in orthopedics such as calcium phosphate (Fig. 1).

The focus of the infection in the bone treated with AMP mixed with carrier was eradicated much more effectively then the focus treated with antibiotics such as vancomycin or gentamicin mixed with the same carrier. Furthermore, AMPs incorporated into model implant made from poly-methylmethacrylate based bone cement prevented the formation of the bacterial biofilm on its surface after the implant was inserted inside the infected bone.

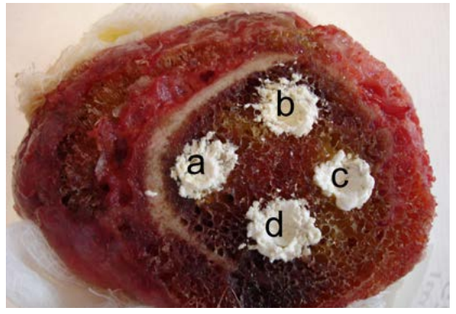

Fig. 1. Infected spongy part of the bone sample filled with a peptide mixed with local carrier (a, b), local carrier alone (c), and local carrier loaded with antibiotic (d).

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DESIGN AND STABILITY STUDIES OF DUAL PEPTIDES SIMULTANEOUSLY TARGETING GHRELIN AND Y_2 RECEPTORS

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Gastrointestinal peptides are mainly involved in the regulation of food intake and energy homeostasis and have emerged as a promising target for new obesity treatments. [1] However, the food regulation system is tightly regulated in interconnected pathways where redundancies can lead to poor efficacy and drug tolerance.[2] Ghrelin and Y_2 receptors both play a central role in appetite regulation inducing opposite effects.[3,4] The Y_2 receptor is involved in the short-term satiety signaling while the ghrelin receptor mediates hunger, promotes weight gain and stimulates energy homeostasis.

In this context, we previously developed a rational multi-targeting approach to simultaneously target the ghrelin and Y_2 receptors and we designed a dual peptide that showed a dual activity *in vitro* but a moderate stability *in vivo*.[5] The simultaneous targeting of ghrelin and Y_2 receptors was based on the co-localisation of both receptors on the same neuronal population, the NPY/AgRP neurons, in the arcuate nucleus of hypothalamus.

In order to increase the stability of the dual peptide, we recently developed new derivatives using two ligation techniques: the Copper-catalysed Azide-Alkyne Cycloaddition (CuAAC) and the oxime ligation. The stability of the dual peptides has been evaluated *in vitro* in human blood plasma and liver.

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TRIDECAPTIN A_1 SELECTIVELY BINDS TO GRAM-NEGATIVE LIPID II

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The number of bacterial strains resistant to current classes of antibiotics is increasing and has led some to warn that we are approaching an "antibiotic apocalypse." Therefore, there is an increasingly urgent need for new structurally and mechanistically distinct classes of antibiotics. The tridecaptins are linear, non-ribosomally synthesized peptides produced by *Bacillus* and *Paenibacillus* species. Although they have been known for decades, their antimicrobial activity was left mostly unexplored until their serendipitous re-discovery by our group.[1-3] In particular, analogues of tridecaptin A_1 ($TriA_1$) show selective activity against Gram-negative bacteria, including against multidrug resistant *Klebsiella pneumoniae* both *in vitro* and *in vivo* (mouse model).[4-6] Furthermore, no persistent resistance develops against *Escherichia coli* exposed to low concentrations of octyl-tridecaptin A_1 (Oct-$TriA_1$) over a one-month period. Our studies have shown that $TriA_1$ selectively binds to the Gram-negative analogue of peptidoglycan precursor lipid II. Nuclear Magnetic Resonance (NMR) and molecular docking studies were used to determine the NMR solution structure of the Oct-$TriA_1$:lipid II complex, identifying a new lipid II binding motif for an antimicrobial peptide. Our studies suggest that $TriA_1$ exerts its bactericidal effect against Gram-negative bacteria through disruption of the proton motive force at the inner-membrane. Furthermore, we have used *in vitro* assays to show that the presence of Gram-negative lipid II in artificial membranes significantly accelerates the disruption of a proton gradient. Based on our studies, we believe tridecaptin A_1 could be an excellent antibiotic candidate.

