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The Binding of Pyrimidinyl Phosphonucleotide Inhibitors to Bovine Seminal Ribonuclease.

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Bovine seminal ribonuclease (BS-RNase) is a potent antitumor agent. However, the antitumor action of BS-RNase is associated with severe side effects such as aspermatogenicity, immunosuppression and embryotoxicity that render the pharmaceutical use of BS-RNase problematic. A resolving key might be the attainment of a delicate balance between effective antitumor activity and side effect actions. A potent and specific BS-RNase inhibitor could assist on that by playing the role of an on/off switch and acting as an antidote to the side effects of the antitumor action of BS-RNase. To initiate structure-assisted inhibitor design studies, we have investigated the binding of five substrate analogs, uridine 2' phosphate (U2´p), uridine 3' phosphate (U3´p), uridine 5' diphosphate (UDP), cytidine 3´ phosphate (C3´p), and cytidine 5´phosphate (C5´p), to BS-RNase by kinetic experiments and X-ray crystallography. The five pyrimidinyl compounds are moderate inhibitors of the enzyme with similar Ki values. In vivo studies on the aspermatogenic effect of BS-RNase in mice after intratesticular injections of BS-RNase and inhibitors C3´p and C5´p have shown that both compounds inhibit significantly the biological action of BS-RNase in mice confirming our hypothesis that ribonucleolytic inhibitors have a potential as pharmaceutics in attenuating the side effects of the anticancer activity of BS-RNase. ADME pharmacokinetic property predictions reveal inhibitors U2´p, U3´p and C5´p as the most promising with respect to oral bioavailability. However, calculations suggest that the number of H-bond acceptors/polar surface areas need to be reduced in future development of more "drug-like" phosphonucleotide derivatives.

Keywords: protein-ligand complexes; ribonuclease; reaction kinetics

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Crystal Structure of the Taz2 Domain of p300 Transcriptional Coactivator.

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Crystals of human p300 peptide (residues 1723-1836) corresponding to the extended zinc-binding Taz2 domain were grown under paraffin oil from a mixture of ammonium sulfate and alcohols. The space group is $I4_132$ with a=155.3 Å. The 2.5 Å data set was collected at the SER-CAT beamline 22-ID at the single wavelength of 1.283 Å. The crystal structure was solved by SAD approach utilizing anomalous diffraction signal of the bound Zn atoms and was refined with REFMAC to an R-factor of 20.1% (R-free=22.7%). The structure comprises a helical bundle held by three Zn fingers and is very similar to the solution structures determined for the shorter peptide [1, 2] corresponding to the evolutionarily conserved Taz2 domain from CBP and p300. Residues 1813-1834 from the current construct form a helical extension of the C-terminal helix and make extensive crystal contact interactions with the peptide binding site of Taz2. The structure thus provides information relevant to the specificity of CBP/p300 interactions with transcription factors.

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Keywords: transcription regulation; zinc finger protein; anomalous diffraction

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Human MST3 in Complex with Mn-ADP: Molecular Switch by Autophosphorylation.

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MST is a subfamily of mammalian serine/threonine kinases related to the yeast sterile-20 protein implicated in regulating cell growth and transformation. The MST3 protein contains a 300-residue catalytic domain followed by a 130-residue regulatory domain, which can be cleaved by caspase. The enzyme is then activated by autophosphorylation, and promotes apoptosis. Here we present five crystal structures of the catalytic domain of MST3, including a complex with ADP and manganese, a unique cofactor preferred by the enzyme, and another complex with adenine. Similar to other protein kinases, the catalytic domain of MST3 folds into two lobes: the smaller N-lobe forms the nucleotide-binding site, and the larger C-lobe recognizes the polypeptide substrate and also participates in catalysis. The bound ADP and Mn ions are covered by a glycine-rich loop and held in place by Asn149 and Asp162. A different orientation is observed for the ligand of the MST3-adenine complex. In the activation loop of all structures, the side chain of the key residue Thr178 has been phosphorylated and sandwiched by Arg143 and Arg176, making the loop well ordered and clearly visible. A core segment containing two prolines,

Pro183 and Pro188, is accommodated in a specific binding pocket. Comparison with other similar kinase structures shows a 180° rotation of the loop and suggests a possible pro-active dimer formation, by which intermolecular phosphorylation may occur. The well-defined protein-ligand interactions further provide additional information for design of potent inhibitors.

