral Blood Cell Populations (PBMC) of Patients with Recurrent *Herpes labialis* and Healthy Controls

Marianthi Kosma[1], Maria Rodi[1], Efi Pasmatzi[2], Alexandra Monastirli[2], Dionysios Tsambaos[2] and Athanasia Mouzaki[1]

[1]Laboratory of Immunohematology, Division of Hematology, Dpt. of Internal Medicine and [2]Dpt. of Dermatology, Faculty of Medicine, University of Patras, Rio 26504, Patras, Greece

Key words: Imiquimolin, Peripheral Blood Cell Populations, *Herpes labialis*

Imiquimod, is an imidazoquinoline, agonist of TLR-7, with antiviral and immunomodulatory activities (1,2). In this study, we isolated PBMC from patients with recurrent *Herpes labialis* and healthy subjects (controls) and cultured them ±imiquimod for 12h. At the end of the culture period, we isolated the PBMC populations $CD14^+$, $CD3^+CD25^+$, $CD3^+CD8^+CD25^-$ and $CD8^-CD4^+CD25^-$ using magnetic beads, extracted their RNA and performed real-time-PCR to estimate the gene expression of cytokines and the transcription factor FoxP3. Our results show that imiquimod increased the baseline expression of IL-6, IL-10, TGF-β1 and to a lesser extend IFN-γ in $CD14^+$ cells, and FoxP3 expression in $CD3^+CD25^+$ cells, of patients, whereas it did not affect the expression of IL-2, IFN-γ andIL-17 in any cell population. We conclude that imiquimod affects specifically monocytes and T regulatory cells whereas it has no effect on Helper and cytotoxic T-cells.

REFERENCES

1. Tsambaos D., Rodi M., Pasmatzi E., Monastirli A., Papadaki H., Mouzaki A.: Long-term remission of recurrent herpes labialis following topical imiquimod application on distant healthy skin:a clinical and immunological study. *Antivir. Ther. 16*: 863-869 (2011)

2. Spruance S.L., Kriesel J.D.: Treatment of herpes simplex labialis. *Herpes 9(3)*:64-69 (2002)

Comparative *in vitro* Evaluation using full Skin and Stratum Corneum in the Transdermal Delivery of Losartan for the Treatment of Hypertension

Alexia-Maria Karagianni[1], Maria-Eleni Androutsou[1,2], Michaila Michalatou[1], Markos Antonopoulos[3], George Agelis[1,2], Theodore Tselios[1], John Matsoukas[1,2]

[1]Department of Chemistry, University of Patras, Rio 26500, Patras, Greece; [2]Eldrug S.A., Platani, Rio, 26504, Patras, Greece, [3]King's College London, School of Biomedical and Health Sciences, London, UK

Key words: Losartan, transdermal delivery, full skin, stratum corneum

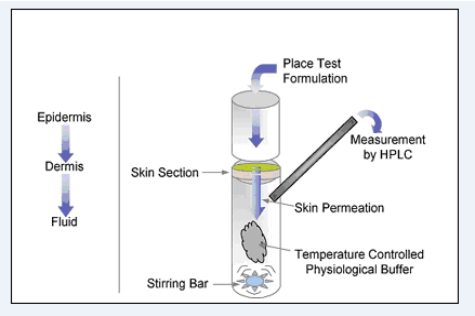


Figure 1: Franz Diffusion Cell (http://home.comcast.net/~rhdesign/2006conrexweb/studies_franz.html)

Transdermal drug delivery systems offer pharmacological advantages compared to other routes of administration. Our objective was a comparative study of transdermal drug delivery using full skin and stratum corneum which is the main barrier of the skin. Various solvents/permeation en-hancers such as azone, terpenes, glycols etc. were used for the better diffusion of losartan through the skin. The results showed that a significant higher flux was achieved using the stratum corneum which appears a threefold in-crease in the diffusion flux (12.8 μg/cm^2* h) com-pared to the experiments using full skin (4.07 μg / cm^2 * h).

REFERENCES

1. Keleb E., Sharma R.K., Mosa E.B., Aljahvi A.Z.: Transdermal drug delivery system-design and evaluation. *Int. J. Adv. Pharm. Sci. 1* : 201-211 (2010)
2. Trommer H., Neubert R.H.H.: Overcoming the stratum corneum: Modulation of skin penetration. *Skin Pharmacol. Physiol. 19*: 106-121 (2006)

Role of *KRAS* Mutations in Neoplasmatic Activation of Nuclear Factor-Kappa B

Chara Apostolopoulou, Antonia Marazioti, Ardiana Moustaki, Theodora Agalioti, Anastasios Giannou, Georgios Stathopoulos

Laboratory for Molecular Respiratory Carcinogenesis, Department of Physiology, School of Medicine, University of Patras, Rio 26504, Patras, Greece

Key words: *KRAS* Mutations, neoplasmatic activation, Nuclear Factor-Kappa B

Introduction: *KRAS* mutations are correlated with activation of Nuclear Factor-kappa B (NF-κB) and promote the progression of cancer. However, the underlying mechanisms of oncogenic activation of NF-κB are not very well understood.
Purpose of this study: Here, we investigate the role of *KRAS* mutations in the constitutive and inducible activation of NF-κB.
Material and Methods: Cancer cell lines isolated from C57BL6 mice with (lung cancer adenocarcinoma LLC, colon cancer adenocarcinoma MC38, pleural mesothelioma AE17) or without (melanoma B16F10, pancreatic adenocarcinoma PAN02) *Kras* mutations were transfected with a plasmid reporter of NF-κB (NF-κB.GFP. Luciferase; pNGL). The expression of *Kras* and inhibitor of NF-κB kinases (IKKs) were modified with RNA interference (RNAi) and/or overexpression plasmids. The activation of NF-κB in cancer cells was studied before and after treatment with more than 50 different ligands.
Results: We find that the constitutive as well as the inducible activation of NF-κB after LPS/TNF is not correlated with the presence of *Kras* mutations in cancer cells. On the other hand, interleukin (IL)-1beta induces the NF-κB activation only in *Kras*-mutant cell lines. Our results indicate that the silencing of *Kras* abrogates the IL1beta-induced NF-κB activation, while the overexpression of Kras mutations in wild type *Kras* cell lines is not sufficient for the induction of NF-κB by IL-1beta. Further, we find that the oncogenic activation of NF-κB in colon adenocarcima cell line MC38 (*ΔKrasG13R*) is mediated *via* the alternative pathway since it is inhibited by RNA interference against *Chuk* and not by RNA interference against *IKBKB* or bortezomib.
Conclusion: KRAS mutations do not affect the constitutive but they configure the inducible activation of NF-κB, possibly playing a key role in

inflammatory interactions between tumor and immune cells.

Sponsors: The present study was supported by European Research Council.

❁

Interactions of Novel Metallo-drugs of Silver(I) Complex towards Intracellular Molecules

C.N. Banti[1], A.D. Giannoulis[1], N. Kourkoumelis[2], A.M. Owczarzak[3], M. Kubicki[3] and S.K. Hadjikakou[1]

[1]Inorganic and Analytical Chemistry, Department of Chemistry, [2]Medical Physics Laboratory, Medical School, University of Ioannina, 45110 Ioannina, Greece.
[3]Department of Chemistry, A. Mickiewicz University, Poznan, Poland
E-mail: shadjika@uoi.gr

Key words: Silver(I) metallo-drugs complex, intracellular milecules, interactions

Salicylic acid ($salH_2$) (or 2-hydroxy-benzoic acid), is a precursor of aspirin, while it has been recently shown that silver complexes of $salH_2$ possess strong antiproliferative activity (1). Lipoxygenase (LOX), on the other hand, is an enzyme which catalyzes the oxidation of arachidonic acid to leukotrienes, in an essential mechanism for the cell life, involving in inflammation mechanism (1). LOX inhibition is found to induce apoptosis (1). Thus, LOX inhibition provides a potential novel target for the treatment and chemoprevention for a number of different cancers. Moreover, the biomedical applications and uses of silver(I) complexes, are related to their antibacterial (2) which appear to involve interaction with DNA (2). Recently, silver(I) complexes have also been studied for their antitumor activity (2,3).
In the course of our studies in the field of metallo-drugs (1-3), we have synthesized and structural characterized a new mixed ligand Ag(I) complex with $salH_2$ and tris-p-tolylphosphine (tptp) ligands of formula $[Ag(tptp)_2(salH)]$ (*1*). The mechanism of inhibition activity of *1* and its ligands, towards the catalytic oxidation of linoleic acid to hydroperoxylinoleic acid by LOX was subsequently studied kinetically and theoretically. The binding properties of *1* on calf thymus (CT) DNA are also evaluated. In addition, the cytostatic activity of *1* against human breast adenocarcinoma cells (MCF-7) and human cervix adenocarcinoma (HeLa) proliferation were measured. The results are compared to that of cisplatin.

REFERENCES

1. Poyraz M., Banti C.N., Kourkoumelis N., Dokorou V., Manos M.J., Simcic M., Golic-Grdadolnik S., Mavromoustakos T., Giannoulis A.D., Verginadis I.I., Charalabopoulos K., Hadjikakou S.K.: Synthesis, structural characterization and biological studies of novel mixed ligand Ag(I) complexes with triphenylphosphine and aspirin or salicylic acid. *Inorg. Chim. Acta 375*: 114-121 (2011)
2. Banti C.N., Hadjikakou S.K.: Anti-proliferative and anti-tumor activity of silver(I) compounds. *Metallomics 5*: 569-596 (2013)
3. Banti C.N., Giannoulis A.D., Kourkoumelis N., Owczarzak A.M., Kubicki M., Hadjikakou S.K.: (2013) submitted for publication

❁

Synthesis and Characterization of New *Fac*-$[M(No)(L^1)(Co)_3]$ Mixed Ligand Complexes

C. Triantis,[1] A. Shegani,[1] C. Raptopoulou,[2] A. Terzis,[2] M. Pelecanou,[3] I. Pirmettis,[1] M. Papadopoulos[1]

[1]INRASTES, [2]IAMPPNM and [3]IB-A, NCSR Demokritos, Agia Paraskevi, Athens, Greece

Key words: *Fac*-$[M(NO)(L^1)(CO)_3]$, synthesis, characterization

In the present work, the synthesis and characterization of new neutral mixed ligand complexes *fac*-$[M(NO)(L^1)(CO)_3]$ is described, where M is Re or ^{99m}Tc, NO is quinaldic acid which acts as a bidentate monoanionic ligand and L^1 is imidazole (im) or tert-butylisocyanide (tisc) or cyclohexylisocyanide (cisc) which acts as a monodentate ligand. Quinaldic acid reacts with the

$[NEt_4]_2[ReBr_3(CO)_3]$ precursor and generates the intermediate aqua complex *fac*-$[Re(NO)(H_2O)(CO)_3]$, *1*. Subsequent addition of the corresponding L^1 produces the mixed ligand complexes *fac*-$[Re(NO)(L^1)(CO)_3]$, *2*: L^1 = im, *3*: L^1 = tisc, *4*: L^1 = cisc. All rhenium complexes have been characterized by elemental analysis, spectroscopic methods and X-ray crystallography. At technetium-99m level, the analogous complexes were prepared in high yield. The stable rhenium and technetium-99m *fac*-$[M(NO)(L^1)(CO)_3]$ complexes may be used as new *building blocks* in Radiopharmaceutical Chemistry.

❁

Synthesis, Characterization and Biodistribution of new *fac*-$[M(NO)(L^1)(CO)_3]$ Mixed Ligand Complexes for Imaging of Alzheimer's Disease

C. Triantis[1], A. Lazopoulos[1], M. Sagnou[2], M. Pelecanou[2], I. Pirmettis[1], M. Papadopoulos[1]

[1]*INRASTES and* [2]*IB-A, NCSR Demokritos,* Agia Paraskevi, Athens, Greece

Key words: *Fac*-$[M(NO)(L^1)(CO)_3]$, Alzheimer's disease

Complexes of ^{99m}Tc, which is the radionuclide of choice in Nuclear Medicine, are studied as potential diagnostic tools for Alzheimer's disease. In the present work the synthesis, characterization and evaluation of the neutral mixed ligand complexes *fac*-$[M(NO)(L^1)(CO)_3]$, where M is Re or ^{99m}Tc, NO is the bidentate ligand quinaldic acid and L^1 is *N*-[4-(benzothiazol-2-yl-phenyl)-3-isocyano-propionamide is presented. The L^1 bears the isocyanide group, which can act as a monodentate ligand, and the 2-(4´-aminophenyl)benzothiazole moiety which binds with high affinity to amyloid plaques of Alzheimer's disease. By employing the *fac*-$[Re(CO)_3]^+$ synthon, the *fac*-$[Re(NO)(L^1)(CO)_3]$, *1*, was prepared by a two step procedure. At ^{99m}Tc level, the analogous *fac*-$[^{99m}Tc(NO)(L^1)(CO)_3]$ complex, *1´*, was prepared in high radiochemical purity. Complex *1´* is stable and lipophilic. Its biodistribution in normal mice showed that it crosses the blood brain barrier to an adequate degree (0.42% ID/g).

