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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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Choline kinase (ChoK) catalyzes the ATP-dependent phosphorylation of choline, the first committed step in the CDP-choline pathway for the biosynthesis of phosphatidylcholine. Overexpression and increased activity of one of the human ChoK isoforms, ChoK alpha have been constitutively reported in malignant cells and tumour tissues. This suggests that the enzyme plays a relevant role in tumorigenesis. Some sets of in vitro and in vivo experiments confirmed that ChoK inhibition is one of the potential novel strateges for the development of new antiproliferative and anticancer drugs. The importance of ChoK for the regulation of cell proliferation has been studied by using an inhibitor hemicholinium-3 (HC-3), which was initially characterized as a lethal, respiratory paralytic agent. Most of the ChoK inhibitors introduced so far are the chemically modified derivatives based on the structure of HC-3. Crystal structures of HC-3 bound human ChoK alpha were determinded with and without ADP. In the crystal structures, HC-3 molecule was well accommodated between the N and C-terminal lobes of ChoK protein, and its one end was placed on the same binding site as a substrate choline, while the other end partially exposed to the solvent. The inhibitor molecule was stabilized mainly through the hydrophobic interactions contributed by the C-terminal lobe. These 3D information provides the first molecular detailed view concerning the mode of inhibitory action and expand our understanding of the factors governing selectivity.

Keywords: tumorigenesis, antiproliferative and anticancer drug, inhibitor

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Fragment screening and structure-based design of adrenaline synthesis inhibitors

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The enzyme phenylethanolamine N-methyltransferase (PNMT) catalyses the biosynthesis of adrenaline, a neurotransmitter linked to the central control of blood pressure. As part of an ongoing international collaboration to develop PNMT inhibitors, we found that the enzyme conceals a cryptic binding site (1-2). This site is revealed upon binding inhibitors that are double the size of the physiological substrate. The changes in active site size and shape are brought about by unfavourable side-chain conformations and rigid-body helix motions, at a modest estimated energetic cost of 2-3 kcal/mol. Our findings further underline the importance of incorporating protein flexibility in structure-based inhibitor design studies, and raise the question of whether such sites are accessible through moderate affinity fragment screening approaches. To address this question, we implemented fragment-based screening by X-ray crystallography for PNMT. We used the ActiveSight library of 384 compounds and found that a number of fragments bind to the PNMT active site. These will now be elaborated to develop potent and selective PNMT inhibitors.

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Keywords: enzyme inhibitor drug design, structure-based drug design, binding enzyme inhibitors

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Design of anti-allergic inhibitors for human hematopoietic prostaglandin D synthase

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Structure-based drug design (SBDD) is not certainly major process in the pharmaceutical company, however, the cost for drug discovery is huge and gradually increased, therefore, the importance of SBDD is thought to be greater and greater. The novel in-silico screening methods of Multiple Target Screening1 (MTS) and Docking score index2 (DSI) using the matrix on the interaction between the protein structures and chemical compounds were developed. To examine the effect of these methods, we selected human hematopoietic prostaglandin D synthase (H-PGDS) as a target. H-PGDS catalyzes the isomerization of PGH2, a common intermediate of various prostanoids, to PGD2, an inflammatory mediator, in the presence of glutathione (GSH). Oral administration of the H-PGDS inhibitor of HQL-79 suppressed antigen-induced eosinophilic accumulation in the lung of wild-type mice and human H-PGDS-overexpressing mice, gliosis and demyelination in twitcher mice, and musclar distrophy in mdx mice4. The optimizing of the known inhibitor4 as well as the screening of a novel lead compound for human H-PGDS by using in silico method are now in progress.
References

Keywords: complex compounds crystal structure, structure-based drug design, antiallergic drugs

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Structure-based drug design in HIV protease- and tRNA-guanine transglycosylase inhibitor development

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