Poster presentations

Topic C

C1 - Peptide and peptidomimetics therapeutics
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P C1 - Synthesis, interaction with phospholipids and biological activity of the laminin active sequence: SIKVAV

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A selective method of cancer treatment by cytotoxic drugs remains an elusive goal. The first step in developing a targeted cancer therapy is generating a ligand that binds to a receptor which is either tumour specific or at least overexpressed in tumour cells. Integrin receptors have been reported as an important characteristic of tumour cells. Several active sequences of laminin were identified from the native protein as potent integrin recognition sites. One of them SIKVAV has been selected to evaluate its usefulness as a potential vector to develop immunoliposomes for targeting cytostatics to tumor cells. In this work, the synthesis as well as the physicochemical study of this sequence as far as its interaction with lipids is described. Results indicate that the peptide sequence is able to form monomolecular layers at the air/ water interface and to penetrate monolayers of dipalmitoyl phosphatidylcholine spread at different surface pressures. Moreover, SIKVAV shows an important affinity for lipid bilayers, coating the surface of liposomes without modifying the microviscosity of bilayers. The presence of this peptide on the surface of liposomes do not promote fusion or aggregation of vesicles. Stability studies carried out with liposomes either loaded of composed of fluorescent markers indicate that SIKVAV do not promote any destabilization of bilayers. Besides, after incubation with erythrocytes the amount of haemogoblin released was not significant. Biological studies carried out include cell proliferation, adhesion and competition assays. The SIKVAV sequence shows no effect in the cell proliferation of different cell lines studied: neither in MCF-7 (breast), HT-29 (colon) and BxPC-3 (pancreas) human carcinoma cell lines nor in B16F10 murine melanoma cell line. However, this peptide like laminin is able to stimulate B16F10 cell attachment in a dose-dependent manner, at EC50 =21.3 and 1.6 mg/ml respectively (Fig.1). We also assessed the ability of SIKVAV to block laminin-mediated adhesion. In these studies, B16F10 cells and the peptide were added to laminin-coated wells. SIKVAV is active in inhibiting laminin-mediated cell adhesion, blocking attachment by more than 20% at a concentration of 0.5 mg/mL. From these results we can conclude that this peptide is able to promote cell adhesion competing for laminin recognition sites. As a summary, the studies here described indicate that coating liposomes with the SIKVAV sequence do not promote any type of destabilization of their structures or leakage of entrapped material. Moreover, interactions between DPPC and peptide, although small, result in negative energies of mixing thus indicating a soft stabilizing process. These characteristics together with biological results suggest that this peptide sequence can be assayed to prepare inmunoliposomes to target drugs to cells expressing its receptor

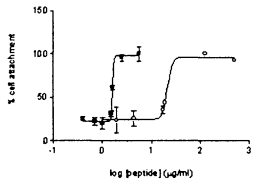

Figure 1. Attachment of B16F10 cells to peptide- and laminin-coated plates.

P C2 - Clot permeable peptide inhibitors of thrombin and factor Xa

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During clotting of human whole blood, the components of blood, including the operating enzymes such as thrombin (factor IIa) and factor Xa, are entrapped in the thrombi. Upon disintegration of thrombi, the enzymes are liberated, and the high local concentration of factors IIa and Xa induce rethrombosis, formation of secondary thrombus.
Clotting of blood plasma proceeds similarly; operating enzymes are entrapped in the clots. Factors IIa and Xa of platelet rich plasma clots can hydrolyze their substrates if they are clot permeable, like Tos-Gly-Pro-Arg-pNA* and Moc-D-Chg-Gly-Arg-pNA having van der Waals volumes of 420 and 454 Å^3, respectively; Bz-Ile-Glu-Gly-Arg-pNA, a factor Xa substrate with a volume of 543 Å^3, cannot be cleaved. Previously we identified some efegatran analogues that inhibited the amidolytic activity of entrapped factors IIa and Xa, e.g. D-Hma-Pro-Arg-H had IC_{50} values of 0.27 and 0.19 μM, respectively [1]; the van der Waals volume of this arginal is ~320Å^3. Further studies led to further analogues, e.g. GYKI-66 430, which has somewhat lower IC_{50} values against factors IIa and Xa within the plasma clots, i.e. 0.12 and 0.10 μM, respectively. Recent studies revealed that the molecular size is only one of the factors that determine the clot permeability and/or the anti-factor activities within the plasma clots. The peptidyl arginals are generally more or much more inhibitory than their agmatine or p-aminomethylbenzamidine congeners of similar size. It is of particular interest that peptidyl arginals that are clot permeable and have improved anti-thrombin and anti-factor Xa activities can substantially reduce the endotoxin-induced mortality of rats, i.e. from 70% to 1-2%.

References
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*Abbreviations: pNA, p-nitroanilino; Chg, cyclohexylglycine; Hma. Hexahydromandelic acid (cyclohexylglycolic acid).

P C3 - Synthetic antidotes against snake neurotoxins

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The efficiency of polyvalent molecules as specific ligands or inhibitors has been demonstrated in different cases where multivalent binding produces high avidity interactions, able to compensate for the low affinity of each binding site. In general, the increase in affinity or the decrease in IC_{50} can be ascribed to the thermodynamic effect of the multivalent interaction between the polyvalent molecules and oligomeric or membrane-anchored targets. In some cases, the efficiency of polyvalent ligands in-vitro has been confirmed by their *in-vivo* effect [1].
In our studies, we have used a tetravalent peptide to inhibit the binding of the snake neurotoxin α-bungarotoxin to the nicotinic receptor. The tetravalent peptide is composed of four copies of a mimotope of the toxin binding site of nicotinic receptors, which competes with the receptor for toxin binding [2-4]. We have here compared the efficiency of the tetrameric peptide with that of the monomeric peptide, in terms of kinetic rates, affinity constant and IC_{50}. At the same time, we have tested their activity *in-vivo*.
We found that when toxin binding is analyzed in solution, each peptide in the tetrameric molecule has essentially the same kinetic rates, affinity and IC_{50} of the monomeric peptide. The tetrameric peptide is clearly more efficient than the monomeric peptide only when immobilization of the toxin produces a polyvalent interaction, which as expected is accompanied by an avidity effect. Nonetheless, the tetrameric peptide is at least 100 times more effective than the monomeric form in neutralizing toxin lethality in mice. Since a polyvalent interaction between the tetrameric peptide and the soluble snake toxin is not expected to happen *in-vivo*, the extremely higher *in-vivo* efficiency of the polymeric peptide in respect to the correspondent monomeric form opens some general interesting perspectives for the use of polymeric peptides as therapeutic agents. The *in-vivo* efficiency of the tetrameric peptide seems not to be due to thermodynamic effects related to polyvalent interactions and could be induced by a different pharmacological behaviour of the tetrameric peptide in respect to the correspondent monomeric peptide. Polymeric peptides could generally have a different clearance than monomeric peptides, which might render the use of synthetic peptides more efficacious in several different therapeutic applications.

