Drug Research Oral Presentation
Senior Scientists' Forum

The binding properties of some novel ruthenium (III) complexes with human serum transferrin

[1]Arsene Andreea L., [2]Uivarosi Valentina, [1]Mitrea Niculina, [1]Dragoi Cristina M., [1]Nicolae Alina

University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Bucharest, Romania
1Department of Biochemistry
2Department of Inorganic Chemistry
andreeanitulescu@hotmail.com

Background. The transferrin cycle has gained increased interest in recent years and it holds promise as an attractive system for strategies of drug targeting to tumor tissues. Indeed, tumor cells exhibit a large demand of iron for their growth and therefore express the transferrin receptor at a high rate. As a consequence, transferrin conjugates that retain a good affinity for the transferrin receptor can preferentially interact with cancer cells. This strategy is exploited nowadays for targeting novel anti-cancer drugs.
Following the success of cisplatin, several metal complexes other than platinum have been considered over the years as possible alternatives to cisplatin, particularly it was found that ruthenium (III) compounds possess antitumor and antimetastatic activities. The literature studies showed the affinity of these complexes for crucial biomolecules (like transferrin) and provide evidence for formation of stable adducts between them.
Objectives. The paper presents the transferrin-binding properties of some ruthenium (III) complexes with quinolones and dimethylsulfoxide, having general formula RuL2(DMSO) mCl3·nH2O ((Ru-nf) L: norfloxacin (nf), m = 1, n = 1; (Ru-cpx) L: ciprofloxacin (cpx), m = 2, n = 2; (Ru-of) L: ofloxacin (of), m = 1, n = 1; (Ru-levo) L: levofloxacin (Levo), m = 2, n = 8; (Ru-pip) L: pipemidic acid (pip), m = 1, n = 2, DMSO: dimethylsulfoxide). In this regard we investigated, in vitro, the interactions of these ligands with human transferrin through spectroscopic techniques, with the ultimate goal of preparing adducts with good selectivity for cancer cells.
Results and Conclusions. By analyzing the obtained experimental results a series of conclusions could be drawn: all studied complexes interact with human serum transferrin; the molar ratio [complex]/[transferrin] strongly influences the binding affinity and our research established that the best interaction between the complexes studied and transferrin is achieved for a molar ratio of 8; the best interaction was registered for ruthenium (III) complex with pipemidic acid (Ru-pip), followed by ruthenium (III) complex with norfloxacin (Ru-nf)

Comparison of dual acting and conventional NSAIDs towards parameters of NO-synthase system and oxidative stress in mucosal membrane of large intestine of rats with experimental ulcerative colitis

[1]Sklyarov A. Ya., [2]Lesyk R. B., [1]Panasyuk N. B., [1]Fomenko I. S., [2]Havryluk D. Ya.
Danylo Halytsky Lviv National Medical University,
69, Pekarska St., Lviv, Ukraine, 79010
[1] Department of Biochemistry
[2] Department of Pharmaceutical, Organic and Bioorganic Chemistry
sklyarov@meduniv.lviv.ua; dr_r_lesyk@org.lviv.net

Background. Although the etiology of ulcerative colitis remains unknown, mounting evidence implicates increased eicosanoids production in the inflammatory process of this disease. In the past few decades several compounds have been developed to block both COX and 5-LOX. Dual COX/LOX inhibitors constitute a valuable alternative to classical NSAIDs and selective COX-2 inhibitors for treatment of inflammatory diseases. Moreover, they appear to be almost exempt from gastrointestinal toxicity. The role of COX/PGs and LOX/LTs systems in the pathogenesis of ulcerative so far remains disputable and leaves much to be elucidated.

Aim of this research was to compare the action of nonspecific COX inhibitor indomethacine, COX-2 selective inhibitor celecoxib, 5-LOX blocker AA-861 and 2-amino-5-(3,5-ditertbutyl-4-hydroxybenzylidene)-thiazol-4-one (2A5DHT), which is the active substance of dual COX-2/5-LOX inhibitor darbufelone on indexes of NO-synthase system and intensity of oxidative stress in the mucous membrane of the large intestine (MMLI) under condition of experimental ulcerative colitis.

