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1st HUNGARIAN-SINGAPOREAN WORKSHOP on DRUG DISCOVERY and BIOMATERIALS

Hungarian Academy of Sciences
Budapest

March 10-11, 2008

PROGRAMME AND BOOK OF ABSTRACTS

Ist HUNGARIAN-SINGAPOREAN WORKSHOP on DRUG DISCOVERY and BIOMATERIALS

Hungarian Academy of Sciences
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organized by the Hungarian Chemical Society,
sponsored by the National Office for Research and Technology (NKTH), Hungary

Chairman: Prof. Péter Mátyus
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PROGRAMME AND BOOK OF ABSTRACTS

Ist HUNGARIAN-SINGAPOREAN WORKSHOP
on
DRUG DISCOVERY and BIOMATERIALS

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Dear Colleagues,

It is a great pleasure and honour for me to extend a cordial welcome to you all at the ***'Ist HUNGARIAN-SINGAPOREAN WORKSHOP on DRUG DISCOVERY and BIO-MATERIALS'.***

This event is perfectly in accordance with the spirit and aims of the Master Collaboration Agreement between Hungary's National Office for Research and Technology (NKTH) and Singapore's Agency for Science, Technology and Research (A*STAR): it will provide an excellent opportunity for the exchange of ideas, and we hope that it will promote successful collaboration between scientists from both countries.

The main topics of the Workshop, which extend over the wide range of drug discovery and biomaterials, will be represented by experts from both sides, not only in parallel, but also in a complementary manner, so as to facilitate the establishment of joint projects.

Besides these scientific aspects of the Workshop, our Singaporean guests will have every chance to enjoy the hospitality and the beauties of our historic capital.

May I wish everyone a memorable stay in Budapest, with new ideas for your own research and fruitful new personal contacts.

Péter Mátyus

President of the Hungarian Chemical Society

Chairman of the 1st HSW

TIMETABLE

Monday, March 10

8:30 *Registration*
9:30 Opening

Session I

9:50	Plenary Lecture (PL-1)	Ying, J.
10:20	Oral Lecture (OL-1)	Zrínyi, M.
10:35	Oral Lecture (OL-2)	Wan, A.
10:50	*Coffee Break*	
11:10	Plenary Lecture (PL-2)	Hudecz, F.
11:40	Oral Lecture (OL-3)	Kurisawa, M.
11:55	Oral Lecture (OL-4)	Yu, H.-h.
12:10	Oral Lecture (OL-5)	Szente, L.

12:30 Lunch break

Session II

14:00	Oral Lecture (OL-6)	Arányi, P.
14:15	Oral Lecture (OL-7)	Penke, B.
14:30	Oral Lecture (OL-8)	Yang, Y.-Y.
14:45	Oral Lecture (OL-9)	Darvas, F.
15:00	Oral Lecture (OL-10)	Gao, Z.
15:15	Oral Lecture (OL-11)	Mező, G.
15:30	*Coffee break*	
15:50	Oral Lecture (OL-12)	Kálai, T.
16:05	Oral Lecture (OL-13)	Gerencsér, J.

Poster Session I
16:30-17:30 (P1–P9)

Tuesday, March 11

Session III

9:15	Plenary Lecture (PL-3)	Chai, C.
9:45	Oral Lecture (OL-14)	Chen, D.
10:00	Oral Lecture (OL-15)	Kéri, Gy.
10:15	Oral Lecture (OL-16)	Bernardo, P.
10:30	Oral Lecture (OL-17)	Martinek, T.
10:45	*Coffee break*	
11:05	Oral Lecture (OL-18)	Lear, M.
11:20	Oral Lecture (OL-19)	Herczegh, P.
11:35	Oral Lecture (OL-20)	Chen, A.
11:50	Oral Lecture (OL-21)	Huleatt, P.

12:15 Lunch break

Session IV

13:45	Plenary Lecture (PL-4)	Sperlágh, B.
14:15	Oral Lecture (OL-22)	Ho, P.
14:30	Oral Lecture (OL-23)	Somsák, L.
14:45	Oral Lecture (OL-24)	Szökő, É.
15:00	Oral Lecture (OL-25)	Verma, C.
15:15	*Coffee break*	
15:35	Oral Lecture (OL-26)	Tóth, G.
15:50	Oral Lecture (OL-27)	Kantchev, E.
16:05	Oral Lecture (OL-28)	Zhang, Y.

Poster Session II
16:30-17:30 (P10–P17)

SCIENTIFIC PROGRAMME

Monday, March 10

8:30 *Registration*

9:30 Opening

Session I

9:50 **Ying, Jackie Y.**
NANOSTRUCTURE PROCESSING OF ADVANCED CATALYSTS AND BIOMATERIALS (*PL-1*)

10:20 **Zrínyi, Miklós**
SMART POLY(AMINO ACID) BASED GELS FOR CONTROLLED DRUG RELEASE (*OL-1*)

10:35 **Wan, Andrew C. A.**
POLYELECTROLYTE COMPLEXES, BIOSIGNALS AND TISSUE ENGINEERING (*OL-2*)

10:50 *Coffee Break*

11:10 **Hudecz, Ferenc**
SYNTHESIS AND BIOMEDICAL APPLICATION OF PEPTIDE BASED BIOCONJUGATES (*PL-2*)

11:40 **Kurisawa, Motoichi**
ENZYME-MEDIATED INJECTABLE BIODEGRADABLE HYDROGELS FOR DRUG DELIVERY AND TISSUE ENGINEERING (*OL-3*)

11:55 **Yu, Hsiao-hua (Bruce)**
DNA BIOSENSING PLATFORMS BASED ON NANOSTRUCTURED CONDUCTING POLYMERS (*OL-4*)

12:10 **Szente, Lajos**
PHARMACEUTICAL APPLICATIONS OF CYCLODEXTRINS (*OL-5*)

12:30 *Lunch break*

Session II

14:00 **Arányi, Péter** (*OL-6*)

14:15 **Penke, Botond**
INTERACTION OF SHORT PEPTIDES AND PEPTIDOMIMETICS AS DRUG CANDIDATES WITH Aβ OLIGOMERS AND FIBRILS (*OL-7*)

14:30 **Yang, Yi-Yan**
POLYMER NANOSTRUCTURES FOR DELIVERY OF THERAPEUTICS (*OL-8*)

14:45 **Darvas, Ferenc**
INTEGRATING FRONTIERS OF ORGANIC SYNTHESIS (FROST) TECHNOLOGIES INTO DRUG DISCOVERY (*OL-9*)

15:00 **Gao, Zhiqiang**
ULTRASENSITIVE ELECTROCHEMICAL BIOSENSORS FOR MICRORNAS (*OL-10*)

15:15 **Mező, Gábor**
DRUG DELIVERY SYSTEMS BASED ON GONADOTROPIN-RELEASING HORMONE (*OL-11*)

15:30 *Coffee break*

15:50 **Kálai, Tamás**
SYNTHESIS AND APPLICATION OF DOUBLE (SPIN AND FLUORESCENT) SENSOR REAGENTS (*OL-12*)

16:05 **Gerencsér, János**
APPLICATIONS OF MULTICOMPONENT REACTIONS (MCRS) IN LIBRARY SYNTHESIS (*OL-13*)

Poster Session I

16:30-17:30 (*P1–P9*)

Tuesday, March 11

Session III

9:15 **Chai, Christina L.L.**
TOWARDS THE DEVELOPMENT OF THE NATURAL PRODUCTS CALOTHRIXINS AS CHEMOTHERAPEUTICS (*PL-3*)

9:45 **Chen, David Y.-K.**
SYNTHETIC ADVENTURES OF ARCHITECTURALLY COMPLEX BIOACTIVE NATURAL PRODUCTS (*OL-14*)

10:00 **Kéri, György**
SIGNAL TRANSDUCTION THERAPY WITH KINASE INHIBITORS (*OL-15*)

10:15 **Bernardo, Paul H.**
TARGETING CANCER: DESIGN, SYNTHESIS AND EVALUATION OF SMALL MOLECULE INHIBITORS OF BCL-X_L USING *IN VITRO* AND *IN SILICO* STUDIES (*OL-16*)

10:30 **Martinek, Tamás A.**
β-PEPTIDE FOLDAMERS AS POTENTIAL DRUG SCAFFOLDS (*OL-17*)

10:45 *Coffee break*

11:05 **Lear, Martin J.**
TOTAL SYNTHESIS PURSUITS IN DRUG DISCOVERY (*OL-18*)

11:20 **Herczegh, Pál**
SYNTHESIS OF ANTIBIOTIC ANALOGS (*OL-19*)

11:35 **Chen, Anqi**
TOTAL SYNTHESIS OF BIOACTIVE RESORCINYLIC MACROLACTONES (*OL-20*)

11:50 **Huleatt, Paul B.**
DEVELOPMENT OF GENERAL METHODOLOGY FOR THE SYNTHESIS OF EPIDITHIOPIPERAZINEDIONES (*OL-21*)

12:15 *Lunch break*

Session IV

13:45 **Sperlágh, Beáta**
P2X RECEPTORS: A NEW THERAPEUTIC TARGET IN CNS DISEASES (*PL-4*)

14:15 **Ho, Paul C.**
PHARMACOKINETICS OF A SUBMICRON LIPID EMULSION OF TRIBUTYRIN AFTER ORAL AND INTRAVENOUS ADMINISTRATION (*OL-22*)

14:30 **Somsák, László**
GLUCOSE ANALOG INHIBITORS OF GLYCOGEN PHOSPHORYLASE AS POTENTIAL ANTIDIABETIC AGENTS (*OL-23*)

14:45 **Szökő, Éva**
STEREOCHEMICAL STUDY ON IN VIVO AND IN VITRO METABOLISM OF SELEGILINE (*OL-24*)

15:00 **Verma, Chandra**
NEW MECHANISMS IN THE P53 PATHWAY FOR THERAPY (*OL-25*)

15:15 *Coffee break*

15:35 **Tóth, Géza**
NEW ENDOMORPHIN ANALOGUES WITH ALICYCLIC β-AMINO ACIDS: INFLUENCE ON BIOACTIVE CONFORMATION AND PHARMACOLOGICAL PROFILE (*OL-26*)

15:50 **Kantchev, Eric A.**
N-HETEROCYCLIC CARBENE-LIGATED PALLADACYCLE AS RATIONALLY DESIGNED, HIGHLY ACTIVE, PRACTICAL CATALYST FOR HECK-MIZOROKI REACTION (*OL-27*)

16:05 **Zhang, Yugen**
NEW DEVELOPMENT OF IMIDAZOLIUM AND N-HETEROCYCLIC CARBENE CHEMISTRY (*OL-28*)

Poster Session II

16:30-17:30 (P10–P17)

PLENARY LECTURES

NANOSTRUCTURE PROCESSING OF ADVANCED CATALYSTS AND BIOMATERIALS

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Nanostructured materials are of interest for a variety of applications. This talk describes the synthesis and properties of two classes of nanostructured materials: nanoparticulate materials and nanoporous materials for catalytic and biomaterials applications. Nanoparticulate materials are made up of crystallites or particles of ~10 nm. They may be generated by various physical and chemical approaches with ultrahigh surface areas. Through controlled synthesis in reverse microemulsions, my laboratory has achieved complex oxide nanoparticles with ultrahigh thermal stability for the effective catalytic combustion of methane. This approach has also enabled us to derive polymeric nanoparticles for the glucose-sensitive delivery of insulin. Through chemical precipitation and additive dispersion, we have also attained nanocomposite systems as highly selective and sensitive semiconductor sensors, bioactive ceramic orthopedic implants, and efficient gene delivery vectors.

My laboratory is involved in the synthesis of novel nanoporous materials with tailored oxidation states, coordination chemistry and electronic structure. We have found that sol-gel processing can be combined with supramolecular templating agents in deriving well-defined mesoporous and microporous transition metal oxides (termed TMS). The compositional flexibility and pore size tailoring of the TMS molecular sieves open new possibilities for catalytic applications beyond the silicate-based zeolitic materials or mesoporous MCM-41. We have also attained mesocellular foams by using triblock copolymers and swelling agents in templating silicate precursors. These ultralarge-pore materials have been used to fixate organometallic ligands for the effective epoxidation, hydroxylation, Heck catalysis and asymmetric hydrogenation. The resulting heterogenized catalysts provide for excellent activity, enantioselectivity and reusability.

