HPLC and theirs structures were identified by ESI-MS.

No 100

Synthesis of Substance P (SP_{6-9}) Analogs Incorporating D-Ttp and Peptoid-peptide Hybrids: Study of their Anti-proliferative Properties *in vitro*

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Synthetic peptides are currently under investigation as possible anti-tumor agents. The Substance P (SP) analog [D-Arg[1], D-Phe[5], D-Trp[7,9], Leu[11]]SP (antagonist D) and also the C-terminal analog [Arg[6], D-Trp[7,9], MePhe[8]]SP_{6-11} (antagonist G) inhibit the tumor growth and cell proliferation of Small Cell Lung Cancer (SCLC) *in vitro* and *in vivo* (1,2). Recently we synthesized the C-terminal analogs [Glp[6], Glu(Bu^t)[11]]SP_{6-11} and [Glp[5], Glu(Bu^t)[11]]SP_{5-11}, which showed significant inhibition in the proliferation of the cancer cell lines HeLa and T47D (3).

In the present study a series of tetrapeptide analogs have been synthesized, based on the sequence of SP_{6-9}, using the stepwise synthesis or the fragment condensation method either in solution or in Solid Phase Peptide Synthesis. All the synthesized analogs

Glp-D-Trp-Phe-Gly-OH *(1)*, Arg-D-Trp-Phe-Gly-OH *(2)*, Glp-D-Trp-Phe-D-Trp-OH *(3)*, Arg-D-Trp-Phe-D-Trp-OH *(4)*, Glp-D-Trp-MePhe-D-Trp-OH *(5)*, Arg-D-Trp-MePhe-D-Trp-OH *(6)*, Glp-NPhe-NPhe-Gly-OH *(7)*, Arg-NPhe-NPhe-Gly-OH *(8)*, Glp-D-Trp-NPhe-D-Trp-OH *(9)*

were purified (HPLC) and identified (ESI-MS, ^{1}H NMR, FT-IR).

The analogs *1-6* are peptides incorporating in their chain D-Trp, whereas the analogs *7-9* are peptoid-peptide hybrids (4), which are oligomeric peptidomimetics containing one or more N-substituted glycine residues. The incorporation of N-substituted glycine in the peptide chain is expected to improve their stability against proteases. Thus the analogs *7-9* have incorporated the peptoid monomer [N(CH_2-Ph)-CH_2-CO-] (NPhe) instead of the amino residue of [HN-CH(CH_2-Ph)-CO-] (Phe).

Subsequently the above tetrapeptides were tested for their antineoplastic properties in several cancer cell lines. The analog *6* showed anti-proliferative activity for the cancer cell lines OAW-42 (human ovarian cancer) and T47D, leaving 67% survival fraction at the concentration of 100 μM. More results concerning the activity of the other peptides, as well as the activity of peptoids, will be presented during the conference.

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No 101

Design and Synthesis of Thrombin Receptor-derived Non-Peptide Mimetics

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Thrombin, a multifunctional serine protease generated at sites of vascular injury plays a central role in blood coagulation. Thrombin is also a powerful agonist for a variety of cellular responses. Most of these biological activities of thrombin are mediated through its specific G-protein-linked functional receptors. According to a novel mechanism of receptor activation, thrombin binds to its receptor's amino-terminal extension, cleaving the peptide bond between Arg_{41} and Ser_{42}. This proteolytic event unmasks a new amino-terminal domain that acts as an anchored

ligand to stimulate receptor function. Recently, the identification of the platelet thrombin receptor opened a new area in the development of agents that may selectively inhibit the effects of thrombin on cells, without affecting fibrin formation. In this regard, we have synthesized a number of novel compounds, which are designed to be analogues of Thrombin Receptor Activating Peptides (TRAP) and carry the pharmacophoric features of Phe and Arg residues present in the active pentapeptide SFLLR. These compounds will be tested in the rat aorta relaxation assay and in platelet aggregation studies. Their biological activity will be evaluated in the *in vivo* system of chorioallantoic membrane.

No 102

Incorporating Salicylic Acid Derivatives with Specific Inhibitory Activity to the Gp-Ib Platelet Receptor

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We have already reported that the combination in the same molecule of dipeptide or tripeptide amides, containing amino acid(s) of RGD sequence and the salicylic-residue 2-RO-C_6H_4-CO~ {where R= H or CH^3CO} at their N-terminal amino group, have shown inhibitory activity on human platelet aggregation stimulated by collagen, ADP or adrenaline [1,2]. Recently, we have described the synthesis and the inhibitory activity on human platelet aggregation of four novel RGD peptide analogs incorporating moiety of salicylic acid derivatives, at their N-terminal end [3]. Here, we present four new synthesized analogs with structural modifications in order to get more potent inhibitors against platelet aggregation.