Results. In an experimental model of inflammatory bowel disease, nonspecific COX inhibition with indomethacin resulted in a significantly worse clinical condition, as compared with acetic acid colitis alone. Under COX-2 inhibition with celecoxib cytoprotective processes in MMLI were enhanced and oxidative stress levels were reduced. 5-LOX inhibition with AA-861 revealed no significant effect on structure-hemorrhagic lesions in MMLI caused by the administration of 4% acetic acid. Concomitantly, iNOS activity was reduced, MDA content displayed a tendency to the decrease. COX-2/5-LOX inhibition by 2A5DHT compound did not cause considerable destructive changes of the MMLI of rats. The activity of inducible nitric oxide synthase (iNOS) declined more than 2 fold as compared to their activity in colitis. The intensity of lipoperoxidation processes was found to be much lower than under the separate effect of celecoxib or indomethcine.

Conclusions. Dual COX-2/5-LOX inhibition with 2A5DHT compound displayed significant cytoprotective effect, manifested by the decreased area of the MMLI lesions, and the decline of NO-synthases activities and the intensity of lipoperoxidation processes. The substance 2A5DHT significantly over exceeds the cytoprotective effects of both selective and non-selective COX/LOX inhibitors and can be used in the treatment of inflammatory bowel disease.

Drug Research Oral Presentation
Yong Scientists' Forum

Design, synthesis and antitumor activity screening of novel heterocyclic derivatives of 4-thiazolidinones based on the hybrid pharmacophore approach

Havrylyuk Dmytro, Zimenkovsky Borys, Lesyk Roman

Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University,
69, Pekarska St., Lviv, Ukraine, 79010
d-havrylyuk@ukr.net; dr_r_lesyk@org.lviv.net

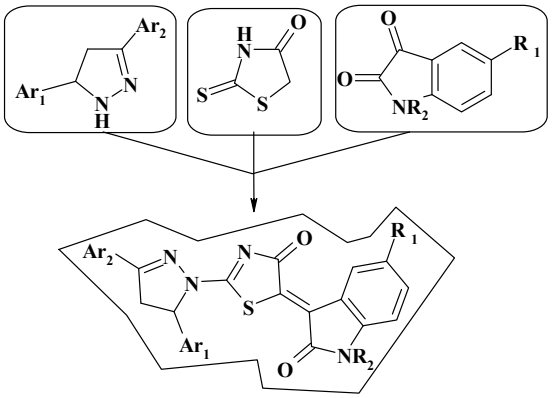
4-Thiazolidinone and 1H-indole-2,3-dione are the privileged scaffolds in modern medicinal chemistry having broad spectrum of biological activity and wide possibility to their chemical modification. The evaluation of antitumor activity is actual and promising for these compounds. The systematic study of 4-thiazolidinones derivatives with heterocyclic fragments in the molecules allowed us to identify a number of high-active compounds as potential antitumor agents [1,2]. The mechanisms of antitumor 4-thiazolidinones can be associated to their affinity to JNK-stimulating phosphatase-1 (JSP-1), tumor necrosis factor TNFα, anti-apoptotic biocomplex Bcl-XL-BH3, integrin αvβ3 receptor, nonmembranic protein tyrosine phosphatase (SHP-2), etc [2]. Besides that, the 1H-indole-2,3-dione derivatives possesses a potent anticancer activity as tyrosine kinase inhibitors and inhibitors of cyclin-dependent kinases (CDKs). From another hand various diazole derivatives (pyrazoles and pyrazolines) as inhibitors of cyclin-dependent kinase, heat shock proteins, vascular endothelium growth factors and P-glycoprotein were identified.

It is known that combination of different bioactive fragments with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects. Therefore, we synthesized non-condensed heterocyclic compounds by linking the main structural unit of the 4-thiazolidinone, 1,3-dihydroindol-2-one and pharmacological attractive pyrazoline moieties and carried out the evaluation of their antitumor activity in vitro.

Antitumor activity screening is conducted within the framework of DTP scientific program of National Institutes of Health (Bethesda, USA). Anticancer activity assays of 18 compounds allowed us to determine the high activity for 16 derivatives on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines in concentration 10-5 M. Subsequently, these compounds were selected in advanced assay against a panel of approximately sixty tumor cell lines at 10-fold dilutions of five concentrations (100μM, 10μM, 1μM, 0.1μM and 0.01μM). Finally, the tested compounds showed a broad spectrum of growth inhibition activity against all human tumor cells in micromolar concentrations at the GI50 level (average logGI50 values <-6.00). Among the tested compounds, Les-3833 was found to be the most active candidate with average logGI50 and logTGI values -7.46 and -6.81 respectively. Currently, the 3

lead-compounds from this group are under in-depth in vivo studies according to the decision of NCI Biological Evaluation Committee. One should note, that mentioned compounds showed no toxicity in Nontumored Animal Toxicity Assays.

