Synthesis and bioevaluation of some new N-substituted pyranophenothiazines

Thi-Thuan Nguyen¹*, Hanh Dufat², Sylvie Michel²

¹ Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Ha Noi, Vietnam
² Laboratoire de Pharmacognosie, UMR/CNRS 8638, Université Paris Descartes, 75006 Paris, France

ABSTRACT

Tuberculosis is a dangerous infectious disease. Because of the enlargement of multidrug-resistant strains (MDR-TB), the need of new drugs becomes more important. In the last research, 2 pyranophenothiazine series had been synthesized and bio-evaluated. Herein, eight N-substituted pyranophenothiazine derivatives from 2 series were designed, synthesized and evaluated for their antituberculosis activities. The designed compounds were prepared by alklylation reactions and all synthesized compounds were confirmed by analysis of NMR and MS spectra. Antimycobacterial activity of the synthesized compounds on Mycobacterium bovis BCG had been determined using the microdilution resazurin assay - The results showed that 4 compounds exhibited a mild inhibiting activity on the tested tuberculosis strains. These structural requirements will be taken into account for the design of further analogues in pyranophenothiazine series.

1. INTRODUCTION

During the last decades, tuberculosis is always a dangerous infectious disease. Because of the enlargement of multidrug-resistant strains (MDR-TB), the need of new drugs becomes more important¹². In order to research new antituberculosis agents, the laboratory of Pharmacognosy (Paris Descarte University) had created a structure hybrid between a phenothiazine and a 2H-chromene. As the result, 2 series of pyranophenothiazine (leading compunds were 1 for angle-pyraño[a]phenothiazine, and 2 for linear-pyraño[b] phenothiazine series) (Figure 1) and their derivatives with different levels of oxidation had been synthetized¹. Regarding in the literature of phenothiazine group, the most effective antituberculosis agent is thioridazine - an N-substituted phenothiazine (Figure 1)⁴, so we intended to form the N-subsituted derivatives in these 2 pyranophenothiazine series. The current paper reports the results obtained from the synthesis, biological evaluation of these new pyranophenothiazine derivatives.
2. MATERIALS AND METHODS

2.1. Materials

The designed compounds were prepared using conventional synthetic methods with all PA chemical agents (from Merck, TCI, Sigma). IR spectra were recorded on a Nicolet 510 FT-IR spectrophotometer as KBr discs. $^1$H and $^{13}$C NMR spectra were recorded on an AC 300 Bruker spectrometer (300 MHz for $^1$H and 75 MHz for $^{13}$C) and an Avance 400 Bruker spectrometer. Mass spectra were recorded on a Nermag R 10-10C mass spectrometer (DIC/NH$_3$) (90eV), or a Hewlett Packard 5890 spectrometer (IE) (70eV), or a ZQ 2000 Waters spectrometer (ESI). The Mycobacterium bovis BCG 1173P2 strain was from Institut Pasteur (Paris, France).

2.2. Methods

2.2.1. Chemistry

The leading compounds (1 and 2) were synthesized according to the pathway depicted in Scheme 1.

Scheme 1: Synthesis of leading compounds of pyranophenothiazine

A demethylation of 2-methoxy phenothiazine (3) had given 10H-2-hydroxy phenothiazine (4), which was put into the reaction with 3-chloro-3-methylbut-1-yne (5) to form 2-(1,1-dimethyl-propargyloxy)-phenothiazine (6) in overall yield of about 50%. This propargylic ether had been cyclized intramolecularly at high temperature to afford the expected products 1 and 2 at the proportion of 2:1 respectively.

The N-substituted derivatives of these leading compounds were synthesized in the second step according to the pathway depicted in Scheme 2.

Scheme 2: Synthesis of leading compounds of pyranophenothiazine
N-substituted derivatives were obtained by alkylation with various alkyl chlorides or chloroalkylamine hydrochlorides derivatives in presence of sodium hydride in anhydrous dimethylformamide to give 4 compounds of each series respectively. The purify of each compound was determined by TLC with 2 different mobile phases. The result should be accepted if there was only one trace on the plate under 366nm and also after spraying the solution of vanillin in H$_2$SO$_4$ concentrated.

