39. Krkine nagrade 39th Krka Prizes

19. simpozij / Zbornik povzetkov

Novo mesto, 16. oktober 2009

www.krkine-nagrade.si

Častni odbor 39. Krkinih nagrad

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Glavni in odgovorni urednik:	dr. Miha Plevnik

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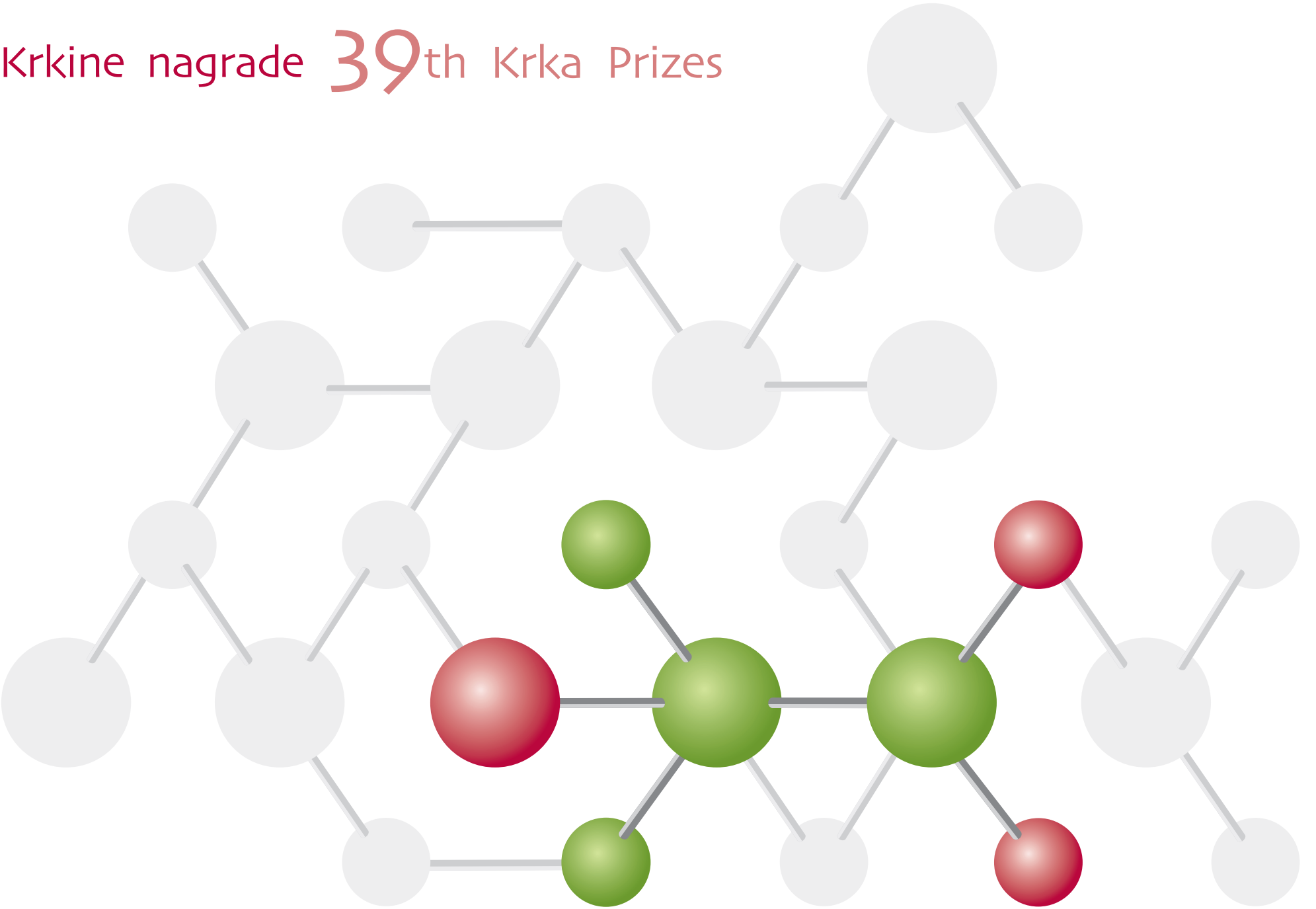
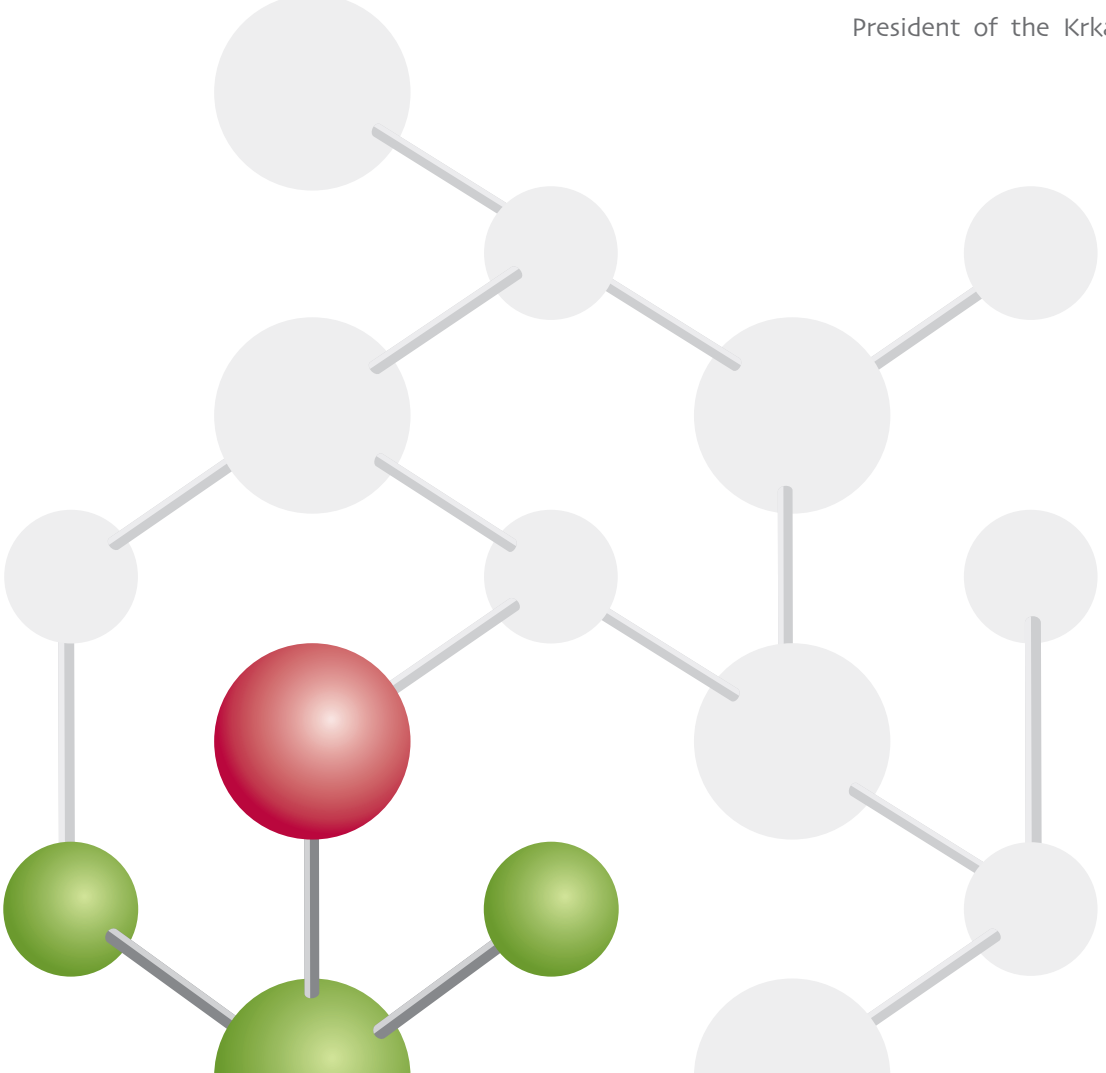


Letošnje Krkine nagrade podeljujemo v zelo dinamičnem obdobju. Minulo leto, v katerem ste pripravljali svoje raziskovalne naloge, je zaznamovala vrsta dogodkov. Ne samo materialni, tudi vrednostni temelji naše civilizacije so se stresli. Znanje in izkušnje bodo pomagale pri okrevanju gospodarstva, izkušnje pa nedvomno že vplivajo na širše družbene pogoje, v katerih živimo in ustvarjamo. Vrsta vprašanj se postavlja o tem, kako naprej, kaj je napredek in kakšen mora biti, da omogoča nadaljnjo resnično in oprijemljivo rast. Najvišji skupni imenovalec pri tem predstavlja znanje. Nova znanja in inovacije, katerih pomen in vrednost uporabniki prepoznajo tudi v najtežjih časih, so tista stalnica v razvoju naše družbe in civilizacije, ki nas vodi naprej. Raziskovanje, razvoj in uporaba novih znanj ter spoznanj so zato potrebni bolj kot kdajkoli. Izobraževanje in vzgoja mladih, navdahnjenih in ustvarjalnih mladih raziskovalcev je zato nedvomno med najpomembnejšimi temelji napredka. V Krki se zavedamo, da so za to potrebne spodbude, zato nadaljujemo s partnerskim delom z akademskimi ustanovami. Krkine nagrade so ena od najpomembnejših vezi, tako z raziskovalci kot mentorji. Najprej naj se vam torej zahvalim za vse delo in energijo, ki ste jo vsi vložili v pripravo vaših del. Ob čestitkah za dosežke pa vam želim, da bi tudi vnaprej vztrajali na težki, a zanimivi poti k novim dognanjem.

Dr. Aleš Rotar
Predsednik Sveta Sklada Krkinih nagrad

This year's Krka Prizes are being awarded in a very dynamic period. The past year in which you have prepared your research projects was marked by a series of events. Not only the material foundations, but the entire value system of our civilization was shaken. Knowledge and experience will help the economy recover. Experience itself undoubtedly already has an impact on the broader social circumstances in which we live and create. Many questions are being asked like what to do next, what progress is, and what it should be to enable future realistic and tangible growth. The highest common denominator in this context is knowledge. New knowledge and innovation, the importance and value of which users identify even in the most difficult of times, are the one constant in the development of our society and civilization, which leads us forward. Research, development and application of new skills and knowledge are therefore needed more than ever. Training and education of young, inspired and creative junior researchers is therefore clearly one of the most important foundations of development. In Krka we recognize the value of incentives, and therefore continue our collaboration with academic institutions. The Krka prizes are one of the most important links with researchers, as well as mentors. First, let me thank you for all the work and energy you put into preparing your research. Along with my congratulations for your achievements, let me express my wish that you all persist on the difficult and interesting road towards new discoveries.

Dr. Aleš Rotar
President of the Krka Awards Council



KRKINI NAGRAJENCI 2009 / KRKA PRIZE WINNERS 2009

Št.	Nagrajenci	Status	Mentor / Somentor(ji)
2236	Jasmina Bevec	dijakinja	Jana Goršin Fabjan
2237	Miha Črnigoj	doktor znanosti s področja biotehnologije	Nataša Poklar Ulrih
2238	Andrej Ferlan	magister znanosti s področja farmacije	Franc Vrečer
2239	Rok Frlan	doktor znanosti s področja farmacije	Slavko Pečar Aleš Obreza
2240	Jurij Gasparič	dijak	Neda Benički Švagelj
2241	Tanja Glavan	univerzitetno diplomirana inženirka kemijskega inženirstva	Aleš Gasparič Marin Berovič
2242	Rade Injac	doktor znanosti s področja farmacije	Borut Štrukelj
2243	Žiga Jeraj	študent farmacije	Odon Planinšek
2244	Janez Jerman	študent farmacije	Franc Vrečer Silvo Zupančič
2245	Lara Jordan	dijakinja	Robert Sekereš Stanka Florijančič
2246	Vanja Kerbler Kocuvan	magistrica farmacije	Mojca Brunskole Tea Lanišnik Rižner
2247	Teja Kitak	študentka farmacije	Rok Šibanc
2248	Tadej Kofjač	magister farmacije	Stanko Srčič Dušan Prelog
2249	Janez Kopač	študent farmacije	Odon Planinšek
2250	Andreja Kovač	doktorica znanosti s področja farmacije	Stanislav Gobec
2251	David Kralj	doktor znanosti s področja kemije	Jurij Svete
2252	Tjaša Krašovec	dijakinja	Robert Sekereš Stanka Florijančič
2253	Zoran Lavrič	magister farmacije	Stanko Srčič Zvonko Trontelj
2254	Urška Lešnik	doktorica znanosti s področja biotehnologije	Hrvoje Petković Lea Pogačnik
2255	Veronika Medic	dijakinja	Matejka Kure
2256	Mateja Mervič	dijakinja	Jana Goršin Fabjan
2257	Nino Metelko	dijak	Neda Benički Švagelj

	Št.	Nagrajenci	Status	Mentor / Somentor(ji)
	2258	Mia Mikec	dijakinja	Robert Sekereš Stanka Florijančič
	2259	Andreja Mirtič	univerzitetno diplomirana biokemičarka	Janez Plavec
	2260	Jerneja Mori	univerzitetno diplomirana mikrobiologinja	Roman Jerala
	2261	Nataša Novina	magistrica znanosti s področja poslovodenja in organizacije	Marko Hočevar Brane Krevs
	2262	Nataša Obermajer	doktorica znanosti s področja farmacije	Janko Kos
	2263	Jasminka Pavlinac	doktorica znanosti s področja kemije	Stojan Stavber Marko Andrej Zupan
	2264	Andrej Perdih	doktor znanosti s področja farmacije	Tomaž Šolmajer
	2265	Malgorzata Piecha	doktorica znanosti o okolju	Polonca Trebše
	2266	Ajda Podgoršek	doktorica znanosti s področja kemije	Jernej Iskra Marko Andrej Zupan
	2267	Irena Pulko	doktorica znanosti s področja kemijske tehnike	Peter Krajnc Aleš Podgornik
	2268	Brigita Radovan	univerzitetno diplomirana mikrobiologinja	Romana Marinšek Logar Gordan Sladič
	2269	Luka Rifelj	dijak	Neda Benički Švagelj
	2270	Zvone Simončič	doktor znanosti s področja farmacije	Vojko Kmetec Ksenija Kogej
	2271	Matej Sova	doktor znanosti s področja farmacije	Stanislav Gobec
	2272	Ervin Šinkovec	univerzitetno diplomirani inženir kemijskega inženirstva	Stane Pejovnik Miran Hvalec
	2273	Tina Šmuc	doktorica znanosti s področja biokemije in molekularne biologije	Tea Lanišnik Rižner
	2274	Marko Tramšek	doktor znanosti s področja kemijske tehnike	Andreja Goršek Đurđa Vasić-Rački
	2275	Peter Trkman	doktor znanosti s področja informacijsko upravljalskih ved	Miro Gradišar
	2276	Tanja Troha	dijakinja	Matejka Kure
	2277	Tina Ukmar	študentka farmacije	Franci Merzel Aljaž Godec
	2278	Uroš Uršič	doktor znanosti s področja kemije	Branko Stanovnik
	2279	Viktor Uršič	doktor znanosti s področja medicine	Jurij Šorli

	Št.	Nagrajenci	Status	Mentor / Somentor(ji)
●	2280	Anamarija Vajs	magistrica znanosti s področja kemije	Anton Meden Marija Grčman
●	2281	Maja Zorec	dijakinja	Jana Goršin Fabjan
●	2282	Aleksandra Zupančič	dijakinja	Matejka Kure
●	2283	Katja Žmitek	doktorica znanosti s področja kemije	Jernej Iskra Marko Andrej Zupan

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»Začni z velikim! Četudi imaš čas, se z malim ne ubadaj. Ne neuspeh, prenizek cilj je greh«

James Russell Lowell

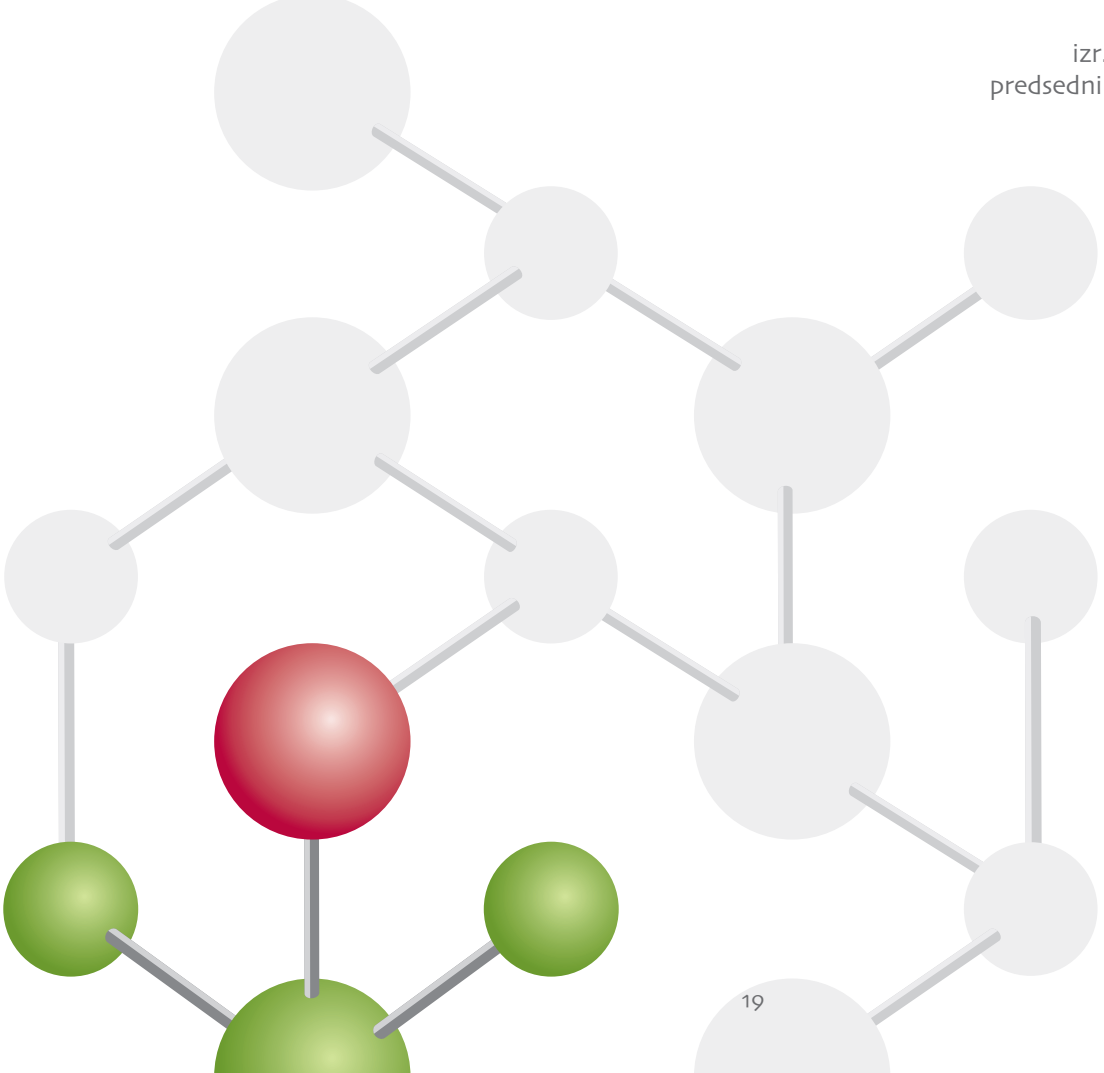
Čas velikih sprememb v gospodarstvu in družbi ponuja priložnost pogumnim, da z novimi idejami in rešitvami prispevajo k preboju gospodarstva. Odziv na letošnji razpis na že 39. Krkine nagrade kaže, da pri nas ne manjka sposobnih raziskovalcev in njihovih mentorjev, ki si upajo in hočejo stopati po neizhojenih poteh do novih visokih ciljev, kot je npr. iskanje zdravilnih učinkovin za zdravljenje bolezni, za katere še nimamo optimalne terapije, optimizacija procesov v razvojno-proizvodni verigi v farmacevtski industriji.

Tudi letos so se na razpis prijavili raziskovalci s svojimi raziskovalnimi nalogami na vseh stopnjah študija kot tudi mladi srednješolci, slednjim je bil v večini primerov to prvi stik z raziskovalnim delom. Naloge so bile na visokem znanstvenem nivoju, želeli pa bi si več aplikativnih znanstvenih raziskav usmerjenih na področja ključna za razvoj generičnih zdravil, katerih izsledki se lahko relativno hitro uporabijo v razvoju generičnih zdravil z visoko dodano vrednostjo, ki niso pomembna samo za uspešno poslovanje podjetij ampak so preko znižanja stroškov za zdravila pomembna tudi za celotno družbo.

Še enkrat se v imenu Znanstvenega odbora pri Svetu Krkinih nagrad zahvaljujem vsem raziskovalcem, ki so sprejeli naš izziv in se prijavili na letošnji razpis. Posebna hvala tudi vsem mentorjem in somentorjem, za njihov trud in pomoč mladim raziskovalcem, pri izvedbi raziskovalnega dela. Na koncu pa se želim zahvaliti vsem recenzentom, ki so v kratkem času, ki so ga imeli na voljo opravili zelo kakovostne recenzije in s tem omogočili izbor nagrajencev.

Letošnje nagrade naj bodo vzpodbuda mladim raziskovalcem in njihovim mentorjem, da si postavijo visoke cilje, kajti na ta način bodo tudi na koncu dosežki veliki in pomembni za razvoj naše družbe.

izr. prof. dr. Franc Vrečer
predsednik Znanstvenega odbora



»Greatly begin. Though thou have time, but for a line, be that sublime. Not failure, but low aim is crime.«

James Russell Lowell

A time of major changes in the economy and society offers opportunities to those brave enough to contribute new ideas and solutions to achieving an economic break through. There have already been 39 responses to this year's competition. The Krka Awards show that we have no lack of researchers and mentors daring to blaze a trail towards ambitious new goals, such as seeking active pharmaceutical ingredients to treat diseases still without an optimal therapy, optimising processes in the development-production chain within the pharmaceutical industry.

This year researchers have again applied to the competition with research tasks at all levels of study, as have secondary school students for many of whom this is a first experience of research work. The work has been of high quality, through we would like more applied scientific research directed towards key areas for generic pharmaceutical development, with results that can be relatively quickly applied in the practical development of generic pharmaceuticals with high added value. These are not only vital to the successful performance of the Krka companies, but by lowering medical costs are also very important for society as a whole.

Once more on behalf of the Scientific Committee of the Krka Awards Council I would like commend all the researchers who accepted the challenge and entered this year's competition. Particularly thanks must go to all the mentors and co-mentors for their hard work and support for junior researchers in their research work. Finally, I would like to thank all the editors who carried out a very high quality review of the work despite the short time available to them, allowing us to make our selection of award winners.

I hope this year's awards will be an encouragement to junior researchers and their mentors to set themselves ambitious targets, because in that way the final results will be great and will serve to further the development of our society.

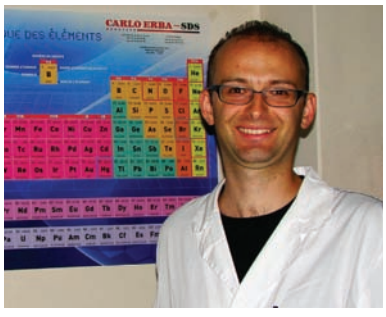
Assoc. Prof. Franc Vrečer, Ph. D.
President of the Scientific Committee

Krkine nagrade za posebne dosežke na področju raziskovalnega dela

Krka Prizes for Special Achievements in Research

Predstavitev nagrajencev

Presentation of prizewinners



Dr. Rok Frlan

Rok Frlan je asistent na fakulteti za farmacijo in trdno verjame, da so zgrešeni poskusi le stopnice do končne rešitve. Prosti čas najraje preživlja s svojo družino v naravi, uživa pa tudi v kolesarjenju in teku.

Bi lahko na kratko opisali svojo raziskovalno pot? Ste že od malega v sebi čutili raziskovalni duh?
Moje zanimanje za naravoslovje najverjetneje izvira iz preživljanja mladosti v idiličnem vaškem okolju, kjer sem imel tudi dovolj možnosti za raziskovanje narave in njenih pojavov. Ker me je zelo zanimala biologija, sem pred vpisom na fakulteto tehtal med medicino in kemijo, vendar sem se odločil za srednjo pot – farmacijo.

Kaj vas navdihuje na vaši raziskovalni poti?
Največji navdih je prispevek mojega raziskovalnega dela k napredku znanosti. Zavedam se, da so odkritja le majhen košček v mozaiku, pri čemer upam, da bodo moja spoznanja prispevala k blaginji celotnega človeštva.

Na kaj ste se osredotočili v svoji doktorski nalogi in kakšni so vaši zaključki?
Cilj doktorske disertacije je bila sinteza novih potencialnih učinkovin za zdravljenje bakterijskih infekcij. Uspelo nam je sintetizirati kar nekaj spojin, ki smo jih testirali in vitro na izoliranih bakterijskih encimih. Ob tem smo ugotovili, da so nekatere spojine izkazovale inhibitorno aktivnost v srednjem mikromolarnem koncentracijskem območju. Spoznanja moje doktorske disertacije so pomemben prispevek k razumevanju odnosa med strukturo in delovanjem spojin pri načrtovanju novih inhibitorjev encimov, udeleženih v biosintezi bakterijske celične stene.

Kako poteka vaš delovni dan? Kako usklajujete poklicno in osebno življenje?
Zjutraj odidem v službo, ko moja družina še spi, in se vrnem domov sredi popoldneva. Po kosilu s partnerko in hčerama odidemo v naravo, če je le mogoče. Poklicno in osebno življenje se trudim čim bolj uravnovesiti, včasih mi vzame več časa eno, včasih drugo, a ob koncu dneva sem zadovoljen.

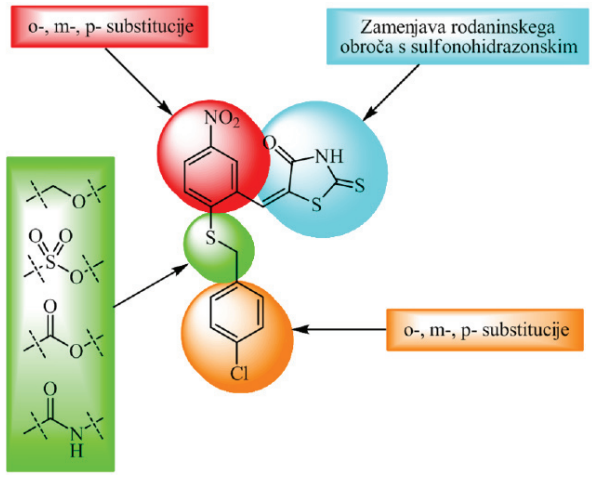
Kako bi se opisali, katere so tiste vrednote, ki jim sledite v življenju?
Našel sem se v svojem delu, ki je hkrati hobi in izziv. Zato ideje in rešitve dobivam ob najbolj neobičajnih priložnostih. Sem vztrajen in se nadvse rad učim, zame so zgrešeni poskusi le stopnice na poti do končne rešitve.

