INTRODUCTION

Dear friends,

Nearly a year has gone by, and the EPSA Students’ Science Publication (ESSP) has already been published twice. And now it is time for this year's third and last edition. The aim of the ESSP has been to give students, who have performed scientific research during their studies, the possibility to publish their abstracts. With the professional support of the European Federation for Pharmaceutical Sciences (EUFEPS), students get the opportunity to develop a new skill by learning how to write an abstract and how to critically analyse their own results. EUFEPS is the reviewing body in this project. Professional researchers with their experience and expertise evaluate each abstract according to the field they belong to, hence each student receives feedback from someone that is familiar with the topic.

We are happy to present you 4 very interesting abstracts dealing with various topics such as: ‘the biochemical analysis of olive leaf extracts as possible treatment for diabetes’, ‘the impact of modifying the Reformatsky reaction on yield and purity of a COX-2 ligand’, ‘the design and synthesis of NOD1 inhibitors’ and ‘the biological analysis of the efficiency of new derivatives in Alzheimer’s Disease’. In addition, as in the previous editions, each student author will share experiences from the time in which they did their research.

Yours in EPSA,

Rebwar Saleh, Science Coordinator 2013-2014
Janice Geers, Science Coordinator 2014-2015
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ACTIVITY OF OLIVE LEAF EXTRACTS ON HEP G2 CELLS IN HYPERGLYCEMIC CONDITIONS
Mirjana Amidžić, Marijana Zovko Končić, Barbara Fumić, Roberta Petlevski, Patricia Marić
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Oxidative stress is one of the key mechanisms in the development and progression of impaired glucose tolerance and insulin resistance. Oxidative stress has deleterious effects on many organs and tissues, hepatic cells being one of the main targets. In Mediterranean countries, olive leaves are used as a traditional medicine for diabetes. It is a rich source of natural antioxidants with many positive effects on human organisms. Among them, the most prominent one is the iridoid glycoside - oleuropein. Therefore, the effects of the olive leaf extracts were investigated on the levels of extracellular lactate dehydrogenase (LDH), intracellular glutathione (GSH) as well as glutathione S-transferase (GST), and α-amylase inhibitory activity in Hep G2 cells in hyperglycemic conditions.

In order to investigate potential benefits of olive leaf consumption in diabetes, its antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, chelating ability and antioxidant activity using α-carotene-linoleate assay. The extracts were prepared using ultrasonic extraction, using ethanol/water in varying ratios at several temperatures. The total content of phenols, flavonoids and phenolic acids was determined spectrophotometrically, and the content of oleuropein by using a combination of Thin Layer Chromatography (TLC) and video densitometry. Obtained results were used for designing experiments for extracts preparation in which content of polyphenols, flavonoids, phenolic acid and oleuropein were maximized and the half maximal effective concentrations (EC50 values) of the performed antioxidant assays were minimal. The conditions giving the highest amounts were OE-25-75* (25°C, 75% ethanol) for antioxidant activity, OE-20-100* (20°C, 100% ethanol) for polyphenols and OE-60-100* (60°C, 100% ethanol) for oleuropein.

In addition, all the extracts have demonstrated notable antioxidant activities. However, neither of the extracts has shown any α-amylase inhibitory activity. The activity of the extracts in Hep G2 cells was dependant on the method used for its preparation. The extract from the OE-25-75* conditions showed membrane protective properties as seen in lower amount of extracellular LDH, even in comparison to non-hyperglycemic conditions. Finally, all the investigated extracts were capable of increasing the GST activity in Hep G2 cells. It could be concluded that olive leaf and its extracts could offer benefits as adjuvant therapy in diabetes.
Q&A OF THE AUTHORS

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Why did you select this topic for your research?
I have been interested in pharmacy since childhood; more specifically in preventive medicine and natural ways to stop disease before it becomes a serious issue. Having always been fascinated by science, I chose to pursue this interest by studying herbal properties at the Department of Pharmacognosy. My perception of science has changed over time as my understanding of the content has increased and subjects that once appeared independent now belong to one coherent field and interact with another.

How do you feel about having coped with the challenges that research can bring?
Challenges in research are numerous, but choosing the right topic and having a basic focus, as well as the right methodology is fundamental. Last, but not least, the challenge is dealing with data we have collected. Having an excellent research team is the best way to cope with any research problem.

What personal skills did you develop as a researcher during your research period?
Above all, adaptability, accuracy and analytical thinking.

