Anti-Tubercular Activity of Disulfiram, an Anti-Alcoholism Drug, against Multi-Drug and Extensively Drug-Resistant *Mycobacterium tuberculosis* Isolates

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Running title

Anti-Tubercular Activity of Disulfiram

Footnote

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Abstract

The antimycobacterial activities of disulfiram (DSF) and diethyldithiocarbamate (DDC) against multi-drug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) clinical isolates were evaluated in vitro. Both DSF and DDC exhibited the potent anti-tubercular activities against 42 clinical isolates of M. tuberculosis, including MDR/XDR-TB strains. Moreover, DSF showed remarkable bactericidal activity ex vivo and in vivo. Therefore, DSF might be a repurposed drug for the treatment of MDR/XDR-TB.
Introduction

According to the updated guidelines of world health organization, the effective medications against multi-drug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) are confined due to only a limited selection of available drugs, therefore, the developments of the novel or repurposed anti-TB drugs against MDR/XDR-TB are strongly desired (8). Disulfiram (tetraethyl thiuram disulfide, DSF), has been used orally in the clinical treatment of alcoholism since 1949 and has been proven to exert an inhibitory effect on aldehyde dehydrogenase \textit{in vivo} with 80% bioavailability and established safety profiles (12, 33). Both DSF and its first metabolite, diethyldithiocarbamate (DDC), were reported to exhibit the growth-inhibitory activity against bacteria, fungi, protozoan, or viruses (2, 17, 22, 25, 26). In the mid-1950s, the tuberculostatic effects of DSF and DDC have been demonstrated \textit{in vivo} using guinea pigs (16). Subsequently, it has reported that DDC enhances the monocyte-induced anti-tubercular activity both in healthy volunteers and human immunodeficiency virus-infected patients \textit{ex vivo} (15). Recently, the anti-tubercular activities of DDC and the nitric oxide synthase inhibitor, pyrrolidine dithiocarbamate (PDTC), against non-replicating \textit{Mycobacterium tuberculosis} (MTB) have been demonstrated (4).
addition, we reported the unique antimycobacterial activities of dithiocarbamates, and also the potent anti-tubercular activity of compounds containing the dithiocarbamate groups, such as dimethyldithiocarbamate (DMDC), DDC, or PDTC (13, 14). More recently, the mode of action of dithiocarbamates against MTB has reported to be β-class carbonic anhydrases (β-CAs), which are considered as possible drug targets (18). However, the mechanism of action of DSF remains unknown.

In the present study, we evaluated the antimycobacterial activities of DSF and its metabolites against MTB, including MDR/XDR-TB clinical isolates, in more detail. Furthermore, the intracellular bactericidal activities of these compounds against a virulent strain MTB H37Rv within macrophages were examined ex vivo and the bactericidal activity of DSF in vivo was determined using the mouse model of chronic TB. Finally, the mechanisms of action of these compounds were investigated by means of gene-overexpressing strains in vitro.
Materials and Methods

Bacterial strains. *M. tuberculosis* H$_{37}$Rv ATCC 25618, *M. tuberculosis* H$_{37}$Ra ATCC 25177, *Mycobacterium avium* ATCC 25291, and *M. avium* ATCC 35718 were purchased from American Type Culture Collection (ATCC, USA). *Mycobacterium smegmatis* JATA 64-01 was provided by Dr. Takahashi (the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Japan). *Mycobacterium bovis* BCG *str. Tokyo* 172 (BCG Tokyo) was purchased from BCG Japan, Co. Ltd. *M. avium* 104 was provided by Dr. Deborah Geiman (John Hopkins University, USA). Clinical isolates of *M. tuberculosis* were isolated in Higashi Nagoya National Hospital (Japan). Mycobacterial strains were cultured in Middlebrook 7H9 broth (Difco, USA) supplemented with 10% ADC (5% bovine serum albumin [fraction V], 2% dextrose, and 0.005% bovine liver catalase), including 0.05% Tween 80, or on Middlebrook 7H11 agar (Difco, USA) supplemented with 10% OADC (5% bovine serum albumin [fraction V], 2% dextrose, 0.005% bovine liver catalase, and 0.05% alkalized oleic acid) at pH 6.6. *Staphylococcus aureus* 209PJC-1, *S. aureus* RN4220, *S. aureus* MF490, *Enterococcus faecalis* ATCC 19433, *Enterococcus faecium* ATCC 19434, *Escherichia coli* JM109, *Klebsiella pneumoniae* ATCC BAA-1705, and *Pseudomonas aeruginosa*
PAO1 were grown on Mueller-Hinton agar (Becton Dickinson, USA).

