obtained using molecular dynamics simulations of XPLOR (Chakravarty, (1995). Ph.D. thesis, University of Bombay, India). Further simulations on nineteen other sulfonamide complexes whose crystal structures were not known, clearly revealed that the loop region comprising of L198, T199, H200, P201 and P202 were crucial for the design of HCAI - specific sulfonamide inhibitors. Several substituted aromatic and benzene sulfonamides were then docked into the active sites of the isozymes to optimise the interactions with these loop residues. Stereospecific substitution of methyl imidazole group in benzene sulfonamide resulted in strong interactions between the imidazole groups of the inhibitor and His 200 as observed from the energy minimised structure of the complex. Since His 200 is non - conserved between HCAI and HCAII, this indicated that the inhibitor would be more specific against HCAI. Energy minimisation of the resultant complex confirmed it. Further substitution of an alkyl chain resulted in additional stable non - bonded interactions with another non conserved active site residue Ala / Val 121. The compound BARCZM1 has been synthesised (Ghosh et al.; To be published) and is being characterized for its inhibitory properties the details of which will be presented.

PS04.12.16 THE CRYSTAL STRUCTURE OF HUMAN α-THROMBIN/ LY178550 COMPLEX: 5-AMIDINOINDOLE-4-BENZYLPIPERIDINE NON PEPTIDAL ACTIVE SITE INHIBITOR. Nickolay Y. Chirgadze, Daniel J. Sall, Robert Hermann, David K. Clawson, V. Joe Klimkowski, Gerald F. Smith, Donetta S.Gifford- Moore, William J Coffman, Eli Lilly and Company, Indianapolis, IN USA

Thromboembolic diseases remain a leading cause of mortality and morbidity in developed societies. Thrombin, a trypsin-like serine protease, is a key mediator in such disease states, primarily through fibrin formation and platelet aggregation.[1] In response to the well documented liabilities associated with warfarin,[2] an industry wide search has been initiated to discover safe and effective, orally active thrombin inhibitors that can be used to treat thrombotic disorders. Over the past few years, a number of very potent and selective inhibitors of thrombin have identified based on the NAPAP, Argatroban (MD-805), or a D-Phe-Pro-Arg structural motifs.[3] In general, however, the peptidal nature of these class of agents is prohibitive of high oral bioavailability.

In an effort to identify non peptidal inhibitors of thrombin which might have a more favorable pharmacokinetic profile than their peptide-related counterparts, we have prepared LY178550 as an initial lead for future structure-based drug design studies. Agent LY178550 consists of two primary components: 1) 5-amidinoindole which has been previously employed as an arginine surrogate in the design of inhibitors of arginine endopeptidases,[4] and 2) a hydrophobic 4-benzylpiperidine tail which has the potential to interact with the well characterized P_3 pocket of the thrombin active site.

A crystal structure of human α-thrombin complexed with LY178550 was determined by X-ray technique at 2.2 Å resolution. A final complex model has crystallographic R-factor of 14.4% with standard deviation from ideal for bond distances of 0.014 Å. A clear well defined electron density was observed for the inhibitor molecule in the active site. The inhibitor main chain has a L-shape and mimics conformation of arginal trypeptides[5]. This poster will describe the X-ray crystallographic study of the interaction of LY178550 with the active site of human α-thrombin.

[1] In *The Thrombin;* 1st ed.; Machovich, R., Ed.; CRC Press Inc.:, 1984.; V1, pp 122.
[2] Smith, G. F. et al. *Thromb. Res.* 1988, *50*, 163-174.
[3] Scarborough, R. M., *Ann. Rep. Med. Chem.* **1995**, *30*, 71.
[4] Geratz, J. D. *et al. Arch. Biochem. Biophys.* **1979**, 197, 551-559.
[5] Chirgadze, N.Y., *et al.,* Amer Crystallog. Ass Meeting, Aug 9-14, **1992**, v.20., 116.

