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# An orphan cytochrome P450 from *M. tuberculosis* as a potential drug target for the metabolization of the antituberculous drug SQ109: *identification and characterization of the resulting metabolite*

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The emergence of the multidrug-resistant tuberculosis (MDR-TB) strains generates the need for efficient drugs. Among the new potential anti-TB, SQ109 showed activity against resistant *Mycobacterium tuberculosis* (*Mtb*) and already advanced to Phase II clinical trials. SQ109 is an N-geranyl-N'-(2-adamantyl)ethane-1,2-diamine, degraded by human liver cytochrome P450 (P450) and by recombinant *Mtb* CYP124A1. In our aim to identify potential substrates to the orphan *Mtb* P450, CYPX, we found that it could bind and metabolize SQ109. The haemoprotein CYPX displays typical UV-visible spectroscopic features which allow measuring binding constants for potential substrates. SQ109 strongly binds ($K_d$ = 2.08 +/- 0.4 µM) to CYPX. *In vitro* enzymatic assays were carried out by reconstitution of the catalytic cycle of CYPX to test if SQ109 was a substrate. The SQ109 unique produced metabolite was analysed by ESI-FT-ICR-MS. The ion signal obtained at m/z 347.30586 ($C_{22}H_{39}N_2O^+$) allowed us to identify an additional oxygen atom in the SQ109 structure. To characterize the oxidation site and its nature, collision-induced dissociation (CID) experiments were performed on non-metabolised and oxidised SQ109 precursor ions. Based on the observed fragment ions, including neutral losses of water molecules, structures were proposed with a hydroxyl group on carbon 4 or 5 on the geranyl moiety. The localization of the hydroxyl is in good agreement with the position of SQ109 relative to the catalytic haeme group, that was obtained by docking of the SQ109 in a 3D homology model of CYPX. Overall, our findings identified *Mtb* CYPX as a new potential drug target which could contribute to the metabolization of the highly potent anti-TB SQ109.