**Drugs and reagents preparation for in vitro and in vivo studies.** Isoniazid (INH), rifampicin (RIF), streptomycin (STR), ethambutol (EMB), ethionamide (ETH), p-aminosalicylic acid (PAS), ciprofloxacin (CIP), and bathocuproinedisulfonic acid disodium salt (BCPS) were purchased from Sigma-Aldrich co., USA. Thiuram, DSF, DMDC, DDC, PDTC, and amikacin disulfate salt (AMK) were purchased from Wako Pure Chemical Industries, Ltd., Japan. Kanamycin (KAN) was purchased from Meiji Seika Kaisha, Ltd., Japan. The bulk powder of disulfiram provided from Mitsubishi Tanabe Pharma Corporation, Japan. S-methyl diethyldithiocarbamate (S-Me-DDC), S-methyl diethylthiocarbamate (S-Me-DTC), S-methyl diethylthiocarbamate sulfoxide (S-Me-DTC sulfoxide), and S-methyl diethylthiocarbamate sulfone (S-Me-DTC sulfone) were purchased from Toronto Research Chemicals Inc., Canada. Hydrophilic or hydrophobic agents were dissolved in distilled deionized water (DDW) or dimethyl sulfoxide, respectively. Before examination, the stock solutions were diluted with assay broth, e.g., 7H9 broth. For in vivo use, RIF and DSF were dissolved or suspended in 5% gum arabic solution.

**Broth dilution test (BDT) and agar dilution method.** The BDT for the determination of MICs was performed as previously described (30). The starting drug
concentration was 100 \( \mu \text{g/ml} \). In the cases of INH and RFP, the concentrations were 10 and 1 \( \mu \text{g/ml} \), respectively. The MIC\(_{90}\) of test compounds against clinical isolates were defined as the antimicrobial concentration that showed the 90% growth inhibition of the strains. The agar dilution method for the determination of MICs using 7H11 agar at pH 6.6 was performed according to the Manual of Clinical Microbiology (21). The starting concentration of drugs, INH, RIF, and CIP, was 3.2 \( \mu \text{g/ml} \), and that of DSF, DDC, STR, EMB, KAN, and PAS was 0.125 \( \mu \text{g/ml} \). The drug susceptibility test for clinical isolates was performed with Broth MIC MTB (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) and the assay was conducted according to the method of provider (32). To determine the synergistic effects of DSF and DDC with metal ions, i.e., \( \text{Zn}^{2+} \), \( \text{Cu}^{2+} \), \( \text{Co}^{2+} \), or \( \text{Al}^{3+} \), the self-made Sauton broths were prepared with or without a metal salt, such as \( \text{ZnSO}_4 \), \( \text{CuSO}_4 \), \( \text{CoCl}_2 \), or \( \text{Al}_2(\text{SO}_4)_3 \). The 10-fold concentrated bacterial culture was next compared to the one used for normal BDT. The MICs against the several bacteria, except for mycobacteria, were determined using the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (31).

