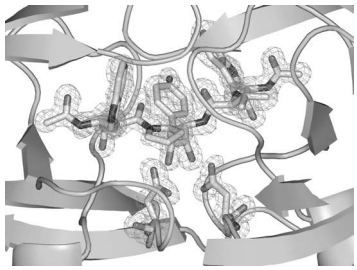
synchrotron radiation, clearly show that the catalytic site is fully occupied by a single ordered molecule (see Figure). This permitted unambiguously the identification of nature and stereochemistry of the bound inhibitor. Furthermore, the clear electron density map, without residuals, suggests that the inhibition constant of this compound should be at least one order of magnitude lower than the constants of the other compounds. The full occupancy of the site indicates that its value is less than 1μM. This biocrystallographic study has allowed a first assessment of inhibition properties without the purification of the mixture and the classic activity assays that are normally conducted on each compound. The co-crystallization strategy could be applied in conjunction with combinatorial chemistry synthesis to discover, by self selection, new potent inhibitors.



Keywords: single-crystal structure analysis, inhibitor binding, isomers

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Crystal Structure of a Disintegrin Heterodimer from *Echis carinatus* at 1.9 Å Resolution

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Disintegrins are a family of small proteins that bind to integrins specifically. Their binding site is characterized by the presence of Arg-Gly-Asp motif which indicates an RGD-dependant mode of interaction with integrins. The disintegrins interfere with the functions of integrins as antagonists. Disintegrin was isolated from the venom of *Echis carinatus* and crystallized in the tetragonal space group $P4_32_12$ with a=b=90.7Å and c=55.5Å. It exits as a heterodimer unlike the low resolution structure which existed as a homodimer with its two subunits related by a two fold crystallographic symmetry. It is interlinked by two disulfide bonds at the N-terminal region and contains 64 amino acid residues in each chain. Each monomer contains three pairs of six antiparallel β-strands and is stabilized by four disulphide bridges. It has been refined to an R-factor of 0.212 and R_{free} of 0.251 for all the data. The two chains of the dimer are anchored at N-terminal but diverge away at their C-termini exposing the Arg-Gly-Asp motif onto opposite directions, thus enhancing their binding efficiency. This is one of its unique features. The structural studies of disintegrins can provide a useful framework for the design of potent antagonists of integrins.

Keywords: disintegrin, heterodimer, drug design

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Structure-assisted Design of Inhibitors Targeting Coronavirus Main Proteases

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Coronaviruses (CoVs) are important etiologic agents of upper respiratory and digestive tract diseases in humans and animals; especially, the severe acute respiratory syndrome (SARS). The viruses are characterized with a highly complex cascade of proteolytic processing the replicative polyproteins to control viral gene expression and replication, which was predominantly mediated by the viral main proteinase (M^{pro}, also called $3CL^{pro}$), therefore, an attractive target for drug development[1].

A series of novel compounds with Michael receptor was designed according to the crystal structures of 3 coronaviruses M^{pro}s. The solved structures of SARS-CoV and porcine transmissible gastroenteritis virus (TGEV) M^{pro}s individually complexed with these compounds revealed that inhibitors possessing *α,β*-unsaturated ester combined with peptidyl-binding elements specific for CoV M^{pro}s undergo a nucleophilic addition of the protease's catalytic Cys, resulting in covalent-bond formation and irreversible inactivation of the viral proteases. One compound in this series has exhibited potent and extensive inhibition effect on 6 CoV M^{pro}s covering all 4 groups within genus *Coronavirus*. Meanwhile, the novel small molecules showed low micromolar concentration of EC_{50} for inhibition of viral replication and very low cell toxicity. We suppose further modification of these compounds assisted with structural information might lead to discover drug candidates against all CoV-associated diseases, including SARS.

[1] Yang H., Yang M., Ding Y., Liu Y., Lou Z., Sun L., Zhou Z., Ye S., Pang H., Gao G., Anand K., Bartlam M., Hilgenfeld R., Rao Z., *Proc. Natl. Acad. Sci. USA*, 2003, **100(23)**, 13190-13195.

Keywords: SARS, main protease, drug design

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Structural Insights into the Substrate Binding Mechanism, Inhibition and Regulation of Pim-1

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Pim-1 is a highly conserved cytoplasmic serine/threonine kinase that was first discovered as a preferential proviral insertion site in Moloney Murine Leukemia Virus (MoMuLV) induced T-cell lymphomas. The expression pattern of Pim-1 is widespread and the protein is over-expressed in a series of tumors but highest expression levels are found in cells of the hematopoietic and lymphoid system. Pim-1 phosphorylates a number of signal transduction proteins involved in the regulation of cell cycle, apoptosis, differentiation and proliferation.

We determined the structure of human Pim-1 in complex with an inhibitor of the bisindolyl maleimide (BIM) class as well as in ternary complex with its consensus peptide (pimtide) and BIM-1/AMPPNP that provides interesting insight into the substrate binding and inhibition of Pim-1 and suggests further applications of BIM-like compounds for treatment of leukaemia and other Pim-1 dependent cancer types.

Structural analysis of the monophosphorylated Pim-1 and auto-phosphorylation studies show that the human Pim-1 kinase activity is not influenced by auto-phosphorylation of activation loop residues. The N-terminus of Pim-1 has been shown to be important for several Pim interacting proteins, it is therefore likely that phosphorylation at Ser8 indicated by phosphorylation mapping plays a role in modulating these interactions.

