MS16.P13

Acta Cryst. (2011) A**67,** C291

New HIV Integrase inhibitors discovered through fragment screening

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HIV integrase is a clinically validated target for the treatment of AIDS with one approved therapeutic on the market. Resistance to treatment by antivirals occurs rapidly, so new therapeutics for novel HIV targets or for different sites on the already clinically validated enzyme targets are needed. We have developed low micromolar inhibitors to HIV integrase at two different sites found on the core catalytic domain. These inhibitors do not bind to the catalytic site and therefore represent new classes of compounds structurally different to the currently marketed therapy.

We developed these inhibitors by fragment screening using surface plasmon resonance (SPR) as the initial method and by x-ray crystallography to determine the binding mode of the compounds. We determined that there were three sites of binding, two of which could be further developed by traditional medicinal chemistry techniques. The biophysical methods were complemented by enzyme assays which showed that the compounds inhibited integrase reactions *in vitro*. The SPR, assay and structural data were utilized to develop more potent enzyme inhibitors.

We identified a new allosteric binding site on integrase as a cleft near the mobile loop. The mobile loop is known to be important for the integration reaction and we confirmed that binding at the new site inhibited the catalytic activity of the enzyme [1]. Using structure based drug design, we were able to develop inhibitors binding at this site that were 45 times more potent integrase inhibitors than the original hits.

Lens epithelium-derived growth factor (LEDGF) is known to bind to HIV integrase and regulate its cellular location. Both short peptides of LEDGF and some small molecules have been shown to bind to integrase and disrupt the interaction of LEDGF with HIV integrase, and as a result these molecules inhibit integrase activity. We have developed novel compounds that bind to the LEDGF binding site of HIV integrase and determined that these inhibit integrase with low micromolar potencies using two *in vitro* biochemical and cellular assays.

I will describe the compounds discovered, the fragment screening protocol used and the structures of these novel compounds in complex with the HIV integrase core catalytic domain.

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Keywords: antiviral, fragment-screening, AIDS

MS16.P14

Acta Cryst. (2011) A**67,** C291

Structure of human glucokinase with disease-causing E339K mutation reveals changes in the ATP binding site

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Human glucokinase (GK) plays an important role in glucose homeostasis. An E339K mutation in GK was recently found to be associated with hyperglycemia. It showed lower enzyme activity and impaired protein stability compared to the wild-type enzyme. Here, we present the crystal structure of E339K GK in complex with glucose. This mutation results in a conformational change of His416, spatially interfering with adenosine-triphosphate (ATP) binding. Furthermore, Ser411 at the ATP binding site is phosphorylated and then hydrogen bonded with Thr82, physically blocking the ATP binding. These findings provide structural basis for the reduced activity of this mutant [1].

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Keywords: human glucokinase, E339K mutation, crystal structure

MS16.P15

Acta Cryst. (2011) A**67,** C291

Branched chain amino acid biosynthesis: A target for biocide discovery

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Plants, bacteria and fungi all synthesize the branched-chain amino acids (BCAA) *de novo*. On the other hand, humans and other animals do not have this ability, obtaining these molecules directly from their diet. As a result enzymes in BCAA pathway are excellent targets for the discovery of novel biocides. Acetohydroxyacid synthase (AHAS) and ketol-acid reductoisomerase (KARI) are the first two enzymes in the pathway. AHAS is the target for a range of commercial herbicides, while several KARI inhibitors are experimental herbicides.

We have determined the structures of the catalytic subunit for the free fungal AHAS [1] and have determined structures of the complexes between five commercial herbicides (sulfonylurea and imidizolinone families) and both plant [2] and fungal AHAS [3], [4]. These structures show remarkable structural flexibility upon binding either inhibitor class. More recently we have shown that the sulfonylureas do have antifungal activity with MIC_{50} values as low as 90 nM.

For plant and bacterial KARIs we find that the binding of NADPH and metal ions to these structures have profoundly different effects [4], [5],[6]. In the case of plant KARI the active site of the free enzyme appears to be completely accessible to the solvent but upon metal ion and NADPH binding the active site becomes progressively occluded. On the other hand, for bacterial KARI the active site of the free enzyme is almost completely closed over. Only upon the addition of the metal ions and NADPH does the active site become accessible to substrate. These structures provide a basis for future rational structure-based discovery of KARI inhibitors.

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Keywords: branched-chain amino acids, antifungal, herbicide