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Drug Discovery and Design

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

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VOLUME 31 ❁ 2017 No 1

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GENERAL INFORMATIONS

REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS INTERNATIONAL EDITION

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

The Journal publishes, in special issues, papers presented at:

- the *Conferences with International Participation Medicinal Chemistry: Drug Discovery and Design organized by the Departments of Chemistry, Medicine and Pharmacy of the University of Patras, Hellas*
- the *Panhellenic Congresses of Pharmacology organized by the Hellenic Society of Pharmacology*

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

INSTRUCTIONS TO AUTHORS

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

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

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

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

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

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

Letter from Guest Editor

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

When we began the Post Graduate Program "Medicinal Chemistry", 19 years ago in our minds we had a vision and a goal. The aim was to establish a research program which would provide: Specialized executives in the vital field of Health, Innovation in the Development of Methods and Products, Innovative Products (active drugs). We also wanted the innovation and the products to be for the benefit of society. Moreover, we would like this program to contribute to the Advancement of Education and the Development of the Country.

Main research interests of the Program are focused on the Organic and Pep- tide Synthesis of Biomolecules, Rational Design with Aided Computer and
Modeling Methods, Biological Evaluations in vivo and in vitro, Molecular Biology, Molecular Medicine, Toxicology, Biomedical Analysis, Pharmacognocy, Pharmacokinetics, Research Methods. The program has organized seventeen (17) Medicinal Chemistry Conferences with International participation. The Program honors each year a distinguished scientist for the important contribution to Bio- medical Research and Science. So far the Program has honored outstanding scientists in the field: Graham Moore, University of Calgary, Canada anf George Kollias, University of Athens, Greece (2015), Harald zur Hausen, Nobel of Medicine, German Cancer Research Center, University of Heidelberg, Germany (2014), Ada Yonath, Nobel of Chemistry, Weizmann Institute of Science, Israel (2013), Kleomenis Barlos, University of Patras, Greece (2012); James D. Watson, Nobel of Medicine and Physiology, Cold Spring Harbor Laboratory, USA (2011); Andrew V. Schally, Nobel of Medicine and Physiology, University of Miami, USA (2010); Dimitrios Nanopoulos, University of Texas, USA (2009); Jean-Marie Lehn, Nobel of Chemistry, Louis Pasteur University, College de France, France (2008); Kyriakos Nikolaou, Scripps Research Institute, USA (2007); Aristidis Patrinos President Synthetic Geonomics Inc., USA (2006), Charalam- bos Gavras, Boston University, USA (2005); Konstantinos Sekeris, University of Athens, Greece (2004); Michael Maragoudakis, University of Patras, Greece (2003), Chris Plat- soukas, Temple University, USA (2002); Athanasios Giannis, University of Leip- zig, Germany (2001); Vasso Apostolopoulou, Austin- Burnet Research Institute, Australia (2000).

After 19 years, the Program with a brilliant path in Greek University, in research, in education, in innovation, and in Connecting with Society, with 250 graduates (with a brilliant professional and academic career), with leading collaborations with outstanding research results, with openness, with large investments in the Program's research, is an Island of Excellence and an Island of Development for the country.

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

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

The Guest Editor
John M. Matsoukas
Professor of Chemistry
Chairman Organizing Committee “Greece Honors James Watson”

MUNICIPALITY OF SPETSES
ANARGYRIOS AND KORGIALENIOS SCHOOL

Under the Auspices
of H.E. the President of Hellenic Republic, Mr Prokopios Pavlopoulos

50 YEARS
1966 – 2016
MOLECULAR BIOLOGY CONFERENCES IN ANARGYRIOS
AND KORGIALENIOS SCHOOL OF SPETSES
since the First Conference with the participation of James Watson &
Francis Crick

17th Medicinal Chemistry Conference

POSTGRADUATE PROGRAM
"MEDICINAL CHEMISTRY: DRUG DISCOVERY AND DESIGN"
DEPARTMENTS OF CHEMISTRY, MEDICINE AND
PHARMACY OF UNIVERSITY OF PATRAS

August 29-31, 2016

Anargyrios and Korgialenios School of Spetses
"From Molecular Biology to Medicinal Chemistry"
Spetses, Hellas

Greetings for 17th Medicinal Chemistry Conference

John Matsoukas
Professor of Chemistry
Chairman Organizing Committee
"Greece Honors James Watson"

I would like to welcome our visitors and thank them heartfully for their participation in this celebration and joy. Professor Watson, Professor Padel, distinguished colleagues thank you very much for the honor of being with us today.

Today is a day of Honor, Joy and Pride for Anargyrios and Korgialenios School of Spetses, for the Graduate Program of Medicinal Chemistry which co-organized the event, for the Postgraduate Research in our country, for our universities, for Greece. Today we celebrate a historic anniversary, the 50 years of Molecular Biology Conferences and the presence of a leading researcher of the 20th Century and of all times, James Watson, and a friend of our country. We honor the researcher whose discovery, a pioneer of Science, changed human thinking and our lives and laid the human kind to a new world, a very different and better world.

In the first Molecular Biology Conference in Spetses, just before fifty years ago, in 1966, James Watson and Francis Crick presented the discovery of the double helix of the DNA. For the immense discovery of the double helix on February 28, 1953, the most important of the 20th century and for many of all time, they had been awarded with the Nobel Prize of Medicine and Physiology in 1962.

In this Conference, apart from Professor Watson, we have the pleasure and honor to have with us Professor Ruth Padel, a Greek scholar and Professor of Poetry at King's College London. Professor Padel is the great-great granddaughter of Charles Darwin and her work is inspired by her ancestor. Two leading scientists, landmarks of two great discoveries, of DNA and Evolution, respectively, meet for the first time today here in Spetses. With this conference we also honor the Postgraduate Research and our graduate students everywhere in Greece and abroad, giving the message that Greece produces innovation and excellence. James Watson's response and presence here, at this conference is a proof of excellence in Greece.

ACCEPTANCE SPEECH FOR THE NOBEL PRIZE IN MEDICINE AND PHYSIOLOGY 1962 SWEDISH ACADEMY, STOCKHOLM CONCERT HALL, 10 DECEMBER 1962 DISCOVERY THE DOUBLE HELIX OF DNA

James D. Watson

Your Majesties, Your Royal Highnesses, Your Excellencies, Ladies and Gentlemen.

Francis Crick and **Maurice Wilkins** have asked me to reply for all three of us. But as it is difficult to convey the personal feeling of others, I must speak for myself. This evening is certainly the second most wonderful moment in my life. The first was our discovery of the structure of DNA. At that time we knew that a new world had been opened and that an old world which seemed rather mystical was gone. Our discovery was done using the methods of physics and chemistry to understand biology.

I am a biologist while my friends **Maurice** and **Francis** are physicists. I am very much the junior one and my contribution to this work could have only happened with the help of **Maurice** and **Francis**. At that time some biologists were not very sympathetic with us because we wanted to solve a biological truth by physical means. But fortunately some physicists thought that through using the techniques of physics and chemistry a real contribution to biology could be made. The wisdom of these men in encouraging us was tremendously important in our success. **Professor Bragg**, our director at the Cavendish and **Professor Niels Bohr** often expressed their belief that physics would be a help in biology. The fact that these great men believed in this approach made it much easier for us to go forward.

The last thing I would like to say is that good science as a way of life is sometimes difficult. It often is hard to have confidence that you really know where the future lies. We must thus believe strongly in our ideas, often to point where they may seem tiresome and bothersome and even arrogant to our colleagues. I knew many people, at least when I was young, who thought I was quite unbearable. Some also thought **Maurice** was very strange, and others, including myself, thought that **Francis** was at times difficult. Fortunately we were working among wise and tolerant people who understood the spirit of scientific discovery and the conditions necessary for its generation.

I feel that it is very important, especially for us so singularly honored, to remember that science does not stand by itself, but is the creation of very human people. We must continue to work in the humane spirit in which we were fortunate to grow up. If so, we shall help insure that our science continues and that our civilization will prevail. Thank you very much for this very deep honor.

REVIEW CLINICAL PHARMACOLOGY AND
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THE TREE OF LIFE:
Charles Darwin – His Thought, Life and Family

Ruth Padel

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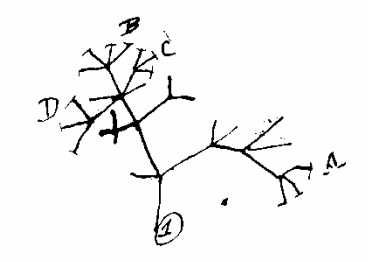

In this illustrated talk the British poet Ruth Padel, Charles Darwin's great great granddaughter, reveals the development of Darwin's idea of evolution in the context of his family and life. She draws on her family, in particular her grandmother Lady Nora Barlow, the first scholar of Darwin's work. Nora Barlow edited Darwin's *Autobiography*, his *Voyage of the Beagle,* and the *Letters* between himself and John Henslow (his geology tutor at Cambridge) written while he was on the Beagle voyage 1831-35. These letters trace the evolution of his ideas about what he came, later, to call the "mutation" of species. The first portrait of Charles is at the age of seven with his little sister. He is holding a living plant in a flower pot. Much of his later work focussed on plants: his own garden in Kent, reflected the insights into the inter-relation of species which began in the tropical forests of Brazil. So it is appropriate that in 1838, when he encapsulated his great insight in a visual image, it took the form of a tree: with the typically modest words accompanying it in his notebook, "I think."

REVIEW CLINICAL PHARMACOLOGY AND
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EDITION 31: 12 (2017)

Engineered Cell Vesicles for Drug Delivery

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Purpose: To isolate, characterize and evaluate engineered cells as vesicles for drug delivery.

Materials and methods: Engineered cell vesicles (CVs) (encapsulating FITC-dextran or Calcein as an aqueous space probe), were prepared following isolation of Human Embryonic Kidney (HEK) cells and B16 melanoma cells. The cell material was engineered to prepare vesicles of nanosize which encapsulate a fluorescent aqueous compartment probe, such as FITC-dextran or calcein, by applying a number of consecutive freeze- thawing cycles. The CVs produced were characterized for their size distribution and surface charge by DLS, and for their FITC-dextran content by measuring Fluorescent Intensity (FI). The lipid concentration was measured by the Stewart assay. Calcein integrity of HEK and B16 CVs in absence and presence of serum proteins was evaluated, as a method to evaluate their applicability for Drug Delivery applications, by measuring the latency of entrapped calcein during incubation in buffer or FCS at 37^0C, for 24h. Finally, the cytotoxicity of the two CV-types towards B16 and HEK cells after 4h of incubation of 200 and 400 nmoles of lipid (from each formulation)/10^6 cells, was measured by the MTT assay.

Results: CV mean hydrodynamic diameters were between 200-300nm with negative charge (-15mV). The retention of calcein in EVs, was stable during 8h of incubation in buffer and FCS, however there was a gradual release of the vesicle-entrapped dye between 8 and 24h. Interestingly, there was no difference of CV integrity in buffer and in presence of proteins (FCS). The cytotoxicity results indicated that both CV types are non-toxic towards the cells evaluated.

Conclusions: The CVs produced are nano-sized, encapsulate good amounts of calcein and FITC, are non-cytotoxic and are considerably stable in presence of proteins, which prove that such engineered cellular vesicles have potential for drug delivery applications.

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REVIEW CLINICAL PHARMACOLOGY AND
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Implementation of Computational Tools for the Design and Discovery of P2X7 Receptor Inhibitors Against the Inflammasome Activation

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The purinergic P2X7 receptor is activated by ATP or its breakdown products, ADP and AMP and it is converted to an inotropic ligand-gated non-selective cation channel. P2X7 is widely distributed throughout the mammalian body and it has an important role in inflammation and immunity. Recent studies indicate that P2X7 receptor is a potential target in common inflammatory diseases. Prolonged activation of the P2X7 receptor drives to the inflammasome formation, mainly of NLRP3, a protein complex which is associated with various chronic and rare inflammatory diseases. In the present study, *in silico* tools including pharmacophore modeling and virtual screening have been implemented to identify hit compounds, as potential antagonist for P2X7, from commercial libraries.

REVIEW CLINICAL PHARMACOLOGY AND
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Mathematical Literature: A New Literary Genre?