❁

Novel Hydroxytyrosol (HT) Analogues as Potential Antioxidants and COX-2 Inhibitors

Charis Semidalas[1], Irene Tsertou[2], Ploumisti Gennaiou[2], Andromachi Tzani[2], Marina Roussaki[2], Christos Nixarlidis[3], Anastasia Detsi[2], Panagiotis Zoumpoulakis[1]

[1]Laboratory of Organic Chemistry, Department of Food Technology, Technological Educational Institution of Athens, Egaleo, Athens, Greece, [2]Laboratory of Organic Chemistry, School of Chemical Engineering, National Technical University of Athens, Athens, Greece, [3]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece

Key words: Hydroxytyrosol (HT) analogues, potential Antioxidants, COX-2 inhibitors

Hydroxytyrosol (HT) is one of the most important phytochemical constituents of olive oil incorporateed in the aglycon of oleuropein. HT possesses a very interesting pharmacological profile ranging from antioxidant, antimicrobial and anti-inflammatory to LDL oxidation inhibition and capacity to reduce the risk of coronary heart disease and atherosclerosis.The aim of the present research project is the design, synthesis, structure elucidation and conformational analysis of novel HT analogues. The structure-activity relationship information extracted by this study will help define the structural and electronic requirements to achieve optimum combined antioxidant and COX-2 inhibitory activity, identify lead compounds and design novel compounds with promising bioactivity profile.

Acknowledgement: This work is supported from the ARHIMIDES2012-2015, SECTORAL OPERATIONAL PROGRAMME, EDUCATIONAND LIFELONG LEARNING – ESPA 2007-2013

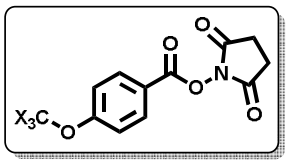
❁

N-Hydroxysuccinimidyl p-Methoxybenzoate as Suitable Derivatization Reagent for Isotopic Dilution Assay of Biogenic Amines in Food

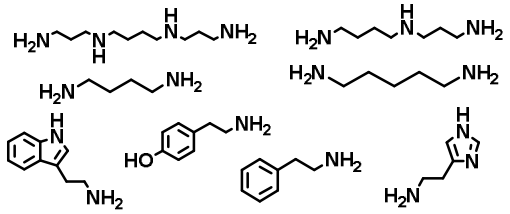
Christos Bakavos[1], Constantinos Athanassopoulos[1], Dionissia Pepe[1], Anna Napoli[2], Fabio Mazzotti[2] and Giovanni Sindona[2]

[1]Synthetic Organic Chemistry Lab., Department of Chemistry, University of Patras, Rio 26504, Patras, Greece
[2]Mass Spectrometry Lab., Department of Chemistry and Chemical Technologies, University of Calabria, Italy

Key words: *N*-Hydroxysuccinimidyl p-Methoxybenzoate, foods



d_0/d_3- 4-MBA-OSu X=H or X=D



Biogenic amines used for analysis

We present a simple methodology for the simultaneous identification and determination of biogenic amines in food matrices, based on the use of a stable isotope-coded derivatization and liquid chromatography tandem mass spectrometry. The tagging reagent was envisaged in *N*-hydroxysuccinimidyl ester of d_0/d_3-4-methoxybenzoic acid (d_0/d_3-4-MBA-OSu), which mainly functionnalizes primary amines and can be prepared easily from commercialy available sources. The identification and structural characterization of tagged biogenic amines was exploited in MALDI MS and MS/MS. Multiple-reaction monitoring in combination with Standard Isotope Dilution (SID-MS) has been applied in the assay of biogenic amines in different food stuffs, providing a method whose reliability is confirmed by the values of accuracy and by the calculated analytical parameters.

REFERENCES

1. Mazzotti F., Benabdelkamel H., Di Donna L., Athanassopoulos C.M., Napoli A., Sindona G.: Light and heavy dansyl reporter groops in food chemistry: amino acid assay in beverages. *J. Mass Spectrom. 47*: 932-939 (2012)

❁

Citrullinated Analogues of MBP_{82-98} in the Immunotherapy of Multiple Sclerosis

Constantina Christopoulou[1], George Deraos[2], George Agelis[2], John Matsoukas[1,2]

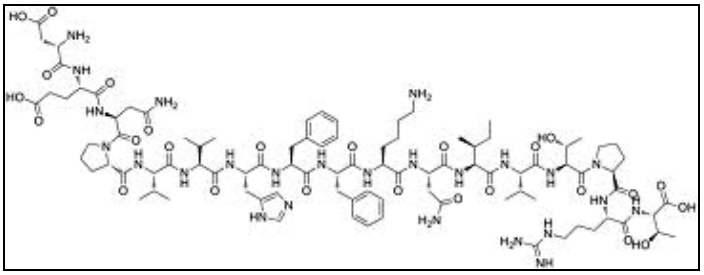
[1]Department of Chemistry, University of Patras, 26500, Patras, Greece, [2]Eldrug S. A., Pharmaceutical Company, 26504, Platani, Patras, Greece

Key words: MBP_{82-98} (Dirucotide), citrulinated analogues, multiple sclerosis

Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the Central Nervous System (CNS). It is caused by the coordinated attack of the immune system against the myelin, resulting in inflammation in axons and the appearance of neurological dysfunction. Several epitopes are involved in the pathogenesis of MS, including the myelin basic protein (MBP). A char-

acteristic of MBP is the large number of post-translational modifications, such as deimination, an enzymatic reaction resulting in the conversion of arginine to citrulline. Citrullination of MBP leads to loss of positive charge of the side group of arginine, causing disruption of MBP around axons. Previous studies in PBMCs (Peripheral Blood Mononuclear Cells) with citrullinated peptides, showed an increase of T-regulatory cells, which are very important for MS patients. In this work, citrullinated modified analogues of the epitope MBP_{82-98} (Dirucotide) were synthesized for the first time, in order to achieve their biological evaluation. The synthesis of these analogues was accomplished using the conventional manner of peptide synthesis as well as a microwave peptide synthesizer.

1. Deraos G., Chatzantoni K., Matsoukas M.T., Tselios T., Deraos S., Katsara M., Papathanasopoulos P., Vynios D., Apostolopoulos V., Mouzaki A., Matsoukas J.: Citrullination of linear and cyclic altered peptide ligands from myelin basic protein (MBP(87-99)) epitope elicits a Th1 polarized response by T cells isolated from multiple sclerosis patients: Implications in triggering disease. *J. Med. Chem. 51*: 7834-7842 (2008)
2. Moscarello M.A., Mastronardi F.G., Wood D.D.: The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple. *Neurochem. Res. 32*: 251-256 (2007)



❁

Molecular Mechanisms of Human Colon Cancer Resistance to Chemotherapy

D. Tsoumas[1], V. Bravou[1], E. Giannopoulou[2], V. Zolota[3], H.P. Kalofonos[2]

[1]Department of Anatomy, [2]Department of Clinical and Molecular Oncology, [3]Department of Pathology, School of Medicine, University of Patras, Rio 26504, Patras, Greece

Key words: Colon cancer, chemoresistance, molecular mechanisms

The development of chemoresistance in colon cancer is a major problem in cancer treatment. Integrin-linked kinase (ILK), an intracellular serine/threonine kinase located in focal adhesions, is implicated in human colon cancer progression (1,2). In this study, we investigate the role of ILK in *chemoresistance mechanisms* in human colon cancer, as well as its significance as a therapeutic target and predictive biomarker. ILK signaling pathways are investigated in cell lines (*in vitro*) and in human colon cancer tissue samples (*in vivo*) in relation with i) response to chemotherapeutic drugs and ii) expression of *cancer stem cell (CSC)* and Epithelial-*mesenchymal transition (EMT)* markers. Also, we will evaluate the impact of *ILK drug-targeting* in chemoresistance, and CSC with EMT marker expression in resistant and non resistant cell lines.

1. Bravou V., Klironomos G., Papadaki E., Taraviras S., Varakis J.: ILK over-expression in human colon cancer progression correlates with activation of beta-catenin, down-regulation of E-cadherin and activation of the Akt-FKHR pathway. *J. Pathol. 208*: 91-99 (2006)
2. McDonald P.C., Fielding A.B., Dedhar S.: Integrin-linked kinase: essential roles in physiology and cancer biology. *J. Cell Sci.121:* 3121-3132 (2008)

❁

Estimation of Pharmacokinetic Parameters of Pyrazinamide in Mice after Allometric Scaling from Human

Charikleia Danika, Iliana Leontari, Andreas Matthios, Mohamed Ahmed S. El Mubarak, Gregory Sivolapenko

Laboratory of Pharmacokinetics, Department of Pharmacy, University of Patras, Rio 26504, Patras, Greece

Key words: Pyrazinamide, pharmacokinetic parameters, mice

Through the allometric scaling, a population pharmacokinetic model was developed, for the estimation of pharmacokinetic parameters in mice, using experimental data from human patients. The drug used for modeling (scaling down) was the pyrazinamide. Using the NONMEN program and via simulations the pharmacokinetic parameters of clearance (Cl), volume of distribution (Vd), absorption rate constant (k) and half-time ($t_{1/2}$) in mice were estimated, which were found to be 0.0123L/h, 0.016L/h, 0.7688 h^{-1} και 0.901h respectively. Afterwards, the optimal dose scheme in mice was estimated after intravenous administrationofpyrazinamide in order to achieve thedesired therapeutic effect. Two possible dose schemes were found: 25 mg/kg per 3 hours and 35 mg/kg per 6 hours. Finally, the idealexperimental protocol of pyrazinamide administration in mice was designed (minimum number of animals, minimum number andexact time points) in order to validatethe population pharmacokinetic model and aLC-MS/MS method was developed as well, for the determination of pyrazinamide in mice plasma.

❁

Study of Novel Bio-inspired Tyrosinase Modulators

Dimitrios Xanthopoulos, Christina Kyrkou, Maria Kokkoti, Eftichia Kritsi, Kyriakos C. Prousis, Spyros Zografos, Theodora Calogeropoulou, Panagiotis Zoumpoulakis

Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Vas. Constantinou, Athens, Greece

Key words: Bio-inspired tyrosinase modulators

Tyrosinase is a copper-containing oxidase which catalyzes the rate limiting step in mammalian melanogenesis and enzymatic browning of mainly fruits and mushrooms. Therefore the discovery and development of tyrosinase inhibitors is of interest in both food and cosmetic applications (1). Bioinspired compounds were synthesized as novel tyrosinase modulators and were evaluated *in vitro* using the isolated enzyme from *Agaricus bisporus* (2). *In silico* docking studies were performed in an effort to rationalize the differences in their biological profiles. Our results suggest that the critical factor for the binding of the novel inhibitors is the interaction between a deprotonated carboxyl group with the coppers of the active site, while the presence of a catechol moiety can be possibly related to agonistic activity.

REFERENCES

1. Chang T.S.: An updated review of tyrosinase inhibitors. *Int. J. Mol. Sci. 10*: 2440-2475 (2009)
2. Sánchez-Ferrer A., Rodríguez-López J.N., García-Cánovas F., García-Carmona F.: Tyrosinase: a comprehensive review of its mechanism. *Biochim. Biophys. Acta 1247(1)*: 1-11 (1995)

❁

Synthesis of Indole Analogues for the Blocking of the Trimolecular Complex Formation

Dora Dimitriou, Marios Konstantinou, Veronica Tsoulougian, Theodore Tselios

Section of Organic Chemistry, Biochemistry and Natural Products, Department of Chemistry, University of Patras, Rio 26504, Patras, Greece

Key words: Indole analogues, trimolecular complex formation, synthesis

Multiple Sclerosis (MS) is an immunologically controlled, inflammatory, demyelinating disease, characterized by destruction of the white matter (myelin) of the central nervous system (CNS), leading to serious medical conditions like paralysis. The main cause of the disease is still un-

clear. However, it is believed to be mediated by an autoimmune T cell response directed to the proteins of the myelin sheath, such as myelin basic protein (MBP), proteolipid protein (PLP) and oligodendrocyte glycoprotein (MOG). The T-cell response is triggered by the formation of the trimolecular complex between the major histocompatibility complex (MHC), the immunodominant myelin protein epitopes and the T cell receptor (TCR). Herein we report the synthesis of non-peptide analogues, with the ability to simulate the immunodominant epitope 83-99 of MBP. These analogues were designed to block the formation of the trimolecular complex and therefore the T-cell activation. More specifically, indole analogues were synthesized with substitution in positions 2 and 4 or 6. The synthesized molecules contain a carboxyl group in position 2 and a phenylamino or benzylamino group in position 4 or 6. The synthesis of the indole ring was achieved by Fischer reaction followed by catalytic hydrogenation, arylation or reductive amination and ester hydrolysis. These molecules were purified using column and liquid chromatography and they were identified by mass spectrometry and ^{1}H-NMR.