SYNTHESIS AND BIOMEDICAL APPLICATION OF PEPTIDE BASED BIOCONJUGATES

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Medium size oligopeptide as well as polypeptide carriers could be utilised for "passive targeting" of covalently attached drugs resulting in altered pharmacokinetics (e.g. accumulation), increased intracellular uptake, decreased non–specific toxicity and immunogenicity.[1,2] We have developed new groups of water-soluble peptid-drug-conjugates in which daunomycin, Dau, methotrexate (MTX), pemetrexed or other anitumour agents are coupled to amphoteric or polycationic branched polypeptides or cell penetrating peptides (e.g. oligoarginine).[3,4] Toxicity, *in vitro* and *in vivo* antitumour activity of conjugates with daunomycin were investigated using sensitive and multidrug resistant, mouse (L1210) and human (HL60 leukaemia) tumors. The antiparasitic activity of the MTX containing conjugates was analysed also *in vitro* and *in vivo* using *Leishmania donovani* infected macrophages and animals. We found that attachment of peptide to the bioactive cargo significantly improved the antitumour or antiparasitic properties of the drug, respectively and also significant reduction of drug related side effects could be demonstrated.

Synthetic peptides comprising linear or continuous topographic epitope sequences of proteins are frequently considered as specific and small size antigens/immunogens. However, it has been demonstrated that short peptides corresponding to the core of the B- or T-cell epitope could possess only limited antibody binding properties or capability to induce specific T-cell responses. One of the promising approaches to maximize immunoreactivity could be the attachment of optimized epitope in multiple copies to oligo- or polypeptide carriers. Here we outline our results on epitope-conjugates with preserved immunspecificity. Our data show that immunorecognition of the epitope could be markedly influenced by the carrier structure and topology. In comparative studies we have studied the T-cell response properties of bioconjugates containing oligopeptides representing a) an immunodominant epitope domain of the 38 kDa *M. tuberculosis* or b) B-cell epitope from *Herpes simplex virus* glycoprotein D.[5,6] We found that the chemical nature of the carrier, the form of epitope presentation have marked effects on antibody recognition as well as the T-cell responses detected.

Acknowledgement: These studies were supported by grants of Medichem 2 (1/A/005/2004), Hungarian Research Fund (K 68285), GVOP (3.2.1 – 2004-04-0005/3.0), and ETT (43/2006).

References:

1. F. Hudecz, Z. Bánóczi, G. Csík, *Med. Res. Rew.* 25, 679 (2005).
2. J. Reményi, G. Csík, P. Kovács, F. Reig, F. Hudecz, *Biochim. Biophys. Acta* 1758, 280 (2006).
3. R. Szabó, et al., *Bioconjugate Chemistry* 16, 1442 (2005).
4. Zs. Miklán, R. Szabó, V. Zsoldos-Mady, Z. Bánóczi, F. Hudecz, *Biopolymers* 88, 10 (2007).
5. Mező, G. et al. *Bioconjugate Chemistry* 14: 1260-1269 (2003).
6. Krikorian, D., et al.: *Biopolymers*, 84: 383-399 (2006).

TOWARDS THE DEVELOPMENT OF THE NATURAL PRODUCTS CALOTHRIXINS AS CHEMOTHERAPEUTICS

Bernardo, Paul H.[a]; Smith, Geoffrey D.[b]; Waring, Paul[b]; Chai, Christina L.L.[a]

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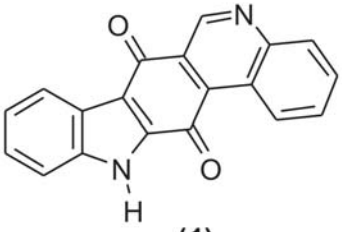
Secondary metabolites continue to provide a plethora of bioactive natural products as lead structures for the development of therapeutics. From these natural products, non-natural compounds are designed in order to study structure-activity-relationships so as to enable a better understanding of the pharmacophore that is required for biological activities. As part of our synthesis programme at the Institute of Chemical and Engineering Sciences and in view of the strong biomedical interest in Singapore, we are developing synthetic routes to a number of natural products and related analogues.



(1)

One such natural product is calothrixin B **(1)**, a secondary metabolite which was isolated from the *Calothrix* cyanobacteria in 1999.[1] Calothrixin B and the *N*-oxide, calothrixin A, have a novel pentacyclic structure comprising the indole, quinone and quinoline moieties. Both calothrixin A and B were shown to inhibit the *in vitro* growth of the chloroquine-resistant strain of the human parasite, *Plasmodium falciparum* in a dose-dependent manner. They were also found to kill several cancer cell lines, all with excellent potency, with IC_{50} values at nanomolar concentrations. This talk will provide an overview of our progress towards the synthesis of the calothrixins and structurally related quinones. In addition, the structure-activity studies of calothrixins and closely related quinones will also be reported.

Reference:

1. Rickards, R. W., Rothschild, J. M., Willis, A. C., de Chazal, N. M., Kirk, J., Kirk, K., Saliba, K., J. and Smith, G. D., *Tetrahedron*, *55*, 13513-20 (1999).

P2X RECEPTORS: A NEW THERAPEUTIC TARGET IN CNS DISEASES

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P2X receptors form a unique family of ligand-gated ion channels conveying the ionotropic actions of extracellular ATP. These receptors have two transmembrane domains and a large extracellular loop; they are permeable to both divalent and monovalent cations, such as Na^{+} and Ca^{2+} and their activation results in a non-selective inward cationic current. Until now, seven different subunits (P2X1-7) of this receptor family have been identified, which form functional receptor-ion channel-complexes in homo- and/or hetero-oligomeric assemblies. Different P2X receptor subunits are expressed throughout the nervous system, and also in non-neuronal tissues. Although their function is far from fully explored yet, their therapeutic utilisation has been raised in many CNS diseases including neurodegenerative diseases and neuropathic pain[1]. Within the P2X receptor family, the homooligomeric P2X7 receptor (P2X7R) has distinguished structural, functional and pharmacological features: (1) Its intracellular carboxy terminal domain is longer (239 amino acids) than those of other P2X receptor subunits; (2) its persistent activation elicits the opening of a membrane pore, which renders the membrane permeable to high molecular weight molecules and ions up to 800 D and eventually leads to cell death; (3) it needs much higher concentrations of ATP, to be activated, than all the other P2X receptors; (4) despite that it was originally cloned from the rat brain, its typical localization is the immunocompetent cells of the CNS and the periphery and is believed to be primarily involved in host-defense reaction. However, a growing number of evidence indicates that its signaling role in the brain is more widespread than previously anticipated[2]. Thus, we showed that the activation of P2X7Rs releases glutamate and GABA from the hippocampus, an effect, which is absent in mice genetically deficient in P2X7 receptors (P2X7 -/-). In addition, we showed that in vitro ischemia increased the expression of the P2X7R protein in GFAP positive astrocytes of a mixed cortical neuron-glia culture, and this upregulation is also manifested in functional terms, as increased efflux of [^{3}H]GABA and an increased facilitation of mIPSC frequency was detected in response to P2X7R activation after the treatment. Moreover, P2X7 receptors appear to be endogenously activated upon ischemia-like conditions contributing to increased excitatory transmitter release and glutamatergic excitotoxicity, as P2 receptor antagonists, such as the selective P2X7R antagonist Brilliant Blue G decreased glutamate release upon combined oxygen-glucose deprivation from rat hippocampal slices. A further area that could be of interest for drug development is mood related disorders, as recent linkage studies revealed the polymorphism of the P2X7R gene in patiens suffering in major depresssion and bipolar disorder. We showed that P2X7 -/- mice do not develop behavioral despair in the Porsolt forced swim test; have altered monoamine (serotonin, norepinephrine and dopamine) levels in mood related brain areas and display decreased stress response to restraint stress. These data altogether strongly suggest that P2X7Rs have a determinant role in the development of depressive behavior.

References:

1. Burnstock, G. Pathophysiology and therapeutic potential of purinergic signaling. *Pharmacol Rev.*, 58:58-86, 2006.
2. Sperlagh, B., Vizi, E. S., Wirkner, K., Illes, P. P2X7 receptors in the nervous system. *Progr. in Neurobiol.*, 78:327-46, 2006.

ORAL LECTURES

SMART POLY(AMINO ACID) BASED GELS FOR CONTROLLED DRUG RELEASE

Gyarmati Benjámin; Némethy Árpád; Gyenes Tamás; Torma Viktória; Szilágyi András; Filipcsei Genovéva; Zrínyi Miklós

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Tremendous efforts have been devoted recently to design biocompatible smart materials for controlled drug release. Stimuli responsive polymers and their hydrogels can be used as effective drug reservoirs due to their environmental sensitivity. pH sensitive polymer gels can be applied as enteric coating materials, site specific targeting or tumor specific delivery system.

We have developed a novel preparation method for synthesis of chemically cross-linked polysuccinimide (PSI) and poly(aspartic acid) (PASP) gels. PSI molecules were cross-linked with different kind of biological molecules: putrescin, lysine and cystamine, and the gels were hydrolyzed in alkali media to get PASP hydrogels. The strategy of syntheses is supposed to be generally applicable for preparation other PSI-based biocompatible and biodegradable polymers.

The pH dependence of equilibrium swelling degree was measured in several media like HCl (0,01–0,1 M) citrate (pH 2–6), imidazole (pH 6,1–8) borate (pH 8,1–11) buffers and NaOH solutions (0,01–0,1 M). The poly(aspartic acid) gels show a volume phase transition around the pK values of poly(aspartic acid).

The synthesized gels contain exclusively amino acids, which makes them promising candidates as base materials of controlled drug delivery systems.

References:

1. V. Torma, T. Gyenes, Z. Szakács, B. Noszály, Á. Némethy, M. Zrínyi: Novel amino acid-based polymers for pharmaceutical applications *Polymer Bulletin* 2007, 59, 311-318.
2. T. Gyenes, V. Torma, M. Zrínyi: Swelling Properties of Aspartic Acid-Based Hydrogels *Colloids and Surfaces* A doi:10.1016/j.colsurfa. 2007.06.
3. T. Gyenes, V. Torma, B. Gyarmati, M. Zrínyi: Synthesis and swelling properties of novel pH-sensitive poly(aspartic acid) gels *Acta Biomater.* 2008. *In press*.

POLYELECTROLYTE COMPLEXES, BIOSIGNALS AND TISSUE ENGINEERING

Wan, Andrew C. A.; Tai, Benjamin C.U.; Chin, Sau Yin; Yao; Xiaosai; Ying; Jackie Y.

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Polyelectrolyte complexation involves the insolubilization of oppositely charged polyelectrolytes by the primary process of charge neutralization in conjunction with secondary chemical interactions such as hydrophobic interactions and Van der Waals forces. Polyelectrolyte complexation is inherently suitable for the generation of matrices for tissue engineering as it is an aqueous-based, room temperature process that allows for the viable incorporation of proteinaceous growth factors and extracellular matrix components. A special case of polyelectrolyte complexation that we have studied and applied to the fabrication of scaffolds for tissue engineering is the formation of fiber by interfacial polyelectrolyte complexation.[1] The initial problem of fibers aggregating into a dense monolith in water was overcome by silica crosslinking of the fibers through incorporation of TEOS. A three-tiered polyelectrolyte complex membrane has also been designed to present biological functionality on an otherwise non-fouling surface.[2] Chemical strategies have been developed to conjugate the cell-adhesive RGD peptide onto polyelectrolyte complex scaffold and membrane forms. Examples will be presented on the application of the scaffolds in liver tissue engineering, epithelial morphogenesis and the encapsulation of pancreatic cells for immunoisolation.

References:

1. A. C. A. Wan, B. C. U. Tai, K. J. Leck and J. Y. Ying, *Advanced Materials 18* (2006) 641.
2. A. C. A. Wan, B. C. U. Tai, K. Schumacher, A. Schumacher, S. Y. Chin and J. Y. Ying, *Langmuir* (2008) *In press*.

ENZYME-MEDIATED INJECTABLE BIODEGRADABLE HYDROGELS FOR DRUG DELIVERY AND TISSUE ENGINEERING

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Injectable *in situ* gel-forming polymeric hydrogel systems have been used extensively for the controlled release of bioactive molecules and the encapsulation of cells. Ideally, the injectable system should form a hydrogel within a narrow range of physiologically acceptable temperatures at a sufficiently rapid rate. In addition, chemical crosslinking is a more versatile method compared to physical methods in the synthesis of the hydrogels due to the mechanical stability and control of degradability. However, the chemical method is unsuitable for application to injectable systems because toxic chemical agents are usually used in hydrogel synthesis and these induce undesirable reactions with bioactive molecules, such as growth factors, and cells.

