Fragment based design of mycobacterial thioredoxin reductase inhibitors: from a fragment screening to novel inhibitors.

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The resurgence of tuberculosis, caused primarily by Mycobacterium tuberculosis (Mtb), and the appearance of multi-drug and extensively drug resistant strains leads to an urgent need for new antituberculotic drugs with alternative modes of action. As part of the thioredoxin system the thioredoxin reductase (TrxR) is essential for thiol redox homeostasis [1]. The mycobacterial TrxR shows a substantial difference in sequence, mechanism and structure to eukaryotic TrxRs leading to the expectation that the mycobacterium tuberculosis TrxR is a selective and promising target for a tuberculosis treatment. The druggability was already shown with a compound class derived from a docking-based virtual screening approach [2,3].

For the identification of new fragment-based starting points and for the investigation of new interaction sites for potential drugs, a crystallographic fragment screening was performed [4]. TrxR crystals that reproducibly showed high-resolution diffraction (~1.7 Å) were soaked with the 96 structurally diverse fragments of the F2X-Entry Screen [5]. The diffraction data were collected at BESSY II [6] processed and refined by a largely automated software pipeline at HZB including the FragMAXapp and hit identification by a multi dataset analysis approach called PanDDA [7, 8]. 40 fragments were found bound to nine binding sites, of which four sites are positioned at binding pockets or important interaction sites and therefore show high potential for possible inhibition.

After a detailed analysis of all fragments and binding sites, two fragments were chosen to be optimized, based on their unique interaction and potential selectivity for the mycobacterial TrxR. Interesting analogues were analysed by the SAR-by-catalogue approach using molecular docking. 24 compounds were purchased for further testing. Compounds with an extension of the second fragment were not purchasable due to the needed exit vector that is not synthetically accessible. Therefore, suitable fragment analogues and promising compounds were computational designed, analysed and synthesised. First biochemical activity assays of the compounds show signs of inhibitory activity. Further studies will include more biochemical and biophysical assays of the evolved compound and further crystallisation to compare experimentally observed and predicted binding poses.

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