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P C4 - p21(WAF1)-Derived octapeptide inhibitors of CDK-cyclin complex: the effect of structural variants of the C-terminal Phe residue

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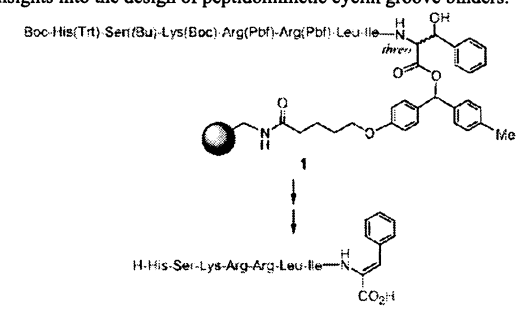
The tumour suppressor protein p21^{WAF1} plays a central role in regulating eukaryotic cell cycle progression *via* its capacity to associate with G1- and S-phase CDK-cyclin complexes. This interaction occurs, in part, with a binding groove located in the cyclin sub-unit. Since the same groove is involved in the recruitment of macromolecular CDK substrates, e.g. pRb and E2F, blockade of this recruitment site would prevent recognition and subsequent phosphorylation of CDK substrates. It was recently established that the octapeptide p21(152-159), His-Ser-Lys-Arg-Arg-Leu-Ile-Phe-NH_2, is a potent competitive binder to the recruitment site in cyclins A and D1, and hence an inhibitor of specific CDK-cyclin complexes. Furthermore, the *C*-terminal Phe residue appeared to be a key determinant in the binding competence of p21(152-159)-NH_2. We herein report the solid-phase synthesis of several structural variants of the parent octapeptide in which the *C*-terminal residue is replaced with a judicious choice of Phe derivatives, specifically homochiral β-phenylserine (Pse), *Z*-dehydrophenylalanine (Dhp) and phenylalaninol. The synthesis entailed the utilization of a robust polymer-supported 5-[4-(4-tolyl(chloro)methyl)phenoxy]pentanoyl linker (GE Atkinson, PM Fischer & WC Chan, *J Org Chem* (2000), **65**, 5048). For example, Fmoc-L-Pse-OH, prepared *via* α-chymotrypsin mediated resolution of *N*-Ac-DL-Pse-OMe, was readily tethered to the linker-resin and the desired peptides were synthesized in high yields and purity. In addition, the Dhp-containing peptide was obtained *via* the dehydration of an assembled resin-bound N^1-Boc,DL-Pse^8-peptide **1**.
The biological activities of the synthetic peptides were established by (i) CDK2-cyclin A kinase assay using pRb as substrate, and (ii) competitive cyclin A binding assay using an immobilized p21(152-159)-NH_2 derivative. The results of these preliminary SAR studies provided insights into the design of peptidomimetic cyclin groove binders.



P C5 - N-Methylated peptides as inhibitors of β-amyloid aggregation: a potential therapeutic strategy for the treatment of Alzheimer's Disease

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Alzheimer's disease (AD) was first reported in 1906 and is currently the most common cause of senile dementia. It has been calculated that in 2005 more than 22 million persons will be affected by AD in the United States.
The mechanism of the disease remains unknown, but it has been demonstrated that β-amyloid family of peptides (βA) is a key feature of the neuropathology of AD. βA is the main component of neuritic plaques, which are involved in neuronal death. Therefore inhibitors of βA aggregation have been proposed as a way of preventing the formation of neuritic plaques (1).
Although the structure of βA fibrils is not well stablished, it is widely accepted that they are composed by βA peptides forming a β-sheet structure. For this reason, our group has proposed using β-sheet disrupting peptides capable of recognising βA to prevent aggregation. We have designed, synthesised and tested some of these small peptides in cell cultures (PC12) using βA(1-42) as neurotoxic peptide. The results obtained showed a statistically significant improvement in cell survival. The kinetics of fibril amyloid formation has been followed analytically using congo red binding, showing the effect of our inhibitory peptides in the aggregating behaviour of βA(1-42).

P C6 - Derivatives of the native antibacterial peptide pyrrhocoricin exhibit desirable pharmacological properties *in vitro* and *in vivo*

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The rapid emergence of bacterial strains that are resistant to current antibiotics is one of the major health problems of today. Proline-rich cationic antibacterial peptides kill bacteria by binding to the 70 kDa heat shock protein DnaK and inhibiting protein folding. Therefore, they may fulfill the required criteria of novel antibacterials, targeted for clinical development. We designed and synthesized a C-terminally dimeric analog of pyrrhocoricin that is protected both at the N-terminus as well as the backbone. Using an optimized in vitro broth dilution assay for peptide antibiotics, native pyrrhocoricin and the designed dimer killed β-lactam or tetracycline resistant clinical isolates of *Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae and Moraxella catarrhalis* in the submicromolar (low μg/mL) concentration range. The designed dimer was active against *Haemophilus influenzae* strains both in the broth dilution and the bactericidal killing assays, and showed improved stability in mammalian sera compared to the native analog. In a murine *H. influenzae* lung infection model using a human clinical pathogen, a single dose of the dimeric pyrrhocoricin analog reduced the bacterial count in the bronchoalveolar lavage from 23100 cfu/mL to 10000 cfu/mL when delivered intranasally. An extensive analysis of the in vivo activities with various peptide delivery routes is currently in progress. This multiply protected peptide is a promising candidate for clinical development, and shows the potential of modified synthetic peptides as drugs, if the lead molecules and the efficacy screening conditions are selected with the special properties of peptides in mind. The most active pyrrhocoricin analogs in vitro feature an increased number of positive charges at the N-terminus. These peptide derivatives are not only more potent than the native peptide against the strains listed above, but very efficiently kill strains unresponsive to the native product such as *Pseudomonas aeruginosa*. However, the dominant mode of action of these analogs may feature membrane disintegration instead of DnaK inhibition.

P C7 - Synthetic peptides mapped on angiostatin K4 domain inhibit endothelial cell migration

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Angiogenesis is a complex process that involves endothelial cell proliferation, migration, basement membrane degradation, and neo-vessel organization. Angiostatin, consisting of four homologous triple-disulfide bridged kringle domains (K1, K2, K3 and K4), has previously been shown to exhibit profound inhibition of endothelial cell proliferation *in vitro* and angiogenesis *in vivo*. It was also demonstrated that angiostatin could suppress the growth of a variety of tumors *via* the blocking of angiogenesis. The characterization of the kringle domains of angiostatin has demonstrated that single kringles of angiostatin play different roles in the inhibition of endothelial cell migration, a crucial process in angiogenesis. In particular kringle 4 (K4), which has only marginal anti-proliferative activity, is among the most potent fragments in inhibiting endothelial cell migration. In contrast the fragment containing kringles 1, 2 and 3 is equivalent to angiostatin in inhibiting endothelial cell proliferation but manifests only a modest anti-migratory effect. The following sequences have been synthesized to investigate the anti-migratory activity of linear and cyclic peptides patterned on K4:

Name	Sequence
K4(358-379)	C Y H G D G Q S Y R G T S S T T T T G K K C
K4 (379-407)	C Q S W S S M T P H R H Q K T P E N Y P N A G L T M N Y C
K4 (407-430)	C R N P D A D K G P W C F T T D P S V R W E Y C

The linear peptides present side-chain protected Cys residues by Acm or SBu' groups, whereas cyclic analogues have been obtained by disulfide bond formation between Cys^{358} and Cys^{379}; Cys^{379} and Cys^{407}, or Cys^{407} and Cys^{430}, respectively. In addition, a 51-mer analogue has been synthesized in a linear form by disulfide bond formation between the C-terminal Cys residue of K4(358-379) fragment and the N-terminal Cys of K4(379-407) peptide, and in a cyclic form by disulfide bone formation between Cys^{358} and Cys^{407} of the linear heterodimer. The anti-migratory activities of synthetic peptides have been examined on endothelial cells in a Boyden chamber-based assay. VEGF has been used as a chemoattractant to induce the migration of endothelial cells. Preliminary data suggest that the linear sequence named K4(358-379) exhibits a potent inhibitory activity of endothelial cell migration with an IC_{50} of 390 nM, comparable to the inhibitory activity of the entire K4 domain (IC_{50} of 500 nM). The cyclic analogue of K4(358-379) does not increase the anti-migratory effect of the peptide, while all the other sequences present an IC_{50} around 1 μM. Conformational investigations are in progress to correlate structural organization and biological activity.