Would you like to share anything else with the students in Europe? Any recommendations?
I would like to encourage students in developing countries to engage themselves in research. Even though they might not be working with the latest equipment, it is possible to obtain good results by using “old-fashioned” methods.
TESTING THE IMPACT OF MODIFIED REFORMATSKY REACTION ON THE SYNTHESIS OF 3,3-BIS-(4-CHLOROPHENYL)-3 HYDROXYPROPIONIC ACID

Department of Pharmaceutical Chemistry, University of Belgrade – Faculty of Pharmacy, Serbia

The objective of this work was the assessment of the impact of modifying the Reformatsky reaction on the yield and purity of 3,3-bis-(4-chlorophenyl)-3-hydroxypropionic acid. This compound has high affinity to the cyclooxygenase 2 (COX-2) active site and it would be benchmarked against ibuprofen. The binding energy of complex formation between COX-2 and derivatives of 3-hydroxy-3-aryl propionic acid were calculated and compared with ibuprofen-COX-2 binding energy. The compound with the best profile was synthesized in two ways. The first one represents the classical Reformatsky reaction:

\[
\text{O} \quad \text{O} \quad \text{O} \quad \text{Br} \quad \text{Cl} \quad \text{O} \quad \text{ZnBr} \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{O} \quad \text{Zn/THF} \quad 65^\circ \text{C} \quad \text{O}
\]

The other represents a modified Reformatsky reaction:

\[
\text{Br} \quad \text{O} \quad \text{Br} \quad \text{O} \quad \text{ZnBr} \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{O} \quad \text{Zn/THF} \quad 65^\circ \text{C} \quad \text{O}
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\]

The other represents a modified Reformatsky reaction:

\[
\text{Br} \quad \text{O} \quad \text{Br} \quad \text{O} \quad \text{ZnBr} \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{O} \quad \text{Zn/THF} \quad 65^\circ \text{C} \quad \text{O}
\]

In both cases acid hydrolysis of the products is carried out and products were extracted:

\[
\text{O} \quad \text{O} \quad \text{O} \quad \text{Br} \quad \text{Cl} \quad \text{O} \quad \text{ZnBr} \quad \text{Cl} \quad \text{Cl} \quad \text{O} \quad \text{O} \quad \text{H}_3\text{O}^+
\]

Calculating binding energy in the formed inhibitor-COX-2 complex was various: binding energy in the ibuprofen-COX-2 complex was -6.90 kcal/mol, in 3-phenyl-3-(4-chlorophenyl)-3-hydroxypropionic acid-COX-a complex was -7.45 kcal/mol, in 3,3-bis-(4-chlorophenyl)-3-hydroxypropionic acid-COX-2 complex was -7.73 kcal/mol. The compound with the lowest binding energy, 3-bis-(4-chlorophenyl)-3-hydroxypropionic acid, was synthesised. Purity of the product after the classical Reformatsky reaction was 44.60% and 95.90% after modified Reformatsky reaction. The purity was examined by using the HPLC method with FID detector. The yield after the modified Reformatsky reaction was 52.74%. The structure of the obtained compound was characterized by LC/MS, IR and NMR spectroscopy.
Based on results of docking studies it was concluded that some derivatives of 3-hydroxy-3-aryl propionic acid have lower binding energy than the ibuprofen in complex with the COX-2 active site. Based on these findings, these derivatives could be more potential inhibitors of COX-2 than ibuprofen, and could exhibit better anti-inflammatory activity. Based on results after synthesis, it was concluded that the synthetic route to obtain 3,3-bis-(4-chlorophenyl)-3-hydroxypropionic acid was improved by using the modified Reformatsky reaction, where the ester, which subsequently will react with the ketone, is the first to be synthesised. In this way a higher level of purity of the product is obtained. As anti-inflammatory effects of the synthesised compound are expected, further studies in vivo will be of high interest.
Q&A OF THE AUTHORS

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Why did you select this topic for your research?
I decided to work on synthesis of non-steroidal anti-inflammatory agents (NSAID), because I found that it is an interesting topic. We started with a target enzyme, and in the end, we had a lot of synthesised molecules with characterised structures and optimised synthetic route.

How do you feel you have coped with the challenges that research can bring?
When I saw unexpected results for the first time, I was really surprised. At first, I repeated the whole experiment, followed by literature data and I checked my methodology. I didn't know what to do next because my theory wasn't proved. Then I changed my hypothesis, modified experiments and that led me to the new results and new important conclusions.