**Serum bactericidal test.** Serum bactericidal test was performed as according to the method of Byrne et al. (4, 23). DSF, DDC, INH, and RIF were administrated orally at 80 mg/kg, 80 mg/kg, 25 mg/kg and 10 mg/kg, respectively. INH and RIF were used as
positive control. The serum bactericidal activity was compared to vehicle control, 5% gum arabic. The serum samples were collected by cardiopuncture. For DSF and DDC, serum was collected at 0.083 and 2 hr after administration in accordance with the $t_{\text{max}}$ value of DDC (0.083 hr) as previously determined in our laboratory. For INH and RFP, serum was collected at 1 hr after administration as previously described (4). For vehicle, serum was collected before and 2 hr after administration. DDC and INH were also intravenously administrated, and serum was collected at 0.083 hr after administration. The pooled serum was heated at 56°C for 40 min in order to inactivate complement. Then serum samples were filtrated using 0.45 μm followed by 0.2 μm syringe filters. *M. tuberculosis* H$_{37}$Rv was cultivated in 7H9 broth supplemented with 10% ADC, including 0.05% Tween 80 and was grown to log phase. The final inoculum was confirmed as $4 \times 10^6$ CFU/well (100 μl) by means of colony assay using 7H11 agar plate supplemented with 10% OADC.

Intracellular anti-tubercular activities of DSF and DDC in the differentiated THP-1 cells. The human acute monocyte leukemia cell line THP-1 (ATCC TIB-202) was purchased from ATCC. THP-1 was maintained in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, including 100 units/ml penicillin G (Meiji Seika Kaisha, Ltd., Japan) and 100 μg/ml streptomycin (Meiji Seika
Kaisha, Ltd., Japan), in a humidified 5% CO₂ atmosphere at 37°C. The intracellular anti-tubercular assay was according to the method as previously described (19).

**Therapeutic efficacy in an experimental mouse model of chronic TB.** In order to examine the therapeutic efficacy of DSF and to determine the therapeutic dose range, an experimental mouse model of chronic TB was used as previously described (19). Mice obtained from the Institute of Cancer Research (ICR) (n=5 per group), were inoculated intravenously with 1×10⁶ CFU/ml of *M. tuberculosis* H₃₇Rv through the caudal vein and allowing the infection to develop for 28 days. The test compounds were then administered orally once daily for 28 days (RIF: 5-20 mg/kg, DSF: 40-160 mg/kg [2-fold dilutions]). The extracted lungs and spleen were homogenized with 1 ml DDW. The bacterial burden (CFU/organs) in either organ was counted using 7H11 agar plates to determine the therapeutic efficacy. Statistical analysis was conducted using Microsoft office excel 2007. Statistical significance was set at a *p* value of <0.05, *; <0.01, **; <0.001, *** compared to vehicle control group.

**Sulfonation mechanisms of DSF and DDC.** The *ethA*- or *ethR*-overexpressing strains were prepared as described previously and were used for MIC determination (1, 6).
Results and Discussion

