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# Molecular Modeling and NMR Conformational Analysis Apllied in the Study of Zn(II) Binding Sites of Biomolecules

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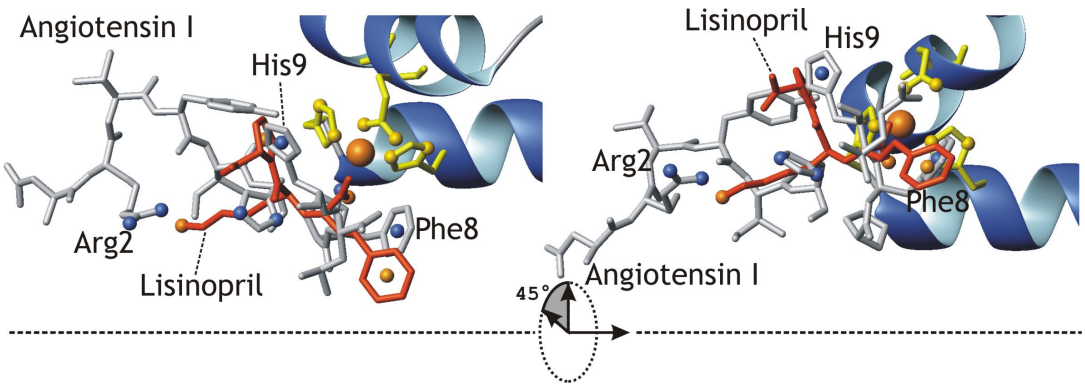
*Key words*: Biomolecules, Zn(II) binding sites, molecular modelling, NMR conformational analysis




The Angiotensin-I Converting Enzyme (ACE) is a *gluzincin* Zinc Metallopeptidase and plays a pivotal role in blood pressure regulation. ACE catalyzes the hydrolysis of the Angiotensin-I (AI) carboxy-terminal dipeptide His-Leu, thus transforming AI to the vassopressor octapeptide Angiotensin-II (AII). ACE is encountered in two distinct forms in humans, the *somatic* and the *testis* form, bearing 2 and 1 Zn(II)-containing catalytic sites, respectively (1,2). Only recently, the X-ray structure of *testis* ACE has been determined (3).

The reconstitution of the polypeptide skeleton bearing the amino acid sequence of the two catalytic sites of ACE, has been performed using synthetic peptides and Zn(II) salt. The solution structures of these constructs have been studied through Molecular Modeling and high-resolution multinuclear NMR spectroscopy.

The NMR models of two ACE catalytic sites may set the basis in order to study their interaction with physiological substrate, such as Angiotensin I and Bradykinin, as well as with various inhibitors (5).

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REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION 20: 394-395 (2006)


# Computational Analysis of the Conformational Features Induced in Peptide Analogues Containing the (S,S)-CXC- Motif

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*Key words*: Peptide analogues, (S,S)-CXC- motif, computational analysis

Cyclization via a disulfide bond is a widely used strategy to design constrained peptide analogues. The CXXC motif, which is the smallest and highly conserved unit in biological systems, has been extensively studied to estimate its propensity to form β-turns. Recently we have reported on the synthesis, activity and conformational preferences of highly constraint RGD analogues containing the (S,S)-CDC- motif (1). The main goal of this study is to explore the influence of the (S,S)-CXC- motif to the relative orientation of the X amino acid and the X-2 or X+2 residue side chains. The structure of the peptides has been investigated by molecular dynamics methods. Our findings indicate that despite the nature of the X residue, there is a preference for an almost *cis* coplanar orientation of the X and either one of the adjacent to Cysteine residue side chains. The 11-membered cyclic structure does not favor any β-turn conformation while the backbone dihedral angles within the cycle are very constraint. The $\chi_3$ angle of the first Cysteine residue is distributed around $+80^{o}$ or $-100^{o}$. It is concluded that the (S,S)-CXC- motif can be incorporated in peptide analogues in which the *cis* coplanar orientation of the corresponding amino acid side chains is desirable. A cluster analysis of the cyclic structures presented here, as well as from other linear RGD containing peptides (2,3), studied by Molecular Dynamics and NMR, further reveals the stabilization effect of the (S,S)-CXC- motif.