We have been developing a simple and biocompatible *in situ* gel-forming system composed of hyaluronic acid-tyramine conjugates using a peroxidase-catalyzed oxidation reaction.[1] We achieved independent control of gelation rate and mechanical property of the hydrogel by utilizing a two-step catalytic coupling mechanism. We demonstrated that a faster gelling hydrogel yielded more localized gelation on the administered site through the subcutaneous injection compared to a slower gelling hydrogel. We also demonstrated that mechanical strength directly influenced hydrogel biodegradibility. Recently, we designed HA-epigallocatechin gallate (HA-EGCG) conjugates for enzyme-mediated hydrogel formation. EGCG, a major component of green tea, has been recognized to have biochemical pharmaceutical effects, including antioxidant, anticarcinogenic and anti-inflammatory properties. These beneficial bioactivities have been attributed mostly to the strong binding ability of EGCG to many biological molecules including peptides and proteins, which affect various enzyme activities and signal transduction pathways. This hydrogel achieved sustained protein release due to protein immobilization against diffusion and enzyme inhibition, which slowed down the degradation, due to the EGCG moiety. We believe that the system will provide great advantages for highly regulated drug delivery and tissue regeneration through the controlled release of bioactive molecules and/or cells.

Reference:

1. M. Kurisawa, J. E. Chung, Y. Y. Yang, S. J. Gao, H. Uyama, *Chemical Communications* (2005) 4312.

DNA BIOSENSING PLATFORMS BASED ON NANOSTRUCTURED CONDUCTING POLYMERS

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DNA biosensors are one of the most promising tools for molecular diagnostics. The completion of the genome project reveals more information about the relationship between genes and diseases. Nucleic acid hybridization between the target and the probe forms the basis for DNA detection. A nucleic acid detection platform provides a solution for applications ranging from point-of-care examinations for pathogen, virus and early cancer detection, to bioinformatics for molecular diagnostics and medicine. Current detection platforms suffer from poor detection limit, complex detection protocols, and heavy intellectual property protection for more general applications. Recent research efforts towards the development of DNA biosensing platforms based on nanostructured conducting polymer will be presented.

Nanostructured conducting polymers (CP), particularly poly(3,4-ethylenedioxythiophene)s (PEDOTs), represent another promising material for use in biosensor applications. A key advantage of CP-based sensors is their amplified sensitivity to the existence of small amounts of analytes. PEDOT has shown great stability in aqueous solution and compatibility with biofluids. Three different approaches involving PEDOT nanobiointerfaces, EDOT intercalators, and label-free biomolecule detection[1] from nanostructured PEDOT are currently being explored. Combined with proper device fabrication and microfluidic system integration, they provide an ideal platform as fast, sensitive and automated biosensors for diagnosis.

Reference:

1. E. Mohamed Ali, E. A. B. Kantchev, H.-h. Yu and J. Y. Ying, *Macromolecules 40* (2007) 6025.

PHARMACEUTICAL APPLICATIONS OF CYCLODEXTRINS

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Past decades have witnessed not only a growing appreciation and practical application of the molecular inclusion phenomena between pharmaceuticals and cyclodextrins (CDs), but also a deeper understanding of the molecular mechanisms behind improved drug delivery and effective targeting achieved by cyclodextrin-based supramolecular complexes. Since 1985, over 35 different CD-containing pharmaceutical products have reached marketing approvals.

The present talk gives a survey on the benefits of cyclodextrin-assisted drug delivery systems and a view on the future trends by using illustrative examples from both marketed and future cyclodextrin-enabled pharmaceutical formulations.

Cyclodextrins in life-cycle management of pharmaceuticals:

makes presently marketed „old" and successful drug products more effective and patient-friendly, creates new markets

enables re-classification of drug actives from class II and IV to class I (in a non-covalent manner, thus no New Chemical Entities, but Improved Chemical Entities)

provides new patent protection for the old drugs in novel forms

Cyclodextrin assist drug delivery across barriers/absorptive mucosae:

CD are mostly used for oral drug delivery systems to improve bioavailability

CDs are able to reverse ABD transporters activity, MDR via temporary interaction with cell membrane lipids opening a new way to anticancer drug delivery (e.g. parenteral and oral taxoid delivery)

CDs can improve peptide and gene delivery by acting as non-viral transfecting agents

Cyclodextrins improve both physical and chemical stability of drugs:

CDs prevent aggregation and maintain monomeric form of peptide/protein actives in liquid pharmaceutical formulation (e.g. insulin, Growth Hormon, kinases etc.)

CDs decelerate hydrophobic clustering and deposit formation

The molecular encapsulation of sensitive active substances improves their resistance against heat, oxidation, hydrolysis etc. thus their shelf life and chemical stability improves

Cyclodextrins assist serum-free biotechnological processes as solubiliser, stabiliser

A number of cyclodextrin-assisted enzymatic conversion processes are in use for manufacturing pharmaceutical actives. (e.g. steroid bio-conversions, cholesterol status management)

Application of cyclodextrins is vaccine production (a recently approved Pertussis vaccine *Daptacel*^{TM} by Sanofi-Aventis)

INTERACTION OF SHORT PEPTIDES AND PEPTIDOMIMETICS AS DRUG CANDIDATES WITH Aβ OLIGOMERS AND FIBRILS

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β-Amyloid peptides (Aβ) play central role in the etiopathology of Alzheimer's disease (AD). The main component contains 42 amino acids (Aβ 1-42; Aβ 42) and shows high tendency for aggregation to oligomers, protofibrils and fibrils. At first Aβ fibrils were considered the most toxic Aβ species forming plaques in the brain. However, the number of the amyloid plaques has only a very little correlation to the severity of the disease. As a consequence, Aβ-oligomers have been considered as the most toxic Aβ 42 – form. According to our experiments both Aβ oligomers and fibrils can be toxic but their effect might be different in receptor level. Aβ 42 oligomers may act in the synapses causing neuronal dysfunction, Aâ fibrils may activate microglial cells causing neuroinflammation. Some peptidomimetics designed and synthesized in our laboratory can protect neurons from the toxic effect of Aβ 42 aggregates; their mechanism of action will be discussed.

POLYMER NANOSTRUCTURES FOR DELIVERY OF THERAPEUTICS

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Drug delivery is as important as the development of new drug entities. The main goal of drug delivery is to transport drugs to diseased sites using a therapeutic dosage. A number of biomaterials based on natural and synthetic materials have been proposed to achieve such a goal. However, the method of delivering drugs to specific cells or cell compartments remains a challenge. The aim of our study is to develop polymer nanostructures for efficient delivery of small molecular anticancer drugs, macromolecular anticancer proteins and genes, and cells to specific tissues, cells or cell compartments, thereby alleviating or eliminating the side effects associated with the use of conventional delivery systems and improving the efficacy of therapeutics.

In this talk, three types of polymer nanostructures will be introduced. One of them is pH-triggered temperature-sensitive core-shell nanoparticles. The structure of these nanoparticles is stable in the normal physiological environment (pH 7.4), but deforms and releases the enclosed drug molecules in an acidic environment. A signal that recognizes tumor cells is conjugated to the shell of the nanoparticles, making them capable of targeting a drug to tumor cells and then releasing it intracellularly for more efficient and safer cancer therapy[1-4]. The second type of polymer nanostructures is self-assembled from biodegradable cationic amphiphilic copolymers, which carry drugs in the core and bind genes/proteins on the positively charged shell. By enabling the co-delivery of drugs and genes/proteins in a single carrier, the synergy of drug and gene therapies may be achieved to combat multi-drug resistant cancers[5,6]. In addition, these cationic nanoparticles have also been proven to deliver anticancer proteins into cells much more efficiently than commercial vectors[7]. The third type of polymer nanostructures is a thermosensitive membrane with nanosized porous structure. The membrane has excellent mechanical properties and stability. In addition, cells attach well onto the membranes at 37ºC but detach from the membranes at lower temperatures without using trypsin[8,9]. These membranes have great potential for use in cell grafting applications for wound healing.

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INTEGRATING FRONTIERS OF ORGANIC SYNTHESIS (FROST) TECHNOLOGIES INTO DRUG DISCOVERY

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Introduction of FROST technologies operating on high pressure, high temperature with flow reactors pre-packed with catalysts, reagents, or scavengers, combined with extensive use of supercritical solvents offers a radical improvement of effectivity in medicinal chemistry both in lab scale and in post-discovery scale-up operations. While FROST technologies offer a drastic reduction of time and manpower required by the chemistry and scale-up side of discovery, their integration with modeling and chemical genomics are largely unsolved.

We performed synthesis in new type of flow reactors, some of which operating up to 200 bars and 350 °C temperature. The flow reactor for using supercritical conditions operated up to 150 bars and 200 °C, respectively. For validating the value of the approach, hydrogenation, Heck and Suzuki coupling reactions, catalytic isomerization, reactions using supercritical CO and other chemical transformations were performed and compared with usual batch reactions or in some cases with microwave-assisted reactions.

Based on the performed ca. 2,000 reactions, we found that using the flow reactors shorter development time and higher synthetic effectivity can be attained. Introducing supercritical conditions led to additional benefits at reactions like greater control over QA/QC, higher PAT compliance and enhanced „green" features.

Extension of the technologies to enzyme-packed reactors and to continuous preparation of nanocrystals are also discussed.

Integration of the technologies with bioanalogous design strategies for target-family based drug discovery are exemplified at designing of matrix metalloprotease (MMP) inhibitors. About 300 known MMP inhibitors were annotated and organized into a ligand space were a „lead evolution tree" was formed. In that tree, substructural changes leading to significant increase in biological effects were revealed by using the EMIL serendipity-enhancing approach. Subtype-specific privileged fragments were extracted to improve activity and/or selectivity. The compounds with preferred activity profile were correlated with sequence homology as well as binding site similarity within the target family, allowing the identification of substructural modification patterns suitable to produce selective inhibitors.

ULTRASENSITIVE ELECTROCHEMICAL BIOSENSORS FOR MICRORNAS

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MicroRNAs comprise a group of non-coding 18–25-nucleotide RNAs. Recent progress in miRNA research has shown that miRNAs regulate a wide range of biological functions from cell proliferation and death to cancer progression. It is widely believed that miRNA expression analysis is the key to its physiological functions. Therefore, there is an urgent need for a reliable and ultrasensitive method for miRNA expression analysis. Unfortunately, the very short length of miRNA renders PCR-based tools ineffective because much shorter primers do not effectively and selectively bind to such short miRNA templates. The inherent miniaturization of electrochemical devices, their compatibility with advanced semiconductor technology and their low cost of production, on the other hand, make them excellent alternatives for developing portable ultrasensitive miRNA biosensors. We have developed chemical amplification-based ultrasensitive miRNA detection systems where the analytical signal correlates directly to the amount of miRNA in the solution. We have performed miRNA quantification on miRNAs extracted from cancer cells and were able to detect miRNA from as little as 10 ng total RNA.

DRUG DELIVERY SYSTEMS BASED ON GONADOTROPIN-RELEASING HORMONE

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The targeting chemotherapy based on the cell specific or overexpressed receptors on tumors might provide a more effective therapeutic approach for cancer treatment. Gonadotropin-releasing hormone (GnRH) receptor expression was identified on different tumors (breast, ovarian, endometrial, prostate, renal, brain, pancreatic, melanomas and non-Hodgkin's lymphomas) with high percentages in specimens.[1] Cytotoxic analogs of GnRH were developed and their *in vitro* and *in vivo* efficacy was demonstrated in the last decade.[2,3] In our research a GnRH analog (GnRH-III; Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-NH_2) isolated from see lamprey was used as targeting moiety. GnRH-III shows antiproliferative activity itself on numerous tumor cells, and the modification of the Lys side chain in position 8 does not influence significantly the biological activity of the hormone.[4] GnRH-III has 500-1000 times less potency on releasing gonadotropin hormones (LH, FSH); therefore, it is a more selective antitumor agent than the human GnRH derivatives. Daunomycin (Dau) and doxorubicin (Dox) as antineoplastic agents were conjugated to GnRH-III through amide, oxime, hydrazone or ester bonds. The effect of the conjugation site, type of bonds and the presence of an enzyme labile spacer between the drug and hormone on *in vitro* antitumor activity was studied using different cancer cell lines (MCF-7 human breast, HT-29 human colon and C26 murine colon cancer cells). Dau-GnRH-III conjugate containing oxime bond was selected for *in vivo* experiments using mice transplanted by C26 colon cancer xenograft. The conjugate showed similar antitumor activity as the free drug, but less toxic side effect and longer survival of the animals were observed in the case of the application of the conjugate.[5] The increased antitumor activity and lower endocrin effect of dimer derivatives of GnRH-III were also demonstrated in our experiments.[4] The dimers had elevated enzymatic stability, as well. Based on these findings our research is further focused on the application of GnRH-III dimers as homing devices in drug delivery systems for targeting chemotherapy.

