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Abstracts Posters

Raman Spectroscopy of Osteoporotic Rat Tibiae

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In the present work, Raman Spectroscopy was employed to study changes in the amount and/or quality of bone were studied in osteoporotic tibiae from female wistar rats compared to healthy controls. Osteoporosis was induced through ovariectomy. The height of the primary phosphate band (PO_4^3, v_1) for the mineral at 959 cm^{-1}, the carbonate peak at 1070 cm^{-1} under the combined phosphate-carbonate envelope 1010-1100 cm^{-1} spectral range, the matrix bands at 855 cm^{-1} (hydroxyproline), 875 cm^{-1} and 920 cm^{-1} (proline), as well as the three major peaks under amide I envelope (1620-1710 cm^{-1}) were measured after proper baselinining and deconvolution. The mineral to matrix ratio [959 cm^{-1} /(855 cm^{-1}+875 cm^{-1}+920 cm^{-1})] was reduced, suggesting decreased mineral quantity in the osteoporotic tibiae compared to controls. Carbonate levels remained stable which implies absence of new bone tissue formation. The mineral to amide I envelope ratio exhibited an increasing trend sug-gesting that amide I cannot be used as collagen metrics as it is subject to polarization effects. Further analysis of the amide I envelope shows that the band changes shape following bone dis-ease, which is a result of the change in the ratio of the peaks lying under the amide I envelope. Therefore, changes in collagen cross-linking accompany reduction of mineral amount and lead to reduced strength and increased fragility in osteoporosis.

Synthesis And Characterization of Novel fac-[M(NO/NS)(P)$(CO)_3$] and [M(NO/NS)$(P)_2(CO)_2$] Complexes (M = Re, ^{99m}Tc)

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Low oxidation state complexes of technetium and rhenium of the general formula *fac*-[^{99m}Tc/^{188}ReL$(CO)_3$], are studied extensively, as a result of the significance of these radiometals in the development of radiopharmaceuticals for imaging (^{99m}Tc) or radiotherapy (^{188}Re). In this work, we focused on the development of neutral mixed ligand technetium and rhenium complexes of the general formula *fac*-[^{99m}Tc/Re(NO/NS)(P)$(CO)_3$] and [^{99m}Tc/Re (NO/NS)$(P)_2(CO)_2$] where NO and NS are 8-hydroxyquinoline and 8-thioquinoline, respectively, as bidentate ligands and P is triphenylphoshpine as monodentate ligand. The synthesis of rhenium complexes *fac*-[Re(NO/NS)(P)$(CO)_3$] was conducted by reacting equimolar amounts of the NO or NS ligand and triphenlyphosphine with the precursor $(NEt_4)_2[ReBr_3(CO)_3]$ or by reacting triphenylphoshpine with the precursor *fac*-$[Re(NO/NS)(CO)_3]_2$. The synthesis of rhenium complexes [Re(NO/NS)$(P)_2(CO)_2$] was conducted by reacting the NO or NS ligand and triphenlyphosphine in molar ratio 1:2 with the precursor $(NEt_4)_2[ReBr_3(CO)_3]$ or by reacting the NO or NS ligand with the precursor *mer, trans*-[Re$(CO)_3(P)_2Cl$]. All the complexes where characterized by IR, NMR spectroscopies and X-ray crystallography. The analogous ^{99m}Tc tracer complexes were prepared by reacting low concentrations of the ligands NO or NS and P (10^{-3}, 10^{-4} M) with the precursor *fac*-[^{99m}Tc$(OH_2)_3(CO)_3]^+$. When the

reaction is conducted at rt for 1hr primarily the monophosphino-substituted complexes *fac*-[^{99m}Tc(NO/NS)(P)(CO)$_3$] are formed, while at 80 ^{0}C for 30 min the bisphosphino-substituted complexes [^{99m}Tc(NO/NS)(P)$_2$(CO)$_2$] are formed quantitatively. Identification of the ^{99m}Tc complexes was done by HPLC chromatographic comparison with the analogous prototype rhenium complexes.

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A Molecular Modeling and NMR Spectrosopy Study of Two New Cytotoxic C2-Substituted Pyrrolo[2,3-f]quinolines

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DNA cross-linking agents are known for their significant antitumor activity, which has been attributed to their ability to form irreparable base pair adducts at precisely defined genomic locations. Most of the reported cross-linking probes involve three or four aromatic chromophores in their skeleton and are of sufficient size to recognize two or three base pairs (1). An extension of this limited sequence recognition, which is attained by the use of larger aromatic heterocycles, has been found to drastically enhance cytotoxicity due to the formation of more rigid irreversible interstrand bonds (2). In the course of our program directed towards the development of new DNA-complexing agents, we have previously reported on the synthesis and cytotoxic profile of a series of C_2-substituted pyrrolo[2,3-*f*]quinolones(3). In an attempt to further explore the activity of these agents we designed and synthesized the pyrrolo[2,3-*f*]quinolines *1* and *2* (Scheme 1). The promising biological properties of the compounds led us to apply a combination of 2D NMR (NOESY) data and Monte Carlo conformational analysis in order to explore the conformational space of these molecules and correlate the obtained results with those of with the controlled release in simulated aqueous gastric and intestinal media.

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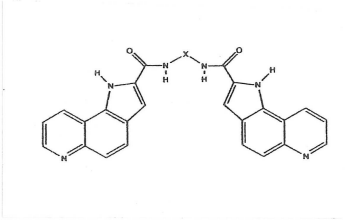

Scheme 1

1. X=$(CH_2)_3NH(CH_2)_4NH(CH_2)_3$, *$N_2$-{[3-(4-{3-[(1H-pyrrolo[2,3-f]quinoline-2-carbonylamino)]propylamino}butylamino)propyl]}-1H-pyrrolo[2,3-f]quinoline-2-carboxamide*
2. X=$(CH_2)_4NH(CH_2)_3$, *N_2-{3-[{4-[(1H-pyrrolo[2,3-f]quinoline-2-carbonylamino)]butylamino}propyl]}-1H-pyrrolo[2,3-f]quinoline-2-carboxamide*

❁

Construction and Expression of Extracellular Recombinant Domains of the Human Muscle Nicotinic Acetylcholine Receptor (AChR) in Insect Cells Aiming at Developing an Antigen Specific Treatment for Myasthenia Gravis

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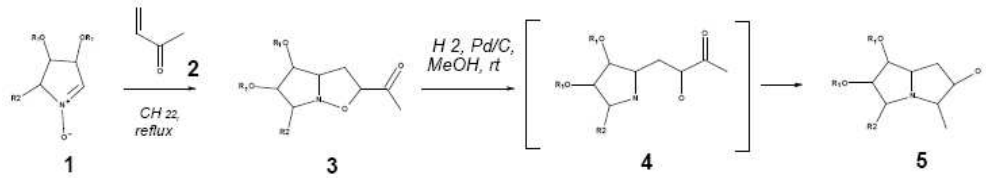
Myasthenia gravis (MG) is an autoimmune disease caused in the majority of patients (85%) by autoantibodies against the human muscle acetylcholine receptor (AChR), a post-synaptic ligand-gated ion-channel located at the neuromuscular junction. Autoantibodies against the AChR cause

loss of the available and functional AChRs leading to muscle weakness and fatigability. An attracttive therapeutic approach is the extracorporeal specific removal of the pathogenic autoantibodies using AChR-based immunoadsorbents. In this study, the N-terminal extracellular domains (ECD) of AChR the subunits α_1 and β_1 were cloned into baculovirus expression vectors and heterologously expressed using insect SF9 cells. Additionally, the two subunits were linked by the use of a flexible peptide linker to form the β_1-α_1 concatamer. The recombinant proteins were expressed as soluble polypeptides, purified and characterized. Furthermore, they were immobilised on sepharose beads in order to test them as immunoadsorbents using sera from MG patients. They were all found to bind anti-AChR autoantibodies, albeit to varying degrees. They, thus, pose as potential candidates for the therapeutic antigen-specific clearance of MG sera.



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Synthesis of Pyrrolizidine Azasugars *via* Combined Application of 1,3-Dipolar Cycloaddition and Reductive Amination

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Azasugars concentrate a great interest since they could find applications in the treatment of various deceases due to their capacity to inhibit glycosidases (1). Their biological activity is re-lated with the stereochemistry of their hydroxy substituents and the development of new stereocontrolled methodologies for their synthesis is an important synchronous synthetic target (2). In connection with our previous studies (3), we present here a short reaction sequence for the synthesis of pyrrolizidine azasugars using as keysteps the 1,3-dipolar cycloaddition and the reductive amination reactions. Asymmetric nitrones *1* derived mainly from sugars react with methyl vinyl ketone *2* to give the isoxazolidine derivatives *3*. Compounds *3* are transformed to the desired pyrrolizidines *5* in one step by reductive amination of the non isolated intermediates *4*. The stereoselectivity of the reactions is controlled by the stereochemistry of the starting nitrones.