REFERENCES

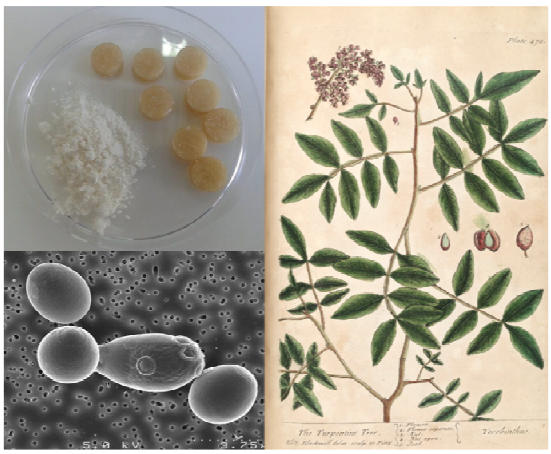
1. Mantzourani E.D. Mavromoustakos T.M., Platts J.A., Matsoukas J.M., Tselios T.V.: Structural requirements for binding of myelin basic protein (MBP) peptides to MHC II: Effects on immune regulation. *Curr. Med. Chem. 12*: 1521-1535 (2005)
2. Mantzourani E.D, Tselios T.V., Grdadolnik S.G., Platts J.A., Brancale A., Deraos G.N., Matsoukas J.M., Mavromoustakos T.M.: Comparison of proposed putative active conformations of myelin basic protein epitope 87-99 linear altered peptide ligands by spectroscopic and modelling studies: the role of positions 91 and 96 in T-cell receptor activation. *J. Med. Chem. 49*: 6683-6691 (2006)
3. Mantzourani E.D. Platts J.A., Brancale A., Mavromoustakos T.M., Tselios T.V.: Molecular dynamics at the receptor level of immunodominant myelin basic protein epitope 87-99 implicated in multiple sclerosis and its antagonists altered peptide ligands: triggering of immune response *J. Mol. Graph. Model. 26*: 471-481 (2007)
4. Spyranti Z., Dalkas G.A., Spyroulias G.A., Mantzourani E.D., Mavromoustakos T., Friligou I., Matsoukas J.M., Tselios T.V.: Putative bioactive conformations of amide linked cyclic myelin basic protein peptide analogues associated with experimental autoimmune encephalomyeli-tis. *J. Med. Chem. 50:* 6039-6047 (2007)

Preparation and Study of Formulations Based on *Pistacia Terebinthus L.* Resin and Probiotic *Saccharomyces boulardii* Cells for Medical Uses

Dionysia Diamantopoulou, Argyro Bekatorou, Michalis Kallis, Maria Kanellaki, Alexios Vlamis

Department of Chemistry, University of Patras, Rio 26500, Patras, Greece

Key words: *Pistacia Terebinthus L.* resin, probiotic *Saccharomyces boulardii* cells, Medical uses



The increasing consumer awareness and scientific evidence has led to an increased demand for wholesome and safer food, free from pathogenic microorganisms, microbial toxins, chemical contaminants, and excessive additives. In this respect, both industry and the research community have recently focused on the development of *functional* foods or food supplements, which claim to provide health-promotion or disease prevention benefits beyond their basic nutritional value. A significant part of the functional food market is represented by *probiotics*, which are foods containing live bacteria that can positively affect human health by altering their intestinal microflora when consumed in adequate amounts. The most frequently used probiotics are lactic acid bacteria, mainly *Lactobacillus* and *Bifidobacterium* spp., as well as some *Enterococcus, Streptococcus, Leuconostoc*, *Saccharomyces* spp., etc. The close relationships between gut microbiota, nutrients, health and disease, have led to increasing interest in using probiotics and *prebio-*

tics, i.e. non-digestible substrates that can be utilized by probiotics in the gut stimulating their growth and viability. The combined use of probiotics and prebiotics is known as *synbiotics*. *Saccharomyces boulardii* is yeast widely used as medicine to prevent and treat intestinal disorders. Plant components, such as mastic resins (*Pistacia lentiscus L.*, *P. terebinthus L.*, etc.), have also been used traditionally and commercially, for their medicinal properties (healing, antioxidant, antibacterial, antiinflammatory) as well as for the treatment of the symptoms of dyspepsia. In this study, (a) liquid (cell suspension in *aq* resin extract) and (b) solid formulations (cells entrapped in resin pellets) of *P. terebinthus* resin (mastic of Paphos) with *S. boulardii* are proposed for possible medicinal uses. The viability of *S. boulardii* in these formulations will be evaluated during storage as well as in acidic solutions simulating gastric acid. The morphology of entrapped cells will be observed by SEM. Finally, the formulations will be analyzed for their antioxidant capacity and total phenolics contents, as well as for sugars, organic acids, and aroma-active volatile compounds by classic and instrumental techniques (HPLC, GC, GC/MS).

REFERENCES

1. Foligné B., Daniel C., Pot. B.: Probiotics from research to market: the possibilities, risks and challenges. *Curr. Opin. Microbiol. 16*: 284-292 (2013)
2. Kavak D.D., Altiok E., Bayraktar O., Ulku S.: Pistacia terebinthus extract: As a potential antioxidant, antimicrobial and possible β-glucuronidase inhitor. *J. Mol. Cat. B: Enzym. 64(3-4)*: 167-171 (2010)

❁

Keratin and Collagen Polypeptide Models as a Means for Assessing the Deterioration of Organic Materials of Cultural Heritage

E. Fotou[1], A. Tsiligianni[1], S. Zevgiti[1], C. Papadopoulos[1], E. Panou-Pomonis[1], G. Panagiaris[2], M. Sakarellos-Daitsiotis[1]

[1]Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece
[2]Department of Conservation of Antiquities and Works of Art, Technological Educational Institution (T.E.I.) of Athens, Egaleo, Athens, Greece

Key words: Cultural Heritage, deterioration, organic materials, keratin, collagen polypeptides

Our study is focused on the development of a reliable and reproducible immunoassay for the evaluation of collagen and keratin-based decay of organic materials constituting natural and cultural heritage. Therefore, polypeptide models consisting of the characteristic amino acid pattern of keratin and collagen were designed, synthesized and identified. Ac-YRSGGGFGYRGGGFGYRS-β-Ala-NH_2, model peptide of the N-terminal part of Type II keratin, [Pro-Cys(Acm)-Gly]$_n$, model peptide of the C-terminal domain of Type I keratin and [Pro-Ser(OBzl)-Gly], model peptide of collagen, were administrated to animals for the induction of specific antibodies (1,2). Anti-[Pro-Ser(OBzl)-Gly]$_n$ and anti-collagen antibodies were tested against collagen isolated from bone, parchment and commercial collagen. Antibodies against Ac-YRSGGGFGYRGGGFGYRS-β-Ala-NH_2, [Pro-Cys(Acm)-Gly]$_n$ and antibodies recognizing the N-terminal, the C-terminal and the internal part of keratin were assayed for their binding to keratin from wool and commercial keratin.

It is concluded that the combined application of antibodies originated from collagen or keratin, as well as from the corresponding peptide models is a valuable tool in determining the extent of the decay of organic materials consisting our cultural heritage.

REFERENCES

1. Berisio R., Vitagliano L., Mazzarella L., Zagari A.: Recent progress on collagen triple helix structure, stability and assembly. *Protein Pept. Lett. 9*: 107-116 (2002)
2. Zevgiti S., Sakarellos C., Sakarellos-Daitsiotis, Ioakimoglou E., Panou-Pomonis E.: Collagen models as a probe in the decay of works of art: synthesis, conformation and immunological studies. *J. Pep. Sci. 13*: 121-127 (2007)

❁

Exploiting ChEMBL Database to Identify Indole Analogues as HCV Replication Inhibitors

E. Vrontaki[1,2], G. Melagraki[1], T. Mavromoustakos[2], A. Afantitis[1]

[1]Department of Chemoinformatics, NovaMechanics Ltd, Nicosia, Cyprus
[2]Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Athens 15771, Greece

Key words: ChEMBL database, HCV replication inhibitors, 3D-QSAR CoMSIA, Molecular docking, Virtual Screening

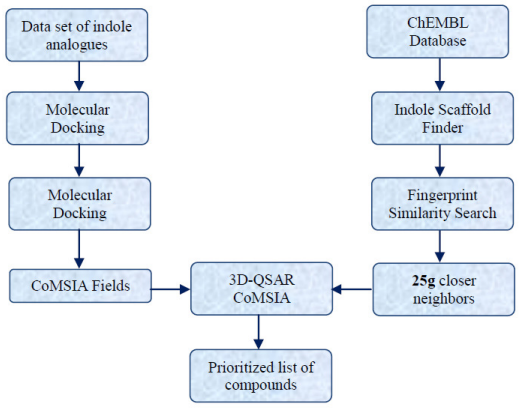
A computational Virtual Screening workflow using ChEMBL database in combination with Molecular Docking, 3D-QSAR CoMSIA and Similarity Search was applied with the aim to identify N-substituted indole analogues acting as inhibitors of HCV replication (Scheme). All the 41 compounds under study were docked in the "Palm II" allosteric site of the crystal structure of the enzyme HCV RNA-dependent RNA polymerase (NS5B GT1b) (1-3). The docking pose of each compound was subsequently used in a receptor-based alignment for the generation of the CoMSIA fields. The best CoMSIA model was derived using the steric, hydrophobic, and H-bond acceptor fields. The statistical parameters of the CoMSIA model are ONC = 6, q^2 = 0.632, SEP = 0.453, SEE = 0.120, F = 131.324 and R^2 = 0.974 for training set and R^2 = 0.727 for validation set, showing a good predictive ability of the model (4). This framework gives insight into the structural characteristics that affect the binding and the inhibitory activity of these analogues on HCV RNA polymerase.

The obtained *in silico* model was subsequently utilized to predict the activity of novel compounds prior to their synthesis and biological tests, within a virtual screening procedure. The ChEMBL database was exploited to dispose compounds containing the indole scaffold that are predicted to possess high activity and thus can be prioritized for biological screening. The approach revealed 18 promising chemistry driven compounds with potential high activity, while three from those have been already tested and possess high potent antiviral activity against HCV infected in hepatoma cells in nanomolar scale (4).

The encouraging results indicate that the proposed methodology can be generalized as a rational drug discovery tool for the identification of new leads for a broad spectrum of diseases.

REFERENCES

1. Karelson M., Dobchev D.A., Karelson G., Tamm T., Tämm K., Nikonov A., et al.: Fragment-based development of HCV protease inhibitors for the treatment of hepatitis C. *Curr. Comput.-Aided Drug Des. 8*: 55-61 (2012)
2. Barreca M.L., Iraci N., Manfroni G., Cecchetti V.: Allosteric inhibition of the hepatitis C virus NS5B polymerase: In silico strategies for drug discovery and development. *Future Med. Chem. 3*: 1027-1055 (2011)
3. Chen G., Ren H., Turpoff A., Arefolov A., Wilde R., Takasugi J., Khan A., et al.: Discovery of N-(4'-(indol-2-yl)phenyl)sulfona-mides as novel inhibitors of HCV replication. *Bioorg. Med. Chem. Lett. 23*: 3942-3946 (2013)
4. Vrontaki E., Melagraki G., Mavromoustakos T., Afantitis A.: Exploiting ChEMBL database to identify indole analogues as HCV replication inhibitors. *Methods* (2014), *in press*.



