POSTERS

No 56

The Effect of two Aza-homo-steroides Esters in the Human Cancer Cell Lines, LNCap and MDA-MB-231

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Homo-aza-steroids are modified steroids that contain -NHCO- group in A, C or/and D ring (lactamic ring) and were experimentally used as carrier molecules for cytotoxic agents against murine tumors and leukemias in order to diminish toxicity and to increase antineoplastic activity. Several of homo-aza-steroidal ester of alkylating moieties showed excellent anti-leukemia results, while the respective unmodified esters were inactive against L1210 murine leukemia. These satisfactory results of homo-aza-steroidal esters, prompted us to study the p-[N,N-bis(2-chloroethyl)amino]-phenylacetate esters of 3β-hydroxy-17α-aza-D-homo-5α-androstan-17-one(*1*) and 3β-hydroxy-12α-aza-C-homo-5α,22-spirostan-12-one(*2*), *in vitro* against MDA-MB-231 and LNCap human cancer cell lines.

In order to determine the *in vitro* cytotoxic effect of the tested compounds 1 and 2, we used two established human cancer cell lines, LNCap FGG prostate carcinoma and MDA-MB-231 breast carcinoma. LNCap FGG cells were cultivated in complete RPMI medium, supplemented with 10% FBS, L-glutamine and antibiotics (penicillin/streptomycin). MDA-MB-231 cells were cultivated in DMEM medium, supplemented with 10% FBS, L-glutamine, antibiotics (penicillin/streptomycin), sodium pyruvate and $NaHCO_3$. Cells were plated in 96-well microtiter plates in a cell density of 4x104 cells LNCap/100 μl medium and 5x104 cells MDA-MB-231/100 μl medium and cultivated for 18 hours at 37 °C in a 5% CO_2 incubator. The cells were treated with the two test compounds in the concentration range of 5, 10, 20, 50, and 100μM/150μl medium for 48 hours at 37 °C in a 5% CO_2 incubator. Both of the compounds tested were dissolved in DMSO (the dilutions of DMSO we used were always higher than 1/100).

The antineoplastic effect of the test compounds was evaluated using the MTT tetrazolium salt assay. The measured effect of the compounds on each cell line was calculated according to the expression % viability OD_{test}/OD_{ctrl}, where OD_{test} the average of optical density measurements of MTT-derived color after 48 hours exposure of cells to the test compounds and OD_{ctrl} the average of optical density measurements of MTT-derived colors after 48 with no exposure of cells to the test compounds.

We tested the compounds *1* and *2* versus two tumors cell lines, LNCap and MDA-MB-231. The compound *1* exhibits a significant cytotoxic effect against both human cancer cell lines, especially at the two higher concentrations used (50 μM and 100 μM). On the other hand, the compound *2* did not exhibit significant cytotoxic effect in any of the cancer cell lines tested. It is worth mentioning that the compound *2* induced significantly cell division at the concentration of 100 μM compared to the positive control.

No 57

Synthetic studies on anti-HIV peptide T-1249

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Therapeutic peptides are specifically manufactured to be used as drugs in the treatment of human disease. One of these is T- 1249, which is a peptide consisting of 39 amino- acids. T-1249, currently in phase II clinical trials, belongs to an entirely new class of antiretrovirals, known as fusion inhibitors. The compound mimics one of two coil-like segments of HIV-1 ´s gp-41 envelope protein that plays a critical role early in the process of infection. Due to its therapeutic importance, we studied the synthesis of T-1249, ap-

plying convergent methods developed recently in our lab.

For our studies the T- 1249 sequence was divided into 4 protected fragments (fig.1). These were synthesized on 2-chlorotrityl resin using Fmoc-amino acids, side chain protected with groups of the tBu/Trt-type. The final sequence was assembled by the sequential condensation of the 21-28, 13-20 and 1-12 fragments on the resin-bound 29-39 fragments.