Kako najraje preživljate svoj prosti čas?
Za protiutež delu v laboratoriju so moji hobiji povezani z naravo, tečem, kolesarim in tudi z družino večino časa preživljamo v naravi. Ob deževnih in mrzlih dneh pa se zabavamo za računalnikom ali s sestavljankami.

NAČRTOVANJE, SINTEZA IN VREDNOTENJE SULFONOHIDRAZIDNIH INHIBITORJEV LIGAZ Mur

Rok Frlan
Fakulteta za farmacijo, Univerza v Ljubljani
Mentor: Slavko Pečar
Fakulteta za farmacijo, Univerza v Ljubljani
Somentor: Aleš Obreza

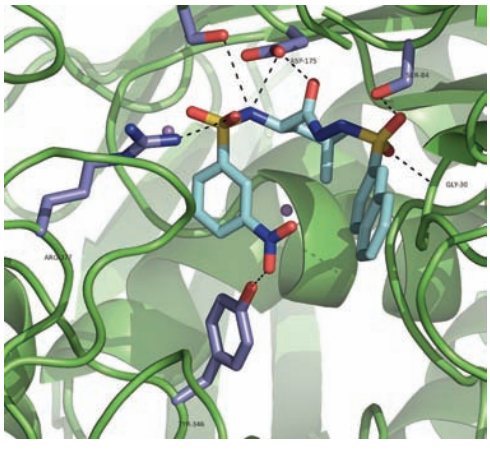
Zaradi naraščajoče rezistence na protibakterijske učinkovine postajata v današnjem času načrtovanje in sinteza novih protibakterijskih učinkovin vedno bolj aktualna. Med najbolj privlačnimi tarčami so encimi, udeleženi v biosintezi peptidoglikana, ki je ključna sestavina bakterijske celične stene, zato lahko vsaka motnja v tej makromolekuli vodi v propad bakterijske celice. Nekatere od začetnih stopenj v biosintezi peptidoglikana katalizirajo encimi iz skupine Mur ligaz (MurC, MurD, MurE in MurF), ki smo jih izbrali kot tarče pri našem raziskovalnem delu.
V okviru doktorske naloge smo ovrednotili hipotezo, da so derivati sulfonohidrazidov lahko potencialni inhibitorji ligaz Mur. Pri tem smo uporabili sodobne pristope farmacevtske kemije, s katerimi smo načrtovali in sintetizirali več strukturno različnih novih sulfonohidrazidnih inhibitorjev Mur ligaz ter jih biokemijsko ovrednotili. V začetnem delu smo se osredotočili zlasti na encim MurC, za katerega ni bilo znanih veliko inhibitorjev, kasneje pa smo načrtovali tudi inhibitorje za ostale ligaze Mur.
Eden od pristopov pri načrtovanju novih spojin je bilo posnemanje strukture znanih inhibitorjev MurC, benziliden rodaninov, ki smo jih uporabili kot spojine vodnice. Tiazolidin-2,4-dionski obroč v spojini vodnici smo najprej nadomestili s strukturno sorodnim necikličnim sulfonohidrazonskim skeletom, nato smo preko modifikacij vseh delov molekule poskušali povečati inhibitorno aktivnost na MurC. p-Klorobenziltio substituent v spojini vodnici smo tako nadomestili z različnimi arilsulfonati, arilamidi in arilestri (Slika 1). Sintetizirali smo serijo spojin, za katere nam je Urad RS za zaščito intelektualne lastnine podelil patent št. SI22254, Evropska patentna organizacija pa je v obravnavo sprejela patent št. EP1845083 (A2). Kljub temu, da je bilo za te spojine sprva ugotovljena inhibitorna aktivnost v mikromolarnem območju, se je kasneje izkazalo, da gre po vsej verjetnosti za t.i. promiskuitetne inhibitorje.

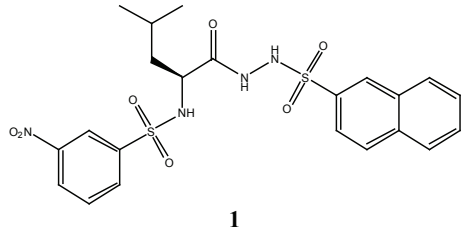


Slika 1: Načrtovanje in strukturne spremembe inhibitorjev MurC s posnemanjem strukture rodaninov kot spojine vodnice.

Vzporedno smo se lotili racionalnega načrtovanja novih inhibitorjev MurC na osnovi strukture aktivnega mesta encima. Z uporabo programske opreme za virtualno rešetanje in na podlagi objavljene kristalne strukture encima MurC s produktom encimsko katalizirane reakcije smo na osnovi sidranja in povezovanja strukturnih fragmentov v aktivnem mestu načrtovali in sintetizirali spojine s sulfonohidrazidnim skeletom, ki naj bi se vezale v vezavno mesto za difosfatni del substrata (oz. produkta). S kritičnim vrednotenjem rezultatov biokemijskih testiranj smo določili osnovne strukturne zahteve za doseganje dobre inhibitorne aktivnosti na MurC.

Prva serija spojin, ki je vsebovala levcinski fragment, je izkazovala inhibitorno aktivnost v srednjem μM koncentracijskem območju in nam je služila kot osnova za nadaljnje delo. Enega prvih sulfonohidrazidnih inhibitorjev MurC (IC_{50} = 240 μM) smo testirali tudi na bakterijskih kulturah, kjer smo ugotovili šibko protibakterijsko aktivnost (MIC = 128 μg/mL, *S. aureus*) (Slika 2).





1

RA(MurC, 250 μM) = 49%
MIC (*S. aureus*) = 128 μg/mL

Slika 2: Predlagana vezava sulfonohidrazidnega inhibitorja **1** v aktivno mesto encima MurC.

Sledeče modifikacije so bile usmerjene tako v povečanje topnosti molekul kot tudi v izboljšanje inhibitorne aktivnosti spojin. V ta namen smo levcin zamenjali s treoninom, sulfonohidrazidni fragment pa smo pri nekaterih spojinah nadomestili s hidrazidnim. Uporabili smo različno substituirane aromatske obročne sisteme z namenom povečanja možnosti tvorbe H-vezi med aktivnim mestom encima in polarnimi skupinami na aromatskih obročih. Eden od poskusov doseganja večje inhibitorne aktivnosti je bila tudi rigidizacija spojine, kjer smo namesto levcina uporabili prolin. Kljub dobremu prileganju v aktivno mesto pa nobena od sintetiziranih spojin v tej seriji ni izkazovala inhibitorne aktivnosti.
V nadaljevanju smo nato sintetizirali spojine, ki so poleg mimetikov difosfatnega dela substrata (oz. produkta) vsebovale tudi krajše peptidne fragmente, ki so posnemali peptidne dele naravnih substratov encimov MurB, MurC, MurD in MurE. Z namenom tvorbe močnejših interakcij z aktivnim mestom encimov smo pri nekaterih inhibitorjih namesto karboksilnega dela peptidnih fragmentov uporabili hidroksamske kisline. Kot mimetik difosfatnega dela substrata (oz. produkta) smo v tem primeru uporabili različne mimetike difosfatnega dela substrata (oz. produkta): sulfonamidokarbamatni, sulfonamidotreonin in sulfonamidotreoninohidrazidni mimetik. Spojine smo rigidizirali s fenilnim obročem, katerega smo substituirali s prej omenjenimi mimetiki difosfatnega dela substratov (oz. produktov) in s peptidnimi fragmenti. Na ta način nam je uspelo sintetizirati več serij spojin, od katerih je ena spojina izkazovala inhibitorno aktivnost na MurC v srednjem μM območju.
Z opisanimi pristopi nam je uspelo sintetizirati kar nekaj spojin, ki smo jih testirali in vitro na izoliranih bakterijskih encimih. Ob tem smo ugotovili, da so nekatere spojine izkazovale inhibitorno aktivnost v srednjem mikromolarnem koncentracijskem območju.
Tekom te doktorske disertacije smo ugotovili, da je sulfonohidrazidni fragment primeren gradnik za načrtovanje inhibitorjev ligaz Mur. Z uporabo pristopov strukturno podprtega načrtovanja smo namreč dosegli, da so se spojine s sulfonohidrazidnim delom v našem modelnem sistemu prilegale v bližino vezavnega mesta za difosfat. eHITS se je pri tem izkazal kot primerno orodje za načrtovanje novih učinkovin, saj nam je omogočil napoved aktivnosti z več kot 50% zanesljivostjo. Z uporabo eHITS in iz rezultatov biokemijskih testov smo spoznali, da se lahko sulfonohidrazidni fragment zamenja s strukturno sorodnim hidrazidnim fragmentom, vendar pri tem tvegamo zmanjšanje topnosti spojin. Prav tako smo ugotovili, da so se kot najbolj optimalne za doseganje inhibitorne aktivnosti izkazale spojine z levcinom v svoji osnovni strukturi. S pomočjo modifikacij v aromatskem delu smo spoznali, da ta del molekule dopušča različne variacije in posledično omogoča izboljševanje inhibitorne aktivnosti tako na MurC kot tudi na MurD.
Omenjena spoznanja, dajejo pomemben doprinos k razumevanju odnosa med strukturo in delovanjem pri načrtovanju novih inhibitorjev encimov, udeleženih v biosintezi bakterijske celične stene.

DESIGN, SYNTHESIS AND EVALUATION OF SULFONOHYDRAZIDE INHIBITORS OF Mur LIGASES

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Due to increasing resistance to antibacterial agents the design and synthesis of new antibacterial agents is becoming a new focal point of the field. Among the most validated targets for new antibacterial compounds are enzymes which are a part of peptidoglycan biosynthetic pathway constituting an essential part of the bacterial cell wall and thus any anomaly in this macromolecule can lead to cell lysis. Some of the initial stages of the peptidoglycan biosynthesis are catalyzed by enzymes from the Mur ligase family (MurC, MurD, MurE and MurF), which were chosen as targets in our research.
We evaluated a hypothesis stating that sulfonohydrazides could be designed to inhibit Mur ligases. Various structurally diverse new sulfonohydrazide inhibitors of Mur ligases were designed, synthesized and biochemically evaluated using modern approaches in medicinal chemistry. Initially, we focused mainly on MurC enzyme for which only few inhibitors had been previously known. However, during our continuous research inhibitors of other Mur ligases were also designed.
One of the approaches was to use benzylidenerodanines as lead compounds which are well-documented MurC inhibitors. Leads were then further modified on the thioxothiazolidin-4-one ring which was first replaced by structurally similar non-cyclic sulfonohydrazone moiety. All other parts of molecule were then modified in an attempt to increase the inhibitory activity on MurC. p-Chlorobenzil substituent in the lead compound was thus replaced by various arylsulfonates, arylamides, and arylesters (**Fig. 1**). We have also acquired the right to the Slovenian patent by the The Slovenian Intellectual Property Office. However, despite the manifested inhibitory activity in the micromolar region it has been afterwards discovered that this inhibitory activity was most probably the result of a nonspecific promiscuous binding activity.

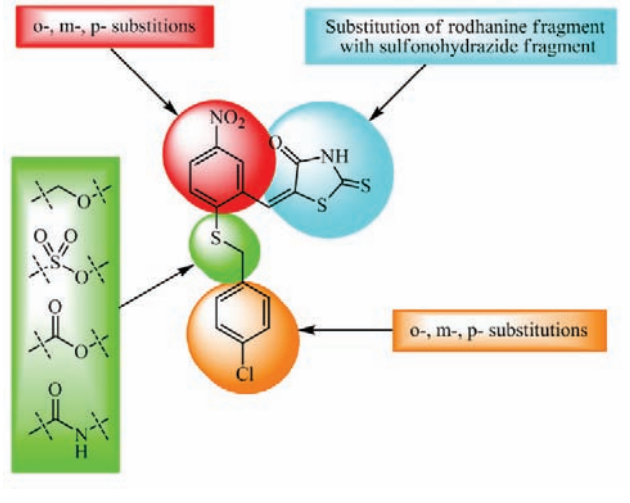
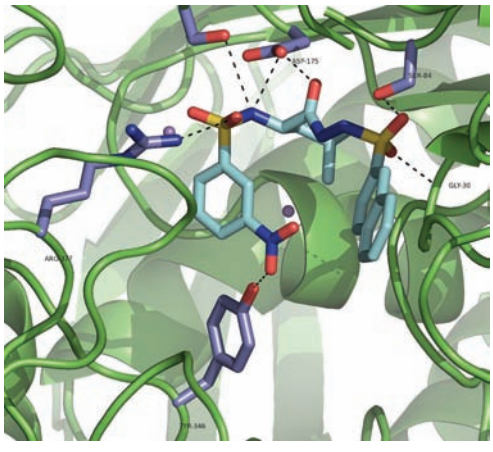
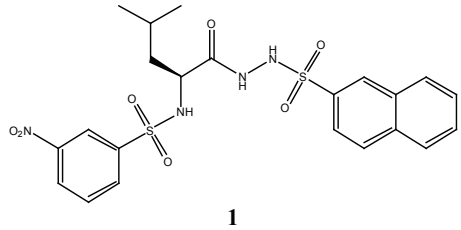


Figure 1: Design and structural changes of MurC inhibitors using benzylidenerodanines as lead compounds

On a parallel line a more rational design based on the structure of the enzyme's active site to new MurC inhibitors was attempted. Using published X-ray crystal structures of MurC complexed with the reaction product, computer based virtual screening and de novo design we prepared new sulfonohydrazide moiety based inhibitors, which, according to our model, were designed to bind into diphosphate binding pocket with sulfonohydrazide moiety. Basic structural requirements for good inhibitory activity against MurC using critical evaluation of biochemical testing results were depicted. The initial hits with leucine as a building block were having the inhibitory activity in the middle micromolar range and were used in further studies.

One of the first inhibitors with IC_{50} of 240 μM was also found to posses weak antibacterial activity with MIC of 128 μg/mL against *S. aureus* ().





1

RA(MurC, 250 μM) = 49%
MIC (*S. aureus*) = 128 μg/mL

Figure 2: Proposed binding mode of sulfonohydrazide inhibitor 1 into active site of MurC.

Further modifications were directed towards increased solubility of compounds as well as towards their increased inhibitory activity. Leucine was therefore replaced by threonine and in some compounds sulfonohydrazide was also replaced by hydrazide. With the aim of increasing the number of H-bonds between the compounds and the enzyme, differently substituted arenes were used. Rigidization of compounds where proline was used instead of leucine was also one of the attempts in increasing the potency. However, despite promising docking results no compound was found active.

In a search for new compounds with the potential to inhibit MurB, MurC, MurD and MurE, we also designed a series of compounds which consisted of peptide mimetic, diphosphate mimetic and phenyl substitute for sugar residue, which orients both mimetics towards the proper conformation and to some level restricts the molecule's degrees of freedom. Sulfonylcarbamates, sulfonamidothreonine and sulfonamidothreoninohydrazide were used as pyrophosphate mimetics. The hydroxamic acid functionality, a well-known metal complex forming group, was also introduced into some of the mimetics in order to form strong interactions with the Mg^{2+} in the enzymes' active sites. This series of compounds was tested and one was found to be active against MurC in middle μM range.

With the procedures described above we managed to synthesize several inhibitors with their inhibitory activities in the middle μM range.

In the course of our scientific research we found sulfonohydrazide to be a suitable fragment in the design of novel inhibitors of Mur ligases. Using structure-based design the compounds were efficiently docked into the vicinity of diphosphate binding pocket. Furthermore, using eHITS we were able to predict inhibitory activity with over 50% accuracy and therefore this docking software proved to be a suitable tool for the design of new inhibitors. According to the results of the eHITS and of biochemical testing it was concluded that sulfonohydrazide fragment could be replaced with structurally similar hydrazide fragment, however, a risk of lower solubility of designed compounds was present. In addition, leucine was found to be the most suitable building block for the inhibitory activity. It has also been established that both phenyl fragments enable several modifications which can be used to tune up the inhibitory activity towards MurC as well as MurD.

These important discoveries, which have been made in the dissertation, contribute significantly to the understanding of the structure activity relationship in the design of novel inhibitors of Mur ligases, which participate in bacterial cell wall biosynthesis.

Dr. Andreja Kovač

Andreja Kovač je asistentka na katedri za farmacevtsko kemijo fakultete za farmacijo, ki v prostem času najraje potuje v oddaljene kraje, kjer spoznava nove kulture in ljudi.

Bi lahko na kratko opisali svojo raziskovalno pot? Ste že od malega v sebi čutili raziskovalni duh?
Že v osnovni šoli sem se začela navduševati za kemijo, hodila k dodatnemu pouku in na različna tekmovanja. To je zagotovo pripomoglo k temu, da sem se po končani gimnaziji odločila za študij farmacije. Svoj raziskovalni duh sem takrat hranila s preučevanjem protitumornega delovanja spojin na osnovi talidomida, za kar sem prejela tudi Krkino nagrado.

Kaj vas navdihuje na vaši raziskovalni poti?
Odkrivanje novega mi predstavlja glavni navdih pri raziskovalnem delu, poleg tega ga iščem tudi v preprostih stvareh, kot so sprehodi v naravi, zanimive knjige ali dobri filmi.

Na kaj ste se osredotočili v svoji doktorski nalogi in kakšni so vaši zaključki?
V doktorskem delu sem se ukvarjala z iskanjem novih inhibitorjev encimov, ligaz Mur in D-alanin:D-alanin ligaze, ki sodelujejo pri sintezi bakterijske stene. Na ta način namreč lahko preprečimo rast in razmnoževanje bakterij. Pri iskanju inhibitorjev smo uporabljali dva različna pristopa, dobili smo nekaj strukturno novih inhibitorjev omenjenih encimov, ki so poleg delovanja na encime imeli tudi protibakterijsko delovanje. Moje celotno raziskovalno delo je potekalo v okviru evropskega projekta INTAFAR, zaradi česar sem imela tudi možnost več mesecev preživeti v laboratorijih v tujini, in sicer v Parizu in Leedsu.

Kako poteka vaš delovni dan? Kako usklajujete poklicno in zasebno življenje?
Družine še nimam, zato se lahko v celoti posvečam raziskovalnemu delu. Kot asistentka na Katedri za farmacevtsko kemijo fakultete za farmacijo pa sodelujem tudi pri pedagoškem procesu.

Kako bi se opisali, katere so tiste vrednote, ki jim sledite v življenju?
Sem pozitivna oseba, ki ima rada izzive. V življenju sledim načelu, da je vsaka izkušnja za nekaj dobra.

Kako najraje preživljate svoj prosti čas?
Moja največja hobija sta potovanja in potapljanje. Če je le mogoče, si vsako leto privoščim daljše potovanje v oddaljene kraje, kjer spoznavam nove kulture, pokrajino, ljudi in svet pod vodo. Prosti čas namenjam tudi športu, predvsem skvošu in plesu, zelo rada pa se družim tudi s prijatelji.

ODKRIVANJE NOVIH INHIBITORJEV BAKTERIJSKIH LIGAZ Z VIRTUALNIM REŠETANJEM IN SINTEZO SUBSTRATNIH ANALOGOV

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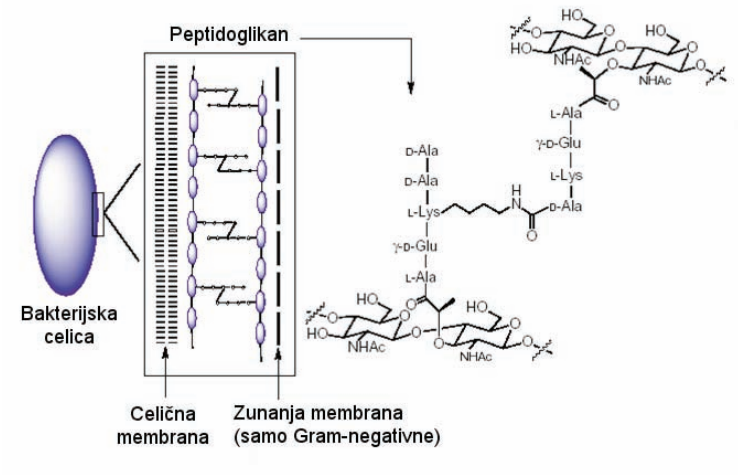
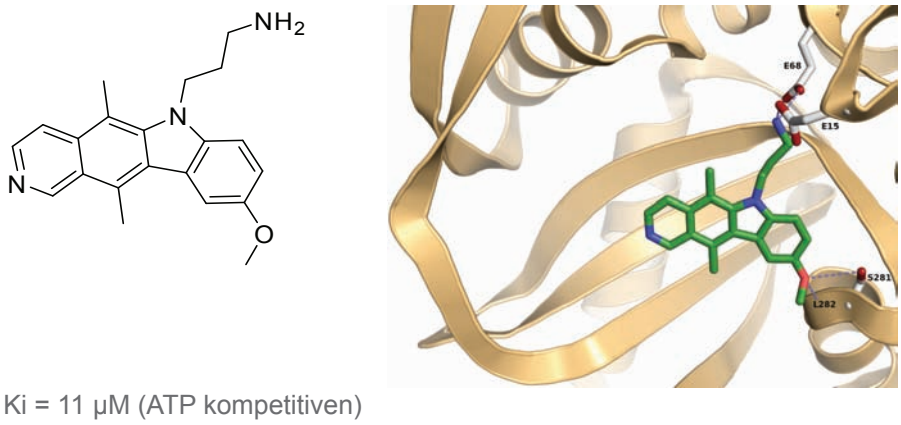
Pojav multi-rezistentnih sevov patogenih bakterij (kot so npr. *S. aureus, E. faecium* in *P. aeruginosa*), ki so odporne na več protimikrobnih učinkovin hkrati (MDR = Multidrug resistant bacteria), predstavlja resen problem v sodobnem zdravstvu. Iskanje novih tarč in sinteza protibakterijskih zdravilnih učinkovin z delovanjem na rezistentne bakterije je zato neizogibno v bitki zoper infekcijske bolezni (1).
Ena od pomembnejših tarčnih mest za razvoj novih protibakterijskih učinkovin je še vedno bakterijski peptidoglikan, ki daje bakterijskim celicam obliko in jih varuje pred visokim osmotskim pritiskom. Sestavljen je iz mreže polisaharidnih verig, ki so prečno povezane s kratkimi polipeptidi. Vpletanje v njegovo sintezo ali strukturo vodi do lize celic in posledično do propada bakterij. Biosinteza peptidoglikana je kompleksen proces, ki vključuje okrog 20 reakcij in poteka v citoplazmi, na notranji ter na zunanji strani celične membrane. Pri tem sodelujejo številni encimi, ki predstavljajo potencialna tarčna mesta za razvoj novih protimikrobnih učinkovin. V zadnjem desetletju je vse več raziskav usmerjenih v znotrajcelične citoplazemske faze biosinteze peptidoglikana, med katerimi zavzemajo pomembno mesto Mur ligaze. Gre za štiri od ATP odvisne ligaze (MurC-MurF), ki katalizirajo zaporedno pripenjanje aminokislinskih ostankov na D-laktoilni del peptidoglikanskega prekurzorja UDP-*N*-acetilmuraminske kisline. Poleg Mur ligaz sodelujejo pri sintezi peptidoglikana še številni drugi pomembni encimi, med njimi tudi D-alanin:D-alanin ligaza (Ddl), ki iz dveh D-alaninov naredi D-Ala-D-Ala dipeptid, ki je substrat za MurF (2).



Slika 1: *Zgradba bakterijske celične stene*

V sklopu raziskav doktorske naloge smo se osredotočili na encime MurC-F in DdlB. Poleg reakcijskih mehanizmov in specifičnosti teh encimov so v literaturi znane še tridimenzionalne strukture apoencimov, oziroma kompleksov encimov s substrati in produkti reakcije, kar nam omogoča racionalno načrtovanje novih zaviralcev Mur ligaz s potencialnim protibakterijskim učinkom. V okviru raziskovalnega dela smo najprej vpeljali v naše laboratorije in *vitro test* z malahitno zelenim za določanje encimske aktivnosti. Test temelji na osnovi določanja fosfata, ki se sprosti pri encimsko katalizirani reakciji in nam omogoča ovrednotenje inhibitorne aktivnosti potencialnih inhibitorjev Mur ligaz in Ddl. Iskanja inhibitorjev smo se najprej lotili z virtualnim rešetanjem NCI-jeve (National Cancer Institute, Ameriški nacionalni inštitut za raziskovanje raka) banke spojin in z naključnim rešetanjem banke spojin Fakultete za kemijo. Pri virtualnem rešetanju smo uporabili dostopne kristalne strukture encimov MurD (*E.coli*), MurF (*S. pneumonie*) in DdlB (*E. coli*) ter računalniške programe za virtualno rešetanje visoke zmogljivosti eHits 6.0 in AutoDock 4.0, ki nam s svojimi cenilnimi funkcijami

omogočijo, da *in vitro* ovrednotimo le manjšo serijo tistih spojin, ki jim program napove najmočnejšo vezavo. Dobili smo nekaj strukturno povsem novih inhibitorjev DdlB in MurD z IC50 v nizkem mikromolarnem območju. Nekateri so poleg inihibitornega imeli tudi protibakterijsko delovanje. S preučevanjem encimke kinetike smo ugotovili, da je večina DdlB inhibitorjev kompetitivna napram ATP (3, 4).



Ki = 11 μM (ATP kompetitiven)

MIC vrednosti

E. coli 1411	32 μg/ml
E. coli SM 1411	8 μg/ml
S. aureus 8325-4	32 μg/ml

Slika 2: *Najmočnejši inhibitor DdlB, ki smo ga dobili z virtualnim rešetanjem NCI-jeve banke spojin s pomočjo programa AutoDock 4.0.*

Nove inhibitorje DdlB diazendikaboksamidnega tipa pa smo odkrili z naključnim rešetanjem domače banke spojin (5). Z uporabo peptidomimetičnega pristopa in poznavanja reakcijskih mehanizmov, ki pri ligazah MurC-F in DdlB poteka preko tetraedričnega prehodnega stanja, pa smo načrtovali in sintetizirali nove spojine s hidroksimetilkarbonilnim skeletom.

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DISCOVERY OF NEW INHIBITORS OF BACTERIAL LIGASES BY VIRTUAL SCREENING AND SYNTHESIS OF SUBSTRATE ANALOGUES

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Appearance of multidrug resistant strains of pathogenic bacteria (MDR = Multidrug resistant bacteria) is a serious medical issue in modern healthcare. A battle against infectious diseases caused by drug-resistant bacteria is therefore inevitable and involves searching for new bacterial targets and the development of new antimicrobial agents (1).
Peptidoglycan, an essential component of the bacterial cell wall which provides the structural integrity necessary for bacterial cells to resist internal osmotic pressure, remains one of essential and well validated targets for antibacterial therapy. Net-like structure of peptidoglycan consists of polysaccharide chains interconnected with short polypeptides. Interference with the synthesis or structure of peptidoglycan results in cell lysis and eventually bacterial death. Biosynthesis of peptidoglycan is a complex process which involves around 20 reactions catalyzed by different enzymes which all represent targets for development of new antibacterial agents. It occurs in the cythoplasm, on the inner side of bacterial cell membrane and on the outer cell membrane. During the last decade, more and more efforts have been devoted to early (cytoplasmic) stages of peptidoglycan biosynthesis, where Mur ligases play an important role. Mur ligases, which consist of four enzymes (MurC-F), assemble the final intracellular peptidoglycan precursor UDP-MurNAc-pentapeptide by successive addition of L-Ala, D-Glu, m-A_2pm or L-Lys, and D-Ala-D-Ala to D-lactoyl part of peptidoglycan precursor UDP-N-acetylmuramic acid. Beside Mur ligases also other important enzymes are involved in the synthesis of peptidoglycan. One of them is D-alanine:D-alanine (Ddl), which is responsible for supplying the MurF substrate, D-alanyl-D-alanine (2).