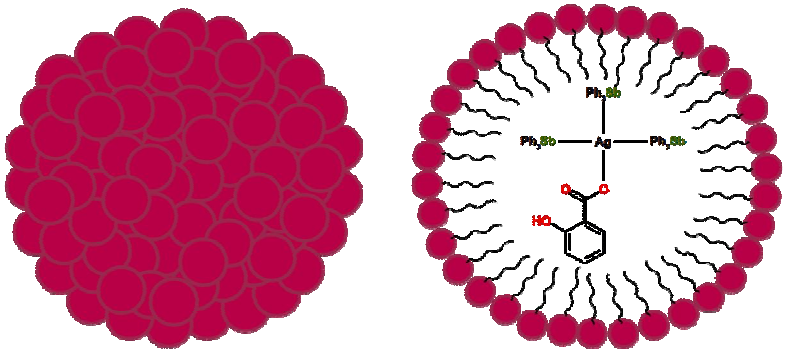
Synthesis, Characterisation and Bioactivity of Hexadecyltrimethyl-ammonium Bromide-silver Complex Micelles in Aqueous Media

E.I. Gkaniatsou[1], C.N. Banti[1], N. Kourkoumelis[2], S. Skoulika[3] and S.K. Hadjikakou[1]

[1]Inorganic and Analytical Chemistry, [3]Section of Physical Chemistry, Department of Chemistry, [2]Medical Physics Laboratory, Medical School, University of Ioannina, 45110

Ioannina, Greece. E-mail: shadjika@uoi.gr; Fax: +30 26510-08786; Tel: +30 26510-08374

Key words: Hexadecyltrimethyl-ammonium bromide-silver complex, synthesis, characterization, bioactivity



Surfactants increase the solubility of poorly water-soluble drugs. Due to their amphiphilic nature, they form micelles in aqueous media with the polar head facing towards the exterior, while the non-polar tail is oriented towards the core of the structure. Micelles often are used as drug carriers with the added benefit of increasing the bio-availability of the drug, as they can remain in the blood stream long enough to provide gradual accumulation in the required area and their sizes allow them to accumulate in areas with leaky vasculature (tumours) (1). In the present study, the cationic surfactant hexadecyltrimethylammonium bromide, an effective antiseptic agent and potential anticancer agent (2), was used for the encapsulation of the silver(I) complex, {[Ag(Ph_3Sb)$_3$(salH)} (compound *1*) (Ph_3Sb = triphenylstibine and $salH_2$ salicylic acid). The compound *1* was synthesized and characterized by m.p., vibrational spectroscopy (FT-IR), ^{1}H-, ^{13}C-NMR, UV-Vis spectroscopic techniques and high resolution mass spectroscopy (HRMS). Single crystal X-ray diffraction analysis was performed for the characterisation of the structure at ambient conditions. The stability of the complex in aqueous solution has been verified by means of ^{1}H-NMR, UV-Vis and conductivity measurements. The solubilisation of the potential anticancer compound *1* (3,4) was investigated by X-ray fluorescence (XRF) and scanning electron microscopy (SEM). The concentration of the complex in the micelle was determined via TG analysis. The *in vitro* cytotoxic activity (cell viability) of the micelle against HeLa (human cervical) and MCF-7 (human breast) cells was evaluated. The cytotoxic activity of the compound *1* has also been tested for comparison.

1. Ito E., Yip K.W., Katz D., Fonseca S.B., Hedley D.W., Chow S., Xu G.W., Wood T.E., Bastianutto C., Schimmer A.D., Kelley S.O., Liu F.F.: Potential use of cetrimonium bromide as an apoptosis-promoting anticancer agent for head and neck cancer. *Mol. Pharmacol. 76*: 969-983 (2009)
2. Torchilin V.P.: Structure and design of polymeric surfactant-base drug delivery systems. *J. Control. Release 73*: 137-172 (2001)
3. Banti C.N., Giannoulis A.D., Kourkoumelis N., Owczarzak A.M., Poyraz M., Kubicki M., Charalabopoulos K., Hadjikakou S.K.: Mixed ligand-silver(I) complexes with anti-inflammatory agents which can bind to lipoxygenase and calf-thymus DNA, modulating their function and inducing apoptosis. *Metallomics 4*: 545-560 (2012)
4. Reis D.C., Pinto M. C.X., Souza-Fagundes E.M., Wardell S.M.S.V., Wardell J.L., Beraldo H.: Antimony(III) complexes with 2-benzoylpyridine-derived thiosemicarbazones: Cytotoxicity against human leukemia cell line, *Eur. J. Med. Chem. 45*: 3904-3910 (2010)

Studies of Oxytocin Analogues on Breast Cancer Cells Proliferation

Efstathia Giannopoulou[1], Constantina-Soultana Kitsou[1], Chrysanthos Petropoulos[2], Jiřina Slaninová[3], Haralambos P. Kalofonos[1], Paul Cordopatis[2], Vassiliki Magafa[2*]

[1]Clinical Oncology Laboratory, Division of Oncology, Department of Medicine, [2]Lab of

Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Rio 26504, Patras, Greece
[3]Institute of Organic Chemistry and Biochemistry, Section of Peptide Biochemistry, Academy of Sciences of Czech Republic

Key words: Oxytocin analogues, breast cancer

The peptide hormone oxytocin (OT) is a cyclic nonapeptide. The widespread allocation of OT receptors (OTRs) in the central nervous system has firmly established OT as a central neurotransmitter with roles in reproductive and social behaviors (1). The last years, a new role of OT in the pathology of cancer is promoted (2). Several tumor types have been reported to express OT receptors (OTRs) including primary breast cancers, endometrial carcinomas, neuroblastomas, glioblastomas. Towards this direction, studies on breast cancer cells proliferation of OT analogues were performed. The peptides were prepared by standard SPPS methods (3), analyzed by analytical HPLC and their identity confirmed by Electro-Spray MS. Results showed that the effect of OT analogues on the prolifera-tion of breast cancer cells depends on the mo-difications of Oxytocin molecule.

REFERENCES

1. Gimpl G., Fahrenholz F.: The oxytocin receptor system: structure, function, and regulation. *Physiol. Rev. 2*: *81:* 629 (2001)
2. Cassoni P., Sapino A., Marroco T., Chini B., Bussolati G.: Oxytocin and oxytocin receptors in cancer cells and proliferation. *J. Neuroendocrinol. 16*: 362 (2004)
3. Fields B.G., Noble L.R.: Oxytocin and oxytocin receptors in cancer cells and proliferation. *Int. J. Pept. Prot. Res. 35:* 161 (1990)

Acknowledgments: The authors thank the University of Patras for the K. Karatheodoris Research Grant (to V. Magafa_2010_D.156)

❁

Conformational Studies of [Mpa[1]] OT Analogues

Eftichia Kritsi[1,2], Constantinos Potamitis[1], Nikos L. Assimomytis[1], Jiřina Slaninová[3], Paul Cordopatis[4], Panagiotis Zoumpoulakis[1*], Vassiliki Magafa[4*]

[1]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece; [2]Section of Chemical Sciences, Department of Chemical Engineering, National Technical University, Athens, Greece; [3]Institute of Organic Chemistry and Biochemistry, Section of Peptide Biochemistry, Academy of Sciences of Czech Republic; [4]Lab. of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, Rio, Patras, Greece

Key words: [Mpa[1]] OT Analogues, Conformational Studies

The peptide hormone oxytocin (OT) is a cyclic nonapeptide. The widespread allocation of OT receptors (OTR) in the central nervous system has firmly established OT as a central neurotransmitter with roles in reproductive and social behaviors (1). In the last years, a new role of OT in the pathology of cancer is promoted. Towards this direction, conformational analysis of OT, [Mpa[1], D-Tyr(Et)[2]]OT (*1*) and [Mpa[1], D-Tyr(Et)[2], D-Thi[3]]OT (*2*) was performed. NMR spectroscopy (1D ^{1}H, 2D TOCSY and 2D NOESY) was employed in order to elucidate the structure of OT and its analogues. Molecular Dynamics simulations under NMR-NOE constraints of OT and the two analogues, provided structure-activity possibly explaining the different biological profiles. Both OT and analogue *1* form a *β*-turn involving the residues in positions 2-5, which is crucial for the biological activity (2). In contrast, the replacement of Ile[3] by D-Thi[3] in analogue *2* induced conformational changes causing the loss of *β*-turn.

REFERENCES

1. Gimpl G., Fahrenholz F.: The oxytocin receptor system: structure, function, and regulation. *Physiol. Rev. 2*: *81:* 629 (2001)
2. Husain J., Blundell T.L., Cooper S., Pitts J.E., Tickle I.J., Wood S.P., Hruby V.J., Buku A., Fischman A.J., Wyssbrod H.R., *et al.,* The conformation of deamino-oxytocin: X-ray analysis of the 'dry' and 'wet' forms. *Philos. Trans. R. Soc. Lond. Biol. Sci. 327(1243)*: 625-654 (1990)

Acknowledgments: The authors thank the University of Patras for the *K. Karatheodoris Research Grant* (to V. Magafa_2010_D.156)

❁

Surface Modified Silica Nanoparticles as Catechin Containing Carriers in Drug Delivery

Eleftherios Halevas, Olga Tsave, Athanasios Salifoglou

Department of Chemical Engineering, School of Engineering, *Aristotle* University of Thessaloniki, Thessaloniki 54124, Greece

Key words: Silica nanoparticles, catechin containing carriers, drug delivery

Sol-gel encapsulation of flavonoids and flavonoid extracts with specific physicochemical properties and biological functions within robust polymer matrices remains a challenging task, despite extensive research in the field of drug delivery systems. Numerous studies have been reported in the field of sol-gel processes, describing a diverse spectrum of physical and chemical packing properties of sensitive biomolecules encapsulated in silica matrix. In this project, classical sol-gel synthesis approaches have been used under mild conditions in order to minimize the denaturing effects on encapsulated active flavonoids. The silica templated matrix was obtained by using two types of surfactants bearing different alkyl chains (*CTAB*, PEG-3000) as structure-directing agents for the silicon oxide framework. An organic precursor of silicic acid (triethoxymethylsilane) was used and processed through competitive hydrolysis and polycondensation reactions under controlled directions, assured by the presence of an oriented template. Silica materials, thus obtained, were used for encapsulation of catechin as the bioactive target molecule. The synthesis of encapsulated nanocompounds was achieved by taking into consideration the specific interaction between the colloidal gel precursors and molecular structure(s) of the selected biomolecule. The main objective was to improve the encapsulation conditions for the specific type of biomolecules, seeking the highest stability and functionality without loosing the quality of the flavonoid properties, particularly antioxidant and optical properties, like fluorescence. The structural properties of the encapsulated samples were studied by FT-IR and UV-Vis spectroscopy, TGA, SEM/EDX analysis, and XRD. The size distribution of synthesised polymeric silica mate-rials were investigated by BET, ζ-potential and particle size analysis.

❁

Identification of GAGs in GAG Blends Using micro-RAMAN Spectroscopy

Eleni Kamilari[1], Christos Kontoyannis[1,2], Fotini Lamari[1] and Malvina Orkoula[1,*]

[1]Department of Pharmacy, University of Patras, [2]ICE/HT-FORTH, Patras, Rio 26504, Patras, Greece

Key words: GAGs, GAG blends, micro-RAMAN Spectroscopy, identification

One of the major components of the extracellular matrix is glycosaminoglycans (GAGs), anionic linear heteropolysaccharides, which regulate several important processes. The methods used for the structural determination of GAGs include isolation, enzymatic digestion and analysis with separation techniques, usually HPLC and capillary electrophoresis. In the present study, micro-Raman spectroscopy was employed for the identifycation of GAGs in their mixtures. The important advantage of the technique lies on the fact that analysis is done simultaneously for all constituents with no need for previous separation. Chondroitin sulfate, heparin and dermatan sulfate in binary and ternary mixtures were studied. The comparison of the spectra of the mixtures to the relative standard spectra resulted in identification and discrimination between GAGs, based on the characteristic vibration frequencies of the functional groups. Deconvolution of peaks using the appropriate software was necessary for the analysis. The identification of heparin was easily done using the peaks at 826 cm^{-1}, 896 cm^{-1}, 1047 cm^{-1} and 1071 cm^{-1}, while for dermatan sulfate the relatively weak vibrations at 809 cm^{-1}, 866 cm^{-1}, 978 cm^{-1} and 1066 cm^{-1} were used. Chondroitin sulfate proved the most difficult component to spot due to a lesser number and weak in intensity characteristic peaks (at 846 cm^{1}, 950 cm^{-1} και 997 cm^{-1}).

