SIXTH FRAMEWORK PROGRAMME
LIFE SCIENCES, GENOMICS AND BIOTECHNOLOGY FOR HEALTH
LIEFESCIHEALTH-6

Specific targeted research or innovation project

LSHC-CT-2006-037733

Normolife

Development of new therapeutic substances and strategies for treatment of pain in patients with advanced stages of cancer

FINAL ACTIVITY REPORT

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Revision [draft, 1, 2]:	

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1. Project execution

Development of new therapeutic substances and strategies for treatment of pain on patients with advanced stages of cancer –
http://www.normolife.eu/

NormoLIFE

Objectives of the project

The objectives of this project have been focused on development of new multi-target antinociceptive compounds that may interact with peripheral receptors expressed in inflamed and/or pathologically modified tissues and suppress generation and transmission of nociceptive signals from the periphery to CNS. Compounds partial penetration into the central nervous system and interaction with receptor systems involved in nociceptive signal transmission and pain perception should result in synergic periphery/CNS pain suppression. Compounds have been especially developed for the prospective treatment of pain of terminal/metastatic cancer.
The project involved general complementary scientific objectives: chemistry, *in vitro* bio-pharmacology and *in vivo* pharmacology that will be accomplished by multidisciplinary integrated teams. Syntheses of new compounds, designed by theoretical (SAR) analysis, have been accomplished in chemical laboratories. Over hundred new compounds have been designed and synthesized on the first stages of the project. These compounds activities have been evaluated in *in vitro* tests of receptor binding to opioid receptors and in *in vitro* functional cellular tests. The results allow to select compounds for in vivo tests in animal, especially developed model of metastatic cancer pain. The final in vivo studies were performed with the compounds that large scale syntheses have been developed and accomplished.

*The **impact** of the project on its industry or research sector*

The accomplishment of the Project resulted with a set of new type of compounds that have been proposed as a new generation of analgesics, especially designed for treatment of various chronic pains, particularly pain of patients with advanced stages of cancer. The designed and synthesized molecules have been further tested in biological and pharmacological in vitro and in vivo tests to select the most promising molecules. To obtain the most accurate answer, the performed standard tests have been especially modified or a new tests have been developed. The data allowed to select several molecules that will be promoted for further development as a medicines. Individual partners and consorcium of the Project already started promoting activities of their own compounds for further studies and/or seek for involvement of industry in further development of this type of analgesics.

11 Partners

- Medical Research Centre, Polish Academy of Sciences, Poland (Coordinator, Work package WP 1,3,9)
- Faculty of Pharmacy, University of Catania, Italy (WP 2,4)
- Department of Natural Sciences, Free University of Brussels, Belgium (WP 2,4,5)
- Department of Chemistry, Warsaw University, Poland (WP 2,4)
- Biological Research Centre, Hungarian Academy of Sciences, Hungary (WP 1,4,5,6)
- Institute of Biology, National Centre of Scientific Research "Demokritos", Greece (WP 1)
- Institute of Organic Chemistry, Polish Academy of Sciences, Poland (WP 2)
- Industrial Chemistry Research Institute, Poland (WP 8)
- Department of Biological Sciences, University of Rostock, Germany (WP 7)
- Pattern Expert, Germany (WP 7)
- Steinbeis-Forschungs- und Entwicklungszentrum GmbH, Germany (WP 9)

Coordination/ Contact

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Figure 1: Normolife Final Meeting, Brussels November 2009

Work performed

The project was organized in 9 work packages (WP). Eight were linked to scientific issues and one WP dealt with management tasks and reporting. All WPs were fulfilled timely and adequately to the working plan. The work performed and derived achievements are highlighted below.

Summary of WP 1- 11

WP 1 – In vitro receptor binding studies

Objectives
In vitro receptor binding studies:
- To assess the specificity and potency of the new compounds for mu, delta and kappa opioid and other neurotransmitter receptors
- To validate the functionality of the new compounds
- To conduct functional assays
- To explain the differences in the physiological responses they induce

The aim of the Workpackage 1 coordinated by NCSR-D was to characterize pharmacological properties of the new compounds developed by different members of the consortium. In this workpackage also Partners 1 and 5 have been involved. Partners LINDEN, VOLCANO, VUB, UM-CHEM provided a number of various compounds. Each compound was tested initially by opioid receptor binding studies using membrane preparations from rat brain or performed in membranes form HEK293 cells stably expressing the δ, μ or the κ-opioid receptors. Depending on the type of the analog (agonist, antagonist) and its affinity towards the selective opioid receptor (μ,δ,κ) we further assessed their pharmacological profile performing functional assays by measuring adenylyl cyclase GTP γ [^{35}S] binding, MAP kinase phosphorylation, the rate of desensitization and internalization of the μ-/and or δ-opioid recepors in cells expressing these receptors. Our data revealed novel compounds such as 1) chameleon peptides (eg “Neuropeptide 2”) with neurotensin and opioid nature, to selectively inhibit adenylyl cyclase inhibition; 2) a chameleon peptides with opioid agonists and tachykinin agonists or antagonists 3) a benzomorhan based compounds (like LP1), a potent δ-and μ-opioid receptor analogs; 4) compounds of VUB partner being extremely potent δ and μ-opioid receptor agonists, 5) peptides derived from UW-CHEM that displayed agonistic propertied for the μ-opioid receptor and prospectively low permeability through blood-brain barrier and .6) collection of chimeric compounds that combine opioid affinities with anticancer acting elements, including platinium analogs exerted also agonistic effects for the μ-opioid receptor as assessed by cAMP accumulation measurements in HEK293 cells.

WP 2 – Structure-activity relationship studies of chameleon compounds

Objectives

1. Molecular structure simulation and analysis of synthetic drugs using computer programs (Macromodel)
2. Crystallization of synthetic product and structural X-ray analysis of crystalline material of synthetic compounds
3. Correlation of biological *in vitro* and *in vivo* data with structure of compounds Structure-activity study of synthetic compounds.

Primary data of optimized structures of chameleon compounds

Aim of WP-2 package was to select several compounds synthesized by Partners 1-4, that the best met project objectives, i.e. they have high potency and similar range of affinity towards three subtypes of opioid receptors mu, delta and kappa and recommend them for further *in vivo* studies. Basis for the structure-activity selection were structural data and results of various biological tests. Structural data comprised: chemical structures of the designed non-peptidic or peptidic compounds of chimeric character, synthesized with systematic variation of one or more substituents, their molecular structure in solution, studied by advanced NMR techniques, and/or solid state structures obtained from X-ray diffraction methods. These data have been supplemented by molecular modeling calculations. Basic biological data available in this work package included affinity and specificity of the above analogs to three subtypes of opioid receptors , functional tests and studies on methabolic pathway of the compounds.
The structure-activity studies involved peptidic chameleon compounds with conformationally restricted benzomorphan system. Systematic permutations of residues in three positions allowed to establish structure-activity relationships for this group of compounds. Successfully, three compounds LP-1, 12 and 13 met project objectives very well: have high and relatively similar affinity to three receptor subtypes, and were established as opioid receptor agonists.

Structural data of various peptidic chameleon compounds or their fragments

A group of tetra- and hexapeptides containing benzoazepine moiety (Aba), introducing β-turn mimicry was designed. Structure-activity studies involving advanced NMR methods, X-ray and in silico docking experiments explained their enhanced potency and selectivity. Analogs of ENDOMORPHIN-1 containing Tcc fragment have been designed. Binding to opioid receptors studies showed that affinity strongly depends on chirality of the two stereogenic centers, what was explained by molecular modeling results.
Several new interesting design strategies have been developed throughout the Normolife project. First approach involved utilization of opioid peptides (like endomorphins or enkephalins) chimerized with Pt(II)-chelating systems of amino acid character, for direct delivery of platinum ions to the human glioblastoma cells (due to the fact that opioid receptors are overexpressed in membranes of cancer cells). These compounds have been proven as an efficient antiproliferative agents, particularly when their solubility in water has been improved significantly by introduction of polyethylene glycol moieties of various length. Another molecular category are new branched peptides (peptide dendrimers) that have been designed and studied in two aspects – interactions with sharing common morphological features, membranes of bacteria and

human melanoma cells, or as a new type of a carrier compounds, improving solubility of opioid peptides (eg. AWL600).

Another problem that has been addressed during design procedures was metabolic stability of the designed opioid peptides. For that reason, a series of β^2-homo-amino acids have been efficiently synthesized and introduced at different positions of the enkephalin sequence. Structure-activity results along with stability in human plasma showed, that substitution of alfa amino acid with beta amino acid in the peptide sequence afforded very potent and selective compounds with prolonged life time, but not in all cases. At the moment no general rule has been found.

In summary, new design strategies, focusing on project objectives have been accomplished, that allowed successful selection of valuable compounds, proposed for applications as a new, potent, versatile analgetics or anticancer agents.

WP 3 – In vivo studies of new compounds

Objectives
In vivo evaluation analgesic activity of selected new compounds in healthy animals and those with implanted cancer cells.

Data of in vivo analgesic activity of selected compounds;
The selected compounds on the basis of in vitro receptor binding and metabolic profile have been tested in vivo healthy animals. The tested compounds were applied systematically (intraperitoneally or intravenously). The test allowed to eliminate inactive compounds after systemic administration and classified sic compounds for next step of studies in animals with implanted cancer cells. The development of proper test for cancer pain has been critical step of the workpackage. After initial studies the animal model proposed in previous stage has been discarded and substituted with new test in which cancer cells were implanted subcutaneously implanted into the plantar region of the left hind paw. Within two weeks local increase sensitivity to thermal stimulus has been observed in the place of cancer growing. The local (Hargreaves test) and general (tai flick) antinociceptive effects have been evaluated. The tested compounds shows antinociception in Hargraves test and tail flick test. In most of the cases the antinociceptive effectiveness has been much stronger in male than female animals. Only compound with strong affinities to opioid kappa receptors showed increased activity in female animals.

Data on influence of cancer proliferation on analgesic effectiveness of selected chameleon compounds
The test of influence of cancer proliferation on analgesic effectiveness has been measured by antinociception provided by compound within period of seven days in cancer developed animals. The studied compound (AWL600) showed high effectiveness of local (Hargraves test) as well as general (tai flick) tests. In the same condition used morphine produced development of tolerance.

The relationships between type of cancer cells and analgesic effectiveness of chameleon compounds
The various effects of newly developed compounds have been observed in in vitro proliferation tests. These effect probably were dependent on interaction with specific receptors (gliblastoma) or nonspecific interaction with cell membranes. However, in vivo antinociceptive test of metastatic cancer, no differences have been observed between

cancer and developed nociception. Also effectiveness of new compounds were dependent on progression of cancer, not type of cancer.

WP 4 – Synthesis of new compounds

Objectives
1. Synthetic hybridization of non-peptidic pharmacophores with modified peptides
2. Synthesis of precursors for tritiation of compounds which hybridize non-peptidic pharmacophores with modified peptides
3. Make available the benzo-azepine mimimetics of tyrosine and phenylalanine, and to use them to prepare chameleon compounds for in vitro and in vivo evaluation.