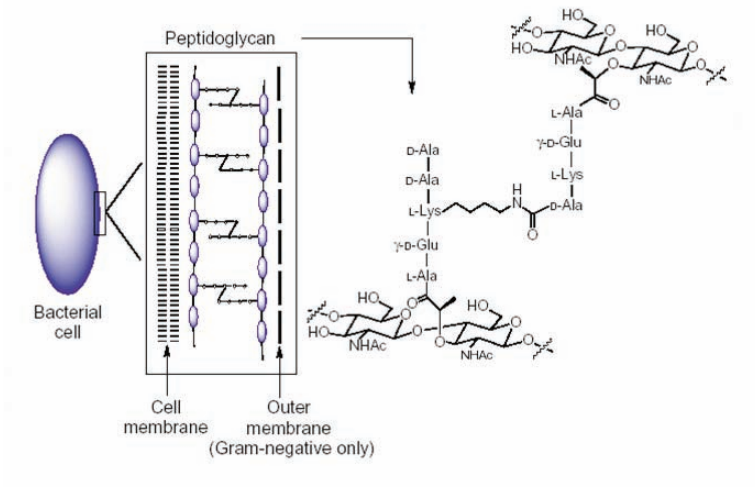
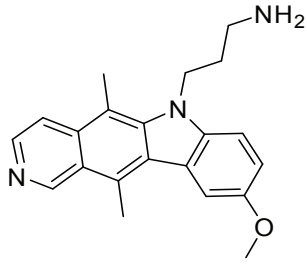
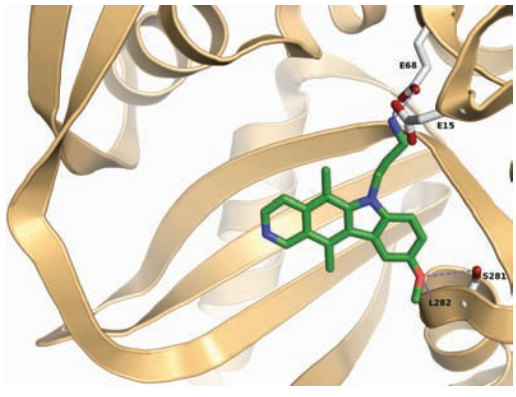


Figure 1: The structure of bacterial cell wall.

In thesis we focused our attention to MurC-F and DdlB enzymes. Their reaction mechanisms, specificity and tridimensional structures are known from the literature and this enabled us to design new inhibitors with potential antibacterial activity. First, we set up in our laboratories the Malachite green *in vitro* assay for the determination of enzyme activity. The assay is based on the determination of the free phosphate released during the enzyme-catalyzed reaction and allows us to evaluate the inhibitory activity of potential inhibitors. Virtual screenig was performed on available crystal structures of MurD (from *E.coli*), MurF (from *S. pneumonie*) and DdlB (from *E. coli*) enzymes using computational tools for structure-based high-throughput virtual screening eHiTS 6.0 and AutoDock 4.0, and the NCI Diversity Set (National Cancer Institute bank of compounds). Only the high-ranked compounds with predicted strong binding affinity were then evaluated *in vitro*. New low-micromolar inhibitors

of DdlB and MurD with novel scaffolds were discovered. Some inhibitors also had promising antibacterial activities against Gram-positive and Gram-negative bacteria. Kinetic experiments revealed that most of the DdlB inhibitors discovered are ATP competitive (3, 4).





Ki = 11 μM (ATP competitive)

MIC values

E. coli 1411	32 μg/ml
E. coli SM 1411	8 μg/ml
S. aureus 8325-4	32 μg/ml

Figure 2: *The most potent inhibitor of DdlB obtained by virtual screening of the NCI Diversity Set using AutoDock 4.0.*

In addition, diazenedicarboxamide inhibitors of DdlB were discovered with screening of in-house bank of compounds from the Faculty of Chemistry and Chemical Technology (5). As Mur ligaes and DdlB share the similar catalytic mechanism which proceeds via the tetrahedral transition-state, compounds with the hydroxymethylcarbonyl fragment were designed and synthesized using the peptidomimetic approach.

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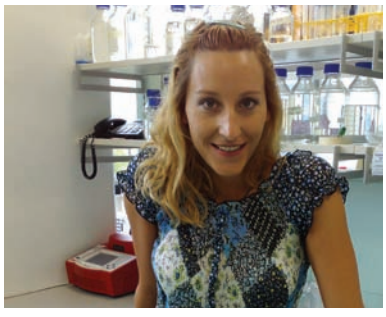

Dr. Urška Lešnik

Urška Lešnik je zaposlena kot raziskovalka v biotehnološkem podjetju Acies Bio in je strastna zagovornica zdrave mere humorja v vsaki situaciji. V prostem času rada igra tenis, teče in jadra ter preživlja lepe trenutke v krogu svoje družine in prijateljev.

Bi lahko na kratko opisali svojo raziskovalno pot? Ste že od malega v sebi čutili raziskovalni duh?
Že od prvih ur biologije na Prvi gimnaziji v Mariboru, ko smo se pogovarjali o začetku življenja, DNA, proteinih, mikroorganizmih in fermentacijah, sem vedela, da me zanima mikro svet. Po končanem dodiplomskem študiju mikrobiologije na biotehniški fakulteti sem v okviru podiplomskega študija Bioloških in biotehniških znanosti, smer Biotehnologija, zaključila doktorat. Njegova tematika se mi zdi zanimiva tako z vidika bazičnega raziskovanja kot tudi z aplikativnega vidika.

Kaj vas navdihuje na vaši raziskovalni poti?
Pri mojih raziskovalnih prizadevanjih me najbolj motivira to, da imajo rezultati raziskav neki širši smisel. Torej, da ne gre le za lastno izobraževanje in izpopolnjevanje, ampak da sodelujem pri razvoju nečesa, kar bo potencialno uporabno in koristno tudi za širšo javnost.

Na kaj ste se osredotočili v svoji doktorski nalogi in kakšni so vaši zaključki?
V okviru doktorskega študija sem klonirala in okarakterizirala skupino genov za biosintezo tetraciklinskega antibiotika kelokardin. Razumevanje genetike klonirane skupine genov in uporaba metod biosinteznega inženiringa nam omogočata konstrukcijo novih biosinteznih derivatov kelokardina, ki predstavljajo potencialne kandidate za medicinsko uporabo, uporabili pa jih bomo tudi kot matrice za nadaljnje polsintezne kemijske pristope. Razvoj novih kelokardinskih analogov bomo usmerili v dizajniranje spojin z izboljšano protibakterijsko učinkovitostjo, potencialno pa tudi s protivnetno, protinevrodegenerativno in protirakasto aktivnostjo.

Kako poteka vaš delovni dan? Kako usklajujete poklicno in zasbno življenje?
Moji dnevi potekajo zelo intenzivno in veseli me, da sem obkrožena z ljudmi, ki razumejo, dovoljujejo in omogočajo, da med poklicnim in zasebnim življenjem nimam strogo potegnjene ločnice.

Kako bi se opisali, katere so tiste vrednote, ki jim sledite v življenju?
Sem zelo odprt, spontan in družaben človek. Imam zaupanje v ljudi in verjamem, da stisk roke nekaj velja. Kljub temu da se resno spoprijemam s problemi, sem tudi strasten zagovornik zdrave mere humorja v vsaki situaciji.

Kako najraje preživljate svoj prosti čas?
Rada počnem stvari, ki mi povrnejo psihofizično ravnovesje, za zbistritev misli tečem, za dvig elana igram tenis, za pomiritev jadram, za dušo se odpravim na krajša ali daljša popotovanja. Za čisto srečo pa najdem čas za družino in prijatelje.

KLONIRANJE GENOV ZA BIOSINTEZO TETRACIKLINOV IZ IZBRANIH BAKTERIJ REDU *ACTINOMYCETALES*

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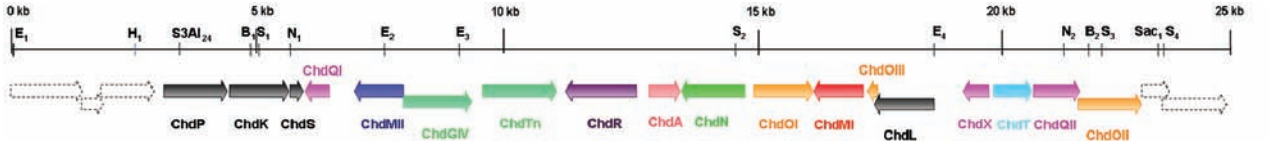
Tetraciklini predstavljajo veliko skupino medicinsko pomembnih antibiotikov z značilno strukturo štirih linearno povezanih šestčlenskih obročev. Naravni tetraciklini, kot so tetraciklin, klortetraciklin, oksitetraciklin in demetilklortetraciklin, so sintetizirani s pomočjo t.i. poliketid sintaz (PKS) tipa II. Klasični tetraciklini preprečujejo sintezo proteinov pri bakterijah, zato so bili kot prvi antibiotiki širokega spektra pogosto neupravičeno uporabljani proti številnim mikrobnim infekcijam, kar je pripomoglo k razširitvi bakterijske rezistence. Kljub temu obstaja manjša skupina tetraciklinskih analogov, med katere spada tudi tetraciklinski antibiotik kelokardin, ki ne delujejo na bakterijski ribosom, ampak delujejo bakteriocidno tudi na sicer tetraciklin-rezistentne seve (1). Poleg tega imajo nekateri tetraciklinski analogi tudi potencial za zdravljenje neinfektivnih bolezni kot so nevrodegenerativne bolezni (Parkinsonova, Huntingtonova, multipla skleroza), arteroskleroza, osteoporoza ter revmatoidna in rakasta obolenja, kar občutno poveča terapevtski potencial tetraciklinskih molekul.

Namen našega dela je bil klonirati, sekvencirati in okarakterizirati novo skupino genov za biosintezo tetraciklinskega antibiotika kelokardina. Pripravili smo kozmidno genomsko knjižico producenta kelokardina, *Amycolatopsis sulphurea*. S pomočjo sonde, ki je kodirala gen iz PKS tipa II (KSα), verižne reakcije s polimerazo in restrikcijskih analiz smo preiskali knjižnico in sekvencirali izbrani kozmid VIIC4. Predvidene odprte bralne okvirje smo določili in analizirali s pomočjo programa FramePlot 4.0 beta. Domnevne funkcije genov smo določili s pomočjo analize BLASTx, podprte z orodjem za iskanje ohranjenih proteinskih domen (Conserved Domain Search na strani NCBI). Klonirano nukleotidno zaporedje vsebuje 18 genov, značilnih za skupine genov PKS tipa II (Slika 1).

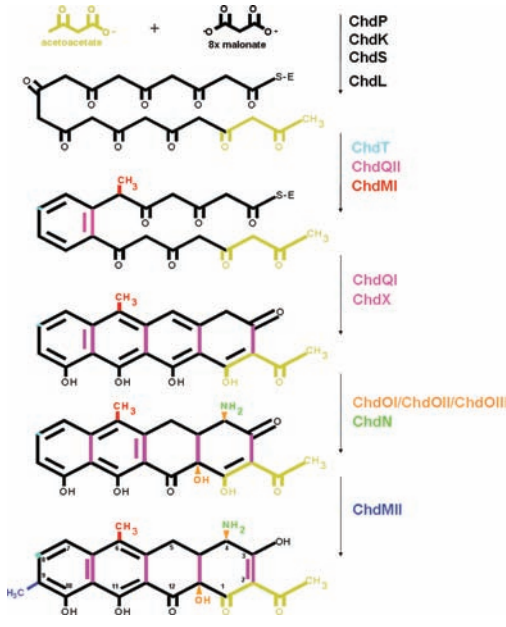


Slika 1: Grafičen prikaz velikosti in organizacije genov v skupini genov za biosintezo kelokardina. Neprekinjena črta nad geni označuje red velikosti, na njej pa so s kraticami označena mesta restrikcijskih encimov, in sicer: E = EcoRI, H = HindIII, S3AI = Sau3AI, B= BglII, S = SphI, N = NcoI in Sac = SacI.

V skladu s kemijsko strukturo kelokardina skupina genov zajema gene za minimalni PKS (KSα, KSβ and ACP), tri gene, vpletene v procese ciklizacije/aromatizacije, dve metiltransferazi, eno aminotransferazo, tri oksigenaze, ketoreduktazo, acil-CoA ligazo, eksporter in transkripcijski regulator, poleg teh pa še gena za glikoziltransferazo in transpozazo (Slika 1).

Iz dobljenega zaporedja DNA smo predvideli biosintezno pot kelokardina. Biosinteza osnovne poliketidne verige poteka s pomočjo t.i. minimalnega PKS (ChdP, ChdK in ChdS), ki katalizira ponavljajoče se dekarboksilativne kondenzacijske reakcije malonata, dokler veriga ne doseže želene dolžine. Keto skupina na 9. C-atomu poliketidne verige se najverjetneje reducira s pomočjo predvidene ketoreduktaze ChdT, domnevna metiltransferaza ChdMI pa predvidoma metilira 6. C-atom v verigi. Dve ciklazi/aromatazi (ChdQI in ChdQII) predvidoma ciklizirata/aromatizirata obroča D in B, medtem ko se drugi obroč (C) najverjetneje zapre sam, obroč A pa najverjetneje s pomočjo ciklaze ChdX. Predvidena aminotransferaza ChdN na 4. C-atom doda amino skupino, metiltransferaza ChdMII pa najverjetneje metilira 10. C-atom poliketida (Slika 2).

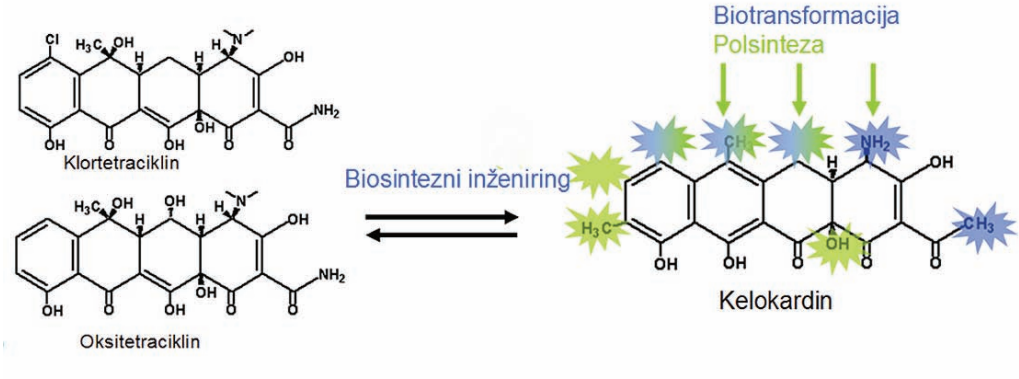
Ime gena	Funkcija gena	Dolžina (ak)
chdP	ketosintaza - alfa	433
chdK	ketosintaza - beta	409
chdS	acil prenašalni protein	88
chdQI	ciklaza/aromataza	302
chdMII	metiltransferaza	335
chdGIV	glikoziltransferaza	399
chdTn	transpozaza	505
chdR	rezistenca (eksporter)	481
chdA	transkripcijski regulator	190
chdN	aminotransferaza	433
chdOI	oksigenaza	404
chdMI	metiltransferaza	341
chdOIII	oksigenaza	69
chdL	acil-CoA ligaza	?
chdX	ciklaza	151
chdT	ketoreduktaza	263
chdQII	ciklaza/aromataza	315
chdOII	oksigenaza	430



Slika 2: Predvidene funkcije genov iz skupine genov za kelokardin in shema biosinteze kelokardina.

V skupini genov za kelokardin najdemo le en gen za rezistenco (eksporter ChdR), kar glede na to, da kelokardin ne deluje na bakterijski ribosom, ne preseneča. V neposredni bližini gena za rezistenco (ChdR) se nahaja edini regulatorni element v skupini genov za kelokardin, ChdA. Ob primerjavi regulatornih mehanizmov iz skupine genov za biosintezo kelokardina z regulatornimi geni iz skupine genov za oksitetraciklin in klortetraciklin (2) smo ugotovili, da je ChdA homolog regulatorjev CtcR in OtrR iz klortetraciklina oz. oksitetraciklina, in da najverjetneje regulira izražanje gena za eksporter.

Razumevanje genetike klonirane skupine genov in uporaba metod biosinteznega inženiringa nam omogočata konstrukcijo tetraciklinskih analogov s širšim spektrom bioloških aktivnosti. Novi biosintezni derivati kelokardina lahko predstavljajo neposredne potencialne kandidate za medicinsko uporabo, lahko pa služijo kot matrice za nadaljnje semi-sintezne kemijske pristope (Slika 3).



Slika 3: Potencialna mesta za biosintezne, biotransformacijske in kemijske pristope modifikacije kelokardina

Novi tetraciklinski analogi, nastali na podlagi kelokardina, lahko imajo izboljšano protibakterijsko učinkovitost, ali pa služijo celo kot protivnetni, antinevrodegenerativni in protirakasti dejavniki, zato smo rezultate, ki so nastali v okviru doktorske disertacije, tudi patentno zaščitili.

1) Copra I. 1994. Antimicrob Agents Chemother, 38(4):637-640.
2) Lešnik *et al*. 2009. *Food Technol. Biotechnol.*, *47(3)*:323–330.

CLONING OF GENES FOR THE BIOSYNTHESIS OF TETRACYCLINES FROM SELECTED BACTERIA OF *ACTINOMYCETALES* GENUS

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Supervisor: Hrvoje Petković
Co-supervisor: Lea Pogačnik
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Tetracyclines are a large group of medically-important antibiotics with a common basic structure of four linearly fused six-membered rings. Natural tetracyclines such as tetracycline, chlortetracycline, oxytetracycline and demethylchlortetracycline are synthesized by the so-called Type-II polyketide synthases (PKS). Classical tetracyclines interfere with protein synthesis in susceptible bacteria. They were the first broad-spectrum antibiotics, often used indiscriminately against a variety of microorganisms, causing a widespread microbial resistance. However, a small group of tetracycline analogs, including chelocardin, has been identified that are not targeting bacterial ribosomes and are exhibiting bactericidal activity even against the tetracycline-resistant strains (1). Moreover, some tetracycline analogs have promising activity for the treatment of non-infectious diseases such as neurodegenerative disorders (Parkinson's, Huntington's, multiple sclerosis), artherosclerosis, osteoporosis, rheumatoide diseases, tumor invasion and metastasis, thus significantly expanding the therapeutic potential of tetracycline-related molecules.

The objective of our research was to clone, sequence and characterize a novel gene cluster encoding an unusual tetracycline antibiotic chelocardin. A genomic cosmid library of a non-streptomyces type II polyketide producer *Amycolatopsis sulphurea* was created and screened with the type II PKS probe (KSα), PCRs and restriction analysis. A selected cosmid VIIC4 was sequenced. The open reading frames were analyzed with the FramePlot 4.0 beta program and putative gene functions were assigned according to the BLASTx analysis, supported with the Conserved Domain searches at the NCBI site. The cloned nucleic acid contained 18 genes typical of a Type-II polyketide cluster (Figure 1).

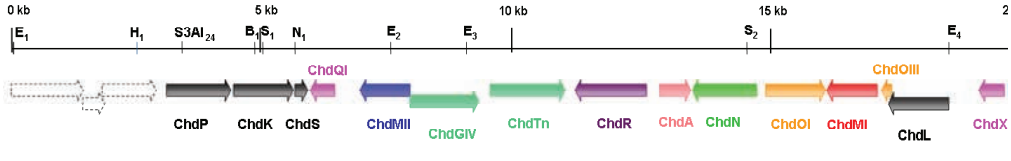

Figure 1: Graphical representation of gene size and organization in the chelocardin biosynthesis gene cluster. Solid line above the genes marks the order of magnitude; above this line abbreviated names for restriction enzymes cleavage sites are marked: E = EcoRI, H = HindIII, S3AI = Sau3AI, B= BglII, S = SphI, N = NcoI and Sac = SacI.

Consistently with the chemical structure of chelocardin, the cluster encodes genes for a typical minimal PKS (KSα, KSβ and ACP), three genes involved in the cyclisation/aromatisation process, two methyltransferases, one aminotransferase, three oxygenases, a ketoreductase, an acyl-CoA ligase, a drug resistance transporter and a transcriptional regulator, as well as a glycosyltransferase and a transposase (Figure 2).

From the DNA sequence obtained, the biosynthetic pathway was proposed. The basic chelocardin polyketide chain is assembled by the action of minimal PKS (ChdP, ChdK in ChdS) which catalyses repetitive decarboxylation reactions of malonate until the chain reaches its final length. The keto group on the C9 position is reduced by the putative ketoreductase ChdT, while a putative methyltransferase ChdMI presumably methylates the C6 position of the nascent chain. Two putative cyclases/aromatases (ChdQI and ChdQII) presumably catalyze the cyclization/aromatization reaction of the rings D and B, while the ring C most probably undergoes cyclization spontaneously. Cyclization of the ring A is most likely catalyzed by the cyclase ChdX. Presumably, the putative aminotransferase ChdN attaches the amino group to the C4 position of the polyketide before the putative methyltransferase ChdMII methylates the C10 position in the final step of biosynthesis (Figure 2).

Gene name	Gene function	Length (aa)
chdP	ketosynthase- alpha	433
chdK	ketosynthase – beta	409
chdS	acyl carrier protein	88
chdQI	cyclase/aromatase	302
chdMII	methyltransferase	335
chdGIV	glycosyltransferase	399
chdTn	transposase	505
chdR	resistence (exporter)	481
chdA	transcriptional regulator	190
chdN	aminotransferase	433
chdOI	oxygenase	404
chdMI	methyltransferase	341
chdOIII	oxygenase	69
chdL	acyl-CoA ligase	?
chdX	cyclase	151
chdT	ketoreductase	263
chdQII	cyclase/aromatase	315
chdOII	oxygenase	430

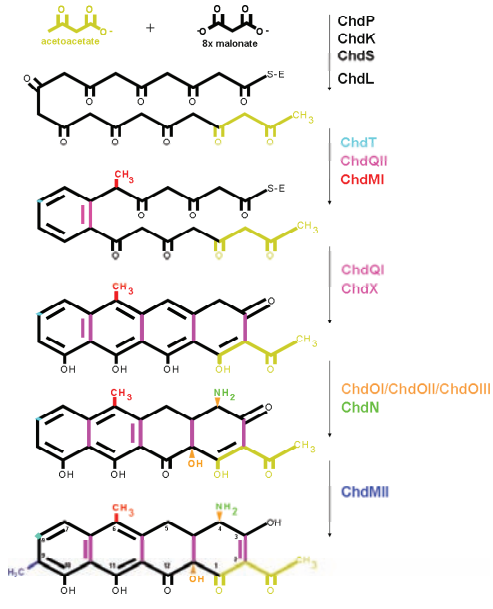

Figure 2: Putative gene functions of the chelocardin genes and the proposed chelocardin biosynthesis pathway

Within the chelocardin gene cluster only one resistance gene (the exporter, ChdR) can be identified, which is consistent with the findings that chelocardin does not interfere with the ribosome. Next to ChdR, the only regulatory element of the chelocardin gene cluster, ChdA, can be found. A comparison of regulatory mechanisms present in the chelocardin gene cluster with regulatory mechanisms of the oxytetracycline and chlortetracycline gene clusters shows that ChdA is homologous to regulators CtcR and OtrR from the chlortetracycline and oxytetracycline clusters, respectively (2). These homologues most likely encode regulators of exporters.

Through understanding of the genetics of the cloned cluster, modified tetracycline analogs with alternative biological activities can be created by applying biosynthetic engineering methods. Such new chelocardin-derivatives are potential candidates for medical use themselves, or they can also serve as new matrices for further semi-synthetic chemical approaches (Figure 3).

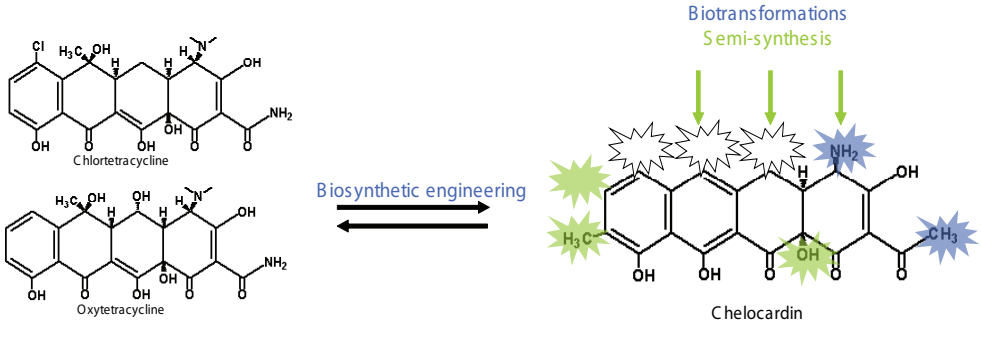

Figure 3: Possible sites for semi-synthetic and biosynthetic transformations of chelocardin

The development of new chelocardin analogs will be aimed at constructing products with improved antibacterial and potential antiinflamatory, antineurodegenerative and anticancer activities, therefore a patent application on the subject of this thesis was filed.

1) Copra I. 1994. Antimicrob Agents Chemother, 38(4):637-640.
2) Lešnik *et al*. 2009. *Food Technol. Biotechnol., 47(3):323–330*.

Dr. Zvone Simončič

Zvone Simončič je vodja skupine razvojnih projektov v Krki in pravi, da ga najbolj motivira raziskovanje številnih vprašanj, s katerimi želi priti do jasnih odgovorov in novih ugotovitev.

Bi lahko na kratko opisali svojo raziskovalno pot? Ste že od malega v sebi čutili raziskovalni duh?
Že od nekdaj sem hotel na vprašanja najti odgovore in z »ne vem« večinoma nisem bil zadovoljen. Da me zanima predvsem naravoslovje, sem začutil v Gimnaziji Novo mesto pri urah kemije, fizike in matematike. V sodelovanju s Krko sem takrat izdelal raziskovalno nalogo in zanjo prejel Preglovo nagrado. Na fakulteti za Farmacijo pa sem kasneje prejel še Krkino nagrado in Prešernovo nagrado, ki sta bili rezultat raziskovalnega dela, ki se mu nisem hotel odpovedati.