Quantitative Analysis of Warfarin Sodium in Syrup Formulation Using Raman Spectroscopy

Evangelia Dimitrokalli[1], Malvina Orkoula [1] and Christos Kontoyannis[1,2]

[1]Department of Pharmacy, University of Patras, [2]ICE/HT-FORTH, Patras, Rio 26504, Patras, Greece

Key words: Warfarin sodium, syrup formulation, Raman spectroscopy

Active Pharmaceutical Ingredient (API) Warfarin Sodium, used as an anticoagulant, can be found in three formulations: as a tablet, oral suspension (syrup) and dilution for injections. In the present study a method of quantitative analysis of API in the syrup was developed, using Raman spectroscopy. API in suspension is both in diluted and in solid form, whereas some excipients are in form of insoluble particles. With the proposed methodology the sample was analyzed without any special treatment (e.g. dilution or separation from placebo or from the insoluble particles) as other analytical techniques require. For the quantitative analysis the ratios of vibration peaks' areas of API (1573 and 1612 cm^{-1}) to the area of two peaks of the excipients at 923 and 1462 cm^{-1}, was calculated. A calibration line was constructed using as standards dilutions of the commercially available syrup. Due to the presence of insoluble particles (API and excipients) and to the small laser's spot area on the syrup a drop the sample was placed on a glass slide with gold reflected surface (mirror) substrate. The slide was then rotated using a home-made apparatus. In this way it was possible to collect the Raman signal of a spectrum from the circumference of a circle formed during sample's rotation minimized undersampling problems.

❁

Binding Assays of Linear and Cyclic Analogues of Myelin Epitopes with HLA-DR2b and HLA-DR4 Alleles

[1,3]G. Deraos, [2]S. Deraos, [2,3]J. Matsoukas, [3]H. Kaldbacher

[1]Derpartment of Biochemistry, University of Tubingen, Germany
[2]Department of Chemistry, University of Patras, Rio 26504, Patras, Greece
[3]Eldrug S.A., Pharmaceutical Company, GR-26504, Platani, Patras, Greece

Key words: HLA-DR2b and HLA-DR4 Alleles, myelin epitopes

Multiple sclerosis (MS) is a $CD4^+$ T cell mediated chronic inflammatory disease of the central nervous system (CNS) characterized by discrete areas of inflammation followed by demyelination and paralysis. Myelin basic protein (MBP) is one of the major autoantigens defined in MS, with enchephalitogenic T cell epitopes, MBP_{83-99} being a major one. A new approach is the introduction of rationally designed cyclic peptides or a non-peptide-mimetic drug molecule resembling in the pharmacophore groups that NMR studies and molecular modeling results in a study with the receptor complex. In this study, linear and cyclic analogues of MBP83-99 and MOG35-55 were tested for their binding ability with HLA-DR2b and HLA-DR4 alleles. The results showed that cyclic analogues could bind with high affinity to HLA-DR2b and HLA-DR4 as well as the linear counterparts.

REFERENCES

1. Matsoukas J., Apostolopoulos V., Kalbacher H., Papini A.M., Tselios T., Chatzantoni K., Biagioli T., Lolli F., Deraos S., Papathanassopoulos P., Troganis A., Mantzourani E., Mavromoustakos T., Mouzaki A.: Design and synthesis of a novel potent myelin basic protein epitope 87-99 cyclic analogue: enhanced stability and biological properties of mimics render them a potentially new class of immunomodulators. *J. Med. Chem. 48(5):* 1470-1480 (2005)

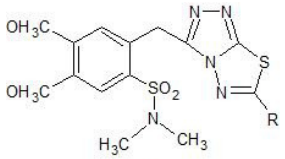
❁

Synthesis and Pharmacological Studies of 3-Sulfonamide-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole Derivatives

Georgios Charitos[1], Charalambos Camoutsis[1], D.T. Trafalis[2]

[1]Laboratory *of P*harmaceutical Chemistry, Department of Pharmacy, University of Patras, Rio 26504, Patras, Greece; [2]Department of Medicine, Laboratory of Pharmacology, University of Athens, Athens, Greece

Key words: 3-Sulfonamide-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives, synthesis, pharmacological studies



I

Heterocycle derivatives of 1,2,4-triazole and 1,3,4-thiadiazole show a broad spectrum of pharmacological properties (1). Also the condensation of these rings, triazole and thiadiazole, gives 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole (2). These compounds show antibacterial, antimicrobial, antiviral, anti-inflammatory and anticancer activity. Based on the above a series of 3-[2-(N-dimethylsulfamoyl)-4,5-dimethoxybenzyl]-6-phenyl-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives with general chemical structure I were synthesized in order to study their anticancer activity.

REFERENCES

1. Kaliappan Ilango: Facile synthesis and cytotoxic activity of 3,6-disubstituted 1,2,4-triazolo-[3,4,b]-1,3,4-thiadiazoles. *European J. Chem:* 5053 (2010)
2. Subrahmanya Bhat K., Jagadeesh Prasad D., Boja Poojary: Synthesis of some new 1,2,4-triazolo[3,4-b]-thiadiazole derivatives as possible anticancer agents. *Phosphorus, Sulfur and Silicon 179*: 1595-1603 (2004)

❁

Synthetic Methodologies for an Amino-derivatization of Aflatoxins such as AFM1 and AFB1

Konstantina Falida, Gerasimos Tsivgoulis

Division of Organic Synthesis, Department of Chemistry, University of Patras, Rio 26504, Patras, Greece

Key words: Aflatoxins, AFM1, AFB1, synthetic methodologies

Aflatoxins belong to the class of mycotoxins and they are among the most toxic and carcinogenic natural substances. Especially the aflatoxins AFB1, AFB2 και AFM1 are the most commonly found and AFB1 is the most carcinogenic of all. As a result, big efforts are made for their detection and quantitation through a variety of techniques, such as ELISA and monoclonal antibodies. These techniques require the synthesis of derivatives of aflatoxins capable to form protein conjugates. Usually, derivatives with carboxylic groups are synthesized through attachment to the carbonyl group of the aflatoxin. Alternatively, derivatives bearing amino groups could be used for attachment to certain cases of proteins. Thus, in the present work methods to obtain such aminoderivatives are studied. In the method that is presented the high cost aflatoxin is connected with the amino containing part at the last step through a well known in the area of aflatoxins reaction with a hydroxylamino derivative.

❁

Binding Affinity towards DNA and Antimicrobial Activity, of new Silver(I) Complex with the Anti-thyroid Drug Methimazole

L Kyros[1], C.N. Banti[1], A. Owczarzak[2], M. Kubicki[2], I. Sainis[3], N. Kourkoumelis[4] and S.K. Hadjikakou[1]

[1]Section of Inorganic and Analytical Chemistry, Department of Chemistry, [3]Cancer Biobank Center, [4]Medical Physics Laboratory, Medical School, University of Ioannina, 45110 Ioannina, Greece, e-mail: shadjika@uoi.gr
[2]Department of Chemistry, A. Mickiewicz, University, ul. Grunwaldzka 6, 60-780 Poznan, Poland

Key words: Methimazole, silver(I) complexes with tertiary phosphines

Methimazole (MMI) is sulfur containing imidazole compound and it is the most common used anti-thyroid drug for the treatment of Graves' disease (1). Because of its antioxidant activity, the coordination of MMI with transition metal ions, is of great interesting (2). Silver(I) complexes with tertiary phosphines were investigated for their tumoricidal properties making possible the assessment of a structure-activity relationship (SAR) (2). Therefore the relation of the complexes' antibacterial activity, with the nature of their ligands, their volume, shape (3), the geometry around silver ion and their interaction with DNA (3) is a matter under consideration. Here we report the synthesis and structural characteriza-tion of the new silver(I) complex with triphenylphosphine and mmi with formula $[Ag_2Br_2(tpp)_2(mmi)_2]$. The antimicrobial activity against *E.Coli* and *P. aeruginosa* and its binding properties towards DNA, lipoxygenase (LOX) and glutathione is studied. The results are compared with the corresponding one found for $[AgCl(tpp)_2mmi]$.

1. Urquiza N.M., Islas M.S., Dittler M.L., Moyano M.A., Manca S.G., et al.: Inhibition behavior on alkaline phosphatase activity, antibacterial and antioxidant activities of ternary methimazole-phenanthroli-ne-copper(II) complex. *Inorganica Chimica Acta 405*: 243-251 (2013)
2. Santini C., Pellei M., Papini G., Morresi B., et al.: In vitro antitumour activity of water soluble Cu(I), Ag(I) and Au(I) complexes supported by hydrophilic alkyl phosphine ligands *J. Inorg. Bioch. 105*: 232-240 (2011)
3. Kyros L., Banti C.N., Kourkoumelis N., Kubicki M., Sainis I., Hadjikakou S.K.: Synthesis, characterization, and binding properties towards CT-DNA and lipoxygenase of mixed-ligand silver(I) complexes with 2-mercaptothiazole and its derivatives and triphenylphosphine. *J. Biol. Inorg. Chem. 19*: 449-464. (2014)

❁

Synthesis and Study of Amphipathic Cationic Peptide Models for the Development of new Antimicrobial Agents

M. Tsirogianni, A. Tsiligianni, K. Chionis, D. Krikorian, A. I. Koukkou, M. Sakarellos-Daitsiotis and E. Panou-Pomonis

Peptide Chemistry Research Laboratory, Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

Key words: Amphipathic Cationic Peptide Models, antimicrobial agents, synthesis

AMPs have a broad-spectrum of action against both Gram (-) and Gram (+) bacteria as well as fungi, and viruses (1-3). In the present study, cationic helical peptides of the following type were synthesized in order to develop new antibiotics: H-RWLRLLWRFLRL-NH_2, $(RWLRLLWRFLRL)_2$K-Ahx-NH_2, XLFDIIKKIAESF-NH_2 (where X = G, A, V, L) and XLLKFIKKLL-NH_2 (where X = G, K, R). Synthesis was performed by the conventional stepwise Fmoc/tBu solid-phase method. The peptides were purified by RP-HPLC and identified by ESI-MS. The above peptides were tested for their antimicrobial activity against Gram(-) bacteria, Gram(+) bacteria and fungi. The peptides were also tested for their hemolytic activity against human erythrocytes.

1. Powers J.P., Hancock R.: The relationship between peptide structure and antibacterial activity. *Peptides 24*: 1681-1691 (2003)
2. Dawso R.M., Liu C.Q.: Properties and application of antimicrobial peptides in biodefense against biological wafare threat agents. *Crit. Rev. Microbiol. 34*: 89-107 (2008)
3. Kang S.J., Kim D.H., Mishig-Ochir T., Lee B.J.: Antimicrobial peptides: their physicochemical properties and therapeutic application. *Arch. Pharm. Res.* 35: 409-413 (2012)

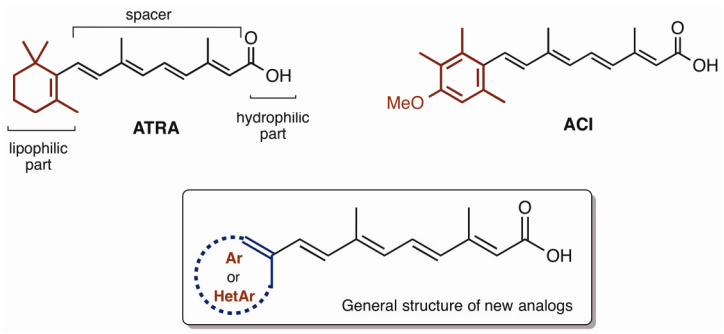
❁

Total Synthesis of New Acitretin Analogs, Suitable for QSAR Studies

M.A. Dima, A. Antoniou, S.E. Bariamis, and C.M. Athanassopoulos.