Chemical objectives of **WP4** were the synthesis of new compounds with peptidic or non-peptidic structure with the aim to obtain new ligands useful in chronic pain treatment. The designed strategy was based on the development of new multitarget compounds to block nociceptive signals formation and transmission more effectively than traditional monotherapies with lower side effects. Moreover, another objective of WP4 was the synthesis of precursors for tritiaton of final compounds. During the Normolife project the partners **VOLCANO, VUB, UW-CHEM** and **BRC** synthesized different libraries of new compounds for further biological and pharmacological evaluations.
Partner **VOLCANO** first synthesized 11 new benzomorphan-based compounds with different substituents on the nitrogen of benzomorphan scaffold to modulate affinity and selectivity versus μ, δ and κ receptors as well as agonist and antagonist profile of obtained ligands. The most promising compound, named LP1, was characterized by a N-propanamide spacer and a phenyl ring in benzomorphan substituent. To modulate affinity and selectivity profile of previous amide compounds and to clarify the role of the amide function Partner VOLCANO synthesized a new series of 8 selected amine analogues. Successively, a series of 17 LP1 analogues were synthesized by replacing phenyl ring of LP1 with different aromatic and heteroaromatic rings. To further explore the SAR of LP1 a series of 7 analogues were synthesized by introducing different p-substituted phenyl ring with lipophylic and/or elettron-attactive and electron-realising substituents and by the utilization of constrained phenyl rings. Finally, taking in consideration the preliminary pharmacological results an acylated phenylpropylamine analogue of LP1 has been identified and synthesized as a possible lead compound.
The main deliverables for partner **VUB** was the preparation of benzoazepine mimetics of phenylalanine and tyrosine, suitably protected for peptide synthesis, and their use to prepare benzoazepine-containing chameleon peptides. Partner VUB developed new synthetic pathways that results in building blocks used in the solid phase synthesis of 19 new benzoazepine-containing peptides. For α-MeAba and non-α-methylated Aba-containing peptides, an asymmetric synthesis of α-MeAba and non-α-methylated Aba has been performed which allowed the determination of the absolute configuration of the isomers obtained. With a collaborator of partner BRC, VUB group prepared a set of 15 hybrid opioid peptides and peptides that contained a brominated Phe residue were be exchanged for tritium by partner 5.

Partner **UW-CHEM** synthesized 9 opioid-platinum chimeric peptides which combine an opioid fragment, based on the biphalin analogue Tyr-D-Ala-Gly-Phe-NH-NH-Phe, and a fragment designed to form complexes of Pt(II) with the aim to obtain compounds that could express analgesic properties and provide anticancer activity. Due to very poor solubility in water of synthesized chimeric peptides, Partner UW-CHEM introduced a

poliethyleneglycol fragment as additional structural elements to improve solubility of synthesized compounds. So, Partner UW-CHEM synthesized new 7 opioid-platinum chimeric peptides using different opioid pharmacophore (like endomorphins or enkephalins) and without the incorporation of hydrazide bridge, which was necessary element of the first chimeric structures. UW-CHEM group synthesized a library of 22 analogues of endomorphins containing different β^2-homo-amino acids and 4 analogues of endomorphins 1 with *cis* and *trans* tetrahydro-β-carbolines synthetic blocks as tryptophan mimetics. Finally, partner UW-CHEM performed the synthesis of a precursor compound for tritiation (Lys-Phe(I)-Phe(I)-Gly-Leu-Met-NH_2) to obtain radioligand Lys-Phe[H^3]-Phe[H^3]-Gly-Leu-MetNH_2 for binding studies of synthesized chimeric opioid-tachykinin peptides (by partner 5, BRC).
Partner **BRC** synthesized 15 *cis*-(*1S,2R*)ACPC/ACHC, *cis*-(*1R,2S*)ACPC/ACHC, *trans*-(*1S,2S*)ACPC/ACHC and *trans*-(*1R,2R*)ACPC/ACHC containing endomorphin-1 and endomorphin-2 analogs. Moreover, partner BRC synthesized a set of halogenated endomorphin derivatives to study the effects of halogenation of Phe^4 on the binding potency and blood brain barrier permeability. Various functional groups (F, Cl, Br, I, NO_2) were applied to replace the para-hidrogen. Additionally 3 Gly^5-extended endomorphins were synthesized to map a possible biosynthetic pathway of endomorphins *in vitro.* Finally, partner BRC prepared 7 precursor peptides as research tools for the synthesis of tritiated opioid or tachykinin receptor ligands. Boc protected diiodo-tyrosine, 3,4-dehydro-proline or 2-amino cyclohexene-1-carboxylic acid (ΔAchc) were used in the peptide syntheses.

WP 5 – In vitro metabolic studies

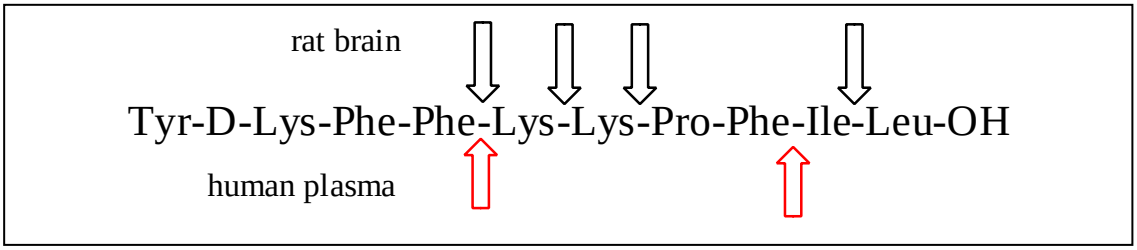
Objectives:

The objective is the determination of the metabolic pathways of the selected compounds in various media.
This work package consists of the determination of the metabolic degradation pathways of six finally selected compounds for *in vivo* studies compounds using HPLC/MS. Respected compounds will be provided by partners who will originally developed them. Incubation with rat serum will be performed. Extraction protocols for the parent compounds and for their metabolites will be worked out. The life time and the degradation products will be quantified by HPLC and identified by mass spectrometry.

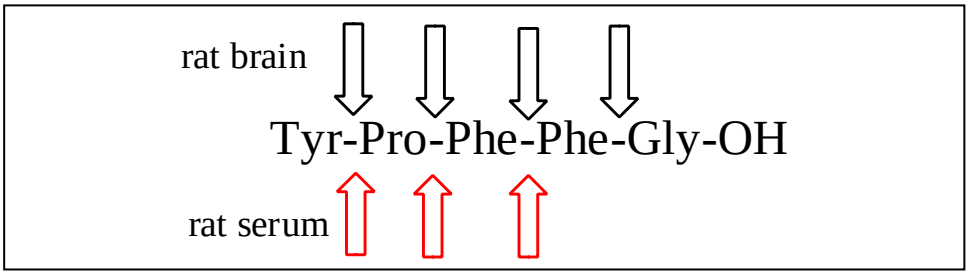
Description of work

The metabolism of compounds that were selected on the basis of their biological profile was determined by partners 3 (VUB), 4 (UW-CHEM) and 5 (BRC). Stability has been studied either in plasma – rat and human – and in rat brain homogenates. The analytical protocol for the incubation of the compounds and for the identification of the metabolites has been worked out. In short, the incubation in human plasma was carried out for different time intervals; the plasma proteins were precipitated and the supernatant containing the parent peptide and the metabolites was analyzed by HPLC. The structure of the metabolites was determined by mass spectrometry. The time course of the disappearance of the parent peptide signal and of the appearance of the metabolite signals allowed the estimation of the half-life ($t_{1/2}$) of the peptide and of its metabolites.For incubation in rat brain homogenates, tritiated peptides have been prepared. The analysis was performes as for the plasma incubations.

A set of opioid-neurotensin hybrids was studied. The metabolic breakdown of **LINDEN 2 (= BVB07)**: H-Tyr[1]-*D*-Lys[2]-Phe[3]-Phe[4]-Lys[5]-Lys[6]-Pro[7]-Phe[8]-Ile[9]-Leu[10]-OH is summarized below:



This indicates a different pathway in plasma than in brain. It liberates an intact opioid part. The degradation in the neurotensin part can be prevented by modifications made in PK7: H-Tyr-D-Lys-Phe-Phe-Lys-Lys-Pro-Phe-Ile-D-Asp-NH_2 and in PK20: H-Dmt-D-Lys-Phe-Phe-Lys-Lys-Pro-Phe-Tle-Leu-OH, which generate both intact opioid and neurotensin parts.
Two higly potent new opioid BVD02: H-Dmt-Me-D-Ala-Phe-MeGly-NH_2 and BVD03: H-Dmt-Me-D-Ala-Aba-Gly-NH_2 were studied and were found to be highly stable in plasma.
A similar study identified the following degradation pathway for glycine-extended endormorphin-2 (EM-2):



A set of six EM-2 analogs and of ten enkephalin (TAPP) analogs was studied, showing that the introduction of β^2-homo-amino acids can influence the metabolic stability and lead to very stable compounds with half life up to 14 hours.
Finally an opioid-tachykinin chimeric peptide: H-Tyr[1]-*D*-Ala[2]-Phe[3]-Gly[4]-Tyr[5]-Pro[6]-Ser[7]-*D*-Ala[8]-Phe[9]-Phe[10]-Gly[11]-Leu[12]-Met[13]-NH_2 was studied. The degradation forms metabolites within less than 1 hour time. LC/MS analysis showed a metabolite H-Tyr[1]-*D*-Ala[2]-Phe[3]-Gly[4]-Tyr[5]-Pro[6]-Ser[7]-*D*-Ala[8]-Phe[9]-OH. The half-life time of the peptide estimated by using the peak area in the HPLC chromatograms (figure) was 4 hours.

WP 6 – Synthesis of tritiated compounds

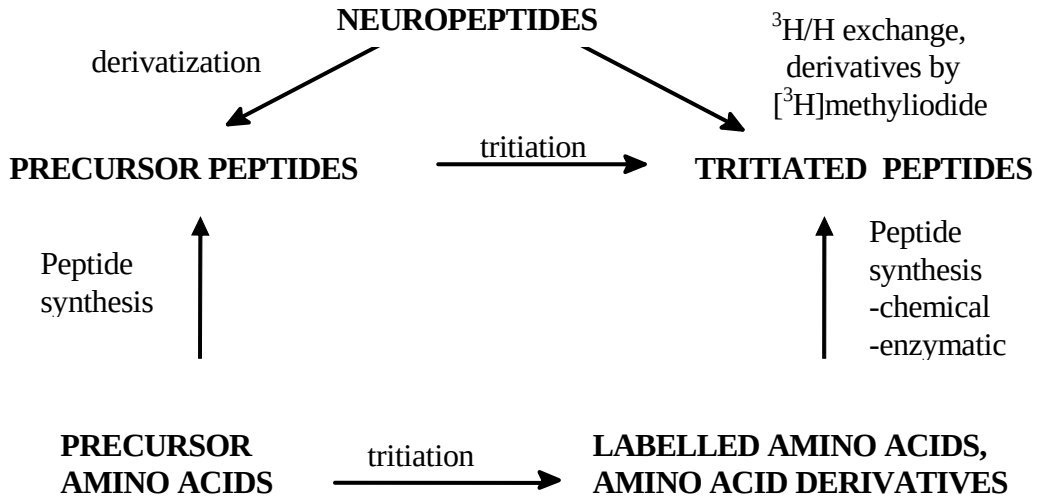
Objectives
Radioligand compounds are necessary tools in the receptor binding and metabolic studies of peptides. Synthesis of tritiated compounds from precursors provided by VOLCANO, VUB, UW-CHEM and BRC laboratory

The tritiated compounds were synthesized using unsaturated or halogenated precursor peptides as you see in Table 2. Diiodo-tyrosine 3,4-dehydro-proline or 2- amino-cyclohexene-1-carboxylic acid were introduced into the precursor peptides. These

precurcors were synthesized by P1, P4 and P5 partners. For labelling tritium gas and PdO catalyst were used. For general protocol scheme see figure below.

During the period of funding radioactive opioid peptides were synthesized for receptor binding studies, investigation of biosynthesis of endomorphin-2 and metabolic studies of novel and promising compounds. In all cases the precursor compounds were synthesized using solid phase peptide synthesis. ^{3}H-tritium gas and the precursors were used as starting materials for the dehalogenation and saturation labeling reactions. Two novel radiolabelled endomorphin analogues (^{3}H-Achc^{2}-endomorphin-2 and ^{3}H-Gly^{5}-endomorphin-2) were developed, prepared and used for receptor binding and metabolic stability studies. ^{3}H-Achc^{2}-endomorphin-2 became a commercial product with the help of Isotope Institute Ltd, Budapest.

A novel tritiated opioid and neurotensin hybrid peptide (LINDEN 2) H-Tyr-D-Lys-Phe-Phe-Lys-Lys-Pro-Phe-Ile-Leu-OH was prepared using a precursor peptide containing pBrPhe in position 3. It was used for metabolic stability studies. Kappa opioid peptide radioligands are not available commercially, so D-Ala^{3} dynorphin (1-11)-NH_2 was developed and produced in tritiated form. Optimization of receptor binding conditions for



this ligand is in progress. A new tritiated Substance P analogue (Lys-3H-Phe-3H-Phe-Gly-Leu-Met-NH_2) was synthesized. Receptor binding conditions of this radioligand are being optimized. Furthermore, special radioimmunoassays were developed for endomorphins and ^{3}H-Tyr-Pro was synthesized, for the investigation of a *de novo* biosynthetic route of endomorphin-2.

