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New Approaches to the Synthesis of Peptides Containing Heterocyclic-Structures

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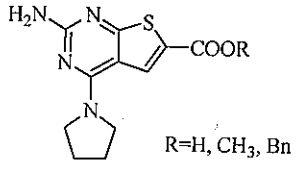
Peptides and proteins play a central role in nearly all-physiological processes that are associated with life. While proteins have biocatalytic functions and are important components of tissues, peptides play an important role in the organism as hormones, neurotransmitters, and neuromodulators. However, their potential as therapeutics is limited by an inherent instability towards proteolytic cleavage, a lack of oral bioavailability and an inability to cross the blood-brain barrier. A good deal of effort has been directed towards overcoming these deficiencies in an attempt to develop new medicinal agents. However, a much more general approach to new therapeutics involves the chemical modification of a peptide in order to introduce those biological and chemical properties that are associated with effective drug action.

There are many approaches to modify a bioactive peptide including substitution of an amino acid with another (peptide analogues), peptide bond surrogate, even preparation of non-peptide compounds (*peptidomimetics*). In our days a very interesting approach is the synthesis of compounds with a peptidic part and a heterocyclic one (*semi-mimetics*).

As a tool for the preparation of semi-mimetics we used the compounds presented in Scheme 1. These compounds are suitable for the substitution of certain amino acids (His and Pro) in a peptide sequence.



Scheme 1. Heterocyclic structures.

The insertion of these compounds in the *N*– or *C*–terminal and even into a peptide chain is performed by solution techniques or solid phase synthesis.

No 94

Role of Thrombin and its Receptors in Metastatic Ability of Human Cancer Cells

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Tumour metastasis is a complex process which consist of: invasion, the ability of an epithelial cancer cell to detach from and invate through extracellular matrix, migration, the movement of the cell in response to chemotactic factors and aptotaxis, the movement of the cell which ought to the touch with chemotactic factors.

In addition, it has been shown that thrombin and/or its receptor is related in tumour progression and metastasis. The aim of this report is to further investigate the role of thrombin and its receptors in tumour metastasis. We used four different cancer cells lines, MDA-231 and MCF-7 from breast and PC_3 and LNCaP from prostate. MDA-231 and PC_3 express high levels of trombin receptor(PAR-1) but MCF-7 and LNCaP express low levels of PAR-1.

We showed that, MDA-231 and PC_3 (highly aggressive cell lines) readily migrate toward NIH 3T3 condition medium (chemotaxis solution), while MCF-7 and LNCaP (less aggressive cell lines) did not. Thrombin did not affect or had a little effect on migration of MDA-231 and PC_3 cells when it was used as chemotactic factor. When these cells were pretreated with thrombin for fifteen mimutes, a significant inhibition was evident. Same results were also obtained when trombin were co-administrated with cells to upper

chamber.On the other hand thrombin promoted the aptotaxis of PC_3 and MDA-231 cells in a dose-dependent way. Furthermore we showed that only MDA-231 and not the MCF-7 cells invade through matrigel. Thrombin had no effect on the migration and aptotaxis of LNCaP and MCF-7 cells.

In conclusion, we showed that the presence of PAR-1 was important for the cells in order to migrate and that the activation of PAR-1 with thrombin inhibited the migration of these cells. Moreover thrombin promoted aptotaxis only to the cells that have high levels of PAR-1. These results emphasize the involvement of thrombin receptors in metastatic ability of human cancer cells.

No 95

Activation of MMPs is Necessary in the α_2-Adrenergic Receptor Induced MAPK Activation in PC12 Cells

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The α_2-adrenergic receptors belong to the super family of G-Protein Coupled Receptors (GPCRs). They mediate effects of the endogenous catecholamines, epinephrine and nor-epinephrine. Three different genes encode the human α_2-adrenergic subtypes (α_{2A}, α_{2B} and α_{2C}), that differ in their ligand binding properties, tissue distribution, chromosomal location and signaling pathways.

Recent studies in the Department of Pharmacology indicated that epinephrine induces the morphological and molecular neuronal differentiation of α_2-adrenergic receptor transfected PC12 cells. A critical step in this process is activation of MAPK. This pathway is involved in differentiation, cell survival and according to recent studies is involved in apoptosis too.