A design for synthesis of 4-thiazolidinones based on the molecular hybridization



Current status of antitumor activity screening by NCI DTP Program

I Phase Prescreening at 60 cancer cell lines in concentration 10^{-5}M	Tested **18** compounds Selected **16** "hits" *in vitro*
II Phase 60 cell lines assay at 10-fold dilutions of five concentrations	Tested **16** compounds Selected **5** compounds for consideration by Biological Committee NCI
III Phase Consideration by Biological Committee NCI	Selected **3** compounds for in-depth study as drug-candidates
IV Phase Advanced biological studies *in vivo*	In-depth study of **3** compounds are in progress

In conclusion, these preliminary results allowed us: to identify the high group potency of synthesized compounds on antitumor activity assays *in vitro*; to select 3 lead-compounds for *in vivo* studies; to determine no toxicity of mentioned compounds *in vivo*.
Consequently, the hybridization of 4-thiazolidinone template with 1,3-dihydroindol-2-one and pyrazoline moieties in one molecule is promising approach in drug-like molecules design.

[1] R. B. Lesyk, and B. S. Zimenkovsky. 2004. 4-Thiazolidones: Centenarian History, Current Status and Perspectives for Modern Organic and Medicinal Chemistry. **Current Organic Chemistry,** 8: 1547-1577.
[2] D. Havrylyuk, B. Zimenkovsky, O. Vasylenko et al. 2009. Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. *European Journal of Medicinal Chemistry*, 44(4): 1396-1404.

Drug Research Young Scietists' Poster Presentation

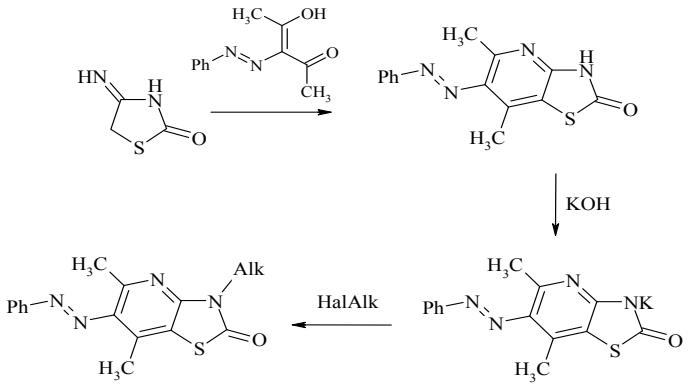
Synthesis, transformations and anti-inflammatory activity study of 3*H*-thiazolo[4,5-*b*] pyridine derivatives

Chaban T. I., Ogurtsov V. V.
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
69, Pekarska St., Lviv, Ukraine, 79010

chemdep@meduniv.lviv.ua

The intensive researches have been focused on five-membered heterocycles condensed with the pyridine ring in recent year as the potential anti-inflammatory drugs. Thiazolo[4,5-*b*]pyridines are ones of the least accessible and in turn the least-studied organic compounds. Their biological activity screening data are also not provided extensively. Studies on biochemical properties of these structures reveals they are possessing antifungal activity, can act as *H*3-histamine and metabotropic glutamate 5 (mGluR5) receptors agonists and also been found to provide high inhibitory activity against epidermal growth factor receptor and a number of other enzymes.

In this work we are reporting the convenient 3*H*-thiazolo[4,5-*b*]pyridine-2-one derivatives synthetic route development. 4-Iminothiazolidone-2 was used as the starting compound, which was treated with fenylazoacetylacetone to form 5,7-dimethyl-6-fenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one. The compound properties allowed to detect the acidic character of the proton in the thiazole ring third position, so its treatment with potassium hydroxide yielded the corresponding potassium salts. The nucleophilic properties of 5,7-dimethyl-6-fenylazo-3*H*-thiazolo[4,5-b]pyridine-2-one were exhibited in its reaction with electrophilic agents to form N-alkylated products. A series of appropriate alkyl halides, monochloroacetic acid and its derivatives were used as alkylating agents.



The structures of 5,7-dimethyl-6-fenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-one and its N-alkyl derivatives were established on the basis of H-NMR spectra.

