Acinetobacter baumannii is one of the species most commonly isolated from clinical specimens and it can be the cause of severe and sometimes lethal, nosocomial infections. It colonizes patients in Intensive Care Units and contaminates inanimate hospital surfaces [1].

Antibiotic resistance to clinically employed β-lactam antibiotics currently is recognized as the most important risk factor for multiresistance. Described resistance mechanisms include hydrolysis by β-lactamases, alterations in outer membrane proteins and penicillin-binding proteins, and increased activity of efflux pumps.

β-lactamases can be divided into four classes (A, B, C, and D) according to their sequence similarities [2]. On the basis of their different catalytic mechanisms, two groups have been established. β-lactamases of classes A, C and D own in its active site serine groups, while class B β-lactamases are metalloproteins that require zinc for their activity. Metallo-β-lactamases often have mosaic structures with additional domains but in general all of them are small enzymes sharing a common four-layer αββα motif with a central β-sandwich and two α-helices on either side. This motif has an intrinsic metal-binding site located at an edge of the β-sandwich and it is occupied by a divalent zinc ion having a tetrahedral array of three histidines and a water molecule. The importance of the zinc ion to β-lactam substrate binding is unquestionable [3].

We present the three-dimensional structure of a new zinc metallo-hydrolase enzyme, extracted from a clinically epidemic multidrug-resistant *A.baumannii* strain. Crystals were grown using the vapour diffusion technique. They belong to space group P1, with cell dimensions a=60.04, b=64.55, c=69.37 Å; α=82.12, β=81.46, γ=75.89 º and four molecules in the asymmetric unit, which diffracted beyond 2.4 Å. We solved the phases by a SAD experiment. Our goal is to understand the role of the two metals ions in the catalytic pathway and get some insights into the mechanism of metallo-β-lactamase hydrolysis. This information could serve as a starting point for the development of new antimicrobial agents given that there is clearly a specific need for new potent class B β-lactamase inhibitors to bridge the existing therapeutic void.

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Keywords: A. baumannii, β-lactamase, zinc.

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Fragment-based drug discovery applied to heat shock protein 90 (HSP90)

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Heat shock protein 90 (HSP90) is a chaperone which guides the folding of proteins into functional configurations affecting stabilisation and activation. Many of these proteins are oncogenes regulating tumour cell growth, survival and apoptosis. Therefore, inhibitors of HSP90 are potentially useful as chemotherapeutic agents in cancer. This poster describes an application of Astex Pyramid™ fragment screening to the ATPase domain of HSP90. The screening identified an aminopyrimidine with affinity in the high micromolar range and subsequent structure-based design allowed its optimisation into a low nanomolar series with good ligand efficiency. A phenolic chemotype was also identified with affinity close to one millimolar. This fragment was optimised using structure based design into a resorcinol lead which has sub-nanomolar affinity for HSP90, excellent cell potency, and good ligand efficiency. This fragment to lead campaign improved affinity for HSP90 by over 1,000,000 fold with the addition of only 6 heavy atoms. Subsequent lead optimisation of the resorcinol lead produced AT13387, a compound that is now in clinical trials for the treatment of cancer.

Keywords: drug, fragment, chaperone

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Structure and binding analysis of active site inhibitors targeting HIV-1 RNase H

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Human immunodeficiency virus (HIV-1) reverse transcriptase (RT) is a multifunctional enzyme containing both polymerase and RNase H functionalities. RNase H is required to generate specific RNA primers and subsequently break down intermediate RNA/DNA hybrids during reverse transcription. This activity is dependent on binding of two divalent metal ions in the active site. Pyrimidinol carboxylic acid and *N*-hydroxy quinazolinedione inhibitors were designed to coordinate these metal ions at the active site. High resolution (1.4Å-2.1Å) crystal structures were determined with the isolated RNase H domain and RT that permit accurate assessment of the metal and water environment. The geometry of the metal coordination suggests that the inhibitors mimic a substrate state prior to phosphodiester catalysis. The inhibitors also engage His539 *via* π stacking interactions and form a polar interaction with Arg557. Surface plasmon resonance studies confirm metal-dependent binding to RNase H and demonstrate that the inhibitors do not bind at the polymerase active site of RT. Comparative analysis of human RNase H1 bound to RNA/DNA substrate suggests that there are differences in the position of the equivalent residues at His539 and Arg557 in human RNase H1. This provides a rational for why these inhibitors selectively target the HIV enzyme over the human enzyme. Additional evaluation of the active site reveals an open protein surface with few additional interactions to optimize inhibitors.

Keywords HIV, antiviral therapy, reverse transcriptase

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Structural basis of fosmidomycin's action on *Plasmodium falciparum*

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The human malaria parasite *Plasmodium falciparum* is responsible for the death of more than a million people each year. The emergence

of strains of this malaria parasite resistant to conventional drug therapy has stimulated the search for antimalarials with novel modes of action. Fosmidomycin [3-(*N*-formyl-*N*-hydroxyamino) propylphosphonic acid] has proved to be efficient in the treatment of uncomplicated *P. falciparum* malaria in recent clinical trials conducted in Gabon and Thailand. It has a novel mode of action through the inhibition of 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), an enzyme of the non-mevalonate pathway of isoprenoid biosynthesis, which is absent in humans. However, the structural detail of the DXR inhibition by fosmidomycin in *P. falciparum* is unknown.