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Maths and Literature, two terms seemingly incompatible, have occupied in recent decades as theorists and critics of literature as scientists and scholars from the field of mathematics. The discussion of their relationship took greater dimension in response to the publication of the novel "Uncle Petros and Goldbach's Conjecture" by Apostolos Doxiadis, who had great success firstly abroad and then in our country. The British journalist Gilbert Adair, based on the work of Apostolos Doxiadis, introduces the term Mathematical Literature. How far back in time is justified the association of those two areas? Today can we say that we are in front of the birth of a new literary genus?

And if so what are the typical traits? Can it be said that the discussion between the two areas expresses the idea of two cultures as noted by Pamela Grossing? The purpose of this announcement is to illuminate the hermetically sealed universe of "Mathematical Literature" and to examine in detail the relationship between the two areas within international and Greek literature. Is the Mathematical Literature able to offer an adequate alternative approach to mathematics released from the purely didactic mathematical reason?

REVIEW CLINICAL PHARMACOLOGY AND
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Detection and Quantification of Nicotine in Saliva Using Raman Spectroscopy

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Detection of substances, such as nicotine, in biological fluids can be the subject of study for many disciplines, such us toxicology. This type of analysis is usually performed with High Performance Liquid Chromatography and Gas Chromatography - Mass Spectrometry. Their advantages include precision, low detection limits, but they are expensive, time-consuming and require expertise. In this study, Raman Spectroscopy is used as an analytical tool for the detection and quantification of nicotine in human saliva samples. Speed and easiness of analysis combined with the possible use of portable systems equipped with optical fibers, are some of the benefits. For this purpose, mixtures of nicotine in saliva of non-smoking volunteers were prepared, in known concentrations, and their spectra were collected. Nicotine was proved to be detectable in levels as low as μg / ml of sample. Detection of nicotine in saliva samples of systemic-smokers volunteers with the proposed methodology was also successful.

REVIEW CLINICAL PHARMACOLOGY AND
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Application of Wireless Electric Stimulation and Pulsed Electromagnetic Radiation in Protein Crystallization

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

In this work two technologies are investigated upon crystallization: the influence of non-contact current transfer using a prototype ion-generator (O^{2-}) device (NCCT device) and the influence of electro-impulses using an electronic patch (RX patch). The use of these two devices is much simpler and easier than other devices with classic electrodes. Also the risk of contamination is minimized.

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

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

REVIEW CLINICAL PHARMACOLOGY AND
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Synthesis and Biological Activity of Ni(II) Complexes With the Antimicrobial Agent Fleroxacin

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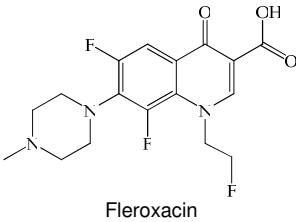
Fleroxacin (figure) is a quinolone antimicrobial drug acting by the inhibition of DNA-gyrase. It has a broad spectrum of activity against a range of Gram-(-) and Gram-(+) bacteria and is mainly used for the treatment of urinary tract infections [1,2]. Despite the limited biological significance of nickel, nickel complexes have reported to exhibit remarkable antimicrobial, antifungal and antioxidant activity [3,4].

We present herein the synthesis and characterization of Ni(II) complexes with the antimicrobial drug fleroxacin in the absence or presence of the N,N'-donor ligands 2,2'-dipyridylamine (bipyam) and 1,10-phenanthroline (phen). The interaction of the complexes with calf-thymus DNA was investigated by UV spectroscopy and DNA-viscosity measurements and by evaluating their ability to compete with ethidium bromide for the DNA intercalation sites. Furthermore, the ability of the complexes to bind to HSA and BSA albumins was studied by fluorescence emission spectroscopy.



Fleroxacin

KEYWORDS: quinolones, fleroxacin, nickel(II) complexes, biological activity

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REVIEW CLINICAL PHARMACOLOGY AND
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Utilizing the Virus-induced Blocking of Apoptosis in an Easy Baculovirus Titration Method

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Baculovirus-mediated protein expression is a robust experimental technique for producing recombinant higher-eukaryotic proteins because it combines high yields with considerable post-translational modification capabilities. In this expression system, the determination of the titer of recombinant baculovirus stocks is important to achieve the correct multiplicity of infection for effective amplification of the virus and high expression of the target protein. To overcome the drawbacks of existing titration methods (e.g., plaque assay, real-time PCR), we present a simple and reliable assay that uses the ability of baculoviruses to block apoptosis in their host cells to accurately titrate virus samples. Briefly, after incubation with serial dilutions of baculovirus samples, Sf9 cells were UV irradiated and, after apoptosis induction, they were viewed via microscopy; the presence of cluster(s) of infected cells as islets indicated blocked apoptosis. Subsequently, baculovirus titers were calculated through the determination of the 50% endpoint dilution. The method is simple, inexpensive, and does not require unique laboratory equipment, consumables or expertise; moreover, it is versatile enough to be adapted for the titration of every virus species that can block apoptosis in any culturable host cells which undergo apoptosis under specific conditions.

REVIEW CLINICAL PHARMACOLOGY AND
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Navigated Drug Delivery in Cancer

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Cancer is a complex and evolving disease and recent diagnosis/treatment options have shifted towards targeted therapies. Tumor targeting ligands (TTLs) are emerging as an important component in customized therapies. TTLs can assist grading tumors and tailoring treatment accordingly through installation of cell-targeting/specific ligands to therapeutic agents. These ligands can be also conjugated to molecular imaging agents to follow expression of the targeted biomarker during treatment.

Herein, we will present our efforts to develop novel anticancer agents and nanoparticles targeting cancer drug targets and tumor microenvironment, examples of selective delivery of drugs to cancer cells through targeting cell surface receptors as also the development of tumor homing peptides.

ACKNOWLEDGEMENT

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

REVIEW CLINICAL PHARMACOLOGY AND
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BMP2/4 Signaling Restricts Coup-TF's Gene Expression to Ventral Ectoderm of the Sea Urchin Embryo

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Coup-TF, a highly conserved gene in all metazoans, plays an important role in organogenesis and neurogenesis during embryonic development. Recent mutagenesis studies from our laboratory indicate that an upstream 320bp module (-212 to -532) contains three elements (-453, -432 and -377), which are responsible for *PlCoup-TF's* late embryonic regulation in the ventral ectoderm (*Kalampoki LG and Flytzanis CN,2014*). The aim of this project is to identify the transcription factor(s) that binds the -453 element and acts as a positive regulator and the factors that bind to -432 and -377 elements and act as negative regulators. These transcription factors seem to be necessary and sufficient for correct quantitative and spatial regulation of the *Coup-TF* gene. Thus, element specific DNA affinity chromatography, using the three cis-acting elements and embryonic nuclear protein extracts, was employed to isolate specific DNA binding proteins. Proteomic analysis was performed for the identification of the corresponding nuclear factors. Following bioinformatics, the transcription factors Otx (orthodenticle related homeo-domain) and Hox7 (homeo-domain) were selected for further study as repressors that bind -377 element and restrict *Coup-TF* gene expression to the vental ectoderm (neurogenic region) of the embryo. The *in vivo* regulatory role of Otx and Hox7 in regards to *Coup-TF* expression was studied in morphant (knockdown) embryos, created by microinjection of specific morpholino antisense oligonucleotides. Otx and Hox7 morphant embryos show delayed development and allow ectopic *Coup-TF* expression in the dorsal ectoderm. *In vitro* translated Otx protein, was tested by EMSA experiments for its *in vitro* binding ability to the -377 *cis*-acting element. The results of these experiments indicate that Otx binds to the -377 element, specifically and with high affinity, suggesting that Otx is the negative regulator of Coup-TF. Furthermore, the *in vivo* role of Hox7, Hox11/13b and IrxA transcription factors was studied, considering that BMP2/4 signaling activates gene expression of these factors in the dorsal ectoderm. Silencing of the aforementioned transcription factors causes expansion of *Coup-TF* gene expression to the dorsal ectoderm of the embryo. As a result BMP2/4 signaling is suggested to restrict *Coup-TF's* expression to the ventral ectoderm by activating the IrxA, Hox7 and Hox11/13b repressors in the dorsal ectoderm of the sea urchin embryo.

REVIEW CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
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The Heart, The Miracle, The Mind. Poetry and Science

Anna Katsigianni

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This paper aims at exploring basic aspects of the interaction between poetry and science during the 20th and the 21st centuries. More specifically it focuses on studying the influence of a wide range of sciences on the poetical discourse. Due to time limitations it has to be confined to a limited number of poets of different literary movements. A tendency to base their poetics upon various scientific fields is the unifying thread of many different poetical temperaments. The dialogue between poetry and science is not new: it can be traced back to Palamas's scientific positivism and the dynamic presence of technological terminology in poetic discourse, or later within the frame of futurism, the most radical aesthetic movement in art, for both content and form. During the 20th century avant-garde, the influential power of science over poetry has been expanded through the use, by the surrealists, of the scientific method of psychoanalysis. In the post-modern era, various options of scientific thinking and discourse dominate many poetical voices investigating the big existential *aporia*.

REVIEW CLINICAL PHARMACOLOGY AND
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hCoup-TF II's Role in Human Endothelial Cells

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Endothelial cells form a single layer that invests all the vessels of the body and play an extremely important role in a variety of homeostatic mechanisms. Two of the most characteristic features of endothelium are the participation in the process of inflammation and its ability to remodel the vascular wall (angiogenesis). Chicken ovalbumin upstream promoter Transcription Factor-II (Coup-TFII), a member of the nuclear receptor superfamily, plays a critical role in angiogenesis during developmental and in pathological conditions. Targeted deletion of the Coup-TF II gene results in embryonic lethality with defects in angiogenesis, lymphangiogenesis and heart development. It is also shown that Coup II is actively involved in the molecular pathway determining vein identity. Because Coup-TF II is also strongly expressed in Human Umbilical Endothelial Cells (HUVECs), we hypothesized that it might play an essential role in the intrinsic function of endothelial cells.

To answer this question, we knocked down Coup-TF II in HUVECs and we examine their ability in sprouting, proliferation and migration. We observed that depletion of Coup-TF II decreased substantially the number of sprouts, the number of proliferating cells and affects endothelial cell migration in a critical way. Another goal in our study was to determine whether the silencing of Coup-TF II affects the ability of endothelial cells to participate actively in inflammation. We already know that endothelial cells play a key role in the ability of immune cells to move to the affected area and the involvement of Coup-TF II in this process was an unexplored field. What we observed was that the inhibition of this transcription factor reduces the response of endothelial cells to proinflammatory agents.

REVIEW CLINICAL PHARMACOLOGY AND
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Effect Crocus Sativus Extract on Acetylcholinesterase Activity of Specific Brain Regions in Adult Male Rats after Induction of Type I Diabetes

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The aim of the present study was to investigate the potential beneficial effect of *Crocus sativus L.* (saffron) styles' extract on brain cholinergic system of adult male rats (Wistar) with streptozotocin-induced type I diabetes. Rats were divided into the following four groups: Control group (**C**): vehicle-injected. Group of diabetic rats (**D**): diabetes was induced with a single intraperitoneal injection of streptozotocin (55 mg/kg, day 0). Group with saffron administration (**Cr**): 60mg/kg of saffron extract intraperitoneally injected every other day. Group of diabetic rats with ongoing administration of saffron extract (**DCr**): treatment started 2 weeks after the induction of diabetes and lasted 10 weeks. After the end of the administration, the rats were sacrificed and specific brain regions (cortex, striatum, hippocampus and cerebellum) were isolated. The activity of G1 and G4 isoforms of acetylcholinesterase (AChE) was determined, in both salt-soluble (SS) and detergent-soluble (DS) fraction respectively, by using Ellman's colorimetric method. The results revealed: a) statistically significant increase in the activity of both AChE isoforms in striatum and hippocampus of the diabetic group (D), which indicates cholinergic system deficiency of those regions, and b) statistically significant inhibition of G4 isoform activity in all brain regions studied, as well as G1 isoform activity of striatum, after administration of saffron extract on the diabetic group (DCr) and the non-diabetic group (Cr), an observation that exhibits a positive effect on the cholinergic system of those regions. Conclusively, the styles' extract of *Crocus sativus L.* in the induced type I diabetes, has a beneficial impact on all brain regions studied respecting G4 AChE isoforms' activity, while the positive effect on G1 isoforms' activity presented tissue-specificity.

