MS9-O4 Crystallographic fragment-screening – Results from the HZB-Marburg collaboration

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In the last decade, fragment-based lead discovery has evolved into a widely applied technique in drug development. While originally pre-screening fragment binding investigations by biophysical methods were mandatory, nowadays complete fragment libraries can be screened by X-ray crystallography, owing to the ever increasing level of automation in diffraction data collection using synchrotron radiation and processing. In this context, it is essential to use high throughput methods, to have good diffraction quality target protein crystals and to work with a high quality fragment library. Thorough crystallographic analysis of protein-fragment complexes and their binding modes reveal detailed structural knowledge to develop fragments (100-200 Da) into new potential lead structures (300-500 Da). Recently, we have started to establish an experimental facility optimized for high throughput fragment screening at the BESSY II storage ring [1]. We have validated our assembled library of 96 fragments against two target proteins. These initial results revealed that this library can identify binding partners at a hit rate of about 10%. In addition we are currently testing several novel techniques to simplify and accelerate sample preparation. The ultimate aim is to make our library in combination with a highly automated beamline [2] available for academic and industrial users. This unique facility for screening experiments and evaluation of bound fragments will enable efficient fragment screening on a much broader basis.

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Keywords: fragment screening, fragment libraries, X-ray crystallography

MS9-O5 Inhibition of human Aldehyde Dehydrogenase1 by the anti-tumor agent Duocarmycin

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Inhibition of human Aldehyde Dehydrogenase1 by the anti-tumor agent DuocarmycinDuocarmycin SA an antibiotic metabolite isolated form Streptomyces, is one of the most potent anti-tumor agents known to date.[1] This highly cytotoxic effect is currently exploited by conjugating duocarmycin to antibodies for specific tumor cell targeting. Two such antibody-drug conjugates (MDX-1203, SYD985) are now in phase I clinical trials against Non-Hodkins lymphoma, breast and kidney cancer.[2] Previous studies revealed that duocarmycins and similar compounds exert their potent anti-tumor properties by DNA alkylation.[3] However, recently an additional proteomic target, aldehyde dehydrogenase 1A1 (ALDH1A1), was identified in human lung cancer cell lines. [4,5] ALDH1A1 is responsible for the oxidative formation of retinoic acid and therefore involved in gene regulation and cell differentiation.[6] Moreover ALDH1A1 is highly expressed in several tumors [7, 8, 9] where its expression is correlated with increased proliferation and poor prognosis. Dissection of the structure-activity relationship of duocarmycin showed that the alkylation subunit alone is sufficient for ALDH1A1 inhibition.[5] Here we present biochemical data as well as the X-ray crystal structure of the ALDH1A1 without and in complex with a Duocarmycin analogue (sdb6). Sdb6 is covalently bound to one of the two active site cysteines, with the cyclopropabenzindole ring of the molecule forming pi-stacking with aromatic amino acid side chains. The structural and biochemical data shed light on the highly specific inhibitory mechanism of duocarmycine on ALDH1A1 and could aid the rational design of ALDH1A1 inhibitors.

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Keywords: aldehyde dehydrogenase 1, antitumor agents, duocarmycin