P C8 - Furin-mediated cleavage of natural and modified gp160 peptides

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Pro-protein bioactivation by limited proteolysis is a general mechanism that is commonly used in hormone, receptor and viral protein maturation. However, the details on how the process of substrate recognition is governed by the specific enzymes have not been fully clarified as yet. As a matter of fact, the presence of a signal-sequence is necessary, but not sufficient, in order to guaranty the enzymatic reaction; moreover, it seems that conformational features might be required for recognition. Individuation of these requirements could be obtained by enzymatic digestion of suitably designed peptide analogues of the substrate. Potential applications of these investigations are the design of enzymatic inhibitors, that could modulate the physiological functions mediated by the bioactive molecules released by proteolysis. In particular, the enzymatic cleavage of an inactive precursor (gp160) to give two proteins (gp120 and gp41), that are fundamental for the infection, is a key-event in the biosynthesis of the HIV virus. The enzyme realizing this essential step, is an endogenous enzyme called furin that recognizes and processes the REKRA site. We have demonstrated that a 19mer synthetic peptide (p498), spanning the gp160 sequence Pro^{498}-Gly^{516} is properly digested by furin. Peptides, carrying in their C-terminal sequence the same cleavage site of gp160, but specifically designed to assume a specific conformation in the N-terminal region, have been synthesized and are now used to investigate the role of secondary structure in determining the cleavage efficiency by furin. In addition, the following analogues have been synthesized in order to explore the importance of positions P_3 and P_2':

The underlined letters represent D-amino acids; Bold characters underlined the basic-X-basic-basic sequences; Ac = acetyl

Name	Sequence
p498	P T K A K R R V V Q R E K ↓R A V G I G
h-REKR	Ac E H V N A I Q E A R R L L N R E K ↓R A V G I G
r-REKR	D P K G V T V T V T Y I V T R E K ↓R A V G I G
r-RPKR	D P K G V T V T V T Y I V T R P K ↓R A P G I G
Pro^{16} r-REKR	D P K G V T V T V T Y I V T R P K ↓R A V G I G
Pro^{20} r-REKR	D P K G V T V T V T Y I V T R E K ↓R A P G I G

Digestion experiments have shown h-REKR is a good substrate, comparable to the native one, while the r-REKR sequence is not processed by this enzyme. Introduction of two Pro residues at positions 16 and 20 improves the characteristics of the substrate and this seems to be due to a balance between the effect brought about by Pro^{16} substitution and Pro^{20} introduction. In fact, modifications limited to position P_2' give poor substrates, whereas modification at position P_3 creates the best substrates among all the proposed sequences, including peptide p498 which represents the natural sequence of gp160. Consequently, we have demonstrated the critical importance of the P_3 position, that was not indicated by Nakayama K. Biochem. J., 327, 625-635 (1997) among the residues involved in substrate recognition. The effect could be due to a specific interaction with the P_3 residue or to the capacity of the substituted amino acid to influence the local secondary structure. Conformational studies using different techniques, i.e. CD, FT-IR mono- and bidimensional NMR, and molecular modeling are in progress.

P C9 - Human glucagon-like peptide-1 amide (hGLP-1(7-36)NH_2) is cleaved by plasma enzymes not only at the N-terminus but also at the C-terminus; development of novel GLP-1 analogs which are highly resistant to enzymatic degradation

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Glucagon-like peptide-1 (GLP-1) is a strictly glucose- dependent insulinotropic hormone, which has received increasing attention as a possible new treatment for type 2 diabetes. However, the potential use of the native GLP-1 as a therapeutic agent is greatly hampered by its short plasma half-life. Previously, it was believed that dipeptidyl peptidase IV (DPP-IV) was primarily responsible for the fast degradation of the native GLP-1(7-36)NH_2 in plasma by cleaving off the His^7-Ala^8 dipeptide from the N-terminus of the hormone [1]. Here we report for the first time that besides the N-terminal degradation caused by DPP-IV, GLP-1(7-36)NH_2 is also extensively digested by plasma enzymes at the C-terminus. In human and rat plasma, the C-terminal cleavages occur at the peptide bond between Gly^{35} and Arg^{36}, and the peptide bond between Lys^{34} and Gly^{35}. Replacement of Ala^8 at the N-terminus of GLP-1 with the $C^{\alpha,\alpha}$-disubstituted glycines, such as α-aminoisobutyric acid (Aib) and 1-aminocyclopentane-1-carboxylic acid (Ac_5c), yields analogs which are resistant to the N-terminal degradation induced by DPP-IV. However, these N-terminal protected analogs (e.g., [Aib^8]hGLP-1(7-36)NH_2 and [Ac_5c^8]hGLP-1(7-36)NH_2) are still subject to enzymatic degradation at the C-terminus. Further substitution of Aib or β-alanine (β-Ala) for the C-terminal Gly^{35} residue results in analogs which are not only stable to DPP-IV but also completely resistant to the C-terminal enzymatic degradation. These analogs with the modifications at both N- and C-termini are highly stable in human, rat or mouse plasma.
For example, [$Aib^{8,35}$]hGLP-1(7-36)NH_2 has a half-life of 9.8h in rat plasma, which is approximately 12-fold longer than that of the native hGLP-1(7-36)NH_2. In the *in vitro* receptor binding assay, [$Aib^{8,35}$]hGLP-1(7-36)NH_2 has high binding affinity to the human GLP-1 receptor with a Ki of ~0.95nM. In the animal models of type 2 diabetes, this analog is significantly more efficacious than the native GLP-1 in stimulating insulin release and lowering blood glucose.
The improved *in vivo* activity of [$Aib^{8,35}$]hGLP-1(7-36)NH_2 is likely due to its enhanced enzymatic stability and increased circulating half-life.

References
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P C10 - Analogues of a potent oxytocin antagonist with truncated C-terminus or shorter side chain of the basic amino acid in position 8

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A potent oxytocin antagonist, (Cyclo S^1-S^6) (S)Pmp-D-Trp-Ile-Gln-Asn-Pen-Pro-Arg-Gly-NH_2 (ANTAG), in which (S)Pmp is β,β-(3-thiapentamethylene)-β-mercaptopropionic acid, is under study as a potential inhibitor of preterm labor. Analogues of ANTAG truncated at the C-terminus were needed to facilitate identification and HPLC analysis of its potential metabolites and to prepare radioactive labeled ligands for pharmacokinetic and pharmacodynamic studies. To this effect the sequence ANTAG $^{1-9}$ acid and the truncated sequences ANTAG $^{1-8}$ acid, ANTAG$^{1-7}$ acid, and ANTAG$^{1-6}$ acid, and their respective amides, were prepared by solid phase peptide synthesis and were characterized. The biological potency of these analogues was studied in the rat uterotonic *in vitro* assay. In general, our results indicate that substantial shortening of the linear peptide tail is accompanied only with a moderate loss of antagonistic potency for both peptide amides and peptide acids. We also synthesized analogues of ANTAG having Lys or basic amino acid with shorter or modified side chains in position 8, namely: [Lys^8]ANTAG, [Orn^8]ANTAG, [Dab^8]ANTAG, [Dap^8]ANTAG, and [Cit^8]ANTAG in which Dab = L-1,4-diaminobutyric acid, and Dap = L-1,3-diaminopropionic acid. All of these analogues were potent oxytocin antagonists but slightly weaker than the parent ANTAG. The most potent antagonists and the relatively neutral [Cit^8]ANTAG were also tested for their activities in the pressor or antidiuretic tests.
Potency of these analogues in these bioassays will be discussed.