REVIEW CLINICAL PHARMACOLOGY AND
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Cobalt(II) Complexes with the Quinolone Gatifloxacin: Synthesis, Characterization and Biological Studies

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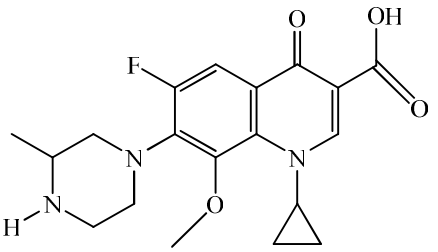
Quinolones are among the most commonly-used antibacterial drugs with a broad spectrum of antibacterial activity against pathogens. Gatifloxacin is a third- generation fluoroquinolone and exhibits improved action in comparison to first-generation quinolones [1]. Its mechanism of action depends on the interference with DNA inhibiting thus the replication process and the protein synthesis [2]. Cobalt compounds play an important role in a plethora of biological processes and as a result it is considered as a biologically active metal [3]. Metal complexes of quinolones have exhibited synergetic activity and are a subject with a numerous research studies [4]. In addition, the presence of N-donor ligands, such as 2,2'-bipyridine, 1,10-phenanthroline and 2,2'-dipyridylamine may increase the biological effect.

In this study the synthesis and the characterization of Co(II) complexes with the antibacterial drug gatifloxacin are presented as well as their interaction with calf-thymus DNA and serum albumins. Furthermore the results of the antibacterial activity of the compounds are presented.



The syntax formula of gatifloxacin

KEYWORDS: quinolones, gatifloxacin, cobalt(II) complexes, biological activity.

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REVIEW CLINICAL PHARMACOLOGY AND
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Raman Spectroscopy: An Analytical Tool for Biological Tissues

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Due to the plethora and the diversity of compounds present in biological tissues identification is a formidable analytical task. Several powerful analytical techniques such as LC-MS are applied but there are several cases that the information that a researcher is seeking e.g. exact location of a material in a tissue or the subtle changes or differences in a biomacromolecule such as collagen requires the use of a versatile tool such as Raman spectroscopy (RS). Despite the inherent weak Raman signal RS offers some unique advantages. As a vibrational technique a Raman spectrum can be used for identification purposes, like an IR spectrum, but unlike IR, RS faces no problem dealing with water, a compound abundant in tissues, since it is a weak Raman scaterer. Other advantages include, but not limited to, its capability of “mapping” an area of a tissue of interest. In this presentation application of RS on tissues such as bone and meniscus will be examined.

REVIEW CLINICAL PHARMACOLOGY AND
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PEGylated Analogues of the Epitope 82-98 of Myelin Basic Protein (MBP) in Research Related to Immunotherapy of Multiple Sclerosis

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

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REVIEW CLINICAL PHARMACOLOGY AND
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Investigation of the Effect of Antitumor Agents on the Mechanical Properties of Breast Cancer Cells During Adhesion

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Introduction

Adhesion of cancer cells is a pivotal step for the progression of cell movement, which may lead to cancer metastasis [1]. This process encompasses deformation of cell membrane and formation of cellmatrix junctions, hence alterations to cell morphology and mechanical properties of cancer cells. This study focus on the effect of antitumor agents to adhered breast cancer cells and correlate with the alteration of mechanical properties.

Materials/Methods

Breast cancer cell lines SKBR-3 (HER-2) and MCF-7 (hormone-depended) were used for the *in vitro* experiments, before and after treatment with antitumor agents 10μg/ml of herceptin (antibody against HER-2) and 10nM of tamoxifen (selective estrogen receptor modulator) respectively. Via micropipette aspiration technique breast cancer cells attached on glass surface coated with collagen-I and after 24h we measured their mechanical properties by aspirating them into the micropipette (radius Rp), applying a range of negative pressures ΔP (0.5-300Pa) and measuring the corresponding cell tongue deformation(L) (Fig.1), therefore, the elastic Young's modulus (E) was determined. Additionally, single cell migration was examined with boyden chamber assay for both cancer cell lines before and after drag treatment.

Results

Mechanical properties of cancer cells were obtained by determining the elasticity of cell membrane. Hence, Young's modulus was calculated through $E = 2(\nu + 1)G$, where elastic shear modulus (G) was determined through the equation of linear elastic solid model:

$$\frac{L}{R_p} = \frac{\varphi_p \Delta P}{2\pi G}$$

since cancer cell regarded as an incompressible elastic half-space, homogeneous isotropic material [2]. Both cancer cell lines reduced their Young's modulus after treatment in a statistically significant way. Regarding single cell migration, antitumor agents reduced in both cancer cell lines their ability to migrate.

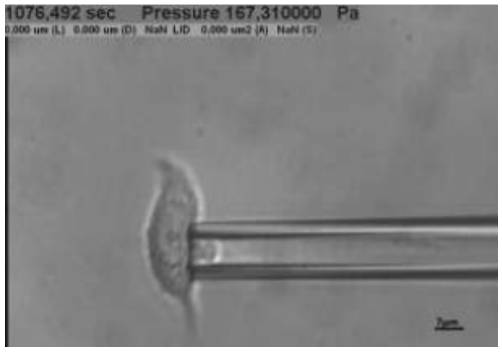

Fig.1 Adhered breast cancer cell (MCF-7) deforms via micropipette aspiration technique.

Discussion

After treatment with antitumor agents tamoxifen and herceptin, we observed reduction of elastic modulus as well as in single cell migration, limiting thus the capacity of cell movement. The use of tamoxifen and herceptin alters the mechanical properties of MCF-7 and SKBR-3 in a way that reduces the metastatic potential of cells.

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REVIEW CLINICAL PHARMACOLOGY AND
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DNA-based Methods for Detection of Allergens in Foodstuffs

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Food allergies are defined as an immune response to food proteins. Food allergens present in foodstuffs have to be labelled according to the European Union. Food industry has established internal threshold values regarding allergens added by recipe, as well as allergens present due to cross-contamination. Many analytical methods have been reported for the detection of various allergens based on its protein or nucleic acid content. However, intensive processed and heat-treated foods are characterized by a higher degradation of proteins compared to DNA. Thus, DNA is preferred due to the fact that is efficiently extracted from food matrices and is less affected by extraction conditions or food processing compared to proteins. In recent years, the number of proposed DNA methods for food allergen detection has been raised. The aim of this study was the development of an analytical method for the simultaneous detection of various nuts that are present even in traces in foods. The method involves DNA isolation from foods, amplification of specific DNA sequences of nuts' genome by Polymerase Chain Reaction (PCR) and a subsequent multiple hybridization assay on spectrally distinct microspheres. These microspheres are carboxylated and contain internally two fluorescent dyes in various ratios producing up to 100 different groups. The amplified PCR products are biotinylated and hybridize to complementary amino-oligonoucleotide probes that are attached to the microspheres. The hybrids are then detected by streptavidin-phycoerythrin conjugate. The analysis of the microspheres is carried out by flow cytometry. The intensity of the measured fluorescence is proportional to the concentration of the corresponding DNA sequence. The method was applied for the detection of peanut, hazelnut and walnut. The limit of detection of the proposed method was 1.4 pM for all the three DNA sequences. The method was also applied successfully for the detection of the three allergens in unprocessed and processed food such as cookies. Different mixtures of cookies were prepared that contained all the three different nuts in proportions varied from 0.01% to 5%. As low as 0.01% of each nut present in the cookie mixtures were detected by this method.

REVIEW CLINICAL PHARMACOLOGY AND
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The Mathematical Universe of Tefcros Michaelides' Literature

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Tefcros Michaelides is a Greek writer and translator with a constant interest in narratives involving mathematics. With his work in education and translation and his contribution in “Thales + Friends”, he is directly involved with the mathematics pedagogy and the need for improved communication tools that commune the mathematical language. This presentation will explore the literary work of Tefcros Michaelides as an open dialogue of science and the history of mathematics, with the narrative means of literature. Can the alloy of literature and mathematics compose a meeting of art and science on behalf of the latter?

REVIEW CLINICAL PHARMACOLOGY AND
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The Mathematical Reasoning as a Field of Narrative Action in the Novel Uncle Petros and Goldbach's Conjecture. The Pursuit of the Beautiful and Truth

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The novel “Uncle Petros and Goldbach’s Conjecture” by Apostolos Doxiadis is a milestone work of the so called Mathematical Literature. Uncle Petros is a mysterious person. Initially rejected by his relatives as a person who has failed in his life until his nephew after several attempts discovers that in the past he was a famous mathematician. He was even so edgy that he decided to devote his whole life to the famous Goldbach’s Conjecture. After his discovery a series of several reactions are triggered. Apostolos Doxiadis on the novel attempts to combine fragments that form the multifaced personality of the main character, a personality entirely tragic which is trapped in the nets of the Beautiful and Truth. The price someone pays when he wants to come closer to the truth becomes evident through the personal story of uncle Petros. George Steiner pointed out that Apostolos Doxiadis’ work is very generous as it offers access to fields that are inherently hermetically closed. The announcement aims to identify the structural similarities between narrative and mathematical proof. The narrative can be seen as a journey with a beginning, middle and end. Can we say that the same applies to the mathematical proof? What about the narratives which are opened to many interpretations? Has the mathematical proof many meanings? Can the mathematical proof be considered as a kind of narrative? What is the role of Mathematical Platonism in this novel? There are all crucial questions that concern both theorists and critics of Literature and scholars of Mathematics and this announcement attempts to examine them in detail.

REVIEW CLINICAL PHARMACOLOGY AND
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The Necessity for New Antibiotics

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

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

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

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

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

REVIEW CLINICAL PHARMACOLOGY AND
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New 3,4-Dihydroquinoxalin-2(1*H*)-one Derivatives as Potential Soluble Guanylate Cyclase (sGC) Activators

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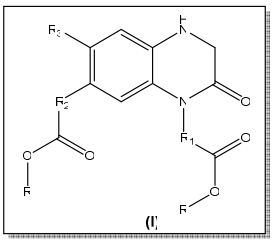
Soluble guanylate cyclase (sGC) is a heterodimeric heme protein which catalyzes the GTP-cGMP conversion, upon binding of nitic oxide (NO) to a prosthetic heme moiety.[1] Several diseases, including systemic and pulmonary hypertension (PH), heart failure, atherosclerosis and thrombosis, are associated with abnormal sGC activity. To overcome these obstacles, direct NO- and heme-independent sGC activators have been developed. These compounds selectively activate the oxidized/heme-free form of sGC upon binding to H-NOX domain.[2]



(I)

Aiming at the discovery of new sGC activators and following a structure-based design approach, we designed and synthesized a library of new 3,4-dihydroquinoxalin-2(1*H*)-one derivatives (I). Introducing several designed molecules to docking calculations, we identified the structural features of these derivatives that mediate residue-specific interactions with sGC H-NOX domain, with the aim to improve binding and sGC activation. Herein, the results of the computational studies and our synthetic efforts towards the target compounds will be presented.

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REVIEW CLINICAL PHARMACOLOGY AND
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Design and In Vitro Evaluation of a Peptide-based Experimental Nanovaccine Against Visceral Leishmaniasis

E. Athanasiou, M. Agallou, M. Margaroni, O. Kammona, C. Kiparissides, E. Karagouni

Visceral leishmaniasis (VL), caused by *Leishmania (L.) donovani* and *L. infantum* protozoan parasites, can provoke overwhelming and protracted epidemics, with high case–fatality rates. Since the arsenal of drugs available is limited and chemotherapy gathers many disadvantages, the development of a highly prophylactic, safe and affordable vaccine is considered as the main tactic of controlling VL. Aim of the present study was the design of chimeric peptides from immunogenic *L. infantum* proteins for encapsulation in PLGA nanoparticles (NPs) and the *in vitro* evaluation of their effect on maturation and functional differentiation of dendritic cells (DCs). Three *L. infantum* proteins (CPA, histone H1, KMP-11) with protective role against VL, were analyzed *in silico* for the prediction of binding epitopes to HLA class I and class II molecules. Peptides including at least one HLA class I-restricted epitope scored very high, as well as adjacent or overlapping HLA class II-restricted epitopes were designed and formed the basis for the synthesis of chimeric peptides with the use of appropriate linkers. Each chimeric peptide was encapsulated in PLGA NPs alone or in combination with an adjuvant, or in PLGA NPs surface modified with an octapeptide (p8) mimicking the TNFα docking region with TNFRII.