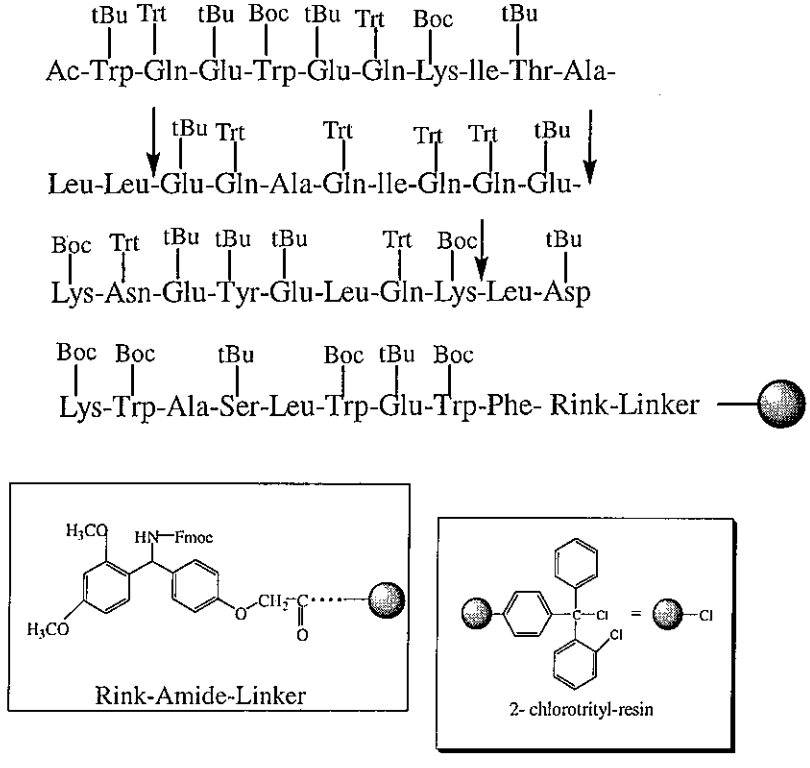

Figure 1

No 58

Identification of Novel Interactants of the Mammalian Acetylotransferase TIP60 by the Yeast Two-Hybrid Approach

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TIP60 was isolated as an HIV-1-Tat interactive protein and has been shown to modestly augment Tat-dependent transcriptional activation. It has also been shown to interact with various cellular transcription factors and belongs to the nuclear histone acetylotransferase (HAT) family. Moreover TIP60 can both activate transcription factors of one signaling pathway (nuclear hormone receptors) and bind to a different transcription factor and inhibit activation of another signaling pathway.

In this report we have used the yeast two-hybrid assay to identify protein interactions with TIP60. Confirming the specificity of candidate clones requires separation and isolation of yeast plasmids, propagation in bacteria and testing

combination of DNA-binding and activation domain hybrids in yeast. We isolated 40 yeast plasmids and we identified three different proteins translated in frame (STAT3β, APOH like protein and BMPR2). We proved that HAT domain of TIP 60 is not required for these interactions. Great deal of additional experimentation is required to verify the above interactions in vivo and further characterize the functional relevance, structure and regulation of the observed interactions and their biological role.

No 59

Design and Synthesis of Histamine Derivatives Analogues as Antagonists to the AT_1 Receptor of Angiotensin II

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In this work the design and synthesis of non-peptide angiotensin II receptor antagonists is reported. The octapeptide angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) that is the major factor of the renin-angiotensin system, plays an important role in the regulation of arterial blood pressure and in the cause of hypertension. The main structural characteristics of the compounds that were synthesized are:

(a) All the compounds contain the phenyl template, upon which the pharmacophoric groups are mounted.

(b) The phenyl ring is joined with an imidazole ring via a methylene group and with the acidic group of tetrazole. The tetrazole group is prefered to other acidic groups not only for its better binding affinity to AT_1 receptor but also because it leads in better oral activity.

(a) At the C-5 position of the imidazole ring there is a guanidyl group or an ethylamine group.

(d) The tetrazole group is protected with the 2-chlorotrityl group (Cltr). The compounds, which were synthesized, contain the crucial pharmacophoric groups of angiotensin II. Thus, the imidazole ring simulates the action of the pharmacophoric group of His, the phenyl group the pharmacophoric group of Phe and the guanidyl group simulates the Arg. Finally, the acidic group of tetrazole simulates the action of the terminal carboxylic group of natural peptide.

No 60

Design and Synthesis of Linear and Cyclic Analogues Based on Human MBP_{87-99} Epitope: Effects on Healthy Peripheral Blood Cells

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Multiple sclerosis (MS) is a chronic specific CD4+T-cell mediated disease of the central nervous system (CNS) characterized by local T cell and macrophage infiltrates, demyelination and loss of neurologic function. MS is widely believed to be an autoimmune disease and to be triggered by CNS-specific CD4 T lymphocytes. Candidate autoantigens include constituents of the myelin sheath such as myelin basic protein (MBP) and proteolipid protein (PLP). Modern approaches towards the therapeutical management of MS involve the design and use of peptide analogues of disease-associated myelin epitopes to induce peripheral T cell tolerance.

In the present report, we designed and synthesized a linear [X^{91}, Ψ^{96}] MBP_{87-99} and a cyclic peptide analogue cyclo(87-99) [X^{91}, Ψ^{96}] MBP_{87-99} based on the human myelin basic protein epitope

(MBP_{87-99}) (Val[87]-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro[99]). Structure-activity studies have shown that Lys[91] and Pro[96] residues are important for encephalitogenicity.