Keywords: kinase structure, phosphorylation, drug design

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Interdomain Communication in HCV Polymerase Abolished by Small-Molecule Inhibitors

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The hepatitis C virus (HCV) polymerase is required for replication of the viral genome and is a key target for therapeutic intervention against HCV. We have determined the crystal structures of the HCV polymerase complexed with two indole-based allosteric inhibitors at 2.3 Å and 2.4 Å resolution. The structures show that these inhibitors

bind to a site on the surface of the thumb domain. A cyclohexyl and phenyl ring substituents, bridged by an indole moiety, fill two closely spaced pockets whereas a carboxylate substituent forms a salt bridge with an exposed arginine side chain. In the apoenzyme, the inhibitor binding site is occupied by a small alpha-helix at the tip of the N-terminal loop that connects fingers and thumb domains. Thus, these molecules inhibit the enzyme by preventing formation of intramolecular contacts between these two domains and consequently precluding their coordinated movements during RNA synthesis. Our structures identify a novel mechanism by which a new class of allosteric inhibitors inhibit the HCV polymerase and open the way to the development of novel antiviral agents against this clinically relevant human pathogen. Furthermore, the structures reveal a mechanism of inhibition, with the inhibitor displacing part of the fingertip loop anchoring fingers to the thumb, which may be relevant also for the inhibition of other viral RNA dependent RNA-polymerases.

Keywords: HCV, NS5B, polymerase

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Structural Parameters Influencing the Affinities and Effectiveness of Ribosomal Antibiotics

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Residing in the large ribosomal subunit and stretching from the site of peptide bond formation, to the other end of the particle, the protein exit tunnel provides the path of the emerging nascent proteins. Being of utmost importance for life, the ribosomal tunnel is targeted by a large number of antibiotics, belonging to the macrolide and ketolide families, which bind to a specific pocket made exclusively of RNA and act by blocking the tunnel, thus hampering nascent protein progression.

High-resolution crystal structures of several antibiotics, belonging to the various branches of these families as well as of compounds possessing characteristic properties of both the macrolides and ketolides, allowed parameterization of the specific contributions of the different nucleotides comprising the macrolide binding pocket. Analysis of these structures shed light on basic issues of antibiotics selectivity and provided the structural basis for the mechanisms of antibiotics resistance.

Comparative analysis of antibiotics binding modes to the eubacterial pathogen model, Deinococcus radiodurans, and to the archaea Haloarcula marismortui, which shares properties with eukaryotes and prokaryotes, showed that despite the overall conservation of the ribosome, phylogenetic and conformational variations in antibiotics binding pocket allow their selectivity, thus facilitating their therapeutical usage.

Keywords: ribosomal tunnel, antibiotics, protein synthesis

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Multiple Inhibitor Co-crystal Structures of the Human Topoisomerase I Covalent DNA Complex bound to a Series of Structurally Diverse Anti-cancer Compounds

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Topoisomerases are ubiquitous enzymes that relieve the torsional stress of DNA generated by nuclear processes such as replication and transcription. All topoisomerases act through a conserved tyrosine residue to cleave the DNA phosphodiester backbone and form a covalent phosphotyrosine intermediate. After cleavage, the broken DNA strand can rotate around the unbroken strand to either wind or un-wind DNA. The phosphodiester backbone is restored in a reversal of the transesterification reaction.

The transient top1-DNA covalent complex is a validated target for the development of anti-cancer compounds. Several structurally diverse families of chemical compounds have been discovered which specifically bind to and trap the transient top1-DNA covalent complex, which eventually results in cell death.

We report the X-ray crystal structures of the human top1-DNA complex bound with representative members of several families of anti-cancer compounds including: camptothecins, homo-camptothecins, indenoisoquinolines, indolocarbazoles and minor groove binding top1 poisons. Two distinct binding sites are identified, one for intercalating compounds such as camptothecin, and another for minor groove binding ligands. The planar nature of the intercalating compounds allows them to stack between DNA base pairs at the site of single-strand cleavage. These new X-ray structures will aid the rational design of completely novel structural classes of anticancer drugs.

Keywords: topoisomerase I, camptothecin, DNA complex

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Fragment-based Screening by X-ray Crystallography: An Alternative to High-throughput Screening

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Screening of libraries of small molecules (or drug fragments) by X-ray crystallography offers an alternative approach to discovering novel active site binders for enzymes, which may be used as a starting point in a drug discovery programme. This method can identify unique fragments with a potency in the millimolar range, and which are not found by most enzyme assay screening methods. Many of these compounds show efficient binding for their size. The use of crystallography as a screening tool gives access to precise structural data on identification of fragment binding, and this information can be used as a starting point for rational optimization of the fragment into a potent inhibitor. This may then be used as a potential lead compound for drug discovery. This method is illustrated with examples from two kinase projects [1].

[1] Hartshorn M.J., Murray C.W., Cleasby A., Frederickson M., Tickle I.J., JhotiH., *J. Med. Chem*., 2005, **48(2)**, 403-413.

Keywords: protein crystallography application, drug discovery and design, kinase

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Inspecting the Pharmacophore of Protein Kinase CK2 with Tetrabromobenzimidazoles

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CK2 is a highly pleiotropic protein kinase whose high constitutive activity is suspected to cooperate to neoplasia. Here the crystal structures of the complexes between CK2 and three new selective tetrabromobenzimidazole derivatives inhibiting CK2 with K_i values between 40 and 400 nM are presented. The ligands bind to the CK2 active site in a different way with respect to the parent compound tetrabromobenzotriazole. They enter more deeply into the cavity establishing halogen bonds with the backbone of Asp114 and Val116 in the hinge region. A detailed analysis of the interactions highlights a major role of the hydrophobic effect in the binding of this class of inhibitors. In contrast polar interactions are responsible for the different orientation of the molecules in the active site which ultimately influences the extent of the accessible surface area buried to the solvent.

Keywords: protein kinases, CK2, inhibitors