The structure of all synthesized compounds had been confirmed by analysing of NMR and MS spectra obtained under the conditions listed in section 2.1.

2.2.2. Bioactivity

Antimycobacterial activity of the synthesized compounds on M. bovis BCG was determined using the microdilution resazurin assay (MRA) [6]. Resazurin salt powder (Sigma) was prepared at 0.01% (wt/vol) in distilled water, sterilized by filtration through a 0.22 µm membrane and stored at 4°C for a week. Drug stock solutions were prepared in dimethylsulfoxide (DMSO) at concentration of 50 mg/mL and frozen until used. The inocula were prepared from M. bovis BCG strains grown in Dubos medium directly in 96-well plates at concentrations from 100 to 0 µL of Dubos medium supplemented with 10% ADC enrichment (Difco). 2 µL of two fold serial dilutions of each drug was prepared in 200 µL of Dubos medium directly in 96-well plates at concentrations from 100 to 0.05µg/mL. Growth controls containing DMSO and isoniazide (from 1 µg/mL to 1 ng/mL) were also included. The plates were covered, sealed and incubated at 37 °C during 8 days, then 30 µL of resazurin solution was added to each well and plates were allowed to incubate at 37 °C for an additional 24 h. A change from blue to pink indicated reduction of resazurin and therefore bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that prevented this color change.

3. RESULTS

3.1 Chemistry

General procedure of N-alkylation: A solution of pyranophenothiazine 1 or 2 (50 mg ~ 0.2 mmol) in anhydrous dimethylformamide (1 mL) was added to sodium hydride (10 mg) under argon at 25 °C. After stirring for 15 min, a solution of propriety alkyl chloride (0.6 mmol) in anhydrous DMF (2 mL) was added and the mixture was stirred for 6 h. The reaction was quenched by addition of a solution of 2.5% NH$_4$Cl in water (20 mL), stirred for 1 h, and then extracted by methylene chloride. The organic phase was dried over anhydrous sodium sulfate, filtered, and then evaporated to dryness under reduced pressure. The obtained greenish residue was purified by chromatography over silica gel eluting with a mixture of methylene chloride and methanol (95/5 v/v) to give the corresponding N-substituted products.

Eight N-substituted pyranophenothiazines had been synthesized. The information of their spectra of $^1$H-NMR, $^1$C-NMR, MS, and IR was given below.

3.3,12-Trimethyl-3,12-dihydropyrano[3,2-a]phenothiazine (7)

The compound 1 reacted with methyl iodide to give the N-alkylated compound 7 as a yellow oil (48.7 mg ~ 93% yield): IR (KBr) $\nu_{max}$ (cm$^{-1}$): 2971, 1589, 1481, 1450, 1271, 1168, 1110, 1016, 802, 755, 728; NMR: (DMSO-d$_6$), $\delta$ (ppm): $^1$H: 7.20 (1H, t, $J$=8 Hz, H$_{10}$); 7.15 (1H, d, $J$=8 Hz, H$_{11}$); 7.10 (1H, d, $J$=8 Hz, H$_{12}$); 6.96 (1H, t, $J$=8 Hz, H$_{13}$); 6.86 (1H, d, $J$=8 Hz, H$_{12}$); 6.62 (1H, d, $J$=9 Hz, H$_{6}$); 6.47 (1H, d, $J$=8 Hz, H$_{7}$); 5.80 (1H, d, $J$=9 Hz, H$_{8}$); 3.49 (3H, s, 3H$_3$); 1.39 (6H, s, 2 CH$_3$); $^{13}$C: 154.0 (C$_{11}$), 147.4 (C$_{10}$), 142.5 (C$_{12}$), 129.6 (C$_{1}$), 127.8 (C$_{7}$), 126.9 (C$_{8}$), 126.7 (C$_{9}$), 126.6 (C$_{10}$), 123.7 (C$_{6}$), 120.1 (C$_{1}$), 119.0 (C$_{12}$), 118.8 (C$_{9}$), 113.5 (C$_{10}$), 111.7 (C$_{5}$), 75.1 (C$_{11}$), 43.0 (C$_{1}$), 27.2 (2 CH$_2$); C$_{15}$H$_{21}$N$_2$OS; Mass: (ES') m/z: 296.1 ([MH$^+$]); Anal. Cal. For C$_{15}$H$_{21}$N$_2$OS (295.3987): C, 73.19; H, 5.80; N, 4.74; O, 5.42, S, 10.85. Found: C, 73.20; H, 5.82; N, 4.76; O, 5.42; S, 10.80.