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BIOLOGICAL EVALUATION OF RADIOIODINATED AMIDATED KYOTORPHIN

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Kyotorphin (KTP) is an endogenous analgesic neuropeptide (L-Tyr-L-Arg) with limited ability to cross the blood-brain barrier (BBB) after systemic administration.[1,2] This behaviour, which narrows its pharmacological applications, has been ascribed to both insufficient lipophilicity and susceptibility to enzymatic degradation.[2] With the aim of increasing the lipophilicity of KTP, several new derivatives have been prepared upon chemical modification of the basic structure. The kyotorphin analogue KTP-amide (KTP-NH_2) presents improved lipophilicity and analgesic activity following administration in animal models.[3] Apart from the ability to cross the BBB, other relevant issues in the development of drugs for the central nervous system are related with the assessment of toxicity and determination of the biological fate. Thus, with the aim of addressing such issues, in this communication, we will report on the radiolabelling of KTP-NH_2 with $^{125}I^-$ as well as on the assessment of the interaction of the resulting radioiodinated peptide with the BBB through permeation studies in bEnd.3 cell lines. We will also describe the biodistribution studies of the radioiodinated peptide in Sprague-Dawley male rats. In addition, to check whether the incorporation of iodine into the tyrosyl residue of KTP-NH_2 would affect its analgesic efficacy, KTP-NH_2 was iodinated with "cold", non-radioactive, iodide and the analgesic efficacy of the resulting mono-iodinated (MIK) and di-iodinated (DIK) peptides was evaluated in acute pain models. The main conclusion drawn from these studies is that although the radioiodinated peptide could translocate the cellular model of the BBB, the accumulation of that species in the brain was not relevant. Significant accumulation of ^{125}I was found in the thyroid, probably reflecting the hydrolysis of the iodine-tyrosine bond by liver deiodinases. The analgesic activity of mono- and di-iodinated KTP-NH_2, evaluated by the hot plate assay, showed a delayed peak of maximal efficacy compared to non-iodinated KTP-NH_2 (30 *vs.* 15 minutes). Overall, the peripheral effects of the peptides cannot be excluded.

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BIOACTIVITY-GUIDED MASS SPECTRAL NETWORKING REVEALS A NEW SET OF ALBICIDIN DERIVATIVES FROM *XANTHOMONAS ALBILINEANS*

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Albicidin is produced by the sugarcane pathogenic bacterium *Xanthomonas albilineans* and is a potent antibiotic specifically targeting the bacterial DNA gyrase with an IC_{50} value in the nanomolar range. The structure of albicidin, which has remained unsolved for more than three decades since its first description, has been solved by a combination of extensive NMR and HR-MS/MS experiments. An inherent problem for the structure elucidation was the low amount of the albicidin production in cell culture. The low production forced us to cultivate several hundred liters in order to purify a few milligrams required for NMR experiments. In comparison, LC-HR-MS/MS has the great advantage of several orders of magnitude and high sensitivity. Thus, this approach requires much less material. But more importantly, purification of compounds is not necessary for structural analysis. Due to the application of targeted LC-MS/MS experiments, we were able to propose the structure of *N*-terminal carba-

moylated and b-methoxylated albicidin derivatives. Both of them are currently chemically synthesized and studied in a medicinal chemistry campaign. In order to search for more pharmaceutically relevant albicidin derivatives, we applied non-targeted LC-HR-MS/MS approaches combined with mass spectral networking, a recently introduced approach for non-targeted MS/MS data-analysis. However, as for the structural elucidation of albicidin, the even lower concentrated derivatives resulted in difficulties to obtain useful MS/MS spectra. Since we typically operate the mass spectrometer in data-dependent acquisition mode (DDA), it inherently selects high abundant compounds of cell extracts for MS/MS scans and possibly ignore low abundant compounds. The same goes for the network analysis which is based on similarity of MS/MS spectra and may not take potential ion species into account. To overcome this problem, we implemented a bioactivity-guided fractionation by solid phase extraction and semi-preparative HPLC. Fractions with antibacterial activity were subsequently submitted to several LC-MS/MS runs in DDA mode with shifted survey scan windows (stitched DDA). Besides the increased amount of MS/MS events per mass range, narrowing down the m/z range of the survey scan also increased the dynamic range of the orbitrap analyzer, which enabled us to significantly increase the method's sensitivity. Finally, we were able to detect a whole series of new albicidin derivatives and to propose their structures based on exact mass determination and MS/MS fragmentation patterns. These new compounds provide great opportunities in lead optimization of albicidin as a new anti-infective drug. Apart from the pharmaceutical relevance, some of the identified by-products give us important insights into the formation of albicidin and its intriguing biosynthetic machinery.

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DEVELOPMENT OF POTENT NEUROMEDIN U RECEPTOR AGONISTS

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Diseases such as diabetes and obesity have become major health concerns worldwide. To address this issue, our group is attempting to contribute through a focus on neuromedin U (NMU), a highly conserved neuropeptide regulator of feeding, energy homeostasis and glycemic control. It exerts its biological effects via two G protein-coupled receptors, NMUR1 and NMUR2. NMUR1 is mostly found in the periphery whereas NMUR2 is most abundant in the central nervous system. Both central and pheripheral administration of the peptide reduce food intake and body weight in rodents. The anorexigenic effect of NMU renders NMUR agonists attractive as potential therapeutics in the treatment of diabetes and obesity [1].

