Novel non-neuroleptic phenothiazines inhibit *Mycobacterium tuberculosis* replication

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Objectives: Phenothiazines are a commercially available class of psychotropic drugs known to show antituberculosis activity. At clinically relevant bactericidal doses, however, the psychotropic drugs produce undesirable side effects in addition to their neuroleptic properties. This study aimed to evaluate rationally designed novel phenothiazines as antimycobacterial drug candidates.

Methods: Remodelling of psychotropic drugs by substitution of characteristic N-alkylamine side chains, important for CNS activity, with N-alkylsulphonates gave novel drug candidates, which were then tested for post-synaptic receptor binding affinity in a radioligand displacement assay. The bactericidal activities were screened using green fluorescent protein (GFP) microplate assays, and the efficacy of intracellular bacillus killing was evaluated by cfu enumeration.

Results: Of the four selected phenothiazine derivatives (PTZ3, PTZ4, PTZ31 and PTZ32) tested, PTZ31 displayed marginal serotonergic activity. The remaining three derivatives did not exhibit dopamine or serotonin receptor binding activity. *In vitro* results showed significant growth inhibition of virulent *Mycobacterium tuberculosis* with MICs of 12.5–25 mg/L. None of the phenothiazine derivatives displayed cytotoxicity in infected primary bone marrow-derived macrophages. Moreover, the phenothiazines showed significant antimycobacterial activity of between 40% and 60% against intracellular (*ex vivo*) *M. tuberculosis*.

Conclusions: We demonstrate that structural modification of the phenothiazine core is possible in a manner that does not affect the ability of the phenothiazine derivatives to inhibit *M. tuberculosis*, but that abolishes undesirable dopamine and serotonin receptor binding.

Keywords: dopamine, serotonin, chemotherapy, thioridazine, antimycobacterial

Introduction

Tuberculosis (TB) is an infectious disease that constitutes a major global public health problem with a reported 8.8 million incident cases globally, most of which occur in Asia and Africa. The duration of standard TB treatment is a minimum of 4–6 months, with patient compliance a major obstacle to disease eradication. The emergence of resistant strains of TB has not only lowered cure rates, but has also considerably decreased treatment options. Additionally, coinfection with HIV has led to adverse drug–drug interactions when TB medication is co-administered with certain antiretroviral therapies. Coinfection with diseases that weaken the immune system significantly increases the chance of progression from latent to active TB. The emergence of multidrug-resistant and extensively drug-resistant strains has become a growing concern, both having high treatment failure rates. Extended chemotherapy is required for both multidrug-resistant and extensively drug-resistant TB (typically 9–12 months); it often has severe side effects and is further complicated by the escalation in drug resistance to first-line drugs.

Phenothiazines are commercially available and in clinical use for the treatment of psychosis. The *in vitro* activities of phenothiazines against *Mycobacterium tuberculosis* have been known for many decades. These drugs affect a large number of targets that are essential for the survival of mycobacteria. More recently, phenothiazines were found to be active in vivo and *in vitro* against resistant strains of *M. tuberculosis* and enhanced the activity of antibiotics. Chlorpromazine was one of...
the first phenothiazine derivatives shown to have antimycobacterial properties and was successfully used to treat a TB patient. A later-developed phenothiazine, thiouridine, showed antimycobacterial activity identical to that of chlorpromazine, but had significantly weaker neuroleptic effects. The extended use of psychotropic drugs has provided substantial evidence of severe side effects when administered over lengthy periods, hence the resistance to their use in anti-TB treatment. Successful results were obtained in a small clinical trial in TB patients in Argentina, where it was claimed that 10 out of 12 extensively drug-resistant TB patients were cured. This positive outcome resulted in strong motivation for the repurposing of thiouridine and the request for broadened trials on antibiotic non-responsive, terminal, extensively drug-resistant TB patients. The successful outcome of clinical trials where thiouridine was used together with linezolid and moxifloxacin illustrated its potential as an alternative to established front-line drugs in combinatorial therapeutic approaches. While it seems that the benefit of extensively drug-resistant TB treatment with thiouridine outweighs the unwanted neuroleptic effects, along with potential side effects, the idea of remodelling phenothiazine into a drug that has only antitubercular activity is a sustainable and attractive alternative.