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Synthesis of Novel Sulfonamide-1,2,4-triazoles, Sulfonamide-1,3,4-thiadiazoles and Sulfonamide-1,3,4-oxadiazoles, as Potential Antibacterial and Antifungal Agents

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The significant antifungal activity of a series of sulfonamide-1,2,4-triazole and 1,3,4-thiazole derivatives against a series of micromycetes, compared to the commercial fungicide bifonazole has been reported (1). These compounds have also shown a comparable bactericidal effect to that of streptomycin but better activity than chloramphenicol respectively against various bacteria (2). In view of the potential biological activity of members of the 1,2,4-triazole, 1,3,4- thiadiazole and 1,3,4-oxadiazole ring systems, and in continuation of our search for bioactive molecules, we designed the synthesis of a series of novel sulfonamide-1,2,4-triazoles, sulfonamide-1,3,4-thiadiazoles and sulfonamide-1,3,4-oxadiazoles emphasizing, in particular, on the strategy of combining two chemically different but pharmacologically compatible molecules (the sulfomamide nucleus and the five member) heterocycles in one frame. Synthesized compounds were tested *in vitro* for antibacterial and antifungal activity and some analogues exhibited very promising results. Conformational analysis was performed for active and less active analogues using NMR spectroscopy and molecular modeling techniques.

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Comparative Studies between Telmisartan and other AT1 Antagonists at Membranes and Receptor Active Site

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AT_1 antagonists (SARTANs) are widely used drugs in the clinical setting today for the regulation of high blood pressure. These drugs interfere in the Renin Angiotensin System (RAS) by preventing the vasoconstrictive hormone Angiotensin II from binding onto the AT_1 receptor. We have postulated that their molecular basis of action involves membrane incorporation and diffusion to the receptor active site. Thus, both the membrane and receptor site are components that determine the AT_1 antagonism. In an effort to comprehend their molecular basis of bioactivity, their effects on the lipid bilayers and receptor site have been studied. Conformational analysis, *in silico* docking studies, Molecular Dynamics simulations, Ala-scanning mutagenesis studies and pharmacophore modeling are performed for telmisartan in order to reveal its crucial structural characteristics and classify the importance of receptor's amino acids for ligand binding. Results of telmisartan are compared with other so far studied SARTANs. The thermal effects of telmisartan on lipid bilayers were studied using differential scanning calorimetry (DSC). Similarly, its effects were compared with other so far studied SARTANs in order to determine the role of the bilayers in the drug action.

Synthesis and Biological Evaluation of Modified Purine Homo-N-Nucleosides Containing Pyrazoline Moiety as Multitarget Agents

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Nucleosides represent a class of compounds that possess very interesting biological activities, especially antiviral and anticancer. Homo-N-nucleosides found to have also antiviral properties. We have reported recently (1) reactions of 9-allylpurines with mesityl nitriloxide and the study of their products as lipid peroxidation and thrombin inhibitors. In continuation of this we like to present here the products *3a,b* of 1,3-dipolar cycloaddition reactions of diphenylnitrilimine (*2*) with 9-allyl-6-chloropurine (*1a*) and 9-allyl-6-morpholinylpurine (*1b*). The analogs *3c-e* prepared from the reactions of piperidine (*4a*), N-boc-piperazine (*4b*) or pyrrolodine (*4c*) with derivative *3a* under MW irradiation. These compounds have been designed as multifunctional/multitarget agents. They are tested as lipoxygenase and lipid peroxidation inhibitors and simultaneously as thrombin inhibitors.

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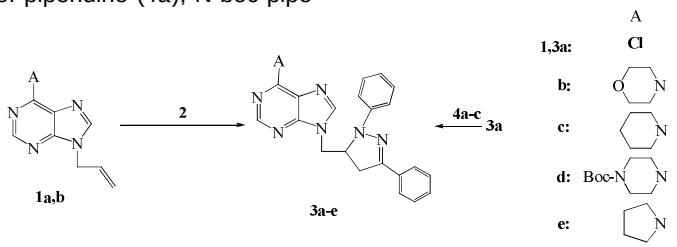

Functional Genomics for the Identification and Characterization of Genes Implicated in the Recruitment and Infltrtion of T-Lymphocytes in Ovarian Tumors

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Ovarian cancer is one of the most common type of cancer among women and the leading cause of death from gynecological malignancies. The molecular basis of ovarian cancer remains quite vague. Still, it is well documented that tumor microenvironment plays key role in tumor progression. For instance, lumphocytic infiltration at tumor sites can result in tumor growth inhibition and has been correlated with an improvement of clinical state and a rise of survival rates of patients with ovarian cancer. In this study we investtigated the gene expression profile of ovarian tumors enriched with CD8+ T lympho-cytes (TIL+ ovarian tumors), by applying a fluorescent version of ADDER (Amplification of Double stranded cDNA 3' End Restriction Fragments), an advanced method of Differential Display. Using this approach we identified for the first time a number of differentially expressed genes in TIL+ ovarian tumors. A significant proportion of these genes correspond to immune response markers, a great many encode for transcription factors while there are also many that correspond to transmembrane and secreted proteins. We are currently validating our results with quantitative Real-time PCR.

New Heterocyclic Arylidene Derivatives with Antioxidant and Anti-inflammatory Activity

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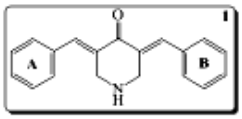

There are many evidences, which led to a close association between chronic inflammation and cancer. It is generally known that chronic inflamemation increase the risk of cancer and treatment with NSAIDs reduce the risk of certain cancer. Also the presence of a variety of inflammatory mediators, including cytokines, chemokines, tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2), matrix metalloproteases (MMP) in tumor tissues, angiogenesis are similar to that in chronic inflammation responses (1). Chalcones is a series of compounds, in which the two aromatic rings are joined by a three carbon, α,β-unsaturated enone system These compounds seem to have cytotoxicity, antitumor, anti-inflammatory, antiplasmodial, immunesuppres-sion, antioxidant and many other biological properties (2,3). Also they posses marked affinity for thiol but not for amino or hydroxyl groups, found in nucleic acids. Since thiols are absent in nucleic acids mutagenic and carcinogenic affects should be absent. Conversion of certain conjugated enones into the corresponding Mannich bases led to significant increases in both the rates of thiol alkylation and cytotoxicity (4,5). In our laboratory we tried to synthesize a series of 3,5- bis (arylidene)-4-piperidones for future development as anti-inflammatory, antioxidant and anticancer agents. These compounds are considered as curcumin analogues. We followed two different ways of chemical synthesis. Following a Claisen-Schmidt condensation between 4-piperidone hydrochloride and the appropriate hetero-aryl aldehyde led to the formation of novel derivatives (6,7). The compounds have been identified using IR, ^{1}H-NMR, ^{13}C-NMR, elemental analyses and mass spectroscopy. The role of lipophilicity is de-trimental for the biological response, thus lipophilicity was determined experimentally as Rm values using RPTLC. We present herein the preliminary results from antioxidant and anti-inflammatory activity tests *in vitro* and *in vivo.* The results are discussed in terms of structural characteristics. Further investigation is in progress for their anticancer activity

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RANP-mdf: Rapid Assignment of Natural Products from Crude PLANT Extracts based on Multi-Dimensional Fingerprint Analysis

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Natural products are privileged structures selected by evolutionary pressures to interact with a wide variety of proteins and other biological targets for specific purposes. The majority of them presents useful biological activities and has become important leads for drugs in a wide variety of therapeutic indications. The chemical space sampled by natural products is vast, and the rapid identification of known compounds (dereplication) is necessary in order to avoid the duplication of effort (1,2). Thus, cheminformatic navigation tools are necessary to map in complex extracts known compounds or/and to predict in accuracy the *families* that novel compounds belongs, without any prior fractionation or isolation processes. Herein, we developed a novel integrative cheminformatics system in order to rapidly assign from simple NMR spectra the identity of natural products that constitute complex plant extracts. This is the first time that a very high number of 2D NMR spectral data of natural products are collected in a database and then used for their direct identification in crude plant extracts, without any preliminary step of fractionation of the extracts, or isolation of the natural compounds.