NMU-8 (H-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2), a natural occurring fragment of NMU, is taken as lead molecule for the synthesis of novel analogues. A first batch of analogues is prepared on basis of the available structure-activity relationships described in the literature [2,3]. Mainly two types of modifications were initially performed, namely chirality switches and the introduction of different *N*-capping groups. In a second set of NMU-ligands, more advanced modifications are performed, such as the introduction of unnatural/constrained amino acids or *N*-alkylated glycines ('peptoid') analogues in the NMU-sequence. A third generation of compounds was synthesized and contains analogues in which the most promising modifications of the previous generations were combined. The *in vitro* characterization of these peptides has been performed by an inositol phosphate accumulation assay. Additionally, the plasma stability of these analogues has been investigated.

The results of the *in vitro* characterization present the discovery of high potency agonists. Compared to NMU-8, more active agonists on both NMURs were discovered. Our experiments revealed, for instance, that acetylation of the *N*-terminus leads in general to an increase of activity. When replacing Tyr[1] by 7-OH-Tic or Dmt, extremely potent agonists for both receptors were obtained as well. Moreover, an improved plasma stability of these compounds is observed. The replacement of Phe[4] by 7-OH-Tic, Dmt, Oic, 1'Nal or 2'Nal leads to ligands with a comparable activity to NMU-8, but an increased plasma stability emerged. The most promising ligands were tested in an *in vivo* model to study their effect on food intake, and promising results were obtained.

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THE MECHANISM OF ANXIOLYTIC-LIKE EFFECT OF GD-23, THE DIPEPTIDE TSPO LIGAND

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The translocator protein (TSPO, 18 kDa) plays an important role for the synthesis of neurosteroids by promoting the transport of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in neurosteroidogenesis. Stimulation of TSPO by appropriate ligands increases the level of neurosteroids [1].

The present study describes design, synthesis and investigation of anxiolytic-like effects of novel dipeptide TSPO ligand, originally designed on the basis of drug-based peptide design method [2] using Alpidem as non-peptide prototype. It is known that Alpidem demonstrates anxiolytic activity [3] and its structure contains all the necessary TSPO ligands pharmacophore elements. Due to these arguments the dipeptide N-carbobenzoxy-L-tryptophanyl-L-isoleucine amide (GD-23) was designed as putative ligand.

Then we used semi-rigid docking with AutoDock Vina 1.1.2 to confirm the similarity of structures of Alpidem and dipeptide GD-23. The conformations that we selected from AutoDock Vina 1.1.2 demonstrated the minimum strain of the valent angles and bonds.

The anxiolytic activities were investigated in Balb/C mice using the illuminated open-field test [4] and elevated plus-maze test in CD-1 mice. The activating effect on locomotor activity in mice was taken as a measure of the anxiolytic activity of compound. GD-23 in the dosage range 0.05-1.0 mg/kg significantly ($p<0.005$) increased total locomotor activity of mice compared with control groups. GD-23 significantly ($p<0.001$) increased the percentage of time spent in the open arms of the maze in the dose range of 0.1-1.0 mg/kg. Anxiolytic effect of GD-23 was abolished by PK11195, a specific TSPO antagonist. It was found that by pretreatment with trilostane, a selective inhibitor of 3β-HSD, or finasteride, a selective 5α-reductase inhibitor, anxiolytic effect of GD-23 was not registered. Results were evaluated as the ratio of the time spent in the open arms of maze to the total residence time of the animals in the open and closed arms. The obtained results demonstrate that the anxiolytic effect is mediated by interaction of the compound GD-23 with TSPO receptor. Hence GD-23 can provide a basis for a new peptide class of fast anxiolytics without side effects of benzodiazepines.

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SYNTHETIC PEPTIDE VACCINES AGAINST FOOT-AND-MOUTH DISEASE: SUCCESS AT LAST

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Peptide-based vaccines would appear as the ideal alternative to conventional (*e.g.*, inactivated whole-virus) vaccines, because they are safe (no infectious agent involved), versatile (readily adaptable to emergent outbreaks) and cost-effective (reliable, reproducible production and scale-up by chemical synthesis; simple storage and transport). These advantages are however offset by problems such as the difficulty in definition and chemical reproduction of epitopes, the usually low immunogenicity of peptides, or the often intricate relation between host-pathogen interaction and immune response, all of which partly explains why only a handful of peptide vaccines have attained therapeutic status.

Among the different pathogens targeted by peptide vaccines, foot-and-mouth disease virus (FMDV), arguably the economically most devastating animal disease worldwide, has received considerable attention. We have recently described vaccine candidates, generically known as B_nT, consisting of a T-cell epitope branching out via a Lys tree into multiple (n= 2, 4) copies of a B-cell epitope.[1,2] This particular arrangement of B and T epitopes on a single molecular platform is shown to confer full protection against FMDV challenge in both swine and cattle.[3] Our presentation will illustrate various important aspects in the development of this synthetic vaccine, particularly issues such as epitope orientation and multiplicity,[4] chemical ligation methods[5] or adjuvanticity as well as provide insights on the uptake of the peptide by immune cells and on its *in vivo* localization.

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