PS04.12.40 DESIGN AND SYNTHESIS OF NOVEL POTENT, NON-PEPTIDE AND ORALLY ACTIVE RENIN INHIBITORS. M. Grütter, J. Maibaum, V. Rasetti, H. Rüeger, R. Göschke, R. Mah, N.C. Cohen, J. Rahuel, F. Cumin, J. Wood, Ciba-Geigy Ltd. Pharmaceuticals Division, Research Dept., CH-4002 Basel, Switzerland

The renin-angiotensis system (RAS) plays a central role in the regulation of blood pressure and in the maintenance of electrolyte balance. In an effort to identify potent small molecule renin inhibitors with good oral bioavailabilityand duration of action we have developed a novel structural concept for the design of inhibitors as potential antihypertensive agents.

Our approach was based on the observation that the S3 and S1 binding sites of renin consitute a large continuous, hydrophobic 'superpocket'. Initially, δ-aminohydroxyethylene dipeptide transition state mimics containing a lipophilic P3 moiety covalently linked to P1 via a spacer group were prepared and found to have moderate *in vitro* potency, although these inhibitors lacked the P4/P2 spanning part of peptide-based inhibitors. These results confirmed the hydrothesis that optimized van der Waals interactions of a hydrophobic (P3-linker-P1)-moiety with the large hydrophobic surface of the complementary binding site could compensate for truncation of the 219 of the enzyme, to the P3-P1 template of these leads dramatically increased *in vitro* potency and afforded inhibitors with subnanomolar IC_{50} values and with high species and enzyme specificity. The iterative optimization process with the assistance of computer-aided molecular modelling and x-ray crystallographic analysis of r-human renin-inhibitor complexes revealed an intriguing common binging interaction of the new compounds to an hitherto unknown non substrate binding site of the renin. Synthesis and SAR data for structually distinct series of inhibitors with favorable physico chemical properties will be presented. *In vivo*, the most potent compounds, CGP 55 128A and CGP 56 346A, induced pronounce and long-lasting blood pressure effects at 1-10 mg/kg p.o. In Na-depleted marmosets.

In summary, the conceptual design described in the present study has led to discovery of completely non-peptide, highly selective and low molecular weight inhibitors of renin with unique structural features.

PS04.14.20 THE SECOND CYCLE OF A PROTEIN ENGINEERING PROJECT: THE REPLACEMENT OF A LOOP IN MONOMERIC TRIOSEPHOSPHATE ISOMERASE (MONOTIM). R.K. Wierenga, N. Thanki, W. Schliebs, J. Ph. Zeelen, R. Abagyan[1], R. Jaenicke[2], EMBL, Postfach 102209, D69012 Heidelberg, Germany, [1]Skirball Institute, NYU, New York, USA, [2]University of Regensburg, Regensburg, Germany

The disorderd loop-1 of monoTIM, including the catalytic lysine-13, has been redesigned with the modelling package ICM, aimed at rigidifying this loop. The new variant is as active as monoTIM and its crystal structure has been determined at 2.6Å resolution.

Protein loops play an important role in molecular recognition. In proteins with the TIM-barrel framework eight loops determine the shape of the active site pocket. In triosephosphate isomerase (TIM), some of these loops (in particular loop-3) are also crucial for formation of very stable dimers. In a first protein design experiment with TIM we designed a new loop-3 which converted the protein into a stable monomeric TIM. The protein, referred to as monoTIM, has 1/1000 fold less catalytic activity than wild type TIM, possibly due to an increased flexibility of some active site loops. In particular loop-1 is highly disordered in different crystal structures of monoTIM(1). Nevertheless, the importance of the catalytic Lys13 of loop-1 for the catalytic activity of monoTIM has been demonstrated by site directed mutagenesis (2). We started a second cycle of protein engineering aimed to rigidify this loop. The actual loop modelling was done with ICM on a seven residue loop. The coordinates of the modelled structure have been deposited at the PDB (1MTM). The variant was made, purified, characterised and crystallised. First insight into the crystal structure of this new variant (at 2.6Å resolution) suggests that loop-1 has indeed been rigidified, whereas Lys13 is still pointing into the active site.

(1). Structure (1995),3,669-679.
(2). Protein Science (1996), 5, 229-239.

PS04.17.47 CRYSTALLIZATION SEPARATES A DISTINCT SHORT FORM OF A POLLEN ALLERGEN WITH BIOLOGICAL ACTIVITY. Weber W, Betzel Ch, Bufe A

Group V major allergen Phl p 5b of timothy grass pollen induces allergic rhinitis and asthma bronchiale. In addition to its allergenicity, a ribonuclease activity has recently been attributed to this 29 kDa protein. The recombinant allergen, though highly purified from a bacterial expression system, has been observed to spontaniously convert to a mixture of various forms with molecular sizes between 10 and 29 kDa. Surprisingly, crystals could be grown from such heterogenous preparations. Single crystals, redissolved and analyzed by SDS-PAGE/immunoblot yielded only one distinct low molecular weight protein which was identified by amino acid sequencing as the C-terminal 13 kDa portion of the allergen. Biological assays with solutions from single crystals demonstrated that both allergenicity as well as RNAse activity are associated with this fragment. The preferential crystal growth of the 13 kDa form (as opposed to the full length protein) indicates the compact conformation of that particular portion of the allergen. In this system crystallization functions as a separation technique which contributes to localize the major functional domain within a larger protein.

Two crystals were used to collect low resolution data up to 4.6 Angstrom on a rotating anode generator. From processing 35 images the space group was evaluated to I4122 with cell dimensions of a = 87.72 Å, b = 87.72 Å., c = 59.61 Å.. 5,581 reflections were merged to a final reduced data set containing 568 reflections and a completeness of 98%. Attempts to collect data to higher resolution using synchrotron radiation are in progress.

PS05.07.09 CRYSTAL STRUCTURE OF [2-(P-(METHOXYPHENYL) HYDROXY) METHYLENE-2H-1,4-BENZOTHIAZINE-3-(4H)-ONE](+). K.A. Nirmala and P. Kumaradhas, Department of Physics, Bangalore University, Bangalore-560 056, INDIA

The title compound $C_{16}H_{15}NO_2S.H_2O$ is a derivative of benzothiazine posses pharmacological activity. The exact structure was determined because of the ambiguity in chemical and physical methods. A single crystal X-ray analysis has been carried out to know about the exact conformation of molecular geometry and crystal packing. The title compound crystalyizeses with water molecule.

Crystal Data: Space group $P2^1/c$, Monoclinic, a=12.050(6)Å, b=5.612(2)Å, c=23.795(4)Å, β=102.46(2)Å, V=1571.2(9)Å^3, Z=4, λ=1.5418Å, D_{cal} =1.350 mg/μ^3 and D_{exp} =1.345mg/μ^3.

The structure has been solved by direct method SHEXLS-86 and it was refined by full matrix least-squares method SHELXL-93 to the final value of R=0.049 and wR=0.11.

The 1,4-benzothiazine is significantly deviated from planarity and having distorted boat conformation. The methoxyphenyl with hydroxy group is pseudo-axial to the 1,4-benzothiazine ring. There is a strong intramolecular hydrogen bonding between the oxygen of water molecule to hydroxy group of the benzothiazine and also there is a strong interamolecular hydrogen bonding between oxygen of the water molecule to carbonyl oxygen. The molecular packing is stabilized by three dimensional hydrogen bonding network. The amide group hydrogen bond with adjacent molecules forms centrosymmetric dimers in the crystal. This is the important facit of the molecular packing.