In accordance with previous reports, DDC exhibited the anti-tubercular activity with the MIC of 1.56-3.13 μg/ml. Despite similar conditions compared to the previous report (4), DSF exhibited the potent anti-tubercular activity with the MIC of 1.56 μg/ml at pH 6.6 using Middlebrook 7H9 broth (Table 1). Importantly, the MIC of bulk powder of DSF provided from Mitsubishi Tanabe Pharma, which is generally used in the clinical treatment of alcoholism in Japan, was comparable to that of chemical reagent, DSF (data not shown). Additionally, the MIC of DSF against BCG Tokyo using 7H11 agar plate was also equivalent to anti-TB drugs, i.e., EMB and KAN (MIC=1-4 μg/ml) (Table S1). Among mycobacterial species, although the antibacterial spectrum of DSF is broad, the antmycobacterial activity of DDC was highly specific to the slow-growing strains such as MTB and BCG Tokyo, indicating that the antibacterial spectrum of DSF is quite distinct from that of DDC (Table 1). Meanwhile, the antibacterial spectrum of DSF against other gram-positive bacteria such as S. aureus (MIC=16 μg/ml), E. faecalis (MIC=32 μg/ml), and E. faecium (MIC=32 μg/ml) was similar to DDC (Table S2). As previously reported, unlike DSF, DDC exhibited the antibacterial activities with MICs of 128 μg/ml against gram-negative bacteria, i.e., E. coli, K. pneumoniae, and P.
DSF, well known as a prodrug, is enzymatically metabolized to DDC in blood followed by activation owing to some reactions such as S-methylation, oxidation, and sulfonation (1). The antimycobacterial activities of the metabolites of DSF, i.e., S-Me-DDC, S-Me-DTC, S-Me-DTC sulfoxide, and S-Me-DTC sulfone, were determined by BDT \textit{in vitro}. The results revealed that S-methylation of the sulphydryl group of DDC led to deactivation (MIC>100 \mu g/ml) in spite of its oxidation, and the metabolites by sulfonation partially restored the antimycobacterial activities (MIC=12.5-25 \mu g/ml) (Table 1). Therefore, it suggests that not only DSF, but also its metabolites, namely, DDC, S-Me-DTC sulfoxide, and S-Me-DTC sulfone are biologically active in the human body, which complicated the assessment of the anti-tubercular activity of DSF after oral administration.

We next further determined the anti-tubercular activities of DSF and DDC against clinical isolates of MTB \textit{in vitro}. As expected, these compounds exhibited the potent anti-tubercular activities against more than 40 clinical isolates of MTB, including MDR/XDR-TB strains (Table 2). The MIC\(_{90}\) of DSF and DDC against clinical isolates were 1.56 \mu g/ml and 3.13 \mu g/ml, respectively (Table 2). Importantly, there was no cross-resistance of DSF or DDC to the almost currently available anti-TB drugs,
including fluoroquinolones such as levofloxacin, sparfloxacin, and CIP (Table S3). Thereby, DSF and DDC may be implemented in future pharmacological regimens against MDR/XDR-TB.

It has been reported that DDC enhances the monocyte-induced anti-tubercular activity \textit{ex vivo} (15). Therefore, in order to confirm whether DSF and its metabolites are effective within macrophages, we determined the bactericidal activities of these compounds against intracellular MTB in differentiated THP-1 cells. As shown in Fig. 1, these compounds exhibited the potent bactericidal activities at 6-30 μg/ml and 10-30 μg/ml, respectively, in a dose-dependent manner, unlike STR and the bacteriostatic drug, EMB. Likewise, S-Me-DTC sulfone, the active metabolite of DSF, exhibited the intracellular bactericidal activity at 30 μg/ml, but not S-Me-DTC, in agreement to the result presented in Table 1 (Fig. 1). These data indicate that DSF, DDC, and S-oxidized metabolites, especially, S-Me-DTC sulfone, are effective against intracellular MTB.

Subsequently, we examined whether DSF and DDC exhibit the bactericidal activities \textit{in vivo} by means of serum bactericidal test. The serum bactericidal activities of INH and RIF were significant in titer of 1:32 as compared to vehicle control. As expected, the serum sample collected at 2 hr after DSF administration orally exhibited bactericidal activity in titer of 1:2, which was comparable to that of DDC administered intravenously,
whereas, the serum sample collected at 0.083 hr after administration exhibited less activity (Table 3). Likewise, the serum sample collected at 2 hr after DDC administration orally exhibited bactericidal activity and that collected at 0.083 hr exhibited less activity at the dose of 1:8 the serum sample from DSF, but not DDC, administrated orally exhibited bactericidal activity. Therefore, DSF showed more potent bactericidal activity as compared to DDC (Table 3). These results also indicated that DDC exhibits the bactericidal activity \textit{in vivo}.