Kaj vas navdihuje na vaši raziskovalni poti?
Zagotovo me najbolj navdihujeta iskanje jasnih odgovorov na jasna vprašanja in težnja po tem, da v raziskavi pridem do neke nove ugotovitve. Motivira me predvsem razrešitev neznanega, čeprav to včasih ni preprosto. Nikoli nisem bil rad obkrožen s preveč neznanimi stvarmi naenkrat.

Na kaj ste se osredotočili v svoji doktorski nalogi in kakšni so vaši zaključki?
V doktorski nalogi smo poizkusili redko uporabljano analizno metodo izotermne mikrokalorimetrije postaviti na zemljevid možnih analiznih metod, ki jih uporabljamo v raziskavah in razvoju zdravil. Uporabnost te metode smo potrdili predvsem na področju zgodnjih t. i. presejalnih stabilnostnih testov, ko na relativno enostaven in hiter način lahko razlikujemo med bolj in manj stabilnimi vzorci. Posledično lahko na ta način skrajšamo razvoj novega zdravila.

Kako bi se opisali, katere so tiste vrednote, ki jim sledite v življenju?
Neučakanost je ena od lastnosti, ki me spremlja že od otroštva, in jo skušam uporabiti kot orožje, da hitreje pridem do rešitev in želenega stanja. Pomembne vrednote, ki jim sledim, pa so tudi poštenost, družina in skrb za svoje zdravje.

Kako najraje preživljate svoj prosti čas?
Moji hobiji so povezani s športom oz. z vsemi oblikami dejavnega preživljanja časa, ki je še najbolje izkoriščen, če sem v družbi najbližjih. Poleg športa pa se rad ukvarjam tudi z glasbo in igranjem v pihalnem orkestru.

UPORABA MIKROKALORIMETRIJE PRI VREDNOTENJU STABILNOSTI ZDRAVILNIH UČINKOVIN IN FARMACEVTSKIH OBLIK NA PRIMERU NEKATERIH ZAVIRALCEV ANGIOTENZINSKE KONVERTAZE

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Stabilnostne študije so pomemben del razvoja vsake zdravilne učinkovine in farmacevtske oblike ter imajo zelo pomembno vlogo pri dokazovanju kakovosti, varnosti in učinkovitosti zdravila v registracijski dokumentaciji. V predformulacijski fazi ter v prvem delu formulacijske faze razvoja zdravil se raziskovalci na eni strani srečujejo z velikim številom potencialnih zdravilnih učinkovin in velikim, skoraj neomejenim številom potencialnih formulacij, v katere vgrajujejo zdravilne učinkovine, na drugi strani pa s pomanjkanjem časa, saj želijo novo zdravilo na trgu predstaviti v najkrajšem možnem času. V sklopu pričujočega dela smo se osredotočili na metodo izotermne mikrokalorimetrije, ki bi lahko zaradi svojih značilnosti doprinesla k hitrejšim in lažje izvedljivim meritvam stabilnostnih lastnosti zdravilnih učinkovin in farmacevtskih oblik iz skupine ACE inhibitorjev.

Izotermna mikrokalorimetrija je nespecifična termoanalizna metoda, ki se v omejenem obsegu že uporablja v farmacevtskem razvoju, vendar je njena uporaba v veliki meri omejena na zelo nadzorovane enokomponentne sisteme ter na kemijske reakcije, ki potekajo po predvidljivih poteh in s predvidljivimi kinetikami. Z rezultati naših raziskav na znanih predstavnikih ACE inhibitorjev in na potencialnem novem ACE inhibitorju se je mikrokalorimetrija izkazala za zelo primerno pri proučevanju stabilnosti teh zdravilnih učinkovin, predvsem v začetnih fazah razvoja zdravilnih učinkovin in farmacevtskih oblik. Pri izotermnih pogojih lahko relativno hitro napovemo določene stabilnostne lastnosti, v nekaterih primerih tudi v kvantitativnem obsegu, kar smo potrdili z vzporednimi študijami s specifičnimi metodami, kot je HPLC.

Z uporabo izotermne mikrokalorimetrije smo na primeru zdravilne učinkovine perindopril v raztopini uspeli kvantitativno ovrednotiti kemični razpad in doseči dobro ujemanje z rezultati specifične HPLC metode, ki je zahtevala predhoden razvoj in validacijo. S simulacijo razvoja novih zdravilnih učinkovin iz skupine ACE inhibitorjev smo sintetizirali novo potencialno zdravilno učinkovino in jo stabilnostno ovrednotili. V skupini zdravilnih učinkovin smo pri preizkušanju stabilnosti v raztopinah opazili in ovrednotili fizikalno spremembo, ki je bila posledica ločitve vodne in oljne faze. Opaženo fizikalno spremembo, ki je nastala zaradi kemične nestabilnosti te zdravilne učinkovine in posledično izločanja lipofilnega razgradnega produkta, smo uporabili za razvoj pristopa hitrega presejalnega preizkusa, ki ga v skupini ACE inhibitorjev lahko uporabimo za razlikovanje med bolj in manj stabilnimi zdravilnimi učinkovinami in farmacevtskimi oblikami na osnovi opazovanja energetskih sprememb.

Študije smo nadgradili s študijami zdravilne učinkovine enalapril maleat v trdnem in njenih farmacevtskih oblik. Znova smo potrdili uporabnost izotermne mikrokalorimetrije kot metode, ki lahko skrajša razvojno fazo novega zdravila. Na primeru dveh stabilnih farmacevtskih oblik, t.j. tablet, z zdravilno učinkovino enalapril maleat, ki se razlikujeta v kvantitativni sestavi, smo z meritvijo, ki je trajala le nekaj dni, uspeli pridobiti podatke o razlikah v stabilnosti obeh, za kar bi sicer po klasičnih pristopih potrebovali več mesecev oz. let rednega stabilnostnega preizkušanja.

S tem izvirnim pristopom smo metodo izotermne mikrokalorimetrije umestili v predformulacijski in formulacijski razvoj zdravil ACE inhibitorjev kot metodo, ki omogoča hitro in relativno enostavno spremljanje dogajanja v posameznih vzorcih ter tako omogoča razlikovanje med bolj in manj stabilnimi zdravilnimi učinkovinami in farmacevtskimi oblikami. V primerjavi z dosedanjo prakso lahko uporaba te metode bistveno skrajša čas v najzgodnejših fazah razvoja, ko je na mizah raziskovalcev veliko število vzorcev potencialnih zdravilnih učinkovin in farmacevtskih oblik.

THE USE OF MICROCALORIMETRY FOR STABILITY EVALUATION OF ACTIVE SUBSTANCES AND DRUG PRODUCTS CONTAINING SOME ANGIOTENSIN CONVERTASE INHIBITORS

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Stability studies are an integral part of the drug development program and have a very important role in the registration documentation for each individual pharmaceutical product and drug substance. There is almost an unlimited number of drug substance samples and drug product samples generated in preformulation and formulation studies, whereas the time available for drug development is limited. In our work we focused on the method of isothermal microcalorimetry, which could bring substantial advantages in performance and duration of these analyses. As a part of these studies drug substances from the group of ACE inhibitors were used.

Isothermal microcalorimetry is a non-specific thermo-analytical method that is in a limited extend already used in pharmaceutical development, especially for analysis of samples with simple degradation reactions and known kinetics. With results presented in our work we confirmed that isothermal microcalorimetry can provide valuable data also when used for drug substances and drug products with simultaneously running complex chemical reactions and physical changes. More and less stable drug substances or drug products from the group of ACE inhibitors can be distinguished with isothermal microcalorimetry. In some cases stability can be determined also in quantitative extend, which was confirmed using specific studies such as HPLC in parallel.

For drug substance perindopril in solutions with different pH we determined degradation rate constant using isothermal microcalorimetry and confirmed it with parallel HPLC study. We also simulated the development of new drug substance structurally belonging to the group of ACE inhibitors and determined its stability properties by using HPLC and isothermal microcalorimetry. One of the important findings regarding thermograms of ACE inhibitors was also a physical change that occurred as a consequence of chemical instability. Broad exothermic peak that was observed in majority of solution samples with pH 2.0 was attributed to phase separation of lipophilic diketopiperazine degradation product. We used this physical phenomenon for the development of a fast approach that can distinguish between more and less stable drug substances from the group of ACE inhibitors and drug products using these substances.

In addition to studies of ACE inhibitors in solutions we performed also stability studies of solid samples of enalapril maleat and its drug products using isothermal microcalorimetry. We again confirmed that this method can shorten the development work of a new drug product. We were able to determine the difference in stability properties of two drug products with different quantitative compositions in a very short time. When using ICH long term stability evaluation to distinguish between the stabilities of these formulations much longer studies would be needed.

Isothermal microcalorimetry was confirmed in preformulation and formulation phase of drug substance and drug product development as a method that enables the rapid and relatively simple determination of stability and thus, distinguishes between more and less stable samples. In comparison with current practice the use of this method can significantly shorten the time in the earliest stages of development.
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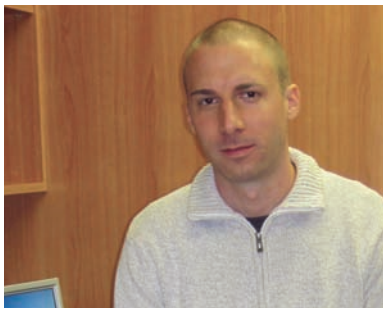

Dr. Uroš Uršič

Uroš Uršič je raziskovalec v operativnem razvoju proizvodnje Mengeš v Leku in je pragmatičen človek, ki trdno stoji na realnih tleh. Njegova življenjska filozofija je, naj vsak živi, kakor hoče.

Bi lahko na kratko opisali svojo raziskovalno pot? Ste že od malega v sebi čutili raziskovalni duh?
Po osnovni šoli v Kobaridu sem se vpisal v Gimnazijo Tolmin in po maturi prišel v Ljubljano, kjer sem bil več let djaven na fakulteti za kemijo in kemijsko tehnologijo, najprej z diplomskim in za tem doktorskim delom. Že od malih nog me je zanimalo naravoslovje, vendar se je moja raziskovalna pot uradno začela z diplomsko nalogo in nato nadaljevala z doktoratom.

Kaj vas navdihuje na vaši raziskovalni poti?
Najbolj zanimiv vidik raziskovalnega sveta je, da mora človek za dobro delo razmišljati nekoliko nekonvencionalno, kar ni vedno lahko, saj smo ljudje precej vpeti v neke standardne modele.

Na kaj ste se osredotočili v svoji doktorski nalogi in kakšni so vaši zaključki?
V svoji doktorski nalogi sem se ukvarjal s sintezo novih polifunkcionalnih reagentov propenoatnega tipa in pregledom možnosti, ki jih ti ponujajo za pripravo novih heterocikličnih spojin. Gre za širjenje znanja o tem, kaj nam ponuja narava.

Kako bi se opisali, katere so tiste vrednote, ki jim sledite v življenju?
Velikokrat so mi rekli, da sem po naravi pesimističen realist, vendar se s tem ne strinjam. Jaz bi se opisal za pragmatičnega človeka, ki stoji trdno na tleh. Moja osebna filozofija je čisto enostavna: vsak naj živi, kakor hoče, samo da pri tem ne sme s svojimi dejanji prizadeti drugih. Seveda je to mejo težko definirati, lahko pa rečem, da je pri meni dokaj liberalna.

Kako poteka vaš delovni dan? Kako usklajujete poklicno in zasebno življenje?
Moj dan se začne s tem, ko me ob šestih zbudi budilka, tako da sem malo pred sedmo v službi. Naš oddelek tako že zgodaj začenja z delom, kar je po svoje zelo dobrodošlo, saj smo zaradi tega nekoliko prej doma. Popoldne poskušam službo čim bolj odklopiti, kot je to sploh mogoče, in uživati v številnih dejavnostih s prijatelji.

Kako najraje preživljate svoj prosti čas?
Pred leti nisem bil pretirano športno dejaven, sedaj pa moje zanimanje vsako leto narašča. Najraje imam tek in badminton, saj je treba za zdravo telo skrbeti. Drugače pa si z veseljem ogledam tudi dober film in določene športne prenose na televiziji, vmes pa rešim še kakšno križanko, da treniram možgane.

NOVI POLIFUNKCIONALNI PROPENOATI KOT REAGENT V SINTEZI HETEROCIKLIČNIH SPOJIN

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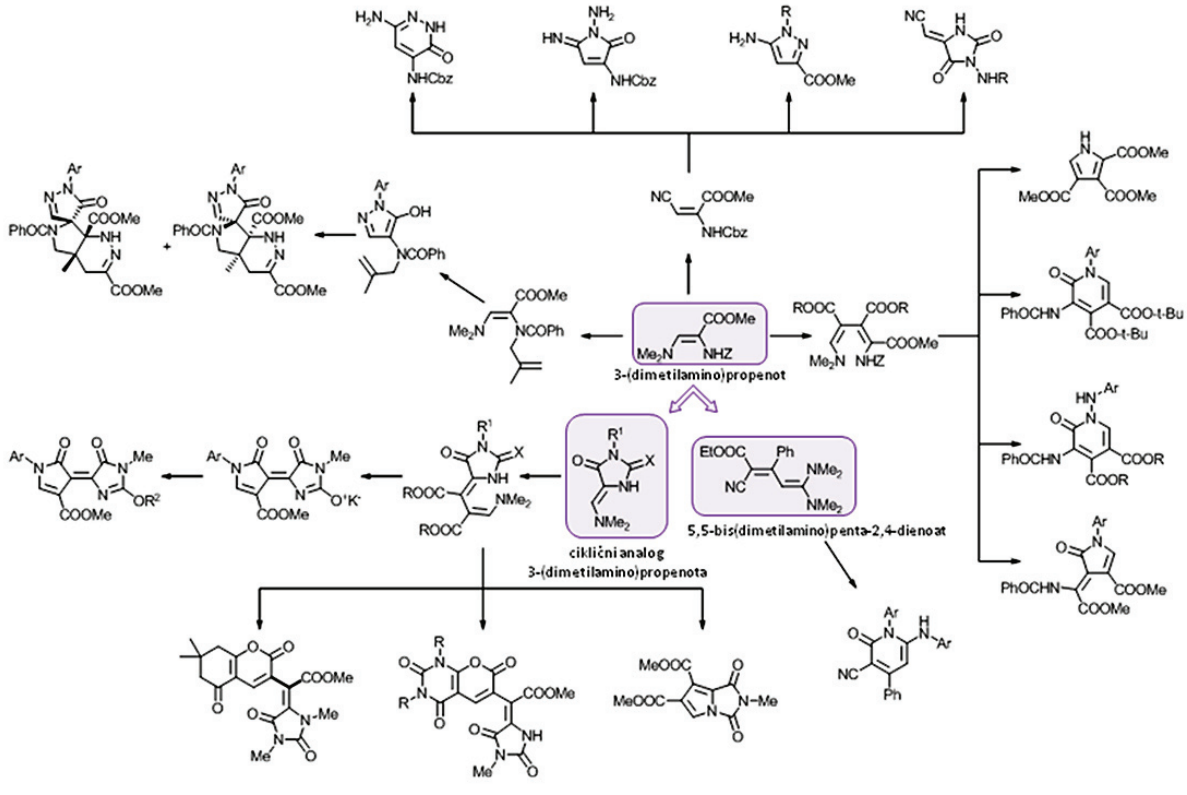
Polifunkcionalni propenoatni reagenti so se izkazali za zelo uporabne v sintezi heterocikličnih spojin, pri katerih je kljub večjemu številu reaktivnih centrov mogoče reakcije voditi selektivno. Pri našem raziskovalnem delu smo razvili nove metode za sintezo visoko funkcionaliziranih 3-(dimetilamino)propenoatnih reagentov in uporabili le-te pri sintezi heterocikličnih spojin.
V prvi fazi našega dela smo preučili reakcije (*E*)-2-[(benziloksikarbonil)amino]-3-cianopropenoata s hidrazini. Reakcija s hidrazinom monohidratom je potekla na ciano in na esterski skupini, pri čemer je nastala zmes piridazina in pirola. Povsem drugače so potekle reakcije z monosubstituiranimi aromatskimi in heteroaromatskimi hidrazini, saj so ciklizacije potekle med estersko in karbamatno skupino, tako da so nastali derivati 3-(arilamino)imidazolidin-2,4-diona. Poleg tega so v določenih primerih s ciklizacijo med ciano skupino in C(2) atomom nastali tudi pirazolski derivati.
V nadaljevanju smo se usmerili v pripravo novih 3-(dimetilamino)propenoatnih reagentov. Ugotovili smo, da 2-acilamino-3-(dimetilamino)propenoati reagirajo z elektronsko revnimi acetileni, kot so acetilendikarboksilati, pri čemer nastanejo (1*E*,3*E*)-1-acilamino-4-(dimetilamino)buta-1,3-dien-1,2,3-trikarboksilati kot edini produkti. Pri teh reakcijah pride najprej do [2+2] cikloadicije acetilenov na propenoate, temu pa sledi retro-elektrociklizacija. Ugotovili smo tudi, da z nesimetričnimi elektronsko revnimi acetileni potečejo cikloadicije regiospecifično, tako da nastane vedno samo en buta-1,3-dienski produkt. Nastali novi dimetilaminopropenoatni reagenti imajo lahko tako do tri esterske skupine, kar omogoča večje število možnih pretvorb, saj po izmenjavi dimetilaminske skupine z dinukleofili lahko poteče ciklizacija na katero koli od treh esterskih skupin.
Ker lahko 5-[(dimetilamino)metilen]imidazolidin-2,4-dione obravnavamo kot ciklične analoge 2-acilamino-3-(dimetilamino)propenoatov, smo izvedli cikloadicije elektronsko revnih acetilenov tudi z njimi. Omenjene cikloadicije so potekle na analogen način kot v prejšnjih primerih, tako da so nastali (2*E*,3*E*)-2-[(dimetilamino) metilen]-3-(2,5-dioksoimidazolidin-4-iliden)sukcinati, vendar so v nekaterih primerih nastale zmesi dveh izomernih produktov, poleg (2*E*,3*E*)-izomerov še (2*E*,3*Z*)-izomerni produkti. Kjub tvorbi zmesi dveh izomerov v nekaterih primerih so tudi v teh primerih cikloadicije z nesimetričnimi acetileni potekle regiospecifično.
Študijo reaktivnosti (1*E*,3*E*)-1-acilamino-4-(dimetilamino)buta-1,3-dien-1,2,3-trikarboksilatov in možnosti pretvorbe le-teh v heterociklične sisteme smo izvedli na primeru reakcij z aromatskimi amini in hidrazini. Z reakcijami med aromatskimi amini in (1*E*,3*E*)-1-acilamino-4-(dimetilamino)buta-1,3-dien-1,2,3-trikarboksilatom, ki ima en metilni ester in dva *terc*-butilna, smo uspešno pripravili piridinonske derivate. Pri tem je očitno sterična oviranost *terc*-butilnih estrov vodila reakcijo do ciklizacije na metilni ester. V primeru reakcij aminov z (1*E*,3*E*)-1-acilamino-4-(dimetilamino)buta-1,3-dien-1,2,3-trikarboksilatom, ki ima tri metilne estre, so nastali izključno pirolovi derivati. Tako smo dokazali, da je mogoče z ustrezno izbiro esterskih skupin voditi reakcije do željenih produktov. Pri reakcijah s hidrazini so neodvisno od vrste esterskih skupin nastali *N*-aminopiridinonski derivati.
Pri reakcijah med aromatskimi amini in (2*E*,3*E*)-2-[(dimetilamino)metilen]-3-(1-metil-2,5-dioksoimidazolidin-4-iliden)sukcinatom so po izmenjavi dimetilaminske skupine in sledeči bazno katalizirani ciklizaciji nastali kalijevi (*E*)-4-[4-(metoksikarbonil)-2-okso-1-aril-1H-pirol-3(2*H*)-iliden]-1-metil-okso-4,5-dihidro-1*H*-imidazol-2-olati kot edini produkti. Te je mogoče direktno O-alkilirati z alkilhalogenidi do (*E*)-metil 4-[2-(alkiloksi)-1-metil-5-okso-1*H*-imidazol-4(5*H*)-iliden]-1-aril-5-okso-4,5-dihidro-1*H*-pirol-3-karboksilatov, pri čemer se ohrani struktura, medtem ko ob dodatku kisline nastanejo zmesi (*E*)- in (*Z*)-izomerov. Na ta način smo pripravili derivate 5-(pirol-3(2*H*)-iliden)imidazola kot povsem novega tipa heterocikličnih spojin.
Kot je značilno za nekatere 3-(dimetilamino)propenoatne reagente, tudi (2*E*,3*E*)-2-[(dimetilamino)metilen]-3-(1-metil-2,5-dioksoimidazolidin-4-iliden)sukcinat in njegov *N*-metilirani derivat reagirata z nekaterimi C-nukleofili. Tako smo uspeli pripraviti derivate pirano[2,3-*d*]pirimidina in kromena. Poleg tega omenjeni sukcinat intramolekularno ciklizira v derivat pirolo[1,2-*c*]imidazola.
Druga metoda za pripravo novih 3-(dimetilamino)propenoatnih reagentov, ki smo jo razvili, je vezava metilalilnega fragmenta na amidni dušik 2-acilamino-3-(dimetilamino)propenoatov s paladijem katalizirano reakcijo s trimetilenmetanom. Ta nov tip 3-(dimetilamino)propenoatnih reagentov omogoča poleg pretvorb značilnih za 3-(dimetilamino)propenoate še cikloadicije na alilno C=C dvojno vez. Na ta način sintetizirani

3-(dimetilamino)-2-[*N*-(2-metilalil)benzamido]propenoat smo s hidrazini najprej pretvorili v derivate pirazola, na C=C dvojni vezi le-teh pa smo nato izvedli cikloadicije 1,2,4,5-tetrazin-3,6-dikarboksilata, pri čemer je cikloadiciji sledila še intramolekularna ciklizacija, tako da so nastali (4*R**,4a'*R**,7a'*R**)-dimetil 1-aril-6'-benzoil-4a'-metil-5-okso-1,1',4',4a',5,5',6',7a'-oktahidrospiro[pirazol-4,7'-pirolo[3,4-*c*]piridazin]-3',7a'-dikarboksilati.
Nazadnje smo se posvetili še 2-ciano-5,5-bis(dimetilamino)-3-fenilpenta-2,4-dienoatu kot tipu spojine z analognimi elementi kot pri 3-(dimetilamino)propenoatnih. Z izmenjavo obeh dimetilaminskih skupin z anilini in sledečo ciklizacijo smo pripravili derivate piridinona.



NEW POLYFUNCTIONAL PROPENOATES AS REAGENTS IN THE SYNTHESIS OF HETEROCYCLIC COMPOUNDS

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Polyfunctional propenoate reagents have proven to be widely applicable in the synthesis of heterocyclic compounds, where, despite the large number of reactive sites, reactions can be performed selectively. In our research work we developed new methods for the synthesis of highly functionalised 3-(dimethylamino) propenoate reagents and used these reagents in the synthesis of heterocyclic compounds.

In the first stage of our research we studied reaction (*E*)-2-[(benzyloxycarbonyl)amino]-3-cyanopropenoate with hydrazines. Reaction with hydrazine monohydrate took place at the cyano and ester group, thus forming a mixture of pyridazine in pyrrol. Reactions with monosubstituted aromatic and heteroaromatic hydrazines proceeded in a completely different way. With these cyclisation took place between the ester and carbamate group to form derivatives of 3-(arylamino)imidazolidin-2,4-dione. In addition to this, in some cases cyclisation took place also between the cyano group and C(2) atom to produce pyrazole derivatives.

We then focused on the preparation of new 3-(dimethylamino)propenoate reagents. We discovered that 2-acylamino-3-(dimethylamino)propenoates react with electron-poor acetylenes such as acetylenedicarboxylates to form (1*E*,3*E*)-1-acylamino-4-(dimethylamino)buta-1,3-diene-1,2,3-tricarboxylates as single products. In these reactions the first step is [2+2] cycloaddition of acetylenes to propenoates, which is then followed by retro-electrocyclisation. We also discovered that cycloadditions with non-symmetrical acetylenes proceed regiospecifically, thus producing single buta-1,3-diene type products. The newly synthesized dimethylaminopropenoate reagents possess up to three ester groups, which offers a wider range of possible transformations, because after the exchange of the dimethylamino group with a dinucleophile cyclisation can take place at any of the three ester groups.

Because 5-[(dimethylamino)methylene]imidazolidin-2,4-diones can be seen as cyclic analogues of 2-acylamino-3-(dimethylamino)propenoates, cycloadditions of electron-poor acetylenes were performed also with them. These cycloadditions proceeded in an analogous manner to those previously mentioned, thus producing (2*E*,3*E*)-2-[(dimethylamino)methylene]-3-(2,5-dioxoimidazolidin-4-ylidene)succinates. However, in some cases mixtures of two isomers were formed, in addition to (2*E*,3*E*)-isomer also (2*E*,3*Z*)-isomer. Despite the fact that in some cases mixtures of two isomers were obtained cycloadditions of non-symmetrical acetylenes proceeded regiospecifically.

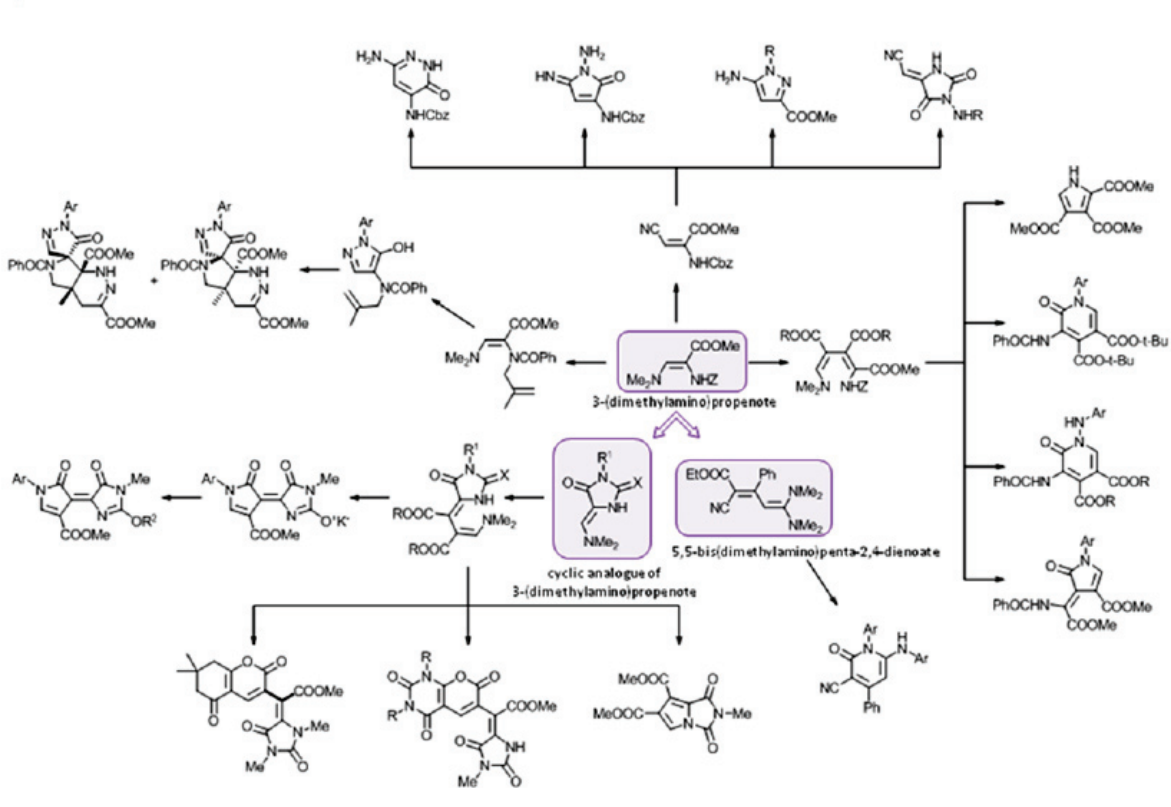
Study reactivity of (1*E*,3*E*)-1-acylamino-4-(dimethylamino)buta-1,3-diene-1,2,3-tricarboxylates and the possibilities of transforming them into heterocyclic systems was performed in the case of reactions with aromatic amines and hydrazines. By reacting aromatic amines with (1*E*,3*E*)-1-acylamino-4-(dimethylamino)buta-1,3-diene-1,2,3-tricarboxylate which possesses one methyl and two *tert*-butyl esters, pyridinone derivatives were successfully prepared. In these cases the steric hindrance of the *tert*-butyl esters led the cyclisations to take place at the methyl ester. On the other hand, in the case of reactions of amines with (1*E*,3*E*)-1-acylamino-4-(dimethylamino)buta-1,3-diene-1,2,3-tricarboxylate which has three methyl esters pyrrole derivatives were formed. In this way it has been demonstrated that by choosing the right ester groups reactions can be led in the desired direction. Reactions with hydrazines proved to be independent of the type of ester groups as in all cases *N*-aminopyridinone derivates were formed.

