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