Keywords: kinase structure; protein conformation; egulation and reaction mechanism of enzymes

FA1-MS07-P04

Is It Possible to Guess Potential Drug Activity from Its Crystal Structure? Agnieszka Skórska-Stania[a], Magdalena Śliwa[a], Barbara J. Oleksyn[a], Kamil Musilek[b,c], Kamil Kuca[b,c], Josef Jampilek[d,e], Robert Musiol[f], Jiri Dohnal[d,e]. *[a]Faculty of Chemistry, Jagiellonian University, Kraków, Poland. [b]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic. [c]Faculty of Science, University of Jan Evangelista Purkyne, Czech Republic. [d]Zentiva a.s., Prague, Czech Republic. [e]Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. [f]Institute of Chemistry, University of Silesia, Katowice, Poland.*
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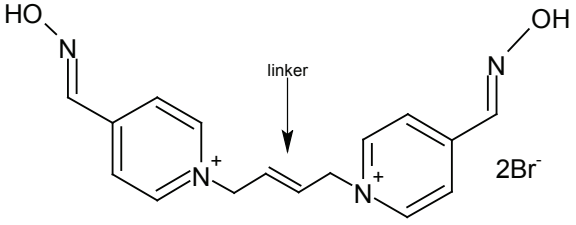
Design of new reactivators of acetylcholinesterase (AChE) gained more interest recently [1]. We have studied crystal structures of two bis-pyridinium oximes (K075 and K282), which can be used as detoxifying agents in case of poisoning with organophosphorous compounds, e.g. sarin, soman, insecticides [2]. Based on the molecular structures of well known AChE reactivators, obidoxime and HI-6, the new potential agents (K075 and K282) were proposed. Their molecules differ in configuration in respect to the double bond between carbon atoms in the linker between two pyridines. The activity of K075 has been determined [3]. It is interesting if the activity of K282 can be predicted by comparison of the crystal structures of both potential reactivators.



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Keywords: drug structure-activity relationships; drug interactions; stereochemistry

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Structural Studies of the Acetylcholine Binding Protein in Complex with Novel Compounds. Line Aagot H. Thomsen [a,b], Thomas Bale [a], Marianne L. Jensen [b], Philip K. Ahring [b], Jette S. Kastrup[a], Michael Gajhede[a]. *[a]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences,University of Copenhagen, Universitetsparken 2, DK-2100, Copenhagen, Denmark. [b]NeuroSearch A/S, Pederstrupvej 93, DK-2750 Ballerup, Denmark.*
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Cys-loop receptors form essential ligand-gated ion channels. In the central nervous system, the Cys-loop receptors mediate neurotransmitters signalling and are involved in fast communication between neurons. The receptors are formed by one to four different subunits in a pentameric complex. The Ligand binding domain is located extracellular with the ligand binding site located in the interface between the subunits and the ion channel pore region is located in the cell membrane of the neuron. Upon binding of neurotransmitter, the receptor undergoes conformational changes, which course the ion channel to open and allow ions to enter the cell. The Cys-loop receptors are implicated in several brain diseases including Parkinson's disease, schizophrenia, depression, Alzheimer's' disease, anxiety and epilepsy [1-3]. Detailed understanding of receptor structure and function is essential for providing a rational basis for the design of new drug allowing new therapeutic strategies for treating such disorders. Threedimensional structures of Cys-loop receptors have proven to be extremely difficult to obtain as the receptors are very
difficult to crystallize. However, a soluble protein forming a very similar pentameric structure, the Acetylcholine Binding Protein (AChBP) from the water snail *Lymnaea stagnalis*, has successfully been expressed and co-crystallized with different ligands [4]. Using the AChBP as a model system for Cys-loop receptors, the main objective is to co-crystallize AChBP with various novel compounds selected by binding affinity.
AChBP has been expressed in *Pichia pastoris* as a soluble protein and subsequently purified using ion exchange chromatography. Crystallization experiments are in progress and the current status of the project will be presented.

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Keywords: cys-loop receptors; AChBP; novel compounds

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How to Design Aurora Kinase A Selective Inhibitors. Magda Kosmopoulou[a], Amir Faisal[b], Chongbo Sun[b], Vassilios Bavetsias[b], Butrus Atrash[b], Nathalie Bouloc[b], Mizio Matteucci[b], Julian Blagg[b], Spiros Linardopoulos[b,c], Richard Bayliss[a]. *[a]Section*