The anti-inflammatory activity of the synthesized compounds was examined. They were evaluated for anti-exudative effect *in vivo* using carrageenin-induced paw oedema test in white rats. Ibuprofen, butadion and diclofenac sodium in their effective therapeutic dose were used as standards. The screening results demonstrate the significant anti-inflammatory action of 3-substituted 5,7-dimethyl-6-phenylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-ones while the nature of the substituent in thiazole ring third position is the defining criteria for the anti-inflammatory activity enhancing. The current research provides a new lead for developing potential anti-inflammatory drugs based on 3*H*-thiazolo[4,5-*b*]pyridine-2-one scaffold.

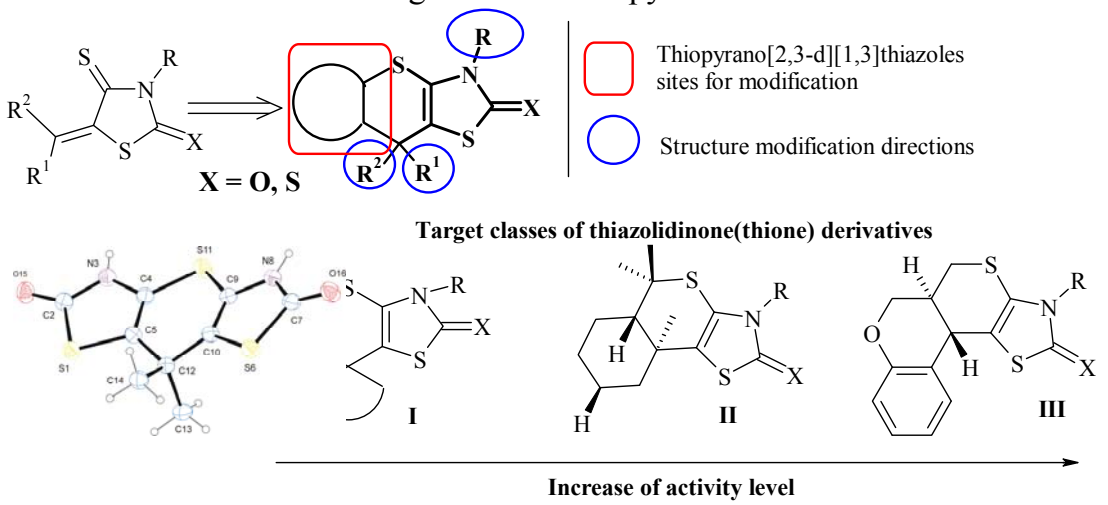
Design of new anticancer agents – thiopyranothiazole derivatives

Kaminskyy Danylo, Kryshchyshyn Anna, Lesyk Roman

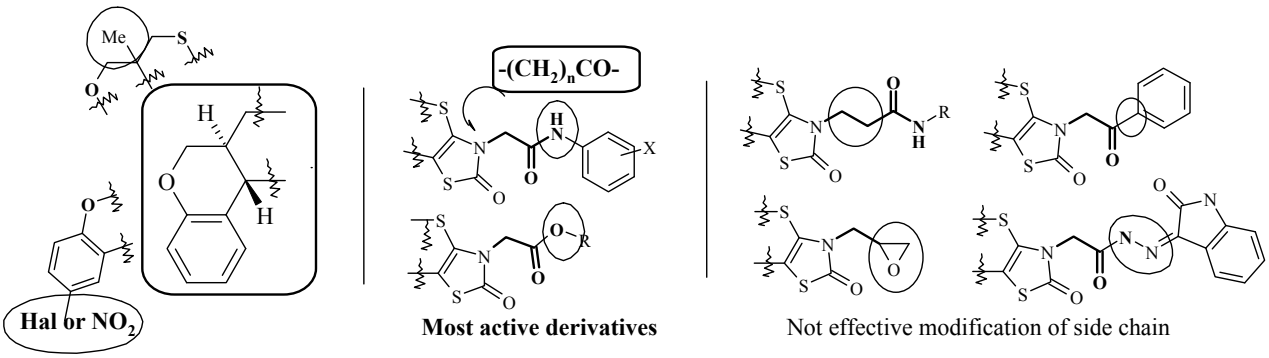
Department of Pharmaceutical, Organic and Bioorganic Chemistry,
Danylo Halytsky Lviv National Medical University,
69, Pekarska St., Lviv, Ukraine, 79010

dankaminskyy@gmail.com; dr_r_lesyk@org.lviv.net

Discovery of 4-thiazolidone derivatives anticancer potential provides wide opportunities for new anticancer agents design using this scaffold. Fused thiopyranothiazoles show special interest as potential source of antitumor agents. The background for our research was determining of the crucial role of substituent existence in the C5 position of basic heterocycle for antitumor activity appearance and the hypothesis that condensed heterocyclic systems possibly imitate the biological activity of their synthetic precursors, namely 5-ylidene-4-thiazolidinones. Previous research held in the department of Pharmaceutical, Organic and Bioorganic Chemistry and SAR data allowed us to outline the directions of rational design of novel thiopyranothiazole derivatives.