3,3-Dimethyl-12-propyl-3,12-dihydropyrano[3,2-a]phenothiazine (8)

The compound 1 reacted with n-propyl chloride to give the N-alkylated compound 8 as a yellow oil (50.5 mg ~ 88% yield): IR (KBr) $\nu_{max}$ (cm$^{-1}$): 2963, 2931, 2872, 1588, 1450, 1429, 1376, 1272, 1219, 1166, 1114, 817, 752; NMR: (DMSO-d$_6$), $\delta$ (ppm): $^1$H: 7.21 (2H, m, H$_{10}$, H$_{11}$);
3,3-Dimethyl-12-(3-dimethylaminopropyl)-1,12-dihydropyran-3,2-aphenothiazine (9)

The compound 1 reacted with 3-chloro-N,N-dimethylpropan-1-amine hydrochloride to give the N-alkylated compound 9 as a yellow oil (38.8 mg – 60% yield): IR: (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 2924, 2854, 1609, 1465, 1379, 1286, 1155, 1151, 1176; NMR: (DMSO-d$_6$), $\delta$ (ppm): $^1$H: 7.23 (2H, m, H$_{18,19}$); 7.15 (1H, dd, $J$=8, 2 Hz, H$_{7}$); 6.99 (1H, t, $J$=8, 2Hz, H$_{6}$); 6.90 (1H, d, $J$=8 Hz, H$_{5}$); 6.55 (1H, d, $J$=9 Hz, H$_{1}$); 6.51 (1H, d, $J$=8 Hz, H$_{5}$); 5.83 (1H, d, $J$=9 Hz, H$_{3}$); 3.91 (2H, br, s, 2H$_{17}$); 2.55 (2H, br, s, 2H$_{16}$); 2.20 (6H, s, 2H$_{21,22}$); 1.68 (2H, m, 2H$_{12,13}$); 1.33 (6H, s, 2 CH$_{3}$); $^{13}$C: 153.8 (C$_{14}$), 145.4 (C$_{11a}$), 142.2 (C$_{12}$), 130.3 (C$_{3}$), 129.2 (C$_{16}$), 127.9 (C$_{19}$), 127.3 (C$_{1}$), 127.1 (C$_{1}$), 124.1 (C$_{1}$), 121.3 (C$_{1}$), 120.6 (C$_{1}$), 119.8 (C$_{1}$), 114.7 (C$_{15}$), 112.2 (C$_{7}$), 75.3 (C$_{5}$), 55.6 (C$_{6}$), 52.6 (C$_{2}$), 44.4, 44.3 (C$_{7a, 7b}$), 27.5, 22.1 (2 CH$_{3}$), 25.7 (C$_{2}$); C$_{22}$H$_{26}$N$_{2}$OS; Mass: (ES$^+$) m/z: 367.2 ([MH]$^+$); Anal. Calcd. For C$_{22}$H$_{26}$N$_{2}$OS (366.5196): C, 72.09; H, 7.15; N, 7.64; O, 4.37; S, 8.75. Found: C, 72.20; H, 7.12; N, 7.76; O, 4.30; S, 8.62.

2,2,11-Trimethyl-2,11-dihydropyran-2,3-b-phenothiazine (11)