WP 7 – Electrophysiological in vitro analysis of compounds interacting with membrane receptors

Objectives

Electrophysiological in vitro analysis of compounds interacting with membrane receptors:

1. Test known agonists for the three receptor types in spinal cord and frontal cortex and define their specific electrical firing pattern changes. (such as DAMGO for mu, deltorphine for delta and dynorphine or a more selective analogue for kappa) are applied individually, in pairs and all three together to determine the patterns changes.
2. Determine dose response curves of 15 designed substances in spinal cord and frontal cortex per year.
3. In another set of experiments with the in vitro inflammation model the pattern changes caused by the compounds under 1. are determined and compared.

Description of work

.The aim of WP7 was to determine substance-specific electrophysiological activity pattern changes measured on frontal cortex and spinal cord neuronal network cultures and analyse the similarities between the pattern changes caused by newly synthesized and by known reference compounds acting at the three opioid receptors.

To this end dose-response curves were first determined for 26 known agonists and antagonists as positive controls. The characteristic network changes obtained by parallel recording from multiple electrodes and over a wide range of concentrations were described by 200 parameters which were used for establishing a data base and for classification based on machine learning algorithms and pattern analysis. The compounds were classified according to their receptors and some could already be classified to be multitarget compounds acting on at least two receptors. Consecutively such substance-specific dose-response curve sets were determined for all 26 new compounds synthesized by various project partners and delivered to P9. This added to the data base the data of 2500 records, i.e. actual individual activity patterns.

To identify the activity patterns of substances, which resemble those of reference compounds, the database was trained with an artificial neuronal network using the machine learning algorithm of the pattern recognition software platform of P10. Correlation analyses of spike train features with analgesic potency were performed for both known and new compounds. As result of this classification an assessment of the predictivity of the algorithms was possible. Then a similarity analysis between all known and all new compounds was performed.

After disclosure of the chemical structure of the newly synthesized substances and their expected mode of action, the correct, non-specific and false classifications were determined. In the classification experiments 50% of the new compounds were classified to have the predicted mode of action. 50% could not be assigned because of a non-specific classification, mainly because the compounds were not directly targeting opiate receptors, but other peptide receptors. Out of the 11 new multi-target substances expected to be possibly multitarget 7 were indeed assigned as such by the classification. This result demonstrates clearly that the mode of action and type of opiate receptor could be assigned correctly in most instances by our assay and the subsequent classification. This proves the explanatory power and informative value of the neurochip technology and the therein affiliated methods for preclinical drug development and for the development of new potent analgesics in particular. Finally,

decision criteria were developed for the prioritization of further development of a given compound. According to these 5 “First choice compounds” with the same or a higher EC50 as Sufentanil could be identified and 6 “Second choice compounds” with an EC50 no more than one order of magnitude smaller than Sufentanil.
Predicting of physiological behavior of drug candidates is a long-standing aim of drug development research. With these results we have for the first time successfully employed a predictive model for the analgesic potency which is not only based on a single parameter but on a description of the global activity changes of whole networks. We showed with these results the feasibility of developing models for the prediction of physiological behavior from an in-vitro model.

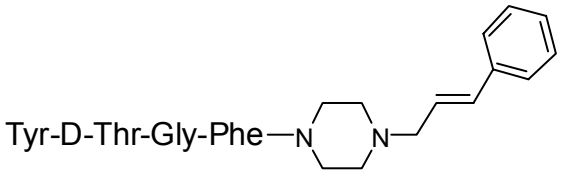
WP 8 – Developing of chameleon drug large scale synthesis

Objectives
Developing of technology and synthesis of 6 new compounds selected for further testing *in vivo* in mouse animal model

In the first period of the project on request of other partners reference peptides have been synthesized by classical methods of peptide syntheses:

Neurotensin: pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
and Substance P: Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH2
The originally planned syntheses of new 6 compounds has been finalized during second period of the project.

Chameleon compounds for in vivo pharmacological and toxicological study. Chameleon compounds for in vivo pharmacological and toxicological study.
During the second stage of the project the new method of synthesis of six compounds have been developed. The methods are new in respect of general approach as well as application in synthesis of particular compounds. The syntheses have been accomplished in gram scale but could be easily scaling-up to technological GMP level. The obtained compounds were available for in vivo and in vitro studies performed by other partners. The compounds synthesized in new methods for large scale, are:
AWL 600 (Biphalin): Tyr-D-Ala-Gly-Phe-NH-NH□Phe□Gly□D-Ala□Tyr
AWL3100 6Htc-Pro-Phe-PheNH2
AA3106 Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-D-Ala-Phe-Phe-Gly-Leu-MetNH2
AA3266 Tyr-D-Ala-Gly-Phe-NH-NH□D-Trp- CO-O-phenyl
AWL5102, [D-Phe-D-Phe-D-NLe-D-Arg-]2=LysNH2
AA5504



Technology of chameleon drug(s) for further promotion and marketing
New compounds synthesized under workpackage 8 have new structures. In addition, the preparation of new compounds (Deliverable D8.1) have been obtained with new strategy of key fragments synthesis starting from N-terminus. Therefore, technology of synthesis of new compounds will be the part of package for promotions and marketing of new compounds. The respective protection of intellectual property it is very important

and critical factor in promotion stages to new compounds. Therefore, key elements of developed technologies have been already covered by patent applications for national (in Poland) and/or international patent. In addition, partners involved in development of particular compounds possess confidential "know-how" that could be available on promotion stages.

WP 9 – Project management and coordination

Objectives
Ensure a smooth flow of the project activities – constant monitoring and steering of the RTD work with the objective to achieve the project goals. Proper and timely execution of all administrative and financial tasks. Elaboration and implementation of corrective actions, where necessary. Faithful interaction with the Commission's representatives. Evaluation of the innovation potential of the project results. Taking care of science and society (e.g. ethics and gender) issues.

Description of work

A **management handbook** were delivered to all project partners to give an overview of the administrational work involved. Since the beginning of the project LINDEN was in close contact with the **scientific project officer** to work on work progress and ensure a high level of common understanding.
At any time the management board with the co-ordinator LINDEN and partner Steinbeis Research Center (SFZ) were in contact with the project partners to distribute and decide on tasks among the work packages. The **development of work package** objectives and achievements are monitored regularly by means of the presentations and minutes of the meetings. In later phases of the project strategy issues, risk and quality management and internal evaluation were major tasks of the project management.
Project partners were reminded of open tasks and deadlines according to the list of deliverables and the agreed work plan. The project partners were informed in time (at least 2 month ago) about requested documents for the first and second periodic as well as for the final EU reporting.

6 Steering Committee meetings were organized about every 6 months with representatives of all project partners: February 11 – 12, 2007 in Warsaw/Poland, September 25, 2007 in Pultusk/Poland, May 23, 2008 in Athens/Greece, Nov, 2008 in Catania/Italy, May, 25, 2009 in Rostock/Germany, Nov, 5-6, 2009 in Brussels, Belgium.

The presented results have been awarded three times with silver and gold medals on international events.

Figure 2: Silver medal for invention "Opioid peptides as carriers of platinum in cancer chemotherapy" awarded at Brussels Eureka 2007

Figure 3: *Gold medal for invention "Opioid-Neurotensin Chimeric Analgesics for Chronic Pain" IWIS, Warsaw, Poland 2008*

Figure 4: *Gold medal for invention "Peptide analgesics, a new generation of analgesics for modern method of applications, Brussels, Eureka 2009"*

2. Dissemination and use

Homepage has been organized under address www.normolife.eu. The page is organized on two levels. First, open to all, is presenting project partners, its mission and successes. The second level is open only for partners of Normolife consortium informing on current information regarding meetings, reports, unpublished results and scientific plans.

2.1 Plan for using and disseminating the knowledge[1]

2.2.1 Exploitable knowledge and its Use

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1. New structures of compounds, potential medicines for pain and cancer treatment	*Chemical substances*	*Pharmacy*	*2011*	Polish Patent Application: *"New, peptide complexes of platinum, synthesis, farmaceutical composition and medical application"* Polish Patent Application P-383412 (2007.09.24)	***Inventors:*** ***Misicka A., Glowinska A., Lipkowski AW.,*** *Owners:* *Warsaw University and Mossakowski Medical Research Centre, Poland*
New structures of cameleon compounds for chronic pain treatment, including cancer pain	*Chemical substances*	*Pharmacy*	*2011*	International PCT Patent Application "Peptide analogues, particularly for the treatment of chronic pain" WO 2009/148343	***Inventors:*** ***Lipkowski AW,*** ***Owner:*** ***Mossakowski Medical Research Centre, Poland***
New technological method of peptide synthesis on large scale	*New technology*	*Pharmacy*	*2010*	International PCT Patent Application "A method of producing an N-blocked biphalin intermediate", WO 2009/093918	***Inventor:*** ***AW Lipkowski*** ***Owner:*** ***Mossakowski Medical Research Centre***
technological method of peptides synthesis on	*New technology*		*2010*	International PCT Patent Application „A method of	***Inventor"*** ***AW Lipkowski*** ***Owner:***

[1] ***Knowledge:*** means the results, including information, whether or not they can be protected, arising from the *project* governed by this *contract*, as well as copyrights or rights pertaining to such results following applications for, or the issue of patents, designs, plant varieties, supplementary protection certificates or similar forms of protection (Article II.1.14 of the contract)

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
large scale				producing a novel opioid peptide", WO 2009/093917	***Mossakowski Medical Research Centre***
New type of peptide molecules	*New chemical substances*		*2011*	"Dendrimeric compounds comprising amino acids, hyperbranched core compound, process for preparation of dendrimeric compounds comprising amino acids and hyperbranched core compound, and use thereof " Polish Pat Appl. P386123	***Inventors: Zofia Lipkowska, Piotr Polcyn, Andrzej W. Lipkowski.*** ***Owner:*** ***Institute of organic Chemistry Polish Acad Sci***
New type of peptide molecules	*New Chemical substances*		*2011*	"Dendrimeric compounds comprising amino acids, hyperbranched core compound, process for preparation of dendrimeric compounds comprising amino acids and hyperbranched core compound, and use thereof " PCT/PL2009/000090	***Inventors: Zofia Lipkowska, Piotr Polcyn, Andrzej W. Lipkowski.*** ***Owner:*** ***Institute of organic Chemistry Polish Acad Sci***

Patent application

Studies performed under the Project resulted in a number of new compounds that could be further developed as an analgesics with special applications for chronic pain, especially for cancer pain. Some results have been published in scientific journal because involved partners represents opinions that these information are important but will not directly resulted in commercial products. Some data are not published and are under discussion on rationale and/or strategies of possible patent applications. Therefore, only fragmental data are already protected by patent applications and are ready for further promotions. These inventions are:

