of drug resistant mutations. Several crystal structures of RT-NNI complexes determined at high resolution (Ren *et al*, 1995, *Nature Struct. Biol.* **2**, 303-308, Ren et al, 1995, *Structure.* **3**, 915- 926) show NNIs bind to a hydrophobic pocket in RT about 10Å from the polymerase catalytic site. Analyses of these crystal structures illustrate the mechanisms of NNI resistance to be mainly a loss of stabilizing van der Waals contacts between the protein and the inhibitor. To dissect out the structural requirements for the design of a potent NNI, we have determined the crystal structures of a series of HEPT analogues covering a wide range of potencies (Hopkins *et al, J.Med.Chem.*, in press). These complex structures reveal conformational changes in the protein some of which correlate with the potencies of the HEPT analogues. The major determinant of increased potency is the improved ring stacking interactions between the 6-benzyl ring of the inhibitors and Tyr181. The conformational switching of Tyr181 into its more exploitable position is caused by steric interactions with the 5-position substituent on the pyrimidine ring. All tight binding NNIs possess groups which throw this conformational switch.

PS04.12.24 STRUCTURE-BASED DRUG DESIGN OF A NOVEL SERIES OF HUMAN CATHEPSIN D INHIBITORS. Angela Y. Lee, Pavel Majer, Sergei V. Gulnik, Jack Collins, Abelardo M. Silva, Narayana T. Bhat and John W. Erickson, Structural Biochemistry Program, SAIC-Frederick, National Cancer InstituteFrederick Cancer Research and Development Center, Frederick, Maryland 217021201

Cathepsin D (Cat D) is a lysosomal aspartic protease implicated in many aspects of pathology such as cancer and Alzheimer disease, thus representing a novel target of therapeutic importance. Development of specific and bioavailable Cat O inhibitors would aid in delineating its role in normal and disease states. Based on our recently solved X-ray structures of human Cat D and its complex with pepstatin A, a new series of inhibitors for Cat D have been designed, synthesized and kinetically characterized. In our attempt to better understand their modes of binding and to aid in our future design, crystallographic studies of human Cat D complexed with some of these inhibitors were carried out. The crystal structure of a complex with a linear statine-based compound will be compared with that of a related cyclic analog, as well as with the model structures.

PS04.12.25 THREE DIMENSIONAL STRUCTURES OF E.COLI PNP COMPLEXES. Chenglong Li and Steven Ealick, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853

We present here several complex structures of E.Coli PNP with its substrates and substrate analogs. Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of purine ribo or 2'-deoxyribonucleosides to the purine and ribose or 2-deoxyribose-1-phosphate. The enzyme has been isolated from both eukaryotic and prokaryotic organisms and functions in the purine salvage pathways. The human and bovine PNPs are specific for the 6-oxo-purines and many of their analogs,and both are trimers with identical subunits. E.Coli PNP represents another class of PNP identified from various sources,which is hexameric,has no sequence similarity with human and bovine PNPs and accepts both 6-amino and 6-oxopurines as substrates. E.Coli PNP has a strikingly different active site and binding features to its substrates compared with that of mammalian PNPs. The phosphate binding site consists of backbone Gly20 and three arginine residues Arg43 and Arg87 from one subunit and Arg24 from the neighboring subunit. The three arginine residue hold the phosphate to form a strong binding net and yet are flexible enough to allow phosphate to initiate the nucleophilic attack due to the long arms of arginine residues. E.Coli PNP does not undergo a large conformational change during the enzymatic reaction like in the case of mammalian PNP. The base binding site consists of residues Phe159,Phe167,Ile206,Val178 and Asp204. It is mainly made of hydrophobic residues and is more open (therefore maybe less specific) than its mammalian counterpart. Another remarkable feature is that the nucleoside binding conformations in the E.Coli and mammalian PNP are totally different. Although the ribosyl and phosphate groups bind to E.Coli PNP and mammalian PNP in similar ways,the purine base is rotated 180 degree about the glycosidic bond. E.Coli PNP has only one H-bonding residue Asp204 and mammalian PNPs have two H-bonding residues Asn243 and Glu 201 near the purine base.

PS04.12.26 THROMBIN COMPLEXES WITH THIAZOLE-BASED INHIBITORS: USEFUL PROBES OF THE S1' BINDING SITE. John H. Matthews*, Raman Krishnan*, Michael J. Costanzo#, Bruce E. Maryanoff#, A. Tulinsky*, * Department of Chemistry, Michigan State University, East Lansing, MI 48824, #The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA 19477

The serine protease thrombin plays a central role in blood clotting with the most prominent function being the conversion of fibrinogen to fibrin in the later stages of the coagulation cascade. A large number of inhibitor-thrombin complexes have been studied by X-ray crystallography. Most of these inhibitors bind to one or the other of the S1-S3 subsites of the active site or the fibrinogen recognition exosite. Less is known of the binding at the S' subsites that involve substrate residues downstream from the point of cleavage. We report here the results of S1'-binding thiazole-containing groups and the implications for the future design of inhibitors. The potent thrombin inhibitor RWJ-50353 is a tripeptide with a D-Phe-Pro-Arg motif (PPACK) with a benzothiazole group. The other inhibitor, RWJ-50215, is related to DAPA, dansylarginine N-(3-ethyl-1,5-pentanediyl) amide, an early member of a class of inhibitors based on the chemical nature of thrombin binding sites and contains a 2-ketothiazole group.

The RWJ-50353-hirugen-thrombin structure was refined to an R value of 0.168 in the (7.0-2.3) Å resolution range with 125 water molecules, while the RWJ-50215 complex converged at an R value of 0.155 in the (7.0-1.8) Å resolution range with 161 water molecules.

Binding in the S1-S3 subsites is similar to the parent compounds PPACK (RWJ50353) and DAPA (RWJ-50215). The benzothiazole in RWJ-50353 and the thiazole in RWJ-50215 bind at the S1' site of thrombin. There they are surrounded by His57, Tyr60A, Trp60D, Lys60F of thrombin, and in the case of RWJ-50215, also by the piperidine ring of the inhibitor. In RWJ-50353, the N1 atom of the benzothiazole forms a hydrogen bond with His57NE2 (2.7 Å) and the indole ring of Trp60D stacks edge onto the face of the benzothiazole ring. The sidechain of Lys60F is displaced from its normal position by the bulky benzothiazole group. Both N1 and O1 of the 2-ketothiazole of RWJ-50215 form hydrogen bonds with the sidechain of Lys60F.