The compound 2 reacted with methyl iodide to give the N-alkylated compound 11 as a yellow oil (45.6 mg – 87% yield): IR: (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 2970, 1633, 1608, 1575, 1494, 1463, 1383, 1367, 1285, 1253, 1188, 1157, 1123, 1021, 881, 841, 760; NMR: (DMSO-d$_6$), $\delta$ (ppm): $^1$H: 7.20 (1H, dt, $J$=8, 1 Hz, H$_{7}$); 7.14 (1H, dd, $J$=8, 1 Hz, H$_{5}$); 6.95 (2H, t, $J$=8 Hz, H$_{18,19}$); 6.83 (1H, s, H$_{5}$); 6.41 (1H, s, H$_{13}$); 6.32 (1H, d, $J$=10 Hz, H$_{6}$); 5.60 (1H, d, $J$=10 Hz, H$_{3}$); 3.28 (3H, s, $J$=6 Hz, 2H$_{20}$); 1.33 (6H, s, 2 CH$_{3}$); $^{13}$C: 153.2 (C$_{14}$), 146.8 (C$_{11a}$), 145.4 (C$_{10}$), 129.1 (C$_{3}$), 128.1 (C$_{3}$), 127.2 (C$_{12}$), 124.5 (C$_{1}$), 123.0 (C$_{16}$), 121.4 (C$_{1}$), 116.2 (C$_{1}$), 115.1 (C$_{1}$), 112.7 (C$_{1}$), 103.8 (C$_{12}$), 76.8 (C$_{7}$), 35.8 (C$_{6}$), 28.1 (2 CH$_{3}$); C$_{14}$H$_{26}$N$_{2}$OS; Mass: (ES$^+$) m/z: 296.1 ([MH]$^+$); Anal. Calcd. For C$_{14}$H$_{26}$N$_{2}$OS (295.3987): C, 73.19; H, 5.80; N, 4.74; O, 5.42; S, 10.85. Found: C, 73.22; H, 5.75; N, 4.68; O, 5.53; S, 10.82.

3,3-Dimethyl-12-(3-piperidin-1-ylpropyl)-1,12-dihydropyran-3,2-aphenothiazine (10)

The compound 1 reacted with 1-(3-chloro-propyl)piperidine hydrochloride to give the N-alkylated compound 10 as a yellow oil (65.3 mg – 88% yield): IR: (KBr) $\nu_{\text{max}}$ (cm$^{-1}$): 1609, 1579, 1466, 1383, 1286, 1158, 1114, 1041, 880, 746; NMR: (DMSO-d$_6$), $\delta$ (ppm): $^1$H: 7.20 (2H, m, H$_{18,19}$); 7.11 (1H, dd, $J$=8, 1 Hz, H$_{7}$); 6.95 (1H, dt, $J$=8, 1 Hz, H$_{5}$); 6.86 (1H, d, $J$=8 Hz, H$_{1}$); 6.53 (1H, d, $J$=10 Hz, H$_{3}$); 6.47 (1H, d, $J$=8 Hz, H$_{2}$); 5.80 (1H, d, $J$=10 Hz, H$_{3}$); 3.93 (2H, br s, 2H$_{17}$); 2.32 (2H, br s, 2H$_{18}$); 2.14 (4H, br s, 2H$_{19}$, 2H$_{20}$); 1.58 (2H, m, 2H$_{12,13}$); 1.40 (6H, br s, 2 CH$_{3}$); 1.29 (6H, br s, 2H$_{14}$, 2H$_{15}$ and 2H$_{21,22}$); $^{13}$C: 153.8 (C$_{14}$), 145.5 (C$_{11a}$), 142.7 (C$_{12}$), 129.8 (C$_{19}$), 128.7 (C$_{19}$), 127.7 (C$_{1}$), 127.2 (C$_{1}$), 126.9 (C$_{1}$), 123.8 (C$_{3}$), 120.9 (C$_{1}$), 114.4 (C$_{10}$), 111.9 (C$_{3}$), 75.1 (C$_{5}$), 55.0 (C$_{6}$), 54.2 (C$_{1}$, C$_{2}$), 52.5 (2 CH$_{3}$, C$_{2}$), 24.4 (C$_{2}$); C$_{22}$H$_{30}$N$_{2}$OS; Mass: (ES$^+$) m/z: 407.2 ([MH]$^+$); 429.2 ([MNa]$^+$); 445.2 ([MK]$^+$); Anal. Calcd. For C$_{22}$H$_{30}$N$_{2}$OS (406.5835): C, 73.85; H, 7.44; N, 6.89; O, 3.94; S, 7.89. Found: C, 73.72; H, 7.42; N, 6.82; O, 4.07; S, 7.97.
2,2-Dimethyl-11-(3-dimethylaminopropyl)-2,11-dihydropyrano[2,3-b]phenothiazine (13)