What personal skills did you develop as a researcher during your research period?
Critical thinking is definitely the most important one. Experience in the lab is also important for preparing experiments (especially in pharmaceutical chemistry), but you can learn it during the practice at Faculty: everyone can make an experiment – you just have to follow instructions. But, if you have critical thinking, you will rationally analyse your problems during the research period, you will try to improve things that are good and minimise things that are not so good.

Anything else you want to share with the students in Europe? Recommendations?
Every research has periods when everything you do goes wrong, but it doesn’t mean that you should give up on everything. Every result is important, although some of them are not matching with the expected ones.
WE WANT YOUR ABSTRACT!

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DESIGN AND SYNTHESIS OF INDOLE SCAFFOLD-BASED NOD1 PROTEIN INHIBITORS
Pekošak Aleksandra
University of Ljubljana, Slovenia

A complex system of the immune system offers several targets for rational design of novel drugs. Our target was the intracellular NOD1 receptor that detects fragments from peptidoglycan, specifically meso-diaminopimelic acid and meso-DAP containing fragments. In response to bacterial ligands the NF-κB pathway is activated, leading to the production of pro-inflammatory cytokines, chemokines and apoptosis, and also potentially to septic shock, chronic inflammatory or autoimmune diseases. At this point NOD1 inhibitors, which could effectively and selectively inhibit the inflammatory response of the immune system, have an important role.

Based on the knowledge of the structure-activity relationship of known NOD1 inhibitors, new compounds were designed with a 2-aminoindole scaffold using "scaffold hopping". The indole scaffold is present in a number of biologically active compounds and is regarded as a "privileged structure" and was therefore used as a benzimidazole mimic.

The scaffold replacement proved satisfactory, based on biological tests measuring the percentage of inhibition of NF-κB activation stimulated with agonist C12iE-DAP on the Ramos-Blue™ cells. The most active compound 13, 1-(4-chlorobenzyl)-indoline-2-iminium chloride, inhibited NF-κB activation even better than the well-known inhibitor Noditinib. A chloro substituent in position 4 in the aromatic ring (R2) and amino group on the indole (R4), which could form hydrogen bonds with active site of enzyme, showed an increased inhibition activity. However, because of the high standard deviation this compound cannot be regarded as a better inhibitor. In conclusion, although compound 13 proved cytotoxic at a high concentration, it represents a good starting point for further optimization.

![Graph: Residual activity NF-κB of NOD1 inhibitors in Ramos-Blue™ cells](image)
Q&A OF THE AUTHORS

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Why did you select this topic for your research?
During my studies I have always been impressed by scientists’ achievements – discovery and development of the most complicated drugs treating various diseases. During my third year of practical lectures, I found my interest in medicinal chemistry so I decided to do further research work at the Chair of Pharmaceutical Chemistry, Faculty of Pharmacy. The immune system and its complexity has been an unexplored area to me, therefore it was an inspirational topic for research work because I had to deal with the synthesis of potential novel immunomodulatory compounds. Therefore I decided to choose this as my master thesis topic.

How do you feel about having coped with the challenges that research can bring?
I consider myself a hard-working and fully dedicated person, so I was very enthusiastic about the research and the results. To be honest, I still remain optimistic that these compounds will contribute to the pharmaceutical research in the future.

What personal skills did you develop as a researcher during your research period?
I learned that novel drugs synthesis requires a lot of time and patience, but on the other hand I found out that lab work is challenging and also addictive as it leaves you wanting to find out more. It was definitely a remarkable experience that contributed to my future career as a researcher.