1. ***"New, peptide complexes of platinum, synthesis, pharmaceutical composition and medical application"***
   This invention is focusing on development of peptide chimeric compounds that express affinity to opioid receptors and in addition carry platinum ion. Specific interaction with opioid receptors may specifically recognize target cancer cells (eg gliomas) that overexpress opioid receptors and internalization of the opioid receptors may successfully transport platinum through cell membrane. The invention is already protected by Polish Patent Application P-383412 and International Patent Application PCT
   The owners of this invention are Warsaw University (Warsaw Poland) and Medical Research Centre Polish Acad Sci (Warsaw Poland). Inventor already started promoting process of this invention. The invention presented on The Belgian and International Trade Fair for Technological Invention "Brussels Eureka", November 24th, 2007, received silver medal
2. ***"Peptide analogues, particularly for the treatment of chronic pain***"
   This invention is focusing on development of peptide chimeric compounds that express affinity to opioid receptors as agonists and neurokinin receptors as antagonists. The analgesic effect is a result of synergic activation of opioid receptor system and partial blocking pain signal transmission by blocking tachykinin receptors. The invention of special structures of compounds that are able penetrate blood-brain barrier are protected by International Patent Application PCT: WO 2009/148343
   The owner of this invention is Medical Research Centre Polish Acad Sci (Warsaw Poland). Inventor already started promotion of the invention. The invention was one of the element of portfolio "Peptide analgesics, a new generation of analgesics for modern method of applications" presented on The Belgian and International Trade Fair for Technological Innovation in Brussels, 2009
3. ***"A method of producing a novel opioid peptide"***
   This invention is focusing on development of new methods of economical synthesis of fragments of opioid peptides in scale that could be scaling up to commercial production of medicines. The two different inventions are already protected by two International Patent Applications PCTs: WO 2009/093918, and WO 2009/093917
   The owner of the invention is Medical Research Centre Polish Acad Sci (Warsaw, Poland)
4. ***""Dendrimeric compounds comprising amino acids, hyperbranched core compound, process for preparation of dendrimeric compounds comprising amino acids and hyperbranched core compound, and use thereof "***
   This invention is focusing on development of new type of branched peptide compounds called dendrimers. Proper construction of molecules allow to generate small multitarget ligands. Invention is already covered by Polish Pat Appl. P386123, and international Patent Application PCT: PCT/PL2009/000090
   The Institute of Organic Chemistry Polish Acad Sci is the owner of the patents. This invention is the part of portfolio offered by owner to pharmaceutical industries. The inventors are going to use this invention as a base for further development of medicines under special grant, which application is under preparation.
5. ***""Peptide analogues, particularly for the treatment of chronic pain".***
   The use is claimed of opioid peptides with a novel structure, which in addition to the pharmacophore contain structural elements that interact with neurotensisn receptors. Due to the synergistic interaction with the additional element, an augmented analgesic activity is obtained, capable of being used for an extended period due to decreased drug tolerance induction. These compounds may be of particular use in the treatment of chronic pain as effective analgesics during inflammation caused by rheumatoid, gout, neurodegeneration, post-operative or post-accidental lesions, or oncogenic lesions. Invention is already covered by International Patent Application PCT. WO 2009148343.
   The owner of the patent is Medical Research Centre Polish Acad Sci. (Warsaw Poland).
   The invention was a base for further development of new analogues also under this project. The general idea of this type of analogues and structures that are protected by

the patent application has been presented at International Warsaw Invention Show, Warsaw 2008, where has been awarded with gold medal.

Under consideration: patent protection for (tentative title):

BVB03 a highly potent and stable opioid peptide analog for the treatment of pain.
Inventors: D. Tourwé, B. Vandormael, I. Georgoussi, D. Weiss, A. Gramowski

2.2.2 Dissemination of knowledge

Results of competition binding assays, in vitro functional assays and in vivo analgesic activity evaluation of new synthesized compounds were presented in 54th National Meeting of the Italian Society of Biochemistry and Molecular Biology (SIB), Catania, in the EFMC: Drugs of the future. Barcelona (Spain) and in the 6th Meeting of the European Network of Doctoral Studies in Pharmaceutical Sciences, Palermo.

A paper describing competition binding results of the first series of compounds and preliminary in vitro and in vivo studies on lead compound LP1 are in progress and will be submitted in the next few months. A revision of previous text of publication has been due to an increase in biological data necessary to complete pharmacological profile of compounds. Other papers on full in vitro and in vivo characterization on lead compounds LP1 and on synthesis and SAR evaluation of a new series of benzomorphan-based ligands will be submitted .

Overview table

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner respon-sible / involved
	Press release(press/radio/TV				
2010.01.13	TVP news interview on Normolife and new cancer pain treatment	Polish TV	Poland		Project Coordinator
2010.01.13	"Cancer analgesics-present and future" Press Conference organized by Polish Drug Registration Office	Journalists specialized in medicine	Poland	45	Project coordinator
2009.02.02	The Parliament, Issue 281, page 28 focussing on World Cancer Day Information on the Project Normolife	Mainly EC Parliament Members and visitors	European Commu-nity, Eu Parliment		Project Coordinator
2007	Normolife," e-newsletter (2007), issue 58, pg 9, http://www.ekt.gr/content/img/product/70715/811.pdf	"Smart drugs for tracing pain", "Ereuna kai Kainotomia			NCSR-D
2007, 05.12	"Agelioforos" Daily Newspaper of Thessaloniki, New smart drugs as pain reliefers		Greece and EC		NCSR-D

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible / involved
	Conferences				
February 2-4, 2007	22nd Annual National **Conference of Indian Society** for Study of Pain Lecture	Anesthesiologists of India	Lucknow. India	1000	1 (LINDEN)
24th Febr. 2007	**8th Pharmacology Symposium**	Research	Athens, Greece	…	6 (NCSR-D)
Sept. 16-20 2007	**Nuove opportunità** nella terapia del dolore	Research	Italy (Chieti)	300 persons	2 (VOLCANO)
Sep. 23-26 2007	**19th Polish Peptide Symposium**	Drug developers, preclinical pharmacology, pharmaceutical Industry Chemists, pharmacologists, physiologists	Polish and international	300	4, 6, 9,10
September 5-8, 2007	**Joint Meeting** of the German Society of Neuropathology and Neuroanatomy and the Polish Association of Neuropathologists with International Participation poster	Neuropathologists and neurochemists	Poland and Germany	300	1 (LINDEN)
November 24th, 2007	**Belgian and International Trade Fair** for Technological Invention "Brussels Eureka"	Research and Technology Inventors	International	500	1, 4
2007, December 7-9	59th Greek **Conference of Biochemistry and Molecular Biology**	Biochemists, Molecular biologists	Athens, Greece		6 (NCSR-D)
8-11 April, 2008	Conference-**ENC Meeting** European Opioid Conference – European Neuropeptide Club Joint Meeting		Ferrara, Italy	250	1, 4, 6
May 18-20, 2008	"**Chimeric peptides**"		Patras, Greece		6 (NCSR-D)
July 2008	2 talks at the 6th Int. **Meeting on Substrate-integrated Micro-electrodes**	Researchers on biosensors, neuro-pharmacology, electrophysiology	international	200	9, 10
Oct	Trade Fair, **Conference**	Drug developers,	internatio	300	9,10

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible / involved
2008	**MIPTEC 2008**, Basel	preclinical pharmacology, pharmaceutical Industry	nal		
July 8-11, 2008	6th International Meeting on Substrate-Integrated Microelectrode Arrays.	Scientists, Pharma	Reutlingen, worldwide	300	P1, URO, PATTERN
July 12 – 16, 2008	6th FENS Forum, European Neuroscience Meeting,	Scientists, Pharma	Geneva, worldwide	6.000	URO, PATTERN
Sept. 2008	30th European Peptide Symposium	Research	Helsinki, Finnland		UW-CHEM
May 2009	Chemsession 2009	Research/ Industry	Warszawa, Poland		LINDEN. UW-CHEM
Sept. 2009	20th Polish Peptide Symposium	Research	Cetniewo, Poland		LINDEN. UW-CHEM, ICHO, ICHP
Sept. 2009	54th National Meeting of the Italian Society of Biochemistry and Molecular Biology (**SIB**).	Research	Catania, Italy		VOLCANO
Sept. 7-12, 2009	World Congress 2009 on Medical Physics and Biomedical Engineering, Munich, Germany	Scientists, Pharma	Munich, Germany, worldwide	500	URO, PATTERN
October 14-16, 2009	19th Annual Conference of the German Society of Cytometry, Leipzig	Scientists, Pharma	Leipzig, Germany, Europe	300	URO, PATTERN
October 17-21, 2009	39th Annual Meeting Society for Neuroscience, Chicago	Scientists, Pharma	Chicago, USA, worldwide	30.000	URO, PATTERN
Nov. 2009	Conference	Research	Palermo, Italy	120 people	VOLCANO
	Publications				
2007	Publication: Z. New Crystalline form of 7-amino-4-methylcoumarin	Pharmaceutical Researchers	International journal		7 (ICHO)
2007	Folia Neuropathol. 45:99-107		International		1 (LINDEN)
2007	Eur J Pharmacol. 563:209-212		International		1 (LINDEN)
2007	Brain Res Bull. 74:119-129		international		3, 5
2007	Cryst. Eng. Comm. 9: 735-739		international		7
2007	Proceedings of the 29th scientific conference of the Hellenic Society of Biosciences	Bisoscientists	international		6 (NCSR-D)

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner respon-sible / involved
2007	New benzomorphan derivatives of MPCB as MOP and KOP receptor ligands	Pharmazie	internarional		2 (VOLCANO)
Nov. 2007	Publication	Research	International journal		2 (VOLCANO)
July 2008	Publication	Research	International journal		2 (VOL CANO)
2008	Tetrahedron 64: 1506-1514				4
2008 submit-ted	Publication: Application of dendrimers in phar-maceutical sciences – perspectives	Pharmaceutical Researchers	National journal		7 (ICHO)
2008	J Med Chem.51:173-177		International		3, 5
2008	J Med Chem. 51: 2571-2574		International		1, 3
2008	Epitheorese Klinikes Farmakologias kai Farmakokinetikes		internatio nal		6 (NCSR-D)
	For further information see also the lists below				
	Posters				
2007, Febr. 24	8th Pharmacology Symposium 2 Posters	Chemists, pharmacologists,	Athens, Greece		6 (NCSR-D)
2007, May 17-19	*Proceedings of the 29th scientific conference of the Hellenic Society of Biosciences*	Chemists, pharmacologists,	Kavala, Greece		6 (NCSR-D)
2007, June, 6-7	Polish Crystallographic Meeting		Wrocław, Poland		7 (ICHO)
2007, June 28–July 3	33rd FEBS Congress & 11th IUBMB Conference, “Biochemistry of Cell Regulation”, 2 posters		Athens, Greece		6 (NCSR-D)
2007, July 8-13	INRC Annual Meeting, International Narcotic Research Conference	Chemists, pharmacologists,	Berlin, Germany		6 (NCSR-D)
2007, Aug 22-27	24th European Crystallographic Meeting		Marakesh , Morocco		7 (ICHO)
Sept. 23-26 2007	Proceedings 19th Polish Peptide Symposium	Research	Poland (Pultusk)	200 people	2 (VOL-CANO)

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible / involved
2007, December 7-9	59th Greek Conference of Biochemistry and Molecular Biology	Biochemists, Molecular biologists	Athens, Greece		6 (NCSR-D)
2008, May 23-25	5th Panhellenic Congress of Pharmacology, Hellenic Society of Pharmacology (HSP)		Athens, Greece		6 (NCSR-D)
2008, May 25-30	Second European Workshop in Drug Synthesis	Research	Italy (Siena)	200 people	2 (VOLCANO)
2008, May 8-10	Euro-CNS 2008 9th European Congress of Neuropathology 1 poster	Neuropathologists and Neurologists	international	300	1 (LINDEN)
May 24-27, 2008	11th Naples Workshop on Bioactive Compounds - New frontiers on search of bioactive molecules - from peptides to drugs	Medicinal Chemists and Pharmacologists	international	150	4 (UW-CHEM)
09.2007	Poster	Research	Poland (Pultusk)	200 people	VOLCANO
05.2008	Poster	Research	Italy (Siena)	200 people	VOLCANO
09.2009	Poster	Research	Catania (Italy)	150 people	VOLCANO
10.2009	Poster	Research	Barcelona (Spain)	200 people	VOLCANO
July 20-23, 2009	2 Posters at the Joint Meeting of the European Club and the Summer Neuropeptide Conference	Neuropeptide Festival 2009	Salzburg, Österreich	200 people	LINDEN
September 6-10, 2009	5 Posters	20th Polish Peptide Symposium,	Władysławowo, Poland	200 people	LINDEN
June, 25-26, 2009	Poster	Polish Crystallographic Meeting	Wrocław, Poland		ICHO
2009	Poster	25th European Crystallographic Meeting	Istanbul, Turkey		ICHO
31 August – 5 Sept., 2008	Poster	30th European Peptide Symposium,	Helsinki, Finland		ICHo
June, 14-18,	.	Dendrimer Symposium	Stockholm,		ICHO