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Structural Elucidation of Antihypertensive Agent BV6 and its Molecular Docking Study at Bace-1

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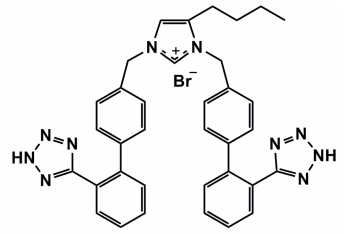

A non-peptide compound, BV6, has been synthesized as AT_1 antagonist and its chemical structure shown in Fig. 1 has been elucidated using a combination of 2D COSY and 2D NOESY experiments. BV6 has been found to mimic the action of losartan. As a promising, bioactive molecule was screened for several receptors in order to explore the possibility to serve as an inhibitor of other targets. The molecular docking results showed binding of BV6 at the human aspartic protease BACE-1. BACE-1 is the initial protease that processes amyloid precursor protein (APP) in the pathway, leading to Aβ proteins. Thus, BACE-1 has emerged as a promising pharmaceutical target for combating Alzheimer desease. These results propose the testing of the molecule for potential beneficial properties against Alzheimer disease.

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Synthesis of Polyphosphorylated Myo-Inositol Dimers with Potential Allosteric Activity of Hemoglobin

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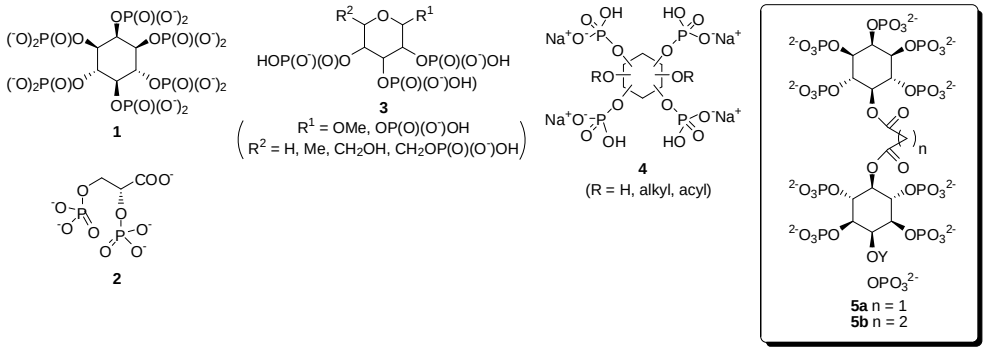

Myo-inositol hexakisphosphate (IHP, *1*) is the strongest allosteric effector of hemoglobin (Hb) in red blood cells (RBCs) identified to date. It displaces Hb-bound 2,3-bisphosphoglycerate (BPG, *2*) by binding to the allosteric pocket with higher affinity, triggering a decrease in oxygen/Hb affinity and subsequently leading, upon entrance into circulating RBCs, to increased and regulated oxygen release upon tissue demand. As numerous ailments involve hypoxia, including cardiovascular diseases and cancer, achieving increased oxygen release is expected to restore normoxia. High effector activity toward Hb, similar to that of IHP, has recently been observed for several structurally related perphosphorylated hexapyranoses and pentapyranoses (e.g. *3*) and inositol tetrakisphosphates (ITPs, *4*). Investigation of the structure-activity relationships in allosteric Hb regulation identified that, among others, the ability of the compounds to lower the Hb affin-

ity for oxygen is directly related to their number of negative charges; that is, a greater number of phosphates corresponds to a higher allosteric effect. These results prompted us to design and synthesize novel *myo*-inositol dimers in which the two inositol rings are linked by a diester bridge, namely decakis phosphates of bis-inositolyl malonate and succinate (*5a* and *5b*, respectively). Preparation of these sodium salts was achieved following a highly efficient seven steps synthetic scheme using parent *myo*-inositol as starting material. The obtained polyphosphates will be further evaluated for their biological activity.

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Biochemical Characterization of Novel Ketolide Antibiotics *in vivo* and *in vitro*

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Ketolides represent the last generation of macrolide antibiotics. They are active against erythromycin resistant strains while maintaining their activity against erythromycin susceptible ones. Telithromycin and cethromycin are the most pioneer compounds among them with telithromycin being already in the market. Both drugs are 3-keto derivatives of erythromycin with a fused 11, 12-cyclic carbamate group. In addition, an alkyl-aryl side chain is linked to the lactone ring either through the N atom of the carbamate group or through the O-6 of the lactone ring. A new version of ketolides is fluoroketolides, ketolides carrying fluorine atoms attached to the lactone ring. In this study we present data for such new compounds that carry an alkyl-aryl chain at O-6 of the lactone ring and one or two fluorides at C-2 or C-13 of the ring. According to our genetic and biochemical studies the new ketolides bind in to the nascent peptide exit tunnel, at a site previously described as the site of interaction of macrolide antibiotics. The presence of the side aryl chain plus fluorine atoms promotes the formation of specific interactions with helix 35 of 23S rRNA causing an increased affinity for ribosomes that may be responsible for the effectiveness of the new ketolides.

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Regioselective Biotransformation of Natural Derived Polyphenols

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Natural products and especially flavonoids cover a very interesting chemical space of biological relevance due to their vast chemical diversity, and fine-tuning for optimal interactions with biological macromolecules through evolutionary selection (1). However, they usually suffer from low bioavailability upon oral administration, probably because of their low lipophilicity. Indeed, recent studies indicated that o-methylated flavonoids illustrated higher intestinal absorption, resistance to hepatic metabolism and better anticancer activity in respect to the hydroxylated compounds (2). In order to achieve regioselectivity in acylation reactions of flavonoids and avoid the tedious protection/deprotection steps required in a chemical synthesis method, due to the numerous reactive hydroxyl groups of this flavonoid, we turned to biocatalysis. We achieved high conversion yields as also high degree of regioselectivity of aglycone flavonoids to relevant esters by enzyme transformations (3). In the frame of this method an increase of the natural biodiversity of flavonoids could be achieved toward the production of more potent bioactive derivatives through biotransformation.

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Synthesis of an HIV-1 Epitope, Identified from an Elite Controller, for Vaccine Purposes

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The HIV-1 epidemic continues spreading around the world with many new infections per day and more than 40 million people living with HIV-1 (1). Development of an effective vaccine is still an urgently needed priority in AIDS. However most of the efforts undertaken have failed so far as the virus has evolved numerous mechanisms to protect its functionally important entry domains from the immune system. Elite controllers (EC) is a subgroup of long term non progressors, which are persons controlling the infection over decades without any antiviral therapy. Based on the phage display technology (2) epitopes for HIV-specific antibodies in EC sera were identified and synthesized. One of the epitopes belonging to the external region of gp41 was synthesized on a Rink amide resin following the solid phase Fmoc technology. Palmitoyl group (3) was incorporated in the α amino terminal group as adjuvant. Immunization experiments for the induction of neutralizing antibodies and the formulation of vaccine candidates are currently in progress.

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Design and Synthesis of Permethylated β-Cyclodextrin/LHRH Analogue Conjugates

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Cyclodextrins are cyclic oligosaccharides consisting of a hydrophobic cavity that is capable of including a variety of hydrophobic compounds via host-guest complexation (1). This property has been extensively explored in the past to change the physicopharmaceutical properties of lipophilic drugs such as water-solubility, bioavailability, improved stability, and effectiveness. Covalent linkage of bioactive peptides to cyclodextrins has also been proposed (2,3) to possibly take advantage of their complexation in terms of solubility and reduced enzymatic degradation, although such conjugates with the relatively large cyclodextrin carrier it's possible to affect the active site recognition process. Luteinizing Hormone Releasing Hormone (LHRH) is a linear decapeptide which is produced in the hypothalamus under the control of neurotransmitter type compounds (4) and it is the central regulator of the reproductive system (5). LHRH agonist analogues, such as Leuprolide, Buserelin are widely used for the treatment of hormone depended cancer and they have found to be more potent than the natural hormone. The goal of this study was the design and synthesis of permethylated β-cyclodextrin/LHRH analogue conjugates with improved biophysical properties, compared to natural hormone, in order to be studied for antitumor activity (6,7).