The compound 2 reacted with 3-chloro-N,N-dimethylprop-1-amine hydrochloride to give the N-alkylated compound 13 as a yellow oil (41.2 mg – 63% yield) IR: (KBr) νmax (cm⁻¹): 2924, 2854, 1609, 1465, 1379, 1286, 1155, 1115, 1074, NMR: (DMSO-d6), δ (ppm): 1H: 7.19 (1H, dt, J= 8, 1 Hz, H3; 7.13 (1H, dd, J=8, 1 Hz, H2); 7.00 (1H, d, J=8 Hz, H1); 6.93 (1H, t, J=8 Hz, H4); 6.86 (1H, s, H6); 6.47 (1H, s, H5); 6.31 (1H, d, J=10 Hz, H7); 5.60 (1H, d, J=10 Hz, H8); 3.85 (2H, t, J=6 Hz, 2H); 2.30 (2H, t, J=6 Hz, 2H); 2.09 (4H, s, 2H); 1.76 (2H, m, 2H); 1.33 (6H, s, 2 CH3); δC: 153.1 (C12), 146.2 (C11), 144.9 (C10), 129.3 (C9), 127.9 (C8), 127.5 (C7), 124.7 (C6), 124.5 (C5), 123.0 (C4), 121.4 (C3); 116.4 (C2), 114.4 (C1), 104.8 (C0), 76.8 (C6), 56.0 (C5), 45.7 (C4, C5), 45.0 (C3), 28.0 (2 CH2), 26.8 (C2); δC: δH2N2OS; Mass: (ES+) m/z: 367.2 ([MH]+); Anal. Cal. For C23H26N6OS: C: 72.09; H: 7.15; N: 7.64; O: 4.37; S: 8.75. Found: C: 71.97; H: 7.22; N: 7.70; O: 4.43; S: 8.68.

3.2 Bioactivity

The antmycobacterial activity was screened on M. bovis BCG using the Microdilution resazurin assay (Table 1).

4. DISCUSSION

As detailed on the last report of Nguyen et al., the leading compounds (pyrano[a]pheno-thiazine - 1, and pyrano[b]phenothiazine - 2) were synthesized by an intramolecule-cyclisation of 2-(1,1-dimethyl-propaglyoxy)-phenothiazine (which was formed from 10H-2-hydroxy phenothiazine and 3-chloro-3-methylbut-1-ynyl). From these raw materials, N-substituted derivatives were obtained by alkylation with various alkyl chlorides or chloralkylamine hydrochlorides in presence of sodium hydride in anhydrous dimethylformamide to give 4 compounds of each series respectively. The structures of these 8 new compounds had been confirmed by spectra of 1H - NMR, 13C-NMR and MS.

In fact, the molecular mass of each compound corresponded to its molecular structure. The 1H-NMR spectra contained the numbers of proton corresponding to their structures, with 2 peaks of 3H at about 1.3 ppm, corresponding to 2 methyl groups on pyran ring: 6 peaks in the aromatic zone (from 8 to 5 ppm) attributed to the 6 proton of the phenothiazine ring. Compare with the leading compound corresponding. 13C - NMR spectra of all 8 new derivatives had been lacking of NH peak and addition of the peaks corresponding to the substituted group.

All structures were confirmed by respective 13C - NMR spectra.
Table 1. Antimycobacterial of the pyranophenothiazine derivatives on *M. bovis* BCG

| Compound | IC<sub>50</sub> (µg/mL) *M. bovis* BCG | Compound | IC<sub>50</sub> (µg/mL) *M. bovis* BCG |
|----------|-------------------------------------|----------|-------------------------------------|
| INH      | 0.4                                 | 1        | >100                                |
| 2        | 25                                  | 7        | 37.5                                |
| 3        | 50                                  | 4        | 50                                  |
| 8        | >100                                | 9        | 12.5                                |
| 10       | 63                                  |          |                                     |

The MS spectre of each compound gave the molecule peak with m/z corresponded closely to the intended molecule mass.