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SYNTHESIS AND APPLICATION OF DOUBLE (SPIN AND FLUORESCENT) SENSOR REAGENTS

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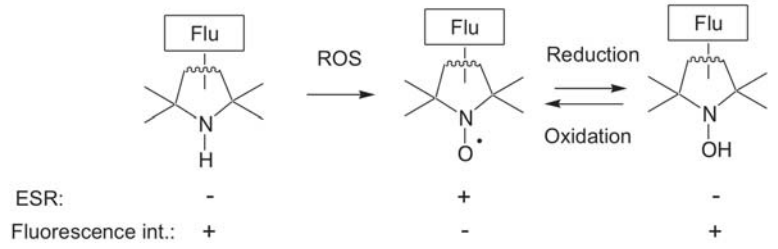
Fluorophores covalently bound to a nitroxides give a unique, redox-sensitive sensor. In these donor-aceptor molecules the paramagnetic stable nitroxide free radical quenches the fluorescence. However, when nitroxide is reduced to hydroxylamine the fluorescence increases.[1] This feature of these donors-aceptor pairs were experienced with coumarin, dansyl, aminophthalimide, BODIPY and Nile red donors.[2] A sterically hindered amine (precursor of nitroxide) covalently attached to a fluorophore offers the detection of reactive oxygen species (ROS) as formation of nitroxide, which quenches the fluorescence.[3,4] This change can be followed both fluorescence and EPR spectroscopy with these double sensor compounds. The synthesis and application of these double sensor reagents will be presented.



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APPLICATIONS OF MULTICOMPONENT REACTIONS (MCRS) IN LIBRARY SYNTHESIS

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Multicomponent reactions (MCRs) have been versatile tools for the synthetic chemists in the preparation of structurally diverse compounds, as well as compound libraries. Since the first reported MCRs the demand for novel and economically powerful MCRs is rapidly increasing. The enormous synthetic possibilities that MCRs offer can be further increased by post-synthesis transformations, which can be furnished by concomitant reaction of a suitably functionalized or protected MCR-product.

Biginelli reaction is one of the most widely used multicomponent reactions in parallel synthesis of combinatorial libraries due to the easy accessibility of the building blocks and the large variability at the diversity introducing sites. The Biginelli three-component condensation reaction will be discussed together with the application of a variety of post-MCR approaches leading to libraries containing novel condensed ring-systems.

The MCRs involving Meldrum's acid – such as the Yonemitsu reaction – generally retain the unique ring-captured malonic acid moiety, which can be released by losing acetone, when reacting with nucleophiles. This reaction is frequently accompanied with partial decarboxylation. In this way various diversity elements can be built into the diverse MCR products. The successful application and extension of the above reaction in library syntheses will be demonstrated.

In the present lecture applications of Biginelli and Yonemitsu reactions in different library synthesis programs will be discussed.

SYNTHETIC ADVENTURES OF ARCHITECTURALLY COMPLEX BIOACTIVE NATURAL PRODUCTS

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Over the decades, the total synthesis of natural products has admirably served as the cradle for the evolution of chemical sciences, and nurtured the discovery of novel synthetic technologies and strategies. In conjunction with the biological activities harnessed by the natural substances, accessing natural and designed compounds by chemical means has cemented its position in bridging biology and medicine. The Chemical Synthesis Laboratory @ Biopolis has been actively pursuing number of architecturally complex natural products exhibiting promising therapeutic potentials. This objective served a dual role in advancing our understanding in chemistry and chemical biology, and training synthetic manpower to support the vibrant biomedical industry in Singapore.

In this talk, progress towards selected targets, together with recently accomplished total syntheses will be described,[1-4] with emphasis on strategic design and recognizing the unique reactivity embedded within the molecular scaffold.

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SIGNAL TRANSDUCTION THERAPY WITH KINASE INHIBITORS

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Signal transduction therapy has become recently a leading area of modern drug research aiming to inhibit and modulate the pathomechanism based validated target molecules in intercellular and intracellular signaling. Proliferation of infected, damaged or malfunctioning cells is very often a key factor in the generation of the pathological state, not only in cancer and infectious diseases but also in inflammation or autoimmune related diseases. Signals of intercellular communication are mediated from the cell surface to the nucleus by a cascade of phosphorylation events, involving the interaction of a big series of proteins: a network, that monitors the environment of the cell and co-ordinates its responses. The malfunctioning network of supporting signals within tumour cells present multiple targets for pharmaceutical interception, which is the basic idea of signal transduction therapy. Selective inhibition of these false proliferative signals via targeting receptor tyrosine kinases and other signaling enzymes, resulting in the induction of apoptosis by depletion of the "survival factors" is one of the most studied and widely accepted concept of modern chemotherapy.[1,2]

We have used the approach of rational drug design to achieve the selective inhibition of certain pathologically relevant signalling enzymes or receptors, like receptor tyrosine kinases, cyclin dependent kinases or some survival factors.

Our novel Nested Chemical Library (NCL) technology is based on a knowledge base approach where focused libraries around 108 cores are used to generate a pharmacophore model. We have established a unique proprietary kinase inhibitory chemistry around these core structures with small focused sublibraries around each core. We have developed nM lead molecules against a series of kinases and various infectious disease targets including Mycobacterium tuberculosis, Influenza and HIV.[1,2] Our recently developed signal inhibiting peptide TT232 has reached Phase II clinical trials against melanoma, while it has also a strong anti-inflammatory activity in various inflammatory models. We have demonstrated that TT232 causes cell cycle arrest and induces apoptosis via the translocation of PKM2 into the nucleus.[3]

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TARGETING CANCER: DESIGN, SYNTHESIS AND EVALUATION OF SMALL MOLECULE INHIBITORS OF BCL-X_L USING *IN VITRO* AND *IN SILICO* STUDIES

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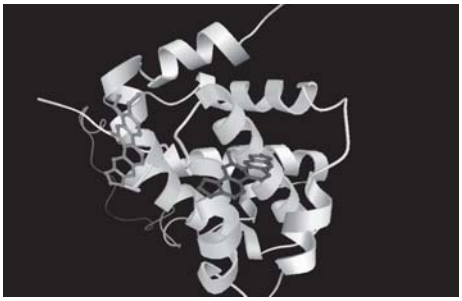

The Bcl proteins act as regulators of apoptosis, or programmed cell death, a process which is crucial for the removal of unwanted or damaged cells. The over-expression of the anti-apoptosis Bcl proteins such Bcl-2, Mcl-1, and Bcl-X_L in cancer cells has been implicated in the resistance of cancers to chemotherapeutics. A landmark paper in 2005 by Oltsdorf *et al.* on the design and synthesis of ABT-737[1] led to renewed interest in the field to discover new potent inhibitors of the Bcl-X_L and other anti-apoptosis proteins.[2]

The natural products chelerythrine and sanguinarine have been shown to induce apoptosis in cells which over-express Bcl-X_L.[3,4] The work presented here focuses on the synthesis of phenanthridine-based analogues of chelerythrine and sanguinarine. This talk highlights some of the computational and biological studies which led to the identification of even more potent inhibitors of Bcl-X_L, which are currently being evaluated and optimized for further studies.

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β-PEPTIDE FOLDAMERS AS POTENTIAL DRUG SCAFFOLDS

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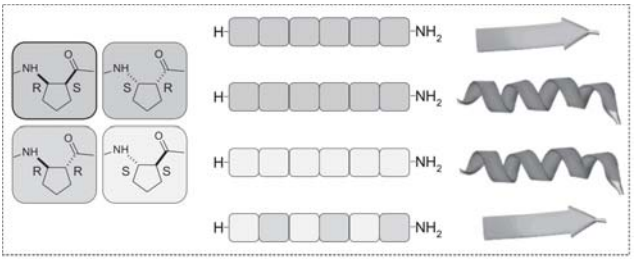
Control over folding and self-assembly is a major current goal of biopolymer and biomimicking polymer research: it can result in complex biopolymer-like activities and new structural classes of nanostructured materials. The self-organizing β-peptides have attracted considerable interest, because they exhibit various stable secondary structure motifs that can be exploited to construct biologically active substances.[1] The folding propensity is influenced by local torsional, side-chain – backbone and long-range side-chain interactions. Although β-peptide foldamers are sensitive to the solvent, the systematic choice of the side-chain pattern and spatiality allows the design of the desired specific secondary structure.



We set out to establish a general approach to the stereochemical control over secondary structures and the secondary structure-dependent self-assembly of the β-peptide units. The studied models were synthetized via standard solid-phase techniques. The self-organizing secondary structures were characterized by using NMR, IR, electronic and vibrational CD spectroscopic methods. The experimental results were evaluated with the help of molecular modelling.

It was found that systematic control of the secondary structure can be attained by tuning the configuration pattern of the backbone atoms of the cyclic β-amino acid residues.[3-5] The tailor-made β-peptide helices and strands are prone to self-assemble into tertiary structure motifs. The application of β-peptide foldamers may open up new directions in the synthesis of organized artificial secondary structures with biochemical functions.

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TOTAL SYNTHESIS PURSUITS IN DRUG DISCOVERY

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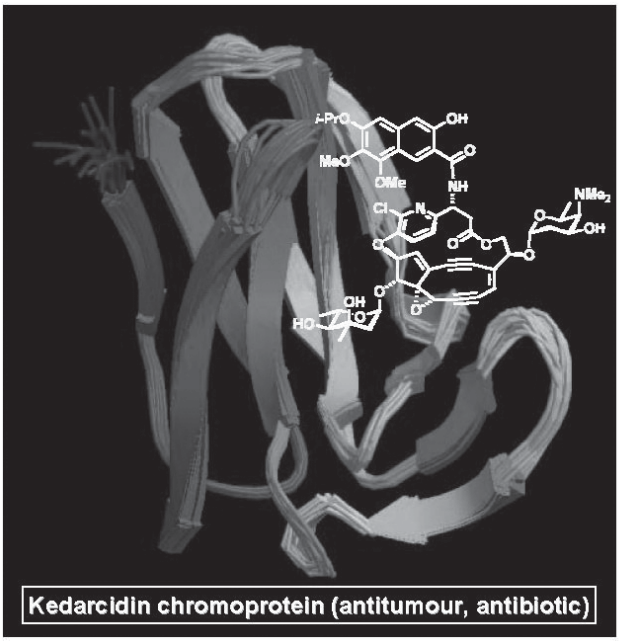
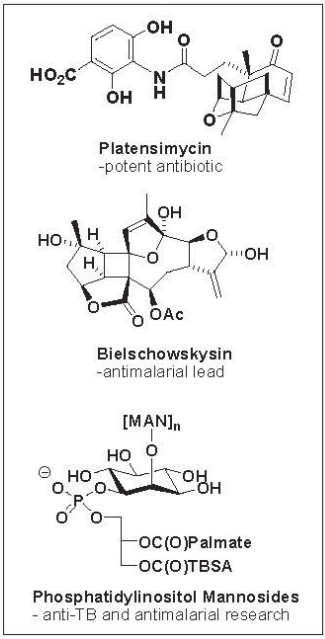
Driven by our passion for synthesis and drug discovery, selected total synthesis pursuits will be highlighted in our efforts to harness the inherent biological activity from natural products. Ongoing work to platensimycin (antibiotic), bielschowskysin (antimalarial) and some complex glycophospholipids of *Mycobacterium tuberculosis* will be briefly highlighted. The majority of this talk will then concentrate on our efforts to understand the chemistry and biology of natural 9-membered enedynes, specifically the kedarcidin chromoprotein.[1-3]





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SYNTHESIS OF ANTIBIOTIC ANALOGS

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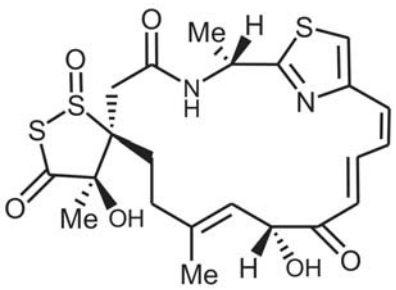
The "warhead" of the DNA-cleaving anticancer antibiotic leinamycin has been built synthetically into nucleosides in order to construct an aiming device for the analog. Cytotoxicities of the new derivatives have been studied in detail.