P C11 - Bicyclic analogues of a potent oxytocin antagonist

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Conformational studies have suggested that oxytocin antagonists (OTAs) are conformationally more rigid than oxytocin or its agonists. The potent OTA, (Cyclo S^1-S^6) (S)Pmp-D-Trp-Ile-Gln-Asn-Pen-Pro-Arg-Gly-NH_2 (ANTAG), in which (S)Pmp is β,β-(3-thiapenta-methylene)-β-mercaptopropionic acid, with a pA_2 = 8.86 in the rat uterotonic assay *in vitro*, has a good potential as an inhibitor of preterm labor. We prepared analogues of ANTAG with less conformational flexibility by creating a second ring between positions 1 and 9, or 4 and 9. Employing solid phase peptide synthesis, we synthesized (cyclo 1-9)[(HN)Pmp^1, Gly^9]
ANTAG, in which (HN)Pmp is β,β-(3-iminopenta-methylene)-β-mercapto-propionic acid, and (cyclo 4-9)[X^4, Gly^9]ANTAG, in which X = Lys, Orn, Dab or Dap (Dab = L-1,4-diamino-butyric acid and Dap = L-1,3-diaminopropionic acid). The analogues were made by usual methods for making OTAs. The second ring was formed by means of the BOP reagent. The molecular weight of these bicyclic peptides was verified by MS as usual. We also made (Cyclo S^1-S^6) (S)Pmp-D-Trp-Ile-Gln-Asn-Pen-Pro-Arg-Gly-NH-CH_2-CH_2-NH_2, ANTAG-ethylenediamine monoamide, and its dimer $(ANTAG)_2$-ethylenediamine diamide. Biological properties of all of these analogues were studied in the rat uterotonic assay in vitro and will be discussed. Two of the analogues (cyclo4-9)[Lys^4, Gly^9]ANTAG and (cyclo 4-9)[Orn^4, Gly^9]ANTAG had the same potency as the parent ANTAG. The most potent antagonists were also tested for their antipressor or antidiuretic activities, and the specificity of the effects will be presented.

P C12 - A journey from bradykinin peptide antagonists to peptide and peptidomimetic antineoplastics

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The kinins, including bradykinin (BK), exercise an important regulatory control in inflammation and in the growth and proliferation of cancers [1]. Bradykinin is known to mobilize calcium from intracellular or extracellular sources, in several tumor cells. Our highly potent, metabolism-resistant B1 and B2 antagonist peptide, B9430 (DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg) (Hyp = *trans*-4-hydroxyproline; Igl=-(2-indanyl)glycine; Oic=octahydroindole-2-carboxylic acid) blocks Ca^{2+} mobilization by BK but does not inhibit the growth of small cell lung cancer (SCLC). Crosslinking B9430 at the N-terminal with a suberimidyl (SUIM) linker (B9870) or N-acylation with 2,3,4,5,6-pentafluorocinnamic acid (B10238) gives potent selectively cytotoxic agents for SCLC cells *in vitro* and *in vivo*. Based on BK antagonist structures, we have also developed a highly potent anti-cancer peptide mimetic, BKM-570 [2,3,4,5,6-pentafluorocinnamoyl-(O-2,6-dichlorobenzyl)-L-tyrosine-N-(2,2,6,6-tetramethyl-4-piperidyl)amide]. BKM-570 is far more potent for growth inhibition of SCLC *in vivo* in nude mice than is the widely used highly toxic chemotherapeutic drug cisplatin [2]. We synthesized a number of analogs of the above-mentioned peptides and peptidomimetics for structure-activity studies to identify the structural features required for good anti-cancer activity. These peptides and mimetics have been tested *in vitro* and *in vivo* against lung cancer and prostate cancer lines and were found to posses high anti-cancer activity in these tests. These results suggest that both lung cancer and prostate cancer are reasonable targets for drugs based on our peptide analogs and mimetics. Syntheses and structure-activity relationships will be discussed.

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P C13 - Design of psychotropic dipeptides starting from the chemical structures of nonpeptide drugs

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Exogenous psychotropic drugs often act via neuropeptide receptors. On the ground of this concept we have developed a novel approach, Drug-based Peptide Design (DPD) [1], for creating potential peptide drugs. The basic steps of this approach are as follows:1) the selection of a non-peptide drug structure for peptide design based on its biological properties and molecular profile; 2) identification of amino acid side chains and peptide bond fragments in the non-peptide drug; 3) construction of the simplest peptide analogs of non-peptide drugs; 4) study of the biological activity of these peptides; 5) most active peptide stereoselectivity examination; 6) identification of peptide analog primary structure in the primary structure of known neuropeptide; 7) peptide analogs modification using data on parent neuropeptide secondary structure; 8) selection of potential drug candidate. Using DPD we have developed high-active nootropics, pyroglutamyl- and prolyl- containing dipeptides pGlu-Gly-NH_2, pGlu-Asn-NH_2, cyclo-Pro-Gly, N-phenylacetyl-Pro-Gly-OEt (GVS-111) as topological analogs of the nootropic drug piracetam (N-carbamidomethyl-pyrrolidone-2). These dipeptides demonstrated stereoselective nootropic activity at dose range of 0.01-10 mg/kg i.p. One of designed dipeptides, cyclo-Pro-Gly, was identified in rat brain [2]. GVS-111 (US Patent 5439930) has been progressed as a clinical candidate for the treatment of moderate cognitive disorders. DPD also allowed us to design a lead dipeptide analog of benzamide neuroleptic sulpiride, Pro-Tyr-NH_2, which is identical to neurotensine fragment NT(9-10). Based on NT structure we have designed N-caproyl-Pro-Tyr-OMe (GZR-123), that is 10 times more active than sulpiride. N-Acyl moiety of the latter plays the role of an important Leu^{13} residue of NT. GZR-123 has clinical potential as a perspective neuroleptic agent devoid of extrapyramide side-effects.

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P C14 - Synthesis and evaluation of prolylisoxazoles as inhibitors of prolyl oligopeptidase.

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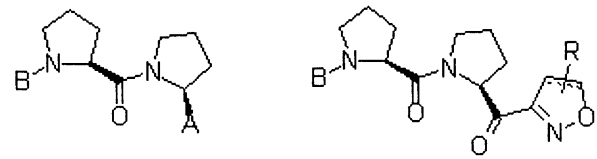
Prolyl oligopeptidase (POP, EC3.4.21.26) is a serine protease. It preferentially cleaves oligopeptides at the carboxyl side of a prolyl residue and is involved in the metabolism of several neuroactive peptides and peptide homones such as thyrotropin-releasing hormone, gonadotropin-releasing hormone, vasopressin, oxytocin and substance P. The enzyme (Tc80 POP) has also been found in *Trypanosoma cruzi*. It plays a role in host cell invasion by the parasite.
It has been demonstrated that POP is involved in cognition disorders and possibly also in anxiety, depression and sexual behaviour. Several inhibitors have been described and claimed as potential drugs in amnesia and cognitive disturbances. These inhibitors often are substrate-like prolylproline peptides (I) where the cleaved amide bond is substituted for electrophilic groups such as aldehyde or ketones or is omitted (A). The terminal amino group is acylated as an amide or carbamate group (B). Several other inhibitors are not substrate related.



