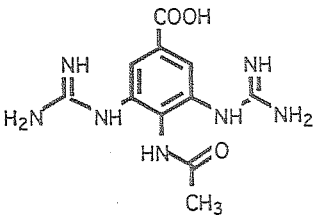
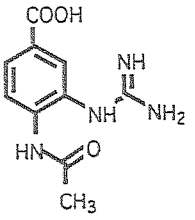
PS04.12.34 CRYSTAL STRUCTURE OF INFLUENZA VIRUS NEURAMINIDASE WITH INHIBITOR 3,5-DIGUANIDINO-4-(*N*-ACETYLAMINO)BENZOIC ACID E. A. Sudbeck,[1] M. J. Jedrzejas,[1,2] S. Singh,[3] W. J. Brouillette,[3] G. M. Air,[2] W. G. Laver,[4] Y. S. Babu,[5] S. Bantia,[5] P. Chand,[5] N. Chu,[5] J. A. Montgomery,[5] D. A. Walsh,[5] M. Luo,[1,2] [1]Center for Macromolecular Crystallography, [2]Department of Microbiology, [3]Department of Chemistry, University of Alabama, Birmingham, AL, [4]John Curtin School of Medical Research, Australian National University, Canberra, Australia, [5]BioCryst Pharmaceuticals, Inc., Birmingham, AL.

We have designed a series of benzoic acid derivatives to fit into the active site of influenza virus neuraminidase and inhibit its enzymatic activity. Because the residues in the active site of neuraminidase are conserved for all known strains of influenza virus, these types of inhibitors have a potential to be highly effective drugs against the virus. One such compound, 3,5-diguanidino-4-(*N*-acetylamino)benzoic acid (**1**), shows measurable inhibition of neuraminidase in activity assays. We have solved the X-ray crystal structure of B/Lee/40 neuraminidase complexed with **1** at -180°C and 2.2Å resolution. The inhibitor interacts with amino acid residues in the active site of neuraminidase through specific hydrogen bonds and hydrophobic interactions. The structure is compared to the complex of neuraminidase with a related inhibitor, 3-guanidino-4-(*N*-acetyl-amino)benzoic acid (**2**). Compounds **1** and **2** interact similarly in the active site of neuraminidase except for a displacement of two structural water molecules by the 5-guanidino group of **1**.



1 (IC50 = 0.07mM)



2 (IC50 = 0.01mM)

PS04.12.35 SIALIDASE INHIBITORS TO PREVENT BACTERIAL VAGINOSIS RELATED PREMATURITY. J. Tsao, C. White, M. Luo, Center for Macromolecular Crystallography, University of Alabama in Birmingham, Birmingham, AL.35294

Bacterial vaginosis (BV) is one of the common vaginal infectious disorder affecting women of reproductive ages, including 15-20% of pregnant women. Many studies have indicated the link between BV and prematurity, but the pathophysiological mechanism are still poorly understood. However, elevated sialidase activity is directly correlated with the prematurity outcomes of pregnant BV patients. In many bacteria infections, bacteria sialidases play an important roles in host cell adhesion and have been implicated in pathogenicity. We have designed three new classes of bacteria sialidase specific inhibitors based in the active site structure of *Salmonella typhimurium* LT2 sialidase. The inhibitors were constructed using two benzoic acid-based lead compounds and targeting towards a hydrophobic pocket located near Trp 128 and a strong anionic pocket located between Asp 100 and Glu 231 in the bacterial sialidase active site. Several synthesized inhibitor compounds show measurable inhibition of sialidase activity. We soaked two of the compounds into the *Salmonella typhimurium* crystals and determined the structure of the complexes. The inhibitors interact with the active site residues through hydrogen binding and hydrophobic interactions. Those complex structures will help to design more effective inhibitors that are specific to the bacterial vaginosis related bacteria sialidase.

PS04.12.36 HIGH RESOLUTION X-RAY STRUCTURE OF BOVINE PURINE NUCLEOSIDE PHOSPHORYLASE WITH INHIBITORS. Weiru Wang and Steven E. Ealick, Section of Biochemistry, Molecular and Cell Biology, 209 Biotechnology Building, Cornell University, Ithaca, NY 14853

Purine nucleoside phosphorylase (PNP, EC 2.4.2.1) is a salvage enzyme important to the T-cell-mediated part of the immune system and as such is an important therapeutic target. This paper describes a series of high resolution x-ray structures of PNP inhibitors-9(((phosphono)-alkyl)-benzyl)-guanines, (((guaninyl)alkyl)-phosphinico)-methyl)-phosphonic acids and 9-deazaguanine derivatives, complexed with bovine PNP. These inhibitors are multi-substrate analogues and are able to bind the purine bind site, the ribose binding site and the phosphate bind site simultaneously in the PNP active site. These competitive inhibitors have high binding affinity with Ki ~10nM and lower. X-ray diffraction data were obtained at low temperature with the Macromolecular Diffraction Facility at the Cornell High Energy Synchrotron Source (MacCHESS). Enzyme-inhibitor structures were refined to resolutions ranging from 1.7 to 2.0 Å with XPLOR. Detailed inhibitor-enzyme interactions were investigated based on these high resolution structures. Residue His64 which is close to the phosphate binding site moves in and out of phosphate binding site surface when different inhibitors are bound. This movement changes the number of positive charge in the phosphate binding site and suggests that electrostatic interactions in the phosphate binding site are different for different inhibitors. A phosphonyl group attached to a 9-deazaguanine derivative interacts with two positively charged residues on the pocket surface without His64 while a free phosphate, or di-phosphate analogue group interacts with three positively charged residues including His64. The difference in electrostatic interactions might contribute to difference in binding energy. Interactions between the inhibitor and enzyme in the purine binding site and the ribose binding site are similar to the results from previous study. Small conformational and positional deviation will be described. Conformational changes of the residues in the PNP active site were also observed. These changes are complementary to the inhibitor conformation and may also contribute to binding energy.

PS04.12.37 STRUCTURES OF *CANDIDA ALBICANS* DIHYDROFOLATE REDUCTASE: HOLOENZYME AND TERNARY INHIBITOR COMPLEXES Marc Whitlow, Andrew J. Howard, David Stewart, Karl D. Hardman (Genex Corp., Gaithersburg, MD 20877); Lee F. Kuyper, David P. Baccanari, Mary Fling, Joseph H. Chan, Robert L. Tansik (Glaxo Wellcome Inc., Research Triangle Park, NC, 27709, USA).

Crystal structures of *Candida albicans* dihydrofolate reductase (DHFR) were determined as part of an effort to develop selective inhibitors for the treatment of systemic fungal infections. The incidence of systemic fungal infections has risen considerably in recent years: *C. albicans* is now the fifth leading cause of microbial infection in the hospital setting, and opportunistic fungal infections present a major problem to AIDS patients. The success of selective DHFR inhibitors as antibacterials and antimalarials prompted us to explore the development of compounds for the selective inhibition of *C. albicans* DHFR (versus human DHFR). The enzyme was cloned, expressed, and crystallized, and its structure was determined as the holoenzyme and in ternary complex with a number of inhibitors. A potent but nonselective inhibitor (a 2,4-diaminopyrrolo[3,2-d]quinazoline) bound to the enzyme as expected. However, a selective class of inhibitor (2,4-diamino-5-arylthioquinazolines) showed unusual binding properties. Two of the most selective inhibitors bound to the enzyme with the 5-arylthio moiety positioned in the site that is normally occupied by the