In the reactions of aromatic amines with (2*E*,3*E*)-2-[(dimethylamino)methylene]-3-(1-methyl-2,5-dioxoimidazolidin-4-ylidene)succinate following the exchange of the dimethylamino group and subsequent base catalyzed cyclisation potassium (*E*)-4-[4-(methoxycarbonyl)-2-oxo-1-aryl-1H-pyrrol-3(2*H*)-ylidene]-1-methyl-oxo-4,5-dihydro-1*H*-imidazole-2-olates were obtained as single products. These can be directly *O*-alkylated with alkylhalogenides to produce (*E*)-methyl 4-[2-(alkyloxy)-1-methyl-5-oxo-1*H*-imidazol-4(5*H*)-ylidene]-1-aryl-5-oxo-4,5-dihydro-1*H*-pyrrole-3-carboxylates, where the conformation is maintained, whereas upon treatment with acid mixtures of (*E*)- in (*Z*)-isomers are formed. In this way derivatives of 5-(pyrrol-3(2*H*)-ylidene)imidazole were synthesized as a new type of heterocyclic compound.

As it is characteristic for some 3-(dimethylamino)propenoate reagents also (2*E*,3*E*)-2-[(dimethylamino) methylene]-3-(1-methyl-2,5-dioxoimidazolidin-4-ylidene)succinate and its *N*-methylated derivate react with certain *C*-nucleophiles. In this way derivatives of pyrano[2,3-d]pyrimidine and chromene were prepared. In addition the mentioned succinate cyclises intramolecularly into pyrrolo[1,2-c]imidazole derivative.

The second method for the preparation of new 3-(dimethylamino)propenoate reagents that was developed is attachment of a methylallyl fragment onto the amidic nitrogen atom of 2-acylamino-3-(dimethylamino) propenoates by palladium catalysed reaction with trimetylenemethane. This new type of 3-(dimethylamino) propenoate reagent offers in addition to the transformations characteristic for 3-(dimethylamino)propenoates also cycloadditions to the allylic C=C double bond. 3-(Dimethylamino)-2-[*N*-(2-methylallyl)benzamido]propenoate prepared in this manner was first transformed into pyrazole derivatives by reacting it with hydrazines. These pyrazoles were then reacted with 1,2,4,5-tetrazine-3.6-dicarboxylate where first cycloaddition to the C=C double bond took place, which was then followed by an intramolecular cyclisation to form (4*R**,4a'*R**,7a'*R**)-dimethyl 1-aryl-6'-benzoyl-4a'-methyl-5-oxo-1,1',4',4a',5,5',6',7a'-octahydrospiro[pyrazole-4,7'-pyrrolo[3,4-*c*] pyridazine]-3',7a'-dicarboxylates.

At the end we focused our attention to 2-cyano-5,5-bis(dimethylamino)-3-phenylpenta-2,4-dienoate as a type of compound with analogous elements as in 3-(dimethylamino)propenoates. By exchanging both dimethylamino groups with anilines and subsequent cyclisation derivatives of pyridinone were obtained.



Krkine nagrade
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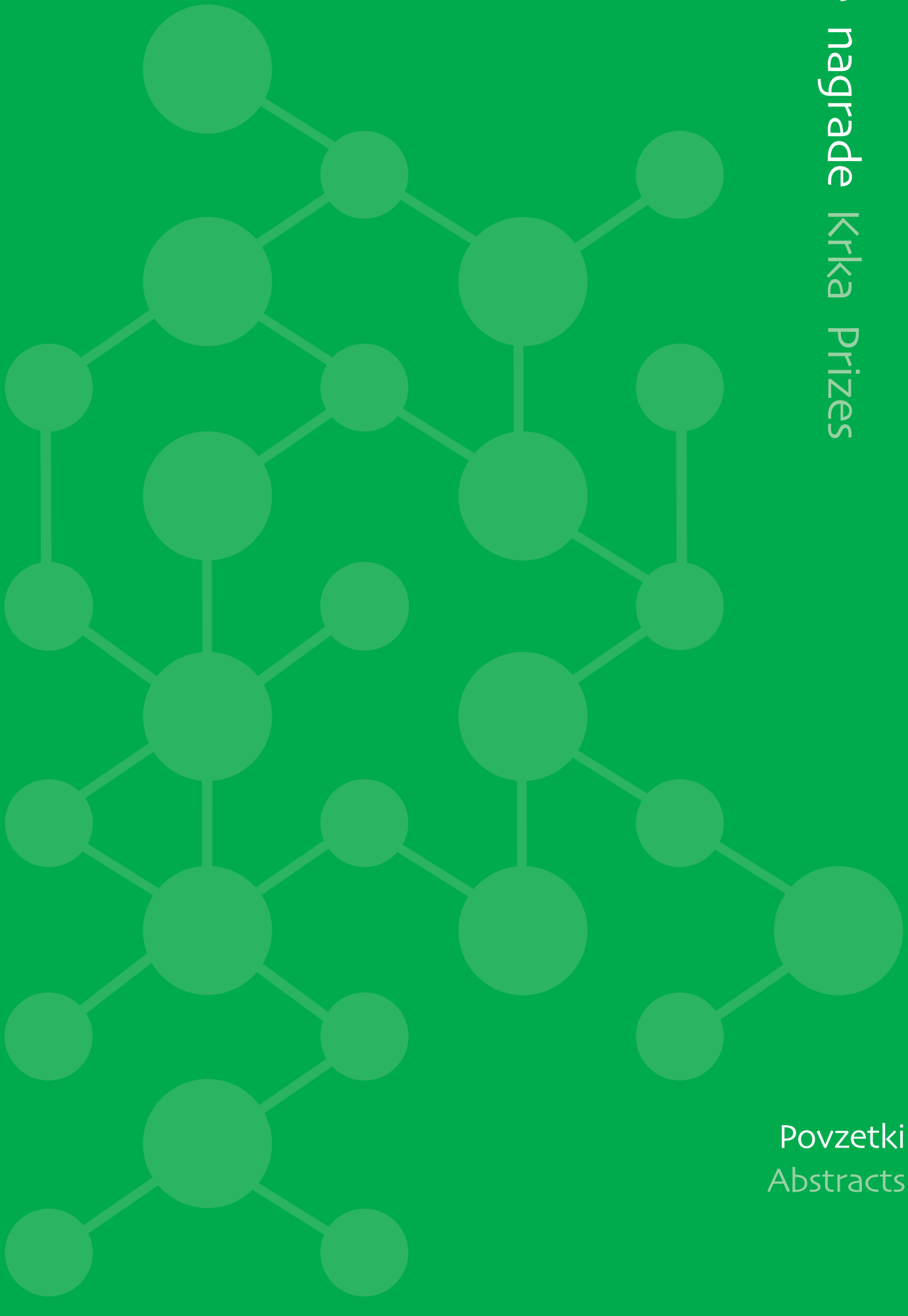
Povzetki

Abstracts



MEHANIZMI STABILIZACIJE MOLEKULE DNA HIPERTERMOFILNE ARHEJE *AEROPYRUM PERNIX IN VIVO* TER *IN VITRO*

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Makromolekule hipertermofilnih arhej so prilagojene na preživetje v okoljih z zelo visokimi temperaturami. V genomu arheje *Aeropyrum pernix* sta poznana dva proteina (Alba1 in 2), ki sta potencialna analoga evkariontskih histonov v arhejah. Proteina smo izrazili v različnih sistemih za izražanje genov in ju okarakterizirali. Preučevali smo tudi medsebojno delovanje proteinov ter različnih molekul DNA pri različnih temperaturah. Raziskali smo vpliv koncentracije NaCl, govejih histonov ter proteinov Alba na termično stabilizacijo različnih molekul DNA. Proteini Alba termično stabilizirajo molekule DNA, vendar v manjši meri kot goveji histoni. Večja termična stabilnost DNA pri arheji *A. pernix* je najverjetneje dosežena s kombinacijo stabilizacije s proteini Alba ter ioni v citoplazmi. Dodatno smo preučevali vzajemen vpliv različnih fluorescenčnih barvil na molekule DNA ter na celice arheje *A. pernix*. Fluorescenčna barvila Hoechst 33258, DAPI in akridin oranž so DNA termično stabilizirala, medtem ko je barvilo $DiBAC_4(3)$ DNA rahlo destabiliziralo. Raziskave so vključevale lokalizacijo fluorescenčnih barvil v celicah ter test živosti celic *A. pernix*. Poleg komercialnega kompleta BacLight™ smo žive in mrtve celice arheje *A. pernix* najbolje ločili z barvanjem s kombinacijo barvil akridin oranž in $DiBAC_4(3)$.

STABILISATION MECHANISMS OF DNA MOLECULE FROM HYPERTHERMOPHILIC *ARCHAEA AEROPYRUM PERNIX IN VIVO* AND *IN VITRO*

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Macromolecules of hyperthermophilic Archaea are adapted to survive in environments with extremely high temperatures. Two potential histone counterpart proteins (Alba1 and 2) were found in *Aeropyrum pernix* genome. These two proteins were expressed in different heterologous expression systems and characterized in its interactions with DNA molecules at different temperatures. We have studied dependence of thermal stability of different DNA molecules regarding to different concentrations of NaCl, bovine histones and Alba proteins. Alba proteins thermally stabilize DNA molecules, however in lesser part than bovine histones. Higher thermal stability of DNA in *A. pernix* cells is most likely accomplished with combination of stabilization with Alba proteins and ions present in the cell cytoplasm. Additionally interactions of different fluorescent dyes with DNA molecules and whole *A. pernix* cells were investigated. Fluorescent dyes Hoechst 33258, DAPI and acridine orange have thermally stabilized DNA while $DiBAC_4(3)$ slightly destabilized DNA. Our experiments included the localization of the dyes in the cells and viability assays of *A. pernix*. Besides using commercially available kit BacLight™, live and dead cells were best distinguished with combination of fluorescent dyes acridine orange and $DiBAC_4(3)$.

UGOTAVLJANJE PROTEKTIVNOSTI FULERENOLA *IN VIVO* V AKUTNI IN KRONIČNI KARDIOMIOPATIJI PRI TERAPIJI MALIGNIH NEOPLAZEM Z DOKSORUBICINOM PRI PODGANAH

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Fulereni so posebna oblika ogljika, ki so jih odkrili sredi osemdesetih let devetnajstega stoletja. Trenutno se odvijajo številne raziskave, v katerih poskušajo odkriti tisto farmacevtsko obliko, ki bi kar najbolje izkoristila biološke lastnosti fulerenov in njihovih derivatov. Danes že izkoriščajo nekatere lastnosti fulerena, kot sta sposobnost odstranjevanja radikalov ter posebna molekulska zgradba. V raziskavah so pokazali, da je fulerenol učinkovit pri zaviranju metastaziranja, zdravljenju cerebralnih sprememb, kot sta Alzheimerjeva in Parkinsonova bolezen, zdravljenju hepatitisa C in aidsa. Fulerenole $C_{60}(OH)_n$, polihidroksi derivate fulerena, intenzivno proučujejo zaradi dobrih antioksidativnih lastnosti. Domnevajo, da delujejo kot lovilci radikalov v bioloških sistemih, pri oksidativnem stresu, ki ga povzroči radioaktivno obsevanje. Za fulerenol $C_{60}(OH)_{24}$ smo dokazali, da ima zaščitne učinke pred z doksorubicinom povzročeno toksičnostjo v živalskih modelih. Potencialno zaščitno vlogo pred kardio-, hepato-, nefro- in pulmotoksičnostjo, povzročeno z doksorubicinom, smo proučevali na modelih podgan s kemijsko povzročenim rakom.
Ključna prednost fulerenola je njegovo dvojno delovanje – ščiti pred učinki sevanja in ščiti pred poškodbami tkiv (srca, jeter, ledvic in pljuč) med protirakavim zdravljenjem (radio- in kemoterapijo). Potrebne pa bodo še nadaljnje raziskave akutnih in kroničnih učinkov na prašičih in nato klinične raziskave na ljudeh.

INVESTIGATION OF PROTECTIVE EFFECTS OF FULLERENOL *IN VIVO* IN ACUTE AND CHRONIC CARDIOMYOPATHY DURING DOXORUBICINE THERAPY OF MALIGN NEOPLASMA IN RATS

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Fullerenes are a type of carbon molecules first discovered in the mid-1980s. Many studies are presently in progress to search for pharmaceutical products that can capitalize on the excellent bioactivity of fullerenes and their derivatives. Thus far, different fullerene properties have been put to use, such as its ability to eliminate radicals as well as its particular molecular structure. Studies have found it to be efficient in suppression of metastases, treatment of cerebral conditions such as Alzheimer's and Parkinson's diseases, type-C hepatitis therapy, and HIV treatment. A polyhydroxilated derivative of fullerene, named fullerenols $C_{60}(OH)_n$, is being extensively studied due to its great potential as an antioxidant. It is proposed that fullerenols may act as free radical scavengers in biological systems, in xenobiotics, as well as radioactive irradiation-induced oxidative stress. It has demonstrated protective effects against cytotoxicity of doxorubicin in animal models, especially by fullerenol $C_{60}(OH)_{24}$.
Different rat models with chemically induced cancer were used to investigate a potential protective role of fullerenol against cardio-, hepato-, nephro- and pulmotoxicity induced by doxorubicin.
The key benefit of fullerenol is its dual function as radio-protector and organo-protector (heart, liver, kidney, and lung) during the anticancer therapy (radio- and chemo-). However, there is a need to carry out further studies, including an acute and chronic investigation in small pigs, and then after human trials.

UPORABA ENAMINONOV V SINTEZI PIRAZOLOVIH DERIVATOV

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Različne 3-(dimetilamino)propenoate in sorodne enaminone smo uporabili v sintezi pirazolovih derivatov, kot so heterociklični analogi aminokislin in pirazolovi analogi histamina. Najprej smo študirali reakcije (*Z*)-2-acetilamino-3-(dimetilamino)propenoata s hidrazini. Pripravili smo ustrezne hidrazone oziroma enhidrazine, ki smo jih nato v prisotnosti baze ciklizirali v 1-substituirane 4-acetilamino-5-hidroksi-1*H*-pirazole kot analoge heterocikličnih aminokislin. Nadaljevali smo s študijo sinteze dveh tipov pirazolovih analogov histamina: a) 1-substituiranih 4-(2-aminoetil)-5-hidroksi-1*H*-pirazolov in b) 1-substituiranih 5-(2-aminoetil)-1*H*-pirazol-4-karboksamidov. Pri pripravi prvega tipa produktov smo izhajali iz pirolidin-2-ona, ki smo ga pretvorili v ustrezne N-zaščitene enamino laktame. Reakcije teh enaminonov s hidrazini in nadaljnja odstranitev N-zaščitnih skupin so privedle do želenih analogov histamina. Zaradi enostavnosti sinteznega postopka smo lahko omenjene spojine pripravili tudi s paralelno sintezo. Drug tip pirazolskih analogov histamina, 5-(2-aminoetil)-1*H*-pirazol-4-karboksamide, smo pripravili iz Boc-ß-alanina. Tega smo najprej s kalijevim monometil malonatom pretvorili v ustrezni ß-keto ester in iz njega sintetizirali ustrezen enaminon. Nadaljnje reakcije tega enaminona s hidrazini so privedle do 1,4,5-trisubstituiranih pirazolov, ki smo jih nato z različnimi kombinacijami selektivnih reakcij odstranitve zaščitnih skupin in amidiranja (*N*-aciliranja) pretvorili v omenjene karboksamide.

APPLICATION OF ENAMINONES IN THE SYNTHESIS OF PYRAZOLE DERIVATIVES

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Various 3-(dimethylamino)propenoates and related enaminones were employed in the synthesis of pyrazole derivatives, such as heterocyclic analogues of amino acids and pyrazole analogues of histamine. First, reactions of (*Z*)-2-acetylamino-3-(dimethylamino)propenoate with various hydrazines were studied. Under mild conditions, these reactions furnished the corresponding hydrazones and enehydrazines, which then underwent base-catalysed cyclisation to yield the desired disubstituted pyrazoles as heterocyclic analogues of amino acids. Next, the synthesis of two types of pyrazole analogues of histamine was studied: a) 1-substituted 4-(2-aminoethyl)-5-hydroxy-1*H*-pyrazoles and b) 1-substituted 5-(2-aminoethyl)-1*H*-pyrazol-4-carboxamides. For the synthesis of the first type of histamine analogues, pyrrolidin-2-one was used as starting material, which was transformed into a suitably protected enamino lactam. Reactions of these enamino lactams followed by removal of the N-protecting group then furnished the desired histamine analogues. This synthetic approach was then also extended to a parallel solution-phese synthesis of the title compounds. The second type of histamine analogues, 1-substituted 5-(2-aminoethyl)-1*H*-pyrazol-4-carboxamides, was obtained starting from Boc-ß-alanine, which was first transformed with potassium monomethyl malonate into the corresponding ß-keto ester, from which the desired enamino ketone was prepared. Reactions of this enaminone with hydrazine derivatives furnished 1,4,5-trisubstituted pyrazoles. Subsequent selective deprotection-amidation (acylation) reactions were then used for a stepwise conversion in the corresponding carboxamides.

MEHANIZEM DELOVANJA IN VLOGA INHIBICIJE KATEPSINA X PRI REGULACIJI IMUNSKEGA ODZIVA

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Katepsin X uvrščamo med cisteinske katepsine, v klan CA papainske družine cisteinskih peptidaz. Vloga katepsina X je pomembna pri imunskem odzivu, ki ga katepsin X uravnava preko integrinskih receptorjev Mac-1 in LFA-1. Katepsin X je ključen pri adheziji makrofagov in dendritičnih celic. Omogoča uspešno zorenje dendritičnih celic, tvorbo citokinov in njihovo migracijo. V limfocitih T katepsin X povzroči aktivacijo LFA-1 receptorja in s tem podaljšanje uropoda ter nastanek nanocevk, ki služijo prenosu informacij med fizično oddaljenimi celicami v obliki prenosa kalcijevih signalov, veziklov in membranskih komponent. Preko aktivacije LFA-1 receptorja katepsin X poveča proliferacijo limfocitov T, preko Mac-1 receptorja pa povzroči supresijo imunskega odziva. Supresija imunskega odziva povzroči nezadovoljiv imunski odgovor, ki omogoča kronične okužbe. Katepsin X kaže vlogo tudi pri vnetni obliki raka dojke, kjer se nakazuje njegova neodvisna prognostična vrednost. Poleg integrinskih receptorjev sta pomembni tarči katepsina X izocima alfa in gama enolazi. Preko cepitve C-terminalnega dipeptida enolaze katepsin X zavre nevritogenezo in zmanjša preživetje nevronskih celic, saj C-terminalni del alfa enolaze služi kot plazminogenski receptor in je aktivacija plazminogena ključna v procesu nevritogeneze. Aktivacija plazminogena je prisotna tudi v tumorskih celicah raka dojke. Protitelo, ki prepoznava zaporedje ključno pri uPA-posredovani aktivaciji plazminogena, zmanjša aktivacijo plazminogena ter invazijo tumorskih celic. Protitelo je zlasti uporabno pri tarčnem ciljanju rakavih celic dojke z nanodelci z vgrajenim cistatinom, ki omogoča inhibicijo katepsina B, znotrajcelične proteolize ter invazije celic.

MECHANISM OF ACTION AND THE ROLE OF INHIBITION OF CATHEPSIN X IN REGULATION OF IMMUNE RESPONSE

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Cathepsin X is a cysteine protease of CA papain family. The role of cathepsin X is important in immune response, where cathepsin X is involved in the activation of integrin receptors Mac-1 and LFA-1. Cathepsin X regulates the adhesion of macrophages and dendritic cells. It enables an efficient maturation of dendritic cells, cytokine production and migration. In T lymphocytes cathepsin X-mediated activation of LFA-1 receptor induces uropod extension and formation of nanotubes, that enable transmission of information between distant cells in the form of calcium fluxes, vesicles and membrane components. The activation of LFA-1 receptor increases the proliferation of T lymphocytes, Mac-1 receptor activation induces immune suppression. Immune suppression triggers inefficient immune response enabling chronical infections. A role for cathepsins X is indicated also in inflammatory breast cancer. We evaluated prognostic value of cathepsin X in sera of patient with early and inflammatory breast cancer, where independent prognostic information is suggested.
Besides integrin receptors, alpha and gamma enolase are important targets for cathepsin X. Proteolytic cleavage of C-terminal dipeptide by cathepsin X reduces neuritogenesis and neuron cell survival. C-terminal part of alpha enolase serves as plasminogen receptor and the activation of plasminogen is crucial for neuritogenesis. Plasminogen activation is found also in breast cancer cells. The antibody, recognizing the sequence involved in uPA-mediated plasminogen activation, significantly reduced plasminogen activation and tumour cell invasion. The antibody could also be used for targeting the cystatin loaded nanoparticles to breast cancer cells, thereby achieving specific cathepsin B inhibition, reduced intracellular proteolysis and cell invasion.

RAZISKAVE REAKCIJSKIH POGOJEV ZA JODO- IN FLUOROTRANSFORMACIJE ORGANSKIH MOLEKUL

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Ekološka ozaveščenost in stremljenje k okolju sprejemljivejšim procesom je v devetdesetih letih pripeljalo do novega načina mišljenja v kemiji, ki ga poznamo pod izrazi 'zelena kemija' ali 'kemija za trajnostni razvoj' in poudarja načelo preventive pred kurativo. Raziskovala sem vpliv reakcijskih pogojev za elektrofilno jodiranje in fluoriranje organskih spojin, pri čemer sem reakcijske spremenljivke prilagajala tako, da bi bile okolju prijaznejše od doslej znanih postopkov. Študirala sem predvsem okolju prijaznejše reagente in reakcijske medije (voda, ionske tekočine, pogoji brez uporabe topila), katerih uporaba vodi do učinkovite, kemoselektivne in regioselektivne pretvorbe s čim manjšim deležem odpadka. Pri izbiri substratov sem bila osredotočena na metoksi substituirane benzenove derivate, ki so pogost strukturni element spojin z bioučinkovitimi lastnostmi. Vpliv reakcijskih spremenljivk na potek pretvorbe sem študirala še na nekaterih drugih aktiviranih aromatih, substratih s prisotno oksidabilno funkcionalnostjo in na manjšem obsegu substratov alkenskega in alkinskega strukturnega tipa.

AN INVESTIGATION OF REACTION CONDITIONS FOR FLUORO- AND IODOTRANSFOMATIONS OF ORGANIC MOLECULES

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The continuously growing concerns for sustainable development and ever increasing stringent environmental demands have in the 90's lead to the emergence of the concept known under the umbrella of 'green chemistry'. Its principles highlight the importance of prevention rather than remediation and thus urge for the development of environmentally friendlier processes. An investigation of the effect of reaction conditions on the iodination and fluorination of organic compounds in an electrophilic manner was carried out by the alteration of the reaction conditions in accordance with the principles of green chemistry. The research was focused on the study of environmentally friendlier reagents and reaction media (water, ionic liquids, solvent-free reaction conditions) and was aimed toward the development of efficient, chemoselective and regioselective transformations that would be environmentally more acceptable than existing methods. The range of substrates comprised mostly methoxy substituted benzene derivatives, often found as structural motifs of bioactive compounds. Moreover, the effect of reaction conditions on the course of transformation was investigated on some other aromatics activated toward electrophilic functionalization, on substrates possessing an oxidizable functional group and on a few substrates with an alkenic and alkynic structural moiety.

DINAMIČNI MODEL ENCIMA MurD IZ *E. COLI* IN *IN SILICO* NAČRTOVANJE NOVIH INHIBITORJEV

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V zadnjih letih je identificiranih vedno več patoloških bakterijskih sevov, ki so odporni na večino znanih protibakterijskih učinkovin, kar kaže na nujnost razvoja novih protibakterijskih učinkovin. Encimi, ki sodelujejo v biosintezi bakterijskega peptidoglikana, so zanimive tarče zaradi možnosti doseganja selektivne toksičnosti. Sinteza peptidnega dela monomerne enote peptidoglikana poteka intracelularno s postopnim dodajanjem aminokislin, ki ga katalizirajo encimi Mur sintetaze. Med njimi encim MurD katalizira reakcijo tvorbe peptidne vezi med D-Glu in prekurzorjem UMA, ki je sklopljena s hidrolizo molekule ATP. Postavili smo kompleksen dinamični model delovanja encima MurD iz *E. Coli* in z *in silico* metodami strukturno podprtega načrtovanja identificirali nove inhibitorje Mur ligaz. Z uporabo tarčne molekulske dinamike (TMD) smo preučevali vezavo substratov v njuni aktivni mesti in analizirali zapiranje C-terminalne domene. Off-path simulacije (OPS) smo uporabili za nadaljnjo določitev energetike zapiranja C-terminalne domene. S QM/MM metodami smo študirali nastanek tetraedričnega intermediata, potencialnega izhodišča pri razvoju Mur inhibitorjev. Dobljeni modeli encimske reakcije so v skladu z reakcijskim vrstnim redom in kažejo, da v reakciji najprej reagira substrat UMA z ATP v acilfosfatni intermediat in da se D-Glu pred reakcijo z acil-fosfatom deprotonira. Izvedli smo simulacije proste energije vezave *N*-sulfonil glutamatnih inhibitorjev, ki kažejo, da imajo najpomembnejšo vlogo pri vezavi tega strukturnega razreda van der Waalsove interakcije. Izhajajoč iz strukturnih podatkov za MurD in MurE smo razvili protokol virtualnega rešetanja za preiskovanje knjižnic molekul. Identificirali smo nov razred dualnih inhibitorjev encimov MurD in MurE, ki kot nadomestek za glutaminsko kislino vsebujejo benzen 1,3- dikarboksilatni fragment.