The aim of the present study is to examine the possible involvement of MMPs in the activation of MAPK by α_2-adrenergic receptor. We employed three different clones of PC12 cells expressing, after transfection, individual α_2-adrenergic receptor subtypes and we used 1,10 Phenanthroline, a specific inhibitor of MMPs, and Western Blotting analysis.

We found that activation of MAPK by epinephrine was almost totally abolished by the inhibitor in all three clones studied, suggesting that MMPs activation is a necessary step in the process.

It is possible that MAPK activation is mediated by transactivation of a tyrosine kinase receptor, expressed in PC12 cells, such as EGF- Receptor or TrkA, through activation of MMPs, as previously described in other cell systems.

No 96

AT_1 Antagonists: *Diffusion in the Membrane Bilayers and Docking on the AT_1 Receptor*

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The AT1 antagonists, derivatives of Losartan (class of SARTANS) are drugs, already approved for the treatment of hypertension, which compete the binding of the hormone Angiotensin II, an octapeptide implicated in the hypertension disease, onto the transmembrane helices of AT1 receptor. Since their binding site is located deep in the transmembrane loops of the AT1 receptor they are anticipated to cause their pharmacological effects after embedding in the phospholipid bilayers and diffusing laterally into the active site of AT1 receptor (Figure 1). For this reason the thermal effects and dynamic properties of SARTANS in phospholipid bilayers were studied in an attempt to understand the relationship between bioactivity, conformation and membrane perturbation. In addition, their docking onto AT1 receptor was studied in order to get more insight in their molecular basis of action. Such studies aim to propose explanation for the different pharmacological properties observed in these molecules and propose novel synthetic ones with optimised pharmacological profile.