A recent review highlighted the versatility of the phenothiazine pharmacophore’s ability to demonstrate a diverse range of bioactivity with minimal structural remodelling. The parent pharmacophore is a 10H-phenothiazine, which consists of a tricyclic ring with a thiomorpholine fused between two benzene rings (Figure 1a). Phenothiazine drugs differ mainly at the C-2 and nitrogen positions (or 10H) of the tricyclic skeleton. The N-alkylated alkylamine moiety is crucial for receptor binding, while the C-2 substituent plays a significant role in pharmacokinetics. The pharmacology of the CNS activity of the phenothiazine class has been well studied. Common features of this compound class include their ability to cross the blood–brain barrier due to their lipophilicity, and, when it reaches the target receptor, the fact that the position and orientation of the side chain nitrogen atom differentiates their affinities for the many dopamine and serotonin subtypes. The antitubercular efficacies of the phenothiazines and a number of other drug candidates in the discovery/development pipeline illustrate the value of drug repositioning and remodelling as an important strategy in the battle against TB. We postulated that phenothiazines could make a significant contribution to anti-TB therapy regimens provided they have no psychotic effects. The objective of this study was therefore to generate new phenothiazine derivatives, PTZ3, PTZ4, PTZ31 and PTZ32, that have reduced or no neuroleptic potential and yet are still able to inhibit virulent M. tuberculosis replication. This study therefore reports on the serotonin and dopamine binding properties of these new phenothiazine derivatives and their antimycobacterial activity.

Materials and methods

Compounds

Isoniazid and thiouridine were purchased from Sigma (St Louis, MO, USA). Modified phenothiazine derivatives were synthesized by the Chemistry Department, University of Cape Town, South Africa. Additional information on the synthesis of the modified phenothiazines is available as Supplementary data at JAC Online.

Neuroleptic potential

The modified phenothiazines (PTZ3, PTZ4, PTZ31 and PTZ32) were sent to Caliper Life Sciences (MA, USA) to be evaluated in a radioligand binding assay in which binding to the dopaminergic receptor subtypes D1, D2 and D3 and the serotonergic receptor subtypes 5-HT1A, 5-HT2A and 5-HT2C was measured.

The experimental conditions for the dopamine and serotonin binding assay, as reported by Caliper Life Sciences, were in accordance with industry standards, utilizing well-established 3H-labelled reference ligands (see Table A in Figure 2). In brief, the dopaminergic receptor subtypes D1, D2 and D3 were prepared from HEK-293 cells expressing human recombinant D1 subtype receptor, CHO cells expressing human recombinant D2 subtype receptor and SF9 cells expressing rat recombinant D3 subtype receptor, respectively. The reactions for the radioligand binding of the dopaminergic receptor subtypes were carried out in 50 mM Tris–HCl (pH 7.4) containing 5 mM KCl and 5 mM MgCl2 at 25°C. The assay conditions for receptor subtype D1 required addition of 1 mM EDTA and 1.5 mM CaCl2, those for receptor subtype D2 required addition of 1 mM EDTA and 120 mM NaCl and those for receptor subtype D3 required addition of 5 mM EDTA, 120 mM NaCl and 1.5 mM CaCl2.

The radioligand binding assays of the serotonergic receptor subtypes 5-HT1A, 5-HT2A and 5-HT2C were done on the bovine hippocampal membrane, rat cortical membrane and pig choroid plexus membrane, respectively. The reaction for the 5-HT1A subtype was carried out in 50 mM Tris–HCl (pH 7.4) at 25°C, that for the 5-HT2A subtype in 50 mM Tris–HCl (pH 7.6) at 37°C and that for the 5-HT2C subtype in 50 mM Tris–HCl (pH 7.7) containing 4 mM CaCl2 and 0.1% ascorbic acid at 37°C. The dopaminergic and serotonergic radioligand binding assay reactions were terminated by rapid vacuum filtration onto glass fibre filters. Radioactivity trapped on the filters was measured and compared with control values to evaluate interactions of the modified phenothiazines with the respective receptors. Assays were performed in duplicate. The average bound radioactivity of the reference compound in the presence of the modified phenothiazines was then expressed as a percentage. The percentage inhibition of radioligand binding is 100% minus the percentage of radioactivity bound.