The *in vitro* evaluation of the nanoformulations was performed in DCs isolated from transgenic mice expressing an interspecies hybrid MHC class I gene with the alpha-1 and alpha-2 domains of the human HLA-A2.1. Encapsulation of chimeric peptides in PLGA NPs resulted in a significant increase in the number of DCs expressing CD40, CD80, CD86, MHC class I and MHC class II molecules, in comparison to DCs pulsed with soluble peptides. MPLA incorporation, as well as surface modification with p8 enhanced maturation, with a significant increase in the number of DCs expressing the hybrid HLA-A2.1 confirming the successful design of chimeric peptides. DCs pulsed with a mixture of p8-modified PLGA NPs or a mixture of PLGA NPs with co-encapsulated MPLA induced strong T cell priming and polarization to $CD4^+$ T_H1 and $CD8^+$ T cell subsets. In conclusion, the above nanoformulations were proved suitable candidates for the development of an experimental vaccine against VL, and the *in vitro* system used could be considered as a useful tool for the screening of candidate vaccine subunits contributing to the reduction of animal use in preclinical studies.

REVIEW CLINICAL PHARMACOLOGY AND
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Multiple Sclerosis and Treatment: Pharmaceutical Remedy and Diet with Exercise

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Multiple Sclerosis (MS) is a chronic inflammatory disease of the Central Nervous System (CNS) characterized by the degeneration of the insulating caps of the nerve cells of the brain and spine.
Most nerve axons are surrounded by an insulating lipoprotein, myelin, which helps in the transmission of nerve impulses. The destruction of myelin disrupts the ability of parts of the nervous system to communicate, resulting in a wide range of symptoms, including physical, intellectual, and sometimes psychiatric problems.

In this study the disease is presented with reference to the pathogenesis and epidemiology of the disease. Environmental factors affecting the development of MS with reference in the etiology and pathophysiology will be analyzed, and a report will take place on the existing therapies and new approaches, involving either pharmaceuticals or vitamin D intake combined with physical exercise.

The ultimate goal is to compare drug treatments and enhance the one that helps to suppress the disease and at the same time improves sectors that make up the patient's quality of life.

KEYWORDS: Multiple Sclerosis (MS), Central Nervous System (CNS), Myelin, Etiology and Pathophysiology of MS, Vitamin D.

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REVIEW CLINICAL PHARMACOLOGY AND
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EDITION 31: 36 (2017)

Towards the synthesis of Tumor Necrosis Factor (TNFα) protein by combination of Solid-Phase Fragment Condensation and Native Chemical Ligation

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The human Tumor Necrosis Factor (TNFα) is a protein that naturally occurs inside the body and consists of 157 amino acids. TNFα is generally considered to be a pro-inflammatory cytokine and its primer role is in host defense against some bacterial infections by initiating the defense response to local injury. For this role, its concentrations in tissues are low and its effects are beneficial. However, at high concentrations, TNFα can lead to excess inflammation and organ injury, so it plays a key role to some immune-mediated inflammatory diseases. The expression of TNFα in the affected tissue is increased, leading to the induction of a variety of direct pathogenic effects and the production of other mediators of inflammation and tissue destruction. Recently, TNFα antagonist monoclonal antibodies have been developed, with a potential role in the treatment of certain inflammatory diseases and certain cancers. The aim of this project is the total chemical synthesis of human TNFα protein by combination of Solid Phase Fragment Condensation (SPFC) and the Native Chemical Ligation methods. The TNFα sequence was divided into two large peptide segments consisting of 69 and 88 amino acid residues. The two segments will be synthesized by combining the Fmoc/tBu-based step-by step Solid-Phase Synthesis (SPS) with the condensation of suitably selected protected peptide fragments on 2-chlorotrityl resin (CTC resin) and subsequently will be joined together by the Native Chemical Ligation method to the entire protein sequence.

REVIEW CLINICAL PHARMACOLOGY AND
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Quantitative Determination of Nicotine in Electronic Cigarette Refill Liquids Using Raman Spectroscopy

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Electronic cigarettes are widely used during recent years as a healthier alternative to cigarettes containing tobacco. These battery operated devices cause vaporization, through suitable apparatus, of solutions known as electronic cigarette refill liquids which consist of humectants, flavoring agents and nicotine at concentrations ranging from 0 to 24 mg/mL. Quantification of nicotine is mainly performed by the techniques of High Performance Liquid Chromatography (HPLC) and Gas Chromatography conjugated with Mass Spectrometry (GC/MS). In the current study, an alternative method, based on Raman spectroscopy, was developed for the quantitative determination of nicotine in solutions used in electronic cigarette devices. Important advantage of the technique, along with the ease of operation and short time of analysis, is the possibility of on line analysis and data acquisition through sealed containers using a portable spectrometer equipped with an optical fiber probe. The proposed methodology was employed for the study of a large number of commercially available electronic cigarette refill liquids. Relative errors from the values indicated in the package were found to be around 10%.

REVIEW CLINICAL PHARMACOLOGY AND
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Detection of Heavy Metals in Electronic Cigarette Refill Liquids

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Electronic cigarettes are battery operated devices that deliver vapor to the user through vaporization of solutions (electronic cigarette refill liquids). These liquids consist of humectants, flavoring agents and nicotine. Heavy metals may enter the refill liquid during the production process or during vaporization, where the liquid is in contact with the metallic resistance of the steaming device at high temperature. Those metals may ultimately be inhaled by the users with potential toxic effects on their health. In the present study, a methodology, based on Total Reflection X-Ray Fluorescence Spectroscopy (TXRF), was developed for the detection and quantitative analysis of heavy metals in refill liquids and their individual components. The proposed methodology was also applied to the examination of aerosols generated from commercially available atomizers after liquefaction in nitric acid solution. Results showed that nickel, lead, copper and chromium were detected in electronic cigarette refill liquids and their individual components and their concentrations were within the range of 0.001 to 0.055 ppm. Cadmium was identified in a sample of electronic cigarette liquid (0.655 ppm), as well as in nicotine and in some kinds of flavoring agents. Nickel, lead, copper and chromium were determined in liquefied aerosols from most atomizers in concentrations within the range of 0.001 to 0.009 ppm, whereas their levels were higher (0.013-0.106 ppm) in liquefied vapors generated from atomizers that had undergone extensive use.

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

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

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Investigating Genetic Disease Architecture through the Human Protein Interactome

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OBJECTIVE
The major objective of medical genetics concerns the elucidation of the genetic basis of the diseases. In the era of systems and network medicine, this effort could be greatly assisted by the investigation of the known disease-gene associations through the protein- protein interaction network (protein interactome) of human. Protein interactions being fundamental for the catalysis and regulation of all major cellular processes, the interactome provides a representation of the intracellular molecular function interconnectivity. In this context, the specific aims of this study are: (a) the determination of a high-reliability gene-disease association set to be used as the basis of our investigation, (b) the projection of this dataset on the human protein interactome and (c) the analysis of the network to reveal biological associations and molecular mechanisms underlying the architecture of the diseases.

PRIMARY SOURCES AND METHODS
Gene-disease associations were mined from the Online Mendelian Inheritance in Man (OMIM) [1] and Universal Protein Resource (UniProt) [2] databases. The final dataset was determined from the intersection of the two primary sources after appropriate normalization. Finally, we linked these associations with the human protein interactome of the knowledge metadatabase PICKLE [3] [4], which has been developed by our group and records ~90.000 interactions for ~13.000 proteins, i.e. ~65% of the human proteome.

RESULTS
The final gene-disease bipartite graph comprises 3.198 associations between 2.257 genes and 3.021 diseases. Investigating these associations through the human protein interactome indicates that most of the disease proteins are peripheral nodes characterized by a small number of direct interactions. The proteins that are considered essential for life are mainly central nodes with a high number of interactions. Mutations in these proteins are rarely associated with inherited diseases, but mostly with diseases of somatic cells, such as cancer. Proteins associated with the same disease or similar diseases are usually adjacent in the network. Finally, distinct diseases associated with overlapping molecular pathways share clinical features, and vice versa.

CONCLUSIONS
The integrated analysis of a high- reliability gene-disease association set through the human protein interactome can further our understanding of the molecular pathophysiology of the diseases leading to the identification of novel potential targets and biomarkers towards the design of more effective drugs and therapeutic protocols.

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REVIEW CLINICAL PHARMACOLOGY AND
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Genomic Medicine in the Post-genomic Era

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

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Role of Pmc1 Ca^{+2} ATPase and Nhx1 Na+ or K^+/H^+ Exchanger on Uptake of U(VI) in *Saccharomyces cerevisiae*

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Uranium (U) is a serious environmental pollutant resulting from various anthropogenic activities, such as mining, nuclear industries, and phosphate fertilizers. It is a highly toxic element, hence its removal from waste waters is of great importance[1-3]. For this purpose, common physicochemical methods (reverse osmosis, ion exchange, etc.) have been employed, but their high cost of application pressed for biotechnological approaches. The ability of microorganisms to remove metal ions from a solution is such an encouraging alternative. Moreover, microbial masses derived as by-products of various agro-industrial processes can be used as low cost biosorbents. Microorganisms can influence the mobility of dissolved metal ions by various processes such as active accumulation, redox reactions and passive sequestration on the cell surface[2-7]. In most biosorption studies, the parameters usually investigated are the initial metal concentration, temperature and pH. The cell metabolic and growth states are also critical[3,4]. The yeast *Saccharomyces cerevisiae* is well known to accumulate heavy metals. Studies have shown that U(VI) accumulates on the cell surface, associating with phosphorous to form uranyl-phosphate minerals, and that it retards cell growth. Also, it was found that U specifically affects *S. cerevisiae* at the molecular level, regulating protein expression and that the higher the metabolic activity, the lower is the accumulation of U. Therefore, specific proteins are necessary for U tolerance and accumulation. Single-gene-deletion strains are very useful tools in screening such proteins. If the specific gene is deleted, the U tolerance and accumulation is expected to be reduced in the strain[5]. In this study, three *S. cerevisiae* strains were studied and compared for U uptake: (a) the wild type BY4741, (b) the *pmc1*Δ (lacking vacuolar Ca^{2+} ATPase involved in depleting cytosol of Ca^{2+} ions and preventing growth inhibition by activation of calcineurin in the presence of elevated concentrations of Ca^{2+})[6], and (c) the *nhx1*Δ (lacking Na^+/H^+ & K^+/H^+ exchanger required for intracellular Na^+ & K^+ sequestration, osmotolerance to acute hypertonic shock, and for vacuolar fusion) [7]. The growth kinetics of all strains in the presence of U(VI) (50 ppm in the culture medium) were monitored at 30°C and pH 3. The inocula were obtained either from freshly grown cultures or from freeze-dried cultures. Growth was observed only in the *pmc1*Δ culture 12 h after inoculation and reaching a stationary phase after 30 h. At the end of this experiment, the U(VI) concentration (determined using Arsenazo III) was lower than 0.1 ppm, which was the detection limit of the method used. Under non metabolic conditions, uptake of U(VI) by the three strains was studied at pH 3 & 4, by equilibrating for 24 h the same amounts of freeze-dried cells (0.025 g) in 20 mL U(VI) solution (50 ppm) of constant ionic strength (I=0.1 M $NaClO_4$). U(VI) concentration was determined in the supernatant, revealing that the strain *pmc1*Δ, which has the highest pH_{pzc}, demonstrated a little lower capacity (about 10%) than the other two strains, which attained U(VI) removal of about 95% of the initial concentration.