A new methodology for covalent linking of biologically active molecules has been elaborated on the basis of conjugate addition and of 1,3-dipolar cycloaddition reaction.

TOTAL SYNTHESIS OF BIOACTIVE RESORCINYLIC MACROLACTONES

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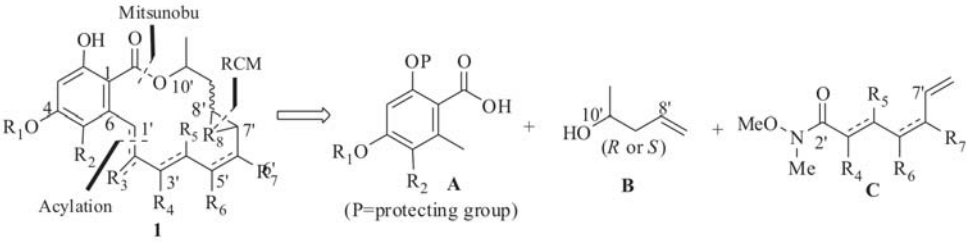
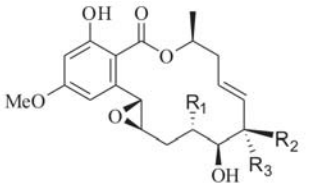
The resorcinylic macrolactone (**1**) is a group of natural products which display potent and diverse biological activities, especially as protein kinases and inhibitors for heat shock protein 90 (HSP90).[1] Particularly noteworthy to this class of natural products is that minor differences in structure or functional groups often result in large changes in their biological profile. In these regards, we are interested in developing an efficient and general approach to the synthesis of these compounds and their analogues as well as carry out structure-activity relationship studies. In this presentation, we report a three component (A-C) general approach for the synthesis of these compounds. This utilises three key reactions, i.e., the Mitsunobu reaction to install the ester linkage, a lithiation-acylation reaction to couple the aromatic portion A with the Weinreb amide C and a ring closing olefin metathesis to form the macrocycle.

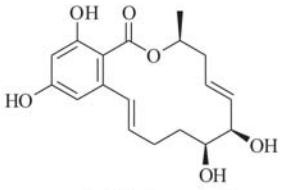


1 **A** **B** **C**

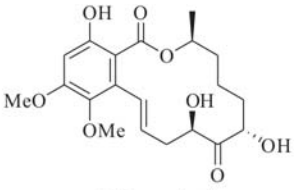
The successful application of this approach is demonstrated in an efficient and practical total synthesis of aigialomycin D (**5**), a potent antitumour and antimalarial natural product.[2] Current work towards the synthesis of other members of the resorcinylic macrolactone, such as aigialomycin A-C (**2**-**4**) and queenslandon (**6**), will also be discussed.



2 Aigialomycin A: R_1=OH; R_2, R_3= O
3 Aigialomycin B: R_1=R_3=OH; R_2=H
4 Aigialomycin C: R_1=R_2=H; R_3=OH



5 Aigialomycin D



6 Queenslandon

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DEVELOPMENT OF GENERAL METHODOLOGY FOR THE SYNTHESIS OF EPIDITHIOPIPERAZINEDIONES

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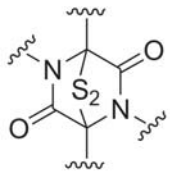
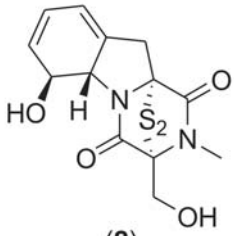
Epidithiopiperazinediones (ETP) are compounds which contain a piperazinedione ring bridged at the α-carbon centres by a disulfide linkage as depicted in the generic structure **1**. Naturally occurring ETP compounds comprise an important class of bioactive secondary metabolites isolated from many different species of fungi.[1] Fungal sources of ETP natural products have been found throughout the tropical and temperate regions of the world in both terrestrial and marine environments.[2] Perhaps the most celebrated member of this class of natural products is gliotoxin (**2**) which has been shown to possess a range of potent activities including antibacterial, antifungal, antiphagocytic, antitumor, antiviral activity and immunosuppressive properties.[2-4] The previously reported syntheses of ETP compounds have drawbacks associated with generality, efficiency, yields and the use of harsh reaction conditions.[5-8] As a result, very few total syntheses of complex ETP natural products have been reported despite the continued isolation of new and interesting members of this class of compounds.[9-11] Thus, there is a need to develop a novel and generally applicable methodology for the synthesis of ETP compounds. This presentation will detail our seminal synthetic work and our ongoing efforts towards the full development of such methodology.



(**1**)



(**2**)

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PHARMACOKINETICS OF A SUBMICRON LIPID EMULSION OF TRIBUTYRIN AFTER ORAL AND INTRAVENOUS ADMINISTRATION

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A submicron lipid emulsion of tributyrin with ability of binding to low-density lipoprotein (LDL) was formulated. The aim of this study was to investigate the pharmacokinetics of the emulsion *in vivo*. The pharmacokinetics of tributyrin and its metabolite, butyrate was evaluated in male Wistar rats after administration with pure tributyrin or tributyrin emulsion. After oral administration, C_{max}, T_{max} and $T_{1/2}$ of butyrate were 87.6 μM, 25.3 min and 63.0 min for the pure tributyrin compared to 1344.5 μM, 8.5 min and 19.8 min for the 10% (v/v) tributyrin emulsion. C_{max} and MRT of tributyrin were 2.74 μM and 87.9 min, 4.2 μM and 132.0 min for pure tributyrin and 10% emulsion, respectively. The bioavailabilities of the pure tributyrin vs tributyrin emulsion were 15.3% vs 65.7% and 34.9% vs 64.5% calculated from butyrate and tributyrin, respectively. After the rats were treated with 17α-ethynylestradiol (a LDL receptor upregulator), the distribution volumes calculated from both butyrate and tributyrin were significantly increased after oral administration or infusion of the 10% tributyrin emulsion. The increased distribution volume after co-administration with a LDL receptor upregulator suggested the increased uptake of tributyrin/butyrate by tissues with increased expression of LDL receptors.

GLUCOSE ANALOG INHIBITORS OF GLYCOGEN PHOSPHORYLASE AS POTENTIAL ANTIDIABETIC AGENTS

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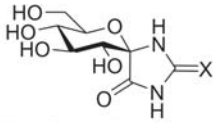
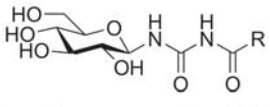
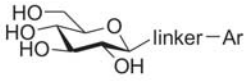
Type 2 (or non-insulin-dependent) diabetes is a severe and prevalent disease spreading epidemically all over the world.[1] Inhibition of glycogen phosphorylase (GP)[2,3] the rate-limiting enzyme of glycogen degradation, has been attracting a lot of interest among several investigational approaches[4] to provide more efficient control of blood glucose levels as compared to the existing therapies. Glucose derivatives binding to the catalytic site of the enzyme represent an intensively investigated group of GP inhibitors: spiro-hydantoins **1** and **2**, as well as acyl-ureas **3** and **4** are among the most active compounds and serve as leads for the design of more efficient derivatives.[5,6]



1 X = O K_i = 3.1 μM
2 X = S K_i = 4.2 μM



3 R = Ph K_i = 4.6 μM
4 R = 2-naphthyl K_i = 0.35 μM



5

The presentation will focus on the synthesis and enzyme kinetic evaluation of derivatives **5**, as to the length and atomic composition of the linker and size and orientation of the aromatic moiety in order to establish structure-activity relationships. Binding modes as revealed by X-ray crystallographic investigation of some of the enzyme–inhibitor complexes will also be presented.

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STEREOCHEMICAL STUDY ON IN VIVO AND IN VITRO METABOLISM OF SELEGILINE

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Selegiline, the *R*-(–)-isomer of the chiral compound deprenyl, is a selective and irreversible inhibitor of monoamine oxidase (MAO) B enzyme. It is widely used in the treatment of Parkinson's disease and other neurodegenerative disorders. Neuroprotective and antiapoptotic activity of the compound in various pharmacological tests has also been reported. These effects are, at least partly, independent of the inhibition of MAO-B, though still stereoselective. Majority of them requires metabolic transformation of selegiline, however, the known metabolites were found only partially effective. Recently, besides desalkylation another metabolic route has been proposed, that results in the generation of *N*-oxidized metabolite.

The objective of our study was to investigate the extent of generation of this new metabolite during metabolism of deprenyl enantiomers both *in vivo* and *in vitro.* Since a new asymmetry center is created during the *N*-oxidation of the prochiral *tert*-nitrogen containing deprenyl, the stereochemistry of the conversion, as well as the metabolic enzymes involved were examined.

Chiral capillary electrophoresis methods have been developed for the simultaneous separation of deprenyl enantiomers and their metabolites. The methods have been validated for the determination of the metabolites in rat urine and microsome preparations. In case of selegiline and *S*-(+)-deprenyl, the *N*-oxidation has been demonstrated *in vivo* and *in vitro* alike. The metabolic transformation has been found stereoselective in rats, and the generation of the *NS*-isomers was preferred. In the *in vitro* metabolism studies, using human flavin-containing monooxygenase enzymes (FMO), both isomers proved to be better substrates for FMO 1, the extrahepatic isoform of the enzyme, than FMO 3. Methamphetamine, desalkylated metabolite of deprenyl, was also converted by FMO: both of its isomers were substrates of FMO 1, but only *S*-methamphetamine was that of FMO 3. Methamphetamine-hydroxylamine formed was further transformed by FMO enzymes; amphetamine-hydroxylamine was identified as the product of a demethylation reaction.

The observed differences between the activities of the FMO isoenzymes can result in significant species variations in deprenyl metabolism. The neuroprotective potential of the *N*-oxidized metabolite of selegiline is to be investigated.

NEW MECHANISMS IN THE P53 PATHWAY FOR THERAPY

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Computer simulations have shown that there are mechanisms in addition to the classically perceived ones that control mechanistically the phsophorylation-dependant interaction between p53 and its negative regulator, the E3-ubiquitin ligase MDM2. The computer predictions have been experimentally verified and are now providing new avenues for targetted therapy.

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NEW ENDOMORPHIN ANALOGUES WITH ALICYCLIC β-AMINO ACIDS: INFLUENCE ON BIOACTIVE CONFORMATION AND PHARMACOLOGICAL PROFILE

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Alicyclic β-amino acids, *cis*-(*1S,2R*)-ACPC/ACHC, *cis*-(*1R,2S*)-ACPC/ACHC, *trans*-(*1S,2S*)-ACPC/ACHC and *trans*-(*1R,2R*)-ACPC/ACHC were introduced into endomorphins[1] in order to examine the conformational effects on bioactivity. Using a combination of different receptor binding techniques, ^{1}H-NMR and molecular modeling it was concluded that Pro[2] replacement by these residues does not lead to the pharmacological inactivation of endomorphins, in accordance with earlier observations[2], but results in proteolyticly stable peptide analogues with similar pharmacological activities. In general, analogues containing *cis*-(*1S,2R*)-ACPC/ACHC showed the highest μ-opioid receptor affinity and selectivity, which were comparable to that of the parent endomorphins. The *in vitro* potency of the synthesized compounds was diverse as measured by competition and GTPγS experiments. The importance of the configuration of alicyclic β-amino acids was also confirmed by molecular modeling studies. Several possible conformations were proposed for compounds containing *cis* alicyclic amino acid isomers, and the bioactive structure is most likely to be found among these. Even considering that conformational studies were done on isolated molecules in aqueous media and therefore the most populated conformational family does not necessarily correspond to the bioactive structure, a higher propensity of various turn structures were found for analogues which also displayed higher receptor affinities. This suggests that these peptides might bind to the μ-opioid receptor in a compact, folded structure rather than extended.