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner respon-sible / involved
2009			Sweden		
July 12 – 16, 2008	Electrophysiological profiling of the opioid receptor system in drug development of new synthetic neuroactive peptides for cancer pain treatment	6th FENS Forum	Geneva, Schwitzerland		URO, Pattern, LINDEN, UW-CHEM
October 12-15 , 2008	A database of substance-specific network activity: Application to functional screening in preclinical drug development.	Miptec,	Basel, Switzerland,		URO, Pattern
June, 2009	Determination of singular activity patterns of functional neuronal networks on microelectrode arrays	The 9th Göttingen Meeting of the German Neuroscience Society,	Göttingen, Germany,		URO, Pattern
October 17-21, 2009	Similarity analysis of antipsychotics and antidepressants using network spike train patterns derived from MEA recordings	39th Annual Meeting Society for Neuroscience,	Chicago, USA, worldwide		URO, Pattern
	Exhibitions				
June 2008	International Invention Exposition	Research and Chemical industry	Warsaw, PKIN		LINDEN, UW-CHEM
October 12-15, 2008	Miptec, Basel	Biotech Industry, Pharma	Basel, worldwide	1.000	URO, PATTERN
October 17-21, 2009	39th Annual Meeting Society for Neuroscience, Chicago	Neuroscientists, Pharma	Chicago, USA, worldwide	30.000	URO, PATTERN
Nov. 2009	Exhibition 200 years of chemistry in Warsaw University	Research and Chemical industry	*Warsaw University Hall*		LINDEN, UW-CHEM
Nov. 2009	The Belgian and International Trade Fair for Technological Innovation	Research and Technology	Brussels, Belgium		LINDEN
	Project web-sites				
	www.normolife.eu		worldwide		LINDEN
Feb-2007,	Website N.C.S.R. “Demokritos”, “Tracing		worldwide		NCSR-D

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner respon-sible / involved
	pain "President's website http://www.demokritos.gr Website N.C.S.R. "Demokritos", Institute of Biology, http://bio.demokritos.gr/Georgoussi/media_eksipna_farmaka.htm				
	http://www.neb.gr/phpBB2/viewtopic.php?f=24&t=2043				NCSR-D

Conferences

- **A Głowińska J Matalińska, AW Lipkowski, A Misicka** Opioid peptides as possible selective carrier vehicles of platinum for anticancer therapy, Int. Warsaw Invention Show, Warsaw, Poland, June 4-5, 2008
- **AW Lipkowski, P Kleczkowska, P Kosson, A Klinowiecka, Z-I Georgoussi, D Tourwe,** Opioid-Neurotensin chimera as a New prospective analgesics for chronic pain., Int. Warsaw Invention Show, Warsaw, Poland, June 4-5, 2008
- **A Gramowski, O Schröder, A Lipkowski, A Misicka-Kesik, D Weiss**: Drug development of new synthetic peptides for cancer pain treatment: Electrophysiological profiling of the opioid receptor system. MEA meeting Reutlingen, Germany, July 8-11, 2008
- **AW Lipkowski,** Chimeric peptides as a new generation of analgesics, 30th European Peptide Symp. Helsinki, Finland, August 31-September 5, 2008
- **Georgoussi Z., Swevers L., and Iatrou K.** "Functional Genomics and Proteomics in identification of new therapeutic leads and novel pharmaceutical targets. 8th Pharmacology Symposium, 24th February 2007
- **Georgoussi Z.** "Novel Signaling pathways mediated by the opioid receptors". 19th Polish Peptide Symposium, Pultusk, Poland 23-27 September 2007.
- **Georgoussi Z.,** "Novel interacting partners modulating opioid receptor signaling" European-Opioid Conference-ENC Meeting, Ferrara, Italy 8-11 April, 2008
- **Georgoussi Z.,** "Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screening novel receptor interacting partners" Patras, Greece, May 18-20, 2008
- **Georgoussi Z.,** *41st European Brain and Behavior Society Meeting and 23rd Hellenic Society for Neuroscience Meeting* με τίτλο ""From G protein-coupled receptor signaling to novel therapeutic targets: the opioid receptor example" Rhodes Island, Greece, 15-18 September 2009
- **Georgoussi Z,** University of Zhejiung title "Novel therapeutic targets of G protein coupled receptors: the opioid receptor paradigm" *Hongzhou, China* 29 October 2009

Conference presentations:

Lecture and oral communications:

- **L Leontiadis, Z Georgoussi** RGS4 protein: a new player in μ- and δ- opioid receptor signaling. Newsletter, Vol. 54, p 16. 59th National Conference of Biochemistry and Molecular Biology, , Athens, Greece. December 7-9, 2007
- **E Georganta, A Agalou, Z Georgoussi,** Interaction of the δ-opioid receptor with STAT5B and Gβγ subunits reveals novel signaling pathways. European Opioid Conference – European Neuropeptide Club Joint Meeting, , Ferrara, Italy, April 8-11, 2008
- **E Georganta, G Mazarakou, A Agalou, Z Georgoussi**, The C-termini of the μ- and δ-opioid receptors differentially bind to Signal Transducers and Activators of Transcription, STAT5A and STAT5B. 5th Panhellenic Congress of Pharmacology, Athens, Greece, May 23-24, 2008
- **Z Urbanczyk-Lipkowska** "Annual report on progress in research grant NORMOLIFE", Institute of Organic Chemistry, PAS, Warsaw, February 2008.
- **AW. Lipkowski:** "Neuropeptide analgesics as prospective new generation of analgesics", 22nd Annual National Conference of Indian Society for Study of Pain, Lucknow, February 2-4, 2007
- **Z Georgoussi:** "Novel signaling pathways mediated by the opioid receptors", 19th Polish Peptide Symposium, Pultusk, September 23-27, 2007
- **L Leontiadis, M-P Papakonstantinou, M Sarris, Z Georgoussi,** "Mapping of the functional domains of opioid receptors responsible for RGS4 protein interaction". 29th Conference of the Hellenic Society for Biological Sciences (HSBS), Kavala, Greece. 17-19 May, 2007,
- **L Leontiadis, M-P Papakonstantinou, Z Georgoussi,** " RGS4 interacts directly with μ- and δ-opioid receptors to regulate their signaling", INRC Annual Meeting, International Narcotic Research Conference, Berlin, Germany July 8-13, 2007
- **Z Georgoussi, L. Swevers, K Iatrou,** "Functional Genomics and Proteomics in identification of new therapeutic leads and novel pharmaceutical targets. 8th Pharmacology Symposium, 24th February 2007
- **Z Georgoussi** "Novel Signaling pathways mediated by the opioid receptors". 19th Polish Peptide Symposium, Pultusk, Poland 23-27 September 2007.
- **Z Georgoussi "**Novel interacting partners modulating opioid receptor signaling" European-Opioid Conference-ENC Meeting, Ferrara, Italy 8-11 April, 2008
- **Z Georgoussi** "Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screening novel receptor interacting partners" Patras, Greece, May 18-20, 2008
- **AW. Lipkowski, A. Misicka, DB Carr:** "Multitarget peptide ligands as prospective new medicines for chronic pain treatment", 19th Polish Peptide Symposium, Pultusk, September 23-27, 2007
- **G Toth,** "Methods for Tritium Labelling of Neuropeptides", 19th Polish Peptide Symposium, Pultusk, September 23-27, 2007
- **D Tourwe,** "Azepinone-constrained Phe and Tyr residues: new scaffolds for the design of peptides and peptidomimetics", 19th Polish Peptide Symposium, Pultusk, September 23-27, 2007
- **A Misicka:** "Targeting cancer cells with neuropeptides platinum complexes", 11th Naples Workshop on Bioactive Compounds - New frontiers on search of bioactive molecules - from peptides to drugs, Naples, May 24-27, 2008
- **AW Lipkowski:** EU Funded Research Projects and Collaboratives. Normolife. Development of new therapeutic substances and strategies for treatment of pain in patients with advanced stages of cancer, 5th Research Forum of the European Association for Palliative Care (EAPC), Trondheim, 28-31 May 2008
- **L Pasquinucci**, Analgesici oppioidi: nuove opportunità nella terapia del dolore. Atti XVIII Convegno Nazionale della Divisione di Chimica Farmaceutica della Società Chimica Italiana, Chieti-Pescara 16-20 Settembre 2007,

- **A Gramowski, O Schröder, D Weiss,** Electrophysiological Profiling of Substance P and the opioid receptor system as reference for new synthetic neuroactive peptides. 19th Polish Peptide Symposium, Sep. 23-26 2007
- **D Weiss, O Schröder, A Gramowski, K Jügelt**: High content analysis of neuro-active compounds' mode of action using neuronal networks grown on microelectrode arrays. 19th Polish Peptide Symposium, Sep. 23-26 2007
- **„Opioid peptide analogu es for cancer pain treatment"**
  AW Lipkowski, B**.** Szaniawska, P. Kosson, A. Klinowiecka, A. Lesniak, J. Janiszewska, K. Kurzepa, Neuropeptide Festival 2009. Joint Meeting of the European Club and the Summer Neuropeptide Conference, July 20-23, 2009
- **Neuropeptide analogu es as prospective adjuvants of cancer pain treatment**
  AW Lipkowski, E. Matyja, B. Szaniawska, A. Lazarkiewicz, P. Kosson, A. Klinowiecka, A. Lesniak, J. Janiszewska, K. Kurzepa, 20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **Analgesici oppioidi: nuove opportunità nella terapia del dolore.**
  Atti XVIII Convegno Nazionale della Divisione di Chimica Farmaceutica della Società Chimica Italiana, Chieti-Pescara September 16-20 2007, 15, CI1.
  Pasquinucci L.
- **In vitro and in vivo pharmacological evaluation of LP1, a benzomorphan-based compound.**
  6th Meeting of the European Network of Doctoral Studies in Pharmaceutical Sciences, Palermo November 16-18 2009, p 36.
  Iemolo A., Parenti C., Aricò G., Al-Khrasani M., Amata E., Scoto G.M. Ronsisvalle G., Pasquinucci L.
- **„Opioid peptide-platinum(II) complexes:synthesis, characterization and in vitro antitumor activity**
  Głowińska A., Tomczyszyn A., Kosson P., Matalińska J., Lipkowski A. W., Misicka A., Proceedings 30th EPS,", 30th European Peptide Symposium, Helsinki, Finnland, September 2008
- **„Synthesis of □2-amino acids and their application in endomorphin analogues"**
  Tymecka D., Kosson P., Lipkowski A.W., Misicka A., 30th European Peptide Symposium, Helsinki, Finnland, September 2008
- **„Neuropeptide analogs as prospective selective carriers of platinum ion in anticancer therapy"**
  Misicka A., Glowinska A., Kalinska K., Jaworski K., Tomczyszyn A., Lazarczyk M., Matyja E., Kosson P., Lipkowski AW., 20th Polish Peptide Symposium, Cetniewo, Poland, September 2009
- **"□2-Homo-amino acid scan of Endomorphin-2 and its D-Ala2-analogue (TAPP)"**
  Tymecka D., Kosson P., Lipkowski AW., Misicka A., 20th Polish Peptide Symposium, Cetniewo, poland, September 2009
- **"Synthesis of tritiated ligand for binding assays to the tachykinin receptors"**
  Tomczyszyn A., Lipkowski AW., Toth G., Misicka A.,20th Polish Peptide Symposium, Cetniewo, Poland, September 2009
- **"Synthesis of water soluble platinum peptide complexes"**
  Głowińska A., Kalinska A., Jaworski K., Kosson P., Lipkowski AW., Misicka A., 20th Polish Peptide Symposium, Cetniewo, Poland, September 2009
- **„Opioid peptides as carriers for platinum ion in anticancer therapy"**
  Głowińska A., Łazarczyk M., Kosson P., Matyja E., Lipkowski AW., Misicka A., *Chemsession 2009, 15.05.2009, Warszawa*
- **„Opioid peptide analogue-polymer carrier compositions for chronic pain treatments"**
  Zbrzezna J., Lesniak A., Tymecka D., Misicka A., Henninck WE., Lipkowski AW., Neuropeptide festival 2009, Joint meeting of the European Neuropeptide Club and the Summer Neuropeptide Conference, Salzburg, Austria, July 2009