DYNAMICAL MODEL OF THE *E. COLI* MurD ENZYME AND *IN SILICO* DESIGN OF NOVEL INHIBITORS

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The increasing incidence of bacterial resistance to the available antibiotics renders the discovery of novel antibacterials urgent. An essential bacterial component, peptidoglycan, is traditionally an optimal target with respect to selective toxicity. In the peptidoglycan biosynthetic pathway Mur ligases catalyze the intracellular construction of its peptide moiety. MurD enzyme in particular catalyses an incorporation of the D-Glu into the UMA precursor coupled with the concurrent ATP hydrolysis. In this work a complex dynamical model of the *E. coli* MurD enzyme was derived along with *in silico* structure-based discovery of novel Mur inhibitors. Targeted molecular dynamics (TMD) simulations were performed to examine the substrates binding process and gain insight into the closure of the C-terminal domain. Off-path simulation (OPS) technique was initiated for the energy evaluation of the TMD-generated pathways. QM/MM molecular modeling approach was utilized, to evaluate reaction pathways leading to the tetrahedral intermediate formation – a frequent drug design starting point. Calculated models confirmed the expected reaction order; first between ATP and UMA resulting in the acyl-phosphate intermediate, followed by the addition of the D-Glu which most likely enters the enzyme reaction in its deprotonated form. Binding free energies calculated for a series of MurD *N*-sulphonyl-glutamic acid inhibitors revealed non-polar van der Waals interactions as the main driving force for the binding of these inhibitors. Based on the available structural data for the MurD and MurE enzymes a virtual screening campaign was performed, resulting in the identification of a novel class of glutamic acid surrogates - benzene 1,3-dicarboxylic acid derivatives possessing dual MurD and MurE inhibitory activity.

ŠTUDIJ STABILNOSTI IN RAZGRADNJE STATINOV V OKOLJU

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Statini so zdravila, ki se uporabljajo za zniževanje ravni holesterola v krvi. Kot izjemno učinkoviti inhibitorji encima HMGCoA (3-hidroksi-3-metil-glutaril-CoA) reduktaze prispevajo k znižanju telesne ravni skupnega holesterola ter lipoproteinov nizke gostote. V medicini se najpogosteje uporabljajo za zdravljenje hiperholesterolemije ter tako prispevajo k manjši umrljivosti zaradi bolezni srca in ožilja. Kljub tridesetletni prisotnosti na trgu ostajajo razgradnja, pretvorbe in učinki statinov ter njihov vpliv na okolje še vedno nepoznani. S prisotnostjo v odpadnih vodah iz čistilnih naprav, ki nastajo zaradi neučinkovitega čiščenja slednjih, namreč lahko predstavljajo pomemben izvor kontaminacije vodnih teles, ki ogroža številne vodne organizme.
V našo raziskavo smo kot modelne spojine vključili tri reprezentativne predstavnike statinov poznane pod imeni simvastatin, lovastatin in pravastatin. Z raziskavo smo želeli:

- razviti novo, selektivno in občutljivo analizno metodo za določanje statinov v vodnih raztopinah. Raziskavo smo dopolnili s testi stabilnosti omenjenih spojin, saj so slednji izjemno občutljivi na različne okoljske pogoje, kot so: sprememba pH vrednosti, temperature, različna topila, sončna svetloba, itd.
- preveriti učinkovitost različnih postopkov razgradnje statinov (NOP - naprednih oksidacijskih procesov kot sta fotoliza in ozonacija)
- določiti strupenost statinov (standardnih spojin) kot tudi njihovih razgradnih produktov, nastalih med zgoraj omenjenimi razgradnimi poskusi

STABILITY AND DEGRADATION STUDIES OF CHOLESTEROL-LOWERING STATIN DRUGS

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Cholesterol-lowering statin drugs are potent inhibitors of HMGCoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase, which are highly effective in reducing total cholesterol and the low-density lipoprotein cholesterol levels in the body. They have been introduced into the market for the past thirty years, but despite of common use the fate and effects of statin in the environment are largely unknown. Statin residues might enter wastewater treatment facilities and the incomplete removal in sewage treatment plants (STP's) might be a major source of discharge of these compounds to the environment, affecting various aqueous organisms.
Three representatives of statin drugs: simvastatin, lovastatin and pravastatin were chosen as a model compounds for our study. Three major goals of the research were:

- development of sensitive and selective analytical methods for the determination of statin compounds in aqueous solutions. Determination studies were combined with stability experiments, since statin are significantly affected by various conditions such as pH, temperature, solvent systems, sun light, etc. application of different degradation techniques (AOP'S - advanced oxidation processes as photocatalytic degradation and ozonation)
- toxicity measurements were performed for standard statin compounds, as well as for by-products, formed during mentioned above degradation experiments. Biodegradation (manometric respirometry test) was involved in evaluation of statin characterisation in terms of their accessibility to microbial degradation and unwanted effects in the environment.

ŠTUDIJ HALOGENIRANJ ORGANSKIH MOLEKUL V VODI IN FLUORIRANIH TOPILIH

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Raziskovala sem uporabo vode in fluornih topil za okolju prijaznejše bromiranje in kloriranje organskih molekul. Študirala sem vpliv reakcijskih pogojev na bromiranje alkil aromatov z *N*-bromosukcinimidom v vodi; ob prisotnosti vidne svetlobe je potekla reakcija na benzilnem mestu, medtem ko je pri aktiviranih aromatih prišlo do funkcionalizacije aromatskega jedra. Z uporabo 4% H_2O_2 in 9% HBr sem pri sobni temperaturi učinkovito in selektivno bromirala aktivirane in neaktivirane aromate. Vodni HBr-H_2O_2 sistem sem uporabila tudi za α-bromiranje različnih karbonilnih spojin. Različne 1,1- in 1,2- alkil in aril substituirane alkene in alkine sem *anti* stereoselektivno bromirala v CH_3CN pri sobni temperaturi z uporabo 48% vodne raztopine HBr, zrakom kot oksidantom in $NaNO_2$ kot katalizatorjem. Raziskovala sem interakcije med molekulami halogenov in fluornih topil s študijem topnosti fluora, klora in broma v nekaterih fluornih topilih v odvisnosti od temperature. Fluorno topilo sem kot membrano uporabila za difuzijsko dodajanje broma v reakcijski sistem in na ta način raziskala bromiranje alkenov ter ovrednotila vlogo fluorne membrane pri samem procesu bromiranja. Pripravila sem nove obnovljive fluorne alkil in fluorne aril jod(III) dikloride in jih uporabila za kloriranje nekaterih modelnih organskih molekul.

THE STUDY OF HALOGENATION OF ORGANIC COMPOUNDS IN WATER AND FLUOROUS SOLVENTS

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The use of water and a fluorous solvent to create environmentally friendly synthetic protocols for the bromination and chlorination of organic compounds was investigated. The effect of reaction conditions on the bromination of alkyl aromates using *N*-bromosuccinimide in water was examined; in the presence of visible light the reaction proceeds at the benzyl position, while activated aromatic molecules react at the aromatic ring. The use of a 4% H2O2 and a 9% aq. HBr enabled the efficient and selective bromination of activated and nonactivated aromates. The aqueous HBr-H_2O_2 system was also applied for the α-bromination of various carbonyl compounds. Various 1,1- and 1,2-alkyl and arly substituted alkenes and alkynes were *anti* steroselectively dibrominated in CH_3CN at room temperature using 48% aqueous HBr, air as an oxidant and $NaNO_2$ as a catalyst. Interactions between halogenes and fluorous solvents were investigated by studying the solubility of fluorine, chlorine and bromine in several fluorous solvents at different temperatures. Fluorous solvent was used as a membrane for diffusion controlled addition of bromine into the reaction system with alkene and a role of fluorous membrane was evaluated in bromination process. New fluorous alkyl and fluorous aryl iodine(III) dichlorides were prepared and used for the chlorination of several model organic compounds, reagent residue was recycled and reused.

POROZNI POLIAKRILATI IN POLI(VINILBENZIL KLORID) ZA IMOBILIZACIJO ORGANSKIH MOLEKUL

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Pri organskih sintetskih postopkih ter pri separacijah biomakromolekul se vse bolj uporabljajo zamreženi porozni polimeri. Visoko poroznost je možno doseči na različne načine, eden izmed njih je s polimerizacijo kontinuirne faze HIPE (high internal phase emulsion) emulzije, ki smo jo med drugim uporabili pri pripravi vinilbenzil kloridnega in akrilatnih tipov (4-nitrofenil akrilat, glicidil metakrilat, etilheksil akrilat) netopnih polimernih nosilcev. Na nove hiperzamrežene polimerne nosilce smo vezali katalizator (4-metilaminopiridin) in preizkusili njegovo katalitsko aktivnost pri reakciji aciliranja in pri Baylis-Hillmanovi reakciji. S hiperzamreženjem (s Friedel-Craftsovo reakcijo) smo močno povečali specifično površino polimernih nosilcev in njihovo katalitsko aktivnost. Polimerne nosilce lahko med drugim uporabimo za čiščenje produktne mešanice; tako smo funkcionaliziran akrilatni nosilec uporabili za odstanitev rutenijevega katalizatorja iz produktne mešanice. S PolyHIPE membranskim modulom smo izvedli separacijo proteinov, njegova vezalna kapaciteta je bila višja kot za primerljive monolitne nosilce.

POROUS POLYACRYLATES AND POLY(VINYLBENZYL CHLORIDE) FOR IMMOBILISATION OF ORGANIC COMPOUNDS

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For organic synthetic procedures and for the separation of biomacromolecules crosslinked porous polymers are increasingly used. High porosity can be achieved in different ways, one of them is polymerisation of the continuous phase of HIPE (high internal phase emulsion), which was among others used to prepare vinylbenzyl chloride and acrylate types (4-nitrophenyl acrylate, glycidyl methacrylate, ethylhexyl acrylate) of insoluble polymeric supports. On the new polymer hypercrosslinked supports a catalyst (4-methylaminopyridine) was immobilised and its catalytic activity was tested by acylation and Baylis-Hillman reaction. Hypercrosslinking (by Friedel-Crafts reaction) strongly increased specific surface area of polymer support and consecutively their catalytic activity was increased. Polymeric carriers may be used, among other, for the treatment and purification of product mixtures, so we demonstrated that by using functionalised acrylate support for the removal of ruthenium catalyst from a reaction mixture. With a PolyHIPE membrane module, we carried out protein separation, and its binding capacity was higher than for the comparable monolithic supports.

NAČRTOVANJE IN SINTEZA HIDROKSIETILAMINSKIH INHIBITORJEV BIOSINTEZE PEPTIDOGLIKANA

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Eno od pomembnejših in aktualnih tarčnih mest pri iskanju novih protibakterijskih učinkovin predstavljajo ligaza D-alanil-D-alanin (Ddl) in ligaze Mur, ki so ključni encimi pri biosintezi bakterijskega peptidoglikana. Ker so se fosfonatni, fosfinatni in sulfonamidni analogi prehodnega stanja že izkazali kot inhibitorji ligaz Mur in Ddl, smo v okviru našega dela načrtovali, sintetizirali in biološko ovrednotili hidroksietilaminske analoge kot nove potencialne inhibitorje encimov MurC do MurF, VanA in Ddl. Pri načrtovanju smo si pomagali s programsko opremo za »*de novo*« načrtovanje nizkomolekulskih inhibitorjev SPROUT in orodjem za sidranje ligandov eHiTS. Najprej smo optimizirali sintezno pot za pripravo hidroksietilaminskega fragmenta, nato pa smo sintetiziranim spojinam določili inhibitorno aktivnost na DdlB, MurC, MurD in MurF iz *E. coli*, MurE iz *S. aureus* in tudi VanA iz *E. faecium*, ki je odgovoren za rezistenco na vankomicin. Derivati 1-fenilsulfonamido-3-morfolinopropan-2-il dihidrogen fosfata so se izkazali kot inhibitorji omenjenih encimov z IC_{50} vrednostmi v mikromolarnem območju. Ker je VanA odgovoren za rezistenco na antibiotik vankomicin, so omenjeni inhibitorji dobre izhodiščne spojine za razvoj učinkovin, ki bi zavirale pojav rezistence na vankomicin. Poleg tega so tudi dobro izhodišče za razvoj novih multiplih inhibitorjev biosinteze peptidoglikana s potencialnim širokospektralnim protibakterijskim delovanjem. Zaradi zaviranja aktivnosti encima MurE iz *S. aureus* pa bi se lahko uporabljale tudi kot učinkovine pri zdravljenju infekcij z MRSA.

DESIGN AND SYNTHESIS OF HYDROXYETHYLAMINES AS INHIBITORS OF PEPTIDOGLYCAN BIOSYNTHESIS

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One of the most important and validated targets for antibacterial drug discovery research are D-alanyl-D-alanine ligase (Ddl) and Mur ligases, which are essential enzymes for the biosynthesis of peptidoglycan. Phosphinate, phosphonate and sulfonamide transition-state analogs are well-known inhibitors of Mur ligases and Ddl. Therefore, we decided to design, synthesize and evaluate the hydroxyethylamines as new potential inhibitors of MurC to MurF, VanA and Ddl. To design a new class of inhibitors we used »*de novo*« ligand design software SPROUT and ligand docking tool eHiTS. In the following step we developed and optimized an efficient synthetic approach to hydroxyethylamines and later on the synthesized compounds were evaluated for their inhibitory activities against DdlB, MurC, MurD and MurF from *E. coli*, MurE from *S. aureus* and also VanA from *E. faecium*, which is responsible for vancomycin resistance. Hydroxyethylamine derivatives of 1-(4-methoxy-phenylsulfonamido)-3-morpholinopropan-2-yl dihydrogen phosphate were found as inhibitors of aformentioned enzymes with IC_{50} values in micromolar range. Since VanA is responsible for vancomycin resistance the aformentioned inhibitors have the potential to be developed into drugs that would reverse bacterial resistance to vancomycin. They also represent an important starting point for development of multiple inhibitors of peptidoglycan biosynthesis with broad-spectrum antibacterial activity. Moreover, due to inhibition of MurE ligase from *S. aureus* this compounds could be developed into antibacterial drugs against MRSA.

URAVNAVANJE DELOVANJA ESTROGENOV IN PROGESTERONA PRI ENDOMETRIOZI IN RAKU ENDOMETRIJA

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Endometrioza in rak endometrija sta zelo pogosti ženski bolezni. Povečana koncentracija estrogenov in/ali zmanjšana koncentracija progesterona sta faktor tveganja za njun nastanek. Delovanje estrogenov in progesterona je uravnavano na pred-receptorski ravni z encimi, ki hormone spreminjajo iz aktivne oblike v neaktivno in obratno, pa tudi na receptorski ravni s količino ustreznih receptorskih izooblik.
Pri ovarijski endometriozi in raku endometrija smo na ravni mRNA, proteinov in celic preverjali izražanje encimov, vpletenih v zadnjo stopnjo sinteze estrogenov in metabolizem progesterona, in receptorjev, preko katerih estradiol in progesteron delujeta. Z našimi raziskavami smo ugotovili, da aromatazna pot ni edina, ki vodi do povečane koncentracije estradiola v endometriotičnem tkivu. Po naših izsledkih lahko estradiol nastane tudi iz neaktivnega estron-sulfata po sulfatazni poti. Pri ovarijski endometriozi je moten tudi metabolizem progesterona. Z LDA ploščicami smo identificirali še ostale procese, ki prispevajo k nastanku endometrioze. Pri raku endometrija sta pri nekaterih bolnicah pomembna aromataza in AKR1C3, estradiol pa lahko nastane tudi po sulfatazni poti. Zmanjšana je tudi aktivnost zaščitnega progesterona.
S študijami smo prispevali k razumevanju nastanka endometrioze in raka endometrija, identificirali smo nove tarče za razvoj zdravil in potencialne biološke označevalce, ki naj bi v prihodnosti omogočili hitrejše odkrivanje bolezni in posameznim bolnicam prilagojeno zdravljenje.

REGULATION OF ESTROGEN AND PROGESTERONE ACTION IN ENDOMETRIOSIS AND ENDOMETRIAL CANCER

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Endometriosis and endometrial cancer are very common women diseases. Risk factors for both diseases include exposure to estradiol unapposed by progesterone. Action of estrogens and progesterone is regulated at the pre-receptor level, by interconversions of active hormones with their inactive counterparts as well as the receptor level by regulation of expression of estrogen and progesterone receptors.
We examined expression of estrogen and progesterone metabolizing enzymes and of estrogen and progesterone receptors at mRNA, protein an cellular level in ovarian endometriosis and endometrial cancer.
Our studies revealed that aromatase pathway has an important role in estradiol formation in ovarian endometriosis. Direct production of estradiol from estrone-sulfate is another pathway for local production of the potent estrogens. Our data also show disturbed progesterone metabolism. LDA expression study identified biological processes that are involved in pathogenesis of ovarian endometriosis. In some endometrial cancer patients up-regulated aromatase and AKR1C3 can lead to increased levels of estradiol. Our data suggest that also sulfatase pathway has an important role, in some patients also metabolism of protective progesterone is disturbed.
Our results provide us with a better and more detailed understanding of endometriosis and endometrial cancer, while newly identified, highly up-regulated genes may serve as potential targets for pharmaceutical development and as biomarkers with the aim of endometriosis and endometrial cancer diagnosis and personalised treatment.

NAČRTOVANJE KONTINUIRNIH REAKTORSKIH SISTEMOV PROIZVODNJE KEFIRNIH ZRN

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V doktorski disertaciji smo podrobneje obravnavali inovativno načrtovanje kontinuirnih reaktorskih sistemov tradicionalne proizvodnje kefirnih zrn z gojenjem v mleku. Osrednji cilj disertacije je bil določiti optimalne volumne reaktorjev v eno-, dvo- ali več-stopenjskih reaktorskih sistemih. V ta namen smo razvili nove matematične izraze in NLP problem za ocenjevanje optimalnih volumnov reaktorjev pri različnih presnovah glede na pH vrednost fermentacijskega medija in kapacitetah proizvodnje kefirnih zrn. Prav tako smo v disertaciji razvili in validirali t.i. TDN model, ki podaja eksplicitno matematično formulacijo za izračun optimalnega volumna *j*-tega reaktorja v kaskadi *N*-tih PMR. Na omenjeno tematiko disertacije do sedaj ni bilo znanstvenih objav, zato smo predlagali nov pristop k razvoju reaktorskih sistemov proizvodnje kefirnih zrn, ki temelji na t.i. strukturirani in eksperimentalno podprti metodologiji načrtovanja. Gre za celovit sklop uporabe kombinacije rezultatov preliminarne raziskave in modeliranja rastne krivulje pri razvoju novih modelov oz. metod ocenjevanja optimalnih volumnov kontinuirnih reaktorjev, ki upoštevajo edinstvene karakteristike kefirnih zrn. Tako v širšem kontekstu disertacija obravnava tudi optimiranje procesnih parametrov šaržnega gojenja kefirnih zrn, izboljšanje postopka aktivacije kefirnih zrn, statistično primerjavo različnih primarnih napovednih modelov rasti klasično ter optimalno aktiviranih kefirnih zrn, ipd.

DESIGN OF CONTINUOUS REACTOR SYSTEMS FOR KEFIR GRAINS PRODUCTION

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This doctoral dissertation describes an innovative design of continuous reactor systems for traditional kefir grains production with cultivation in milk. The main goal of the dissertation was to determine the optimal volumes of reactors in one-, two- or multi-stage reactor systems. For this purpose a new mathematical expressions and NLP problem for estimating the optimal volumes of reactors at different conversions with regard to pH value of fermentation media and kefir grains production capacities have been developed. In doctoral dissertation, a so-called TDP model, presenting explicit mathematical formulation for calculating the optimal volume of *j*-th reactor in cascade of *N* CSTRs, has also been developed and validated. Up to date, no scientific studies concerning the dissertation topic have been published. Therefore, the new approach for development of reactor systems for kefir grains production, which is based on so-called structured and experimentally supported design methodology, has been proposed. Summarily, the new models or methods for estimating the optimal volumes of continuous reactors, which consider unique kefir grains characteristics, have been developed by integrated usage of results of preliminary research and growth curve modeling. Consequently, in extended context the dissertation also includes optimization of process parameters of batch kefir grains cultivation, improvement of kefir grains activation procedure, statistical comparison between predictive growth models of classically and optimally activated kefir grains, etc.

MANAGEMENT OSKRBOVALNE VERIGE

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V raziskovalni nalogi obravnavam področje managementa poslovnih procesov v posameznem podjetju in predvsem v oskrbovalni verigi. V zadnjih letih namreč v večini panog (tudi farmacevtski) konkurenca ni več med posameznimi podjetji, ampak med oskrbovalnimi verigami. Pri tem so pomembni novi pristopi za povečanje učinkovitosti izvajanja procesov (pretočni časi, stroški, zmanjšanje variabilnosti), njihove fleksibilnosti in obvladovanje tveganj (slednje je še posebej pomembno za farmacevtsko industrijo, kjer je varnost ključnega pomena). Glavni prispevki te raziskovalne naloge na teh področjih so (poleg temeljitega pregleda in klasifikacije obstoječe literature): 1. teoretična podlaga in identifikacija kritičnih dejavnikov uspeha managementa poslovnih procesov v podjetju. Pretekle izkušnje namreč kažejo, da je večina tovrstnih naporov neuspešnih, v poglavju pa teoretično analiziram in na študiji primera predstavim dejavnike, ki vplivajo na uspeh, 2. pristop za prenovo medorganizacijskih poslovnih procesov: v poglavju predstavim pristop za modeliranje, prenovo in merjenje učinkov (tako prenove procesov kot sprememb pri zagotavljanju ustreznega nivoja zalog) ter poudarim pomen iskanja globalnega optimuma za celo verigo, 3. pristop za merjenje koristi (znižanje stroškov, pretočnih časov) ter potencialnih tveganj (angl. value-at-risk) informatizacije oskrbovalnega procesa. Pristop je prikazan na študiji primera naročil velikega podjetja, 4. nova metoda za identifikacijo in management tveganj, povezanih z dobavitelji v oskrbovalnih verigah. Na podlagi analize poslovanja posameznega dobavitelja in volatilnosti njegovega okolja napovemo verjetnost slabšega poslovanja tega podjetja. Pristop je prikazan na študiji primera ameriške multinacionalke.

SUPPLY CHAIN MANAGEMENT

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This research tackles the field of business process management in a company and a supply chain. In recent years the competition in most industries (including pharmaceutical) is no longer among single companies but between supply chains. Therefore new approaches for increase in efficiency of business processes (lead times, costs, reduction of variability), their flexibility and risk management (this is of outmost importance for pharmaceutical industry where safety is a key concern). The main contribution of the presented research report are (in addition to a thorough review and classification of existing literature): 1. the theoretical foundation and identification of the critical success factors of business process management in a single company. Past experiences have namely shown that most of business processes redesign projects ended with a failure. Our research thus theoretically analyses the main influencing factors and show that with a case study, 2. the approach for redesign of inter-organizational business processes: in this chapter a new approach for modelling, redesign and performance measurement of process redesign and the changes in inventory management are shown. Special emphasis is given to the importance of searching for the global instead of local optima; 3. the approach for measurement of benefits (cost and lead time reduction) and potential risks (value-at-risk) of e-procurement implementation. The approach is illustrated with a case study of a large company, 4. a new method for identification and management of risks connected to suppliers. Based on the analysis of performance of a supplier and the volatility of its environment the probability of its sub-par performance (or even bankruptcy) is predicted. The approach is shown with the case study of a multi-national company from the USA.

MOŽNOSTI TVORBE BIOFILMA IN VIRULENTNIH DEJAVNIKOV PRI PROTI METICILINU ODPORNI BAKTERIJI *STAPHYLOCOCCUS AUREUS*

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Stafilokoki tvorijo biofilme na različnih materialih. Namen našega dela je bil raziskati sposobnosti tvorbe biofilma v različnih inkubacijskih pogojih glede na prisotnost kisika pri izolatih MRSA osamljenih v osemletnem obdobju v Bolnišnici Golnik – KOPA in oceniti povezavo med sposobnostjo tvorbe biofilma in prisotnostjo specifičnih virulentnih dejavnikov glede na genetsko ozadje samih izolatov. Za oceno sposobnosti pritrjanja bakterijskih celic na podlago smo uporabili test z mikrotitrsko ploščico. Prisotnost specifičnih virulentnih dejavnikov smo preverjali z verižno reakcijo s polimerazo in ugotovili, da tri genetske determinante (*fnb*A, *ica*A in *hlb*) prevladujejo pri naših izolatih. Tvorba biofilma pri *ica*A pozitivnih izolatih je v vseh testiranih inkubacijskih atmosferah bila značilna. Ugotovili smo, da je učinek specifične kombinacije virulentnih dejavnikov na povišano adherenco pri naših izolatih kumulativen. Značilno višjo sposobnost adherence naših izolatov na podlago smo opazili pri zmanjšani koncentraciji kisika. Ugotovili smo tudi, da genetsko ozadje nima značilnega vpliva na sposobnost tvorbe biofilma. S pomočjo gelske elektroforeze v utripajočem električnem polju smo ugotovili, da je v naši bolnišnici prisotnih več različnih tipov MRSA, od katerih trije prevladujejo. Večina naših izolatov MRSA je podobnih Južnonemškem klonu in imajo tip I stafilokokne kromosomske kasete. Izvor naših izolatov ni klonalnen. Upamo, da bodo rezultati našega dela pripomogli k boljšem poznavanju genetskega ozadja bakterije MRSA v Sloveniji.

BIOFILM FORMATION ABILITIES AND VIRULENT FACTORS IN METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Staphylococci are known to form biofilms on a variety of materials. Therefore the aim of our study was to determine the influence of low nutrient medium and three different incubation atmospheres on in-vitro biofilm formation capability in well defined MRSA strains. In the present study, we also investigated the relationship between biofilm – forming capacities and virulence determinants of MRSA isolates according to its genetic background. We used the *in vitro* microtiter plate assay to quantify biofilm formation. The presence of several virulence determinants was investigated by polymerase chain reaction assay and found three determinants (*fnb*A, *ica*A and *hlb*) to be predominant among these isolates. Enhanced biofilm formation was confirmed in *ica*A- positive MRSA isolates in all three tested incubation atmospheres. We concluded that the MRSA isolates with specific combination of virulence determinants had greater capacities for biofilm formation than did those lacking these genes. The biofilm – forming capacities of MRSA isolates grown in lower oxygen concentrations were significantly greater than those grown in aerobic conditions. Genetic background of our MRSA isolates doesn't influence its biofilm forming abilities. Results of this study showed that multiple strains of MRSA were present in our institution simultaneously during longer time period, even though certain MRSA pulsotypes, based on PFGE, clearly predominate. Most of our isolates were found to harbour SCC*mec* type I and are visually very similar to the Southern German clone that has been one of the most successful contemporary MRSA clones in Europe. Since no similar study with larger number of MRSA isolates from Slovenia exist, we hope that the results of our study will contribute to better understanding of genetic background of MRSA in Slovenia.