PS04.12.17 INFLUENZA B/LEE/40 NEURAMINIDASE: X-RAY STRUCTURE OF ENZYME COMPLEXED WITH 4-GUANIDINO-Neu5ac2en. N. Y. Chirgadze, J. M. Colacino, K. A. Staschke, K. Briner, W. J. Hornback, J. E. Munroe, R. Loncharich, W. G. Laver*, Lilly Research Laboratories, Indianapolis, IN. USA, *The Australian National University, Canberra, Australia

Neuraminidase from influenza B/Lee/40 was crystallized and complexed with the potent and selective influenza neuraminidase inhibitor, 4-guanidino-Neu5Ac2en[1], by soaking the crystal in a concentrated solution of the inhibitor. Crystals suitable for X-ray have been obtained from PEG. They belong to tetragonal $P42_12$ space group containing one subunit per asymmetric unit. The enzyme-inhibitor complex crystal structure was determined by X-ray technique. An experimental data has been collected up to 2.8 Å resolution with an R_{merge} Of 10.4%. The crystal structure has been refined using a molecular dynamic procedure to yield a current crystallographic R-factor of 16%. The electron density of the inhibitor in the active site is well-defined and interpretation of the electron density distribution reveals an interaction between the C-4 guanidinium moiety of the inhibitor with the glutamic acid at position 117 which lies within a pocket of the active site of the neuraminidase. Similar results have been obtained using influenza A N9 neuraminidase[2]. Computational techniques are being used to analyze the enzyme-inhibitor interaction in terms of H-bond strengths.

[1] von Itzstein M. et al. (1993) *Nature* 363:418-423.
[2] Varghese et al. (1995) *Protein Sci.* 4:1081-1087.

PS04.12.18 RESISTANCE OF INFLUENZA A AND B VIRUSES TO 4- GUANIDINO-Neu5Ac2en. N. Y. Chirgadze[1], J. M. Colacino[1], K. A. Staschke[1], A. Baxter[1], G. Air[2], A. Bansal[2], E. Garman[3], J. Tang[1], W. J. Hornback[1], J. E Munroe[1], W. G. Laver[4], [1]Lilly Research Laboratories, Indianapolis, USA, [2]University of Alabama, Birmingham, USA, [3]University of Oxford, UK, [4]Australian National University, Canberra, AU

The reassortant influenza viruses, A/NWS-G70c (N9 neuraminidase [NA]) and B/HK/8/73 (HG) (B/Lee/40 NA), were selected for resistance to 4-Guanidino-Neu5Ac2en (4-GuDANA). The NA of resistant viruses was >200-fold more resistant to 4-GuDANA than was the NA of parental viruses. Resistant A and B viruses displayed 5% and 0.5%, respectively, of the parental NA activity yet both were able to undergo multicycle replication in MDCK cells and grow to equal titer in embryonated eggs. The expression by these viruses of NA activity in MDCK cells over a 72 hour period was extremely low relative to that of parental viruses. Sequence analysis revealed a single mutation in the NA gene leading to the change of a conserved Glu 119 (N9 numbering) to Gly for both A and B viruses. Glu 119 lies in a pocket beneath the active site of the enzyme and has been shown to interact with the C-4 guanidinium moiety of 4-GuDANA. The NA from 4- GuDANAr A/NWS-G70c has been crystallized. Although these crystals grew to only 0.2 mm in the largest dimension, data from low temperature (100K) X-ray diffraction experiments were collected with a merging Rvalue on intensities of 6.2% to 2.0 Å resolution. These data revealed the absence of the glutamate residue at amino acid position 119. We have been unable to obtain NA crystals of X-ray diffraction quality from the 4- GuDANAr B virus. In addition to the above viruses, wild-type influenza B/Lee/40 was selected for resistance to 4-GuDANA and the identical nucleotide change leading to the Glu to Gly alteration in the NA was found. Attempts to select a reassortant N2 virus (A/NWS-Tokyo) for resistance to 4- GuDANA have so far been unsuccessful. In preliminary experiments, 4- GuDANAr A/NWS-G70c was able to induce pyrexia in ferrets indicating that viruses with low levels of an altered NA retain pathogenicity, at least in this model of infection.