showed that safranin bound at this same site. We have found that safranin also inhibits hGR in a noncompetitive fashion but it binds about 16 times less tightly (Kis=453 mM, Kii=586 mM with respect to GSSG) than XAN, and does not preclude the binding of GSSG in the crystal. Despite our relatively detailed crystallographic results pertaining to the interactions XAN makes with the enzyme, the inhibition mechanism remains unclear. Although in structure-based drug design competitive inhibitors are usually targetted, XAN's binding to a well defined site in hGR that is not well conserved among homologs of hGR suggests that noncompetitive inhibitors could also serve as lead compounds for structure-based drug design, in particular as components of chimeric inhibitors.

**PS04.12.31 STRUCTURE AND INHIBITION OF PLASMEPSIN II, A HEMOGLOBIN-DEGRADING ENZYME FROM *P. FALCIPARUM*.** A. M. Silva[1], A. Y. Lee[1], S. V. Gulnik[1], P. Majer[1], J. Collins[1], T. N. Bhat[1], P. J. Collins[1], R. E. Cachau[1], K. E. Luker[2], I. Y. Gluzman[2], S. E. Francis[2], A. Oksman[2], D. E. Goldberg[2] and J. W. Erickson[1] (1)Structural Biochemistry Program, National Cancer Institute/SAIC, Frederick, Maryland 21702, USA. (2) Howard Hughes Medical Institute, Departments of Molecular Microbiology and Medicine Washington University School of Medicine, St Louis , Missouri 63110, USA

*Plasmodium falciparum* is the major causative agent of malaria, a disease of worldwide importance. Resistance to current drugs such as chloroquine and mefloquine is spreading at an alarming rate and our antimalarial armamentarium is almost depleted. The malarial parasite encodes two homologous aspartic proteases - plasmepsins I and II - which are essential components of its hemoglobin degradation pathway and are novel targets for antimalarial drug development. We have determined the crystal structure of recombinant plasmepsin II complexed with pepstatin A. This represents the first reported crystal structure of a protein from *P. falciparum*. The crystals contain molecules in two different conformations, revealing a remarkable degree of interdomain flexibility of the enzyme. The structure was used to design a series of selective low molecular weight compounds that inhibit both plasmepsin II and also the growth of *P. falciparum* in culture.

**PS04.12.32 CRYSTAL STRUCTURES OF TWO THROMBIN COMPLEXES WITH NOVEL PEPTIDE MIMETIC INHIBITORS.** Robert St. Charles, Peggy Vanderhoff-Hanaver, Doug Boatman*, Cy Ogdu*, A. Tulinsky, Department of Chemistry, Michigan State University, East Lansing, MI 48824, *Molecumetics, Bellevue, WA 98005

Thrombin is a trypsin-like blood serine protease specialized in catalyzing the proteolysis of fibrinogen to fibrin monomers during the final stage of the coagulation cascade. Synthetic active site inhibitors of thrombin represent potentially useful antithrombotic agents and are showing promise as alternatives to current heparin-based therapeutics.

The crystal structures of human α-thrombin complexed separately to two similar peptidomimetic active site inhibitors (MOL124-1 and MOL126-1) have been solved to 2.1 Å resolution and refined using restrained least squares with R-factors less than 0.18. Ternary complexes were prepared by soaking crystals of hirugen-inactivated athrombin (space group C2) in 2 - 7 mM solutions of each synthetic inhibitor over a period of 5 days. Both inhibitors resemble D-Phe-Pro-Arg chloromethylketone (PPACK) in structure and bind in a similar fashion within the catalytic site. As with other active site inhibitors of thrombin, the arginyl side chain of the P1 residue of each inhibitor is bound within the primary specificity pocket of the protein making salt-bridged hydrogen bonds with the acidic side chain of Asp 189. Moreover, this arginine residue forms an expected hemiketal with Ser 195 in both complexes. The P2 residue of each mimetic possesses a novel bicyclic structure that constrains the precleavage portion of the inhibitor in an extended, substrate-like conformation, and interacts with the S2 site much like the proline residue of thrombin-bound PPACK. An aromatic P3 residue, D-phenylalanine, occupies the aryl binding site in both complexes. In MOL124-1, this residue is acylated at the amino-terminus, and makes several stabilizing interactions with the protein. One of the two inhibitors (MOL126-1) possesses an aromatic, α-ketoamide-linked P1' residue that is hydrogenbonded to His 57 and makes numerous apolar interactions within the S1' and S2' sites of thrombin. This residue, absent in MOL124-1, is responsible for enhanced inhibitory activity.

**PS04.12.33 STRUCTURE BASED DISCOVERY OF A NEW CLASS OF ENZYME INHIBITORS.** Thomas J. Stout†, M. Paola Costi‡, Daniela Barlocco‡, Marcella Rinaldi‡, Brian Shoichet*, K.M. Perry*, I.D. Kuntz*, Robert M. Stroud† Departments of Biochemistry† and Pharmaceutical Chemistry*, University of California, San Francisco, San Francisco, CA 94143-0448 USA Dipartimento di Scienze Farmaceutiche Universitá Degli Studi di Modena 41100 Modena - Via Campi, 183 Italy‡

Thymidylate Synthase (TS) is an important target for the development of anti-cancer therapeutics. TS forms a biosynthetic bottleneck for the production of the DNA nucleotide thymidine (T) by catalyzing the reductive methylation of **dUMP** to **dTMP**. As such, many inhibitors of TS have been developed as mechanism based substrate or co-factor analogues, but currently less than five have been approved for use as anti-cancer therapeutics. We have used structure-based modeling methods and single-crystal X-ray crystallography to discover an entirely new series of TS inhibitors unrelated to substrate or co-factor (1). First generation leads have subsequently been sequentially improved through cycles of design, synthesis and crystallography to yield TS inhibitors of improved potency and specificity.

These phthalid-based compounds display a new mode of general enzyme inhibition through non-specific occlusion. The crystal structures show multiple modes of ligand binding which vary significantly with small modifications to the ligands. In addition, kinetic analysis shows concentration dependent inhibition, implicating a non-specific mode of binding. Indeed, the hydrophobic non-specific interactions found within the crystal structures appear to predominate over potential hydrogen bonds. The combined occupied volumes of five different enzyme-inhibitor complexes map out a new ligand-accessible space which can now be used for future structure-based design of inhibitors with additional binding specificity.

1. Shoichet, B. K., Stroud, R. M., Santi, D. V., Kuntz, I. D., & Perry, K. M. (1993) *Science 259*, 1445-50.