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In *vitro* and *in vivo* Studies of two Novel Organometallic Technetium99m Complexes Bearing Bifunctional Chelators, for Application in Targeted Radiopharmaceuticals

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Technetium-99m and rhenium-188 radiopharmaceuticals have important applications in Nuclear Medicine SPECT imaging and in radiotherapy, respectively. Organometallic *fac*-[Tc/ReL$(CO)_3$] complexes have gained significant attention in radiopharmaceutical chemistry, since they exhibit kinetic inertness and they are conveniently synthesized at the tracer level. The successful development of targeted diagnostic and therapeutic radiopharmaceuticals involves the bifunctional chelating agent (BFCA) strategy for suitable radiolabeling of biomolecules. In the present work we present the *in vitro* and *in vivo* studies of two novel technetium complexes of the general formula, *fac*-[^{99m}Tc(NSO)$(CO)_3$]. SNO is tridentate ligands, L^1 and L^2, which are thioether derivatives of 3-(1H-imidazol-4-yl)-propanoic acid with thioglycolic acid in position 3 and 2, respectively. Both *fac*-[^{99m}Tc$(CO)_3$] complexes bear a second free carboxylic group that can function as an anchor to conjugate amine containing biomolecules. The synthesis, characterization and structure of the ligands and complexes were reported in our recent works. Studies of the *in vitro* stability showed that the technetium-99m complexes were stable in 1mM L-histidine and 1mM L-cysteine over 24 hours. *In vivo*, biodistribution experiments in mice showed that the complexes exhibit fast blood clearance and no significant uptake in tissue. One hour post injection, all the activity is eliminated primarily via the urinary system. HPLC analysis of rat urine confirmed that the complexes were not metabolized, as expected. Our data indicate that the *fac*-[^{99m}Tc$(L^1/L^2)(CO)_3$] complexes developed, can be successfully applied for the design of targeted radiopharmaceuticals

Identification and Quantification of Hypericum Perforatum Constituents by a Newly Developed High Performance Liquid Chromatography Method

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Hypericum perforatum is widely known for its pharmacologic properties, its constituents (i. e. hypericin, hyperforin, flavonoids) are considered to be responsible for anti-depressant and anti-inflammatory effects. For the quality control of the methanolic and aqueous extracts of *Hypericum perforatum* an HPLC-UV method was developed and validated. A great variety of elution gradients were examined using methanol, acetonitrile and organic buffers and the final optimum conditions were selected based on the intensity and the good resolution of the chromatographic peaks. More than 15 natural products were completely separated by a linear gradient of 10mM ammonium acetate (pH=4.5) -acetonitrile-methanol in one run within 60 min on a C-18 Luna column. The chromatographic method was validated using commercially available standards of chlorogenic acid, rutin, hyperoside, quercetin, isoquercetin and hypericin. Linearity graphs were constructed for each compound and the repeatability values were within acceptable limits. The developed method can be applied towards the quality control of hypericum extracts and the characterization of the composition of *Hypericum* taxa, which have not been studied.

^{99m}Tc-Neuromedin C Analogs in GRPR^{+} Tumor Imaging

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Gastrin releasing peptide receptors (GRPR) have become attractive targets for diagnostic imaging and radionuclide therapy with radiolabeled bombesin analogs due to their high expression in major human cancers, as in prostate and breast cancer. While originally engaged in the development of bombesin radiopeptides we have recently expanded our search toward Neuromedin C (NMC)-based radioligands. The prototype radiotracer, [^{99m}Tc]SAR-NC1 ([^{99m}Tc-N$_4$]NMC), displayed promising properties in GRPR^{+}-models but limited metabolic stability. To improve biological profile, we synthesized a series of SAR-NC analogs by single Gly^{24} (dAla^{24}, βAla^{24}, Sar^{24}) or double Gly^{24} (dAla^{24}) / Met^{27} (Leu^{27}, Nle^{27}) substitutions in the SAR-NC1 motif. The resulting SAR-NC analogs displayed high GRPR-affinity (IC_{50} in the 0.36-13.2 nM range) during competition binding assays in PC-3 cell-membranes against [^{125}I-Tyr^{4}]BB. [^{99m}Tc]SAR-NCs were easily obtained in >95% labeling yields and >1 mCi/nmol specific activities by mixing SAR-NCs, $^{99m}TcO_4^-$, $SnCl_2$ and citrate anions in alkaline medium. All radiopeptides efficiently internalized in PC-3 cells via a GRPR-mediated process. Analysis of the *ex-vivo* blood 5 min pi of [^{99m}Tc]SAR-NCs by HPLC revealed a great impact of attempted modifications on radiopeptide survival in the mouse bloodstream with the doubly dAla^{24}/Leu^{27}-substituted radiotracer displaying the highest stability (>50%). Biodistribution in PC-3 bearing SCID mice showed that dAla^{24}-[^{99m}Tc]SAR-NC displayed the most favorable pharmacokinetic profile in terms of high tumor uptake and fast background clearance.

Complexes of Pt(II) and Pd(II) with Benzothiazole Derivativesa as Anticancer with Combined Activity

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The development of new antitumor agents that will be less toxic and more selective is one of the more active research fields of Medicinal Chemistry. Compounds within the 2-(4'-aminophenyl)-benzothiazole class represent extremely potent and selective antitumor agents (1). Recent evidence has demonstrated a correlation between cell sensitivity to benzothiazoles and the extent of DNA adduct formation (2). On the other hand, several metal complexes have been found to be effective drugs in the treatment of cancer (3), with cisplatin the most representative among them (4). We present herein the synthesis, structural characterization, and evaluation of DNA interactions of novel Pt(II) and Pd(II) complexes with 2-(4-aminophenyl)benzothiazole derivatives. The complexes are anticipated to retain the selectivity profile of the benzothiazole moiety, as well as exhibit synergistic cytotoxicity due to the DNA targeting properties of both the metal core and the benzothiazole pharmacophore. The complexes were successfully synthesized and characterized by ^{1}H and ^{13}C NMR spectroscopy. Circular dichroism and UV-Vis studies revealed strong interactions of the novel complexes with DNA, suggestive of DNA penetration and structure alteration. Thermal denaturation studies equally suggest strong DNA binding through significant reduction of % hyperchromicity, premelting effects and DNA dehelixing.

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Development of Analytical Techniques for the Qualitative and Quantitative Determination of Allantoin and Glycolic Acid in Snail Secretions and Cosmetic Creams

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Snail mucus has a significant economic-commercial interest due to the presence of valued chemical components such as allantoin and glycolic acid. These compounds are used as active ingredients in pharmaceutical and cosmetic products. For the quantitative and qualitative analysis of these two substances appropriate analytical methodologies were developed. High performance liquid chromato-graphy (HPLC) se-paration of both compounds was achieved on a Synergi RP (Phenomenex®) column within 6 min. After a careful study of the mobile phase and extraction medium composition, final separation and extraction conditions were selected. The developed method analytical characteristics (i.e. linearity, precision, accuracy, stability) have been validated. An effort is also made for the analysis of the mucus by non destructive techniques such as Raman and ATR without the use of separation techniques.

Synthesis of Antimicrobial Cationic Peptides for the Development of New Antibiotics

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Antimicrobial cationic peptides (AMPs) have been found to have activity against both Gram negative and Gram positive bacteria as well as fungi, eukaryotic parasites viruses, and most importantly, they seem to be effective against many strains of antibiotic resistant. Synthetic cationic peptides are a class of positively charged small peptides with *amphipathic* helical conformation. In the present study, cationic helical peptides of the following type were synthesized in order to develop new antibiotics:

X-RWLRLLWRFLRL-NH_2, X-RWLRLWRRFLRL-NH_2, X-RWLRLLLRLLRL-NH_2, X-RWLKLLWRFLKL-NH_2, X-KWLKLLWKFLKL-NH_2

where X = H- , Ac- , Ahx-

Synthesis was performed by the conventional stepwise Fmoc/tBu solid-phase method. The peptides were purified by HPLC and identified by ESI-MS. The above peptides were tested for their antimicrobial activity against gram(-) bacteria, gram(+) bacteria and fungi. The peptides were also tested for their hemolytic activity and proteolytic stability. The conformational characteristics of the peptides models were evaluated by circular dichroism spectroscopy (CD).

