New preclinical data on adjunctive antibacterial therapies

Synthetic analogues of pyridine-n-oxide disulfides from *Allium stipitatum* demonstrate potent anti-tubercular activities and inhibit mycobacterial drug efflux pumps

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**Objectives:** Drug resistance in *Mycobacterium tuberculosis* is an issue of great public health concern and new molecules with pleiotropic modes of action are urgently required to tackle this challenging menace. Pyridine-N-oxide alkaloids possessing disulfide functional groups were isolated from the bulbs of *Allium stipitatum*, which belongs to the genus *Allium* with common members like garlic, onion, leeks and chives. The objective of this research was to produce a series of synthetic analogues based on the naturally isolated pyridine-N-oxide and evaluate their antibacterial activities against a panel of mycobacteria. Their efflux pump inhibitory effect was also evaluated because drug efflux is one of the well-established mechanisms that contribute to antibiotic resistance in mycobacteria and the identification of efflux pump inhibitors is therefore an attractive target in antimicrobial therapy.

**Method:** The chloroform extract of macerated *Allium stipitatum* bulbs was separated on silica gel by Vacuum Liquid chromatography (VLC) to obtain fractions. The proposed structure of the naturally isolated compound 2-(methyldithio) pyridine-N-oxide was confirmed by S-methylthiolation of commercial 2-thiopyridine-N-oxide. The synthetic analogues were produced by adopting the method of Kitson and Loomes, briefly, the appropriate aromatic thiol purchased from Sigma Aldrich were S-methylthiolated using S-methylmethanethiosulphonate and back extracted with dichloromethane. The synthesized compound was then characterized by the use of spectroscopic techniques, UV, IR, HRESIMS, 1D and 2D NMR. A number of whole-cell phenotypic bio-assays including spot culture growth inhibition assay, drug efflux assay which interrogate all of the endogenous drug targets simultaneously in specific physiological contexts, were used along with appropriate positive and negative controls.

**Results:** A selection of five compounds from the methyldisulfide chemical class, methyldisulfides, 3-(benzylthio)-5-(methyldisulfanyl)-4H-1,2,4-triazol-4-amine, 2-(methyldisulfanyl)thienol(2,3-d)pyrimidine-4-amine, 7-fluoro-2-(methyldisulfanyl)benzo(d)thiazole, (E)-3-(methyldisulfanyl)-5-styril-4H-1,2,4-triazole, 4-ethyl-5-(methyldisulfanyl)-4H-1,2,4-triazole-3-ol, showed antimycobacterial activities at clinically-relevant concentrations when tested against *M. aurum*, *M. bovis* BCG, *M. tuberculosis* H37Rv and multi-drug resistant strains of TB-clinical isolates. In addition, the synthetic compounds inhibited mycobacterial drug efflux mechanisms, which are reported here for the first time for this class of compounds. The synthesized methyldisulfides are novel chemical scaffolds that have potential as templates for the design of new drugs against TB.

**Conclusion:** This study suggests that synthesized methyldisulfides are new scaffolds, which can lead to potentially new drugs against tuberculosis (TB). The inhibition of efflux pumps by these compounds is promising as it would be a way to improve the efficacy and/or extend the clinical utility of existing antibiotics.