Previously, the tuberculostatic effects of dithiocarbamates and thiuram disulfides have been examined in experimental tuberculosis of guinea pigs (n=10) (16). Whereas this report is considerably important for the drug development against TB, only the evidence, based on the pathological findings without bacteriological examination in lungs, had been shown. This prompted us to evaluate the bactericidal activity of DSF \textit{in vivo} in the mouse model of chronic TB. In the DSF-administrated group, significant CFU reductions were observed both in lungs and spleen at 80-160 mg/kg ($P<0.01$) compared to vehicle control group (Fig. 2). The pulmonary CFU reduction of DSF at 80 mg/kg was similar to that of RIF at 10 mg/kg (Fig. 2). Whereas it has been reported that DSF possessed the tuberculostatic effect at 20 mg/kg in the experimental tuberculosis of guinea pigs, there was no significant bactericidal activity both in lungs and spleen of
mice treated with DSF at 40 mg/kg (Fig. 2) (16). Taken together, these results suggest that DSF would exhibit the therapeutic effect on TB infection. According to the previous reports, DSF could be administered at 6 g without considerable harms in human and intriguingly, it has demonstrated that DSF and its metabolites preferentially transfer to lungs compared to plasma, brain, or liver, suggesting that the use of DSF is feasible for the treatment of TB, especially for MDR/XDR-TB (7, 28).

The currently available anti-TB drugs, i.e., isoniazid, streptomycin, ethambutol, and p-aminosalicylic acid, have hitherto known as metal chelators (9-11). Intriguingly, for example, it has reported that PAS may exert synergistic effect with copper ion on anti-tubercular activity (4). β-CAs, reported as the drug target of dithiocarbamates, belong to metalloenzymes family and include Zn ion at the active site when catalyzing enzymatic reaction (18). On the other hand, dithiocarbamates, e.g., DDC and PDTC have been known as metal chelators and have reported to possess the synergistic effects with copper ion on killing activity against protozoan, i.e., *Plasmodium falciparum* and *Toxoplasma gondii* (5, 20). Thus, to assess whether the metal ions, namely, Zn\(^{2+}\), Cu\(^{2+}\), Co\(^{2+}\), or Al\(^{3+}\), affect the anti-tubercular activities of DSF and DDC, we prepared the metal ion-containing Sauton broth medium and determined the MICs of these compounds. Increased anti-tubercular activities of these compounds was observed in
CuSO₄-containing broth in a dose-dependent manner, unlike ZnSO₄, CoCl₂, or Al₂(SO₄)₃, indicating that the anti-tubercular activities of these compounds are dependent on small amounts of copper ion (Table S4). Consequently, these results indicate that DSF and DDC exert synergistic effects with copper ion (>1 nM), unlike Zn²⁺, Co²⁺, or Al³⁺. For this roundup, the anti-tubercular activities of these compounds were determined using the copper ion chelator, bathocuproine disulphonic acid (BCPS). BCPS were reported to inhibit the activation of signal transduction and the cell growth activated by DSF in melanoma via chelating copper ion (3). As expected, the anti-tubercular activities of these compounds were suppressed by BCPS in 7H9 broth, normally supplemented with 4 μM Cu²⁺ ion and similar results were obtained for INH and ETH (Table 4). These results suggest that these compounds possess the synergistic effects with a small amount of copper ion, existing in human body (29).

Based on our knowledge, β-CAs have been reported as the drug targets of sulfonamides and sulfamates (24). Thereby, we considered that dithiocarbamates required sulfonation via intracellular enzymes e.g., monooxygenase (EthA) for activation (1, 6). Meanwhile, DDC is catalyzed by monooxygenase CYP2E1 in the human body. Hence, we hypothesized that DDC may possess the mechanism of activation relying on the monooxygenase EthA and studied whether ethA expression...
was required for antimycobacterial activities of DSF and DDC. As control results, the 

*ethR* -overexpressing strain exhibited the high levels of resistance to ETH, whereas the 

*ethA* -overexpressing strain was hypersusceptible to ETH (Table S5). However, the 

MICs of both DSF and DDC against either *ethR* - or *ethA*-overexpressing strain were 

equivalent to those of wild type strain (BCG pMV261), indicating that the 

anti-tubercular activities of these compounds do not depend on *ethA* expression (Table 

S5). Albeit the mechanisms of sulfonation of these compounds currently unknown, this 

mechanism via monooxygenase must be necessary to exert the antimycobacterial 

activities.