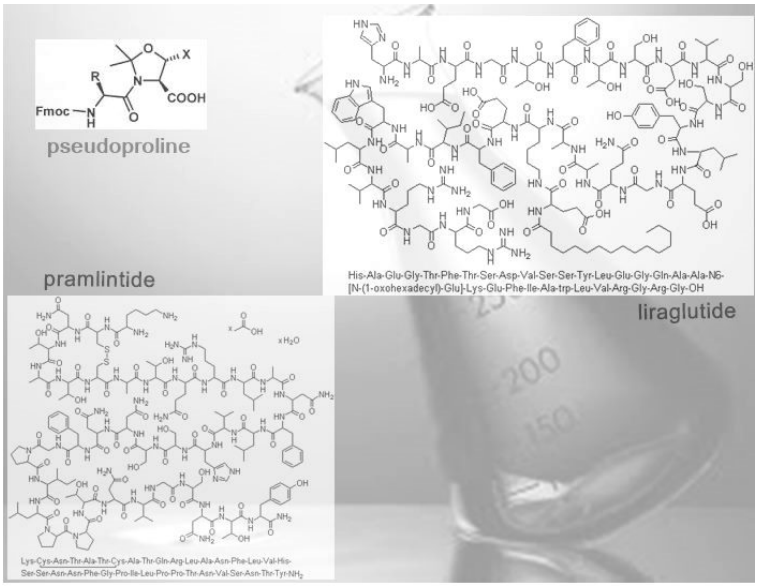
Department of Chemistry, Laboratory of Organic Synthesis, University of Patras, 26500 Patras, Greece

Key words: Acitretin analogs, QSAR studies, synthesis



Naturally occurring retinoids and their synthetic analogues (e.g. acitretin), play an important role in many biological functions, such as vision, growth, reproduction and cell differentiation. They are administered as drugs of choice in dermatological disorders, in chemoprevention and cancer treatment. Moreover retinoic acid (ATRA) is reported as stem cell and cancer stem cell differentiation factor (1), Recently, we have reported convergent methodologies for the synthesis of acidic retinoids, leading to acitretin (ACI) and analogs (2). Based on these protocols we present here in this work, the total synthesis of new acitretin analogs, bearing aromatic/ heteroaromatic rings with changes in the lipophilic part, suitable for QSAR studies. Thus, we expect that the biological evaluation of the latter, both as anticancer and cancer stem cell differentiation agents should reveal *key* structural elements, responseble for their activity and lead us to the rational design of more potent analogs.

1. Dean M., Fojo T., Bates S.: Tumor stem cells and drug re-sistance. *Nat. Rev. Cancer 5*: 275-284 (2005)
2. Magoulas G., Bariamis S., Athanassopoulos C., Haskopoulos A., Dedes P., Krokidis M., Karamanos N., Kletsas D., Papaioannou D., Maroulis G.: Syntheses, antiproliferative activity and theoretical characterization of acitretin-type retinoids with changes in the lipophilic part. *Eur. J. Med. Chem. 46*: 721-737 (2011)



Study of Usability of Pseudoprolines for the Synthesis of Pramlintide's and Liraglutide's Fragments

Maria Georgoulia[1], Danae-Androniki Zisimopoulou[1], Charikleia Barlou[2], Dimitrios Gatos[1]

[1]Department of Chemistry, Section of Organic Chemistry, Biochemistry and Natural Products, University of Patras, Rio, Patras, Greece; [2]CBL Patras, 26000, Patras, Greece

Key words: Pseudoprolines, pramlintide's, liraglutide's Fragments, synthesis

The aim of this project was to study the reduction of resin's loading of esterified pseudoprolines on 2- chloro-trityl chloride resin in order to cogitate and realize the synthesis of fragments of antidiabetic drugs, Liraglutide and Pramlintide, by replacing a dipeptide in the peptide sequence with the corresponding pseudoproline in order to increase the solability of the peptide and avoid racemization in the C terminus of Pramlintide's fragment at the fragment coupling (1). The fragments of the drugs were synthesized by fragment coupling and step by step method using the technique of solid phase peptide synthesis in 2-chloro-trityl chloride resin. The results of this work will help to simplify the synthesis of these fragments of drugs and develop them as pharmaceutical products.

1. http://en.wikipedia.org/wiki/Pseudoproline

❁

Synthesis of pyrrole analogues for the inhibition of T cell activation

Marios Konstantinou, Dora Dimitriou, Theodore Tselios

Department of Chemistry, University of Patras, 26504 Rio, Patras, Greece

Key words: Pyrrole analogues, inhibition of T cell activation, Synthesis

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) which leads to serious pathologies such as paralysis. The main theory for the disease is one of the autoimmune responses of encephalitogenic T cells directed from the proteins of the myelin sheath such as myelin basic protein (MBP), proteolipid protein (PLP) and oligodendrocyte glycoprotein (MOG). The response of T-cells activated by the formation of the trimolecular complex between the major histocompatibility complex (MHC or HLA), immunodominant epitopes of myelin proteins and receptor encephalitogenic T-cells (TCR). Herein, we report the synthesis of pyrrole analogues in order to block the formation of the trimolecular complex and to inhibit the activation of encephalitogenic T cells. The pyrrole analogues were synthesized using the 2 or 3 carboxyl pyrrole as starting material followed by N benzylation, amidation and guadinylation. The synthesized molecules were purified using liquid chromatography and they were identified by mass spectrometry and ^{1}H-NMR.

1. Mantzourani E.D. Mavromoustakos T.M., Platts J.A., Matsoukas J.M., Tselios T.V.: Structural requirements for binding of myelin basic protein (MBP) peptides to MHC II: Effects on immune regulation. *Curr. Med. Chem. 12*: 1521-1535 (2005)
2. Mantzourani E.D, Tselios T.V., Grdadolnik S.G., Platts J.A., Brancale A., Deraos G.N., Matsoukas J.M., Mavromoustakos T.M.: Comparison of proposed putative active conformations of myelin basic protein epitope 87-99 linear altered peptide ligands by spectroscopic and modelling studies: the role of positions 91 and 96 in T-cell receptor activation. *J. Med. Chem. 49*: 6683-6691 (2006)
3. Mantzourani E.D. Platts J.A., Brancale A., Mavromoustakos T.M., Tselios T.V.: Molecular dynamics at the receptor level of immunodominant myelin basic protein epitope 87-99 implicated in multiple sclerosis and its antagonists altered peptide ligands: triggering of immune response *J. Mol. Graph. Model. 26*: 471-481 (2007)
4. Spyranti Z., Dalkas G.A., Spyroulias G.A., Mantzourani E.D., Mavromoustakos T., Friligou I., Matsoukas J.M., Tselios T.V.: Putative bioactive conformations of amide linked cyclic myelin basic protein peptide analogues associated with experimental autoimmune encephalomyelitis. *J. Med. Chem. 50:* 6039-6047 (2007)
5. Potamitis C., Matsoukas M.T., Tselios T., Mavromoustakos T., Golič Grdadolnik S..: Conformational analysis of the MBP83-99 (PHE91) and MBP83-99 (TYR91) peptide analogues and study of their interactions with the hla-dr2 and human tcr receptors by using molecular dynamics. *J. Comput. Aided Mol. Des. 25*: 837-853 (2011)

❁

Design of Peptide Mimetics Based on the Conformational Demands of the Trimolecular Complex TCR-Antigen-HLA(MHC)

Mary Patricia P. Yannakakis, Minos Timotheos Matsoukas, Theodore V. Tselios

University of Patras, Department of Chemistry, Rion Patras, 26504, Greece
e-mail: yannakakism@gmail.com

Key words: TCR-Antigen-HLA(MHC), multiple sclerosis

Multiple Sclerosis (MS) is an immunologically controlled, inflammatory, demyelinating disease, characterized by destruction of the white matter (myelin) of the Central Nervous System (CNS) (1). The main cause of the disease is still unclear. However, it is believed to be mediated by an autoimmune T-cell response which is triggered by the formation of the trimolecular complex between the Major Histocompatibility Complex (MHC/HLA), the immunodominant myelin protein epitopes such as the Myelin Basic Protein (MBP) and the T-cell receptor (TCR) (2). In this project a detailed mapping of the interactions that occur during the creation of the trimolecular complex MHC-peptide antigen-TCR took place. The mentioned procedure provides the conformational characteristics that are essential for the rational design of inhibitors (3,4). The new molecules were designed to prevent the formation of the trimolecular complex and the further multiplication and activation of T-cells that are involved in MS. Several Molecular Docking simulations took place as well as Molecular Dynamic experiments which were carried out by using the MOE software in a LINUX operating system and the crystal structure (pdb: 1YMM) (5,6).

REFERENCES

1. Mantzourani E.D. Mavromoustakos T.M., Platts J.A., Matsoukas J.M., Tselios T.V.: Structural requirements for binding of myelin basic protein (MBP) peptides to MHC II: Effects on immune regulation. *Curr. Med. Chem. 12*: 1521-1535 (2005)
2. Mantzourani E.D., Tselios T.V., Grdadolnik S.G., Platts J.A., Brancale A., Deraos G.N., Matsoukas J.M., Mavromoustakos T.M.: Comparison of proposed putative active conformations of myelin basic protein epitope 87-99 linear altered peptide ligands by spectroscopic and modelling studies: the role of positions 91 and 96 in T-cell receptor activation. *J. Med. Chem. 49*: 6683-6691 (2006)
3. Wucherpfennig K.W.: The first structures of T cell receptors bound to peptide-MHC. *J. Immunol. 185*: 6391-6393 (2010)
4. Li Y., Huang Y., Lue J., Quandt J.A., Martin R., Mariuzza R.A.: Structure of a human autoimmune TCR Bound to a myelin basic protein self-peptide and a multiple sclerosis-associated MHC class II molecule. *EMBO J. 24*: 2968-2979, 2005
5. Hahn M., Nicholson M.J., Pyrdol J., Wucherpfennig K.W.: Unconventional topology of self peptide-major histocompatibility complex binding by a human autoimmune T cell receptor. *Nat. Immunol. 6*: 490-496, 2005
6. Wucherpfennig K.W.: T cell receptor crossreactivity as a general property of T cell recognition. *Mol. Immunol, 40*: 1009-1017 (2004)

Transdermal Delivery of Sartans: An Alternative Approach in the Treatment of Hypertension

Michaila Michalatou[1], Maria-Eleni Androutsou[1,3], Theodore Tselios[1], George Agelis[1,3], Demetrios Vlahakos[2], John Matsoukas[1,3]

[1]Department of Chemistry, University of Patras, Rio 26500, Patras, Greece; [2]Department of Internal Medicine, *Attikon* University Hospital, GR-12462, Athens, Greece; [3]Eldrug S.A., Pharmaceutical Company, 26504 Platani, Patras, Greece

Key words: Sartans, transdermal delivery, hypertension

A new therapeutic approach for the inhibition of the Renin-Angiotensin system is the transdermal administration of AT1 antagonists (Sartans) which has many advantages over the conventional routes of administration. Our objective was to investigate the *in vitro* transdermal delivery of a new synthesized Sartan (BV6Na) in human skin using franz cells and various solvents/permeation enhancers in an effort to find the most appropriate delivery system. Moreover, the produced suspension was *in vivo* evaluated, recording the antihypertensive response of Wistar rats after 3, 6, 8 and 24 hours of transdermal administration. The results showed that the synergistic effect of three permeation enhancers significantly improves the permeability of the substance. The maximum quantity of the Sartan which penetrated the skin was 4.82 μg/cm^2, while antihypertensive effect was achieved in the period of 6 to 8 hours after the transdermal administration. According to these results, the transdermal route of administration may be feasible and represent a new therapeutic approach for the treatment of hypertension.

Acknowledgments: This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program Education and Lifelong Learning of the National Strategic Reference Framework (NSRF) - Research Funding

Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

REFERENCES

1. Sinha V.R., Kaur M.P.: Permeation enhancers for transdermal drug delivery. *Drug Dev. Ind. Pharm. 26*: 1131-1140 (2000)

2. Nishida N., Taniyama K., Sawabe T., Manome Y.: Development and evaluation of a monolithic drug-in-adhesive patch for valsartan. *Int. J. Pharm. 402*: 103-109 (2010)

3. Vikas S., Seema S., Gurpreet S., Rana A., Baibhav J.: Penetration enhancers: a novel strategy for enhancing transdermal drug delivery. *Int. Res. J. Pharm. 2*: 32-36 (2011)

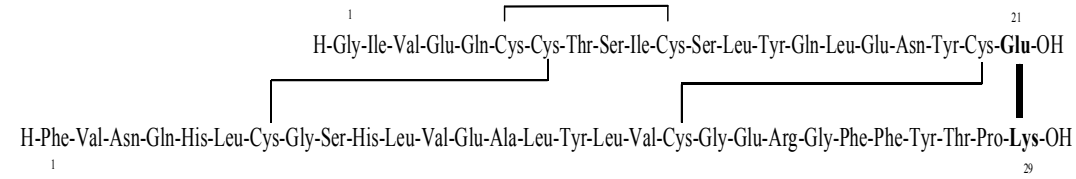
❁

Study of Synthesis of Single-Chain Insulin Derivatives

Nikoleta Parveri, Dimitrios Gatos, Kleomenis Barlos

Section of Organic Chemistry, Biochemistry and Natural Products, Department of Chemistry, University of Patras, Rio 26500, Patras, Greece

Key words: Single-chain insulin derivatives, synthesis



Insulin is a hormone which is considered to be a relatively *short* protein, consisting of two polypeptide chains (A and B) which overall contain 51 amino acids, 21 in A chain and 30 in B chain. It has three disulfide bonds, from which two of them hold the two chains, while the third locates in A Chain. Insulin plays a key role in the metabolism of carbohydrates of the body and its deficiency causes the disease of diabetes (type 1 or type 2). In this research project design and synthesis of a modified single-chain insulin analog was formed, with main object the increase of its stability against proteolytic enzymes, while maintaining its biological activity. For this purpose in this synthesis the Asn A21 is replaced by Glu, without any change in bioactivity, while Thr B30 is absent. Apart from the three disulfide bonds containing in the molecule of insulin, another amide bond was developed between the side carboxyl group of Glu and the side amino group of Lys B29, which will provide the stability of the molecule against hydrolysis, converting it into a long-acting insulin.