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REVIEW CLINICAL PHARMACOLOGY AND
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Coup-TF and the Neurogenic Gene Regulatory Network in Sea Urchin *Paracentrotus lividus*

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Coup TF (Nr2F1), an orphan member of the nuclear receptor super family, is an essential factor in neurogenesis of all metazoans as well as sea urchin embryos. Coup-TF is expressed in the oral ectoderm (OE) of the embryo, which includes the two major neurogenic territories, the ciliary band (CB) and the anterior neural ectoderm (ANE). The temporal and spatial expression patterns of regulatory genes are required for building a gene regulatory network (GRN). In this study we analyzed the expression profile throughout early development of ANE and OE genes such as *Six3*, *Fez*, *Ets4*, Sip1, FoxQ2, Not, SynB and *Gsc*, by qPCR and whole mount in situ hybridization (WMISH). The current OE-GRN model for the sea urchin embryo, which includes the least studied GRN-subgroup of the anterior neural ectoderm, does not encompass Coup-TF. We aim to identify the regulatory interactions between Coup-TF and the aforementioned genes in order to place Coup-TF within the ANE sub-circuit of the embryonic GRN. Thus, we performed a series of experiments with specific anti-Coup-TF morpholino injected embryos that result in severe phenotype and loss of endogenous Coup-TF mRNA as judged by WMISH. Such repression of Coup-TF in developing sea urchin embryos, results in the absence of serotonergic neurons and diminishing or ectopic expression of a number of key neurogenic genes of the ANE and CB. A provisional GRN that indicates the specific interactions between Coup-TF and other neural ectoderm regulatory genes will be presented.

REVIEW CLINICAL PHARMACOLOGY AND
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The Genomic Response of the Mouse Thyroid to Iodine Overload, and the Role of the Nrf2 Antioxidant System

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Background
Despite mice being workhorses of mammalian genetics, the tiny size of their thyroid has precluded generalized use in studies of iodine effects on the thyroid, which have been traditionally performed in rats. There is also paucity of in vivo gene expression analyses of the thyroid's response to iodine using next-generation sequencing technologies. Employing a custom extraction protocol optimized for miniscule samples, we characterized the genomic response of the mouse thyroid gland to an iodine challenge in wild-type (WT) mice. In parallel, by testing mice lacking the transcription factor Nrf2, we investigated the role of this major antioxidant response system in thyroidal gene expression and in response to iodine.

Methods
Male 3 months-old male C57Bl6J WT or Nrf2 knockout (KO) mice were exposed to 0.05% sodium iodide in their water for 7 days. Thyroid gland was excised and used for RNA preparation. RNA-seq was performed by Exiqon. The fold-change cutoff was set to 1.5. Pathway analysis of the differentially expressed genes (DEG) was performed using the Ingenuity Pathway Analysis (IPA) software.

Results
Nearly 1700 genes were differentially expressed in response to iodine; most were up-regulated. Highly enriched pathways include those related to fibrosis; integrin signaling; leukocyte extravasation; inflammation (IL-1, IL-6, IL-8) and the acute phase response; production of reactive oxygen species and nitric oxide; and the Nrf2-mediated antioxidant stress response.
Nearly 500 genes were differentially expressed between WT and Nrf2-KO mice. Highly enriched pathways were related to glutathione-mediated detoxification, xenobiotic metabolism, and the Nrf2 antioxidant response; all were down-regulated in the KO. Nfr2 also impacted the expression of thyroid-specific genes including the sodium-iodide symporter and thyroglobuline.

Conclusion
These data provide a rich foundation for understanding the adaptation mechanisms to iodine challenge such as the escape from the Wolff-Chaikoff effect, as well as the role of oxidative stress in thyroid physiology.

REVIEW CLINICAL PHARMACOLOGY AND
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Development of Ultrasensitive Molecular Methodology for the Identification and Evaluation of New Prostate Cancer Molecular Markers

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According to the International Exhibition for cancerous diseases presented by the IARC (International Agency for Research on Cancer), prostate cancer is the second most common diagnosis in men and the fifth most common cancer overall. This data necessitates early diagnosis, prognosis and monitoring response to treatment of patients with prostate cancer. This can be accomplished by the development of hypersensitive molecular methodologies aiming to the identification of new molecular markers. In this work, a multiplex amplification of different DNA sequences is developed by using multiplex quantitative polymerase chain reaction (PCR) for the simultaneous detection and quantitative analysis of selected genes' mRNA. The assay is based on a multianalyte hybridization assay which takes place on the surface of spectrally encoded microspheres.The microspheres contain two fluorescent dyes in different ratios, generating up to 100 different groups.To circumvent the problem of variation in the efficiency of the multiplication of DNA sequences, DNA internal standard are used. Internal standards are sequences equally sized with their respective DNA-targets,differing only in an area of 24 bases located in the middle of their sequence, allowing subsequent discrimination. After amplification by multiplex PCR, the products undergo a process of simultaneous multiple detection on the surface of discrete microspheres. All DNA amplification products and internal standards are biotinylated and let to hybridize to their-respective complementary oligonucleotide - probesthatare attached to specific sets of fluorescent microspheres. The hybrids are then detected by streptavidin - phycoerythrin conjugate via streptavidin-biotin interaction. Finally, the microspheres are analyzed by flow cytometry using two laser beams. The first laser beam is used for the classification of microspheres into groups, thereby identifying the corresponding gene, while the second laser beam excites the phycoerythrin molecules, whose fluorescence is directly related to target concentration. The method has been successfully applied to eight genes from the Kallikrein family, which are known to be involved in prostate cancer expression.

ACKNOWLEDGEMENTS

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REVIEW CLINICAL PHARMACOLOGY AND
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Development of PLGA Nanoparticles Loaded with a Mutated MOG Peptide Analogue for the Inhibition of EAE

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Multiple Sclerosis (MS) is an autoimmune disease whereby the myelin of the Central Nervous System (CNS) is destroyed by the immune system, leading to serious medical conditions and paralysis.[1] The disease is triggered by the stimulation of encephalitogenic T-cells *via* the formation of the trimolecular complex between the Human Leukocyte Antigen (HLA), an immunodominant epitope of myelin proteins and T-cell Receptor (TCR). Myelin Oligodendrocyte Glycoprotein (MOG) is located on the external surface of myelin and has been implicated in MS induction. The peptide MOG_{35-55} is widely used to induce Experimental Autoimmune Encephalomyelitis (EAE, an experimental model of MS) in mice and to investigate its immunopathological mechanisms *in vivo*. In this study, a conformational analysis of a MOG_{35-55} mutated peptide analogue [$MOG_{35-55}(Ala^{41})$] capable to bind HLA and the TCR was carried out, in order to explore the interactions developed during the formation of the trimolecular complex.[2] The $MOG_{35-55}(Ala^{41})$ peptide was developed in order to trigger immune tolerance against MOG_{35-55} and inhibit EAE.[3] Primary TCR contact Arg^{41}, was replaced by Ala and the peptide was loaded in poly (lactic-co-glycolic) acid nanoparticles (PLGA-NPs) to protect it from enzymatic degradation improving its bioavailability *in vivo*.[4] Moreover, the encapsulated peptide was released slowly form PLGA-NPs during a period of several weeks which favors induction of immune tolerance *in vivo*. EAE was induced in C57BL/6 mice. Treatment with $MOG_{35-55}(Ala^{41})$ PLGA-NPs was carried out and empty PLGA-NPs were used as control. Results showed that vaccination with $MOG_{35-55}(Ala^{41})$ PLGA-NPs displayed a significant protective effect against EAE and decreased the severity of the disease symptoms, whereas the control treatments displayed no effect.

KEYWORDS: Multiple Sclerosis (MS), Myelin Oligodendrocyte Glycoprotein (MOG), poly (lactic-glycolic) acid nanoparticles (PLGA-NPs), Experimental Autoimmune Encephalomyelitis (EAE)

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REVIEW CLINICAL PHARMACOLOGY AND
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Specific Wavelength Dependent Photo-switching or DNA Photo-cleaving Activity of *E*-Acyl-hydrazones

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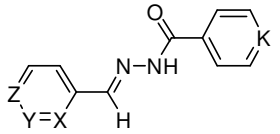
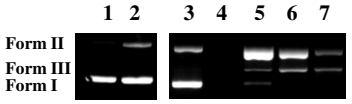
Acyl hydrazones are photo-switchers with the ability to isomerize and switch from *E* to *Z* conformation upon irradiation at specific wavelengths.[1] These compounds posses various biological activities, among which the antimicrobial actibity is notable.[2] The emergence of bacterial resistance to antibiotics, and a possible photodynamic inactivation of pathogenic bacteria via photo-sensitizers[3] has prompted us to study the photo-chemical interaction of *E*-Acyl-hydrazone derivatives **1**-**5** with DNA. DMSO solutions of the compounds at a concentration of 500 μM were individually incubated with supercoiled circular pBR322 plasmid DNA and irradiated at 312 nm for 30 min. No DNA photo-cleaving activity was observed for any of the compounds. The DNA-binding of **1** and **3** was monitored with by a series of spectroscopic and viscosity experiments with calf-thymus (CT) DNA and showed significant intercalation; however, this interaction with CT DNA did not seem to prevent the *E* to *Z* isomerization reaction. Nevertheless, when the same *E*-Acyl-hydrazones **1**-**5** were irradiated at 365 nm for 2 h a significant DNA photo-cleavage was observed. Although mechanistic experiments are expected to clarify the mode of action, the fact that hydrazones may be photo-chemically cleaved via homolysis of the N-N bond, makes us to assume that the DNA photo-cleavage of Acyl-hydrazones may follow the same route. Thus, it seems that *E*-Acyl-hydrazones are wavelength dependent specific photo-switchers and DNA photo-cleavers. Their DNA photo-cleaving activity may be used in novel antimicrobial therapeutic approaches.



1: X=N, Y=CH, Z=CH, K=C-NO_2
2: X=CH, Y=N, Z=CH, K=C-NO_2
3: X=CH, Y=CH, Z=N, K=C-NO_2
4: X=CH, Y=CH, Z=C-NO_2, K=C-NO_2
5: X=CH, Y=CH, Z=C-NO_2, K=N



Form II	6	-	86	72	48	
Form III			7	28	52	

Lane 1: DNA control; Lane 2: DNA control + UV;
Lane 3: DNA + UV + **1**; Lane 4: DNA + UV + **2**;
Lane 5: DNA + UV + **3**; Lane 6: DNA + UV + **4**;
Lane 7: DNA + UV + **5**.

KEYWORDS: DNA photocleavers, photodynamic inactivation, acyl-hydrazones, antimicrobial therapies

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REVIEW CLINICAL PHARMACOLOGY AND
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Comparative Study in POAG or OH Patients between the Original Latanoprost Eye Drops Brand and Two Similar Generics as to the Efficacy and Safety Profile

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Clinical Study

Purpose. This randomized, prospective, interventional study aimed at evaluating the efficacy and safety profile between two generic prostaglandins (Lataz, Xalaprost) and the corresponding original anti-glaucoma eye drops (Xalatan).

Material and Methods. 50 patients with POAG or OH, who had not previously received another antiglaucomatous treatment and were not treated for ocular surface disease, were divided randomly into three groups depending on the drops that they instilled (Xalatan, Xalaprost, Lataz) and after they were studied over 16 weeks for a total of 6 visits. At each visit the mean Applanation Tonometry values [3 measurements each 5' in three different times during office hours (8:00 to 12:00 - 15: 00)] and TBUT were measured. In addition, the OSDI questionnaires were collected from the patient so as to be evaluated.

Results. From the results analysis there is not a statistically significant difference in the mean deviation values in IOP and TBUT in both the 1st and 5th visit between the three collyria. Both generic eye drops showed mean percentage IOP reduction comparable to the standards of PGAs, although the original Xalatan presented the best safety profile, followed by the generic Lataz and least was the generic Xalaprost.

Conclusions. The three drops achieved statistically significant reduction in IOP. No significant difference was recorded in the effectiveness of such generic prostaglandin compared to the original. Least but not last, no patient had a change of antiglaucomatous action for development of ocular surface disease.

KEYWORDS: Latanoprost, POAG, generics

REVIEW CLINICAL PHARMACOLOGY AND
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Search for Infectious Factors Contributing to the Etiology of Human Breast Cancer

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Epidemiological data indicate a potential relation between the consumption of bovine meat and dairy products and the incidence of breast and colon cancer. Specifically, the consumption of milk and dairy products originating from specific cattle species seems to play a significant role in the development of breast cancer. Our group has recently isolated a number of novel circular single-stranded DNAs from bovine sera of healthy cattle and commercially available dairy products, as well as from brain and serum samples of patients suffering from Multiple Sclerosis. This project aims at the identification of episomal DNA agents in breast cancer. Therefore, total DNA is extracted from breast cancer tissue samples as well as healthy controls by different extraction methods. Subsequently, the DNA is subjected to Rolling Circle Amplification (RCA) using random primers in order to specifically amplify circular DNA molecules, which are then identified by Next Generation Sequencing and *de novo* assembly. In parallel, specific primers are used in order to identify such circular DNAs related to the already isolated DNA agents by conventional PCR. Resulting DNA fragments are subsequently subcloned and sequenced. Positive results would provide useful information about potential risk factors of bovine origin contributing to the pathogenesis of breast cancer, which would allow for novel therapeutic strategies for fighting this disease.