To assess their activity in humans, we studied their effect on cellular proliferation and cytokine synthesis in human peripheral blood T-cells. Peptides influesed actively certain cell type proliferation. Linear analogue inhibited INF-γ synthesis in the absence of mitogen while it induced in the presence of the mitogen.

No 61

Design, Synthesis and Conformational Properties of Linear Analogues of Human Myelin Basic Protein Epitope MBP_{1-11}

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Experimental Allergic Encephalomyelitis (EAE) is induced in experimental animals immunized with Myelin Basic Protein (MBP). In EAE, residues 1-11 of myelin basic protein are the dominant disease-inducing determinants in PL/J and $(PL/JxSJL/J)F_1$ mice. Analogues of disease-associated epitopes have been identified which alter disease progression upon co-immunization. Based on the Human MBP_{1-11} sequence [Ala[1]-Ser[2]-Gln[3]-Lys[4]-Arg[5]-Pro[6]-Ser[7]-Gln[8]-Arg[9]-His[10]-Gly[11]] (analogue *1*) we designed and synthesized linear analogues in which Lys at position 4 was replaced with Ala, [Ac-Ala[1]-Ser[2]-Gln[3]-Ala[4]-Arg[5]-Pro[6]-Ser[7]-Gln[8]-Arg[9]-His[10]-Gly[11]] (analogue *2*) or Tyr, [Ac-Ala[1]-Ser[2]-Gln[3]-Tyr[4]-Arg[5]-Pro[6]-Ser[7]-Gln[8]-Arg[9]-His[10]-Gly[11]] (analogue *3*). In these analogues the N-terminus was acetylated. For the synthesis of linear MBP analogues, we resorted to the Fmoc/tBu methodology utilizing the 2 chlorotrityl chloride resin. Design was based on the NOEs connectivities and Molecular Modeling low energy 3-D structures of the above peptides. In bioscreening assays we found that these linear analogues were not effective in inducing or inhibiting EAE in Lewis rats in contrast to PL/J mice, where these analogues are active. However, it was noticed that analogue *2* when co-injected with guinea pig MBP_{72-85} gave a maximum clinical score for two additional days, in contrast to analogues *1*, *3*, which didn't alter agonist effect caused by MBP_{72-85}.

No 62

Synthesis of Imidazole-based Non-peptide Angiotensin II Receptor Antagonists As Potent Antihypertensives

N. Giatas[1], M.-E. Androutsou[1], A. Zoga[1,3], P. Roumelioti[1], P. Zoumpoulakis[2], T. Mavromoustakos[2], D. Vlahakos[3], E. Iliodromitis[3] and J. Matsoukas[1]

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The Renin-Angiotensin System (RAS) plays a pivotal role in blood pressure regulation and electrolyte homeostasis. The octapeptide angiotensin II (AII) is the biologically active component of the RAS, responsible for the development and maintenance of hypertension, as well as for congestive heart failure. Our research group has been engaged in the conformational analysis

study of AII, as well as in the design and synthesis of novel peptidomimetic analogues. The accumulated experimental evidence for AII in DMSO supports a bioactive conformation characterized by i) a Tyr^4-Ile^5-His^6 bend, ii) a major His^6-Pro^7 trans conformer, iii) a cluster of the side chain aromatic rings of the triad key amino-acids Tyr^4, His^6, Phe^8, and iv) a charge relay system between Tyr^4 hydroxyl, His^6 imidazole and Phe^8 carboxylate.

In our laboratory we have designed, synthesized and bioassay novel imidazole based Angiotensin II receptor antagonists, which bear similar pharmacophoric groups as in Losartan-type compounds, but with a different orientation of the imidazole ring substituents. Our analogues were intravenous administered in anesthetized rabbits in order to investigate their biological activity, using Losartan as a reference standard.

No 63

Studies towards the Synthesis of Chiral Polyamines Inconporating a Tetrahydrofuranyl Ring as Conformation Constraint

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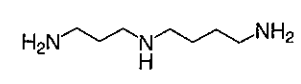
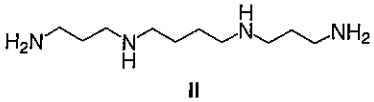
Conformationally restricted *N*-tritylated analogues (*VII-IX*) of spermidine (*I*) and spermine (*II*) were prepared using the isolable *active* ester Trt-βAla-OSu as an N-C_3 synthon and the diamine *III* or the peptide ester *IV*. The latter were readily obtained from the *N*-tritylated chiral *trans*-amino acid (*V*) and the *cis*-amino ester (*VI*), which in turn where prepared in several steps from the commercially available amino acid L-Met.