A series of prolylprolylisoxazoles and -isoxazolines (II) have been synthesized. These compounds where prepared with Boc-prolylaldehyde as starting material and the heterocyclic ring was constructed by a nitrile oxide cycloaddition to the appropriate alkyne or alkene.
These compounds were tested against both human ant trypanosomal POP. The most active compounds inhibit as well human POP as trypanosomal POP with K_i values in the nM range. They inhibit the invasion of mammalian cells by trypomastigotes of *T.cruzi* in a dose dependent way.

P C15 - Synthesis and characterization of new quinazolyl amino acid derivates as potential bioavailable antitumor agents

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Recently a great number of tyrosine kinase inhibitory quinazoline derivatives have been developed. These compounds are potential antitumor agents, at least 16 derivatives are already in clinical and preclinical development stage presently. Most of the compounds are analogues of the very first PD 153035 which showed EGF RTK inhibitory activity in the subnanomolar range. The common part of the analog compounds is the 4-phenyl-amino-6,7- disubstituted quinazoline nucleus. Since the original lead had poor bioavailability (low water solubility), the structural changes were aimed to develop more soluble compounds. This modified molecules may have better ADME parameters. Modification of the substituents in 6,7 position gave the best results. The water solubility was enhanced by morpholino-propyl, N-methyl-piperidinyl-methyl, triazolyl-ethyl and other tertiary and secondary amine substituents in most cases. We have found several new kinase inhibitory quinazolines substituted in position 2 also and having alkylamino and aralkylamino groups in position 4 instead of the traditional phenylamino substituent. There were publications about selective inhibitory activity depending on the configuration of the chiral substituent on the amino group in position 4. We set as an aim to develop new, patentable quinazoline derivatives with better bioavailability than the original leads, and/or enhancing the selectivity of the compounds on different kinases. We have not found any publications in the patent and scientific literature about amino acid and peptide derivatives which practically can fit the above requirements. We used SPOC and FMOC synthetic strategy for the synthesis of quinazol-4-yl amino acids. The key intermediates, 3H-quinazolin-4-ones were prepared in microwave assisted reactions in high yields. The 4-chloro quinazoline series was prepared with the method published in the literature ($POCl_3$, 4-dimethylamino-pyridine catalyst). 4-Chloro quinazolines are imidoyl chloride like acylating agents, therefore the resin bound and deprotected amino acids could be substituted with the quinazoline nucleus on the N-terminal amino groups. Both D and L amino acids were used parallel in order to map the effect of chirality on the biological action. The compounds were tested in MTT assay. Surprisingly, almost all of the amino acid derivatives of 6,7 disubstituted quinazolines showed antitumor activity. We will discuss the structure-activity relationship, the effects of quinazoline substituents and the amino acid part of the compounds.

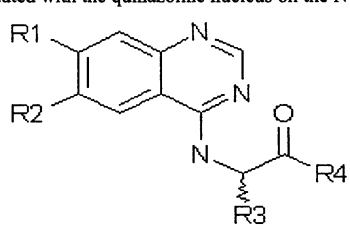

Fig. 1 - Quinazolyl amino acid derivatives

P C16 - Role of cholinergic system in the cardiovascular effects of intracerebroventricularly-injected angiotensin II; participation of vasopressin

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Renin-angiotensin system is essential for the regulation of body fluid homeostasis and blood pressure. Since the existence of this system in the brain has been shown recently, studies have been focused on the central effects and function of the system. Here, participation of central cholinergic system in the effects of intracerebroventricular (i.c.v.) injection of angiotensin II (Ang II) on blood pressure and heart rate was studied in conscious, freely moving rats. Three series of experiments were performed. Series 1 was performed to investigate the cardiovascular effects of i.c.v. Ang II. For this purpose, Ang II (50, 100 ng/10 µL; i.c.v.) or saline (10 µL; i.c.v.) was injected. Series 2 was carried out to observe the role of cholinergic system in the cardiovascular effects of i.c.v. Ang II. Rats were injected with a nicotinic receptor antagonist mecamylamine (25 mg/10 µL; i.c.v.) or a muscarinic receptor antagonist atropine (5mg/10 µL; i.c.v.) or saline (10 µL; i.c.v.), followed 15 minutes later by Ang II (100 ng/10µL; i.c.v.) or saline (10 µL; i.c.v.). Series 3 was performed in order to investigate the effect of blockade of vasopressin V_1 receptors on blood pressure and heart rate. Rats were injected with a specific antivasopressor antagonist of vasopressin (B-mercapto B, B-cyclopentamethylenepropionyl, O-Me-Tyr, Arg)-vasopressin (10 µg/kg) or saline through the arterial cannula and 5 minutes later received either Ang II (100 ng/10 µL; i.c.v.) or saline (10 µL; i.c.v.). The changes in blood pressure and heart rate were observed in all series. Ang II dose-dependently increased blood pressure and decreased heart rate. Both atropine and mecamylamine (i.c.v.) pre-treatments prevented the cardiovascular effects of Ang II. Pre-treatment with a vasopressin V_1 antagonist also prevented the cardiovascular responses to Ang II. Our data suggest that the central pressor effect of Ang II is mediated in part by central acetylcholine via both muscarinic and nicotinic receptors, and vasopressin participates in this effect through V_1 receptors.

P C17 - Development of lead compounds focusing on prevention of infective endocarditis using combinatorial peptide libraries

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Infective endocarditis is often caused by oral indigenous bacteria introduced into the blood stream. Such bacteremia occurs after tooth extraction, and occasionally even after brushing of the teeth. Abnormal or damaged heart valves including artificial valves are the most susceptible to the infection, while normal valves can be infected only by some aggressive bacteria when present in large numbers. With the rapid improvement in techniques in cardiac surgery that have resulted in improved cure-ratios, the number of people at high risk from endocarditis is also rapidly increasing. To prevent this infection, antibiotics are currently used, although the antibacterial spectra are inevitably limited. In addition, repeated administration of antibiotics is generating resistent mutant bacteria that is becoming a serious social problem. Thus, the development of more effective and safer drugs for prevention of this disease is indispensable. The ability of bacteria to adhere to extracellular matrix proteins, which are exposed at the wound endocardia, is considered critical for infection. Inoue et al. have indicated that experimental endocarditis was induced by an oral *viridans streptococcus, Granulicatella (Abiotrophia) adiacens,* and the pathogenicity is related to its adhering activities to fibronectin [1]. A partially purified fibronectin adhesin from this organism inhibited not only binding of this species but also other species responsible for endocarditis [unpublished results]. The purpose of our present study is the development of candidate compounds, which can interfere with the adherence of microorganisms. For this "one peptide on one bead" and "position scanning" methods have been employed to construct peptide libraries. Peptides were prepared by the Fmoc-SPPS and several monoclonal antibodies prepared by us were used for screening.