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REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION 20: 395 (2006)


# Molecular Mechanisms Implicated in the Development of Atherosclerosis: The Role of Extracellular Matrix Proteoglycans

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*Key words*: Atherosclerosis, development, extracellular matrix, proteoglycans, molecular mechanisms

The extracellular matrix (ECM) proteoglycans are molecules that accumulate in atherosclerotic lesions. Their unique molecular features create highly interactive molecules that bind growth factors, enzymes, lipoproteins and a variety of other ECM components to influence fundamental biological events implicated in vascular disease. Proteoglycan synthesized mainly by arterial smooth muscle cells (ASMC) and their synthesis is regulated by specific growth factors and cytokines. The expression of proteoglycans especially that of versican, is markedly upregulated after vascular injury and versican is a prominent component in restenotic lesions.

The interaction of hyaluronan with versican creates an expanded viscoelastic pericellular matrix that is required for ASMC proliferation and migration. Proteoglycans are also accumulated in advanced atherosclerotic lesions, at the borders of lipid-filled necrotic cores as well as at the plaque-thrombus interface, suggesting roles in lipid accumulation, inflammation and thrombosis. Furthermore, the expression of specific versican isoforms influences the assembly of ECM and regulates elastic fiber fibrillogenesis, which is of fundamental importance in ECM remodeling during vascular disease and the development of aneurysms.

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REVIEW OF CLINICAL PHARMACOLOGY AND
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# The Molecular Basis of Hypertension by the Use of Biophysical Methods

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*Key words*: Hypertension, Molecular Basis, Biophysical Methods

In this lecture an atomic level approach of hypertension will be presented. Particular emphasis will be given to:

● The modelisation of the 3D structure of the ACE_N domain of *s*ACE, based on the X-ray structure of testis ACE (*t*ACE).

● Sequence and structural comparison between ACE_N and ACE_C and of other proteins of the gluzincin family highlights key residues that could be responsible for the peptide hydrolysis mechanism.

● Structural models of the interactions of nine ACE inhibitors (lisinopril, captoril, enalaprilat, ramiprilat, quinaprilat, peridoprilat, fosinoprilat, keto-ACE and RXP 407) both to ACE_C and ACE_N catalytic sites were generated by automated computational docking. Pharmacophore refinement, at the atomic level was achieved, which might provide an improved basis for structure-based rational design of second-generation, domain-selective inhibitors.

● Structural studies of the bioactive hormone Angiotensin II (AII) and its inactive precursor AI and implication for the receptor bound conformation trough studies of the monoclonal antibody Fab131 and the homology modeled structure of the GPCRs $AT_1$.

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REVIEW OF CLINICAL PHARMACOLOGY AND
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# Initial Experience Using Bone Marrow Derived Stem Cells for Cardiac Reparation Following Large Myocardial Infarction

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*Key words*: Bone marrow derived stem cells, large myocardial infarction, cardiac reparation, initial experience

Recent reports using intramyocardial (surgical or percutaneous) or intracoronary administration have focused on bone marrow derived stem cells (BMSC) for myocardial regeneration. The latter method of deliver employs an angioplasty balloon catheter, through which BMSC can be slowly infused into the appropriate vascular territory. We describe our preliminary experience using this treatment in patients with old non-viable anterior myocardial infarction and congestive heart failure.

We have treated 12 patients, 10 males, age 50±9, who had experienced an old large anterior myocardial infarction 49±38 months before BMSC treatment. The absence of viability was documented by Tl-201 reinjection scintigraphy and low-dose dobutamine echocardiogram. BMSC were obtained with bone marrow biopsy from the iliac crest during same day procedure, and subsequently processed to isolate a subpopulation (CD +133 cells), known of its capability to differentiate to endothelial cells and probably to cardiomyocytes. A significant number of CD +133 BMSC ($21\pm17x10^6$) were delivered into the coronary artery via a angioplasty balloon catheter. In a group of patients BMSC were labeled in order to document adherence to the myocardium. All patients underwent clinical follow-up, echocardiogram and Tl-201 studies 4.5±2.1 months after treatment.

There were no complications during the procedure or during follow-up. There was no improvement in the global left ventricular ejection fraction, but a significant improvement was noticed in the segmental contractility of the anterior infracted segment. The size of the left ventricle diminished overtime, consistent with favourable remodeling of the heart. These beneficial changes have been associated to the improvement of perfusion in the anterior wall, as shown from the repeat Tl-201 scintigrams.

Infusion of selected BMSC in the coronary artery supplying a large old myocardial infarction results in improvement of local perfusion, segmental myocardial contractility and favourable cavity remodeling. Larger studies are necessary to confirm the safety of this new method of cardiac reparation and the most efficient way to ensure full functional behavior of the implanted cells.