SINTEZA, LASTNOSTI IN BIOLOŠKE AKTIVNOSTI TETRAOKSANOV IN SORODNIH PEROKSIDNIH DERIVATOV

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Raziskovala sem pretvorbe ketonov in aldehidov v substituirane dihidroperokside in 1,2,4,5-tetraoksane (TO). Razvila sem nov postopek sinteze *gem*-dihidroperoksidov (DHP), monoperoksiketalov in *bis*-peretrov iz ketonov in aromatskih aldehidov z uporabo joda kot katalizatorja. Ob prisotnosti 10 mol% joda v CH_3CN so se s 30% H_2O_2 v DHP pretvorili tako ciklični kot aciklični ketoni ter aromatski aldehidi z dobrimi izkoristki. V metanolu so ob prisotnosti 5 mol% joda nastali hidroperoksiketali. ^{t}BuOOH je reagiral analogno H_2O_2, nastali so ustrezni pereterski derivati. Študija relativnih hitrosti dihidroperoksidacije substituiranih benzaldehidov je dala Hammettovo zvezo z dobro linearnostjo in reakcijsko konstanto ρ -2.76, kar kaže na relativno dobro razvit pozitiven naboj prehodnega stanja v hitrost določujoči stopnji reakcije. Selektiven nastanek DHP v CH_3CN kljub prisotnosti vode kaže, da je jod sposoben razlikovati med hidroksi in hidroperoksi skupino ter nukleofiloma H_2O_2 in H_2O. Oksidacijski sistem H_2O_2 / fluorirani alkohol (TFE, HFIP) sem uporabila za neposredno s HBF_4 in MTO katalizirano sintezo tetraoksanov iz ketonov in raziskala vpliv strukture ketonov na pretvorbo v TO ter reakcijo preizkusila tudi na aldehidih. Izbranim sintetiziranim TO sem določila antimalarijsko aktivnost in s spreminjanjem dolžine alkilnih verig raziskala vpliv polarnosti, s spreminjanjem substituiranosti alkilnih verig pa vpliv steričnih interakcij okrog farmakoforne peroksidne skupine na njihovo antimalarijsko aktivnost.

SYNTHESIS, PROPERTIES AND BIOLOGIC ACTIVITIES OF TETRAOXANES AND RELATED PEROXIDIC DERIVATIVES

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Transformations of ketones and aldehydes into substituted dihydroperoxides (DHP) and 1,2,4,5-tetraoxanes (TOs) were studied. A novel procedure for preparation of *gem*-dihydroperoxides, monoperoxyketals and *bis*-perethers from ketones and aromatic aldehydes using iodine as a catalyst is discussed. In the presence of 10 mol% of iodine in CH_3CN cyclic and acyclic ketones as well as aromatic aldehydes were converted with 30% H_2O_2 to DHPs with good yields. In the presence of 5 mol% of iodine hydroperoxy- ketals were formed in methanol and ^{t}BuOOH reacted in a similar manner, producing the corresponding perether derivatives. A study was also made of the relative kinetics of dihydroperoxidation from which the Hammett equation gave a reaction constant (ρ) of -2.76 indicating the strong positive charge development in the transition state. In acetonitrile the iodine catalyst is able to discriminate between hydroxy and hydroperoxy as leaving groups and H_2O_2 vs. H_2O in addition to a carbonyl group. The oxidation system H_2O_2/ fluorinated alcohol (TFE, HFIP) was applied to the direct HBF_4 and MTO catalyzed synthesis of 1,2,4,5-tetraoxanes from ketones. This included investigating the influence that the structure of ketones has on their transformation. Finally, this study determines the antimalarial activities of selected tetraoxanes and investigates the influence of polarity by varying the alkyl chain and the influence of the steric environment around the pharmacophoric peroxide bond by the introduction of substituents.

VREDNOTENJE VPLIVA IZBRANIH PROCESNIH PARAMETROV NA SPROŠČANJE MODELNE UČINKOVINE IZ HIDROFILNIH OGRODNIH TABLET

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Preučevali smo vpliv količine prečiščene vode pri vlažnem granuliranju, vpliv časa gnetenja mokrega granulata ter vpliv trdnosti izdelanih tablet na sproščanje modelne zdravilne učinkovine K-0408 iz hidrofilnih ogrodnih tablet s hipromelozo kot polimerom za zagotavljanje podaljšanega sproščanja. Ugotovili smo, da se modelna zdravilna učinkovina sprošča iz ogrodnih tablet s kinetiko 1. reda in dokazali, da poteka mehanizem sproščanja preko obeh procesov, tako difuzije kot tudi erozije ogrodja. V sklopu raziskav smo dokazali, da je proces izdelave preučevanih ogrodnih tablet dovolj robusten, saj znotraj testiranega območja izbrani faktorji ne izkazujejo značilnih vplivov na konstanto hitrosti sproščanja modelne zdravilne učinkovine in tudi ne na delež sproščene modelne učinkovine po izbranih časih. Prečiščena voda je najpomembnejši faktor, ki vpliva tako na privzem vode kot tudi na obseg erozije in rast granul med procesom granuliranja. Drugi faktor po pomembnosti je trdnost tablet, ki vpliva tako na privzem vode kot tudi na obseg erozije in na zmanjšanje poroznosti tablet. Gnetenje ima med vsemi preučevanimi faktorji najmanjši vpliv, in sicer le na povečanje privzema vode v tablete in zmanjšanje erozije po 1h testiranja. Ugotovili smo, da je od hitrosti privzema vode v tablete odvisna hitrost nastanka zaščitne gelske plasti in s tem povezane začetne erozije tablete.

EVALUATION OF INFLUENCE OF SELECTED PROCESS PARAMETERS ON MODEL DRUG RELEASE FROM HYDROPHILIC MATRIX TABLETS

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The influence of the amount of purified water for wet granulation, kneading time of wet granulate and hardness of tablets on the release of model drug K-0408 from hydrophilic matrix tablets containing hypromellose as a prolonged-release polymer were studied.
It was established that the model drug is released from the hydrophilic matrix tablets following first-order release kinetics and it was proven that the release mechanism of the model drug involves both processes, i.e. diffusion and matrix erosion.
It was established that the manufacturing process of the studied hydrophilic matrix tablets was sufficiently robust as within the tested levels of the selected factors, there were no significant influences on the model drug release rate constant or on the percentage of the model drug released within a selected time periods.
Purified water is the most important factor that affects tablet water uptake, the extent of tablet erosion and granule growth during the wet granulation process. The second important factor is tablet hardness, which affects tablet water uptake, the extent of tablet erosion and reduction of tablet porosity. Among all the factors studied, kneading time had the smallest influence, which was seen only in increased tablet water uptake and reduced tablet erosion after 1 hour of testing.
It was established that the rate of gel layer formation and the related initial tablet erosion depend on the rate of tablet water uptake.

CELOVITO ANALIZIRANJE USPEŠNOSTI POSLOVANJA SKUPINE TERME KRKA PO VIDIKIH URAVNOTEŽENEGA SISTEMA KAZALNIKOV

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Uravnoteženi sistem kazalnikov je učinkovita metoda za celovito analiziranje uspešnosti poslovanja podjetja, ki omogoča preoblikovati strategijo podjetja v splet finančnih in nefinančnih kazalnikov, s katerimi merimo in presojamo uspešnost poslovanja podjetja. Ta kompleksni model v vsakem trenutku pokaže, kje se podjetje nahaja in kako uspešno je pri uresničevanju svojih strateških ciljev, zagotavlja pa tudi obvladovanje sprememb, ki podjetju omogočajo rast in razvoj ter doseganje konkurenčnih prednosti kakor tudi njegovo uspešno poslovanje na dolgi rok. V svojem magistrskem delu sem oblikovala predlog uravnoteženega sistema kazalnikov za skupino Terme Krka na osnovi celovite analize uspešnosti poslovanja skupine v letih 2003-2007. Ugotavljala sem vzročno-posledične povezave med izbranimi strateškimi finančnimi in nefinančnimi kazalniki uspešnosti poslovanja skupine Terme Krka, pripravila predloge za izboljšanje uspešnosti poslovanja in oblikovala sistem poročanja o uresničevanju strateških ciljev in kazalnikov. Ta sodoben sistem strateškega poslovodenja bi lahko postal nov način uresničevanja strategije in bi pomenil celovito prenovo strateške usmerjenosti skupine Terme Krka kakor tudi spremembo mišljenja vseh njenih zaposlenih.

COMPREHENSIVE BUSINESS ANALYSIS OF THE GROUP TERME KRKA BY THE BALANCED SCORECARD VIEWS

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A balanced scorecard is a method for analyzing the overall performance of a company, enabling enterprises to transform strategy into financial and non-financial indicators that measure and assess the performance of the company. This complex model at any time shows where the company is and how well the company is achieving its strategic objectives, it also provides managed changes that allow the firm to grow and develop, and the achievement of competitive advantages as well as its long-term business success. In my Master's thesis I have a proposal for a Balanced scorecard for the Terme Krka group based on a comprehensive analysis of the business success of the group in the years 2003-2007. I evaluated the cause and effect relationship between selected strategic financial and non-financial indicators showing the operational performance of Terme Krka, make proposals to improve its business performance and create a system of reporting on the achievement of strategic objectives and indicators. This modern system of strategic management would become a new way of implementation of strategy and would be a comprehensive renovation of the strategic orientation of Terme Krka, as well as changing the thinking of all of its employees.

RENTGENSKA STRUKTURNA ANALIZA RAZLIČNIH SOLI GATIFLOKSACINA

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Vzgojili smo monokristale ter določili in analizirali strukture še ne preučene skupine soli (oksalat, p-toluensulfonat, sulfat, hidroklorid, 3,5-dinitrosalicilat in citrat) in hidrata gatifloksacina z rentgensko strukturno analizo. Uporabili smo klasičen pristop rentgenske strukturne analize na monokristalih. Strukture smo rešili z uporabo direktnih metod, grobe strukturne modele rešenih struktur pa smo izboljšali z minimizacijo razlike med izmerjenimi in izračunanimi amplitudami. Nato smo analizirali kristalne strukture ter izvedli primerjavo. Raziskovane strukture so z izjemo gatifloksacin hidrata zgrajene iz gatifloksacinijevih ter kislinskih anionov. V nekaterih strukturah so prisotne še molekule topila (voda). Prosti torzijski koti, ki določajo razlike v konformaciji gatfloksacina v različnih soleh so trije. Gre za kote med sistemom kinolinskih obročev gatifloksacina ter piperazinskim obročem, metoksi skupino in ciklopropilno skupino. Za vse raziskovane strukture je značilna intramolekularna vodikova vez med OH skupino karboksilne skupine in karbonilnim kisikom na sistemu kinolinskih obročev. Pri intermolekularnih vodikovih vezeh sodelujejo dušikov atom piperazinskega obroča, kislinski anioni in v nekaterih primerih še molekule vode. Poleg osnovnih strukturnih podatkov je podan kratek opis obravnavanih struktur.

X-RAY STRUCTURE ANALYSIS OF DIFFERENT GATIFLOXACIN SALTS

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The aim of this work was to grow single crystals and to determine and analyse the structures of new gatifloxacin hydrate and salts with the following acids: oxalic, 3-aminobutanoic, p-toluenesulfonic, sulphuric(VI), hydrochloric, 3,5-dinitrosalicylic and citric acid. Classical approach of the single crystal X-ray structure analysis was used. The models of solved structures were refined by minimisation of difference between measured and calculated amplitudes. The refined structures were then analysed and compared. The studied structures (except gatifloxacin hydrate) are composed of protonated molecules of gatifloxacin and acid anions. Additional molecules of solvent (water) are also present in some structures. There are three torsion angles, which define the gatifloxacin conformation. These are the torsions between the quinoline ring system and the piperazine ring, the methoxy group and the cyclopropyl group. There is a characteristic intramolecular hydrogen bond between the hydroxy group of the carboxylic group and the carbonyl oxygen, bound on the quinoline ring system in all structures. The intermolecular hydrogen bonds are formed among the protonated nitrogen of the piperazine ring and the acid anions as well as the water molecules in some cases. In addition to the basic structural data this work also contains a short description of the studied structures.

INDUKCIJA BIOSINTEZE ESTERAZE NA INDUSTRIJSKEM GOJIŠČU

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Encimi imajo dandanes velik spekter uporabe. Najdemo jih v prehrambeni industriji, pri proizvodnji detergentov in prav tako v farmacevtski industriji. Največ pozornosti pa se trenutno namenja prav uporabi encimov v organski sintezi, kar nam odpira nove in nove možnosti za načrtovanje preprostejših poti za sintetične procese, predvsem pa nove poti za rešitev okoljevarstvenih težav. Encimi esteraze pripadajo široki skupini hidrolaz. Sposobne so spodbujati širok spekter kemijskih reakcij. Stabilne in aktivne so tudi v organskih topilih. So katalizatorji reakcij, pri katerih se cepi ali tvori esterska vez spojine. S pomočjo substrata SL nam je uspelo optimizirati industrijsko gojišče za indukcijo esteraze iz mirkoorganizma MOE. Ugotovili smo pozitiven vpliv dodatka sojinega olja in glicerola k osnovnemu gojišču za uspešnejšo rast MOE in hitrejši potek biotransformacije. Uspešno je potekala tudi fermentacija v pilotnem merilu. Dosegli smo kar 99,8% konverzijo pretvorbe substrata SL s koncentracijo 10 g/l v produkt HSK.

INDUCTION OF ESTERASE BIOSYNTHESIS IN THE INDUSTRIAL MEDIUM

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Enzymes today have a large range of applications. We find them in the food industry, in detergent manufacturing and also in the pharmaceutical industry. Most attention is currently devoted to the use of enzymes in organic synthesis, which opens up new possibilities for designing a simpler route to synthetic processes and, above all, new ways to solve environmental pollution problems. An esterase is a hydrolase enzyme that is able to promote a wide range of chemical reactions, is stable and also active in organic solvents. These enzymes are reaction catalysts that cleave or form ester bonds. We succeeded in optimizing the industrial medium for the induction of esterase from microorganism MOE, using the SL substrate. We found a positive influence of the addition of soybean oil and glycerol to the basic medium, for a more effective and faster growth MOE and to accelerate speed of biotransformations. We also had sucess in the pilot-scale fermentation, where we achieved a 99.8% conversion of the SL substrate, with the concentration of 10 g/l, into the HSK product.

FLAVONOIDI KOT INHIBITORJI REKOMBINANTNE TRIHIDROKSINAFTALEN-REDUKTAZE IZ GLIVE *CURVULARIA LUNATA*

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Melanin ščiti temno pigmentirane glive pred stresnimi dejavniki iz okolja in je pomemben virulentni dejavnik patogenih gliv. Encimi, ki sodelujejo pri biosintezi glivnega melanina, predstavljajo zanimiva tarčna mesta za razvoj fungicidov in novih antimikotikov s selektivnim delovanjem. V glivi *Curvularia lunata* nastaja melanin iz 1,8-dihidroksinaftalena po pentaketidni poti. V okviru raziskovalnega dela smo proučevali strukturno različne flavone in derivate stilbena kot inhibitorje rekombinantne trihidroksinaftalen-reduktaze (3HNR). Spremljali smo od NADP(+) odvisno oksidacijo nefiziološkega substrata 2,3-dihidro-2,5-dihidroksi-4H-benzopiran-4-ona. Pri 40 µM koncentraciji substrata so bili najboljši inhibitorji 3HNR flavoni s hidroksilnima skupinama na mestih 5 in 7 ter metoksi skupino na mestu 4´ (acacetin, IC_{50} 4,9 µM in diosmetin, IC_{50} 5,5 µM) ter derivat stilbena dietilstilbestrol z IC_{50} 10 µM. Kinetične študije so pokazale, da dietilstilbestrol zavira delovanje 3HNR po mešanem tipu inhibicije s Ki 3,7 µM. *In vivo* preizkus inhibicije je pokazal, da ima flavon baikalein vpliv na rast in v manjši meri tudi na pigmentacijo glive *C. lunata*. Da bi dobili še bolj učinkovite inhibitorje 3HNR, ki bi bili uporabni kot spojine vodnice novih antimikotikov, so potrebne dodatne raziskave povezave med strukturo inhibitorjev in njihovim delovanjem.

FLAVONOIDS AS INHIBITORS OF RECOMBINANT TRIHYDROXYNAPHTHALENE REDUCTASE FROM FUNGUS *CURVULARIA LUNATA*

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Melanin protects dark-pigmented fungi from environmental stresses and serves as an important virulence factor in pathogenic fungi. The enzymes of melanin biosynthesis thus represent interesting targets for the development of fungicides and new selective antimycotics. In *Curvularia lunata* melanin is produced from 1,8-dihydroxynaphthalene via the pentaketide pathway. Here, we have examined structurally different flavones and stilbene derivatives as inhibitors of recombinant trihydroxynaphthalene reductase (3HNR) by following the NADP(+)-dependent oxidation of a non-physiological substrate, 2,3-dihydro-2,5-dihydroxy-4H-benzopyran-4-one. At 40 µM substrate concentration the most potent inhibitors were 5,7-dihydroxy-4´-methoxyflavones (acacetin, IC_{50} 4.9 µM and diosmetin, IC_{50} 5.5 µM) and stilbene derivative diethylstilbestrol (IC_{50} 10 µM). Kinetic studies confirmed that diethylstilbestrol acts as a mixed type inhibitor of 3HNR, with Ki value of 3.7 µM. *In vivo* inhibition study showed that flavone baicalein affects mainly fungal growth and less pigmentation. Our results support the need for further structure-activity based studies in the search for more potent inhibitors that can be used as lead compounds for future antimycotics.

DOLOČANJE KONČNE TOČKE SUŠENJA V VRTINČNOSLOJNEM GRANULATORJU Z UPORABO TEHNOLOGIJE BLIŽNJE RDEČE SPEKTROSKOPIJE (NIR)

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Prisotnost vlage ključno vpliva na fizikalno-kemijske lastnosti zrnc, kot so pretočnost, stisljivost, tvorba agregatov, kemijska in mikrobiološka stabilnost. Dovoljena vsebnost vlage v zrncih je zato za posamezen izdelek predpisana. Med procesom granulacije, t.j. v fazi sušenja je potrebna natančna določitev končne točke sušenja. Termogravimetrično določanje končne točke sušenja s halogenskim sušilnikom podaljša čas izdelave granulata tudi do 15%, zato smo raziskali smiselnost uvedbe spektroskopskega merilnika vlage, s katerim vsebnost vlage določamo indirektno in sicer z merjenjem absorbance. Pred izvedbo meritev naključnih vzorcev smo aparaturo umerili. Za 4 izdelke iz redne proizvodnje smo izdelali umeritvene krivulje in sicer tako, da smo z referenčno metodo granulatu določili vsebnost vlage, ki smo jo nato dodatno pomerili še spektroskopsko z NIR. Po umeritvi aparata smo za vsak izdelek iz proizvodnje odvzeli naključni vzorec in mu določili vsebnost vlage. Ugotovili smo, da je uvedba merilnika za tri izbrane izdelke smiselna, v primeru enega pa je prišlo do odstopanj, zato bi bilo potrebno opraviti še nekaj dodatnih raziskav, saj nam bo to omogočilo skrajšanje časa potrjevanja končne točke sušenja granulata in večjo ekonomičnost proizvodnje.

DRYING END POINT DETERMINATION IN FLUID BED GRANULATOR USING NEAR INFRARED (NIR) TECHNIQUE

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The presence of moisture plays a key role in providing proper physical-chemical characteristics of granules, as are flow properties, compressibility, formation of aggregates, chemical and microbiological stability. Consequently the allowed moisture content in granules is prescribed for an individual product. During the granulation process, i.e. in the stage of drying, precise end point of drying must be determined. Because thermogravimetric method for assessing end point of drying with halogen dryer prolongs duration of granulate manufacture even up to 15%, we have researched a reasonability of introducing a spectral moisture meter, with which we can indirectly, through absorbance measuring, determine moisture content. Prior to measurement testing of random samples, we have calibrated apparatus. We have created calibration curves for 4 products in regular production by means of reference method, where we have firstly assessed moisture content for granulated form and afterwards made an additional spectroscopy measurement of a product with near infrared (NIR). Following the stage of calibration apparatus, we have taken a random sample for each product in production and determined its moisture content. Results of the study lead us to the conclusion that the introduction of a NIR for the three selected products was successful. Though we noted distortions in the case of one of the selected product, we are aware of necessity for execution of further research, since such method can allow us to shorten the duration of determination of the end point of drying granules and enable a grater economy of production.

UPORABA JEDRSKE KVADRUPOLNE RESONANCE ZA KVALITATIVNO IN KVANTITATIVNO PROUČEVANJE POLIMORFIZMA NIFEDIPINA IN PIROKSIKAMA

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Preverili smo sposobnost ^{14}N jedrske kvadrupolne resonance (JKR), da razlikuje med različnimi oblikami piroksikama ter nifedipina. Kot referečni metodi smo uporabili DSC ter FT-IR. Pri piroksikam I smo odkrili dve resonančni frekvenci pri 2587 ter 3439 kHz. Pripravili smo tudi dve dodatni obliki piroksikama s prekristalizacijo iz 1,2-dikloroetana ter etanola. Spektra obeh oblik se pri 2587kHz bistveno razlikujejta od spektra oblike I. Pri obeh frekvencah smo opazovali tudi odvisnost naraščanja intenzitete signala od volumskega deleža oblike I v različnih zmeseh s piroksikam monohidratom. Linearnost je bila jasno izražena z veliko močjo povezave. Pri nifedipinu I smo odkrili resonančne frekvence pri 2170 ter 2590 kHz. Amorfna oblika nifedipina pri 2590 kHz ni imela odziva. Zatem smo pri tej frekvenci opazovali nastajanje nifedipina I. Prikaz odvisnosti intenzitete signala od časa je razkril sigmoidno krivuljo, katere navidezni plato je bil odvisen od temperature. Dodatno smo z metodo JKR študirali še različne oblike sulfanilamida. ^{14}N JKR metoda je sposobna razlikovati med posameznimi oblikami učinkovin, ob primerno močnem signalu je ustrezna tudi za morebitna kvantitativna opazovanja. Pri tem smo prikazali tudi novo možnost uporabe ^{14}N JKR za proučevanje dinamičnih procesov kristalizacije iz amorfnega stanja.

UTILIZATION OF NUCLEAR QUADRUPOLE RESONANCE FOR QUALITATIVE AND QUANTITATIVE STUDY OF NIFEDIPINE AND PIROXICAM POLYMORPHISM

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We tested the capability of ^{14}N nuclear quadrupole resonance (NQR) to discern between different forms of piroxicam and nifedipine. As reference methods DSC and FT-IR were employed. Two resonant frequencies of piroxicam I were discovered at 2587 and 3439 kHz. Additional samples of piroxicam were prepared with recrystallization from 1,2-dichloroethane and ethanol. Spectra of both recrystallized forms differed significantly from the spectre of form I at 2587 kHz. At both resonant frequencies dependence of signal intensity on a volume fraction of piroxicam I in mixtures with piroxicam monohydrate was examined. Clear linear relationship was established with large value of correlation coefficient. Two NQR resonant frequencies of nifedipine I were discovered at 2170 and 2590 kHz. No signal was recorded for amorphous form of nifedipine at 2590 kHz. Transition from amorphous form to nifedipine was subsequently studied at 2590 kHz. Dependence of signal intensity on time expressed a sigmoid curve, whose apparent steady state value was temperature dependent. Additionally different sulfanilamide samples were studied with the NQR method. ^{14}N NQR is capable od differentiating between different forms of active ingredients. When the signal intensity is high enough, quantitative measurements are also possible. Additionally a new possibility of ^{14}N NQR application for the study of dynamical crystallization processes was demonstrated

STRUKTURNE ŠTUDIJE KVADRUPLEKSA PROMOTORSKE REGIJE GENA *iTSN*1 V RAZTOPINI

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V diplomskem delu smo s pomočjo NMR meritev, CD in UV spektroskopije proučevali stukturne značilnosti zaporedja d[GGGAGCGAGGGAGCG] promotorske regije gena *itsn*1. Gen *itsn*1 je povezan z boleznimi, kot sta Downov sindrom in karcinom dojke. Opazovali smo vpliv koncentracije NaCl na zvitje kvadrupleksa. Proučevali smo dimerizacijo kvadrupleksa ITSN1 in jo potrdili z merjenjem translacijskega difuzijskega koeficienta. Ugotavljali smo temperaturno odvisnost disociacije dimera in stabilnost posamezne enote kvadrupleksa. S pomočjo UV spektrofotometrije smo opazili dvostopenski talilni prehod kvadrupleksa ITSN1. Pokazali smo, da so interakcije med obema enotama dimernega kvadrupleksa šibkejše kot pa interakcije, ki povezujejo posamezen monomerni kvadrupleks. Posebna značilnost kvadrupleksa sta Watson-Crickova bazna para, ki predstavljata najstabilnejšo asociacijo posameznega monomernega kvadrupleksa. Skupaj tvorita G•C•G•C tetrado, ki je v zamaknjeni obliki. Takšna postavitev G•C•G•C tetrade ob prisotnosti Na^+ ionov do sedaj še ni bila opisana. CD spektroskopija kaže na paralelno in antiparalelno ureditev verig. Na podlagi različnih NMR eksperimentov smo določili usmerjenost verig in orientacijo tetrad v smeri urnega kazalca. Vsak spekter, ki smo ga posneli pri določeni koncentraciji, nam je dal košček sestavljanke, da smo na koncu lahko določili topologijo kvadrupleksa ITSN1.

STRUCTURAL STUDIES OF QUADRUPLEX FROM PROMOTER REGION OF GENE *iTSN*1 IN SOLUTION

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In diploma work we investigated stuctural characteristics of sequence d[GGGAGCGAGGGAGCG] from promotor region of the gene *itsn*1 with NMR measurements, CD and UV spectroscopy. Gene *itsn*1 is associated with diseases such as Down's syndrome and breast carcinoma. We observed the impact of NaCl concentration on the folding of quadruplex. We studied dimerization of quadruplex ITSN1 and confirmed it by measuring the translation diffusion coefficient. We observed the temperature dependence of dimer dissociation and the stability of each unit of quadruplex. Using UV spectrophotometry we observed two-stage transition of quadruplex ITSN1. We show that the interaction between the two units of quadruplexes dimer is weaker than the interactions that link the individual monomer qaudruplex. A special features of quadruplex are the Watson-Crick base pairs which represent the most stable association of each monomer quadruplex. Together they form G•C•G•C tetrad which is in the sheared form. Till now such G•C•G•C tetrade in the presence of Na^+ ions have not been described. CD spectroscopy showed a parallel and antiparallel arrangement of strands. On the basis of various NMR experiments, we determine the direction of the strands and orientation of tetrads, all oriented clockwise. Each spectrum which was recorded at a certain concentration gave us a piece of the puzzle that helped us to determine the topology of quadruplex ITSN1.