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New Mixed Ligand Silver(I) Complex with Aspirin and Triphenylphosphine Ligands which Can Bind to LOX and CT-DNA, Modulating their Function. Study of its Cytostatic Activity

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Aspirin (aspH) (o-acetyl-salicylic acid) was the first member of the class of drugs known as non-steroidal, anti-inflammatory drugs (NSAIDs) discovered (1). A number of epidemiological studies have indicated that long term aspirin/NSAID use is associated with 30-50% reduction in risk of colorectal cancer or adenomatous polyps or death from colorectal cancer (1). Lipoxygenase (LOX), on the other hand, is an enzyme which catalyzes the oxidation of arachidonic acid to leukotrienes, in an essential mechanism for the cell life, involving in inflammation mechanism (2a). LOX inhibition is found to induce apoptosis (2b)**.** Thus, LOX inhibition provides a potential novel target for the treatment and chemo-prevention for a number of different cancers. Moreover, the biomedical applications and uses of silver(I) complexes, are related to their anti-bacterial action (3) which appears to involve interaction with DNA (3). In the course of our studies in the field of metallotherapeutics (4), we synthesized and structural characterized a new mixed ligand Ag(I) complex with aspirin (AspH) and triphenyl-phosphine (tpp) ligands of formula {[Ag(tpp)$_3$(asp)](dmf)} (*1*). The mechanism of inhibition activity of *1* and its ligands, towards the catalytic oxidation of linoleic acid to hydroperoxy-linoleic acid by LOX was subsequently studied kinetically and theoretically. The binding properties of *1* on calf thymus (CT) DNA are also evaluated. In addition, the cytostatic activity of *1* against leiomyosarcoma cells (LMS) and human breast adenocarcinoma cells (MCF-7) proliferation were measured. The results are com-pared to that of cisplatin.

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Mononuclear *vs* Dinuclear Cu(II) Complexes: Synthesis, Pharmacochemical and Anticancer Activity

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In the last decade, a great deal of research has been devoted to the study of different types of anti-inflammatory/antioxidants which may at least minimize the deleterious effects induced by reactive oxygen species (ROS). The relative potency/efficacy of mononuclear versus dinuclear moreover polynuclear Cu(II) complexes is limited and this is a subject of our interest. We have undertaken a study on the coordination chemistry, charachterization, pharmacochemical and anticancer activity of new mononuclear and dinuclear Cu(II) complexes with thiophen-2-yl saturated carboxylic acids (1), an emerging class of antioxidants-antiinflammatories, different tripodals and N-donors (Figure). *In vitro* studies of free ligands and their respective copper complexes, include: a) soybean lipoxygenase inhibition, b) interaction with 1,1-diphenyl-2-picryl-hydrazyl stable free radical, c) the HO˙ mediated oxidation of DMSO, d) inhibition of lipid peroxidation and e) scavenging of superoxide anion radicals. Human colon, ovarian, breast and lung cancer cell lines were selected for anti-cancer screening. For the cell cycle and apoptosis experiments, were selected the more active compounds at IC_{50} concentrations.

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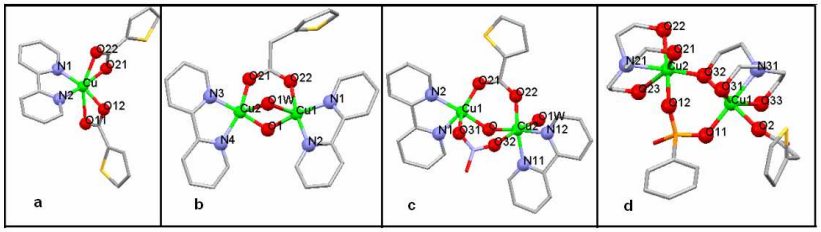

Figure: a) $[Cu(bipy)(C_4H_3S\text{-}COO)_2]$, b) $[Cu_2(bipy)_2(OH)(H_2O)(C_4H_3S\text{-}CH_2COO)]$ $2NO_3$, c) $[Cu_2(bipy)_2(OH)(H_2O)(C_4H_3S\text{-}COO)(NO_3)]$ $(NO_3)(H_2O)$, d) $[Cu_2(H_3tea)(H_2tea)(C_4H_3S\text{-}CH_2COO)(PhPO_3)]$ $(H_2O)(CH_3CN)$

Microwave Solid Phase Peptide Synthesis of Two MBP Analogues on CLTR-Cl Resin and Conjugation Thereof with Polysaccharide Mannan

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Herein, we report the synthesis of two Altered Peptide Ligands (APLs) of Myelin Basic Protein [$(Lys\text{-}Gly)_5\text{-}MBP_{83\text{-}99}(Phe^{91})$, $(Lys\text{-}Gly)_5\text{-}MBP_{83\text{-}99}(Tyr^{91})$] implicated in Multiple Sclerosis (MS). In these APLs, Lys^{91} of MBP has been replaced by Phe or Tyr, since the residue 91 is critical for the contact with the T cell receptor in the trimolecular complex (MHC-Ag-TCR). The $(Lys\text{-}Gly)_5$ is the bridge through which the analogues were conjugated with polysaccharide mannan from *Saccharomyces Cerevisiae* via the formation of Schiff bases. Mannan has successfully been used as a peptide carrier to the mannose receptor of macrophages and dendritic cells, while experiments have shown that peptide analogues conjugated with the oxidized or reduced form of mannan lead to Th1 or Th2 immune response respectively (1). The linear protected analogues were synthesized with Microwave Irradiation using the Liberty™ Microwave Peptide Synthesizer of CEM on 2-chlorotrityl chloride resin (CLTR-Cl) (2-4)]. Fmoc deprotection was achieved with 20% Piperidine in DMF, while for the coupling reactions were used HOBt/DIC in DMF. The final crude products were purified by semi-prep RP-HPLC and identified by ESI Mass Spectrometry.

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doi: 10.1007/s00726-010-0753-6

Prediction of Pharmacokinetics Properties of some Analogues with Antifungal and Antimicrobial Activity

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The prediction of the pharmacokinetic profile of some analogues (Figure 1) (1-3) with antifungal and antibacterial activity was performed in this thesis using VolSurf (4). The aim of this study is to propose novel structures that might possess better pharmacokinetic profile as antifungal and

antibacterial compounds. VolSurf is a computational procedure that is using 3D molecular field maps and it is specifically designed to produce descriptors related to pharmacokinetic properties. The molecules were projected on the following precalculated ADME models: Caco-2 cell permeability, plasma protein affinity, blood-brain barrier (BBB) permeation and drug-water solubility. It is predicted that most of the studied compounds can be transported across the intestinal epithelium. The same profile for the affinity to the plasma-protein was observed. Regarding to the BBB model, some molecules were able to cross the blood brain barrier and some molecules failed. For the drug-water solubility model, all compounds possessed low to medium aqueous solubility. This study shows that improvement in the synthesis can be achieved regarding the ADME properties.

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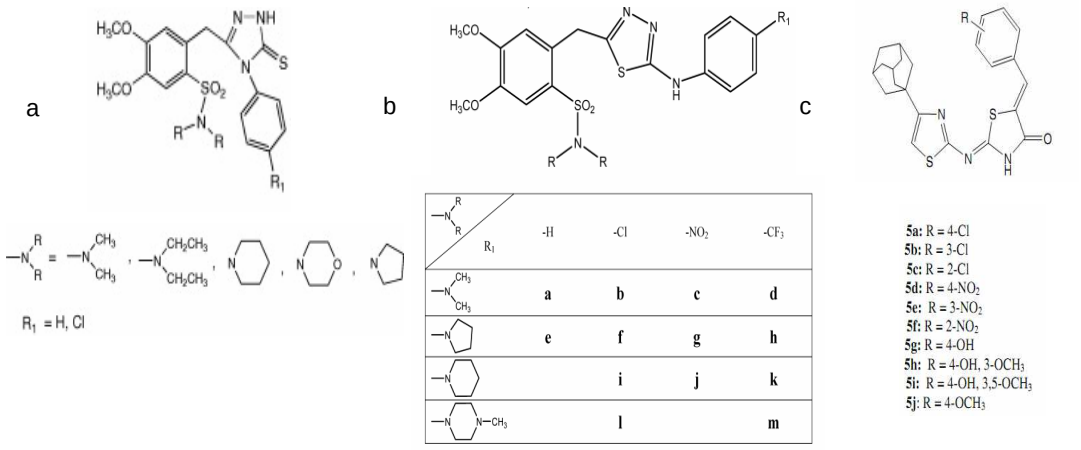

Figure 1: a. Sulfonamide-1,2,4-triazole derivatives, b. Sulfonamide-1,2,4-thiadiazole derivatives, c. 4-thiazolidinones derivatives

Thermal and Dynamic Properties of Drug AT_1 Antagonist Olmesartan in Lipid Bilayers

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Angiotensin II receptor blockers (ARBs) have been designed to inhibit the binding of angiotensin II (AII), onto the AT_1 receptors. Olmesartan medoxomil belongs to the antihypertensive class of ARBs. This drug is an ester prodrug of the active metabolite (olmesartan), which is deesterified in the gastrointestinal tract. In this study, solid state NMR (ssNMR), Raman spectroscopy, x-ray diffraction as well as Differential Scanning calorimetry (DSC) were applied to investigate the thermal and dynamic properties of olmesartan in Lα-dipalmitoylphosphatidylcholine (DPPC) bilay-

ers in the absence and presence of cholesterol. Cholesterol is a major component of the cell plasma membrane and its role is essential to establish proper *membrane permeability* and *fluidity*. The aim of this research work is to study olmesartan/lipid interactions in a temperature range from 20°C up to 50°C covering all mesomorphic phases. The results will be compared with those obtained with other AT_1 antagonists in order to investigate the role of lipid bilayers in the drug action.