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P C18 - Inhibition of islet amyloid polypeptide (IAPP) amyloid formation and cytotoxicity via structure-based, selective N-methylation of amide bonds of amyloid core sequences

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Protein self-assembly into cytotoxic amyloid aggregates is strongly associated with a number of cell-degenerative diseases. Pancreatic amyloid is found in more than 95% of type II diabetes patients and its formation correlates with the pathologic sequelae of the disease. Pancreatic amyloid consists of aggregated islet amyloid polypeptide (IAPP), a 37-residue peptide hormone that is synthesized in the β-cells of pancreas and in its soluble form acts as an insulin counterregulator. We have shown that IAPP amyloid formation proceeds via a nucleated conformational transition of soluble, mainly random coil polypeptide into aggregated β-sheets [1]. Most recently, we have identified the penta- and hexapeptides FGAIL and NFGAIL, or IAPP(23-27) and IAPP(22-27), respectively, as the shortest IAPP sequences that are still able to aggregate into β-sheets and cytotoxic amyloid [2].
Here, we applied the structural model of NFGAIL amyloid fibril as a template and a minimalistic design strategy to design analogues of IAPP amyloid core sequences that were rationally N-methylated on the one "side" of the β-strand. In contrast to the native sequence peptides, the N-methylated analogues were not able to aggregate into β-sheets and were neither amyloidogenic nor cytotoxic. This was demonstrated by various biophysical methods including EM, CD, FT-IR, congo red staining and polarization microscopy and cell viability assays. The N-methylated analogues were found by CD, FT-IR and EM to interact with the native amyloidogenic sequences and completely inhibit amyloid formation. Most importantly, N-methylated IAPP(20-29), or SNNF(N-Me)GA(N-Me)ILSS, also inhibited cytotoxicity of IAPP(20-29). The N-methylated peptides are thus the first reported inhibitors of IAPP amyloid formation and cytotoxicity. Together, these data and recent results on β-amyloid and prion protein peptides suggest that the principle of a minimal and selective N-methylation of backbone amide bonds may find application to the design of inhibitors of amyloid formation and cytotoxicity by other amyloidogenic polypeptides too.

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P C19 - Biological activity of the immunomodulatory peptide SCV-07 against murine tuberculosis

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SCV-07 (gamma-D-glutamyl-L-tryptophan) has been shown to have a broad spectrum of immunostimulatory activities both *in vitro* and *in vivo* [1]. In the current study the biological activity of SCV-07 in a model of induced experimental tuberculosis was investigated. Infection of white wild type mice with disseminate tuberculosis, was performed by injection of I. bovis 8 suspension into the tail lateral vein. SCV-07 treatment began on Day 20, consisting of 5 daily intraperitoneal injections of doses of 0.01, 0.1, 1.0. and 10 μg/kg. The results suggest that SCV-07 treatment during isoniazid tuberculosis therapy influences the severity of the disease and the strength of the immune responses. In this study, SCV-07 significantly decreased both the lung weight index and the lung damage index. M. bovis growth in spleen culture was also decreased. By 24 days after treatment, production of IL-2 was restored to the uninfected animal level. Production of both basal and stimulated INF-γ production by both thymic and spleen cells, as well as circulating levels in serum, was increased by SCV-07 treatment. At the same time points, IL-4 production was decreased in the same cell types and serum. These changes, increase of INF-γ and decrease of IL-4 production, suggest that SCV-07 is elaborating a shift of T-helper cells to a Th1-like immune response. Other immune parameters were improved as well, with increases seen in Con A stimulated thymic cell proliferation as early as 4 days after SCV-07 treatment. By 24 days after treatment, proliferative responses for both thymic and spleen cells were restored to nearly the uninfected animal responses. SCV-07 also stimulated macrophage function, with an improvement of peritoneal macrophage ingesting and killing ability.
SCV-07 therapy decreased the severity of experimental disseminated tuberculosis in mice and increased the effect of isoniazid therapy. SCV-07 at 0.1 μg/kg was the optimal dose. Per oral SCV-07 therapy demonstrated some effects, but will require additional studies.

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P C20 - The power of attraction: Dmt as the universal opioid determinant

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Dmt (2',6'-dimethyl-L-tyrosine) enhances binding and bioactivity of opioidmimetic antagonists and agonists to their receptors. H- Dmt-Tic-OH (1,2,3.4-tetrahydroisoquinoline-3-carboxylic acid) is the smallest recognized δ-opioid antagonist, orders of magnitude more potent than Tyr cognates. C- Terminal modifications had high μ-affinities (K_i =0.2-0.5nM) with marginal effects on δ-binding (K_i =<0.1nM). Hydrophobic *(tert*-butyl, 1-adamantane) or aromatic groups (benzyl, phenyl, 1H-benzimidazole-2-yl) shifted the activity from a potent δ antagonist to a potent δ agonist or to compounds with mixed δ-antagonist/μ agonist or δ-agonist/μ-agonist properties. Linkers between Tic and the third aromatic nucleus facilitated this change: H-Dmt-Tic-NH-CH_2-Bid is a potent δ agonist (pEC_{50} = 9.90) while H-Dmt- Tic-Gly-NH-CH_2-Bid remained a δ antagonist (pA_2 = 9.0); H-Dmt-Tic-Gly-NH-Ph exhibited δ- and μ-agonism ($EC_{50}\delta$= 8.52, $EC_{50}\mu$ = 8.59) and H-Dmt-Tic-Gly-NH-Bzl had mixed δ-antagonist ($pA_2\delta$ = 9.25) and μ-agonist ($EC_{50}\mu$ = 8.57) activities. Modifications to Bid decreased the enhanced μ-affinities (K_i=5-10nM) without affecting δ-affinities (K_i=0.04nM). While Tic appeared to be required for high δ-receptor binding and antagonism, its replacement by piperidine and C-terminal Bid or Bzl groups slightly affected μ affinity ($K_i\mu$ = 1-2 nM) but δ affinities fell sharply (K_i=80-120 nM). Pyrrolidine and diethyl derivatives were unsatisfactory. Symmetric opioidmimetics containing N-terminal Dmt residues separated by an alkyl group [H-Dmt-$(CH_2)_n$-Dmt-H, where n = 2-8] or a pyrazinone ring and linkers (lysine, ornithine, diaminobutyric acid) had only high μ affinities (K_i = 0.04-0.05 nM) as a function of the length of the linker. High agonist activities were obtained in vitro (EC_{50}=3-5nM) with potent icv and sc bioactivity comparable to morphine. These compounds might have potential clinical and therapeutic applications for the reduction of post-operative pain or in alleviating cancer-induced pain. Our data suggest that Dmt is a key residue in the appearance and maintenance of high activity for δ- and μ-opioid receptors, and receptor selectivity depends on the C-terminal residues. The distance between aromatic centers is crucial for the aligning the these nuclei in the receptor. Furthermore, the methyl groups of Dmt suggests a possible stabilization of the residue enabling H bond formation between the OH group and a side chain(s) in the receptor.

P C21 - Potentiating effect of distant sites in cyclic peptide antagonists of the Grb2-SH2 domain

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The epidermal growth factor receptor (EGFR) mediated cellular signaling pathway contributes to cellular processes important to cancer development and progression, including cell proliferation, apoptosis and metastasis. On cellular activation, Tyr phosphorylated segments on EGFR provide a docking site for the Grb2 protein that mediates downstream signaling. We have developed nonphosphorylated cyclic peptides with a unique amino acid sequence that inhibits the EGFR/Grb2-SH2 domain association with selectivity (Y.-Q. Long et al, BBRC., 264, 902-908, 1999). In structure/activity studies, we report here the importance of hydrophobic residue at the Tyr+5 site, in the prototype thioether cyclized peptide. Both acidic and basic amino acid substituents are disfavored at this position, and replacement of Met with beta-tert-butyl-Ala was found to improve the antagonist properties.
We have also modulated the polarity characteristics of the thioether linkage in G1TE by incorporating the polar sulfoxide linker, and also the positively charged N-methyl sulfonium ion linker, as well as replacing the sulphur atom with a -CH_2- moiety. Biacore based binding affinity studies consistently indicate on several analogs that the faster eluting (HPLC) diastereomeric sulfoxide linked peptide isomers provide superior Grb2-SH2 domain antagonists, with the best agents exhibiting binding affinities in the range of 300 nM (IC_{50}). Thus, modulation of functional group polarities provides a convenient method for pharmacophore optimization, and possibly providing agents with improved transport properties.