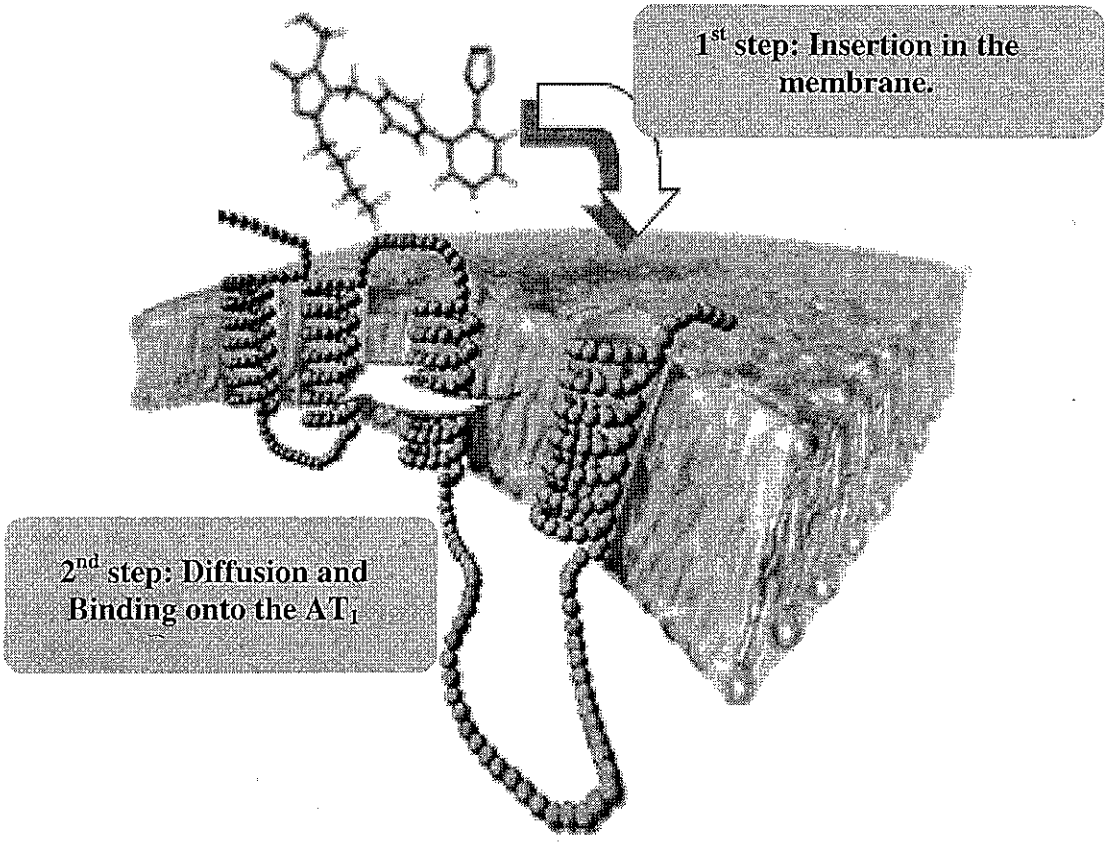

Figure 1: Proposed mechanism of action for the AT_1 antagonist losartan

No 97

Effect of Thrombin Peptide TP508 on Thrombin-induced Cellular Events

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One facet of α-thrombin's multifunctionality is related to its well-characterized activity as a serine protease and another to a group of functions that are not dependent upon protease activity. It has been proposed that thrombin acts through two independent receptor components to induce cellular effects: (i) the proteolytically activated G-protein receptors for thrombin (PAR-1, PAR-3 and PAR-4) and (ii) a nonproteolytically active receptor component, NPAR.

Prior studies have shown that synthetic peptides representing the domain of thrombin responsible for high-affinity binding to fibroblasts stimulate chemotactic and proliferative signals through a nonproteolytic mechanism. One of these peptides, TP508, corresponding to a highly conserved region of the B-chain of the human prothrombin sequence that extends from amino acids 508 to 530, appears to mimic many of the normal effects of thrombin in initiating wound healing even though it has no proteolytic activity and does not appear to activate the proteolytically activated thrombin receptor (PAR-1).

It has been proposed that TP508 initiates signals upon its binding to NPAR that are distinct from those initiated by PAR-1 peptide SFLLRN

and that these two types of signals act in concert to induce certain cellular events but may also be antagonistic to each other.

As part of an ongoing effort to characterize the double signal model for thrombin cellular effects we studied the effect of TP508 on some well-known thrombin-induced PAR-1 mediated cellular responses. TP508 decreased the basal and thrombin-induced prostacyclin production and mitogen activated protein kinase p42/44 in HUVECs whereas it did not affect the basal and thrombin-increased inositol phosphates turnover.

Work is now underway in our laboratory to further define the molecular mechanism by which TP508 interferes with thrombin's PAR-1 mediated actions and to exclude the possibility of a direct molecular interaction between TP508 and thrombin that could account for the inhibitory action of this peptide. It is possible that the well-known bell shape that characterizes many of the cellular dose responses to thrombin is explained by the existence of TP508 in thrombin's molecule which may act as an internal brake to thrombin's cellular actions in high concentration of thrombin.

No 98

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 99

Synthesis of Analogues of Insect Diuretic Hormone Locusta-DH

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Locusta diuretic hormone (Lom-DH) is a neuropeptide that has been isolated from the locust Locusta migratoria. It belongs to the CRF-related diuretic peptides and has the following amino acid sequence:

MGMGPSLSIVNPMDVLRQRLLLEIARRRLRDAEEQIK ANKDFLQQI-NH_2

Lom-DH is a potent stimulant of fluid secretion and cAMP production by locust Malpigian tubules and it is released into the haemolymph from corpora cardiaca.

We synthesized a series of analogues in order to obtain information for the active core of the molecule. These analogues are the N-terminal 1-5 pentapeptide acid, 6-12 heptapeptide acid and amide respectively and the 13-23 untecapeptide acid. In all analogues Met was replaced by its isosteric ester Hse(Me) since it does not affect the activity of Lom-DH. The synthesis of above analogues was performed in the solid phase using the stepwise strategy on the 2-Cl-Clt-resin and the Rink-2-Cl-Clt-resin. For the synthesis we used the Fmoc/Bu^t methodology for protection and the DIC/HOBt for the coupling of amino acids. The purity of the analogues was estimated by