REVIEW OF CLINICAL PHARMACOLOGY AND
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# From Angiotensin to Sartans a New Generation of Anti-Hypertensives

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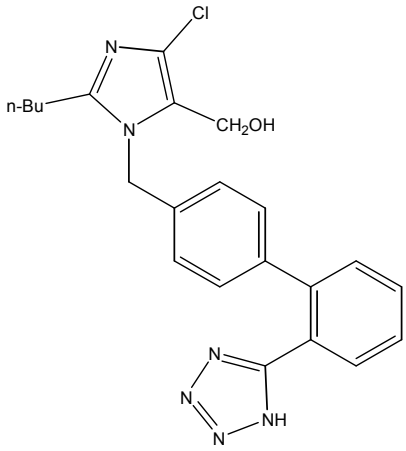
*Key words*: Antihypertensives, angiotensin, losartan




*LOSARTAN*

Losartan was the first drug of the Sartan series to be marketed. Using molecular models of Angiotensin II and mimetic technology developed early in 1990's researchers from Patras and Calgary were able to design and synthesize Losartan analogues, which were found to be strong inhibitors. One of them was equally potent to Losartan with longer period of action. However analogue, named Vivartan, was not further developed as synthesis was not cost effective. Latest efforts with structural modifications have reduced steps to achieve a cost effective synthesis of related compounds with strong potency. This presentation will describe methods to design potent ANG II receptor antagonists.

REVIEW OF CLINICAL PHARMACOLOGY AND
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# Selective Phosphinic Inhibitors of Angiotensin Converting Enzyme (ACE)

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*Key words*: Angiotensin converting enzyme, selective phosphinic inhibitors

Somatic angiotensin – converting enzyme (ACE) contains two homologous domains, each bearing a functional active site with different substrate specificity and different activation requirements (1).

The *in vivo* contribution of each active site to the release of angiotensin II (Ang II) and the inactivation of bradykinin (BK) - two peptide hormones that play a key role in the regulation of blood pressure - is still unknown.

To gain insights into the functional roles of these two active sites, two phosphinic inhibitors RXP407 (2) and RXPA380 (3), were designed and synthesized. These two phosphinic compounds are able to selectively inhibit only one active site of ACE. We will present here: a) the synthetic strategy of the two selective phoshinic inhibitors using combinatorial chemistry and b) the *in vitro* and *in vivo* results.

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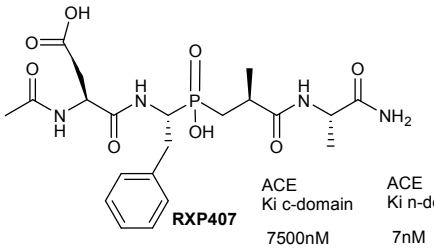
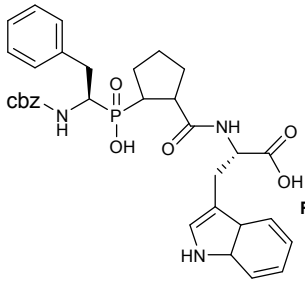


**RXP407**

| ACE Ki c-domain | ACE Ki n-domain |
|---|---|
| 7500nM | 7nM |



**RXPA380**

| ACE Ki c-domain | ACE Ki n-domain |
|---|---|
| 3nM | 10000nM |

REVIEW OF CLINICAL PHARMACOLOGY AND
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# The Role of Polymorphism of $A_2$-Adrenergic Receptors in Drug Design

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*Key words*: $A_2$-Adrenergic receptors, polymorphism, drug design

Until recently pharmacological intervention at the level of GPCR and $\alpha_2$-adrenergic receptor was based on three premises:
(a) The pharmacological action was directed to the ligand binding site of the receptor.
(b) The receptor molecule was considered invariant across the population.
(c) Each physiological function was considered to be controlled by a distinct receptor subtype.

All these tenets are under revision. The characterization of signal transduction pathways initiated upon activation of GPCRs allows today the targeting of intracellular effectors for more selective and efficient action. Meanwhile, most GPCRs are polymorphic with the variant forms displaying different biochemical properties.

Alpha$_2$-adrenergic receptors ($\alpha_2$-ARs) are a representative system in this context. All three known $\alpha_2$-ARs subtypes ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$) are polymorphic. Genetic variation occurs in the functional third cytoplasmatic loop, thus entailing disruption of the biochemical phenotype of the receptor. Data will be presented showing how genetic differences yield biochemical differences in receptor function correlating with clinical phenotypes.