❁

New 2+1 Mixed Ligand Rhenium Complexes of the General Formula FAC-[RE(OO)(L)$(CO)_3$]

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The organometallic core $[M(CO)_3]^+$ (M=Tc,Re) reacts readily with a variety of ligand combinations, allowing the design of complexes with desired properties. In this study, the *2+1* mixed ligand system was employed through the use of the $[NEt_4]_2[Re(H_2O)_3(CO)_3]$ synthon, in which the three water ligands are substituted by one bidentate and one monodentate ligand. The ligands chosen were maltol and kojic acid as bidentate ligands with OO donor atom groups, and imidazole (L_1), isocyanocyclohexane (L_2) and triphenylphosphine (L_3) as monodentate L ligands. Both bidentate and monodentate ligands can be easily derivatized/modified with attachment of a pharmacophore of interest to generate complexes with targeted action. The synthesis takes place through the intermediate formation of the *fac*-$[Re(OO)(H_2O)(CO)_3]$ precursor complexes. The monodentate ligands imidazole and triphenylphosphine reacts readily with the precursor complex in refluxing methanol to generate the corresponding *fac*-$[Re(OO)(L)(CO)_3]$ complex, while for the isonitrile ligand the reaction takes place at ambient temperature. HPLC analysis of the reaction mixture showed the formation of a single product. All complexes were isolated and fully characterised by elemental analysis, IR and NMR spectroscopies. The structure of the *fac*-[Re(maltol)(imidazole)$(CO)_3$] complex was confirmed by X-ray crystallography.

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Studies toward a Novel Total Synthesis of Ecteinascidin-743: New Reaction of TMS-Imines with TMS-Nucleophiles

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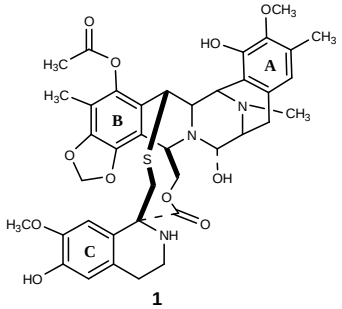

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Ecteinascidin 743 (Figure 1) isolated from the Caribbean tunicate *Ecteinascidia turbinate* (1) is arguably the most potent cytotoxin known as indicated by its evaluation against the National Cancer Institute's human *in vitro* cell line panel including melanoma, non-small-cell lung, ovarian, renal, prostate, and breast cancer, demonstrating *potencies* ranging from 1 pM to 10 nM (2). In fact, the antiproliferative activity of Et 743 is greater than that of Taxol, camptothecin, adriamycin, mitomycin C, cisplatin, bleomycin, and etoposide

by 1-3 orders of magnitude, propelling trabectidin (*1*) to become the first marine anticancer drug to be approved (October 2007) in the European Union (EU) (3) as a first-line treatment for soft tissue sarcomas. The complexity of molecular architecture, the remarkable biological activities, and the restricted natural availability (1.0 g from about 1.0 ton of tunicate) have made *1* an exceedingly attractive synthetic target for total synthesis (4). Our studies toward the validation of key elements of our retrosynthetic analysis will be presented including the general and useful reaction of the title.

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Enzymatic Synthesis of Novel Antioxidant Hybrids for the Prevention of Hypertension-Induced Cardiovascular Damage

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Oxidative stress is a well-known mechanism that is responsible for the development of vascular damage (1). A diverse set of pathogenic stimuli exert their effects through an increased local synthesis of reactive oxygen species. These active metabolites can modify the normal balance among proliferation and apoptosis in heart and arterial walls. We focused on the development of a new class of antioxidant derivatives by conjugating to them hypertension protective fragments. To afford stereoselectivity and regioselectivity in the reaction products we used enzyme catalysts (lipases) in non-conventional media (2). Factors affecting the effectiveness of the biolactalytic processes were investigated. Biological experiments to determine the dual bioactivity character of these analogues are in progress.

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Design, Synthesis and Evaluation of Efficient Peptide Catalysts for Direct Asymmetric Aldol Reactions

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Organocatalysis is regarded as the application of small organic molecules as catalysts to a variety of organic processes and has recently become very popular for the synthesis of chiral molecules (1). Successful examples of organocatalytic reactions exists, including aldol, Mannich, Michael and hetero Michael reactions as well as α-functionalisations of carbonyl compounds. The aldol reaction is a powerful carbon-carbon forming reaction that has been used in the synthesis of numerous molecules and pharmaceuticals, like the heart disease drug lipitor (atorvastation) (2). We will present an array of different efficient peptide-based organocatalysts for assymetric aldol reactions that were designed on the basis of the active site of a much larger enzyme (type I aldolase), acting via a similar enamine-type mechanism.

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Synthesis, Competition Binding and Conformational Studies of New Analogues of C-Terminal Hexapeptide of Neurotensin

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Neurotensin (NT), a tridecapeptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) was first isolated from bovine hypothalamus. NT displays a wide spectrum of biological actions in the central nervous system as well as the periphery of different mammalian species (1). At central level, NT plays the role of neurotransmitter and/or neuromodulator, whereas in the anterior pituitary it regulates hormone secretion. In addition NT plays an important role in the function of the digestive tract and the cardiovascular system of mammals as well as in the regulation of growth of normal and cancer cells. The plethora of NT actions is mediated through its binding to NT recaptors (NTRs). The NTRs are plasma membrane proteins which belong to the family of G-protein coupled receptors (2). The C-terminal hexapeptide fragment of NT [NT(8-13)], which contains the necessary structural requirements to bind and elicit biological effects at NT receptors, is an obvious lead compound for development. However NT(8-13) is rapidly degraded by peptidases. Therefore, it is important to synthesize functional NT analogues with stabilized bonds against metabolic deactivation. Based on the above, we herein report the synthesis of new linear and cyclic analogues of NT with modifications in the basic structure of the C-terminal part of the molecule needed for function in order to improve the metabolic stability. The analogues contain D-Tyrosine(Ethyl) [D-Tyr(Et)] or D-1-naphtylalanine [1-Nal] in position 11, D-Arginine in position 8 or 9 and L-Lysine in position 8 or 9. They were synthesized by the Fmoc/Bu^t solid phase methodology (3) utilizing 2-chlorotrityl chloride resin and are now being tested for their functional properties in binding studies. Competition radioligand binding studies were performed in membrane homogenates from HT-29 cells endogenously expressing NTRs (4). A series of NMR spectra including ^{1}H, 2D TOCSY and 2D NOESY were recorded on a Varian 600MHz spectrometer for two cyclic analogues in order to elucidate their structure. The conformational properties of the two peptides, as well as similarities and differences between them are currently studied using NMR spectroscopy and Molecular Dynamics simulations.

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B and T Cell Epitopes of Hemagglutinin (HA1) from H1N1 and H5N1 Influenza Viruses for Immunization Studies

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The possibility of an outbreak of H5N1 and H1N1 avian influenza virus is not considered a remote possibility if uncontrolled. Therefore, there is a great deal of interest in examining the various factors involved in the entry of the virus to the host cells, the interspecies transmission and the identification of B and T cell epitopes (1). Understanding of the immune response after infection

with the virus is critical for the design of vaccines, drugs and diagnostics.Using a bioinformatics approach, named informational spectrum method (ISM), it was found that HA1 proteins, although highly variable molecules, encode conserved information, which might determine receptor-binding preferences (2). B and T cell epitopes, either free or ligated to an artificial carrier were synthesized and are currently used in immunization experiments to understand the host humoral and cellular immune responses.

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Methodologies towards the Discovery Novel Trypanothione Synthetase Inhibitors with Potential Antileishmanial Activity

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Protozoan parasites of the trypanosomatidae family are the causative agents of various forms of Leishmaniasis (Leishmania species). Trypanothinone Synthetase (TryS) is the sole enzyme responsible for the biosynthesis of trypanothione in the human pathogenic parasites *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major.* Therefore, TryS inhibition is considered to be a particularly attractive strategy to fight leishmanial infections. A model of Leishmania major-TryS has been produced, based on the crystal structures of Leishmania major - Trypanothione Synthetase Amidase (TSA) (1) and E. coli – glutathionylspermidine synthetase (GSPS). Based on the docked complex of the TryS with glutathione, a pharmacophore model was generated. Pharmacophore-based *in silico* screening of commercially available compound libraries in combination with molecular docking studies has led to the identification of putative inhibitor candidates.