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N-HETEROCYCLIC CARBENE-LIGATED PALLADACYCLE AS RATIONALLY DESIGNED, HIGHLY ACTIVE, PRACTICAL CATALYST FOR HECK-MIZOROKI REACTION

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In recent years, bulky, *N*-aryl-substituted N-heterocyclic carbenes (NHCs) have emerged as high-performance ligands in Pd-mediated cross-coupling reactions. However, complexation of NHCs to Pd is not trivial. While catalytically active, catalytic brews prepared *in situ* from NHC precursors (e.g. imidazolium salts) and common sources of Pd do not allow control of the catalyst yield and chemical composition, resulting in waste of precious metal and poor reproducibility. Therefore, the use of well-defined, monoligated complexes of bulky NHCs with Pd has become the dominant strategy to overcome this drawback. Palladacycles ligated with NHCs are excellent candidates for the development of air stable, highly active cross-coupling catalysts. However, the current synthetic methods for the preparation of NHC-palladacycle adducts require the use of highly moisture- and air-sensitive isolated carbenes and purified palladacycles, which are often prepared in low yields under harsh conditions. Therefore, the development of a more practical method employing easily accessible materials will be necessary if the potential of NHC-palladacycles in catalysis is to be fully realized. Recently, we have developed a novel protocol for NHC-palladacycle synthesis relying on a one-pot, three-component, sequential reaction between imidazolium salts, $PdCl_2$ and *N*-benzyldimethylamine. The preparation of NHC-palladacycle adducts by this methodology will be presented, as well the use of these complexes as well-defined precatalysts in Heck-Mizoroki reactions of highly substituted aromatic and heterocyclic substrates.

NEW DEVELOPMENT OF IMIDAZOLIUM AND N-HETEROCYCLIC CARBENE CHEMISTRY

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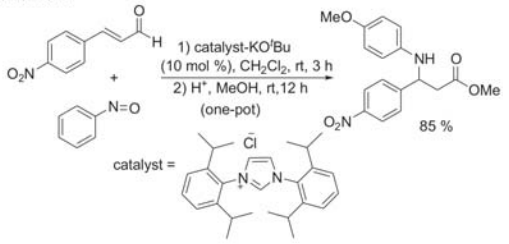
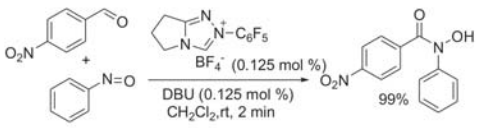
N-heterocyclic carbenes (NHCs) have attracted considerable interest in recent years for their versatile chemistry. The striking similarity of electron-rich organophosphanes PR_3 and NHCs, and NHCs' excellent σ-donating properties allow them to be employed both as nucleophile catalysts and as ligands of choice for a wide range of transition metals. Here, we have developed a novel *N*-heterocyclic carbene catalyzed one-pot synthesis of *N*-PMP protected β-amino acid esters by reaction of enals and nitrosobenzene, followed by an acid-catalyzed esterification and Bamberger-like rearrangement of the intermediate *N*-phenylisoxazolidin-5-ones. We have also developed a powerful NHC-catalyzed amidation of aldehydes with nitroso compounds to form a variety of *N*-arylhydroxamic acids. The reaction can be carried out with a catalyst concentration as low as 0.125 mol%.[1] The first NHC-based transition metal catalysts have been developed for C-S coupling reactions. Ni-NHC catalysts showed good to excellent activities towards various aryl halides in C-S coupling reactions. The new catalysts were inexpensive, easy to synthesize and environmentally friendly. They could be excellent candidates to replace Pd-PR_3 for this reaction. It was also found that the electronic and steric characteristics of NHC ligand greatly affected the catalytic activities.[2]

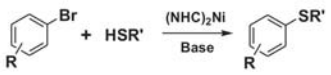
Scheme 1



Scheme 2



Scheme 3



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POSTERS

STUDY OF INTRACELLULAR CALPAIN FUNCTION AND ACTIVITY

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Calpains are intracellular cysteine proteases whose catalytic site is analogs to that of papain-like protease domain. Calpains are of considerable interest because of its implication in numerous physiological and pathological events. Our aim is to study the intracellular function of calpains. For this purpose cell-penetrating calpain substrate and calpain activator peptides were developed.

Our early results suggested that a peptide substrate, *Dabcyl*-TPLKSPPPSPR-*EDANS*, based on the calpain cleavage sequences is suitable for developing a new cell-penetrating calpain substrate[1]. This compound with the Dabcyl and EDANS fluorophores as a FRET pair is specific for calpain even in cell lysate, but unfortunately has poor cell-uptake. Therefore we have elongated this sequence at the *C*-terminal with heptaarginine unit possessing cell-penetrating activity. In order to preserve the necessary distance between the two FRET partners, we inserted a Glu residue between the substrate and heptaarginine parts of the peptide. Thus, the cell-penetrating substrate, *Dabcyl*-TPLKSPPPSPRE(*EDANS*)R_7 was synthesized. This peptide not only retained the substrate property, but was better substrate of Calpain B enzyme. Cell-uptake studies showed that the conjugate enters COS-7 cells more efficiently than the peptide substrate without heptaarginine.

On the other hand the specific activation of intracellular calpain may be a suitable tools to study its function. For this, we synthesised a new group of cell-penetrating calpastatin-peptide conjugates with the activating capacity of m-calpain intracellularly. In these constructs, peptides related to the calpastatin A or C subunit was covalently conjugated to the C-terminal of penetratin via amide, thioether, or disulfide bond[2]. These conjugates were prepared by solid-phase synthesis and/or by chemical ligation. Our results show that conjugates with different bonds possess essentially the same level of activation. Internalization experiments with fluorophore at the N-terminal of penetratin and/or 5(6)-carboxyfluorescein (cf) labeled conjugates show that these constructs are taken up by COS-7 cells. We also noticed that the conjugate even with a disulfide bond between the components seems to be stable and activate m-calpain after intracellular translocation under the conditions studied. To the best of our knowledge, this is the first report to describe conjugates with an m-calpain activating effect on isolated enzymes and more importantly within living cells after transmembrane delivery. Thus, these conjugates seem to be appropriate as molecular tools to activate intracellular m-calpain and to study calpain functions in living cells.

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CONTROL OF DRUG RELEASE BY SMART MEMBRANE

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The idea of intelligent and smart materials evolved in the late 1980´s with the purpose of establishing a new area in material science to take into account the interrelation between materials and natural (external) environment. The term smart materials refer to a class of monolith and composite media having inherent intelligence together with self-adaptive capabilities to external stimuli. This newly developed concept aims to create artificially designed systems possessing sensor-, processor- and actuator functions internally in the material itself. Magnetic field responsive materials are specific subsets of smart materials which can adaptively change their physical properties due to external magnetic field, respectively. At present there are several adaptive materials that can actuate or alter their properties in response to a changing environment. Certain polymer gels represent one class of these materials. Their unique properties based on volume phase transition as a response to a small change of external conditions make such systems useful.

Stimuli-responsive membranes have attracted a widespread interest in the past decade, where the period and rate of transfer can be controlled by an external influence (e.g. temperature, pH, electric or magnetic field etc). The most significant weakness of all these external stimuli-responsive membranes is that their response time is too slow.

In order to enhance the response time of the membrane we prepared a stimuli-responsive composite – gel membranes containing ordered micro channels.

A composite-heterogeneous gel membrane consisting of magnetic polystyrene/ poly(N-isopropylacrylamide) (MPS-PNIPA) core-shell magnetic latex particles dispersed within a PVA network were developed and characterized as a temperature - responsive membrane in this study. The core – shell magnetic latex particles containing poly(N-isopropylacrylamide) in the shell were prepared by encapsulation of magnetic polysyrene core using a precipitation polymerization process. This membrane can act as „on - off switches" or „permeability valves". The duration and the rate of the mass transfer can be controlled by external triggers like temperature or magnetic field. The channels are designed to contain ordered array of stimuli responsive core-shell type gel beads that can change their size according to external stimuli.

By varying the thickness of the shell it is possible to tune the permeability of the membranes over a wide range. Since there is a great diversity of external triggers to control the permeability of solutes, this concept will also be used to develop smart membranes whose pores can open and close by electronically induced external triggers such as magnetic field.

SUPERCRITICAL CARBON DIOXIDE AND CONVENTIONAL EXTRACTION OF BETULIN DERIVATIVES FROM BETULACEAE AND PLATANACEAE SPECIES

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Lupane-type pentacyclic triterpenes, like betulin/betulinol, betulinic acid and lupeol have recently been investigated for their various pharmacological and medicinal properties[1]. High anti-inflammatory, antiviral and antitumor effect of betulin and betulinic acid were described[2]. Betulinic acid has specific cytotoxicity on several tumor cell lines by inducing apoptosis in cells. More betulinic acid derivatives are proved to be potent and highly selective inhibitors of HIV-1. It was also revealed that lupeol and betulin suppress superoxide generation in human neutrophils.

Birch bark as the main source of betulin is well-known, however quantitative data have not been published yet. The aim of our work was to exhibit alternative sources of these triterpenes, and analysing their occurrence both qualitatively and quantitatively in the different plant extracts prepared by supercritical fluid extraction (SFE) and traditional Soxhlet extraction.

The betulin, betulinic acid, lupeol and β-sitosterol content in the bark of *Betula pendula* Roth, *Alnus glutinosa* (L.) Gaertn. and *Platanus hybrida* Brot. was examined by flame ionization detector- and mass spectrometry-coupled gas chromatography (GC-FID, GC-MS), by high-performance liquid chromatography (HPLC) and by TLC-densitometry. Furthermore, the effect of the two extraction methods on the extraction yields was compared.

All four compounds were identified in the bark extracts by TLC-densitometry as well as by GC-MS comparing the mass spectra and retention time with those of pure standards. The main components were betulin and lupeol. Betulinic acid seemed to be a minor constituent, while β-sitosterol occured in considerable amount both in the extract of the leaves, fruits and bark of the Betulaceae and Platanaceae plants. Alnus bark and leaves as promising new sources of lupane-type trierpenes and phytosterols were quantitatively studied. Phytosterols were measured by GC-FID method using 5-α-cholestan-3-on as internal standard. Since betulin proved to be unstable in gas chromatographic model experiments, it was determined by reversed phase (RP-HPLC) Supercosil^{TM} LC-18 column using acetonitril-water 80:20 (v/v) as mobile phase instead of gas cromatography. Detection was accomplished with UV detection at λ= 210 nm. In comparison of the extraction methods, SFE (CO_2) and methanolic Soxhlet extracts seemed to be the best sources of the examined compounds.

Acknowledgement

This work was supported by GVOP 3.1.1.-2004-05-0397/3.0.

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EUPHORBIA SPECIES AS A SOURCE OF STRUCTURALLY DIVERSE BIOACTIVE DITERPENES

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Plants of the Euphorbiaceae family accumulate a high diversity of structurally unique diterpenes, which have attracted great interest from biogenetic, pharmacologic and toxicologic points of view. Characteristic constituents of the family include the tigliane, ingenane and daphnane diterpenes, referred to overall as phorboids,[1] which have protein kinase C-activating and vanilloid receptor type 1 agonist effect.[2,3] Besides these compounds, considerable attention has been paid to the macrocyclic diterpenes, e.g. lathyranes, jatrophanes, which occur exclusively in the Euphorbiaceae family. Pharmacological studies have revealed their antineoplastic, PGE_2-inhibitory, antiviral and propyl endopeptidase-inhibitory activities.

In the course of our studies the chemistry and pharmacology of Hungarian *Euphorbia* species were investigated with special regard to the diterpene-type compounds. Highly functionalized new jatrophane, lathyrane, pepluane and euphoractine-type diterpenes were isolated from *Euphorbia esula, E. salicifolia, E. peplus, E. serrulata, E. platyphyllos* and *E. villosa*. The structures were established by HRESIMS, advanced two-dimensional NMR methods. Stereochemical studies and conformational analysis were performed by means of NOESY experiments, and the absolute configuration of some compounds was determined by X-ray crystallography.

The isolated diterpenes were investigated as concerns the reversal of MDR in mouse lymphoma cells *in vitro*, using the Rhodamine 123 exclusion test. The results show that the structurally related diterpenes differ significantly in the inhibition of the efflux pump activity of Pgp in tumour cells. Compounds derived from *E. peplus*, *E. serrulata, E. platyphyllos* and *E. villosa* displayed strong activity in reversing the MDR, while compounds obtained from *E. esula* and *E. salicifolia* revealed only weak potency. The antiproliferative activity of the compounds was evaluated on drug resistant and drug sensitive L5178 mouse lymphoma cells and found that some compounds exhibit cell growth inhibitory effect. Within the set of *Euphorbia* diterpenes jatrophanes appears the most powerful Pgp inhibitors, and are promising to improve drug therapy in multidrug resistant cancer.