The bioactivity of synthesized compounds was tested on *M. bovis* BCG. From the results shown in Table 1, almost of *N*-substituted derivatives were more potent than the non-substituted compounds (1 and 2). The side chains of alkylamine propyl (compounds 9, 10, 13, 14) with the IC<sub>95</sub> from 0.068 mmol/mL to 0.016 mmol/mL seemed to be more favorable to the antituberculosis activity than alkyl simple chains (compounds 7, 8, 11, 12 – IC<sub>95</sub> more than 0.12 mmol/mL), liked as in the reports of Scalacci *et al.*<sup>7</sup> and Ramprasad *et al.*<sup>8</sup>.

However, the antituberculosis activities of all synthesized compounds were only mild, and less than isoniazid (INH) – the most common antituberculosis agent actual (0.003 mmol/mL). Anyways, Amaral L. *et al.*<sup>4</sup> had reported that phenothiazine analogues could be used to enhance the activity of other antituberculosis agents, especially in the case of multi-drug resistance (MDR) with 4 different proposed mechanisms (1: enhanced killing activity of the human macrophage; 2: inhibition of the over-expressed efflux pump system of the antibiotic resistant *Mycobacterium tuberculosis* infecting the organism; 3: concentration of phenothiazine analogue to levels compatible with the MIC and MBC of the agent; and 4: inhibition of the oxygen consumption of the *Mycobacterium* sp.). This effect was observed not only on some ‘old’ phenothiazines, such as thioridazine, but also on some new phenothiazine-analogue potential antituberculosis agent, such as DG70<sup>†</sup> which was presented a mild activity on *M. bovis* BCG and *M. tuberculosis* H37Rv but good activity on some MDR strains (TDR31, TDR116, TDR36, TDR91).

Because of that, the mild activity of the synthesized compounds might be promised a better antituberculosis agent of other analogues, especially on MDR strains – the most challenge of treatment now.
5. CONCLUSION

In conclusion, 8 new pyranophenothiazine derivatives had been prepared. Their structures were confirmed by analysis of spectral data (1H-NMR, 13C-NMR and MS). Their antibacterial activity was evaluated on M. bovis BCG. The compounds with side chains of alkyl amine propyl had a mild antituberculosis activity. These structural requirements will be taken into account for the design of further analogues in pyranophenothiazine series, and MDR strains will be involved into the test for the most active compounds.

Conflicts of interest (If any)
None to declared

Funding
None to declare

Ethical approval
None to declare

Article info:
Received August 29, 2018
Received in revised form January 9, 2019
Accepted March 29, 2019

REFERENCES

1. Kumar D, Negi B, Rawat BS. The anti-tuberculosis agents under development and the challenges ahead. Future Med Chem. 2015. 7(15):1981-2003.
2. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. Lancet, 2010. 375(9728):1830-43.
3. Nguyen TT, Dufat H, Michel S, Prado S, Saint-Joanis B, Janin Y. Synthesis and bioevaluation of new pyranophenothiazine derivatives. Pharm Sci Asia. 2017. 44(4):227-37.
4. Amaral L, Martins A, Spengler G, Hunyadi A, Molnar J. The mechanism by which the phenothiazine thioridazine contributes to cure problematic drug-resistant forms of pulmonary tuberculosis: recent patents for “new use”. Recent Pat Antiinfect Drug Discov. 2013. 8(3):206-12.
5. Bycroft BW, Johnson AP, Landon W. The reaction of skatole with 3-chloro-3- methylbut-1-yne: A novel ring expansion involving an allenic carbene. J Chem Soc. 1969. D:463a.
6. Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2002. 46(8):2720-2.
7. Scalacci N, Brown AK, Pavan FR, Ribeiro CM, Manetti F, Bhakta S, et al. Synthesis and SAR evaluation of novel thioridazine derivatives active against drug-resistant tuberculosis. Eur J Med Chem, 2017. 127: p. 147-158.
8. Ramprasad J, Nayak N, Dalimba U. Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents. Eur J Med Chem, 2015. 106:75-84.
9. Sukheja P, Kumar P, Mittal N, Li SG, Singleton E, Russo R, et al. A novel small-molecule inhibitor of the Mycobacterium tuberculosis demethylmenaquinone methyltransferase menG is bactericidal to both growing and nutritionally deprived persister cells. MBio, 2017. 8(1). pii: e02022-16.