REVIEW OF CLINICAL PHARMACOLOGY AND
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# Phospholamban's Role in Idiopathic Dilated Cardiomyopathy

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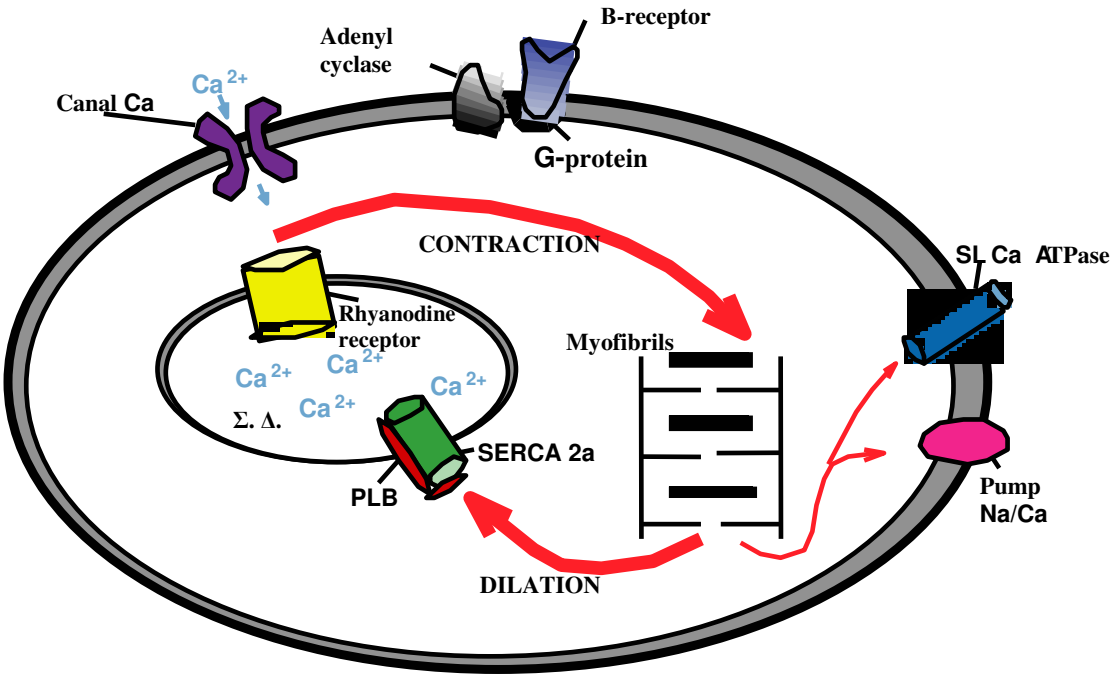
*Key words*: Idiopathic dilated cardiomyopathy, phospholamban

Idiopathic dilated cardiomyopathy is characterized by dilatation and impaired contraction of the left ventricle or both ventricles as a result of intrinsic cardiomyocyte dysfunction. Altered cardiomyocyte $Ca^{2+}$ cycling is widely recognized as contributing to impaired con-

tractile performance in human and experimental heart failure, including idiopathic dilated cardiomyopathy (1). Coordinated regulation of cytosolic $Ca^{2+}$ by the sarcoplasmic reticulum (SR) of cardiomyocytes is required during each cycle of cardiac contraction and relaxation. During contraction the SR serves as a reservoir from which $Ca^{2+}$ is released into the cytosol via the ryanodine receptor. Sequestration of $Ca^{2+}$ from the cytosol into the SR lumen and, thus, relaxation of the heart is mediated by a sarcoplasmic reticulum Ca-ATPase pump (SERCA2a, Figure). The activity of SERCA2a is reversibly regulated by phospholamban (PLN), a 52-amino-acid phosphoprotein. The role of PLN in the regulation of cardiomyocyte contractility has been elucidated through the development of genetically engineered mouse models in which, PTL ablation resulted in significant increases in cardiac contractile parameters, while PLN overexpression was associated with depressed systolic function (2). Therefore the PTL gene was screened in dilated cardiomyopathy patients and two different mutations were identified recently. The first mutation entailed a conversion of arginine to cysteine (PLN-R9C) in PLN and it was linked to the dominant inheritance of dilated cardiomyopathy in a large American family (3) and the second was a T116G point mutation which resulted in conversion of leucine 39 to a premature stop codon (L39stop) in PLN, that was identified in two Greek families with hereditary dilated cardiomyopathy (4). These data emphasize the role of PTL in cardiomyocyte dysfunction and dilated cardiomyopathy development.