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A TEMPERATURE SENSITIVE HYDROGEL VALVE FOR MICROFLUIDIC APPLICATIONS

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Microfluidic applications are getting more and more sophisticated for chemical and biological analyses. The possibility of routing and controlling liquid flow can be of great importance in such applications. A temperature controlled valve is presented here for flow control. The material of the valve is an N-isopropylacrylamide based temperature sensitive hydrogel which is located in a microfluidic T junction. The gel is positioned in such a way, that when swollen (at room temperature) it obstructs the fluid flow. When heated above the critical temperature (40 degrees Celsius) the hydrogel undergoes a phase change thus shrins which in turn opens the channel for the fluid flow. The time dependent swelling and deswelling of the hydrogel in the microchannel is being investigated experimentally. Furthermore a numerical analysis is being done using a simple elastic model for the gel phase changes.

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EFFICIENT INTRACELLULAR DELIVERY OF FUNCTIONAL PROTEINS AND DRUGS USING BIODEGRADABLE AND CATIONIC CORE/SHELL POLYMER NANOPARTICLES

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Protein therapeutics has emerged as an important R&D sector for effective treatment against a broad range of human diseases, including cancer, autoimmune diseases and metabolic disorders. We have successfully developed a non-viral protein-delivery system that utilizes electrostatic availability of cationic core/shell nanoparticles to introduce functional proteins into viable cells. These nanoparticles, self-assembled from a biodegradable and amphiphilic copolymer poly{*N*-methyldietheneamine sebacate)-*co*-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate} P(MDS-*co*-CES), are attractive protein carriers because of their convenience in fabrication and structural stability that enables prolonged circulation in blood and slow elimination by the recticuloendothelium system. In this study, the cationic nanoparticles were fabricated to deliver an anticancer protein, lectin A, into MDA-MB-231, HeLa, HepG2 and 4T1 cancer cell lines. The polymer concentration was optimized, in which the polymer did not have significant cytotoxic effects, while at the same time, was of sufficient amount for lectin A to be efficiently delivered for maximum cancer cell eliminating effects. The optimal polymer concentrations were identified to be 20, 50, 40 and 100 ppm (i.e. mg/L) with IC_{50} values of lectin A as 0.2, 0.5, 10 and 50 mg/L for MDA-MB-231, HeLa, HepG2 and 4T1 cells, respectively. P(MDS-*co*-CES) nanoparticle-mediated lectin A delivery displayed a much higher anticancer effectiveness as compared to a commercially available lipid-based protein carrier, BioPorter. In addition, P(MDS-*co*-CES) nanoparticles had an added advantage over BioPorter with their ability to deliver lectin A and exert cytotoxic effects even under the interference of serum proteins. Confocal images revealed nucleus localization of lectin A when delivered *via* P(MDS-*co*-CES) nanoparticles. Next, in our preliminary studies on codelivery of herceptin and paclitaxel using P(MDS-*co*-CES) nanoparticles, synergistic anticancer effects have been observed in MCF7 and T47D cells. These nano-sized particles show great potential to serve as carriers for intracellular delivery of biologically active proteins.

MICROSTRUCTURE-PROPERTY-SYNTHESIS CORRELATIONS FOR MICRO- AND NANOSIZED BIOPOWDERS

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During the past decade, micro- and nanosized powders of different composition, morphology and properties have been successfully applied in biomedicine and many other biology related fields. Recent developments in synthesis methods including ability to produce single and composite micro- and nanoparticles with unique shape, narrow particle size distribution, controlled bulk and surface compositions and other special, application oriented characteristics, have made these particles even useful materials for a range of new applications. Among the perceived applications much current interest is concentrated on such applications as targeted drug delivery, diagnostics, tissue repair, detoxification of biological fluids, hyperthermia etc.

In order to produce tailor–made micro- and nanopowders for any of the above applications, a solid scientific and technological knowledge and a huge arsenal of up-to-date research and characterization facilities are required.

Similarly to other structural and functional materials there are strong and complicated interactions among composition, microstructure, properties and synthesis routes of particular micro- and nanosized powders. In this presentation a review is given on these interactions, on selected models as examples.

KINETIC MEASUREMENTS ON FLOW CYTOMETER: SIMULTANEOUS MONITORING OF INTRACELLULAR PROCESSES IN SOLUBLE CELLS

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Flow cytometry (FACS) enables the sequential determination of intracellular analytes and pH values in millions of cells loaded with specific ionophore dyes over a period of time. This provides a unique opportunity to measure intracellular processes simultaneously in different kinds of millions of cells during cell activation. However, FACS is not utilized widely to this purpose as there was no reliable method to analyze and describe kinetic alterations. With current software one is able to estimate some parameters, but individual measurements cannot be compared on an objective way. Our innovation is the development of multiple FACS assays and a platform for mathematical characterization of measured data.

We created two FACS assays on BD FACS Aria instrument. The first one monitors calcium flux, generation of reactive oxygen species and mitochondrial membrane potential, while the second one monitors mitochondrial calcium flux, nitric oxide generation and plasma membrane potential in CD4+ and CD8+ lymphocytes simultaneously before and after the administration of a lymphocyte activator.

The generated millions of FACS data are handled by our unique statistical approach. The first step is to fit functions to the kinetic alteration of each of measured intracellular analytes. For this purpose, different kinds of functions are fitted to the median values of kinetic measurements and F-test is used to find the best one. To determine the confidence interval of parameters of the function fitted to the median values, data are boot-strapped to generate 1000 different functions. This also provides an opportunity to statistical comparison between individual measurements.

This technique may be used to investigate purposes (i.e. to test the impact of any agent (such as immunmodulatory drugs) on cellular processes in lymphocytes) and to diagnostic purposes (i.e. to test the alteration of lymphocyte activation characteristics in disease).

SYNTHESIS OF STEROIDAL OXAZOLIDONES, AS NOVEL POTENTIAL INHIBITORS OF 17α-HYDROXYLASE-$C_{17,20}$-LYASE

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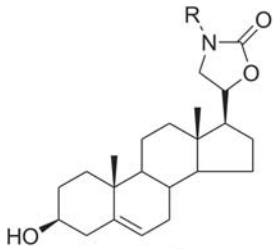
Prostatic cancer is the second leading cause of cancer-related mortality worldwide. 17α-Hydroxylase-$C_{17,20}$-lyase ($P450_{17\alpha}$) is a key enzyme regulating the androgen biosynthetic pathway. Inhibition of this enzyme can block androgen synthesis at an early stage, and may therefore be useful in the treatment of prostatic carcinoma.

We recently set out to synthesize a novel series of steroidal oxazolines, tetrahydrooxazin-2-ones and dihydrooxazines, in which there is a heterocycle containing two heteroatoms at position 17β of androst-5-en-3β-ol[1,2]. We report here the syntheses of a variety of steroidal compounds with the common structural feature of a C-17 2-oxazolidone on the steroid skeleton (**1**, **2**), as presumed inhibitors of $P450_{17\alpha}$.

The inhibitory effects of these compounds on rat testicular $C_{17,20}$-lyase were investigated with an *in vitro* radioincubaton technique.[3]



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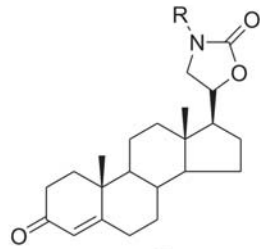

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Acknowledgment

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SYNTHESIS AND QSAR MODELLING OF CRYPTOLEPINE AND δ-CARBOLINE DERIVATIVES

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Malaria is still one of the most widely spread disease worldwide. Despite the efforts for developing new agents, only a few effective synthetic and natural antimalarial drugs were discovered in the past years. In addition, development of resistance makes the traditional antimalarial agents ineffective, therefore there is a continuous need for new medicines.

In this presentation we report on QSAR study on known antimalarial compounds, and the synthesis of new diazines with possible antimalarial effect.

In our QSAR modeling, we were focusing on cryptolepine derivatives and δ-carbolines. In the study we included 31 compounds with *in vitro* antiplasmodial activity data (IC50) against resistant K1 P. falciparium strain[1]. Two groups of models (model A and model B) were developed using the two lowest energy conformers of the reference compound. Automatic flexible alignment was used for the superposition, then manual modifications were also employed for further refinement. QSAR models were generated using the COMFA modul of SYBYL with PLS (Partial Least Square). Leave-one-out (LOO) and leave-multiple-out (LMO) cross validation schemes were used for model validations, whereas external validations were also carried out in order to check the predictive power of the models.

As another direction of our antimalarial research, benzo[*f*]phthalazin-4(3*H*)-one, and its isomer: benzo[*f*]phthalazin-1(2*H*)-one as analogues of δ-carboline were synthesized. These compounds have also recieved much attention, due to the structure-elucidation of cytotoxic compound *Samoquasine A* isolated from the plant Annona squamosa;[2] *Samoquasine A* was identified as benzo[*f*]phthalazin-4(3*H*)-one. Since, its structure has not been proven fully, we elaborated an unambigous synthetic pathway for the synthesis of the proposed structure. Our method was based on a Pd-catalysed cross-coupling reaction of a halodiazine in combination with a methodologically novel condensation reaction for the ring closure.

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TOWARDS PROCESS DEVELOPMENT OF *L*-PROLINE CATALYSED ASYMMETRIC HYDROXYMETHYLATION OF CYCLIC KETONES

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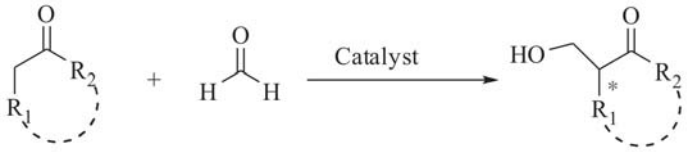
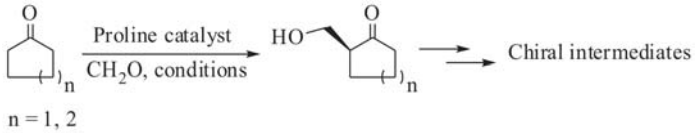
Hydroxymethylation reactions involve the aldol reaction of carbonyl compounds with formaldehyde to introduce a functionalised C1 unit at the α-position of the substrate (**Scheme 1**). The products are valuable intermediates, particularly when they are in chiral form. The asymmetric version of hydroxymethylation is particularly challenging[1-2] due to the nature of formaldehyde which is either in its polymeric or hydrated form.



Scheme 1

Recent reported α-hydroxymethylation of cyclohexanone using *L*-proline[3] and its derivatives[4] provides a mild and efficient way for enantioselective α-hydroxymethylation. In view of the high enantioselectivity obtained, its use of environmentally benign organocatalysts and cheap starting materials, highly valuable products for potential pharmaceutical applications, this reaction was selected as an exploratory process development project (**Scheme 2**).



Scheme 2

This poster presents our efforts towards the development of a process for the α-hydroxymethylation of cyclic ketones. Optimisation on *L*-proline based catalysts, forms of formaldehyde, solvents, reaction time and additives will be discussed.

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GOLDILOCK'S EFFECT IN PHASE LABELING: SUPERLIGHT FLUOROUS METHODOLOGY

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The continuous need for creating novel compounds with functional and structural diversity gives an impetus for method development in synthetic chemistry. One of the pivotal tasks in this field is to develop methodologies which substantially increase the speed of compound synthesis by means of accelerated isolation. To fulfil this demand a broad assortment of solid- and solution-phase tagging approaches have been developed.[1] Furthermore, the phase tagging of reagents or catalysts are of great interest for use in large scale preparation also because of the cost and margin pressure on industrial scale production and the growing emphasis on clean (or less polluting) operation. Despite of the promising results, the relatively high cost of the tagged chemicals and/or their tedious, multistep synthesis still slows their spread.

Recently, the fluorous chemistry as an efficient solution-phase tagging methodology was developed, which based on the unique oleophobic and hydrophobic character of perfluoroalkyl compounds.[2] This technique was successfully employed in catalysis, parallel synthesis and biological sciences. In the initial biphasic applications perfluoroalkane solvents were utilized for catalyst recovery. However, several technological concerns - mainly the cost and persistence propensity of perfluoroalkanes - initiated a further refinement of the protocol.