P C22 - Identification of peptidomimetic HTLV-1 protease inhibitors containing allophenylnorstatine as a transition-state isostere

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Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that is associated with adult T-cell leukemia (ATL). HTLV-1 codes for a virus-specific aspartic protease responsible for processing the *gag* and *gag-pol* polyproteins and for the proliferation of the retrovirus. Since this process is essential for a retrovirus replication, it is believed that this protease is one of the major targets for the chemotherapy of ATL. This idea is also strongly supported by the current results that HIV protease inhibitors had a great contribution for the successful treatments of AIDS. For the last decade, we had great success in developing HIV-1 protease inhibitors, named "KNI compounds", based on the concept of "transition-state mimic". We have discovered that an unnatural amino acid, allophenylnorstatine (Apns), which has a hydroxymethylcarbonyl (HMC) isostere, effectively provided a unique interaction with the active site of HIV-1 protease essentially similar to that of the substrates [1]. To obtain lead compounds in the development of HTLV-1 inhibitors, it would be significant to know whether our HIV-1 protease inhibitors with Apns are effective in HTLV-1 protease inhibition. In the present study, we focused on the establishment of evaluation systems using both chemically synthesized and recombinant HTLV-1 proteases and evaluation of our developed HIV-1 protease inhibitors. An HTLV-1 protease analogue containing a thioether bond between Gly^{60} and Gly^{61} was synthesized by a chemical ligation method, and a recombinant protease with His-tag at the N-terminal of the intact sequence was expressed in *Escherichia coli*. Both purified proteases specifically hydrolyzed a synthetic HTLV-1 substrate, Ala-Pro-Gln-Val-Leu/Phe(p-NO_2)-Val-Leu-His-Pro-Leu [2]. Hence, we evaluated the inhibitory activity of HIV-1 protease inhibitors against HTLV-1 protease using these proteases and found that KNI-727, which is a dipeptide-derived peptidomimetic with strong HIV-1 inhibitory activity, exhibited relatively potent activity. This result would be useful for the further modification in the development of anti-ATL drugs.

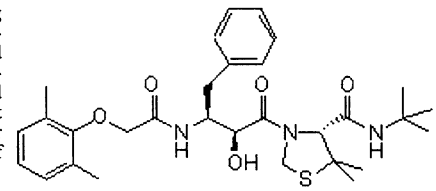

Figure 1. Structure of HIV-1 Protease Inhibitor, KNI-727

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P C23 - Investigations on the effect of integrin-specific peptides on angiogenesis

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Since the discovery of the cell adhesion sequence Arg-Gly-Asp (RGD) in fibronectin [1] it has been shown that the RGD sequence serves as a binding site of many adhesion proteins to integrin receptors [2]. The RGD sequence incorporated into small peptides can mimic these adhesion proteins and can regulate cell-cell and cell-matrix interaction. It has been demonstrated very early that the conformation of such peptides and the way the RGD portion is presented to the receptor have a tremendous influence on potency and integrin selectivity [3]. These studies have established that changes in stereochemistry within the RGD portion and conformational restriction by cyclization can be used to alter binding affinity and selectivity. The $\alpha_5\beta_1$ receptor plays is important for cell migration, tumor invasion and metastasis. The integrin $\alpha_{IIb}\beta_3$ plays a major role in platelet aggregation and is the most thoroughly studied integrin receptor. The receptor $\alpha_v\beta_3$ is a related integrin containing the same β_3 subunit and has attracted considerable interest due to its possible involvement in osteoporosis, angiogenic ocular disorders and cancer. The $\alpha_v\beta_5$ receptor is involved in angiogenic effects as well and the effects of peptides that are selective for this receptor will enable us to elucidate the involvement of these two receptors, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, for angiogenic events. This has considerable interest in pharmaceutical research as well as in tissue engineering. In this paper we summarize our studies on the synthesis, binding potency and biological activity of RGD containing peptides. We present a series of potent peptides that bind specifically to integrin receptors as well as potent non-selective peptides. The $\alpha_v\beta_3$-specific peptides c[(Mpa)-Arg-Gly-Asp-Asp-(t-BuG)-Cys]-NH_2 and c[Arg-Gly-Asp-Asp-(t-BuG)-(Mamb)], the $\alpha_v\beta_5$–specific peptide Gly-c[(Pen)-Phe-Arg-Gly-Asp-Ser-Phe-Cys]-Ala, the $\alpha_5\beta_1$-specific peptide Gly-c[(Pen)-Arg-Ala-Arg-Gly-Asp-Asn-Pro-Cys]-Ala and the potent but non-specific peptide Ac-c[(Pen)-Tyr(Me)-Ala-Arg-Gly-Asp-Asn-(Tic)-Cys]-NH_2 were investigated [4-6]. We report the biological activity of those peptides in *in vivo* and *in vitro* assays and study the effect of these peptides on angiogenesis. These peptides present ideal tools to study the influence of receptor-specificity on biological affects such as angiogenesis, tumor growth and metastasis.

Compound	$\alpha_v\beta_3$	$\alpha_v\beta_5$	$\alpha_5\beta_1$	$\alpha_{IIb}\beta_3$
c[Arg-Gly-Asp-Asp-(t-BuG)-(Mamb)]	3	20	42	240
c[(Mpa)-Arg-Gly-Asp-Asp-(t-BuG)-Cys]-NH_2	20	210	390	70
Gly-c[(Pen)-Phe-Arg-Gly-Asp-Ser-Phe-Cys]-Ala	270	46	180	3400
Gly-c[(Pen)-Arg-Ala-Arg-Gly-Asp-Asn-Pro-Cys]-Ala	0.52	330	0	30
Ac-c[(Pen)-Tyr(Me)-Ala-Arg-Gly-Asp-Asn-(Tic)-Cys]-NH_2	2	6	2	190
c[Arg-Gly-Asp-D-Phe-Val]	26	11	5	960

Tab. 1: Binding affinities of RGD-containing peptides to integrin receptors IC_{50} (nM)

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P C24 - The synthesis and biological effects of alkylating RGD-peptides

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The RGD peptide sequence, which can be found in most extracellular matrix component is the general adhesion site for the integrin receptor family. $\alpha_v\beta_3$ integrin receptors expressed on the endothelial cells of newly formed blood vessels play a key role in angiogenesis. For the inhibition of harmful angiogenic processess selective integrin receptor antagonists have been developed (Kessler et al).
We addressed the question how the introduction of an alkylating moiety into an $\alpha_v\beta_3$ integrin receptor antagonist would influence its adhesion and its antiproliferative properties? As cytotoxic agent we chose melphalan and chlorambucil, both having a carboxyl group capable of covalent coupling to the peptide amino terminus in the case of linear peptides or to side chain amino group of cyclic peptides. As peptide carriers we chose linear and cyclic RGD avb3 integrin receptor selective peptide sequences and for comparison a non selective sequence, too. The linear peptides were synthesized either in solution or on solid phase, cyclisation was performed in solution, melphalan and chlorambucil were coupled as their pentafluorophenyl esters. Only cyclic RGD-peptides inhibited human endothelial vascular cell (HUVEC) adhesion to vitronectin. In the HUVEC cell proliferation assay none of the linear peptides nor their alkylating conjugates show antiproliferative effect comparing with melphalan and chlorambucil control. There was no increase or decrease in proliferation inhibition in the case of the cyclic conjugates, when compared to the unconjugated cyclopeptides, indicating that on one side the chemical modification of the cyclopeptides does not influence the avb3 integrin receptor recognizing ability, however on the other side it does not induce additional proliferation inhibition in *in vitro* conditions.