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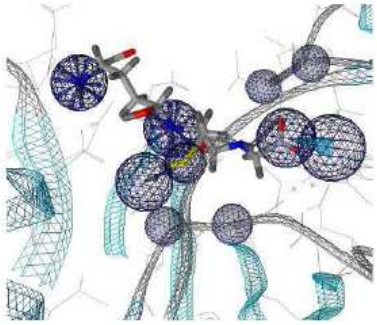

Conformation of Angiotensin II TFA Salt in Amphoteric Environment: NMR Spectroscopy and Molecular Dynamics Simulations

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The octapeptide hormone Angiotensin II (AII) is the primary active agent produced by the rennin-angiotensin cascade, directly correlated to hypertension. It plays a central role in the regulation of blood pressure and electrolyte homeostasis (1). The conformational analysis of AII TFA salt is

studied in amphoteric environment (DMSO), which better simulates the biological environment. Assignment of AII TFA salt was achieved by 2D TOCSY and ROESY NMR spectra, while critical ROE intramolecular distances were used as constraints at Molecular Dynamics (MD) simulations. The results of these simulations reveal that AII adopts a folded conformation caused by the backbone bend at the residues Tyr4, Ile5 and His6, as their chains are oriented on one side of a plane defined by the peptide backbone, while the Val3 and Pro7 are oriented at the opposite side of the plane. The stabilization of the folded conformation can be explained by hydrophobic interacttions between Val3 and Pro7 side chains and by a hydrophobic cluster formed by the Tyr4, Ile5 and His6 side chains. This conformation of AII is similar to the one previously described in aqueous environment (2).

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Unexpected Side Reactions in the SPPS of Cys-contained Peptides

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In the past the formation of peptide side products upon the decomposition of resin linkers during cleavage of the peptides in the Fmoc strategy has been referenced (1,2). Synthesis of several cys-containing biologically active peptides on specific solid supports afforded significant amounts of side-products. We used a battery of analytical tools and monitored the occurrence of an unexpected alkylation on cystein residues as a result of resin decomposition under TFA treatment. The sequence dependence of this alkylation as also reaction optimization efforts will be presented (3).

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Docking Studies of Novel Synthetic Analogs at Monoglyceride Lipase

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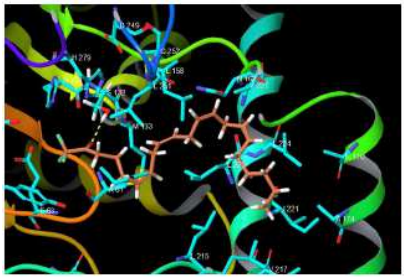

Molecular docking of the synthetic analog ATFMK in the active site of MAGL receptor

2-Arachidonylglycerol (2-AG) possesses a wide range of pharmacological properties including the modulation of neurotransmitter release, control of neuroinflammation and regulation of cancer cell growth to stress-induced analgesia. Monoglyceride lipase (MGL) plays an important role in its metabolism by *in vivo* hydrolyzing it. To restore its pharmacological properties MAGL inhibition might be beneficial. We are applying docking studies aiming to formulate novel structures with higher binding scores as potential synthetic new leads. One significant problem to apply rational

design in the inhibition of MAGL enzyme is the use of various biological essays which in some cases lead to controversial results. To overcome this difficulty we have applied docking results at the molecules subjected to identical biological assays. Another problem encountered in the application of docking studies is the presence of only one x-ray crystallographic result in which MAGL is co-crystallized with JZL-184. Such an absence of crystallographic data make difficult the decision of the exact mode of action for novel compounds as they found to approach in a different ways the active or allosteric sites.

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Synthesis and Biological Evaluation of Fused Pyrido[f]Coumarin Derivatives

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Fused pyridocoumarins possess interesting biological activities (1). Fused pyrido[f]coumarin derivatives are received in this work. The 6-aminocoumarin derivatives *1a-d* prepared by reduction of the corresponding 6-nitrocoumarin derivatives and with propargylation (2) by propargyl bromide (*2*) resulted to the 6-propargylaminocoumarin derivatives *3a-d*. MW irradiation of the later in DMF in the presence of BF_3/Et_2O led to the fused pyrido[f]coumarin derivatives *4a,c,e*. The received new compounds are tested as antioxidants (3) and for their influence on lipid peroxidation (3).

Acknowledgements: This work was supported by the Program HRAKLEITOS II (T. Symeonidis)

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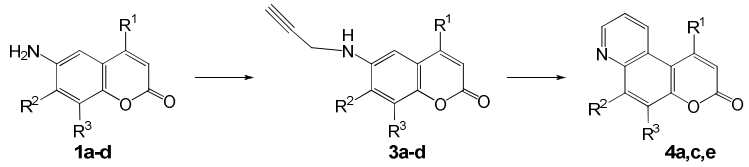

1,3,4 a: $R^1=R^2=R^3=H$
b: R^1= COOMe, R^2-R^3= CH=CH-CH=CH
c: R^1= H, R^2-R^3= $(CH_2)_4$
d: R^1= COOMe, R^2-R^3= $(CH_2)_4$
e: R^1= H, R^2-R^3= CH=CH-CH=CH

❁

Synthesis and Biological Evaluation of ^{111}In-Labeled [DOTA^0,Arg^4,DTrp^8,DCys^{14}]SS14

A. Tatsi[1,2], T. Maina[1], B. Waser[3], R. Cescato[3], P. J. Marsouvanidis[1], E. P. Krenning[4], M. de Jong[4], J. C. Reubi[3], P. Cordopatis[2], B. A. Nock[1]

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We have recently been engaged in the development of *pansomatostatin* radioligands capable to target $hsst_{1\text{-}5}^+$-tumors *in vivo*. One of our first analogs, [^{111}In-DOTA^0,DTrp^8]SS14 ([^{111}In]AT2S), a true pansomatostatin radioligand, could target such tumors only to a moderate extent due to fast

breakdown in the blood of mice. We now attempted further stabilization of this motif by replacing Cys^{14} by $DCys^{14}$ in the peptide ring. This modification was previously reported to enhance biological activity in a series of *in vivo* assays. Further substitution of Lys^4 by Arg^4 aimed to reduce renal values. The resin-anchored DOTA-Ala^1-Gly^2-Cys^3-Arg^4-Asn^5-Phe^6-Phe^7-$DTrp^8$-Lys^9-Thr^{10}-Phe^{11}-Thr^{12}-Ser^{13}-$DCys^{14}$ sequence was cleaved from the resin and deprotected with TFA. Cyclization of Cys^3-$DCys^{14}$ was achieved by iodine oxidation. During $hsst_{1\text{-}5}^{+}$-autoradiography, AT8S exhibited good affinity to all $hsst_{1\text{-}5}$ with the respective IC_{50}s (nM) determined as follows: $hsst_1$=38±18; $hsst_2$=1.6±0.5; $hsst_3$=16±6.2; $hsst_4$=2.8±0.3; and $hsst_5$=25±11. By HPLC analysis of blood collected 5 min after injection of [^{111}In]AT8S in mice 10% of the original peptide was still detected. In SCID mice bearing AR4-2J tumors, [^{111}In]AT8S specifically localized in the $rsst_{2A}^{+}$ implants (1.43±0.41%ID/g *vs*. 0.24±0.05%ID/g – block, at 4 h pi). By introduction of a second D-amino acid in the 12-member ring of [^{111}In]AT8S, the pansomatostatin character was preserved while *in vivo* stability moderately increased and tumor uptake remained sub-optimal. Despite reduction of kidney values by half as compared to [^{111}In]AT2S further pharmacokinetic improvements are warranted.

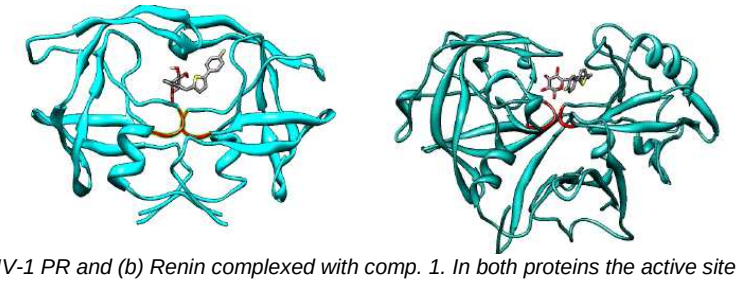

Figure 1: (a) HIV-1 PR and (b) Renin complexed with comp. 1. In both proteins the active site is coloured red

The Interactions of Drugs With Aspartic Proteases

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Two proteins belonging to the family of aspartic proteases, HIV-1 protease and renin, are complexed with the same ligand (compound *1*) are studied in order to identify the differences and similarities in their mode of action. As members of the same family of proteins, they share common structural and functional characteristics. The catalytic site (Asp-Thr-Gly) for both proteins resides in a cavity (Figure 1). Molecular dynamics simulations and MM/PBSA methods were employed for the study of the interactions between the proteins and the ligand. The aim of this study is to identify the similarities and differences of the binding motif of compound *1* at the two aspartic proteases.

