Two natural adducts from *Morus nigra* as inhibitors of Mycobacterium tuberculosis PtpB

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INTRODUCTION. *Mycobacterium tuberculosis* (Mtb), the main causative agent of tuberculosis (TB), secretes two protein tyrosine phosphatases, termed PtpA and PtpB, which contribute to Mtb's ability to survive and replicate within host macrophages by interfering with host signaling pathways. Furthermore, both enzymes have been reported as essential virulence factors of Mtb. PtpB is secreted into host cell cytosol during Mtb infection where apparently contributes to the attenuation of apoptosis and interferon γ (IFNγ)-mediated signal transduction pathways. Increasing evidence of PtpA and PtpB importance for Mtb infection has attracted the interest as promising candidate targets for novel anti-TB treatment. **OBJECTIVES:** The aim of this work was to investigate the inhibitory activity of natural compounds isolated from *Morus nigra* against PtpB. **MATERIAL AND METHODS**: The recombinant PtpB was expressed in *Escherichia coli* BL21(DE3) and purified by metal affinity chromatography. The compounds were extracted from *M. nigra* roots with acetone and further purified by chromatography. The catalysis of PtpB was determined spectrophotometrically using *p*-nitrophenyl phosphate as substrate. Mass spectrometry, intrinsic fluorescence and isothermal titration calorimetry assay were performed to corroborate the inhibition mechanism. **RESULTS AND DISCUSSION:** From the crude extract in acetone we isolated eight compounds A-H, which displayed significant inhibition of PtpB activity with IC_{50} values between 0.36 and 8.42 µM. The best inhibitory effects were achieved by compound C (K_i = 0.13 ± 0.07 µM) and compound B (K_i = 0.11 ± 0.04 µM). These two compounds exhibited competitive mechanism of action, as also corroborated by the proteolysis protection of the PtpB active site in the presence of compounds. Intrinsic fluorescence and ITC results showed favorable binding profiles and indicated that both compounds have only a single binding site in PtpB, with K_d values in agreement with the K_i. **CONCLUSIONS:** We identified novel natural products as potent inhibitors of PtpB, which might contribute to future search for new anti-TB drugs.

Keywords: *Mycobacterium tuberculosis*, PtpB inhibitors, natural products
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