structures and discuss further design strategies for highly selective family 3 glycoside hydrolase inhibitors.

Keywords: glycosyl hydrolases, antibiotic resistance, structure-based drug design

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The crystal structure of AKR1C1 in complex with an active-site inhibitor

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Hydroxysteroid dehydrogenases (HSDs) regulate a wide range of physiological processes including reproduction, development and homeostasis. AKR1C1, is a 20α-HSD involved in the conversion of progesterone to 20-hydroxyprogesterone. Increased activity of AKR1C1 in the endometrium and in breast tissues leads to the formation of tumor-promoting metabolites and to the development of endometriosis, breast cancer and endometrial cancer. At present, there are few known inhibitors that specifically bind and inhibit the adverse actions of AKR1C1. Here we present the first crystal structure of AKR1C1 in complex with potent inhibitor 3,5-dichlorosalicylic acid (IC_{50} = 44 nM). The crystal structure was solved at a resolution of 1.8 Å, with clear electron density corresponding to the inhibitor bound in the active site. The details of the enzyme-inhibitor interactions and selectivity against members of the AKR1C subfamily will also be discussed. The structural information obtained from this study will help speed up the drug design process for the development of more selective and potent compounds that can be used in the treatment of endometriosis and cancer.

Keywords: aldo-keto reductases, enzyme inhibitors, drug design

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Eimeria tenella lactate dehydrogenase as a target for anti-parasitics

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Eimeria species are parasitic Apicomplexa protozoans that cause gastrointestinal coccidiosis infections in birds, and are associated with general morbidity and intestinal lesions. These parasitic infections lead to major losses within the broiler industry, a situation that is deteriorating due to emerging resistance to available therapeutics. By analogy with other Apicomplexa obligate parasites, in the intracellular stages of its lifecycle the Eimeria parasite relies heavily on glycolysis for ATP production, and hence on homolactic fermentation - the action of lactate dehydrogenase (LDH) - to restore the NADH/NAD^+ balance. These parasites are therefore extremely sensitive to LDH inhibition. In common with the LDH of the malaria causing parasite Plasmodium falciparum (PfLDH), Eimeria tenella LDH (EtLDH) has a characteristic five amino acid insert in a loop directly adjacent to the active site. As a consequence, we reasoned that compounds we have previously designed to specifically inhibit PfLDH should cross-react with EtLDH. We are therefore undertaking crystallisation and structural analysis of EtLDH and its inhibitory complexes in order to explore the possibility of targeting EtLDH for novel veterinary therapeutics.

Keywords: lactate dehydrogenase, Eimeria tenella, enzyme inhibitors

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Crystal structure and structure based drug design of HU (histone like protein) from *M.tuberculosis*

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HU is an architectural protein for bacterial chromosome compaction and organization.HU is one of the most ubiquitous proteins in the bacterial cell.It is a small basic protein, binding non-specifically throughout the nucleoid. While the sequence of HU is highly conserved through most of the bacterial species, the HU in M.tuberculosis has a sequence longer than other HUs whose crystal structures have been elucidated (Anabaena (1P71): 94 residues). It has a sequence length of 214 amino acid residues suggesting the presence of an extra domain.As the protein has a possible role in overall gene architectural modification and a global control of gene expression, it makes this HU an important candidate for structural study. For the first time a complete functional N-terminal Domain of HU from M.tuberculosis [H37Rv] (1st 100 amino acid residues) containing the sub-domains for DNA binding and dimerization was crystallized,the crystals diffracted to 2.04Å. Crystal unit cell contains biological dimer of N-terminal region of HU protein. The structure was solved by molecular replacement method. The final *R*cryst and *R*free are 20.6% and 25.0%. As the sequence is highly conserved among the other important mycobacterium species, like M.leprae, M.bovis, M.smegmatis etc., this structure is a good representative HU structure for the whole class and serves as an attractive target for drug design. Two types of drug molecules, one which can interfere with DNA-binding and other with HU dimerization, were designed computationally, utilizing the solved crystal structure of HU. The designed compounds interact with HU with high binding energies, as estimated computationally and could serve as lead molecule for drug design. Details of the 3-D models of HU-drug interactions will be discussed.