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Comparative Study of Behavioral Indicators and Activity of Brain Acetylcholinesterase's Isoforms in Adult and Aged Mice

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The aim of the present study was to investigate the behavioral indicators, anxiety / fear and social dominance and the ratio of the activity of two acetylcholinesterase (AChE) isoforms (G1, G4) in the brain regions of adult versus aged on both male and female mice. Initially, we investigated the behavioral index anxiety/fear with the open field test in adult (3-4 months old) and aged (15-16 months old) mice. The results exhibited anxiogenesis in the aged mice in both sexes. While, the female mice were found to be more stressed than male ones in both age groups. The social dominance behavior was evaluated by using the tube-dominance test in order to compare the two age groups of the male mice as well as the genders differences in each age group. The results support that the adult male dominate over the aged mice. Moreover, in the adult groups, male mice were found to be dominated by the female mice. However, when comparing the aged groups, no gender-dependent dominance motif has occurred between sexes. As Acetylcholine (ACh) is associated with the behavioral manifestations in these aged groups, the activity of the two AChE isoforms (the enzyme that catabolizes the ACh) was determined using Ellman's colorimetric method, in selected brain regions (the cerebellum, cerebral cortex, hippocampus and striatum). Also, the ratio of the activity of these two isoforms in each brain region was evaluated, since this ratio has been associated with the pathophysiology of each tissue. The results demonstrated a reduction of activity of AChE's isoforms through aging in both sexes. AChE in the selected brain regions did not show a uniform pattern of activity in both sexes, irrespective of age. The ratio of the two isoforms (G1/G4) was found to be decreased in the aged group in the hippocampus and striatum regions in both sexes, as well as in the cerebral cortex and cerebellum in female aged mice. In the comparison between genders, a decrease of the ratio in the cerebellum, cerebral cortex and striatum was observed only in the aged group. Conclusively, the increase of anxiety -like behavior- on the aged mice in both sexes and reduced dominance-behavior of aged male mice was accompanied by a decrease in the activity of both AChE isoforms in all brain regions that have been studied. Also, in the aged mice a reduction of the ratio of the two isoforms (G1/G4) occurred, in the hippocampus and striatum. The above results suggest that the studied behavioral indices are associated with a differential specific tissue pattern of changes of the isoforms of AChE; an observation that demands further research.

REVIEW CLINICAL PHARMACOLOGY AND
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The Effect of Isoflavones in Osteoporosis Prevention

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Background: Isoflavones found abundantly in soybeans are structurally similar to estrogen and thus able to exert weak estrogenic effects. Fermentation of soymilk with probiotic bifidobacteria metabolises daidzein into equol. Findings from earlier clinical studies on the potential positive effects of isoflavones on cardiovascular and osteoporosis risk factors in postmenopausal women have been highly variable and inconclusive.

Aim: The objective of this study was to examine the effects of ingesting a fermented soymilk containing viable bifidobacteria on serum lipid profiles, bone turnover markers and levels of FSH, LH and SHBG in postmenopausal women.

Methodology: Thirty-six post-menopausal women were involved in a randomised, double-blind, placebo-controlled, parallel study involving a 12-week supplementation of fermented soymilk (FS), non-fermented soymilk (NFS) and casein-milk (CAS). Subjects in the FS and NFS group ingested the same dosage of isoflavone at 80 mg per day. Populations of *B. animalis* in fermented soymilks were 10^7 to 10^8 viable cells per mL. At baseline and endpoint, hormones, lipids, osteocalcin and β-CrossLaps were analysed in serum and deoxypyridinoline in urine.

Results: There was no significant differences in either BMI (P=0.24) or bodyweight (P=0.14) between the three groups. After 12 weeks of milk supplementation, there was no significant change in mean bodyweight from baseline measurements for any of the groups. Supplementation of FS and NFS caused a decrease in the levels of FSH and LH (P>0.05). Moderate rises in SHBG were observed in the groups consuming the NFS and FS. There were no significant differences between baseline and endpoint means of total cholesterol, triglyceride and LDL-cholesterol (P>0.05). Ingestion of FS showed a trend toward a reduction in bone resorption, with urinary DPD decreasing by 2.4 nmol/mmol of CRE, but no significant difference was evident between baseline and endpoint means of the FS group (P=0.16). In contrast to the increase in DPD excretion shown by women in the NFS and CAS group (10.3% and 3.3%, respectively), women consuming fermented soymilk showed a 17.3% reduction in the urinary excretion of DPD (P=0.05).

Conclusion: Preliminary findings show that FS may prevent bone loss in postmenopausal women. However, further studies with a larger number of subjects per group are required to evaluate trends showing a positive effect on hormones, bone formation and cardiovascular disease risk.

REVIEW CLINICAL PHARMACOLOGY AND
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Expression of Hyaluronidases in Head & Neck Cancers

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Hyaluronidases (Hyals) are a class of six different enzymes with similar activity being implicated in cancer. In head & neck tumors, various isoforms of Hyals are found to be involved in cancer growth and progression. Among these, PH-20 and Hyal-1 seem to play a key role. To further elucidate the implication of Hyals in Head & Neck cancer, the present work focused on the examination of their expression in laryngeal cancer patients, and in relation to the anatomic site of cancer. Samples of 24 patients subjected to laryngectomy were obtained, two from each patient, one from the central part of cancer (C) and a second from a macroscopically normal part (N) and applied to mRNA isolation for the examination of Hyals expression. Fourteen samples were from glottic (G) and ten from superglottic (SG) cancer. Thirteen of the patients were of stage III and eleven of stage IV. RT-PCR analysis showed that the majority of enzymes largely differentiated between C and N samples. The expression of Hyal-1-wt, Hyal-1-v1, Hyal-1-v2, Hyal-2-wt, Hyal-3-wt was increased about 6-, 2-, 4-, 2- and 2-times, in C samples compared to N, respectively. On the other hand, Hyal-1-v5 expression was decreased to 10% in C samples compared to the one of N. PH-20 and Hyal-3-v3 expression was similar in both C and N samples. Hyal-1-v3, Hyal-1-v4, Hyal3-v1 and Hyal3-v2 were not detected. Depending on the anatomic site, PH-20 and Hyal-3-v3 were expressed in SG by 150% and 50% higher than in G samples, respectively, whereas all other enzymes detected showed lower expression in SG samples. The obtained data suggested that analysis of the various isoforms of Hyals might be a useful tool for head & neck cancer diagnosis. In addition, certain enzymes seemed to be expressed characteristically in either G or SG cancer. More data, however, is required, especially of early cancer patients, to verify the applicability of this analysis.

REVIEW CLINICAL PHARMACOLOGY AND
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Study for the Development and Differentiation of Cell Populations After Treatment with Microcurrent and Electromagnetic Fields

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The benefits of electromagnetic radiation in the medical literature in recent years, recorded with the therapeutic effects of clinical studies. This led the interest of many sciences to focus on investigating the biochemical mechanisms that affect and ultimately contribute therapeutic, and finally to apply techniques using electromagnetic energy. Wireless micro-electrostimulation or electrostimulation, and theapply of pulsed electromagnetic fields are new technologies whose effects impressed. The clinical results from the apply of wireless electrostimulation attracted our interest, as there was accelerated healing on ulcers treatment, by the generetion of new collagen fibers and myofibroblasts, by decreasing inflammation and by reducing the granulation mass.

The aim of this thesis was to study the impact that may have the administration of pulsed electromagnetic fields on cell lines PC3 (prostate cancer cells) and LLC (Lewis Lung carcinoma), as we assessed the clinical results of the implementation of wireless micro-electroon patients . We wanted to see how and if the morphology of these cells is affected by this technique, and also investigated the course of evolution of the proliferation of these cells and how the apply of pulsed electromagnetic waves may or may not affect this course.

We administered at different times, with the same conditions each time (intensity and energy of the device), pulsed electromagnetic fields (PEMF) and studied the effect of this technique to mitosis and proliferation of these cell lines, and we also tested whether the morphology of these particular cells is influenced, in cell culture level.

The results of this thesis are discussed in the second part giving the potentials of these experiments in other protocols as well. Specifically, with the completion of this work we found that a single dose of pulsed electromagnetic fields using the device PEMF ring by itself, is impossible to cause a change in the morphology of the cells used in this thesis, and no change in proliferation of the aforementioned cell lines was observed as well.

REVIEW CLINICAL PHARMACOLOGY AND
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Mannan-conjugated Myelin Peptides Induce Dendritic Cell (DC)-driven Tolerogenic Response

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Introduction: The induction of immune tolerance using dendritic cells (tDCs) is a therapeutic strategy for multiple sclerosis (MS). We explored the potential of tDCs loaded with mannan-conjugated myelin peptides for MS immunotherapy.

Materials and Methods: Peripheral blood monocytes and T-cells were isolated from 2 patients with remitting-relapsing MS and 2 age/sex-matched controls. tDCs were generated from monocytes cultured with IL-4/GM-CSF/vitD3 for 6d. The resulted tDCs were loaded with myelin peptides conjugated with mannan (or peptide alone) and co-cultured with T-cells±IL-2 for 3 rounds of peptide stimulation (total of 25d). Cells were analyzed by flow cytometry to determine the phenotype of tDCs and the resulting T-cell populations. The cytokine profiles of DCs and T-cells consisting of IL-1β, IL-2, IL-4 IL-6, IL-8, IL-10, IL-12p70, IL-17A, TNF-a and IFN-γ, were measured by CBA.

Results: tDCs showed a semi-mature phenotype and secreted low to zero levels of proinflammatory cytokines. After d3 of co-culture the lymphocytes were >90% CD3+CD4+, and after the 1st antigen presentation all were differentiated into memory (CD3+CD4+CD45RO+) cells. At the end of the culture period, in the point with the mannan-myelin-peptide-loaded-tDCs (i) Tregs were higher than in cultures with tDCs+peptide alone, (ii) T-cells displayed the least activation potential and (iii) the cytokine profile was antinflammatory.

Concusions: Our results display that the generated DCs are both phenotypically and functionally optimized to induce tolerance in vitro, in healthy subjects and patients. In the context of myelin antigen presentation, the conjugation of myelin peptides with mannan is clearly superior in tolerogenic effect compared to the unconjugated peptide.

This study indicates that mannan-myelin peptide loaded tDCs can be eventually used as immunotherapy in MS patients.

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REVIEW CLINICAL PHARMACOLOGY AND
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Interactions of AT1 Antagonists in the Crystallized AT1 Receptor with the View to Finding Innovative Molecules with Improved Pharmacological Profile

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The structure of the human angiotensin II receptor type1 (AT_1R) was determined by serial femtosecond crystallography at an X-ray free-electron laser (SFX at XFEL) in a complex with the selective antagonist ZD7155 which is the precursor of the antihypertensive molecule of candesartan.AT_1R has a transmembrane α-helix(7TM) with an extracellular N-terminus, three intracellular loops (ICL 1-3), three extracellular loops (ECL 1-3), one amphoterix helix VIII and an intracellular C- terminus.

Because of the different chemical structure of the commercially available AT1 antagonists, the nature of the interactions of each inhibitor with the active side of the AT_1R is different. However, most of them bound in a similar way and are involved in interactions with the three critical aminoacids Arg167, Trp84, και Tyr35 that are also involved at the binding of ZD7155.