Barlos K., Gatos D., Hatzi O., Koch N., Koutsogianni S.: Synthesis of the very acid-sensitive Fmoc-Cys(Mmt)-OH and its application in solid-phase peptide synthesis. *Int. J. Peptide Protein Res. 47*: 148-153 (1996)

❁

Ex vivo Evaluation of the Effect of Cu(II) Metallotoxin on the Rat Sciatic Nerve

Olga Tsave[1,2], Athanasios Salifoglou[2], George Theophilidis[1]

[1]Laboratory of Animal Physiology, Department of Biology, School of Natural Sciences, [2]Department of Chemical Engineering, School of Engineering, *Aristotle* University, Thessaloniki 54124, Greece

Key words: Cu(II) Metallotoxin, rat Sciatic nerve, effect

Copper is an essential trace element encountered in a variety of aerobic organisms. As a metal ion, it is intimately linked with many cellular enzymes and proteins of variable function(s), including ceruloplasmin and superoxide dismutase. Copper appears in two biologically relevant oxidation states, Cu(I) and Cu(II). As a redox active metal ion, it contributes to the production of free radicals and Reactive Oxygen Species (ROS) through Fenton and Fenton-Weiss reactivity. The very same properties, however, that render copper a quintessential metal ion might

also turn it into a metallotoxin. To this end, changes in the cellular physiological levels of copper lead to chemical reactivity inflicting severe perturbation upon its homeostasis and concomitant increase in cell death due to oxidative stress. The purpose of the present work was a) the assessment of the effect of the soluble form of cis-copper glycinate on the isolated sciatic nerve fibers, and b) to assess the potential neuroprotective action of known chelators and antioxidant molecules (NAC, EDTA, QA) against Cu(II) toxicity, on rat sciatic nerve. The methodology employed in this investigation was based on the sciatic nerve of the rat isolated in a three-chamber recording bath which consists of stimulation, incubation and recording chamber. As an indication of the vitality of the nerve fibers the compound action potential (CAP) was recor-ded every second for over 24 h. To achieve direct contact of the nerve fibers with the specific form of soluble Cu(II), the epineurium was re-moved. The recording bioelectric response, CAP, was effected when the part of the nerve in the incubation chamber was exposed to various concentrations of Cu(II). The collective results indicate a) a clear concentration-dependent copper toxicity between 75 to 500 μM, linked to the emerging ROS, b) neuroprotective effect of N-acetylcysteine (NAC) dependent on the concentration of Cu(II) c) clear neuroprotective action of the metal chelator molecule EDTA, and d) no neuroprotective effect by quinic acid (QA).

❁

Sequential Oligopeptide Carrier as Cell Penetrating Transporter for Intracellular Molecular (Drug) Delivery

Ornela Tzouba, Dimitris Krikorian[1], Eugenia Panou-Pomonis, VasilisTsikaris, Maria Sakarellos-Daitsiotis

Peptide Chemistry Laboratory, Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece

Key words: Sequential oligopeptide carrier, cell penetrating transporter, intracellular molecular, drug delivery

The development of new therapeutics and diagnostics that act only where needed is a major challenge to the cell membrane penetration and selectivity of the intracellular drug delivery.Our study is focused on the development of new Cell Penetrating Molecular Transporters (CPMT), which overcome the above barriers and provide extracellular enzymatic resistance, redox sensitive pro-drug linkages and intracellular release of the biomolecule or drug. A multifunctional foldamer carrier Ac-(Lys-Aib-Gly)$_4$, named SOC (Sequential Oligopeptide Carrier), which incorporates lysine for covalently anchoring the bioactive molecules, will be applied as CPMT. SOC carrier adopts helical conformation and has been extensively used in our laboratory as a multifunctional scaffold (1). Selected molecules will be conjugated to the Lys$N^{\varepsilon}H_2$ groups *via* the formation of an oxime carrier-biomolecule linkage. Reduction of the oxime linkage, by using a Cytochrome Reductase system, isolated from pig liver microsomes, will permit the intracellular release of the biocargo (2).

1. Sakarellos-Daitsiotis M., Krikorian D., Panou-Pomonis E., Sakarellos C.: Artificial carriers: a stratedgy for constructing antigenic/immunogenic conjugates. *Curr.Top. Med. Chem. 6*: 1715 (2006)
2. Heberling S., Girreser U., Wolf S., Clement B.: Oxygen-insensitive enzymatic reduction of oximes to imines. *Biochem., Pharmacol. 71*: 354-365 (2006)

❁

Footprinting Analysis and Molecular Dynamics of Polyamine-chloramphenicol Conjugate Binding on the Prokaryotic Ribosome

Ourania N. Kostopoulou[1], George E. Magoulas[2], George Papadopoulos[3], Dionysios Papaioannou[2] and Dimitrios L. Kalpaxis[1]

Laboratory of Biochemistry, School of Medicine[1] and Laboratory of Synthetic Organic Chemistry, Department of Chemistry[2], University of Patras, Rio 26500, Patras, Greece. Department of Biochemistry and Biotechnology[3], University of Thessalia, Larisa, Greece

Key words: Polyamine-chloramphenicol, prokaryotic ribosome, footprinting analysis

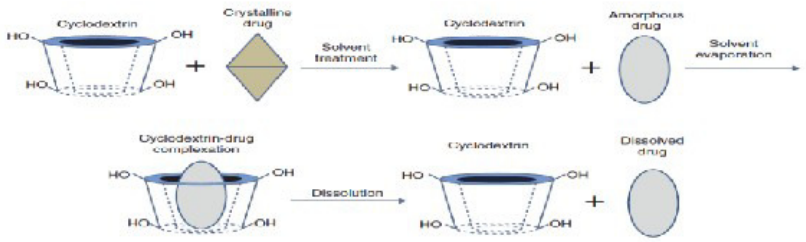
The antibiotic chloramphenicol (CAM) inhibits protein biosynthesis by targeting the peptidyl transferase (PTase) center on the large ribosomal subunit. CAM has been widely used clinically, despite its adverse effects on hematopoietic system. The presence of active trans-membrane transporters for polyamines in cells and the positive effect that polyamines exert on CAM binding to the ribosome gave us the impetus to synthesize and evaluate polyamine-CAM conjugates. Results from cell-free system experiments using a series of polyamine-CAM conjugates showed that the most effective compound was KA240, in which a dibenzylated spermidine (SPD) substitutes for the dichloro-methyl edge of CAM. They also indicated that KA240 behaves as a slow-binding inhibitor, following a two-step mechanism. For the characterization of the initial (CI) and final (C*I) complex between KA240 and ribosome, we applied time-resolved footprinting analysis. The data revealed that KA240, after a transient initial binding within the catalytic center, causes a π-stacking interaction with nucleotides U2585 and U2586 of 23S rRNA through its dibenzylated edge, while preserving its accommodation within the catalytic center. Given that U2585 possesses a critical role in the rotary motion of aminoacyl-tRNA towards the ribosomal A-site, our results suggest that KA240, except for competively inhibiting the PTase center, impedes functional accommodation of ribosomal substrates and simultaneously reduces the binding energy for CAM binding to the ribosome. Molecular dynamics confirmed the results of time-resolved footprinting analysis, strengthening the importance of KA240 as an important lead compound for further studies.

Increment of Solubility and Release of Brinzolamide, preparing Solid Dispersions with a Modified Cyclodextrin

S. Giannakopoulou[1], P. I. Siafaka[1], M. Betsiou[1], I. Koutri[2], Ch. Kiziridi[2], E. Karavas[2], D.N. Bikiaris[1]

[1]Laboratory of Organic Chemical Technology, *Aristotle* University, Thessaloniki, Greece; [2]*Pharmathen S.A*, Pharmaceutical Industry, Dervenakion Str. 6, 153 51 Attiki, Greece

Key words: Brinzolamide, solubility, release, solid dispersions, modified cyclodextrin



In recent years, the hydrophobic active substances have led researchers in new formulation approaches to enhance bioavailability and dissolution rate. The active substance Brinzolamide belongs to the class of carbonic anhydrase inhibitors, which causes reduction of intraocular pressure in patients suffering from glaucoma. The technique of inclusion complexation using cyclodextrins, as part of solid dispersions, is considered as a promising technique for increasing the solubility of hydrophobic active substances. In the present study, inclusion complexes of Brinzolamide: Hydroxypropyl-beta-Cyclodextrin, in various molecule ratios, was prepared using the technique of freeze drying. The effect of Hydroxypropyl-beta-Cyclodextrin on Brinzolamide solubility and association constant Kc was determined by phase-solubility studies *via* Higuchi & Connors method. The techniques used for characterization of the inclusion complex is the Fourier transform infrared spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), X-ray crystallography (XRD) and Scanning Electron Microscopy (SEM), while Dissolution experiments were performed in a Distek Dissolution Apparatus at 37 ^{o}C using as dissolution medium simulated organic tears. The determination of dissolution rate and phase solubility studies was carried out *via* HPLC method.

1. Taupitz T., Dressman J., Buchanan C., Klein S.: Cyclodextrin-water soluble polymer ternary complexes enhance the solubility and dissolution behaviour of poorly soluble drugs. Case example: Itraconazole. *Eur. J. Pharm. Biopharm. 83*: 378-387 (2012)
2. Jansook P., Stefánsson E., Thorsteinsdóttir M., Sigurdsson B.B., Kristjánsdóttir S.S., Bas J.F., Sigurdsson H.H., Loftsson T.: Cyclodextrin solubilization of carbonic anhydrase inhibitor drugs: formulation of dorzolamide eye drop microparticle suspension. *Eur. J. Pharm. Biopharm. 76:* 208-214 (2010)
3. Palma S.D., Tartara L.I., Quinteros, D, Allemandi D.A., Longhi M.R., Granero G.E.: An efficient ternary complex of acetazolamide with HP-ss-CD and TEA for topical ocular administration. *J. Control. Release 138*: 24-31 (2009)

Docking Studies on Coumarin and Quinolinone Derivatives against sPLA$_2$ GIIA

Sofia Vasilakaki, Thomas Mavromoustakos, George Kokotos*

Department of Chemistry, University of Athens, Panepistimiopolis, Zografou 15771, Athens, Greece

Key words: Coumarin, quinolinone derivatives, sPLA$_2$ GIIA docking studies

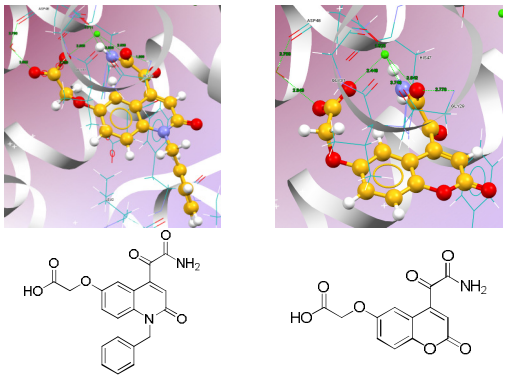


Figure 1. The binding mode of two representative coumarin and quinolinone derivatives in the sPLA$_2$ GIIA active site.

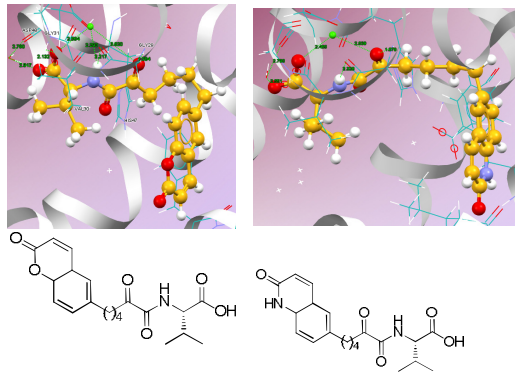


Figure 2. The binding mode of two representative2-oxoamides derivatives in the sPLA$_2$ GIIA active site.

