MS7-04 **Discovery of novel allosteric inhibitors of HCV NS3/4a enzyme via structure-based drug design**

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A reliable, oral cure for hepatitis C infection is still a major unmet medical need. The approval of the NS3 protease inhibitors, telaprevir and boceprevir, in 2011 has been a welcome step in the right direction, but removing the need for pegylated interferon and ribavirin to suppress the emergence of resistance mutations remains an issue. Future treatment is expected to require a combination of drugs acting via different mechanisms. By applying our proprietary fragment screening platform, Pyramid™, to the crystal structure of the full length NS3/4a protein we identified a novel allosteric site at the protease-helicase interface, distinct from the active site targeted by the recently launched drugs boceprevir and telaprevir. Using structure-based design strategies, the initial weakly binding fragment hits were optimised against the full length NS3/4a protein, resulting in leads with low nM potency. Potent compounds demonstrated robust anti-viral activity when evaluated in the cell-based genotype 1b sub-genomic replicon assay, with a wide cytotoxicity window. The physicochemical properties of these early leads were then optimised to gain suitable pharmacokinetics for oral dosing. The resulting key compounds with sub 10nM potency in the replicon assay have MW <400 and cLogP <2 - a distinct contrast to many known protease inhibitors. During this conference we will show the allosteric binding site with bound compounds and the rationale for targeting this site. Key replicon data, including combination studies, will be shown along with pharmacokinetic data for our preclinical compounds.

Keywords: protein crystallography drug design; fragment screening; antiviral drug

MS7-05 **Enzyme inhibitors as tuberculosis drugs – a structure guided approach.** Steffi Munack[a], Kathrin Roderer[b], Jurate Kamarauskaite[b], André van Eerde[a], Peter Kast[b] and Ute Krengel[a] *[a]Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, N-0315 Oslo, Norway, [b]Laboratory of Organic Chemistry, ETH Zźrich, HCI F333, Wolfgang-Pauli-Strasse 10, CH-8093 Zźrich; Switzerland.*
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Tuberculosis (TB) still is a devastating disease. Severe problems arise due to the emergence of multi and extremely drug resistant strains caused by the lack of compliance or access to medication especially in developing countries. [1] New TB drugs are urgently needed, preferably with novel modes of action. We aim to develop new antimicrobials to overcome the problem of drug resistance addressing chorismate mutases (CM), enzymes that play a central role in the biosynthesis of aromatic amino acids in bacteria, fungi and plants. The absence of this shikimate pathway in humans turns the enzymes into potential drug targets against bacterial infections. *Mycobacterium tuberculosis* features two CMs, one secreted enzyme (*MtCM) [2] that appears to be connected to the virulence and pathogenicity of the bacteria [3] and one intracellular enzyme (MtCM). The latter possesses just weak CM activity unless activated by another shikimate pathway enzyme, the DAHP synthase (MtDS), forming a non-covalent protein complex [4]. The solved crystal structures of the enzymes build the basis of the project. A publication describing the "Ligand based virtual screening and biological evaluation of inhibitors of chorismate mutase (Rv1885c) from *Mycobacterium tuberculosis* H37Rv" served as initial inspiration. [5] Intensive structure activity relationship studies have been undertaken starting from the presented lead compounds. In contrast to the published results, the lead compounds as well as the various analogues did not possess any inhibitory effects towards the target enzymes. We are now proceeding with identification of hit compounds via high throughput or small therapeutic fragment library screening followed by modifications of those molecules to optimize binding and inhibition properties and generate leads for the drug design process. The general course of action consists of synthesis of discovered lead compounds, evaluation of those molecules in chorismate depletion and thermofluor assays, co-crystallization with the target enzymes to reveal the detailed binding mode and further structural modifications by means of organic synthetic chemistry based on those findings. In a cyclic approach, we aim for the development of highly specific enzyme inhibitors, tailor-made for the target enzymes, which could find their application as TB drug. The strategy of structure based drug design and recent results of a high throughput library screening (ChemBioNet and in-house FMP libraries) at the Leibniz-Institute for Molecular Pharmacology, FMP Berlin, will be presented.

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