In conclusion, DSF is effective against MDR/XDR-TB and exhibits the intracellular 

bactericidal activity within macrophages and kills TB in mice, indicating that DSF 

might be a repurposed drug for the treatment of MDR/XDR-TB. Further investigation 

on the mechanisms of action is now required to identify the potent drug targets, which 

will be important for the design of novel anti-tubercular drugs.
Acknowledgements

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are active against growing and nongrowing persister *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **51:**4495-4497.


FIG. 1. Intracellular anti-tubercular activities of each agent in differentiated THP-1 cells. The bactericidal activities against intracellular *M. tuberculosis* H37Rv of disulfiram (DSF), diethylidithiocarbamate (DDC), S-methyl *N*-*N*-diethylthiocarbamate (S-Me-DTC), and S-methyl *N*-*N*-diethylthiocarbamate sulfone (S-Me-DTC sulfone) were assessed by the amount of CFU reduction as compared to anti-tubercular drugs, i.e., isoniazid (INH), rifampicin (RIF), streptomycin (STR), and ethambutol (EMB). Error bars represent means ±SD (n=3). Experiments were performed in triplicate and were carried out more than three times and representative data were shown.
FIG. 2. Therapeutic effects of rifampicin (R) and disulfram (D) in an experimental mouse model of chronic TB. ICR mice were inoculated intravenously with *M. tuberculosis* H37Rv. After 28 days, test compounds were administered orally once daily for 28 days, and then lungs and spleen were extracted and number of colonies in organs (log$_{10}$CFU/organisms) were determined using 7H11 agar plates. Rifampicin: 5-20 mg/kg, disulfram: 40-160 mg/kg, vehicle: administered only 5% arabiagum. Error bars represent means ± SD (n=5). The Student’s t-test was used to compare different treatment groups. Statistical significance was set at a *P* value of <0.05, *, <0.01, **; <0.001, *** compared to vehicle control group. NS, not significant.
<table>
<thead>
<tr>
<th>Strain</th>
<th>DSF</th>
<th>DDC</th>
<th>S-Me-DDC</th>
<th>S-Me-DTC sulfoxide</th>
<th>S-Me-DTC sulfone</th>
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<tbody>
<tr>
<td><em>M. tuberculosis</em> H37Rv</td>
<td>1.56</td>
<td>3.13</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>25</td>
</tr>
<tr>
<td><em>M. tuberculosis</em> H37Ra</td>
<td>1.56</td>
<td>3.13</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>25</td>
</tr>
<tr>
<td><em>M. bovis</em> BCG <em>str.</em> Tokyo 172</td>
<td>3.13</td>
<td>6.25</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>50</td>
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<td><em>M. avium</em> subsp. <em>avium</em> ATCC 25291</td>
<td>25</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
<td><em>M. avium</em> subsp. <em>hominissuis</em> 104</td>
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<td>&gt;100</td>
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<td><em>M. smegmatis</em> JATA 64-01</td>
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<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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</table>

*The antimycobacterial activities of each agent were determined by broth dilution test using Middlebrook 7H9 broth containing albumin, dextrose, catalase, and Tween 80 at pH 6.6. Experiments were performed in duplicate. These results are representative of three separate experiments. DSF, disulfiram; DDC, diethylthiocarbamate; S-Me-DDC, S-methyl N,N-diethylthiocarbamate; S-Me-DTC, S-methyl N,N-diethylthiocarbamate.*
TABLE 2. Anti-tubercular activities of DSF and DDC against drug-susceptible and resistant clinical isolates of *M. tuberculosis*