Here, we report the crystal structures of *P. falciparum* DXR (PfDXR) in its inhibitor-free ternary complex, fosmidomycin-bound quaternary complex, and FR900098 (*N*-acetyl derivative of fosmidomycin)-bound quaternary complex at 1.86, 1.9, and 2.15 Å resolution, respectively [1], [2]. The disordered loop region in the inhibitor-free form is well defined in the inhibitor complexes. Fosmidomycin and FR900098 bind to PfDXR in a similar manner, in which each inhibitor is buried in the active site and is shielded from the solvent environment. The methyl group of FR900098, which is not found in fosmidomycin, is accommodated in the active site by the induced-fit movement of the side chain of Trp296 belonging to the flexible loop, which is disordered in the inhibitor-free form. These flexible features demonstrate that the active site of PfDXR has the ability to accommodate an additional functional group of fosmidomycin derivatives. The quaternary complex structures revealed that a *cis* arrangement of the oxygen atoms of the hydroxamate group of the bound inhibitor is essential for tight binding of the inhibitor to the active site metal. We expect present structures to be useful guides for the design of more effective antimalarial compounds.

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Structural Investigation of Pim1 Kinase

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The incidence and death rate of prostate cancer, the most common type of malignancy diagnosed in men in developed countries, is rapidly increasing. Unfortunately, the low response rate to current treatment regimes makes treating prostate cancer extremely difficult. The expression of Pim1 kinase, a serine/threonine kinase, is reported to be upregulated in the prostate. Overexpression has been correlated with unsuccessful clinical outcome in prostate cancer patients. Pim1 is also overexpressed in other human leukemia including acute myeloid leukemia and hematopoietic malignancies.

Pim1 plays a role in cell survival/apoptosis, differentiation, and proliferation. It adopts a constitutively active conformation so is presumed to be linked to cell proliferation and development of tumorogenesis. Specific regions of Pim1 have been identified as essential for activity. The conserved Lys67-Asp186 salt bridge is responsible for constitutive activity and the formation of the Lys67, Asp186, Glu89, triad is crucial. Mutation of Lys67 results in inactivation.

Pim1 binds to its natural substrate, ATP, through a single hydrogen-bond interaction with Glu121, in the hinge region (formed by Leu120, Glu121, Arg122, Pro123, Glu124, Pro125, Val126 and Gln127). The glycine rich P-loop comprises residues Leu44, Gly45, Ser46, Gly47, Gly48, Phe49, Gly50, Ser51 and Val52. The P-loop is flexible and may play a role in specificity and selectivity, it may affect the affinity of inhibitors via induced fit binding modes. Available structures of Pim1 inhibitor complexes show, generally, this region adopts a fairly conserved conformation. The binding of AMP-PNP, however, causes the P-loop to be significantly displaced. It has been reported that this displacement destabilizes the protein, resulting in an unfavorable conformation.

Currently, therapies that target Pim1, mostly employ ATP competitive inhibitors. A consequence of targeting the ATP binding site of Pim1 is that these compounds also tend to interact, non-specifically, with the ATP binding site of many other kinases. This lack of specificity results in a significant reduction in efficacy of the compound and thus its potential in clinical use. Unspecific binding is also often responsible for the emergence of many unwanted side effects.

To date, no small compounds targeting Pim1 have successfully progressed through to clinical use because of lack of specificity. Thus, the design of drugs that can specifically inhibit Pim1 activity is the focus of our research. Recently, we employed insilico-screening techniques to identify potential inhibitors. The crystal structures of these protein-inhibitor complexes were subsequently solved. Interestingly, these compounds cause the P-loop to adopt a completely novel conformation upon binding. Unlike the conformational change caused by AMP-PNP interactions, this loop position seems to be stabilized by additional interaction with the protein. These new inhibitors have relatively high affinity, as well as specificity, and have been shown to be effective *in vitro* and *in vivo*. We believe that the interactions these compounds make with the protein, and the consequent conformational repositioning of the P-loop, may be responsible for the high potency and specificity achieved for this series of compounds. Investigations into their potential as drug candidates are currently under investigation.

Keywords: Kinase, Rational Drug Design, Disease

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Crystal structure of Cbl-b TKB domain in complex with Cblin (Cbl-b inhibitor)

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Cbl-b is RING-type ubiquitin ligase established as a negative regulator of receptor tyrosine kinase signaling in variety of cells. In previous studies, we showed that the Cbl-b-mediated ubiquitination and degradation of IRS-1 (insulin receptor substrate 1), a key intermediate of skeletal muscle growth, contribute to muscle atrophy during unloading [1]. Furthermore, we found that a pentapeptide, named "Cblin (Cbl-b inhibitor)", mimetic of tyrosine 608-phosphorylated IRS-1 inhibited Cbl-b-mediated IRS-1 ubiquitination and degradation under in vitro and in vivo experimental conditions [1]. Cbl-b contains N-terminal TKB domain (tyrosine kinase binding domain) which mediates direct binding to phosphorylated tyrosine residues of substrates. We predicted that Cblin directly bound to TKB domain of Cbl-b resulting in inhibition of the interaction between Cbl-b and IRS-1. However, the direct binding of Cblin to Cbl-b was not investigated. Thus, in order to evaluate the mechanism of inhibition of IRS-1 ubiquitination by Cblin, we determined the crystal structure of the Cbl-b TKB domain in complex with Cblin at resolution of 2.8 Å.