P C25 - The search for correlation between some physicochemical parameters and activity of series of peptidic antagonists of excitatory amino acid receptors

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Previously we have obtained several amino acidic anticonvulsants, probably antagonists of excitatory amino acid receptors (EAA)[1]. One of them, picolinic acid benzylamide (Pic-BZA), showed especially profitable properties [2]. Looking for the others we have designed structures of series of its analogues taking under consideration their some QSAR parameters as well as these of Pic-BZA as a strongly active reference. We have applied HyperChem 4.5 Hypercube Inc. Program. Semiempirical CNDO method was used for a single point calculation and Polak-Ribier algorithm for geometry optimization. Log P values were calculated as a measure of biological barrier penetration. Polarizability, van der Waals and solvent-accessible surface areas and van der Waals and solvent-accessible surface-bounded molecular volume were calculated as the parameters determining the receptor affinity. Partial charge of the N atom and local minimum energy of the molecules were calculated as the parameters determining antagonistic activity of the synthesized compounds against the EAA receptors. Four compounds were synthesized and pharmacologically examined in mice and rats by maximal electroshock seizure test (MES test), subcutaneous Metrazol test (sc MET test) and neurotoxicity test (TOX test). Neurotoxicity of all synthesized compounds was low. Anticonvulsant activity of two compounds was high while of other two was low. We have compared the QSAR parameters of all copmpounds with the results of pharmacological tests. The comparison showed correlation between physicochemical parameters and anticonvulsant activity. This correlation suggests, that it is possible to predict the activity of the anticonvulsants of similar structure without their synthesis and pharmacologicasl examination. The high activity and low neurotoxicity of two designed and obtained Pic-BZA analogues qualifies them as a new promising anticonvulsants.

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P C26 - A bactericidal domain of lysozyme with helix-loop-helix structure present a strong antimicrobial activity

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Lysozyme an enzyme with muramidase activity possesses bactericidal properties. Previously we have presented evidence that chicken egg white lysozyme affects both Gram-positive and Gram-negative bacteria independently of its muramidase activity. More recently we reported that the digestion of lysozyme by clostripain yielded a peptide, IVSDGNGMNAWVAWR (residues 98-112) with moderate bactericidal activity but without muramidase activity. This internal peptide is part of a helix-loop-helix domain having the sequence DITASVNCAKK IVSDGNGMNAWVAWRNR (sequence 87-114 of chicken lysozyme) located at the upper lip of the active site cleft of lysozyme. The helix-loop-helix (HLH) structures are known motifs commonly found in membrane-active and DNA-binding proteins. In order to evaluate the contribution of the HLH peptide to the antimicrobial properties of lysozyme, the HLH sequence and its secondary structure derivatives of chicken- and human-lysozyme were synthesized and tested for antimicrobial activity against several bacterial strains. We found that both, the chicken and the human HLH peptide, this latter having the sequence DNIADAVACAKRVVRDPQGIRAWVAWRNR (residues 87-115) , were strong bactericidal against Gram-positive and Gram-negative bacteria as well as the fungus *C. albicans*. The bactericidal activity of the N-terminal helix of HLH was directed essentially only against the Gram-positive bacteria while the bactericidal activity of the C-terminal helix was directed against to all the investigated strains. Outer and inner membrane permeabilization studies provided evidence that the HLH peptide and its C-terminal helix domain kill Gram-negative bacteria by crossing the outer membrane via self-promoted uptake and causing damage to the inner membrane through channel formation. The results obtained in this study offer a new strategy for the design of potential antimicrobial drugs in the treatment of infectious diseases.

P C27 - Innovative biocompatible immunoadsorbents containing immobilized selective glycopeptide antigens for multiple sclerosis therapy

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We previously demonstrated that the glycopeptide CSF114, containing β-D-glucopyranosyl residue linked to an Asn residue, is able to detect specific antibodies (Abs) by ELISA on sera of patients affected by Multiple Sclerosis (MS), the most known demyelinating autoimmune disease of the central nervous system. We discovered that the specificity of the autoAb recognition is absolutely independent by the peptide sequence, but it requires the presence of Asn(Glc). The first selective Ab ligand CSF114, rationally designed, is able to detect the highest Ab titre in MS patients sera. The Abs were demonstrated to correlate with the disease activity, therefore they are possibly involved in the demyelination process. These results suggested that the use of the synthetic glycopeptide as a selective antigen might represent a simple experimental system not only to investigate auto-reactive Ab responses in MS, but also to explore new therapies for this autoimmune disease. Therapeutic Plasma Filtration (TPF) is an innovative methodology for removing toxic elements from the blood such as Abs. The ability to remove Abs and other immunologically active substances from the blood has led to the use of TPF as a therapy for neurological conditions, in which autoimmunity is believed to play a role. Since modern apheresis techniques aim to provide more specific elimination, according to clinical needs, we present herein our results on the development of specific immunoadsorbents for the selective removal of circulating Abs with a putative demyelinating role. Synthetic peptides to specifically remove Abs, have not been yet so much used as affinity ligands. Moreover, no selective TPF was performed up to now, for MS, probably because no selective antigen was identified for this disease. For this reason we decided to investigate the possibility to develop this technique using our glycopeptide. We selected two different biocompatible matrices as immunoadsorbents (Sepharose, POEPOP [1] and we report the chemical pathway to immobilize on the matrix our synthetic peptide antigen. The efficiency of this immunoadsorbents containing the immobilized synthetic peptide for specific removal of auto-Abs putatively involved in the pathogenesis of MS, will be described.

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P C28 - Bicyclic guanidinium derivatives as dimerization inhibitors of HIV-1 protease

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By targeting the highly conserved antiparallel β-sheet formed by the interdigitation of the N- and C-terminal strands of each monomer, dimerization inhibitors of HIV-1 protease may be useful to overcome the drug resistance observed with current active-site directed antiproteases. Sequestration of the monomer by the inhibitor (or destabilisation of the dimer interface) prevents the correct assembly of the inactive monomers to active enzyme (Fig. 1A).

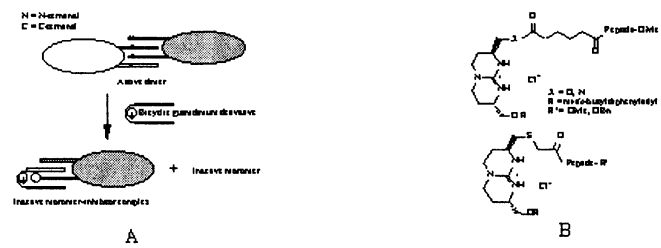

Figure 1. A. Postulated mechanism for dimerization inhibition of HIV-1 protease. B. Structure of bicyclic guanidinium derivatives.

The dimer structure is stabilized by the extended antiparallel β-sheet and a significant hydrophobic area. Bicyclic guanidimium derivatives has been designed to target this dimeric interface. They combine an interfacial peptide strand, a specific binding site (a bicyclic guanidinium unit) for the C-terminal carboxylic acid of HIV-1 protease momomer, and an hydrophobic residue (Fig. 1B). Neither the peptide strand nor the bicyclic guanidinium unit on their own had significant inhibitory properties. Several compounds were found to act as dimerization inhibitors (Kid = 150 nM). By incorporating the bicyclic guanidinium as a specific binding site for the C-terminal carboxylic unit, these postively charged molecules constitute a novel class of dimerization inhibitors.