<table>
<thead>
<tr>
<th>Clinical isolates</th>
<th>No. of strains</th>
<th>Agent</th>
<th>MIC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>DS-TB</td>
<td>20</td>
<td>DSF</td>
<td>0.78-1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDC</td>
<td>1.56-3.13</td>
</tr>
<tr>
<td>DR-TB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22</td>
<td>DSF</td>
<td>0.78-1.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDC</td>
<td>1.56-6.25</td>
</tr>
</tbody>
</table>

Experiments were performed in duplicate. These results are representative of two separate experiments. DS-TB, drug-susceptible TB; DR-TB, drug-resistant TB; DSF, disulfiram; DDC, diethyldithiocarbamate.

<sup>a</sup>DR-TB includes multi-drug resistant TB (n=13) and extensively drug-resistant TB (n=5). Drug-resistant profiles were depicted in Table S3.
<table>
<thead>
<tr>
<th>Titer</th>
<th>Agent</th>
<th>Log reduction (Δlog$_{10}$ CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DSF</td>
<td>DDC</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>p.o.</td>
</tr>
<tr>
<td></td>
<td>hr</td>
<td></td>
</tr>
<tr>
<td>1:2</td>
<td>1.84</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>±0.03</td>
<td>±0.01</td>
</tr>
<tr>
<td>1:4</td>
<td>1.55</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>±0.003</td>
<td>±0.11</td>
</tr>
<tr>
<td></td>
<td>1.41</td>
<td>1.49</td>
</tr>
<tr>
<td>1:8</td>
<td>±0.01</td>
<td>±0.1</td>
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<tr>
<td></td>
<td>1.16</td>
<td>1.18</td>
</tr>
<tr>
<td>1:32</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>±0.02</td>
<td>±0.03</td>
</tr>
</tbody>
</table>

Each value (sample-vehicle control) represents mean SD (n=3). N.D., not detected (>5.0 Δlog$_{10}$ CFU/ml). Hyphen, <1.0 Δlog$_{10}$ CFU/ml. DSF, disulfiram; DDC, diethylthiocarbamate; INH, isoniazid; RIF, rifampicin. p.o., per os; i.v. intravenous injection.
<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (μg/ml) using 7H9 broth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCPS (-)</td>
</tr>
<tr>
<td><strong>thiuram disulfide</strong></td>
<td></td>
</tr>
<tr>
<td>Thiuram</td>
<td>0.78</td>
</tr>
<tr>
<td>DSF</td>
<td>1.56</td>
</tr>
<tr>
<td><strong>dithiocarbamate</strong></td>
<td></td>
</tr>
<tr>
<td>DMDC</td>
<td>1.56</td>
</tr>
<tr>
<td>DDC</td>
<td>3.13</td>
</tr>
<tr>
<td>PDTC</td>
<td>0.4</td>
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<tr>
<td><strong>anti-TB drug</strong></td>
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</tr>
<tr>
<td>INH</td>
<td>0.08</td>
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<tr>
<td>RIF</td>
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<tr>
<td>STR</td>
<td>0.39</td>
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<tr>
<td>EMB</td>
<td>1.56</td>
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<tr>
<td>ETH</td>
<td>3.13</td>
</tr>
<tr>
<td>CIP</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Experiments were performed in duplicate. These results are representative of three separate experiments. DSF, disulfiram; DMDC, dimethyldithiocarbamate; DDC, diethylthiocarbamate; PDTC, pyrroolidine dithiocarbamate; INH, isoniazid; RIF, rifampicin; STR, streptomycin; EMB, ethambutol; ETH, ethionamide; CIP, ciprofloxacin; BCPS, bathocuproinedisulfonic acid.