Mycobacterial culture

M. tuberculosis strain H37Rv was obtained from the Trudeau Mycobacterial Culture Collection (Trudeau Institute, Saranac Lake, NY) and grown at 37°C in Middlebrook 7H9 broth (Difco Laboratories, USA) supplemented with 10% oleic acid albumin dextrose catalase (OADC) (BD and Company, USA) and 0.5% Tween 80 (Merck, USA). Stock concentration was calculated by culture of the bacteria on Middlebrook 7H10 agar (Difco Laboratories, USA). Cultures were placed in semi-sealed plastic bags and incubated for 21 days at 37°C, after which colonies were counted. M. tuberculosis strain H37Rv.gfp (kindly provided by Joel Ernst, University of San Francisco, CA, USA) was cultivated similarly, with 25 mg/L kanamycin in the broth.

GFP microplate assay

Antimicrobial susceptibility testing was performed in black 96-well plates (Greiner Bio-One, Germany). Frozen H37Rv.gfp was thawed, spun down at 10000 rpm for 5 min, the supernatant removed and the pellet resuspended in Middlebrook 7H9 broth with 25 mg/L kanamycin. To each experimental
Figure 1. (a) Molecular structure and numbering of a 10H-phenothiazine. (b) Synthesis of phenothiazine derivatives from commercially available C-2 substituted 10H-phenothiazines. (c) Phenothiazine class of psychotropic drugs and related structures of phenothiazine compounds tested. Note variation at position C-2 of the molecules. MIC values\(^{14}\) of psychotropic drugs are given in brackets beneath the structures.
well, 100 μL of H37Rv.gfp was added at a concentration of 1 x 10^6 cfu/mL. Outer wells were filled with sterile water to minimize evaporation in experimental wells.

Test compounds and controls were first dissolved in water and subsequent 2-fold dilutions were made using 7H9 broth supplemented with 25 mg/L kanamycin. Wells containing compound only (at the highest concentration) were used to detect fluorescence by compounds and broth (vehicle control). Relative fluorescence readings were measured at the indicated timepoints.

Ex vivo activity of phenothiazine derivatives

Primary bone marrow-derived macrophages (BMDMs) were cultured from the femurs of 6–8-week-old C57Bl/6 mice. The cultures were maintained in RPMI medium (Sigma) supplemented with 20% fetal calf serum (FCS) (Gibco, Germany), 30% L929-conditioned medium, 10 mM L-glutamine (Gibco, Germany), 100 μg/mL streptomycin and 100 U/mL penicillin (Gibco, Germany) for 8–10 days at 37°C with 5% CO₂. Confluent cells were seeded at a concentration of 1 x 10^6 cells per well in a 96-well plate.

Macrophages were infected with M. tuberculosis (H37Rv) at a multiplicity of infection of 5:1 (M. tuberculosis:cells) for 4 h. Infected cells were washed with BMDM medium (2 mM L-glutamine, 2% FCS, 10% L929 medium, RPMI) to remove extracellular bacteria, then the initial number of intracellular bacilli was determined by lysing the macrophages and plating the supernatant on Middlebrook 7H10 agar for cfu enumeration.

Two sets of cultures were prepared to evaluate intracellular drug activity and cell viability simultaneously. The infected macrophages were incubated with medium containing isoniazid (1 mg/L), thioridazine (3 mg/L), PTZ3 (25 mg/L) or PTZ4 (25 mg/L) in triplicate. After 5 days of treatment, macrophages were either lysed for cfu determination (% inhibition), or incubated with Cell-titer Blue reagent (Sigma) for 4 h then read on a fluorimeter for cell viability. Percentage inhibition was calculated relative to untreated macrophages and percentage viability was calculated relative to uninfected macrophages.

Results

Preparation of N-propylsulphonates of phenothiazine involves (i) the preparation of the phenothiazine anion and (ii) the reaction of this anion with the cyclic alkyl sulphonate 1,3-propane sultone, as generally described in United States Patent number 7855287 (Figure 1b). The parent phenothiazine and the 2-chlorophenothiazine were N-alkylated with 1,3-propane sultone to provide the respective new sodium 3-(10H-phenothiazine-10-yl)propane-1-sulphonates (PTZ3, PTZ4, PTZ31 and PTZ32) (Figure 1b).