I



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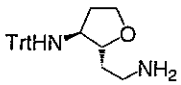

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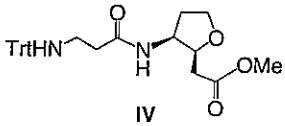
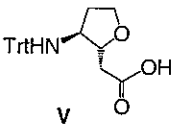
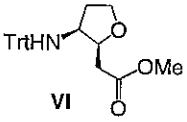


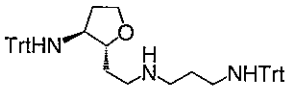
IV



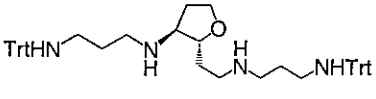
V



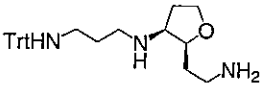
VI



VII



VIII



IX

No 64

Synthesis of the Insect Neuropeptides[His^7]-Corazonin and Proctolin

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Corazonin is a neuropeptide that has been isolated from the acridid *Schistocersa americana*. It is a member of seven neuropeptide families with myostimulatory properties and has the following amino acid sequence:

H-pGlu-Thr-Phe-Glu-Tyr-Ser-His-Gly-Trp-Thr-Asn-NH_2

Corazonin is present in a limited number of neurosecretory cells of the brain of *L.migratoria and S.gregaria* and it is released into the hemolymph from the corpora cardiaca. It is responsible for the induction of different body colors (mainly black) in the locusts. Because of that it was named "dark color-inducing neurohormone" (Lom-DCIN and Scg-DCIN).

This undecapeptide amide was synthesized step by step synthesis in the solid phase on linker-Clt-resin,(the linker of Rink-Bernatowitz has been attached on Clt-resin by esterification) using the Fmoc/Bu-t methodology and the activation was achieved by DIC/HOBt. Removal of linker and Bu-t group was achieved by treatment with TFA solution containing scavengers (anisole, EDTH).

In the same group of myostimulatory families belongs and another neuropeptide with the name *Proctolin.* It is a pentapeptide with the following sequence:

H-Arg-Tyr-Leu-Pro-Thr-OH

It was isolated from the American cockroach *Periplaneta Americana.* This peptide shows biological activity in insects and some of its analogues showed significant biological effects in rats. Proctolin was synthesized step by step in the liquid phase, using the method of mixed carbonic acid anhydrides.

Using the above methodologies we established the optimum conditions for the synthesis of the peptides in high yield and purity. The latter was estimated by RP-HPLC and its structure was identified by ESI-MS.

No 65

Studies on the Convergent Synthesis of Hirudin

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Hirudin variant 1 (HV1) is a small protein consisting of 65 amino acids and it has three disulfide bridges. It is found in the saliva of leeches. Hirudin has anticoagulant activity because it is a potent inhibitor of thrombin, an enzyme basic to the blood coagulation cascade. It has earned the attention of many researching groups because it can find several clinical applications such as treating conditions in which thrombosis can occur. It is used as a drug since 1998 by the name *refludan,* for the treatment of heparin induced thrombocytopenia type II. Hirudin is obtained through biotechnological methods. Its chemical synthesis has not been reported.

Our purpose is to study the chemical synthesis of hirudin. Until now we have obtained HV1 by the convergent method and we also managed to form selectively two of the three disulfide bridges. These first results are very important because they show that it is possible to synthesize a protein, with disulfide bridges, using chemical methods and not only biotechnologically.

No 66

Solid-Phase Synthesis of Peptide Amides Using the 2,4-Dimethoxybenzydrylamine resin

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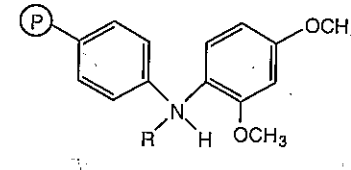
2,4-Dimethoxybenzydrylamine resin was proved to be very useful for the synthesis of peptide amides using the Fmoc/^{t}Bu strategy. The effectiveness of this chemistry is shown with the synthesis of Salmon I Calcitonine. Cleavage of products was effected with mild acidolysis using trifluoroacetic acid (TFA) solutions in dichloromethane (DCM) and triethylsilane (TES) as scavenger. The acid lability of 2,4-Dimethoxybenzydrylamine resin has been also studied and compared with the acid lability of Rink-linker-aminomethyl resin.



R= H, Alkyl

2,4-Dimethoxybenzydrylamine resin