N-3 Unsubstituted compounds (**I-III**) were obtained using methods developed by us and lie in iso- and thiorhodanines usage as starting materials in the Knoevenagel, Diels-Alder and domino-Knoevenagel-*hetero*-Diels-Alder reactions as well. It is worth to mention that compound **I** belongs to the new class of 4-thiazolidinone derivatives and is obtained for the first time.



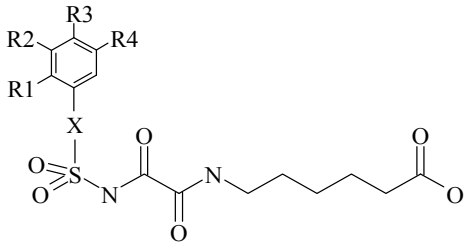
Anticancer activity evaluation of synthesized compounds was carried out within the Development Therapeutic Program (NCI, NIH, USA). As evidenced by the results of research the level of anticancer activity of tested compounds increases when passing from compound **I** to **III**. The derivatives of isorhodanine (X=O) have higher activity level than thiorhodanine isosteres (X=S). The best way of molecules optimization is introduction of the substituent in the position 3. This approach allows achieving a significant increase of the level and/or selectivity of the antitumor effect of the tested compounds in comparison with N-unsubstituted analogues. Among mentioned derivatives the highest anticancer activity was observed for the compounds with N-arylacetamide or ester fragments. Complication or simplification of given fragment led to activity level decreasing. Introduction of the nitro group or Br atom in the position 10 of the compound **III** is needed for the anticancer activity realization unlike introduction of the methyl group in the position 5a.

Anti-inflammatory, analgetic and diuretic activity prediction for 6{[({[phenyl]sulfonyl}amino)-(oxo)acetyl]amino}hexanoic acid derivatives by RDF descriptors approach

[1]Klenina O. V., [2]Tsepelevsky A. A.

[1]- Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
69, Pekarska St., Lviv, Ukraine, 79010
[2]- National pharmaceutical university, Kharkiv, Ukraine
olena_klenina@yahoo.com

QSAR analysis methodology makes use of the molecular descriptors offering valuable information about the structure of molecules, which is used later in the elaboration of the predictive models. This allows cost savings by reducing the laboratory resources needed, and the time required to create and investigate new drugs with certain desired biological activity. In this study, RDF descriptors were used to predict anti-inflammatory, analgetic and diuretic activity for a series of 21 compounds 6-{[({[phenyl]sulfonyl} amino)(oxo)acetyl]amino}hexanoic acid derivatives, which were synthesized at National pharmaceutical university, Kharkiv, Ukraine. They possess significant diuretic activity (the Berlin's test, %), 17 of them possess analgetic (%) against acetic acid induced writhing, and 13–anti-inflammatory (%) against histamine induced rats paw oedema activities. Furocemide, hydrochlorothiazide, metamizole sodium and diclofenac were used as standards.



X = CH_2 or none
R_1 = H, Br, NO_2 **R_2** = H, NO_2, COOH
R_3 = H, CH_3, COOH, NH_2, NO_2, Cl, Br, OCH_3, $COOCH_3$, $COOC_2H_5$, CH_2NH_2, $NHCOOCH_3$, $CH_2NHCOCH_3$, $NHCONHC_6H_{11}$(cyclo)
R_4 = H, Cl, Br

Firstly geometry optimization of compound using semi-empirical PM3 method included in HyperChem 7.5 was carried out. The DRAGON computer software web version 3.0 was used to calculate 150 RDF descriptors. The atomic masses, van der Waals volumes, Sanderson electronegativities and polarizabilities were used as bond weightings. The mathematical models were obtained by means of the Multiple linear Regression Analysis implemented in BuildQSAR software. The GA was used as the variable selection strategy. Regression model's predictive power was validated by calculating Q^2 values. The statistical significance was determined by the regression correlation coefficient, the Fischer ratio and the standard deviations. In order to avoid collinearity, Randic orthogonalization procedure was carried out.