- "**Application of alicyclic beta-amino acids for the development of potent endomorphin analogues"**
  Attila Keresztes, Mária Szűcs, Attila Borics, Ferenc Fülöp, Katalin E. Kövér, Géza Tóth; Straub Days, BRC-HAS, Szeged, Dec 5-8, 2008.
- **"New tritiated neuropeptide analogues as research tools"**
  Géza Tóth, Attila Keresztes, Judit Farkas, Jayapal Reddy Mallareddy, Erzsébet Szemenyei
- **Design of novel tetra-branched peptide dendrimers: antimicrobial and anticancer activity vs 3D structure**
  Z. Urbanczyk-Lipkowska
  Dendrimer Symposium 6, 14-18.06.2009, Stockholm, Sweden
- **Leontiadis L., Papakonstantinou M-P. and Georgoussi Z**. " RGS4 interacts directly with μ- and δ-opioid receptors to regulate their signaling", INRC Annual Meeting, International Narcotic Research Conference, Berlin, Germany July 8-13, 2007
- **Leontiadis, L., Georgoussi Z.** (2007). RGS4 protein: a new player in μ- and δ- opioid receptor signaling. Newsletter, Vol. 54, p 16. 59^{o} National Conference of Biochemistry and Molecular Biology, December 7-9, Athens, Greece (Oral Presentation).
- **Georganta, E. Agalou A. and Georgoussi Z.** (2008). Interaction of the δ-opioid receptor with STAT5B and Gβγ subunits reveals novel signaling pathways. European Opioid Conference – European Neuropeptide Club Joint Meeting, April 8-11, Ferrara, Italy (Oral Presentation)
- **Leontiadis L.J., Georganda E-M, Papakonstantinou M-P, Fourla D-D, Sarris M., Agalou A., Georgoussi, Z.** (2008). Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screening novel receptor-interacting partners. 6th Hellenic Forum on Bioactive peptides, 18th-20th May, Patra, Greece
- **Georganta, E. Mazarakou, G. Agalou A. and Georgoussi Z.** (2008). The C-termini of the μ- and δ- opioid receptors differentially bind to Signal Transducers and Activators of Transcription, STAT5A and STAT5B. *Epitheorese Klinikes Farmakologias kai Farmakokinetikes,* Vol. 22 (2), p 152-155. 5th Panhellenic Congress of Pharmacology, May 23-24, Athens, Greece (Oral Presentation)
- **Fourla D.D. and Georgoussi Z.** (2008). Novel opioid analogs with bifunctional activity for the μ- and δ-opioid receptors. 3d Normolife meeting, N.C.S.R. "D", May 23rd.
- **Leontiadis L. and Georgoussi Z.** (2008). RGS4: A novel interacting protein essential for μ- and δ- opioid receptor signaling. 33rd FEBS Congress & 11th IUBMB Conference, June 28th- July 3rd, Athens, Greece.
- **Leontiadis, L.J. Papakonstantinou, M-P. Sarris M. and Georgoussi Z.** (2008). RGS4 regulates opioid receptor signaling. I.N.R.C., July 13-18, Charleston, SC, USA
- **Leontiadis, L.J. Papakonstantinou, M-P. Sarris M. and Georgoussi Z**. (2008). RGS4 protein interacts with μ- and δ- opioid receptors to modulate their internalization fate. 22nd Conference of the Hellenic Society for Neuroscience, October 16-19, Eugenides Foundation Athens, Greece
- **Georganta, E., Agalou A. and Georgoussi Z.** (2008). Stimulation of the δ-opioid receptor triggers novel signaling pathways leading to transcriptional activation. 16th -19th October, 22nd Conference of the Hellenic Society for Neuroscience, Eugenides Foundation Athens, Greece
- **Georganta, E., Agalou A. and Georgoussi Z.** (2009). New signaling pathways induced by activation of δ-opioid receptor. 9th Pharmacology Symposium, 8th March, Athens, Greece
- **Georganta, E., Agalou A. and Georgoussi Z.** (2009). Novel dynamic complexes between the δ-opioid receptor, STAT5B and G proteins. 23^{d} Annual Meeting of the Hellenic Society for Neuroscience, 13th-14th September, Rhodes Island, Greece.
- **Georganta, E., Agalou A. and Georgoussi Z**. (2009). STAT5B transcription factor forms functional complexes with the δ-opioid receptor and selective G protein subunits. 60th Greek Conference of Biochemistry and Molecular Biology, 20th–22nd November, Athens, Greece.

- **Papakonstantinou M.-P., Sarris M., Nikolos F., Leontiadis L.J., Fourla D.D. and Georgoussi Z**. (2009). RGS2: A new modulator of opioid receptor signalling. 60th Greek Conference of Biochemistry and Molecular Biology, 20th–22nd November, Athens, Greece.
- **Gramowski A, Schröder O, Lipkowski A, Misicka-Kesik A, Weiss DG:** Electrophysiological profiling of the opioid receptor system in drug development of new synthetic neuroactive peptides for cancer pain treatment. 6th FENS Forum, Geneva, Schwitzerland July 12 - 16. Abstract # (2008)
- **Schroeder OH-U, Gramowski A, Jügelt K, Teichmann C, Weiss DG:** A database of substance-specific network activity: Application to functional screening in preclinical drug development. Miptec, Basel, Switzerland, October 12-15 (2008)
- **Olaf H.-U. Schroeder, Alexandra Gramowski1, Konstantin Jügelt, Dieter G. Weiss.** Determination of singular activity patterns of functional neuronal networks on microelectrode arrays. The 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany, June (2009)
- **Jügelt K., Schroeder O.H.-U., Weiss D.G. & Gramowski A**. Similarity analysis of antipsychotics and antidepressants using network spike train patterns derived from MEA recordings. 39th Annual Meeting Society for Neuroscience, Chicago, USA, October 17-21, Abstract # 390, 265 (2009)
- Annual Meeting of Radiochemists of Hungary, Pécs, Hungary, October 14-16, 2009

Posters

- **L Pasquinucci, A Marrazzo, O Prezzavento, G Ronsisvalle,** Benzomorphan-based opioid ligands with mixed mu agonist/delta antagonist activity. 19th Polish Peptide Symposium, Pultusk Poland, September 23-27, 2007
- **L Pasquinucci, E Amata, G Ronsisvalle**, Development of new benzomorphan LP1 analogues, II EWDSy – Second European Workshop in Drug Synthesis, Siena Italy, May 25-30, 2008,
- **LJ Leontiadis, Z Georgoussi**, RGS4 protein interacts with the carboxyl-terminal tails of the μ- and δ-opioid receptors. 8th Pharmacology Symposium, Athens, Greece, February 24, 2007
- **I-M Georganda, Z Georgoussi,** Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation. 8th Pharmacology Symposium, , Athens, Greece, February 24, 2007
- **E Georganta, Z Georgoussi**, Functional complexes between δ-opioid receptor, STAT5B and Gβγ subunits. The 29th scientific conference of the Hellenic Society of Biosciences, p 48-49, Hellenic Society of Biosciences, Kavala, Greece, May 17-19, 2007
- **E Georganta, Z Georgoussi,**"Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation". INRC Annual Meeting, International Narcotic Research Conference, Berlin, Germany, July 8-13, 2007
- **AW Lipkowski, A Misicka, DB Carr, P Kosson**: Peptide analgesics, a new generation of analgesics for modern methods of applications. The Belgian and International Trade Fair for Technological Invention "Brussels Eureka", November 24th, 2007
- **A Misicka, A Glowinska, P Kosson, J Matalinska, AW Lipkowski:** Opioid peptides as carriers of platinum ion in cancer chemotherapy. The Belgian and International Trade Fair for Technological Invention "Brussels Eureka", November 24th, 2007
- **E Georganta, Z Georgoussi,** Functional multi-protein complexes between the δ-opioid receptor, STAT5B and Gβγ subunits of G-proteins. 59th Greek Conference of Biochemistry and Molecular Biology, , Athens, Greece, December 7-9, 2007
- **LJ Leontiadis, M-P Papakonstantinou, M Sarris, Z Georgoussi,** "RGS4 and RGS2 differentially modulate opioid receptor signaling". 5th Panhellenic Congress of Pharmacology, Hellenic Society of Pharmacology (HSP), Athens, Greece, May 23-25, 2008

- **LJ Leontiadis, Z Georgoussi,** "RGS4 protein: A novel interacting protein essential for μ- and δ- opioid receptor signaling". 33rd FEBS Congress & 11th IUBMB Conference, "Biochemistry of Cell Regulation"., Athens, Greece, June 28–July 3, 2008
- **E Georganta, A Agalou, Z Georgoussi**, STAT5B forms tight complexes with the δ-opioid receptor and Gβγ subunits. 33d FEBS Congress & 11th IUBMB Conference "Biochemistry of Cell Regulation", Athens, Greece, June 28–July 3, 2008
- **Leontiadis, L.J., Georgoussi Z**., (2007) RGS4 protein interacts with the carboxyl-terminal tails of the μ- and δ-opioid receptors. *Epitheorese Klinikes Farmakologias kai Farmakokinetikes* 25 (1), 72-74. 8th Pharmacology Symposium, February 24, Athens, Greece (Poster)
- **Georganda, I-M. Georgoussi Z**. (2007) Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation. *Epitheorese Klinikes Farmakologias kai Farmakokinetikes*, Vol. 25 (1), p 28-29. 8th Pharmacology Symposium, February 24, Athens, Greece (Poster)
- **Leontiadis, L. Papakonstantinou, M-P. Sarris, M. Georgoussi Z**. "Mapping of the functional domains of opioid receptors responsible for RGS4 protein interaction". 29th Conference of the Hellenic Society for Biological Sciences (HSBS), 17-19 May, 2007, Kavala, Greece.
- **Georganta, E. Georgoussi Z.** (2007).Functional complexes between δ-opioid receptor, STAT5B and Gβγ subunits. *Proceedings of the 29th scientific conference of the Hellenic Society of Biosciences,* p 48-49, Hellenic Society of Biosciences, May 17-19, Kavala, Greece (Poster)
- **Georganta E. and Georgoussi Z.** (2007) "Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation". INRC Annual Meeting, International Narcotic Research Conference, July 8-13, Berlin, Germany (Poster)
- **Georganta E. and Georgoussi Z.** (2007). Functional multi-protein complexes between the δ-opioid receptor, STAT5B and Gβγ subunits of G-proteins. *Newsletter,* Vol. 54, p 102. 59th Greek Concerence of Biochemistry and Molecular Biology, December 7-9, Athens, Greece (Poster)
- **Leontiadis, L.J. Papakonstantinou, M-P. Sarris M. and Georgoussi Z.** (2008) "RGS4 and RGS2 differentially modulate opioid receptor signaling". *Epitheorese Klinikes Farmakologias kai Farmakokinetikes,* Vol. 22 (2), p 214-216. 5th Panhellenic Congress of Pharmacology, Hellenic Society of Pharmacology (HSP), May 23-25, Athens, Greece (poster).
- **Georganta, E., Agalou A. and Georgoussi Z**. (2008). STAT5B forms tight complexes with the δ-opioid receptor and Gβγ subunits. 33d FEBS Congress & 11th IUBMB Conference "Biochemistry of Cell Regulation", June 28-July 3, Athens, Greece (Poster)
- **D Niedziałek Z Urbanczyk-Lipkowska,** „Polymorphism or polytypism – crystallization experiments leading to formation of a new crystal form of 7-amino-4-metylocoumarin (C120)", Polish Crystallographic Meeting, , Wrocław, Poland, 6-7June, 2007
- **D Niedziałek, Z Urbanczyk-Lipkowska**, „Coumarin 120 - crystallization experiments leading to a new type of polymorphism" 24th European Crystallographic Meeting, Marakesh, Morocco, August 22-27, 2007
- **„Assessment of analgesic Potenzy of novel neuropeptide analogues In a Mouse model of neuropathic pain"**
  A Lesniak, P. Kosson, J. Rafalowska, R. Gadamski, AW Lipkowski, „Neuropeptide Festival 2009. Joint Meeting of the European Club and the Summer Neuropeptide Conference, July 20-23, 2009
- **"Opioid peptide analogue-polymer Cartier compositions for chronic pain treatments."**
  Zbrzezna, A. Lesniak, D. Tymecka, A. Misicka, WE Hennink, AW Lipkowski, Neuropeptide Festival 2009. Joint Meeting of the European Club and the Summer Neuropeptide Conference, July 20-23, 2009