In this poster, we report our further extension of fluorous chemistry: a simple, cost-saving tagging approach that takes advantage of the usage of the smallest possible fluorous phase tag; the trifluoromethyl group, combined with water induced change in phase distribution. This method preserves all relevant properties of the light fluorous methodology, but avoids the usage of costly and bioaccumulating long-chain perfluoroalkyl tags.

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APPLICATION OF A NEW ENANTIOPURE CHIRAL CROWN ETHER BASED CHIRAL STATIONARY PHASE IN ENANTIOSEPARATION OF RACEMIC PRIMARY ORGANIC AMMONIUM SALTS

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There is a requirement for chiral drugs to use as pure enantiomers today, thus the quick and cheap separation of enantiomers is a great challenge in drug development. Crown ethers immobilized on a solid matrix are promising packing materials which can be used in chiral liquid chromatography (e.g. HPLC[1]). Optically pure chiral pyridino-, phenazino-, acridino- and acridono-18-crown-6 type ligands and their discrimination between the enantiomers of chiral primary organic ammonium salts have thoroughly been studied using ^{1}H NMR, photoluminescence spectroscopies, calorimetric titration and X-ray crystallography. Electronic circular dichroism (ECD) spectroscopy was also used in examining enantiomeric recognition of aralkyl ammonium salts[2].

The present poster reports on testing the new chiral stationary phase (CSP, Figure 1) with a variety of racemic primary aralkylammonium salts, mainly aromatic amino acids and derivatives using HPLC.

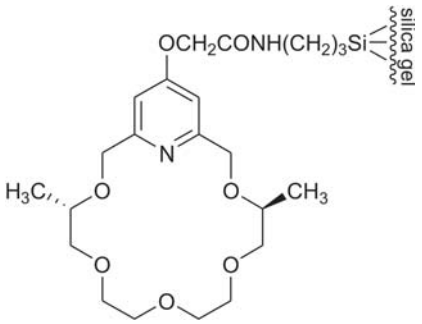

Figure 1. Structure of the new chiral stationary phase (CSP)

Acknowledgement

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A CONVERGENT APPROACH TO THE CONSTRUCTION OF THE CORDYPYRIDONE FRAMEWORK

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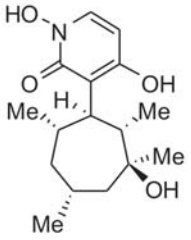
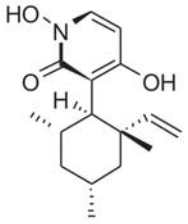
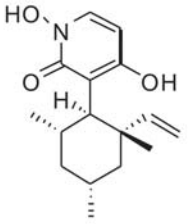
Natural products containing the *N*-hydroxypyridone motif are increasingly being recognized for their pronounced bioactivity. Clardy *et al*. have recently reported the isolation of akanthomycin from the entomopathogenic fungus *Akanthomycin gracilis* and established its antibacterial activity against *Staphylococcus aureus*.[1] The structurally related cordypyridones A and B were isolated from the same fungus. The antimalarial behaviour of the atropisomeric cordypyridones A and B has been previously established by Isaka *et al.* against *Plasmodium falciparum*.[2] In addition, cordypyridone A (8-methyl-pyridoxatin) was isolated from a culture of OS-F61800 and shown to induce erythropoietin gene expression in human cells.[3] This presentation describes a successful, convergent approach to the construction of the cordypyridone framework. The key step in our strategy involves the coupling of a protected pyridone derivative with an appropriately functionalised cycloalkane moiety. We have identified suitable coupling conditions for the key synthetic step and developed access to highly substituted cycloalkanones.



Akanthomycin



Cordypyridone A



Cordypyridone B

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PRACTICAL HECK-MIZOROKI REACTION PROTOCOL MEDIATED BY *N*-HETEROCYCLIC CARBENE (NHC)-LIGATED PALLADACYCLE AS A RATIONALLY DESIGNED CATALYST

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The ever-expanding use of Pd-mediated organic transformation in academia and industry has precipitated the need for more sophisticated catalysts. Recently, *N*-aryl-substituted *N*-heterocyclic carbenes (NHCs) have emerged as highly active ligands in Pd-mediated cross-coupling reactions. On the other hand, palladacycles serve as stable Pd (II) precursors for active Pd atoms: when submitted into the reaction conditions, the palladacycle moiety is degraded with rate dependent on the temperature and the nature of the cyclometallated ligand and the co-reactants. Therefore, palladacycles ligated with NHCs are excellent candidates for development of air stable, highly active cross-coupling catalysts. We shall present our recent results on the use of a NHC-ligated palladacycle as a precatalyst for the Heck-Mizoroki reaction. This rationally designed, single component precatalyst was synthesized on a multigram scale by a novel, one- pot, three-component, sequential reaction between *N*,*N*-dimethylbenzylamine, $PdCl_2$ and the corresponding imidazolium salt as the NHC precursor, is stable to air, moisture and long term storage and can be conveniently dispensed as a stock solution in NMP. A wide selection of challenging aryl bromides, such as containing two ortho-substituents, multiple methoxy groups, free anilines, phenols, aldehydes or N- and S-heterocycles as well as aryl triflates were explored. Less common alkenes (vinyl sulfones, vinyl phosphonates, acrylonitrile and cyclohexanone) were also coupled in moderate to excellent yields. Our Heck-Mizoroki reaction protocol employs a readily available catalyst; couples a wide range of highly functionalized aryl and heteroaryl substrates at a moderate catalyst loading level (0.5 or 2 mol%); does not require extensive substrate-by-substrate optimization; can be deployed with standard laboratory techniques (even in air) and requires minimal workup.

SEMICARBAZIDE-SENSITIVE AMINE OXIDASE AS THERAPEUTIC TARGET: INSIGHTS INTO THE BINDING PROCESS

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Semicarbazide-sensitive amine oxidases (SSAOs) belong in the family of copper-containing amine oxidases; they are sensitivity to inhibition by semicarbazide. SSAOs differ from the other main group of mammalian amine oxidases (AOs) with respect to cofactor, subcellular distribution (mitochondrial membrane vs. cell surface), substrates/inhibitors and biological function. The cofactor of SSAO is a unique 2,4,5-trihydroxyphenylalanyl based quinone ('topaquinone'). Increased SSAO levels have been identified in various pathological conditions, suggesting the potential utilitity of inhibitors. Paradoxically, substrates of the enzyme have recently also been taken into consideration as potential drugs for treatment of SSAO-related disorders.

In part of our studies to develop new inhibitors and substrates of SSAO, we now report on computational docking of some substrates and inhibitors of human SSAO into the binding site by using AutoDock (AutoDock, Scripps Research Institute).

In a docking study with series of aralkyl amine substrates, a leucine residue (Leu-469) located adjacent to the active site was identified as a gate-control residue, moreover, a correlation between binding energies and experimentally obtained data was also obtained.[1] In line with these key features of the previous findings, our procedure, in which the binding site and the binding process itself were investigated without any artifacial constrains[2,3], allowed us to identify some new features of the interaction:

i) the flexibility of Leu-469 residue is necessary for successful docking of substrate;
ii) aromatic residues (Tyr-384, Phe-389, Tyr-394) in the active site form a hydrophobic pocket, the interaction of which with substrates possessing apolar residues may be energetically significant;
iii) in the first step of binding, a decisive interaction occurs with the involvement of Asp-386 and amino group of the substrate.

We confirmed that the flexible side chain of topaquinone (TPQ-417) rotates from the "inactive" (on-Cu) to the "reactive" (off-Cu) conformation and also found that the energetically best docking conformations and interaction energies are in line with the previously published investigations.[1]

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BIFUNCTIONAL CINCHONA BASED THIOUREA ORGANOCATALYSIS IN ASYMMETRIC SYNTHESIS

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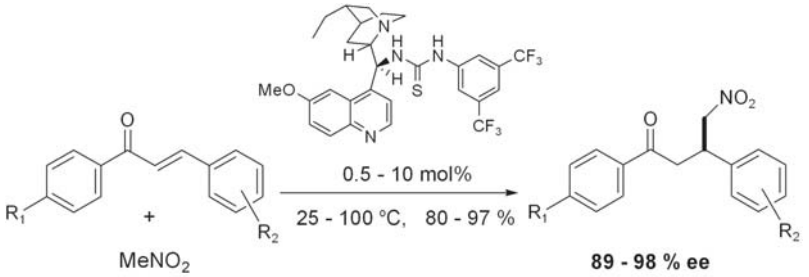
The conjugate addition of a stabilized carbanion to α,β-unsaturated carbonyl compounds is one of the fundamental C-C bond forming reactions in organic synthesis. In the case of nitroalkanes, the products of the 1,4-addition to enones are also useful intermediates for variety of further elaborated structures such as aminoalkanes, aminocarbonyls, and pyrrolidines. As a result, considerable effort has been directed toward the development of catalytic asymmetric versions of this process over the past several years.[1]

Recently, we have reported that a new bifunctional thiourea organocatalyst efficiently promote the Michael reaction between nitromethane and chalcones with high level of enantioselectivity.[2]



Herein, we report the scope and limitation of this methodology and its application in asymmetric synthesis of biologically important targets. Moreover, a plausible mechanism will be presented on the basis of kinetics data, NMR experiments, and theoretical calculations. The detailed understanding of the mechanism also revealed some important new principle of bifunctional organocatalysts.

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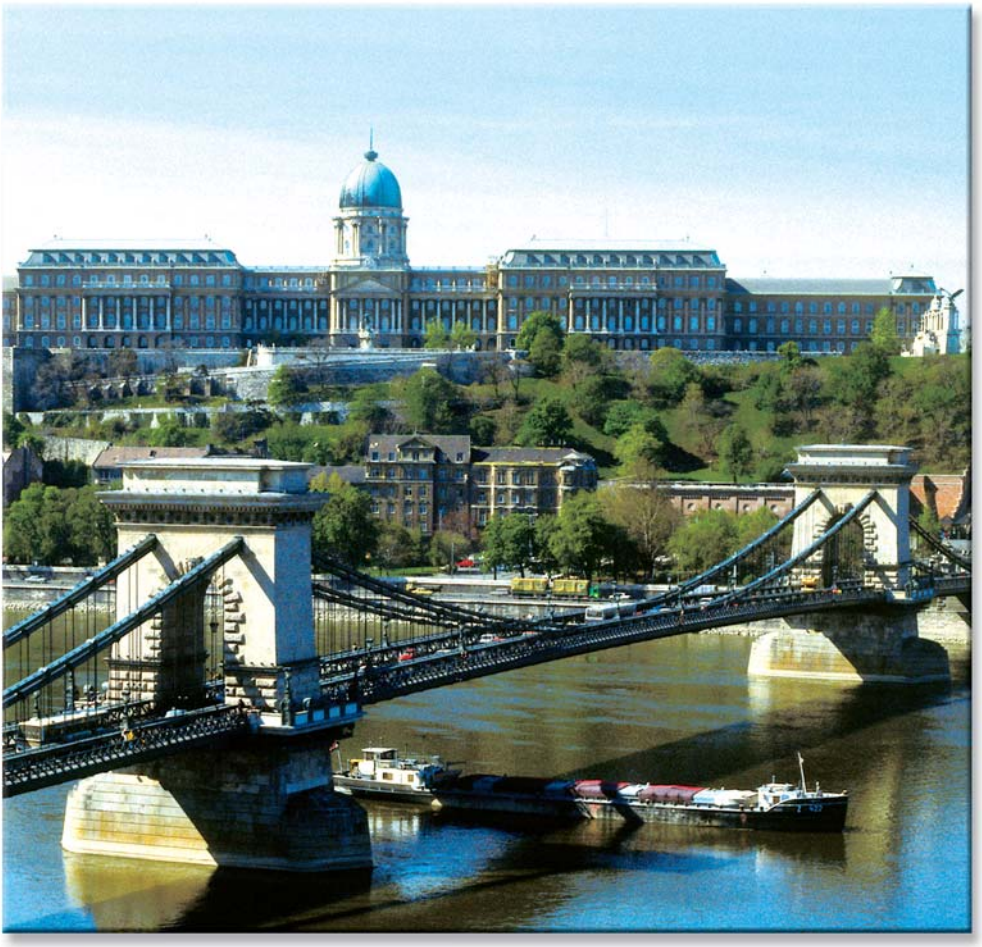

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