Obtained three-variables modes for diuretic activity contain RDF075m, RDF115m descriptors both making positive contribution and RDF145(u,e,p) descriptors making negative contributions (r=0.714-0.802, F=5.90-9.62, Q^2=0.27-0.37). This corresponds to a radius of 7.5 to 14.5 Å. Two-variables QSAR models involving anti-inflammatory activity contain positively contributing RDF130m and negatively contributing RDF060(m,e,p) descriptors (r=0.853-0.867, F=12.03-13.63, Q^2=0.402-0.664). This corresponds to a radius of 6.0-13.0 Å. One- and two-variables models involving analgetic activity contain positively contributing RDF040(u,e,p), RDF140(p,v) and negatively contributing RDF060(u,e,v) descriptors (r=0.842-0.88, F=17.09-24.03, Q^2=0.628-0.709), which corresponds to a radius of 4.0 to 6.0 and 6.0 to 14.0 Å. In this sense a spherical molecular volume could have certain restrictions to the addition of bulky substituents.

Expression of regulatory genes in streptomycetes and overproduction of nogalamycin, doxorubicin and aranciamycin

[1]Klymyshin D., [1]Honchar M., [2]Nimets O., [3]Chaikovska A., [3]Kychabska M., [1]Kolysnyk H., [2]Fedorenko V.

1Institute of Animal Biology, Stoosa str. 38, Lviv, Ukraine, 79034;
2Ivan Franko National University of L'viv, Hrushevskuy str. 4, Lviv, Ukraine, 79005;
3Danylo Halytsky Lviv National Medical University, Pekarska str. 69, Lviv, Ukraine, 79010
dedima@rambler.ru

Streptomycetes are an abundant source of many commercially valuable natural products, including clinically important antibiotic, anticancer, and antiviral compounds along with agents utilized in agricultural, veterinary, and food industries. Doxorubicin, nogalamycin and arancyamycin are the anthracycline-type antitumor drugs produced by Streptomyces peucetius ATCC 27952, S. nogalater Lv65 and S. echinatus DSM40730. They act against the cancer cells of some leukemia; breast, stomach, lung, ovary, and thyroid cancers; soft tissue sarcoma; multiple myeloma and other cancer types. Bacterial strains isolated from nature usually produce only discrete amounts of particular secondary metabolites; production of a given metabolite of interest must therefore be improved in order to meet commercial requirements for its isolation. Most secondary metabolite biosynthetic pathways involve specific activator genes and global regulators. These regulators are considered important targets for metabolic engineering efforts.

In this work, regulatory studies for anthracycline producing strains improvement including investigations of regulatory mechanisms that act in the production of antibiotics during their biosynthesis in the parental strains are discussed.

Expression of global regulators in heterological hosts is an effective approach in overproduction of clinically important drugs. That is why we used different global and site-specific regulators to improve production of doxorubicin, nogalamycin and arancyamycin. AfsS, RelA, AbsA2 pleiotropically regulate antibiotic biosynthesis in S. coelicolor. Overexpression of these genes in S. coelicolor and related species causes antibiotic stimulatory effects in the host organism. In order to express these regulators in S. peucetius, S. nogalater and S. echinatus srains a set of plasmid constructions were obtained. Regulatory genes were subcloned in pKC1218E plasmid under the control of the erythromycin promoter and transferred into streptomycetes by conjugation from Escherichia coli ET12567 (pUB307). Our data shows that expression of afsS and absA2 in S. peucetius, S. nogalater and S. echinatus had no significantly enhancing on antibiotics production. Amplification of relA on a high-copy-number plasmid conferred overproduction of doxorubicin, nogalamycin and arancyamycin in these streptomycetes. We observed previously that multiple copies of site-specific regulator snorA coused to overproduction of nogalamycin in S. nogalater. We overexpressed this gene in S. peucetius and S. echinatus. Multicopies of snorA caused S. peucetius and S. echinatus to overproduce doxorubicin and aranciamycin, indicating that this regulator stimulates antibiotic production in the heterological hosts.

Overexpression of global and site-specific regulators is a good strategy for generating of industrial strains at the molecular genetic level. This in turn led us to study regulatory genes critical for secondary metabolite overproduction. Such experiments followed by detailed investigation of regulatory networks would contribute to strain improvement in forthcoming research.

This work was partly supported by WUBMRC Grant.