Selective $Beta_2$-Adrenergic Activation *via* a Combination of Atenolol/Formoterol Administration Increases Significantly the Coronary Blood Flow in Isolated Rat Hearts

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Background and purpose: Adrenergic agonists have been used for decades in cardiology in a variety of clinical scenarios involving low cardiac output as well as during and after cardiac surgery. Commonly used adrenergic agonists have a variety of specifities, but rely primarily on $beta_1$-

adrenergic activation to stimulate cardiac function. Knowledge is limited regarding the application of $beta_2$-agonists to this end, even though their vasodilation effect may be beneficial. This study was conducted in order to assess the efficacy of one such $beta_2$-adrenergic agonist, formoterol, in augmenting cardiac function.
Experimental approach: The hearts of 10 fully anesthetized female Wistar rats were excised surgically, and subsequently kept functional in an *ex vivo* isolated heart preparation (Langendorff apparatus). After placement on the Langendorff apparatus, hearts were subjected to a dose of the $beta_1$-blocker atenolol, and 5 minutes later to a combined formoterol/atenolol dose. Atenolol was used to minimize the $beta_1$-agonist action of formoterol. Left ventricular pressure (LVP), heart rate (HR), and coronary flow (CF) were monitored throughout the experimental procedure.
Key Results: CF analysis (n=10) showed a mean increase of 15% ($p<0.05$) after formoterol/atenolol administration (compared to baseline and post-atenolol values, separately). This effect lasted at 20 minutes post-administration. Identical testing was performed to assess LVP (n=9) and HR (n=8) variance. Here, statistically significant differences included an early 41% increase in LVP and a late (>20minutes post-administration) 27% increase in HR. Correlation testing showed that CF increase was independent to LVP and HR increases.
Conclusions and implications: Our results indicate that the $beta_2$-agonist formoterol not only successfully increases heart rate and contractility, but also increases coronary flow, most likely by means of $beta_2$-mediated coronary vasodilation. This pharmacological profile may prove to be especially beneficial in situations where cardiac output must be increased, while adequate myocardial oxygen delivery needs to be maintained.

Association of Subtypes and Psychopathology of Schizophrenia with Polymorphism 5-HTTLPR in the Serotonin Transporter Gene Regulation Region

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A cohort study conducted to investigate whether the 5-HTTLPR genetic polymorphism is associated with the subtypes of schizophrenia, positive and negative symptoms (measure by PANSS scale), general psychopathology, or depression (measured by CDS) that schizophrenic patients develop. Transporter-facilitated uptake of serotonin (5-hydroxutryptamine or 5-HT) has been implicated in psychiatric disorders and is the side of action of widely used uptake-inhibiting antidepressant and antianxiety drugs. Human 5-HT transporter (5-HTT) gene transcription is modulated by a common polymorphism in its upstream regulatory region. The short variant (s) of the polymorphism reduces the transcriptional efficiency of the 5-HTT gene promoter, resulting in decreased 5-HTT expression and 5-HT uptake. The association study in a sample totalling 65 schizophrenic patients, 30 with the s/l genotype, 22 with the s/s and 13 with the l/l revealed no statistic significant relevance between the 5-HHLPR genotype and schizophrenic phenotype, showing that although 5-HTT has long been suspected to play a role in behavioral and psychiatric disorders, it is not the only one responsible, but there is a complex interaction between environmental and genetic factors that generates the phenotype of schizophrenia.

Designed Fabrication of Multifunctional Microcontainers for Biological Applications

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In recent years, the design of multifunctional polymeric materials in the suhmicrometer size has been considerably improved due to their wide applications in the fields of biomedicine (1).

Particularly, hollow polymeric micro- and nanospheres have attracted a great deal of attention due to their wide range of applications. These structures have potential utility in encapsulation and controlled release of various biomolecules such as genes, peptides, and drugs (2).

A variety of multt stimuli-responsive microcontainers have been synthesized that are capable of conformation al and chemical changes on receiving an external signal. These changes are accompanied by variations in the physical properties of the polymer. The signal is derived from changes lit the materials' environment, such as a change in temperature (3) or in pH (4), in the present work, we present the synthesis of novel muitiresponsive composite microcontainers with thermal, pH and magnetic responsive characteristics. Specifically, pH and thermal responsive microcontainers were prepared using the distillation precipitation polymerization method with magnetic nanoparticles (Fe_3O_4) encapsulated either in the shell or in the core. Daunorubicin hydrochloride (DNR) was loaded into the microcontainers and release profile was studied by UV-Vis Spectroscopy.These novel hybrid microstructures were characterized with transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), vibrating sample magnetometry (VSM), X-ray diffraction (XRD) and FT-IR spectra.

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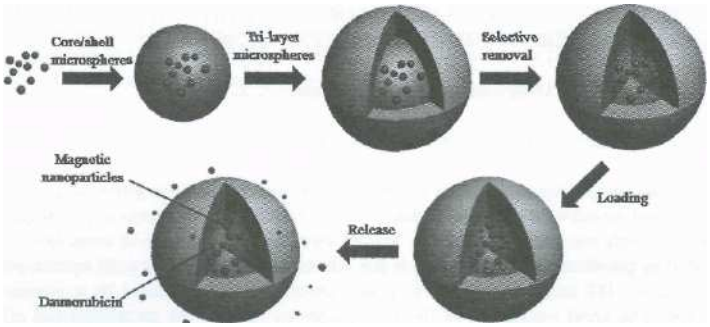

Smart Nanoparticles as New Drug Delivery Systems: Bioapplications

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In recent years, organic nanocontainers have been used widely for bioapplications, such as encapsulation for controlled release of drugs and specific delivery. This enormous progress was followed by hollow nanospheres synthesis. Traditional strategies to synthesize hollow nanospheres, mainly use polymer, silica beads, emulsion droplets and organic aggregates as templates (1,2). However, such nanocontainers are usually complicated for biological application, hut organic nanocontainers are more promising for this scope. For these reasons, significant progress has been made in the design and synthesis of hollow polymer panicles by a variety of physical and chemical methods. Synthetic microspheres have special properties, including pH-sensitive, thermo-sensitive, redox-sensitive and dual thermo-responsive and pH, have been prepared by different techniques with a broad spectrum of applications (3,4). In this work, we have synthesized both, mi-celles and nanocontainers which present specific pH, temperature and magnetic properties. These novel nanocontainers were characterized structural with FT-IR spectroscopy and morphological with transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS). The magnetic properties were studied *via* vibrating sample magnetometry (VSM) and X-ray diffraction (XRD). The synthe-

sized nanocontainers were studied for their loading and release behavior of two drugs, Daunorubicin and Leuprolide. These two drugs are known in medicine about their uses in a broad spectrum of cancer types, such as breast and prostate. Biological evaluation of the synthesized nanocontainers is under investigation (5-7).

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The Effects of BV6 at Lipid Bilayers and at the AT_1 Receptor Site

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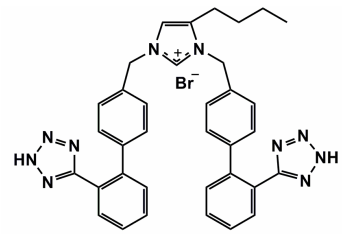

Figure 1: Chemical structure of BV6

BV6 (Figure 1) is a synthetic promising molecule designed as AT_1 antagonist. Biological tests showed that it has similar properties with losartan. The high activity of the molecule triggered our interest to study its interactions in the lipid bilayer and examine its role in its bioactivity. In particular, we have performed Differential Scanning Calorimetry: X-ray diffraction, Raman and solid state Nuclear Magnetic Resonance spectroscopy studies to reveal its thermal and dynamic effects in lipid bilayers. In addition, docking and Molecular Dynamics studies complemented the biophysical results in order to study its interactions at the receptor site. The obtained results were compared with those of losartan in order to show the similarities and differences in the two sites and explain their pharmacological properties.