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REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION 20: 402-403 (2006)


# Angiotensin II Receptor Blockade in Cardiovascular Disease

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*Key words*: Cardiovascular disease, angiotensin II, receptor blockade

Angiotensin II (Ang II) has long been known as a potent vasoconstrictor and stimulator of adrenal steroid synthesis. The coronary, renal and cerebral vasculatures are far more sensitive to the pressor effect of Ang II than vasculature of musculocutaneous tissues. Experimental and clinical work conducted many years ago established the fact that Ang II excess plays a causal role in myocardial and renal damage and is a major contributor to functional deterioration in chronic heart failure. Ang II receptor blockade or angiotensin-converting enzyme (ACE) inhibition were found to increase regional blood flows to vital organs and exert cardioprotective and renoprotective effects. Early studies employed polypeptide analogs/antagonists to Ang II, administered parenterally. Subsequent, far more extensive research work, ranging from basic investigation to multicenter clinical trials, was conducted mostly with ACE inhibitors, whose effects are partly mediated via withdrawal of Ang II and partly via potentiation of bradykinin (which also accounts for some of the adverse reactions to ACE inhibition). More recently Ang II has been recognized to have trophic and mitogenic actions in selected tissues, thus causing hypertrophy and/or proliferation of cardiomyocytes and vascular smooth muscle cells. Chronic ACE inhibition, even in non-hypotensive doses, was shown to reverse left ventricular hypertrophy and vascular wall thickening in hypertensive subjects.

The recent advent of oral Ang II receptor blockers (ABR's) has given new impetus to this field, because comparative experiments using an ACE inhibitor and a selective $AT_1$ receptor antagonist suggest that the systemic hemodynamic effects of ACE inhibition are mostly due to suppression of Ang II. The favorable hemodynamic properties of ACE inhibition have already established this class of drugs as a treatment of choice in hypertension and congestive heart failure. All ARB's tested so far have the same clinical efficacy in the treatment of these conditions, without the ticklish dry cough which is the most common cause of intolerance to ACE inhibition.

Comparative studies of ARB's against an ACE inhibitor revealed similar decreases of

systolic and diastolic blood pressure and the same response rates on monotherapy, with better tolerability of ARB in all age groups. It has now been shown by several clinical trials that ARB's have long-term cardioprotective and renoprotective effects similar to those of ACE inhibitors in patients with hypertension, ischemic heart disease or diabetes mellitus. Both classes of drugs have also been found to diminish the incidence of new onset type 2 diabetes in patients with hypertension or heart failure.

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# Misconceptions in Structural Peptide Chemistry: An Analysis from a Basic Organic Chemistry Perspective

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*Key words*: Structural peptide chemistry, analysis

The concept of conformation has played a central role in various applications of Organic Chemistry. In this presentation, various misconceptions in conformational structural peptide chemistry are analyzed. More specifically the hypothesis and the conclusions of previous $^{17}O$ NMR studies on the detection of: (a) discrete conformational states in peptides and (b) of both oxygens of the carboxylic group of Boc-[$^{17}O$]Tyr(2,6-diClBzl)-OH in DMSO-$d_6$ solution are reconsidered based on elementary concepts of undergraduate organic chemistry. Furthermore, it is demonstrated that the $^{17}O$ shielding time scale is not advantageous compared to that of $^{1}H$ NMR and, thus, it is not suitable for the detection of discrete hydrogen bonded conformational studies on peptides. $^{17}O$ NMR spectroscopy is prone to interpretation errors due to formation of $^{17}O$ labeled impurities during the synthetic procedures. The appearance of two discrete resonances at 340 and 175 ppm of this protected amino acid is not now attributed: (a) to the reduction of the intramolecular conformational exchange rate, due to the effect of intramolecular hydrogen bonding of the hydroxyl part of the carboxyl with the carbonyl oxygen of the Boc-group, and (b) to the effect of solvent viscosity, suggested in the mentioned study. The cause of this phenomenon is attributed to a strong hydrogen bonding of the polar proton acceptor solvent DMSO with the carbonyl group, which effectively reduces the proton exchange rate, thus becoming slow on the $^{17}O$ NMR time scale.

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