The tricyclic aromatic core structure of the phenothiazine derivatives synthesized differed from that of the reference phenothiazines (promazine, chlorpromazine, fluophenazine and thioridazine) in the terminus of the side chain. The amine functionality of the modified phenothiazines was replaced with a sulphonate group (Figure 1c). In addition, the individually synthesized phenothiazine derivatives varied from each other by the substituent at the C-2 position of the tricyclic skeleton. The substituent at the C-2 position of PTZ3, PTZ4, PTZ31 and PTZ32 was identical to the C-2 substituent of promazine, chlorpromazine, fluophenazine and thioridazine, respectively. The phenothiazine products thus obtained were of high purity as determined by LCMS chromatograms and NMR spectra (Figures S1 to S16, available as Supplementary data at JAC Online).

As a key objective of the study, we had the neuroleptic potential of the new modified phenothiazine derivatives, PTZ3, PTZ4, PTZ31 and PTZ32 tested in a dopamine and serotonin receptor radioligand binding assay offered as a commercial service by Salie et al.
Caliper Life Sciences, MA, USA. The results demonstrated abolishment of binding to the dopaminergic-receptor subtypes D1, D2 and D3 and the serotonergic receptor subtypes 5-HT1A, 5-HT2A and 5-HT2C (Figure 2). PTZ31 was the only phenothiazine derivative to show marginal binding at the serotonergic receptor subtype 5-HT1A. Nonetheless, the antimycobacterial efficacy of these phenothiazines remained to be tested.

Therefore, PTZ3, PTZ4, PTZ31 and PTZ32 were then evaluated against M. tuberculosis to assess their bactericidal/bacteriostatic activity. The benchmarked, commercially available phenothiazine thioridazine was chosen as a point of reference. Outcomes were thus compared with thioridazine and the first-line TB drug isoniazid, which were used as positive control compounds against which mycobacterial inhibition could be measured. The results obtained show that all the modified phenothiazines displayed a concentration-dependent inhibitory effect on the growth of M. tuberculosis-H37Rv.gfp (Figure 3). The MICs of isoniazid and thioridazine were similar to published data. Significant antimycobacterial activity was demonstrated for PTZ3 and PTZ4, which exhibited on MIC of 12.5 mg/L (Figure 1b), while 2-fold higher MICs were observed for PTZ31 and PTZ32 at 25 mg/L. Therefore, this study convincingly demonstrated that the novel phenothiazine derivatives retained antimycobacterial inhibitory potential, but had the additional advantage of potentially no neuroleptic effects.

Next we investigated whether the test compounds could traverse cell membrane systems and access intracellular bacilli to inhibit replication. We infected primary BMDMs with M. tuberculosis and treated infected cell cultures for 5 days with PTZ3 or PTZ4, the phenothiazine derivatives having the lowest MIC values. Simultaneously, cell viability was determined as an indicator of each compound’s toxicity in infected macrophages. The percentage cell viability was calculated based on the ratio of infected cells cultured in the presence or absence of the compounds relative to the viability of uninected cells. The inhibition of intracellular bacterial growth by either PTZ3 or PTZ4 was calculated and expressed as a percentage of the value obtained in untreated cell cultures.

We confirmed the bactericidal effects of both isoniazid and thioridazine, which inhibited >90% of intracellular M. tuberculosis growth at 1 and 3 mg/L, respectively (Figure 4a). Moreover, both PTZ3 and PTZ4, tested at 25 mg/L, showed significant antimycobacterial activity of between 40% and 60% (Figure 4a). It is noteworthy that all the infected macrophages were almost completely viable (>95%) after drug treatment (Figure 4b), indicating that the modified phenothiazines were not toxic to macrophages at their MICs.

Discussion
Phenothiazines are known to exert their psychotropic effects by binding to post-synaptic receptors—dopamine and serotonin receptors, among others. Despite their known antibacterial properties, broad clinical application of phenothiazines has been restricted for this purpose primarily due to their neuroleptic non-therapeutic effects. This study reports on the lack of psychotic potential of a novel set of phenothiazine derivatives and their efficacy against virulent M. tuberculosis. We have demonstrated that it is possible to separate the antimycobacterial activity from the psychotropic activity of phenothiazines by increasing their polarity. This results in increased solubility of the phenothiazine derivatives, which possibly reduces their ability to cross the blood–brain barrier.