- **Neuropeptide analogu es as prospective adjuvants of cancer pain treatment"** AW Lipkowski, E. Matyja, B. Szaniawska, A. Lazarkiewicz, P. Kosson, A. Klinowiecka, A. Lesniak, J. Janiszewska, K. Kurzepa,
  20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **Intrathecal administration of novel opioid neurotensin hybryd peptides results In long-standing antinociception In tail-flick test In rats,** P. Kleczkowska, P. Kosson, D. Tourwe, AW Lipkowski, 20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **Analgesic activity of opioid peptides In mice bread for stress induced analgesia,** A Klinowiecka, P. Kosson, B. Gajkowska, M. Sacharczuk, AW Lipkowski,
  20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **New tachykinin-opioid chimeric analogues as potential new analgesics, P.** Kosson, A. Klinowiecka, DB Carr, AW Lipkowski
  20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **Assesment of analgesic Potenzy of an opioid peptide analogue biphalin In a Mouse model of neuropathic pain,** A Lesniak, P. Kosson, J. Rafalowska, R Gadamski, AW Lipkowski
  20th Polish Peptide Symposium, September 6-10, 2009, Władysławowo, Poland
- **Benzomorphan-based opioid ligands with mixed agonist/ antagonist activity.**
  Proceedings 19th Polish Peptide Symposium, Pultusk Poland, September 23-27, 2007, pp 110 .
  Pasquinucci L., Marrazzo A., Prezzavento O., Ronsisvalle G.
- **Development of new benzomorphan LP1 analogues**
  II EWDSy – Second European Workshop in Drug Synthesis, Siena Italy, May 25-30, 2008, P01.
  Pasquinucci L., Amata E., Ronsisvalle G.
- **In-vitro evaluation of a new benzomorphan-based ligand.**
  54th National Meeting of the Italian Society of Biochemistry and Molecular Biology (**SIB**). Catania, 23rd – 27th September, 2009, p. 143.
  Pasquinucci L., Prezzavento O, Amata E, Ronsisvalle S, Parenti C, Aricò G, Iemolo A, Scoto G.M, Georgoussi Z, Ronsisvalle G.
- **Synthesis and in vitro opioid receptor studies on benzomorphan-based compounds: LP1, a potent and long-acting antinociceptive.**
  EFMC: Drugs of the future. Barcelona (Spain), 4th-6th October, 2009, p. 92.
  Pasquinucci L., Prezzavento O, Amata E, Arena E, Ronsisvalle S, Parenti C, Aricò G, Scoto G.M, Ronsisvalle G.
- **"From precursor to peptide: Evidence for endomorphin-2 biosynthesis?"**
  Jayapal Reddy Mallareddy, Balázs Csibrány, Attila Keresztes, Géza Tóth
  Meeting of the Hungarian Neuroscience Society, Budapest, Hungary, January 22-24, 2009
- **"Investigations of Endomorphin-2 Biosynthesis"**
  Jayapal Reddy Mallareddy, Attila Keresztes, Géza Tóth
  Joint Meeting on Medicinal chemsitry, Budapest, Hungary , June 24-27, 2009.
- **"Structural Comparison of -Opioid Receptor Selective Peptides Revealed Four Important Conformational Parameters of Bioactivity."**
  Attila Borics, Géza Tóth
  30th European Peptide Symposium, Helsinki, Finland, 31 August – 5 September, 2008.
- **"Endomorphin analogues with *p*-substituted phenylalanine in position 4: Influence of physicochemical properties on pharmacological profile."**
  Géza Tóth, Attila Keresztes, Andriy Maslyanko, András Rónai
  Joint Meeting on Medicinal chemsitry, Budapest, Hungary, June 24-27, 2009.
- **pH indicators – molecular structure and proton distribution**
  25th European Crystallographic Meeting, Istanbul, Turkey,
  Z. Urbanczyk-Lipkowska, P. Kalicki

- **X-ray studies of fluorescent compounds crystallized from solutions of different pH.** 51 Polish Crystallographic Meeting**,** Wrocław, 25-26.06 2009.
  Przemysław Kalicki, Zofia Urbańczyk-Lipkowska.
- **-lactam moiety functionalized ammphiphilic dendrimeric peptides – design of new antibiotics and inhibitory agents.**
  Dendrimer Symposium 6, 14-18.06.2009, Stockholm, Sweden.
  Marta Sowińska, Aleksandra Rajnisz, Jolanta Solecka, Zofia Urbańczyk-Lipkowska:
- **Electrophysiological profiling of the opioid receptor system in drug development of new synthetic neuroactive peptides for cancer pain treatment**
  6th FENS Forum, Geneva, Schwitzerland July 12 - 16. Abstract # (2008)
  Gramowski A, Schröder O, Lipkowski A, Misicka-Kesik A, Weiss DG
- **A database of substance-specific network activity: Application to functional screening in preclinical drug development.**
  Miptec, Basel, Switzerland, October 12-15 (2008)
  Schroeder OH-U, Gramowski A, Jügelt K, Teichmann C, Weiss DG
- **Determination of singular activity patterns of functional neuronal networks on microelectrode arrays.**
  The 9th Göttingen Meeting of the German Neuroscience Society, Göttingen, Germany, June (2009)
  Olaf Schroeder, Alexandra Gramowski1, Konstantin Jügelt, Dieter G. Weiss.
- **Similarity analysis of antipsychotics and antidepressants using network spike train patterns derived from MEA recordings**
  39th Annual Meeting Society for Neuroscience, Chicago, USA, October 17-21, Abstract # 390, 265 (2009)
  Jügelt K., Schroeder O.H.-U., Weiss D.G. & Gramowski A.

Publications

- **D Niedzialek, Z Urbanczyk-Lipkowska**, New Crystalline form of 7-amino-4-methylcoumarin (coumarin 120) – a polymorph with (1:1) valence tautomers" *CrystEngComm.,* 2007, 9, 735-739.
- **I-M Georganda, Z Georgoussi,** Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation. Epitheorese Klinikes Farmakologias kai Farmakokinetikes, 2007, 25, p 28-29
- **E Georganta, Z Georgoussi** Functional complexes between δ-opioid receptor, STAT5B and Gβγ subunits. Proceedings of the 29th scientific conference of the Hellenic Society of Biosciences*,* (2007), p 48-49
- **E Georganta, G Mazarakou, A Agalou, Z Georgoussi,** The C-termini of the μ- and δ-opioid receptors differentially bind to Signal Transducers and Activators of Transcription, STAT5A and STAT5B. Epitheorese Klinikes Farmakologias kai Farmakokinetikes*,* 2008*,* 22; 152-155
- **LJ Leontiadis, M-P Papakonstantinou, M Sarris, Z Georgoussi,** "RGS4 and RGS2 differentially modulate opioid receptor signaling". *Epitheorese Klinikes Farmakologias kai Farmakokinetikes,* 2008, 22, 214-216.
- **L Pasquinucci, M Iadanza, A Marrazzo, O Prezzavento, S Ronsisvalle, GM Scoto, C Parenti, L De Luca, G Ronsisvalle,** New benzomorphan derivatives of MPCB as MOP and KOP receptor ligands. Pharmazie, 2007, 62, 813-824.
- **M Łazarczyk**, **E Matyja, A Lipkowski,** Substance P and its receptors -- a potential target for novel medicines in malignant brain tumour therapies (mini-review). Folia Neuropathol. 2007, 45:99-107.
- **P Wojciechowski, M Szereda-Przestaszewska**, AW **Lipkowski,** Supranodose vagotomy eliminates respiratory depression evoked by dermorphin in anaesthetized rats. Eur J Pharmacol. 2007, 563:209-212

- **E loja, D Tourwé, I Kertész**, **G Tóth**, **A Borsodi, S Benyhe**. Novel diastereomeric opioid tetrapeptides exhibit differing pharmacological activity profiles. Brain Res Bull. 2007, 74:119-129
- **C Tömböly, S Ballet, D Feytens, KE Kövér, A Borics, S Lovas, M Al-Khrasani, Z Fürst, G Tóth, S Benyhe, D Tourwé,** Endomorphin-2 with a beta-turn backbone constraint retains the potent micro-opioid receptor agonist properties. J Med Chem. 2008, 51:173-177
- **S Ballet**, **A Misicka**, **P Kosson**, **C Lemieux**, **NN Chung, PW Schiller**, **AW Lipkowski**, D **Tourwé,** Blood-Brain Barrier Penetration by Two Dermorphin Tetrapeptide Analogues: Role of Lipophilicity vs Structural Flexibility. J Med Chem. 2008, 51: 2571-2574
- **K Pulka, P Kulis, D Tymecka, L Frankiewicz, W Wilczek, W Kozminski, A Misicka** Diastereoselective Pictet-Spengler condensation of tryptophan with a-amino aldehydes as chiral carbonyl components, Tetrahedron 2008, 64: 1506-1514
- **P Polcyn, J Janiszewska, AW Lipkowski, Z Urbańczyk-Lipkowska,** Application of dendrimers in pharmaceutical sciences – perspectives", 2008, submitted to *Polimery* (in polish).
- **L. Pasquinucci et al.** Benzomorphan-based opioid ligands with mixeḍ agonist/ antagonist activity. *J. Med. Chem., in press*
- **Pasquinucci L., Iadanza M., Marrazzo A., Prezzavento O., Ronsisvalle S., Scoto G.M., Parenti C., De Luca L., Ronsisvalle G** New benzomorphan derivatives of MPCB as MOP and KOP receptor ligands. *Pharmazie*, 2007, 62 (11), 813-824.
- **Conformationally constrained opioid ligands: the Dmt-Aba and Dmt-Aia versus Dmt-Tic scaffold.**
  S. Ballet, D. Feytens, R. De Wachter, M. De Vlaeminck, E.W. Marczak, S. Salvadori, C. de Graaf, D. Rognan, L. Negri, R. Lattanzi, L. H. Lazarus, D. Tourwé G. Balboni, *Bioorg & Med Chem Lett.* **2009**, 19, 433-437.
- **Syntheses, methods of identification and selected medical applications of dendrimers. *POLIMERY,* 2009**, 54 (6): 407-41
  *P*olcyn P, Janiszewska J, Lipkowski, AW, Urbanczyk-Lipkowska Z6
- **Influence of ring substitution on the conformation and beta-turn mimicry of 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one peptide mimetics**. ***TETRAHEDRON* 2009**, 65 (11): 2266-2278
  De Wachter R, Brans L, Ballet S, Van den Eynde I, Feytens D, Keresztes A, Toth G, Urbanczyk-Lipkowska Z, Tourwe D..
- **Design of Antimicrobially Active Small Amphiphilic Peptide Dendrimers *MOLECULES*, 2009**, 14 (10): 3881-3905
  Polcyn P, Jurczak M, Rajnisz A, Solecka J, Urbanczyk-Lipkowska Z.,..
- **New Crystalline form of 7-amino-4-methylcoumarin (coumarin 120) – a polymorph with (1:1) valence tautomers" *CrystEngComm,* 2007**, **9**, 735-739
  Niedzialek D, Urbanczyk-Lipkowska Z..
- **Berkecz R., Ilisz I., Misicka A., Tymecka D., Fülöp F., Choi H.J., Hyun M.H., Péter A.,** *"HPLC enantioseparation of ß2-homoamino acids using crown ether-based chiral stationary phase"* J. Sep. Sci. 3 (2009) 981-987
- **Pataj Z., Berkecz R., Ilisz I., Misicka A., Tymecka D., Fulop F., Armstrong D., Peter A.,** *High-Performance Liquid Chromatographic Chiral Separation of 2-Homo-Amino Acids*", Chirality 21 (2009) 787-798
- **Ilisz I., Pataj Z., Berkecz R., Misicka A., Tymecka D., Fulop F., Choi HJ., Hyun MH., Peter A.,** *"High-performance liquid chromatographic enantioseparation of b2-amino acids using a long-tethered (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase*,J. Chrom. A Online July (2009)
- **Głowińska A., Tomczyszyn A., Kosson P., Matalińska J., Lipkowski A. W., Misicka A.,** Proceedings 30th EPS, *„Opioid peptide-platinum(II) complexes:synthesis, characterization and in vitro antitumor activity"*, Proc. of 30th European Peptide Symposium, Peptides 2008

- **Tymecka D., Kosson P., Lipkowski A.W., Misicka A.,** *„Synthesis of 2-amino acids and their application in endomorphin analogues“*, Proc. of 30th European Peptide Symposium, Peptides 2008
- **Géza Tóth, Attila Keresztes**, **Attila Borics,** Mária Szűcs, Ferenc Fülöp: New Endomorphin Analogues Containing Alicyclic -Amino Acids: Influence on Bioactive Conformation and Pharmacological Profile. Proceedings of the 1st Hungarian-Singaporean Workshop on Drug Discovery and Biomaterials, Hungarian Academy of Sciences.
- **Borics, G. Tóth**: Structural comparison of μ-opioid receptor selective peptides and peptidomimetics. revealed four important conformational parameters of bioactivity. Peptides 2008: Proceedings of the 30th European Peptide Symposium. p. 344-345.