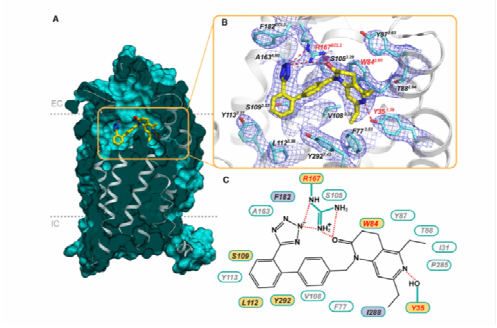

Figure 1: A) Binding region of ZD7155 in AT_1R. B) Three-dimensional illustration of ZD7155 interactions with aminoacids' region that refrain up to 4 A. C) Two-dimensional illustration of ZD7155 interactions with aminoacids' region that refrain up to 4 A[1].

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

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REVIEW CLINICAL PHARMACOLOGY AND
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Cloning, Expression and Purification of the Extracellular Domain of the Growth Hormone Releasing Hormone Receptor

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G protein coupled-receptors (GPCR's) comprise the largest family of cell membrane proteins of the human genome and play a significant role in signal transduction by detecting extracellular stimuli and triggering intracellular downstream cascades to mediate physiological processes. Although all GPCR's share a common seven-transmembrane topology, they greatly vary in ligand recognition and function. Class B, one of the GPCR's families, in which the Growth Hormone Releasing Hormone Receptor (GHRHR) belongs to, consists of fifteen members who are key players in hormonal homeostasis and are interesting drug targets for the treatment of several metabolic disorders and nervous system diseases. Structurally, class B GPCRs consist of a large N-terminal extracellular domain (ECD) and a transmembrane domain (TMD) comprising the GPCR signature of seven membrane-spanning a-helices. Various structures of the ECDs of class B GPCRs in complex with their peptide ligands have been determined by X-ray crystallography and Nuclear Magnetic Resonance (NMR), and have provided information about structural mechanisms of ligand recognition and selectivity.[1] However little is known about the structure of GHRH, which is involved in the condition of dwarfism. Here, we describe the process of cloning, expression and purification of the ECD of GHRHR, which has also recently been found to play a significant role in cancer therapy,[2] aiming to determine its structure in solution through NMR. Hence, it will be feasible to examine the molecular determinants underlying the interaction between the ECD of GHRHR and its natural hormone (GHRH). The development of this particular assay is expected to contribute in the structural information regarding GHRHR for the development of novel antagonistic compounds.

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REVIEW CLINICAL PHARMACOLOGY AND
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Discovery of Novel Immunosuppressants Targeting Calcineurin through Pharmacophore Virtual Screening

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The fast growing number of protein crystal structures that serve as drug targets is being extensively used for virtual screening approaches to identify novel small molecules as potential hits. Pharmacophore modeling is a well-established method for screening large databases for drug design. Calcineurin (CN), a serine-threonine phosphatase involved in T cell signaling, dephosphorylates multiple phosphoserines on nuclear factor of activated T cells (NFAT), a transcription factor, leading to its nuclear translocation and activation. Using the crystal structure of CN bound to a peptide [1], we developed a multi-pharmacophore model and performed a virtual screening of the ZINC database (~12 million compounds) in order to identify potential inhibitors of CN. The selected hit molecules were purchased and pharmacologically tested for binding, NFAT dephosphorylation, cytotoxicity and gene expression. Four of them displayed comparable binding to the peptide and were found to have immunosuppressant activity through induction of NFATc-dependent gene expression. Conclusively, this study has resulted in the first non-peptidic, non-cytotoxic, potential immunosuppressant inhibitors targeting the CN-NFAT substrate interaction site [2].

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REVIEW CLINICAL PHARMACOLOGY AND
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Math and Literature: Related Stories. Readings of a hermetic world

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Mathematical texts are texts, which refer directly or indirectly either to numbers either to mathematics. Mathematics is located in literature texts since antiquity, not only in Greek but also in worldwide antiquity. There are texts, which are based on mathematics exclusively, like *Surya Sintchanta*, on the marriage of Hermes and Literature, Gulliver's Travels e.t.c. To begin with a historical flashback, this announcement is aiming to familiarize the audience with Mathematics and Literature as well to texts, that have dense or few references to numbers and mathematics. Finally, there will be an analysis of Apostolis Doxiadis book "Logicomix", focused on highlights.

REVIEW CLINICAL PHARMACOLOGY AND
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Designing Potential Drug Delivery Silicon Based Nanoparticles

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Using *ab initio* density functional theory (DFT) and fundamental chemical analogies in a bottom up approach, we build powerful theoretical models with promising drug delivery capabilities due to their biocompatibility, biodegradability, low toxicity and solubility. Besides core/shell silicon nanoparticles, and other building blocks of larger porous systems, such as mesoporous silica nanoparticles (MSNs), we use borane and carborane based drug delivery systems as prototypes for the design of homologous silicon and silicon-carbon structures. Our present results are very promising, and highly suggestive that the borane and carborane drug delivery advantages are fully transferable for the silicon based candidates, without high toxicity.

REVIEW CLINICAL PHARMACOLOGY AND
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Mannan-conjugated Myelin Peptides Protect Mice against Autoimmune Encephalomyelitis without Altering T Cell Trafficking into the Central Nervous System

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

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Topobioistosterism in ALR2 Inhibitors: The Case of 4-pyrrolyl-3-fluorophenol

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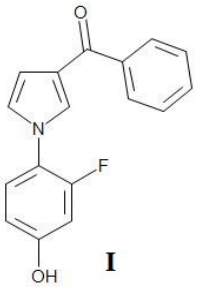
Aldose Reductase (ALR2) plays a significant role in the development of long term diabetic complications and many compounds have been synthesized over the last years due to this purpose. Based on the already known active scaffold of the substituted pyrrolyl-2-fluorophenols, new analogues with 3-flurophenol I, II and III respectively are studied regarding their activity over the enzyme ALR2. Moreover, transferring the fluoro substitute in position 3 in respect with the phenol hydroxyl group reduces acidity and consequently implements cell membrane permeability. All these three compounds were synthesized from 1-fluoro-2-nitrobenzene after Bamberger και Clausen-Kaas reactions in a row. Then the desired analogues were finally synthesized with Friedel Crafts (I and II) and reaction with trifluoroacetic anhydrite (III) accordingly. The biological activity of the compounds was tested via an in vitro procedure with the enzyme, which was delivered at first from homogenized murine eyes. The results were very encouraging since the analogues I and III in the concentration of 10 μM showed inhibition of the enzyme above 50%. Finally, the shift of the fluoro substitute to position 3 proved to be sufficient for the increase of action of the reference compounds.



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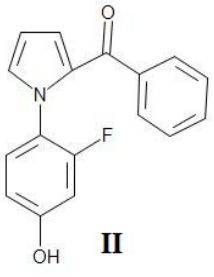

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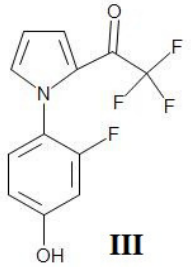

III

REVIEW CLINICAL PHARMACOLOGY AND
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Studies on the Effects of a Protein Kinase C (PKC) Modulator in Prostate and Colon Cancer Cell Lines

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Cancer is a group of diseases caused by a complex combination of molecular mechanisms. Trying to point out and target possible molecules that play a key role in cancer is challenging. During my Erasmus+ practice, I participated in the research conducted by a group in the Division of Pharmacology and Pharmacotherapy, University of Helsinki, where protein kinase C (PKC) is studied as a potential drug target for cancer therapy. PKC is a family of serine/threonine kinases and its isoforms play a significant role in many different signal transduction pathways. An interesting perspective is that opposite to what was speculated in the past, it is the activation rather than inhibition of certain PKC isoforms that can be a solution against cancer progression. Our aim was to study 5-(hydroxymethyl)isophalate analogue HMI-1a3, a C1 PKC modulator, regarding its effect against prostate and colon cancer cell lines and if senescence is implicated in its mechanism of action. The effect of HMI 1a3 on the viability of the cell lines was studied with MTT assay and Western blotting was utilized to investigate the expression of senescence markers p16 and p21 in prostate cancer cell lines. Treatment with HMI 1a3 decreased the viability of all the studied cancer cell lines. In DU145 and PC3 prostate cancer cell lines HMI 1a3 induced an increase in p21 expression, indicating that HMI 1a3 treatment directs these cells to senescence.

The study was supported by Jane and Aatos Erkko Foundation

REVIEW CLINICAL PHARMACOLOGY AND
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Synthesis of New Pyrroloazepinones as Core Structures of Potential BET Inhibitors

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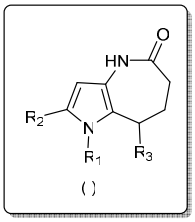
Bromodomain and extra terminal domain (BET) proteins recognize and bind to acetylated histones and are involved in a number of DNA-centered processes, including regulation of gene expression. Aberrant acetylation levels of lysine residues have been associated with inappropriate transcription of disease-promoting genes in cancer. Targeting these proteins with small molecules has led to the discovery of new bioactive compounds with potential therapeutic applications in cancer[1-2], inflammatory disease[1-2], acute leukemia[3] , NUT-midline carcinoma[4], atherosclerosis[5] and lymphoma. Importantly, the majority of BET inhibitors feature a thieno-, benzo-diazepine or triazepine core as part of their structure[1-2].



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Based on these data, we have focused on the generation of new pyrroloazepinone containing scaffolds with potential BET inhibitory activities. To this direction, we designed and synthesized new pyrroloazepinone derivatives (**I**) as core structures of new BET inhibitors. In this study, the results of our endeavors towards the construction of the basic scaffold (**I**) as well as the synthesis of various analogues will be presented.

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REVIEW CLINICAL PHARMACOLOGY AND
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Wireless Microcurrent Stimulation and Pulsed Electromagnetic Fields: Clinical Applications for Wounds and Cellular Studies

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Electrical stimulation is a well-known method for wound healing, giving beneficial outcomes. However, the difficulty in use and the pain provoked to the patient from the electrodes applied, limit dramatically its use. However, two innovative technologies, Wireless Microcurrent Stimulation (WMCS) and Pulsed Electromagnetic Fields (PEMF) overcome the above restrictions. The aim of this study is to evaluate the use of WMCS and PEMF in the healing of complicated and hard-to-heal wounds. We here present patients with pressure ulcers, venous stasis ulcers, diabetic foot wounds and burns that were treated with the W200 device (WMCS- Wetling®, Denmark) and the consumable, wearable PEMF device (Actipatch®, USA) as well. The W200 device was directed (adjusted) to a distance of about 10–15 cm straight onto the wound, with an intensity of 1,5μA. Each therapy lasted 1 hour daily. The patients that used in parallel or separately the PEMF technology applied the device so that the therapeutic area was within the loop, for at least 8 hours daily or usually for almost 24 hours. Selected cases are presented. The patients demonstrated substantial improvement up to complete healing, regardless the underlying cause or the extension of the wound (ulcer, burn e.t.c.). Although the duration of the therapy varies, in the majority of the cases the therapeutic outcome was optimal. We have additionally studied the effect of PEMF technology on cell lines PC3 (prostate cancer cells), LLC (Lewis Lung carcinoma), and human epithelial cells. We have observed the effect of this technology to mitosis, proliferation and migration.

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Computational Study of GK470 Inhibitor of Cytosolic Phospholipase A_2 Group IVA

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Phospholipases A_2 (PLA_2s) is a super-family of enzymes that cleave a fatty acid from the *sn*-2 position of phospholipids. The group IVA cytosolic phospholipase A_2 (GIVA $cPLA_2$) hydrolysis of phospholipids substrates, such as phosphatidylcholine (PC), has a high substrate specificity for arachidonic acid at the *sn*-2 position. It has been shown [1] that GIVA $cPLA_2$ activity leads to the upregulation of other PLA_2 subgroups and promotes the production of pro-inflammatory mediators in many tissues.

GK470 [2] is a new thiazolyl ketone compound and a potent inhibitor of GIVA $cPLA_2$. In particular, it exhibited an $X_i(50)$ value of 0.011 mol fraction in a mixed micelle assay and an IC_{50} of 300 nM in a vesicle assay. In a cellular assay using SW982 fibroblast-like synoviocytes, it suppressed the release of arachidonic acid with an IC_{50} value of 0.6 μM. In a prophylactic collagen-induced arthritis model, GK470 exhibited an anti-inflammatory effect comparable to the reference drug
methotrexate, whereas in a therapeutic model, it showed results comparable to those of the reference drug Enbrel. In a continuation to our previous computational studies on GIVA $cPLA_2$ inhibitors, here we represent the molecular dynamics simulation of GIVA $cPLA_2$-GK470 complex in AMBER. MM-PBSA method was used to calculate the binding free energy terms of the complex and principal component analysis (PCA) was used to explore the structural fluctuations (modes) of the protein (Figure 1). In addition, cluster analysis based on PCA results was performed (Figure 2). According to the results, GK470 occupies the same region of the active site with the AX007 inhibitor,[3] while Van der Waals forces seem to be the main energy contributor of the total free energy of the complex.