Metabolic engineering by interspecies protoplast fusion of angucycline producing streptomycetes

[1]*Kobylyanskyy A.*, [2]*Luzhetskyy A.*, [2]*Bechthold A.*, [3]*Fedorenko V.*

[1]Institute of Cell Biology,
14/16, Drahomanov St., Lviv, Ukraine79005
[2]Albert-Ludwigs-Universität, Institut für Pharmazie,
19, Stefan-Meier-Straße, 79104 Freiburg, Germany
[3]Ivan Franko National University, Department of Genetics and Biotechnology,
4, Hrushevskyy St. Lviv, Ukraine 79005
kob.anton@googlemail.com

Angucyclic antibiotics are quinone natural polyketide products bearing a characteristic four-ring frame of aglycon moiety, which is assembled in an angular manner. There are few hundreds of them characterised, all possessing antitumor activity. The majority of them are actinomycetes' secondary metabolites. For three last decades protoplast fusion has been successfully used to modify the phenotypic traits of streptomycetes. It allows achieving of high frequency gene transfer and recombination. Recently this process was optimized towards a recursive multiparental fusion and combined with high-throughput screening, which greatly accelerated the process of strain development.

Our approach involves genome shuffling of *Streptomyces* strains by means of protoplast fusion. These parental strains carry antibiotic resistance marker insertions in angucycline biosynthetic pathway genes. It stipulates recombination between them, leading to the formation of chimerical clusters, consistent of two parental ones. By applying this technology we aimed to provide the production of novel biologically active secondary metabolites and extend our knowledge about recombination events and enzyme substrate specificity. Fused mutants should be able to restore the missing glycosylation or oxygenation steps owing to complementation of metabolic pathways by the enzymes of both parental metabolic pools. Flexibility of polyketide modifying enzymes allows introduction of new functional groups or additional sugars in unusual for both parents' products positions.

Obtained recombinants displayed different morphology and secondary metabolites spectra in comparison with parental strains. The most remarkable was recombinant of *S. fradiae* Δ*urdQ/R* (olivose-biosynthesis deficient mutant of *S. fradiae* Tü2717) and *S. globisporus* M12 (oxygenase gene mutant of landomycin E producer *S. globisporus* 1912) called *Rec5*. It accumulates 40 angucyclic compounds, among which four are novel, i.e. glycosilated tetrangomycin and tetrangulols.

Metabolic engineering by means of interspecies protoplast fusion with consequent screening of antibiotic resistant recombinants is fruitful in case of angucycline producing mutants with damaged terminal polyketosynthetic steps. As a result of one fusion we obtained a recombinant producing a whole collection of compounds. It is a promising method to proceed in strain modification when inductive mutagenesis reaches deadlock. Beside pure combinatorial profit, a great deal of data on gene functions and nature of enzymes' interaction had been won.

The cytoprotective effect of hexapeptide arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine in experimental gastric lesions in rats

Nasadyuk Christina, Sklyarov Alexandr
Danylo Halytsky Lviv National Medical University
69, Pekarska St., Lviv, Ukraine, 79010
sklyarov@meduniv.lviv.ua

Background. Recent research showed the high gastroprotective activity of some olygopeptides in particular glyprolines, BPC 160 and hexapeptide arginyl-alfa-aspartyl-lysyl-valyl-tyrosyl-arginine (AALVTA). The positive effects of olygopeptides on gastric mucosa (GM) could be explained by their multidirectional influence on homeostasis, intercell communication, receptors of γ-***aminobutyric*** acid cooperating with the systems of dopamine, NO and prostaglandins [Ilic S. et al. 2009; Samonina G. et al. 2008; O. Orlovsky, 2007].

Objective and methods: Aim of research was to study the cytoprotective action of AALVTA in interconnection with the systems of NO-synthases (NOS), cyclooxygenases (COX) and lipooxygenases (LOX) in experimental gastric lesions (GL) in rats. GL in rats were induced by intraperitoneal application of epinephrine (2 mg/kg). Experimental animals were pretreated with AALVTA (1μg/100g) solely and in combination with COX/LOX blocker darbufelon (20 mg/100g) and iNOS blocker aminoguanidine (20 mg/kg).