Keywords: Mycobacteria, histone like protein, structure aided drug design

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Structure-based design of anticancer prodrug PABA/NO

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Glutathione S-transferase (GST) is a superfamily of detoxification enzymes, represented by GSTa, GSTm, GSTp, etc. GSTa is the predominant isoform of GST in human liver, playing important roles for our well being. GSTp is overexpressed in many forms of cancer, thus presenting an opportunity for selective targeting of cancer cells. Our structure-based design of prodrugs intended to release cytotoxic levels of nitric oxide in GSTp-overexpressing cancer cells yielded PABA/NO, which exhibited anticancer activity both in vitro and in vivo with a potency similar to that of cisplatin (Findlay et al. Mol. Pharmacol. 2004, 65, 1070-1079). Here, we present the details on structural modification, molecular modeling, and enzymatic characterization for the design of PABA/NO. The design was efficient because it was on the basis of the reaction mechanism and the structures of related GST isozymes at both the ground state and the transition state. The ground-state structures outlined the shape and property of the substrate-binding site in different isozymes, and the structural information at the transition-state indicated distinct conformations of the Meisenheimer complex of lead compounds in the active site of different isozymes, providing guidance for the modifications of the molecular structure of lead molecules. Two key alterations of a GSTa-selective compound led to the GSTp-selective PABA/NO.

Keywords: structure-based drug design, anticancer prodrug, PABA/NO

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Structure of the LBD of rat VDR in complex with a non-seco-steroidal vitamin D3 analogue YR301

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The vitamin D receptor (VDR) is a ligand-inducible hormone receptor that mediates 1a,25(OH)2D3 action, determining the calcium and phosphate metabolism, induces potent cell differentiation activity and has immunosuppressive effects. Most analogues of 1a,25(OH)2D3 have been used clinically for some years. However, there is a risk of potential side effects, which limits the use of these substances. (2S)-3-[4-(3-{4-[(2R)-2-hydroxy-3,3-dimethylbutoxy]-3-methylphenyl}pentan-3-yl)-2-methylphenoxy] propane-1,2-diol (YR301) has only strong activity in evaluated four stereoisomers of a novel synthetic non-seco-steroidal vitamin D3 analogue LG902378. To understand the strong activity of YR301, the crystal structure of YR301 with the vitamin D receptor ligand-binding domain (VDR LBD) at 2.0 A was solved and compared with the structure of the rat VDR LBD-1a,25(OH)2D3 complex. YR301 and 1a,25(OH)2D3 share the same position and the diethyl-methyl groups occupy a similar space to CD rings of 1a,25(OH)2D3.YR301 has two characteristic hydroxyl groups which contribute to its potent activity. One is 2'-OH of YR301 which is hydrogen bonding to NE2 of both His 301 and His 393. Another is 2-OH of YR301 which is interacting with OG of Ser233 and NH1of Arg270. Each hydroxyl group of YR301 exactly corresponds to 25-OH and 1-OH group of 1a,25-(OH)2D3, respectively. The terminal hydroxyl group (3-OH) of YR301 is hydrogen bonded to Arg270 directly and also interacts with OH of Tyr232 and the backbone NH of Asp144 via water molecules indirectly. The substitution of the water molecules might be helpful for the design of more potent compounds.

Keywords: nuclear receptors, vitamin D, structural drug design

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Structural studies of glutathione S-transferase complexed to commonly used chemotherapy agents

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Glutathione S-Transferases (GSTs), phase II detoxification enzymes, primarily function to remove toxic compounds from the cell [1]. They are, however, overexpressed in many cancers and shown to be deleterious to successful chemotherapy treatment by reacting with anti-cancer drugs. GSTs, therefore, have been identified as an attractive target for inhibitor drug design to increase the efficacy of treatment [2]. Drug resistance remains a limiting factor in cancer chemotherapy and thus understanding its mechanism represents an important step in improving cancer treatment. Many reports correlate over-expression of GST and reduced sensitivity to chemotherapy [1]. GSTs are hypothesised to catalyse conjugation of GSH to anti-cancer drugs forming inactive conjugates. This action represents one of a number of possible mechanisms involved in resistance to current chemotherapy treatment. One of the major aims of this work is to determine the 3D structures of these complexes and subsequently pursue structure-based drug design of human GST pi class enzyme (hGSTP1-1) with the aim of discovering effective and specific inhibitors. I have solved the structure of GST with multiple metal based anti-cancer drugs. The structure of the hGSTP1-1/drug complexes reveals a novel ligand binding site. The identification of this site represents a new means by which GST may be contributing to the development of resistance to chemotherapy treatment, in addition to detoxification by GSH conjugation, by sequestering the drugs at this novel site. This information, in conjunction with successful fragment screening, will be used in the design of novel, therapeutic GST inhibitors.
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Keywords: anticancer drug structural study, enzyme inhibitor drug design, enzyme structure

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Crystal structure of human choline kinase in complex with hemicholinium

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