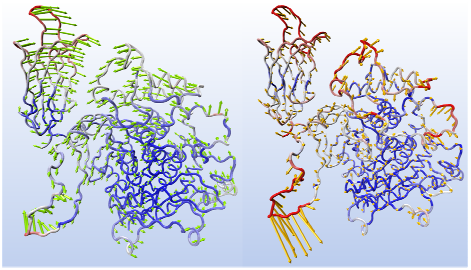

Figure 1. *The green and orange arrows represent eigenvectors pointing to the direction of protein's motions across Mode 1 and Mode 2, respectively.*

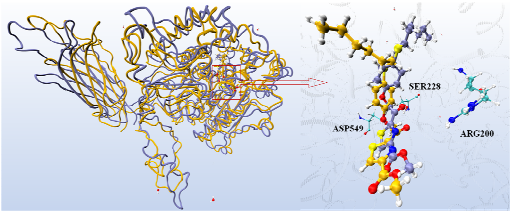

Figure 2. *Alignment of the first two clusters based on PCA results (cluster1:blue, cluster2:yellow).*

ACKNOWLEDGEMENTS: 'This work was supported by computational time granted from the Greek Research & Technology Network (GRNET) in the National HPC facility "ARIS" under project ID: pr001008-COMPINFLAM.'

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REVIEW CLINICAL PHARMACOLOGY AND
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Bottom-up Approach for the Biochemical Interaction of Drug Delivering Nanoparticles with Tamoxifen and Other Anticancer Drugs

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Using ab initio Density Functional Theory (DFT) with the hybrid PBE0 functional we have studied at the molecular level the interaction of tamoxifen with glycine, tyrosine and other amino acids, in order to build a theoretical bot-tom-up theoretical and computational “machinery” for the full multiscalar description of the drug delivery process and activity. We have located several active sites and we have calculated interaction energies, which are of the order of 10-13 Kcal/mole, depending on site and orienta-tion. This is of the same order of magnitude with the can-didate nanoparticles, such as Metal organic frameworks (MOFS) and carbon nanotubes. This project is currently under way.

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REVIEW CLINICAL PHARMACOLOGY AND
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A Systems Approach to the Teaching and Learning of Science: The Role of Discipline-Based Education Research

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The discovery and investigation of the form and function of DNA and, more recently, of other biomolecules, ushered a new era in the study of life. The complexity of wellness and disease is so significant, that simple linear causal chains are inadequate to describe the behavior of many real-world processes—a systems approach is necessary. Today, systems biology promises breakthroughs in a variety of cutting edge questions, including those associated with P4 medicine: medicine that is predictive, preventive, personalized, and participatory. In this talk, I will argue that a similar systems approach is necessary to foster science literacy for informed decision-making in a democratic society, increase the number of well-qualified STEM professionals, provide skills for an ever-growing set of STEM-related careers, and preserve the riches of a particular way of human knowing. In the United States and Europe alike, there are huge challenges to this vision. Students, especially those belonging to groups that have been traditionally underserved in science, lose their innate scientific curiosity and turn away from science, as a result of a multitude of factors including the “game” of school science from Kindergarten to graduate school. Approaching the learning and teaching of science disciplines from a scholarly and systems perspective has demonstrated positive effects on student learning, student interest, and student self-efficacy. A multivalent, research-validated, systems approach to science education has the potential to help us move toward achieving our ambitious goals.

REVIEW CLINICAL PHARMACOLOGY AND
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Monitoring Bone Reconstruction in Fractures of Animal Model Femurs Using Raman Spectroscopy

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[2]Department of Mechanical Engineering and Aeronautics, University of Patras
[3]Institute of Chemical Engineering and High Temperature Chemical Processes, FORTH, GR-26500, Patras, Greece

In this work, the reconstruction of animal models' femur in the vicinity of a fracture was monitored, at the molecular level, using Raman spectroscopy. Particularly, changes in the chemical composition of bone tissue were recorded with time. For this purpose, four groups of male Wistar rats were used, which simultaneously underwent a surgery of artificial fracture of the right femur. The left femur was used as a control for each animal. The groups were sacrificed at different times. Spectra were collected in a distance of several millimeters at both sides of the fracture, using a micro-Raman spectrometer. Intensities of the characteristic peaks of the bone's main components, apatite and collagen, were measured. Although the presence of collagen vibrations was evident in the spectra, even very close to the fracture, the ratio of inorganic to organic material was found reduced in the first weeks and gradually increased in time, which proves the delayed calcification of the collagen network during bone's regeneration. On the other hand, the non reducible to reducible cross-links ratio in collagen network was increased in the same area, indicating decreased elasticity and increased brittleness of the bone in the fracture area during healing period.

REVIEW CLINICAL PHARMACOLOGY AND
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A Pharmacometabolomics-guided Pharmacogenomics Strategy in Precision Medicine

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Inter-individual variability has been a major hurdle to optimize disease management. Precision medicine holds promise for improving health and healthcare via tailor-made therapeutic strategies. Herein, we outline the paradigm of Pharmacometabolomics-guided pharmacogenomics. We envisage merging pharmacometabolomic and pharmacogenomic data (to address the interplay of genomic and environmental influences) with information technologies to facilitate data analysis as well as sense- and decision-making on the basis of synergy between artificial and human intelligence. Humans can detect patterns, which computer algorithms may fail to do so, whereas data-intensive and cognitively complex settings and processes limit human ability. We propose that better-informed, rapid and cost-effective -omics studies need the implementation of holistic and multidisciplinary approaches.

KEYWORDS: Pharmacogenomics, Pharmacometabolomics, Precision Medicine.

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DNA Biosensors for Visual Genotyping of Single Nucleotide Polymorphisms

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DNA analysis has found a wide spectrum of applications including pharmacogenetics, detection of mutations, detection of various pathogens, diagnosis and monitoring of disease. The progress in this field is remarkable. Indeed, for many years, these analyses required tedious and time consuming procedures based on radioactive isotopes. On the contrary, DNA sensors are small and portable devices that enable simple, rapid and low cost DNA analysis without the need of radioactivity. The development and the functional aspects of DNA sensors reveal noteworthy examples of molecular recognition and self-assembly as well as intelligent ways of optical communication between the ‘nano’ world and the ‘macro’ world.

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Targeted Delivery of Drug Molecules

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

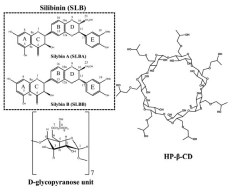

Figure 1: Structure of SLB (top, left) and the cyclic HP-β-CD (right) consisting of seven D-glycopyran segments.

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

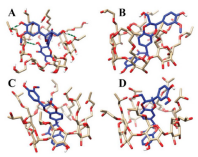

Figure 2: SLBA-HP-β-CD complex: (A) as predicted initially by GlideXP (hydrogen bonds are shown in green), (B) after 100 ns of MD simulation, (C) after 200 ns, and (D) at the end of the simulation.

The physiological conditions, the aqueous solubility and dissolution characteristics of the complex at pH states simulating those of the upper gastrointestinal tract have been applied and showed better properties. The antiproliferative activity of silibinin–HP-β-CD complex comparatively to silibinin in MCF-7 human cancer cells, MTT assays has been increased. Another example is the way AT_1R antagonists interact with their active site. They can either directly approach the receptor or they can be intercalated into the membrane core and then diffuse to the active site of the receptor. Thus, the putative role the membrane bilayer core could adopt in this action remains largely uncharted. Drug:membrane interaction studies aid to understand the role of lipid bilayers in drug action (Figure 3). Molecular Dynamics is used to explore the molecular basis of action of AT1 antagonists. Biophysical and *in silico* studies using formulations of drugs incorporated in cyclodextrins or liposomes are in progress to understand the release of drugs in membrane bilayers and the way they can reach the receptor site.

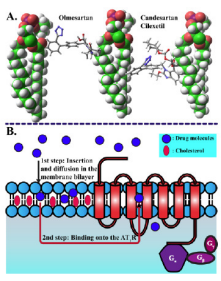

Figure 3: (A) Olmesartan (left) and candesartan cilexitil (right) positioned in one leaflet of membrane bilayer. As it can be observed candesartan cilexitil is positioned deeper in membrane bilayers owing to its more hydrophobic nature. However, both molecules as they are amphipathic adopt polar and non-polar interactions. (B) Two step mechanism of action of ARBs. In the first step ARBs are postulated to embed themselves in the lipid matrix and in the second step are laterally diffused to the active site of AT1R.

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Conformational Study of Proteins by Heteronuclear NMR Spectroscopy

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Conformational study of proteins is of high importance in the field of Medicinal Chemistry. It provides the opportunity to map the target protein in order to rationally design bioactive molecules and learn more about their mechanism in molecular level. Conformational Analysis of a Protein with Heteronuclear Nuclear Magnetic Resonance Spectroscopy was conducted in this study, a technique that becomes increasingly popular in structural characterization of low molecular weight proteins and domains as well as kinetic parameters of the intermolecular interaction. The protein studied was the R957C mutant of the RING domain E3 Ligase Arkadia. E3 Ligases, especially Arkadia play an important role in the specific degradation of proteins through the Ubiquitin-Proteasome pathway. These mutations may alter their conformation and lead to disastrous consequences for the Cell. In the present study a conformational analysis with high resolution analysis was conducted, resulting in a very good compliance of NOE correlations and torsion angle restraints, suggesting that the mutated R957C RING preserve the native ββα topology. Conformational study was also conducted with methods that use chemical shifts for prediction of three dimensional structure of the protein. The results were compared with traditional approaches. Although these methods are still under evaluation, it seems that they perform quite accurate predictions during the early stages. Further study of the dynamic behavior of the protein based on NMR experiments revealed a well folded, rigid domain with disordered tails in both ends and the protein exists in solutions as a monome.

REVIEW CLINICAL PHARMACOLOGY AND
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Comparison of Use and Misuse of Antibiotics Between Male and Female Students of University of Patras'

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Introduction: The misuse of antibiotics leads to development of antimicrobial resistance to antibiotics. This resistance is considered to be a major problem of public health that puts in risk the use of medicines. Avoiding the non-rational use of antibiotics is the best way to face the durability.

Purpose: The aim of this study is to compare the knowledge, behavior and use of antibiotics between male and female students of University of Patras'.

Materials and Methods: We made a questionnaire that has 32 questions, open and close type. Subsequently, a pilot test of the questionnaire in the area of the University of Patras accomplished where 10 students answered and commented the questions for checking the validity of its content. At the beginning of October 2015 were asked 814 students from each department of University of Patras. The participation in this study was voluntarily and anonymous. Finally, we did statistical analysis of answers by using the statistical package IBM SPSS, edition for Windows.

Results: The questionnaire was answered totally by 814 students, where 71.4% were women and 28.6% were men. The 90.3% of male students and the 92.8% of female students said that they know the definition of antibiotics. The 47.0% of male students and the 47.3% of the students have interrupted the therapy when the symptoms disappeared. The 69.8% of male students and the 65.1% of female students declared that they know that is antimicrobial resistance to antibiotics is a public health problem. The 11.6% of male students believe that that antibiotics have bad genetic effects compared to 17.5% of female students.

Conclusions: Despite the fact that male students as much as female students are informed about health issues and know that antimicrobial resistance is a public health problem, they don't receive their medication rationally.

Suggestions: The results of this research can contribute in creating educational workshops open to all students of all departments of the University of Patras. Also, to set up in each hospital special working groups responsible for the use of antibiotics. Finally, to exist more strict prophylaxis measures in hospitals of the country.

KEYWORDS: resistance, antibiotics, bacteria, students, University.

ACKNOWLEDGEMENT

The present work was financially supported by the «Andreas Mentzelopoulos Scholarships University of Patras».