Would you like to share anything else with the students in Europe? Any recommendations?
I found topic selection particularly important, especially if you would like to fully exploit your potential. As you will have to devote quite some time to research, do not forget to select a topic that you really enjoy working with and as a result you will contribute to science and also to people’s health.
BIOLICAL ASSAYS OF ACETYLCHOLINESTERASE INHIBITORS
Małgorzata Girek1, Paulina Olszewska2, Elżbieta Mikiciuk-Olasik2, Paweł Szymański1
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Alzheimer’s disease (AD) is one of the most frequent neurodegenerative disorders and the most common cause of dementia in modern society. The disease occurs predominantly in people above 65 years old. Cholinergic system plays the main role in learning process and is progressively damaged during the illness. Inhibitors of acetylcholinesterase and butyrylcholinesterase enhance the level of acetylcholine in nerve cells and are the main target in AD. The aim of the study was to examine the acetylcholinesterase and butyrylcholinesterase inhibition by the new series of tetrahydroacridine derivatives. Twenty three compounds were analysed and the three best inhibitors were examined to define the type of inhibition and their kinetic value. The Ellman’s test is a common biological assay. With Ellman’s test, it is possible to measure the concentration of thiols in the sample. First acetylthiocholine was hydrolysed by enzymes and thereafter the thiocholine reacted with Ellman’s reagent DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) to produce an ion, which had a yellow color. The quantity of ion was measured by UV spectrophotometry. To determine the half maximal inhibitory concentration (IC50) in Ellman’s test the diverse concentrations of new inhibitors were used. To specify the type of inhibition diverse concentrations of substrate (acetylthiocholine) were added. The values of the IC50 were based on absorbance results. Among twenty three compounds three derivatives of tetrahydroacridine containing three different groups of substituents – iodobenzoic acid, fluorobenzoic acid and dimethylaminobenzoic acid (having the lowest IC50 values) were chosen. As the reference compounds tacrine and rivastigmine were used. The IC50 values of the tested compounds were significantly lower than the reference compounds. The derivative of tetrahydroacridine containing dimethylaminobenzoic acid has been proven to be a competitive type of inhibitor. The derivatives of tetrahydroacridine with iodobenzoic acid and fluorobenzoic acid moiety are uncompetitive types of inhibitors.
Biological assays suggest that the new compounds can be a new potential drug used in treatment of AD. The next step of our research will be concentrated on cytotoxicity and inhibition of beta-amyloid aggregation by selected compounds.
Q&A OF THE AUTHORS

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Why did you select this topic for your research?
I’ve started in cooperation with the Department of Pharmaceutical Chemistry, Drug Analyses and Radiopharmacy at the Medical University of Lodz during my 3rd year of Pharmaceutical studies. I’ve found out more about projects they have run and I chose Alzheimer’s disease (AD) because I considered it as the most interesting. Before pharmacy I wanted to study neurobiology, therefore being the major reason why I found this topic the most promising and merging to my concerns. AD is incurable and it offers many possibilities to work on. My thesis was based on biological assays of enzymes, which I preferred to the synthesis of new derivatives. I worked both acetylcholinesterase and butyrylcholinesterase, which behaved diversely. In vitro experiments may be unpredictable.

How do you feel about having coped with the challenges that research can bring?
I’ve looked through many publications about AD. Our research are a significant part of developing novel derivatives which could be used one day in AD treatment. In June we examined the ability of three selected inhibitors to inhibit β-amyloid aggregation. All researches are very promising and I’m proud to be a part of the team who worked on it.

What personal skills did you develop as a researcher during your research period?
My research period lasted 4 months. I must have become a more precise and careful person. Each day I prepared solutions and dilutions with a very specific concentration. I can’t have done any mistakes with even one drop, because µl is a very small amount and error could lead to repetition of measurement. Perseverance and patience are the major character traits which helps during research. I learned a lot from mistakes and nowadays I feel much more experienced and confident than I was at the beginning of this year.
Would you like to share anything else with the students in Europe? Any recommendations?
If anybody is interested in research, it’s important to initiate contact with professors at the university even before thesis as it provides you with better opportunities to fulfill your aims. Being part of the science team is an invaluable experience which can open many doors. Checking every aspect of studying may be significant in determining your professional path.
EUROPEAN FEDERATION FOR PHARMACEUTICAL SCIENCES (EUFEPS)

The European Federation for Pharmaceutical Sciences is a voluntary association of pharmaceutical scientists, established in 1991 to advance research in the pharmaceutical sciences in Europe. This can be achieved by promoting cooperation between national, regional and European societies or associations which aim at the advancement of pharmaceutical sciences, and by promoting cooperation between and with other pharmaceutical organisations and between individual pharmaceutical scientists.

The Mission

EUFEPS exists to help to meet the challenges and seize the opportunities created by the consolidations occurring both within Europe and globally, driven on by a combination of rapid advances in science and technology, economic pressures, and by political will. Within this frame, EUFEPS’s role and contributions are amply expressed in its mission statement “EUFEPS serves and advances excellence in the pharmaceutical sciences and innovative drug research in Europe”. Spearheading a number of initiatives, EUFEPS works with its membership, throughout the nations of Europe.