Results: The rats introduced to epinephrine developed severe GL, accompanied by increase of the activity of total NOS (by 133%) in GM in particular due to the increase of iNOS activity (6 times), increase of NO (by 50%) and decrease of the level of L-arginine in plasma (by 37%). Pretreatment with AALVTA led to 50% decrease of the area of GL, 56% decrease of the activity of total NOS and 62% decrease of iNOS, 31% decrease of the content of NO in GM as well as tendency to increase of the level of L-arginine in plasma. Pretreatment with AALVTA under conditions of iNOS blockage by aminoguanidine in rats introduced to epinephrine provided decrease of the damaged area of the stomach by 61% compared to mono action of AALVTA, enhanced blockage of iNOS, decrease of NO in GM and increase of the level of L-arginine in plasma. The administration of COX/LOX blocker darbufelon on the background of AALVTA in animals introduced to epinephrine didn't provide significant cytoprotection and showed tendency to increase of the activity of eNOS.

Conclusions: AALVTA provides cytoprotection in experimental GL in rats in particular through the modulation of the activity of NOS, which is more expressed under the conditions of iNOS blockage by aminoguanidine and is not enhanced by the dual COX/LOX blocker darbufelon. So the mechanisms of the cytoprotective effect of AALVTA in interconnection with the systems of COX/LOX need further profound investigations.

Changes in PUFAs due to amaranth oil introduced at the background of experimental colitis

Panasyuk N.B., Sklyarov O.Ya.
Danylo Halytsky Lviv National Medical University
69, Pekarska St. Lviv, Ukraine, 79010
sklyarov@meduniv.lviv.ua

Introduction: Ulcerative colitis is the most common pathology of large intestine. Development of ulcerative colitis is accompanied by structure-hemorrhagic changes and increase in proanti inflammatory cytokins, proapoptic proteins and prooncogens. In ulcerogenic colitis, balance between pro-oxidant and antioxidant systems becomes impaired and activity of inducible NO-synthase enhanced. Role of the short-chain and long-chain fatty acids in cytoprotective mechanisms in the mucous membrane of digestive organs is essential because these acids are the source for synthesis of prostaglandins and leukotrients

Material and methods: Experimental colitis was modeled by 1ml of 4 % acetic acid injected into rats' large intestine. Amaranth oil were given per os in the dose of 0.2 ml/100 g of body weight Then were determined: area and degree of destructive changes in the mucous membrane of large intestine, alterations in lipoperoxidation processes (MDA), activity of SOD, catalase, NOS, iNOS, cNOS, NO in MMLI and L-arginine contents in blood. Fatty acids were determined by the method of norm setting, peak areas of methyl derivatives of the fatty acids were measured and their content calculated in percentage. Obtained results were processed by the variation statistics method at Student's t-criterium determined.

Results: Injection of acetic acid caused ulcerative defects, erosions, hemorrhages in the MMLI. There were observed: 2.5-fold enhancement of general NOS activity ($P<0.05$), cNOS – by 21%, iNOS – 6.9-fold ($P<0.001$), NO content increased by 64%. Blood L-arginine decreased by 50% ($P<0.05$), MDA content increased 2.2-fold ($P<0.001$), SOD activity – by 71% and catalase – by 54%. Content of polyunsaturated acids (linoleic, arachidonic) in the blood decreased by 15-34 %, content of unsaturated acids (oleic, linoleic, arachidonic) decreased up to 13 % versus the contents in intact animals. On introduction of amaranth oil at the background of ulcerogenic action of acetic acid, lesion area in MMLI diminished – necrotic changes, ulcers, erosions were absent; and only solitary punctuate hemorrhages were found at the background of hyperemized mucous membrane, macroscopic signs of colitis were absent at all, content of MDA products decreaced by 34 % ($p<0.05$), SOD activity – 1.4-fold ($p<0.01$), catalase by 20%, and iNOS (by 48%, $P<0.05$), cNOS activity changed slightly, NO content in the MMLI decreased by 44% ($P<0.05$), concentration of L-arginine in blood plasma increased. Content of polyunsaturated acids in the blood increased by 26 %, content of unsaturated acids increased up to 16 % versus the contents in colitis animals. Most pronounced was the increase of linoleic acid whose content in amaranth oil is 52 %. Content of saturated fatty acids in the blood increased by 24 % in the animals with colitis and decreased up to 19 % versus the contents in colitis animals. Protective effect of amaranth oil is likely to be associated with the contents of unsaturated fatty acids in this oil (palmitic – 23 %, palmitoleinoic – 0.18 %, stearic – 1.3 %, oleinoic– 22.6 %, linoleic – 52.4 %, linolenoic – 0.39 %). **Summary:** Thus, obtained results of our investigation prove that amaranth oil displays cytoprotectiveeffect associated with the participation of w-3 and w-6 fatty acid