PRIPRAVA IN IZOLACIJA HIMERNIH FLAGELINOV ZA AKTIVACIJO IMUNSKEGA ODZIVA

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Helicobacter pylori je po Gramu negativna bakterija, ki pri večini gostiteljev ne povzroča kliničnih simptomov, kljub temu jo pri manjšem deležu bolnikov povezujejo z nastankom gastritisa, peptične razjede in celo raka na želodcu. Ta običkan patogen se uspešno izogiba imunskemu odzivu, tudi z izmikanjem prepoznavanja flagelina preko Toll-u podobnega receptorja 5 (TLR5). Za aktivacijo receptorja TLR5 sta potrebna amino- (N) in karboksi- (C) končna dela flagelina bakterije kot je *Escherichia coli*, medtem ko notranja, hipervariabilna regija ni ključna pri signaliziranju preko receptorja TLR5, ampak je pomembna za določanje antigenosti flagelina. V diplomskem delu smo pripravili nov himerni flagelin, tako da smo N- in C-končna dela flagelina *H. pylori* zamenjali z N- in C-končnima deloma flagelina *E. coli*, ki aktivira receptor TLR5. Na C-končni del smo dodali še multiepitop in RGD tripeptid ter histidinski rep za izolacijo proteinov s kelatno kromatografijo. Himerni flagelin smo izražali v *E. coli*, ga izolirali in očistili. Dokazali smo, da sta, tako himerni flagelin kot himerni flagelin z multiepitopom, sposobna aktivacije TLR5 signalne poti. Aktivacijo pridobljenega imunskega odziva smo testirali *in vivo* na miškah, na katerih smo pokazali odziv serumskih IgG protiteles z ELISA testom. Naši rezultati so pomembni pri razvoju novega in učinkovitega cepiva proti človeškemu patogenu *Helicobacter pylori*.

PREPARING AND ISOLATION OF CHIMERIC FLAGELLIN FOR ACTIVATION OF IMMUNE RESPONSE

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Helicobacter pylori is Gram-negative bacterium that in most hosts does not cause clinical symptoms, however a subset of patients develops gastritis, peptic ulcer, and even gastric cancer. This flagellated pathogen escapes immune clearance by avoiding detection by the flagellin receptor Toll-like receptor 5 (TLR5). For activation of TLR5 amino- (N) and carboxy- (C) terminal segment of flagellin of bacteria such as *Escherichia coli* are required. In contrast, it is known that the hypervariable region is not mandatory for TLR5 signaling but is involved in flagellin antigenicity. We therefore constructed a chimeric flagellin by replacing the N- and C-terminal segment of *H. pylori* flagellin with segments from TLR5 activating flagellin from *E. coli*. We expected that such an engineered chimeric flagellin would be able to activate innate immune response by TLR5 signaling pathway. To the C-terminal segment we also added multiepitope, tripeptide sequence RGD and His-tag for Ni-NTA purification. Chimeric flagellins were produced in *E. coli*, isolated and purified. We demonstrated that both chimeric flagellin and chimeric flagellin with multiepitope were able to activate TLR5. The activation of adaptive immune response was tested *in vivo* on mice, where we have shown increased serum IgG antibody response by ELISA. Our results are important for developing a new and effective vaccine against human pathogen *Helicobacter pylori*.

OSAMITEV MIKROORGANIZMOV Z IZBRANIMI ENCIMSKIMI AKTIVNOSTMI

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Encimi omogočajo potek metabolnih poti v mikroorganizmih. Preverili smo prisotnost mikroorganizmov z o-demetilaznimi, monooksigenaznimi, esteraznimi in nitrilaznimi encimi. Okoljske vzorce smo obogatili v minimalnem gojišču z dodatkom selektivnih substratov. S presejalnimi testi smo kvantitativno ovrednotili encimske aktivnosti kot posledico pretvorbe substratov in nastanka obarvanega ali fluorescirajočega produkta. O-demetilazno aktivnost smo odkrili pri 29 sevih. Najbolj aktiven encim ima *C. testosteroni* s specifično aktivnostjo 3,7 ΔA/g mol. Pri *M. lutues, M. sedentarius, C. aquaticum, S. sanguis, E. aerogenes, S.spiritivorum, S. pneumoniae, S. maltophilia., P. stutzeri, A. denitrificans* smo identificirali o-demetilazno aktivnost, o kateri predhodno niso poročali v literaturi. Od 10 izoliranih sevov smo pri *S. uberis, A. faecalis, A. urinae, S. maltophilia* na novo odkrili monooksigenaznim encim. Največjo specifično monooksigenazno aktivnost ima *Stenotrophomonas maltophilia* (2×10^{-8} mol/g min). Esterazno aktivnost smo med 8 sevi na novo odkrili pri *S. sanguis, O. anthropi, A. urinae, S. uberis*. Najbolj aktiven sev je *C. jeikerum*, s specifično aktivnostjo 1190 /g. Prisotnost nitrilaz smo med 4 sevi na novo identificirali pri *C. jeikeium* in *S. maltophilia*. Najbolj aktiven nitrilazni encim spodoben razgradnje alifatskih nitrilov smo odkrili pri *P. aeruginosa*. Specifična aktivnost je bila 879 g NH_4/ mg proteinov v min. Ti izolirani vzorci z dokazanimi encimskimi aktivnostmi lahko pripomorejo k poenostavitvi proizvodnjih procesov, njihovi ekonomičnosti, okolju sprejemljivejši sintezi organskih snovi, uničenju nezaželenih snovi, pretvorbi substrata v bolj polarno, topno ali aktivno obliko za nadaljnje procese.

ISOLATION OF MICROORGANISMS WITH CHOSEN ENZYME ACTIVITIES

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Enzymes are catalysts in living cells. We were screening environment samples for the biocatalysts with o-demethylase, monooxygenase, esterase and nitrilase enzymes. The use of carefully selected substrate results in either colored or fluorescent quantification of products. This allow us to identify active microorganisms. We isolated 29 microorganisms with an active o-demethylase activity. The most active enzyme was detected in C. testosteroni with specific enzyme activity of 3,7 ΔA/g mol. In *M. lutues, M. sedentarius, C. aquaticum, S. sanguis, E. aerogenes, S. spiritivorum, S. pneumoniae, S. maltophilia., P. stutzeri and A. denitrificans* enzyme activity has not been previously reported in scientific literature. From 10 monooxygenase active strains, *S. uberis, A. faecalis, A. urinae* and *S. maltophilia*, have not been previously reported as active. The specific activity of *S. maltophilia* is the highest from all (2×10^{-8} mol/g min). Esterase activity was newly discovered in *S. sanguis, O. anthropi, A. urinae* and *S. uberis*. *C. jeikerum* has the most active enzyme from all of organisms studied (1190 /g). The presence of nitrilase was indentified in *C. jeikeium* and *S. maltophilia* and previously not reported in the literature. The most active nitrilase strain is *P. aeruginosa* showing the specific activity of 879 g NH_4/ mg proteins min. Simple environment samples allow discoveries of new microorganisms with enzyme activities, identification of new enzyme types and new metabolic pathways. The power of this method is to create an ideal biocatalyst for the majority of applications used in the industry and in the field of science.

DELOVANJE RAZLIČNIH KOZMETIČNIH PREPARATOV PROTI AKNAM NA *STAPHYLOCOCCUS AUREUS* IN NJIHOVO DELOVANJE NA RAZLIČNE TIPE MLADOSTNIŠKE KOŽE

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Staphylococcus aureus ali zlati stafilokok je kroglasta bakterija, ki pri človeku povzroča različne okužbe kože in notranjih organov. Je fakultativni anaerob in Gram pozitivna bakterija. Začetna faza našega raziskovalnega dela je bila, da smo na 32. različnih sredstvih za čiščenje kože preiskovali, ali določeno sredstvo zavira rast bakterije *Staphylococcus aureus*. Testirali smo vrsto izdelkov različnih proizvajalcev, med njimi tudi preparate blagovne znamke VITASKIN (VITASKIN gel za čiščenje, VITASKIN matirajoči vlažilni fluid, VITASKIN ekstra blago čistilno mleko, VITASKIN seboregulacijski fluid, VITASKIN čistilno mleko in tonik, VITASKIN kumarično čistilno mleko ter VITASKIN ekstra blag tonik). S pomočjo metode bakteriograma smo ugotovili, da imajo čistilna sredstva različnih proizvajalcev različen učinek na omenjeno bakterijo. Pri večini ni bilo inhibicijskih con, kar pomeni, da sredstva na bakterijo ne delujejo in s tem ne zavirajo njene rasti, medtem ko osvežilni negovalni kremni gel druge znamke in VITASKIN ekstra blag tonik najbolje zavirata bakterijsko rast, saj se pojavijo največje inhibicijske cone. V drugem delu naše raziskovalne naloge smo raziskovali delovanje tistih čistilnih sredstev za kožo, kjer so se pojavile inhibicijske cone. Iz treh različnih tipov kože smo izolirali izolat A, B in C, ki smo ga nacepili v gojišče A, B in C. Glede na to, da ima vsak človek različno mikrofloro na obrazu, so ista sredstva različno delovala na različne izolate.

EFFECTS OF VARIOUS ACNE SKIN CARE PRODUCTS ON *STAPHYLOCOCCUS AUREUS* AND ON DIFFERENT TYPES OF YOUTH SKIN

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Staphylococcus aureus or the golden staphylococcus is a spherical bacterium, which causes various human infections of skin and internal organs. It is a facultative anaerobic and Gram-positive bacterium. In our research, we took 32 different skincare products and examined whether they prevent *Staphylococcus aureus* from further growing. On the basis of the bacteriogram, we discovered that skincare products of various brands have different effects on the bacterium. The products from VITASKIN brand, namely VITASKIN Cleansing Gel, VITASKIN Matt Moisturizing Fluid, VITASKIN Extra Mild Cleansing Milk, VITASKIN Seboregulating Fluid, VITASKIN Cleansing Milk and Tonic, VITASKIN Cucumber Cleansing Milk and cleansing milk of other brand had no zones of inhibition. Therefore, these products do not affect the bacterium and, consequently, do not prevent its growth. However, three other products, among them VITASKIN Extra Mild Tonic, are the best in growth prevention, because the largest zones of inhibition are produced here. In the second part, we examined those skincare products that include zones of inhibition. We isolated the A, B and C isolate from three types of skin, and inoculated them into the A, B and C medium. Given that each person has a different microflora on his or her face, the same products had different effects on the isolates.

PREŽIVETJE SPOR BAKTERIJE VRSTE *BACILLUS SUBTILIS* V SIMULIRANIH POGOJIH PRAZNEGA IN POLNEGA ŽELODCA

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Pri izbranem sevu bakterije *Bacillus subtilis* (CBS117162) smo preučili njegovo odpornost proti solni kislini, eni od ključnih sestavin želodčnega soka, in sicer pri različnih vrednostih pH, s katerimi smo posnemali situacijo praznega in polnega želodca.
V svojih poskusih smo kolikor je to mogoče na enostaven in učinkovit način, posnemali pogoje, ki vladajo v prebavilih monogastričnih živali.
S tem smo prispevali dodatne dokaze, da ostane kultura probiotičnega seva *Bacillus subtilis* viabilna tudi po prehodu skozi želodec, saj v pogojih simuliranega polnega želodca, število živih celic ostane na isti ravni kot pred obravnavo.

VIABILITY OF *BACILLUS SUBTILIS* SPORES IN SIMULATED CONDITIONS OF AN EMPTY AND FULL STOMACH

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We explored resistance of the selected strain of *Bacillus subtilis* (CBS117162) for the hydrochloric acid, one of the major components of gastric juice, at different pH values simulating conditions within empty and full stomach.
Our experiments were designed as simple and as efficient as possible to simulate conditions in the intestine of the monogastric animals.
We participated additional evidence for the excelent viability of the probiotic strain *Bacillus subtilis* since the level of viable cells remains equal to the initial count also after the simulated stomach passage.

ANALGETIKI V VSAKDANJEM ŽIVLJENJU

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Bolečina je neprijeten občutek, ki otežuje naše življenje in jo je zato potrebno odpraviti. Paracetamol, acetilsalicilna kislina, ibuprofen, naproksen… To so le nekatere izmed številnih zdravilnih učinkovin, ki nam pomagajo pri lajšanju bolečine. Protibolečinska zdravila ali analgetiki na različne načine delujejo na periferni in centralni živčni sistem. Ločimo opioidne in neopioidne analgetike. Med prve uvrščamo učinkovine, kot so morfin, metadon, tramadol in z njimi lajšamo močnejše bolečine. Med neopioidnimi analgetiki sta najbolj pogosti učinkovini paracetamol in acetilsalicilna kislina, ki pa se ju uporablja predvsem pri blažjih bolečinah. Analgetiki blažijo tako akutno kot kronično bolečino. V vsakdanjem življenju imajo torej velik pomen, saj ob pravilni uporabi izboljšujejo njegovo kakovost. A kako dobro so ljudje seznanjeni z navodili za pravilno uporabo oz. kako pogosto jih upoštevajo? To sta bili le dve izmed vprašanj, postavljenih v naši anketi, s katero smo želele ugotoviti odnos ljudi do analgetikov in zdravil nasploh ter kakšne so razlike med spoloma.

ANALGESICS IN EVERYDAY LIFE

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Pain is the unpleasant feeling, which makes our life difficult and that is why we try our best to make it go away. Paracetamol, acetylsalicylic acid, ibuprofen, naproxen... These are only few of many active ingredients, which help us to cope with the pain. Painkillers or analgesic drugs act in various ways on the peripheral and central nervous systems. We distinguish between opioid and non-opioid analgesics. The opioids, such as morphine, methadone, tramadol are used for relieving severe pain. Non-opioids – most used are paracetamol and acetylsalicylic acid – relieve mild pain. Analgesics are helpful in cases of chronic and acute pain. Painkillers have an important role in everyday life because when used properly they can greatly improve our life quality. But how informed are people with instructions for use and how often do they follow them? These were just two of the questions put in our questionnaire where we wanted to find out people's relation to analgesics and medicaments in general and what are the differences between male and female users of drugs.

INGVER, ČUDEŽNA KORENIKA?

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Ingver se v zadnjem času veliko pojavlja v različnih prehrambenih izdelkih, omenja se v časopisnih člankih in kulinaričnih revijah in velja za precej eksotično rastlino.
V raziskovalni nalogi smo preučevale izvor, poznavanje in uporabo ingverja. V literaturi in na spletu smo poiskale informacije o ingverju, zdravilnih učinkovinah v ingverjevem olju, njegovi uporabi in učinkovanju na zdravje ter dobro počutje.
Ker smo pri tem ugotovile, da je po nekaterih virih ingver skoraj čudežna rastlina, smo izvedle anketo o poznavanju in možnostih uporabe ingverja v zdravilne namene. Iz rezultatov ankete je razvidno, da ljudje slabo poznajo možnosti uporabe ingverja, bi pa ga v velikem deležu uporabljali kot pomožno zdravilo za raznovrstne tegobe, če bi bil seveda na razpolago v primerni obliki. Okus sveže korenike namreč ni lastnost, ki bi anketirance prepričala v uporabo nepredelanega.
V iskanju primernih oblik za uporabo ingverja smo v eksperimentalnem delu naredile test občutljivosti kože na ingverjevo olje in pripravile ekstrakt ingverja, ingverjev sirup, tinkturo ingverja ter ingverjevo kremo.

GINGER, MAGIC RHIZOME?

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Ginger has lately been appearing quite a lot in different provisions, it has been mentioned in newspaper articles and in culinary magazines, and is regarded as a rather exotic plant.
The research studies the origin, the use and the knowledge about ginger. We have used the literature and internet to seek information about ginger, medicinal properties of ginger oil, its use and effect on health and general well-being.
Since we have discovered that according to some sources ginger is considered an almost magic rhizome, we have conducted a survey about the knowledge about and possible uses of ginger for medicinal purposes. The results of the survey show that people are not very familiar with the possible uses of ginger, but the majority would use it as an additional medicine for various difficulties – if it were available in a suitable form. Namely, the taste of fresh rhizome is not the property which would convince the people questioned to use it unprocessed.
In our search for the appropriate forms of ginger we have performed a test of skin sensitivity on ginger oil and prepared a ginger extract, ginger syrup, ginger tincture and ginger cream.

e - Krkine nagrade e - Krka Prizes

VPLIV POLARNOSTI TOPIL PRI KRISTALIZACIJI MODELNE ZDRAVILNE UČINKOVINE K-2605/05 NA FIZIKALNE LASTNOSTI DELCEV

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Pogoji kristalizacije vplivajo tako na notranjo strukturo, kot tudi na zunanjo obliko kristalov, kar posledično vodi v različne fizikalno-kemijske lastnosti substance. Namen naloge je bil preučiti vpliv različnih kristalizacijskih topil (čista organska topila) na izbrane fizikalno-kemijske lastnosti modelne zdravilne učinkovine K-2605/05.
Pojavnost polimorfizma smo ugotavljali z rentgensko praškovno difrakcijo, IR spektroskopijo, termičnimi analizami ter helijevo piknometrijo. Z uporabo topil z različno polarnostjo za kristalizacijo smo uspeli izolirati novo polimorfno obliko K-2605/05. Z opazovanjem vzorcev modelne zdravilne učinkovine, kristalizirane iz različnih topil, pod optičnim in elektronskim mikroskopom ter z analizo porazdelitve velikosti delcev smo opazili razlike v zunanji obliki in velikosti kristalov. Z inverzno plinsko kromatografijo smo ugotovili, da vzorci, prekristalizirani iz bolj nepolarnih topil, izkazujejo višje nepolarne komponente površinske energije. Pri rezultatih analiz intrinzične hitrosti raztapljanja in kinetične topnosti pa smo opazili predvsem višje vrednosti pri vzorcih z novo kristalno obliko. Razlike med ostalimi vzorci so sicer bile opazne, vendar jih nismo mogli nedvoumno povezati s polarnostjo topila, uporabljenega pri kristalizaciji.

THE INFLUENCE OF SOLVENT POLARITY ON CRYSTALLIZATION OF MODEL ACTIVE INGREDIENT K-2605/05 ON PHYSICAL PROPERTIES OF PARTICLES

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Crystallization conditions affect both the crystal form, as well as the crystal habit and the influence is reflected in different physicochemical characteristics of the crystals. The purpose of the study was to examine the effect of different crystallizing solvents (pure organic solvents) on selected physicochemical properties of a model active ingredient K-2605/05.
Polymorphism was studied with X-ray powder diffraction, IR spectroscopy, thermal analysis and helium pycnometry. A new polymorphic form was obtained by crystallization of the K-2605/05 from various solvents differing in their polarity. By observing the samples of the model drug with optical and electron microscopy and analysis of the particle size distribution, differences in outer shape and particle size of the crystals were observed. With inverse gas chromatography we found that the samples crystallized from more non-polar solvents show higher non-polar components of surface energy. Intrinsic dissolution rate and kinetic solubility studies brought out mainly higher values for samples with the new crystal form, while differences between other samples were noticable but difficult to relate to crystallization solvent polarity.

SOLUBILIZACIJA SLABO TOPNIH ZDRAVILNIH UČINKOVIN

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Na tržišču je vedno več zdravilnih učinkovin BCS razreda II, ki so slabo topne in dobro permeabilne. Njihova biološka uporabnost je zaradi tega omejena s hitrostjo raztapljanja. Hitrost raztapljanja lahko povečamo predvsem preko dveh mehanizmov: to je s povečanjem površine učinkovine, ki je v stiku s tekočino in zvišanjem nasičene topnosti. Cilj farmacevtskega tehnologa je pripraviti takšno farmacevtsko obliko, ki bo slabo topni učinkovini omogočala hitro raztapljanje in s tem posledično izboljšanje biološke uporabnosti. To lahko dosežemo z naslednjimi pristopi: tvorba soli in uravnavanje pH, tvorba trdnih disperzij, zmanjšanje velikosti delcev, tvorba različnih polimorfov in amorfov, priprava formulacij, ki temeljijo na lipidih, tvorba kokristalov, adsorpcija na nosilce, uporaba površinsko aktivnih snovi in druge.

SOLUBILIZATION OF POORLY SOLUBLE DRUGS

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More and more BCS class II drugs are coming to the market - these drugs are poorly soluble and have good permeability. Their bioavailability is therefore limited by their dissolution rate. Dissolution rate can be in general increased using two approaches: with the increase of surface area of the drug and with increase of its solubility. The goal of pharmaceutical technologist is to prepare such a dosage form with poorly soluble drug that will have sufficient dissolution rate to achieve required bioavailability of the drug. That can be achieved with the following methods: salt formation and pH adjustment, formulation of solid dispersions, particle size reduction, preparation of various polymorphous and amorphous forms, lipid based formulations, co-crystal formation, using adsorbents or surface active agents and others.

SOLUBILIZACIJA TEŽKO TOPNIH ZDRAVILNIH UČINKOVIN S POMOČJO IZDELAVE KOKRISTALOV

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Namen najine naloge je bil raziskati inovativne formulacijske in tehnološko-procesne pristope k solubilizaciji slabo topnih zdravilnih učinkovin na primeru trdnih peroralnih farmacevtskih oblik. Kot enega od inovativnih procesov za izboljšanje solubilizacije pri trdnih peroralnih farmacevtskih oblikah sva opisala kokristalizacijo in tvorbo kokristalov. Zelo široka definicija definira kokristal kot večkomponentni kristal ali kristal, ki vsebuje dve različni molekuli, ki sta med seboj povezani z eno izmed nekovalentnih interakcij, med katerimi pa je najpomembnejša H-vez. Kokristali naj bi bili narejeni iz reaktantov, ki so pri sobni temparaturi v trdnem agregatnem stanju. Opisala sva tudi razvoj kokristalov in njihove fizikalno-kemijske lastnosti, med katerimi lahko omeniva izboljšano topnost in hitrost raztapljanja v primerjavi z izhodno težko topno spojino, s tem pa se poveča tudi biološka uporabnost, kar je ključnega pomena za farmacevtsko industrijo. Raziskovala sva tudi možnosti njihove izdelave ter njihove ostale pozitivne lastnosti za uporabo v farmacevtski industriji.

SOLUBILIZATION OF POORLY SOLUBLE ACTIVE INGREDIENTS BY FORMULATING COCRYSTALS

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The purpose of our research was to research innovative formulational and technological approaches of solubilizaton of poorly soluble active ingredients in the case of oral solid dosage forms. One of the innovative approaches to improve solubilization of solid oral dosage forms is by formulating cocrystals. Very wide definition defines cocrystal as multicomponent crystal or crystal which contains two different molecules, which are bonded together by one of the non-covalent interaction. The most important interaction is H-bond. Cocrystals have to be made out of the reactants which are solid at room temperature. We have also described evolution of cocrystals and their physical-chemical properties. One of them is better solubility and the velocity of solubing in compar to the starting poorly soluble compound. As a result, the biological applicability is increased whitch is very important for pharmaceutical industry. In our research we have also talked about possibilities how to make them and about their other positive prefrences for use in the pharmaceutical industry.

VPLIV PROCESNIH PARAMETROV KRISTALIZACIJE NA LASTNOSTI UČINKOVINE S HIPERTENZIČNIM DELOVANJEM

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Kristalizacija je pomembna kemijska in tehnološka operacija s katero iz produkta odstranimo nečistoče, zagotovimo pravilno polimorfno obliko ter želeno velikost in obliko delcev. Raziskovalna naloga predstavlja proučevanje vplivov procesnih parametrov kristalizacije na polimorfizem ter velikost in obliko delcev organske spojine irbesartan (2-butyl-1-[2´-(tetrazol-5-yl)biphenyl-4-ylmethyl]-1,3-diazaspiro[4,4]non-2en-5-one), ki se v farmacevtski industriji uporablja kot učinkovina za zdravljenje bolezni srca in ožilja, centralnega živčevja, glavkoma, retinopatije ter diabetične nefropatije. Za molekulo irbesartan sta značilni dve polimorfni obliki (polimorfna oblika A in polimorfna oblika B). S spreminjanjem procesnih parametrov kristalizacije sem določil pogoje za nastanek obeh polimorfnih oblik.

INFLUENCE OF CRYSTALLIZATION PROCESS PARAMETERS ON CHARACTERISTICS OF PHARMACEUTICAL COMPOUND USED FOR TREATMENT OF HYPERTENSION

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Crystallization has become one of the most important unit operation in chemical and pharmaceutical industry, which can insure product quality characteristics, right polymorphic transformation and right particle size. In this work process parameters which affect the crystallization of irbesartan, 2-butyl-1-[2´-(tetrazol-5-yl)biphenyl-4-ylmethyl]-1,3-diazaspiro[4,4]non-2en-5-one, were studied. Irbesartan is a pharmaceutical compound which is useful in the treatment of cardiovascular diseases, in the treatment of diseases of the central nervous system, in the treatment of glaucoma and diabetic retinopathy and diabetic nephropathy. Irbesartan exists in the solid state as two distinct forms (form A and form B) which were studied in this work.

PASIVNI PREHOD IONIZIRANIH MOLEKUL SKOZI CELIČNE MEMBRANE: V KOLIKŠNI MERI LAHKO MOČNI ELEKTROLITI PREHAJAJO CELIČNE MEMBRANE?

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V delu je predstavljen matematični model za opis transmembranskega transporta disociirajočih molekul, ki vključuje disociacijsko ravnotežje in možnost nastanka ionskih parov ter od velikosti molekule odvisen difuzijski koeficient. Model je rešen numerično, in sicer za različne vrednosti disociacijskih in asociacijskih konstant. Rezultati modela so pokazali, da v primeru majhnih molekul, ki močno disociirajo, transport ionskega para, čeprav je v primerjavi s prenosom nedisociirane oblike zelo počasen, predstavlja poglaviten prispevek k celokupni količini prenešene snovi. Ta prispevek je zanemarljiv v primeru šibko disociirajočih molekul. Model je mogoče poljubno nadgraditi, tako da vključuje določene dodatne fiziološke procese, kot npr. aktivni efluks molekul, morebitna asociacijska ravnovesja nedisociiranih oblik, asociacijska ravnovesja z mobilnimi prenašalnimi molekulami, itd. Vrednosti asociacijskih ter disociacijskih konstant je mogoče izračunati s pomočjo računalniških simulacij, kar omogoča vključitev dejanskih parametrov specifičnih molekul v model transmembranskega transporta.

PASSIVE TRANSPORT OF IONIZED MOLECULES THROUGH CELL MEMBRANES: IN WHAT EXTENT ARE STRONG ELECTROLYTES ABLE TO CROSS THE CELL MEMBRANE?

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A mathematical model is constructed to describe the transport of ionizable molecules through the cell membrane, which takes into account the dissociation equilibrium, the possibility of ion-pair formation and a size dependent diffusion coefficient. The model is solved numerically for several different values of the dissociation and ion-pair association constants. The results predict that ion-pair transport, although being significantly slower than the transport of the undissociated form, represents the dominant contribution to the total amount of transferred substance in case of small molecules which dissociate strongly. This contribution is negligible in case of weakly dissociated molecules. The model can be upgraded to include additional physiological processes, such as active eflux, association equilibrium between undissociated forms, association with mobile carriers, ect. Actual values of association and dissociation constants can be calculated by means of computer simulations, which enable the construction of transport models, which are designed for specific drug molecules.

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