European Dimension

EUFEPS is unique being the only pan-European organisation that represents, under one umbrella, all the pharmaceutical sciences and pharmaceutical scientists engaged in drug research and development, drug regulation and drug policy making. The existence of such an umbrella platform facilitates the highly innovative, integrative and interdisciplinary approaches that are essential if we, in Europe, are to deliver to our citizens safe, effective, economic and timely medicines. The ultimate benefits are an improving health, quality of life, and wealth of our continent.
EUFEPS is recognised by the European Commission, as representing the integrative pharmaceutical sciences within Europe. EUFEPS is also recognised by the EMEA as a neutral scientific resource for independent opinions on draft regulatory guidelines, while EUFEPS works with other European organisations, such as EFPIA, to help identify and promote training to meet industrial needs. EUFEPS has the ambition to provide a forum for policy development in the pharmaceutical sciences, particularly in relations to the discovery and development of new drugs and their introduction into the market, but also as to medicines usage. This includes policies on and leadership development as to: research, education & training, profiling and regulatory affairs.

In addition, EUFEPS plays an active and influential role also in the global arena. It is recognised by the USA FDA and it works actively with its sister organisation AAPS to develop co-sponsored meetings and workshops that run alternatively in Europe and the USA, and is developing links with Asian scientists. Through involvement with its Board of Pharmaceutical Sciences, EUFEPS is also working with FIP to advance the pharmaceutical sciences globally.
Upcoming Conferences organised by EUFEPS:

Global Bioequivalence Harmonisation Initiative
February 2015
For more information see: www.eufeps.org

EuPAT7
May 17-18, 2015, Graz, Austria
organised by EUFEPS
For more information see: www.eupatqbd.org

EUF EPS Annual Meeting 2015
June 2015, Geneva, Switzerland
For more information see www.eufeps.org

6th BBBB Conference on Pharmaceutical Science: “Strategies to improve the quality and performance of modern drug delivery systems”
September 10-12, 2015, Helsinki, Finland
For more information see www.eufeps.org
The seventh pan-European Science Conference on QbD and PAT Sciences brings together pharmaceutical scientists and engineers from industry, academia and regulatory agencies to discuss recent developments and future trends in the field of pharmaceutical product and process development. The focus of the 7th EuPAT conference will be on future and emerging trends in the pharmaceutical sector, including continuous manufacturing; the development and supply of personalised medicines and the design and manufacture of nano-structured, pharmaceutical products. Regulatory uncertainties, technical challenges and barriers to adoption which exist in each of these focus areas will be explored and debated in detail.

The conference builds on the success of the EuPAT series established in 2006 and will be organised by EUFEPS, the Graz University of Technology and the Research Center for Pharmaceutical Engineering (RCPE). The main meeting will be held on May 18-19, 2015, in Graz, Austria, and is organised back-to-back with a training day centred on “the Basics of Pharmaceutical Manufacturing with a focus on Hot-Melt Extrusion - Experiments and Modelling”, which is scheduled to commence on May 17, 2015, at the Graz University of Technology, Graz, Austria. The attendees of EuPAT 7 are welcome to register for the pre-conference day and vice versa.

Session topics at EuPAT 7
- PAT and QbD in continuous manufacturing for drug compounds and products
- Development and production of nano-structured drug products
- Manufacture and supply of personalised medicines
- Sensor – new probes and imaging solutions for innovative control

Invited Speakers at EuPAT 6
Prof. A. Florence, Strathclyde, Glasgow UK
Prof. B. L. Trout, MIT, Cambridge MA, USA
Prof. R. Duncan, Cardiff UK
Prof. G. Storm, Utrecht University, Utrecht NL
Dr. S. Stegemann, Capsugel, Bornem BE
Dr. A. C. Beal, Chief Patient Offices, Sanofi
Prof. A. Zeitler, University of Cambridge, Cambridge UK
AFTERWORD

This edition was made possible by both Science Coordinator 2013-2014 Rebwar Saleh and Science Coordinator 2014-2015 Janice Geers.

Rebwar: ‘This edition was planned to finalise as part of the handover period between us, the Science Coordinators. I thought it was important that the next Science Coordinator felt safe and comfortable with the project before handling it all alone. So by doing this edition together, I hope that Janice will be able to see all the strong sides, and also the weaker sides of the project so that when the edition is done, she will know how to improve and further develop the publication. I am very happy with all her efforts, and I am sure she will bring this “baby publication” further on. Lastly, this publication would never be as good as it can be without the help of EUFEPS, who put the quality stamp on the abstracts. Also, our members who choose to utilise this publication, Thank you!’

Janice: ‘I am really happy to be part of this edition so early in my mandate. It was a great opportunity to learn about dealing with deadlines, cooperating with EUFEPS and guiding the students. I really would like to congratulate the students for their great effort and very nice result! I would also like to thank EUFEPS for their dedication to our project.’