- **Cs. Tömböly**, S. Ballett, D. Feytens, K. E. Kövér, A. **Borics, S.** Lovas, M. Al-Khrasani Z. Fürst, **G. Tóth**, S. Benyhe, D. Tourwé: Endomorphin-2 with a β-Turn Backbone Constraint Retains the Potent μ Opioid Receptor Agonist Properties *J.Med.Chem*. 51., 173-177 (2008) Impact factor: 4.895
- **E. Szemenyei** I. Barna, Z. Mergl, **A. Keresztes**, Z. Darula, E. Kató, **G. Tóth**, A. Rónai: Detection of a novel immonoreactive endomorphin 2-like peptide in rat brain extracts *Regulatory Peptides* (2008) 148., 54-61 Impact factor: 2. 422
- **Keresztes**, M. Szűcs, **A. Borics,** K. E. Kövér, E. Forró, F. Fülöp, **C. Tömböly**, A. Péter, A. Páhi, G. Fábián, M. Murányi, **G. Tóth:** New Endomorphin Analogues Containing Alicyclic -Amino Acids: Influence on Bioactive Conformation and Pharmacological Profile *J.Med. Chem.* (2008) 51, 4270-4279 Impact factor: 4.895
- M. Botros, T. Johanson, Q. Zhau, G. Lindeberg, **C. Tömböly, G. Tóth**, P. Le Greves, F. Nyberg, M. Hallberg: Endomorphins interact with the substance P (SP) aminoterminal SP 1-7 binding in the ventral tegmental area of the brain *Pepides* (2008) 29, 1820-1824 Impact factor:2.368
- Rónai, K. Király, A. Szebeni, **E. Szemenyei**, Z. Prohászka, Z. Darula, **G. Tóth**, I. Till, B. Szalai, E. Kató, I. Barna: Immunoreactive endomorphin-2 is genarated extracellularly in rat isolated L4,5 dorsal root ganglia by DPP-IV *Regulatory Peptides* (2009) 157, 1-2. Impact factor: 2. 422
- R. De Wachter, L. Brans, S. Ballet, I. Van den Eynde, D. Feytens, **A. Keresztes, G. Tóth**, Z. Urbanczyk-Lipkowska, D. Tourwé: Influence of ring substitution on the conformation and -turn mimicry of 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one peptide mimetics *Tetrahedron* (2009) 65, 2266-2278. Impact factor:2.869
- **Borics, G. Tóth**: Structural comparison of -opioid receptor selective peptides confirmed four parameters of bioactivity. *J. Mol. Graph. Model.* (2009) in press. Impact factor:2.347
- **M. Lukowiak, P. Kosson, W.E. Hennink, A.W. Lipkowski**, *The synthesis and pharmacological properties of new fluorescent opioid peptide analogue.* Pharmacol Rep. (2009) 61, 517-521
- **Wojciechowski P,** Szereda-Przestaszewska M, **Lipkowski AW**, *Respiratory and cardiovascular effects of biphalin in anesthetized rats*., Eur J Pharmacol. (2009) 602, 50-53
- **L.J. Leontiadis , M. Papakonstantinou and Z. Georgoussi** (2009). Regulator of G protein signaling 4 confers selectivity to specific G proteins to modulate μ- and δ-opioid receptor signaling. *Cellular signalling*, 21, 1218-1228.
- **E-M. Georganta, A. Agalou, Z. Georgoussi.** (2009). Novel dynamic complexes between the δ-opioid receptor, STAT5B and G proteins are implicated in STAT5B phosphorylation. *Submitted*
- **Gramowski A, Schröder O., Lipkowski A., Misicka-Kesik A, Weiss DG**: Drug development of new synthetic peptides for cancer pain treatment: Electrophysiological profiling of the opioid receptor system. 6th International Meeting on Substrate-Integrated Microelectrode Arrays. Reutlingen, Germany July 8-11 (2008)

- **Schroeder O., Gramowski A., Jügelt K. & Weiss D.G**. Quantitative determination of electrical activity pattern changes caused by neuro-active compounds in single neurons and whole neuronal networks in vitro by microelectrode arrays. 19th Annual Conference of the German Society of Cytometry, Leipzig, Germany, October 14-16, Abstract # (2009)
- **Gramowski A, Schröder O, Jügelt K, Lipkowski A, Misicka-Kesik A, Weiss DG**. Drug development of new synthetic peptides for cancer pain treatment: Electrophysiological profiling of the opioid receptor system. 6th Int. Meeting on Substrate-Integrated Microelectrodes, 2008 217-220, ISBN 3-938345-05-5
- **Schroeder O H-U, Gramowski A, Jügelt K, Teichmann C, Weiss DG.** A database of substance-specific neuronal network activity: Application to functional screening in preclinical drug development. Proceedings MipTec Basel 2008, 4 p, in press
- **Schroeder O H-U, Gramowski A, Jügelt K, Weiss DG.** Concentration-dependent multi-parametric functional screening of CNS drugs with neuronal networks on microelectrode arrays. Proc. World Congress 2009 on Medical Physics and Biomedical Engineering, Sept. 7-12, Munich, Germany, 3 p in press

Publications in preparation :

- **Synthesis, biological evaluation and automated docking of constrained analogs of the opioid peptide H-Dmt-D-Ala-Phe-Gly-NH_2 using the 4- or 5-methyl susbstituted 4-amino-1,2,3,4-tetrahydro-2-benzazepin-3-one scaffold**
  Rien De Wachter[1], Chris de Graaf[2], Atilla Keresztes[3], Geza Toth[3], Bart Vandormael[1], S. Ballet[1], Géza Tóth[3], Didier Rognan[2], Dirk Tourwé[1]
  [1]Organic Chemistry Department; Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; [2]Bioinformatics of the Drug, CNRS UMR 7175-LC1, Université Louis Pasteur Strasbourg I, Illkirch F-67401, France and [3] Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

- **BVD03: a superpotent opioid tetrapeptide analog.**
  Bart Vandormael[1], Iro Georgoussi[2], Attila Keresztes[3], Geza Toth[3], D. Weiss[4], A. Gramowski[4], D. Tourwé[1]
  [1]Organic Chemistry Department; Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium; [2] National Centre for Scientific Research Demokritos, Greece [3] Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary and [4]University of Rostock, Germany

- Four other full publications in neuropharmacology and pain journals are under preparation.

Ph. D. Theses
At Biological Research Centre, Hungarian Academy of Sciences, Hungary

Attila Keresztes
"Endomorphin Analogs Containing Alicyclic □-Amino Acids: Influence on Conformation and Pharmacological Profile." 2008

Erzsébet Szemenyei
"Synthesis and radioactive labelling of biologically active peptides, peptide and protein fragments." 2008

At Medical Research Centre, Polish Academy of Sciences, Poland
Piotr Kosson
"Aktywnosc przeciwbolowa związków wykazujących agonistyczne dzialanie na receptory opioidowe i tachykininowe" (Analgesic activity of compounds with agonist affinity to opioid and tachykinin receptors), in preparation to defence (February 11, 2010)

Marzena Lazarczyk,
Wplyw analogow peptydowych na proliferacje I migracje komorek ludzkich glioblastoma T98 in vitro **(**The influence of peptide analogues on proliferation and migration of human glioblastoma T98G cell growth *in vitro).* September 2009

Exhibitions

The Belgian and International Trade Fair for Technological Innovation, Misicka A, Lipkowski AW, Glowinska A, "*Opioid peptides as carriers of platinum in cancer chemotherapy*", Brussels, Belgium, November 2007

Exhibition on the occasion of 200 years of chemistry in Warsaw University, Misicka A., Lipkowski AW., Glowinska A., *"Neuropeptide analogs as prospective selective carriers of platinum ion in anticancer therapy",* Warsaw University Hall, November 2009

International Invention Exposition, Głowinska, A., Matalinska J., Lipkowski AW. Misicka A. *"Neuropeptide analogs as prospective selective carriers of platinum ion in anticancer therapy",* Warsaw, June 2008

International Invention Exposition, AW Lipkowski P. Kleczkowska, I. Georgoussi, D. Tourwe*, "Opioid-Neurotensin Chimeric Analgesics for Chronic Pain",* Warsaw, June 2008

The Belgian and International Trade Fair for Technological Innovation, Lipkowski AW, "Peptide analgesics, a new generation of analgesics", Brussels, Belgium, November 2009

6.2.3 Publishable results

The new information but "not patentable" in opinion of Consortium or already protected by patent applications are published (See 6.2.2). However, the intellectual properties of "patentable" results obtained within project are only partially protected. (see the list of patent applications). The proper action regarding other results are under preparation.

- Result description: "New, peptide complexes of platinum, synthesis, pharmaceutical composition and medical application"

Possible market applications: In cancer treatment
Stage of development: preliminary in vitro pharmacological data
Contact detailes: A. Misicka, Department of Chemistry, University of Warsaw, e-mail: misicka@chem.uw.edu.pl
Intelectual property rights granted or published:
Polish Patent Application P-383412**,** and International Patent Application (PCT**),** WO 2009,041841

- Result description: "Peptide analogues, particularly for the treatment of chronic pain",

Possible market applications: In cancer pain palliative care treatment
Stage of development: preliminary in vitro and in vivo pharmacological data
Contact detailes: A.W. Lipkowski, Medical Research Centre, Polish Academy of Sciences, Pawinskiego Street 5, 02106 Warsaw, Poland, e-mail: andrzej@lipkowski.org"
Intelectual property rights granted or published:
International Grant Application (PCT): WO 2009/148343

- Result description: "A method of producing a novel opioid peptide"

Possible market applications: In development of new chimeric analgesics
Stage of development: preliminary in vitro and in vivo pharmacological data
Contact detailes: A.W. Lipkowski, Medical Research Centre, Polish Academy of Sciences, Pawinskiego Street 5, 02106 Warsaw, Poland, andrzej@lipkowski.org"
Intelectual property rights granted or published:
Two International Patent Applications PCTs: WO 2009/093918, and WO 2009/093917

- Result description: "Dendrimeric compounds comprising amino acids, hyperbranched core compound, process for preparation of dendrimeric compounds comprising amino acids and hyperbranched core compound, and use thereof "

Possible market applications: Pharmaceuticals
Stage of development: chemical synthetic data and preliminary in vitro pharmacological data
Contact detailes: Z. Lipkowska, Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka Street, Warsaw, Poland, e-mail: ocryst@icho.edu.pl"
Invention is covered by Polish Pat Appl. P386123, and international Patent Application PCT: PCT/PL2009/000090

- Result description: ""Peptide analogues, particularly for the treatment of chronic pain".

Possible market applications: Pharmaceuticals
Stage of development: chemical synthetic data and preliminary in vitro and in vivo pharmacological data

Contact detailes: A.W. Lipkowski, Medical Research Centre, Polish Academy of Sciences, Pawinskiego Street 5, 02106 Warsaw, Poland, e-mail: andrzej@lipkowski.org

Intelectual property rights granted or published:
covered by International Patent Application PCT. WO 2009148343.