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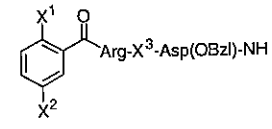

Analog 1	X^1: OH,	X^2: CH_3	X^3: Gly
Analog 2	X^1: OMe,	X^2: H	X^3: Gly
Analog 3	X^1: SH ,	X^2: H	X^3: Gly
Analog 4	X^1: OH,	X^2: H	X^3: β-Ala

No 103

Synthesis of Peptides which Imitate Specific Amino Acid Sequences of the Extracellular Domain of the beta Chain of the High Affinity Il-2 Receptor (Il-2R) to be used for the Suppression of t-Cell Activation

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The regulatory cytokine IL-2 is exclusively expressed by T helper lymphocytes upon stimulation by antigens or mitogens. IL-2 binds to its high affinity receptor IL-2R are which is the surface of T-cells, the expression of which is also restricted to activated T-cells. Binding of IL-2 to its receptor

initiates a signal transduction cascade leading to lymphocyte proliferation and secretion of IL-2. Thus, IL-2R is an attractive target for the selective inhibition of autoreactive T-cells in autoimmune diseases and alloreactive T-cells in transplantation. Molecules that interfere with the binding of IL-2 to its high affinity receptor can block the proliferation of activated T-cells, and the resulting secretion of IL-2. Given that IL-2 binds to its receptor via the extracellular domain of the β chain of the complex IL-2R, synthetic peptides mapping epitopes of the domain recognized by anti-IL2Rβ chain monoclonal antibodies (mAbs), were synthesized and tested for their ability to prevent binding of IL-2 and inhibit the proliferation and cytokine secretion of mitogen-stimulated peripheral blood T-cells.

Solid phase synthesis was applied for the creation of 2 peptides with a primary structure corresponding to the epitopes M^{107}-E^{118}, Y^{178}-Q^{199} of the extracellular domain of the IL-2Rβ chain and of another arbitrarily selected amino acid sequence non-homologous to the IL-2Rβ ectodomain serving as a control peptide. Peripheral blood mononuclear cells (PBMC) isolated from peripheral blood of 16 healthy volunteers were cultured with various concentrations of peptides and the mitogen phytohaemaglutinine (PHA). The inhibitory effect of the peptides on cell proliferation was evaluated by cell counting (Sysmex cell counter) and colorimetric proliferation immunoassays. Cell activation was assessed by immunophenotyping using anti-CD4, CD25 and CD69 mAbs. At least 10.000 cells from each sample were analysed with flow cytometry using a Coulter EPICS-XL-MCL cytometer, and the data were processed using the XL-2 software. The amount of IL-2 in culture supernatants was measured by IL-2 ELISA.

PHA-induced T-cell proliferation was significantly inhibited by the M^{107}-E^{118} peptide. The most effective peptide concentration was 500nM. Cell counting revealed a reduction of the PBMC populations by 27,95% and a reduction of the lymphocyte populations by 30,33%. The amount of IL-2 in the culture supernatants was decreased by 56,65%. Given that IL-2 is produced by antigen activated T-cells, its reduction reflects a suppression of activated T-cells, and this was also confirmed by immunophenotyping. The peptide Y^{178}-Q^{199} had a similar, though milder, effect on cell proliferation but did not inhibit significantly IL-2 secretion by the PHA-activated cells, and flow cytometry showed that activation indicators were not suppressed. The control peptide had no effect on any of the activation parameters tested.

Experiments with T-cells isolated from autoimmune patients are under way to investigate whether these peptides and especially M^{107}-E^{118} are promising for further development as putative inhibitors of T-cell activation in disease states.

No 104

Design and Synthesis of Two Novel Cyclic GnRH Analogues: Biological and Conformational Studies

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Gonadotropin-Releasing Hormone (GnRH) stimulates the synthesis and release of Gonadotropin Hormones (GtH) and is considered to be the key regulator of reproduction. Based on the knowledge of the pharmacophoric groups of the hormone (pGlu, His, Trp, Tyr) as well as the conformational model which is characterized by a ring cluster (His, Tyr, Trp) and a ß-turn (Tyr-Gly-Leu-Arg) stabilized by a D-amino acid at position 6 we designed and synthesized two cyclic GnRH analogues namely

1. Cyclo(4,9) $pGlu^1$-His^2-Trp^3-Lys^4-Tyr^5-$(D\text{-}Trp)^6$-Leu^7-Arg^8-Glu^9-Gly^{10}-NH_2,
2. Cyclo(4,9) $pGlu^1$-$(D\text{-}Phe)^2$-Trp^3-Lys^4-Tyr^5-$(D\text{-}Trp)^6$-Leu^7-Arg^8-Glu^9-Gly^{10}-NH_2

by connecting side chains of residue Lys and Glu which replaced the least important for activity residues Ser and Pro at positions 4 and 9 of the native hormone. The cyclization was carried out using O-benzotriazol-1-yl N,N,N,N′-tetramethyluronium tetrafluoroborate (TBTU), 1-hydroxy-7-azabenzotriazole (HOAt) and collidine. Cyclic

analogues are more stable substances compared to linear ones in terms of degradation retained aromatic aminoacids Trp, Tyr, His and Arg, important for activity. The cyclic analogues of GnRH were tested for stimulation of gonadotropin gene expression in the goldfish pituitary. Cyclic analogue 1 showed considerable activity as it is expected because it mimics the clustering of His^2-Trp^3-Tyr^5 as in GnRH. Cyclic analogue 1 was tested in proliferation assays with human cancer cell lines (breast, ovaries).