Secretory phospholipase A$_2$ (sPLA$_2$) is an enzyme related to inflammatory symptoms as it contributes to arachidonic acid metabolism. It belongs to the PLA$_2$ superfamily, which cleaves *sn-2* acyl-chain of glycerophospholipids, releasing arachidonic acid. It has been proved that activity of sPLA$_2$ group IIA is associated with functional coronary stenosis (1), carotid atherosclerosis (2), multifarious proatherogenic actions in the vessel wall (3) and certain types of cancer (4).
Methyl Varespladib and Me-Indoxam are heterocyclic substituted systems that have shown potent inhibitory activity against sPLA$_2$ including GIIA (5). On the basis of these premises, we decided to study the heterocyclic systems of coumarin and quinolinone substituted by an α-ketoamide group in the 3 or 4 position, a 2-methoxyacetic acid group in 5 or 6 position and a phenyl group in the case of quinolinone analogues. Docking calculations were performed in the active site of sPLA$_2$ GIIA (PDB:1KQU), using the docking program GOLD 5.2. The structures were sketched using Sybyl 8.0 molecular modeling package and optimized using the Powell energy minimization algorithm of Sybyl 8.0. Structures substituted in 4 and 6 positions presented a better binding mode and they were able to create hydrogen bonds with the amino acids of the active site and interactions with Ca^{2+} which is involved in the catalytic mechanism (Figure 1).
Also, long chain α-ketoamide derivatives of α-amino acids have been proved a novel class of inhibitors of sPLA$_2$ GIIA by Kokotos et al (6). Based on this, we designed and docked some α-ketoamide derivatives of valine (Figure 2). Most of the structures docked, had the desirable binding motif leading us to the conclusion that *in vitro* studies of these molecules should be carried out.

Acknowledgmends: This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program *Education and Lifelong Learning* of the National Strategic Reference Framework (NSRF) - Research Funding

Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

REFERENCES

1. Muller O., Ntalianis A., Wijns W., Delrue L., Dierickx K., Auer R., Rodondi N., Mangiacapra F., Trana C., Hamilos M., Valentin E., De Bruyne B., Barbato E., Bartunek J.: association of biomarkers of lipid modification with functional and morphological indices of coronary stenosis severity in stable coronary artery disease. *J. Cardiovasc.Transnl. Res. 6*: 536-544 (2013)
2. Jashari F., Ibrahimi P., Nicoll R., Bajraktari G., Wester P., Henein M.Y.: Coronary and carotid atherosclerosis: similarities and differences. *Atherosclerosis 227*: 193-200 (2013)
3. Rosenson R., Gelb M.H.: Secretory phospholipase A2: a multifaceted family of proatherogenic enzymes. *Curr. Cardiol. Rep. 11*: 445-451 (2009)
4. Murakami M., Lambeau G.: Emerging roles of secreted phospholipase A(2) enzymes: an update. *Biochimie 95*: 43-50 (2013)
5. Dennis E.A., Cao J., Hsu Y.H., Magrioti V., Kokotos G.: Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem. Rev. 111*: 6130-6185 (2011)
6. Mouchlis V.D, Magrioti V., Barbayianni E., Cermak N., Oslund R.C., Mavromoustakos T.M., Gelb M.H., Kokotos G.: Inhibition of secreted phospholipases A□ by 2-oxoamides based on α-amino acids: Synthesis, in vitro evaluation and molecular docking calculations. *Bioorg. Med. Chem. 19:* 735-743 (2011)

❁

Using Raman Spectroscopy for the Study of Collagen Network in Animal Model Femurs

Stefani Fertaki[1], Dionysis Papachristou[2,3], Christos Kontoyannis[1,4] and Malvina Orkoula[1,*]

[1]Department of Pharmacy, [2]Department of Anatomy-Histology-Embryology, School of Medicine, University of Patras, Rio 26500, Patras, Greece
[3]Department of Pathology, University of Pittsburgh, School of Medicine, Pittsburgh, PA, USA
[4]ICE/HT-FORTH, Patras

Key words: Collagen network, animal model femurs, Raman Spectroscopy

Raman spectroscopy is a vibrational, non-destructive analytical technique with *in vivo* capabilities, suitable for chemical characterization of biological tissues at molecular level. In the present work, it was employed for the evaluation of the collagen network quality in femurs of animal models. Collagen secondary structure is reflected at the vibrations under the amide I envelope (1600-1720 cm^{-1}) in a bone spectrum. It is known that non-reducible cross-links resulted in a band at 1668 cm^{-1} and reducible cross-links in a band at 1690 cm^{-1}. Two groups of animals were used in the study, the wild-type and the knock-out. Calculation of the band area ratio [1668 cm^{-1} / 1690 cm^{-1}], revealed a substantial decrease (29%) for the knock-out mice compared to the wild-type. This may arise from a decrease in the amount of the non-reducible (trivalent) cross-links due to decreased formation of them from the reducible (divalent), and/or an increased amount of the reducible or increased formation of them. Thus, fibers appear less strongly interconnected in knock-out mice than in wild-type, which can have a serious impact on bone fracture durability.

❁

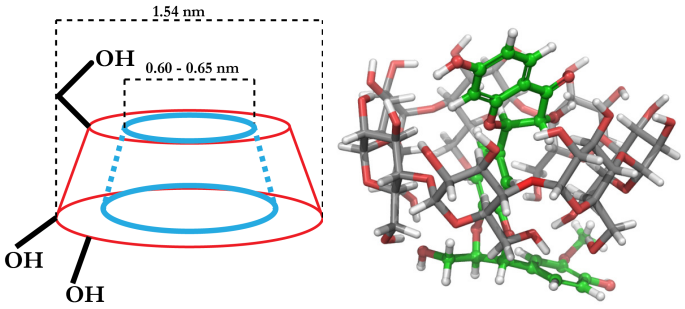
Interactions of Silybin A with Cyclodextrin Derivatives using Solid and Liquid State NMR Spectroscopy, Differential Scanning and Isothermal Titration Calorimetry as well as Molecular Dynamics Simulations

T. Kellici[1,2], D. Ntountaniotis[1], G. Leonis[3], M. Chatziathanasiadou[2], A. Tzakos[2], J. Baldus[4], C. Glaubitz[4], G. Valsami[5], E. Archontaki[1], K. Viras[1], M.G. Papadopoulos[3], *T. Mavromoustakos*[1]

[1]Chemistry Department, [5]Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou 15771, Athens, Greece; [2]University of Ioannina,

Chemistry Department, 45110 Ioannina, Greece; [3]Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece; [4]Institute of Biophysical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

Key words:



The toroidal shape of β-CD (left) and the proposed complex of silybin A (in green) with β-cyclodextrin (gray) (right).

Milk thistle has been used since ancient times to treat a range of liver and gallbladder disorders, such as hepatitis, cirrhosis as well as other gastrointestinal problems. The bioactive phytochemical of this plant is silymarin while the main ingredient of the silymarin complex is silybin, which is present as a mixture of two diastereomers A and B. Silymarin/silybin is considered to be a very safe compound and there are only few reports on its adverse effects. It has lately received attention due to its anticancer and chemopreventive actions. Additionally, references in bibliography have mentioned neuroprotective and cardioprotective activities. As the interest on this compound keeps growing, the limited bioavailability as well as its low water solubility remain the main problems for wider use. The utilization of cyclodextrins (CDs) and their synthetic derivatives have been extensively exploited to improve certain properties of the drugs, such as solubility and bioavailability. The enhanced drug activity and selective transfer or the suppression of side effects can be achieved by inclusion complex formation. Since Silybin possesses five hydroxyl groups and three aromatic rings, CDs are particularly suitable for the increase in solubility as well as for the protection of the compound. In the present study we have used both solid state and high resolution liquid NMR spectroscopy as well as Molecular Dynamics calculations to detect the molecular interactions of silybin A with β-CD and 2-hydroxypropyl-β-CD. A description of the (silybin-CD) system is offered in the right side of the following scheme.

❁

Design and Synthesis of Cyclic Alter Peptide Analogues (APLs) based on the Immunodominant 35-55 Myelin Oligodendrocyte Glycoprotein Epitope (MOG)

A.Tapeinou, M. Agelidakis, E. Kokkali, J. Matsoukas, T. Tselios

Department of Chemistry, University of Patras, GR-26500, Rio, Patras, Greece

Key words: Cyclic Alter Peptide Analogues (APLs), Immunodominant 35-55 Myelin Oligodendrocyte Glycoprotein Epitope (MOG)

Multiple Sclerosis (MS) is considered to be an autoimmune disease characterized by an influx of T cells, B cells and macrophages through the disrupted blood brain barrier and induce inflamemation and demyelination (1). Although the etiology of MS remains unclear, there is evidence that T cells recognizing encephalitogenic epitopes of myelin proteins, such as MOG, playing a pathogenic role in the induction of MS (2). Modern approaches for the treatment of MS involve the design and synthesis of altered peptide analogues of immunodominant myelin epitopes. Cyclization of peptides is of great interest, since the limited stability of linear restricts their potential as

therapeutic agents (3). Herein, we designed and synthesized cyclic peptides by mutating crucial TCR contact residues based on the MOG_{35-55} immunodominant epitope. In particular we have synthesized cyclic APLs by mutating Arg^{41} with Ala or Arg^{41} and Arg^{46} with Ala. The peptides were synthesized in solid phase following the Fmoc/tBu methodology and the DIC, HOBt as coupling reagents. Cyclization was achieved using TBTU, HOAt and collidine reagents. The purity of final products was verified by RP-HPLC and their identification was achieved by ESI-MS.

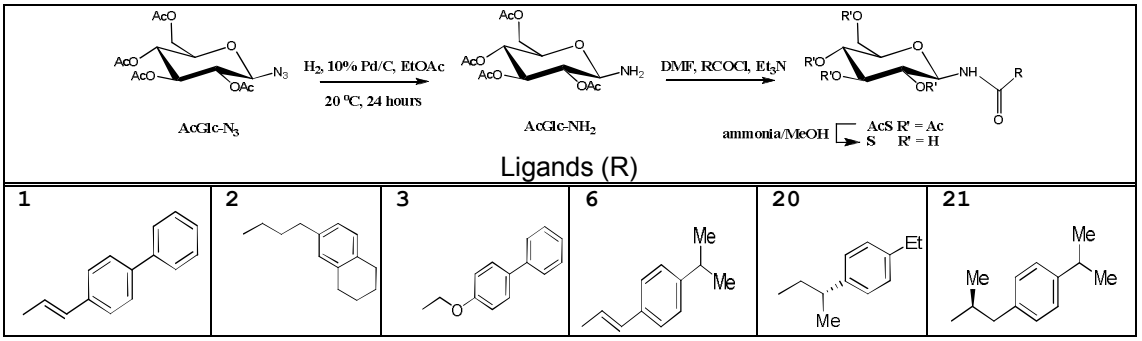
1. Steinman L.: Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. *Cell 85*: 299 (1996)
2. Lutterotti A., Yousef S., Sputtek A., K.H., Stellmann J.P., Breiden P., Schulze S.R.C., Bester M., Heesen C., Schippling S., Miller S.D., Sospedra M., Martin R.: Antigen-specific tolerance by autologous myelin peptide-coupled cells: A phase 1 trial in multiple sclerosis. *Sci. Transl. Med. 5*: 188 (2013)
3. Tselios T., Probert L., Daliani I., Matsoukas E., Troganis A., Gerothanassis I., Mavromoustakos T., Moore G. and Matsoukas J.: Design and synthesis of a potent cyclic analogue of the myelin basic protein epitope MBP72-85: Importance of the ALA81 carboxyl group and of a cyclic conformation for induction of experimental allergic encephalomyelitis. *J. Med. Chem. 42*: 1170-1177 (1999)

❁

N-(*β*-D-Glucopyranosyl) Amides as Glycogen Phosphorylase Inhibitors: Synthesis and Biological Assessment

Vanessa Parmenopoulou[1], Stella Manta[1], *Stavroula Zissopoulou[1]*, Athina Dimopoulou[1], Nikolaos Kollatos[1], Evagelia Geladari[1], Anastassia L. Kantsadi[2], Dimitri Komiotis[1]

[1]Laboratory of Bio-organic Chemistry, [2]Laboratory of Structural & Functional Biochemistry, Dpt. of Biochemistry & Biotechnology, Larissa, Greece



Glycogen phosphorylase is a validated target for the development of type 2 diabetes treatments. For this reason, *N*-acyl-*β*-D-glucopyranosyl amides were synthesized and their inhibitory potency was assessed *in vitro*. Their inhibition constants' values ranged from 5 to 377 μM.

Somsak L.: Glucose derived inhibitors of glycogen phosphorylase. *Comptes Rendus Chimie 14*: 211-223 (2011)