In full knowledge of the minimum structural requirements of phenothiazines for neuroleptic properties, the rationale applied here was to change the polarity of the molecule in the region of the side chain nitrogen. This amine functionality is crucial for affinity to post-synaptic receptors. It is known that the tertiary amine functionality on the side chain would be positively charged at physiological pH. Exchange of the tertiary amine for groups such as carboxylates, phosphonates or sulphonates (pKa 2–5) would result in a change in the charge at the position of the nitrogen. It is accepted that a sulphonate, phosphonate or carboxylate will be negatively charged at physiological pH. Thus the introduction of side chain sulphonate groups, while retaining the core tricyclic structure, yielded novel phenothiazine derivatives that lacked affinity for dopamine and serotonin receptors, and hence was expected to abolish the neuroleptic potential of the new phenothiazine derivatives. The lipophilicity of phenothiazines is well known to facilitate diffusion across the blood–brain barrier. Thus, replacing this hydrophobic amine moiety with a sulphonate group not only results in a change in polarity at a critical receptor recognition site of the molecule, but also increases the aqueous solubility of the molecule and decreases its potential ability to cross the blood–brain barrier.

The affinity of phenothiazines for dopaminergic receptors is in part due to their three-dimensional similarity to histamine and dopamine. Active neuroleptic derivatives of phenothiazine require specific substitution of the hydrogen atom attached to the C-2 carbon atom by a variety of chemical groups, such as chloro, trifluoromethyl, thiomethyl and acetyl, whereas substitutions at the N-10 nitrogen include an aminoalkyl, aliphatic chain. Thus, the pipiderine ring of chlorpromazine is analogous to thioridazine, it is surprising to note that the phenothiazine side chain demonstrates the strongest antipsychotic action as well as the most CNS side effects. The presence of electron rich substituents attached to the C-2 carbon atom is crucial for the neuroleptic activity of phenothiazine. Electronegative atoms or groups attached to the C-2 carbon atom of the phenothiazine ring intensify their antipsychotic activity.

Previously, it was reported that several phenothiazines could be rated according to antimycobacterial potency as follows: thioridazine > fluophenazine > chlorpromazine > promazine. If we consider that PTZ3 is analogous to promazine, PTZ4 is analogous to chlorpromazine, PTZ31 is analogous to fluophenazine and PTZ32 is analogous to thioridazine, it is surprising to note that the order of antimycobacterial activity of PTZ3, PTZ4, PTZ31 and PTZ32 does not follow the order of potency of the compounds to which they are analogous.

The abolishment of dopamine and serotonin receptor affinity was also observed by others, but to a limited extent. Phenothiazine drugs all have a basic side chain at the 10-position and in this particular study the distal nitrogen was retained whilst adding diverse substituents with both an alkyl and a benzyl linker. The molecules thus synthesized retained the tertiary amine functionality on the side chain and had greater lipophilicity than the reference drugs, which in the study referred to were chlorpromazine.
and trifluoperazine. Thus, the major structural difference in their phenothiazine derivatives was the linker between the tricyclic ring system and the amine functionality on the side chain. Phenothiazine derivatives that displayed the lowest MICs (2–3 mg/L in an Alamar Blue assay) still retained 40%–50% dopamine-binding affinity. However, the study demonstrated that steric bulk at the distal end of the side chain almost completely abolished dopamine and serotonin binding, as was reported for a derivative (MIC 15 mg/L) analogous to trifluoperazine (MIC 6–12 mg/L).25

Figure 3. Antimycobacterial activity of isoniazid, thioridazine, PTZ3, PTZ4, PTZ31 and PTZ32 directly against H37Rv.gfp using the GFP microplate assay. (a) Isoniazid and thioridazine were tested at concentration ranges of 0.0125–0.1 and 0.7812–25 mg/L, respectively. (b) The modified phenothiazines were tested at a concentration range of 6.25–25 mg/L. By measuring the fluorescence of H37Rv.gfp for 12 days, the effects of drug treatment on M. tuberculosis growth were shown in a concentration-dependent manner. All experiments included drug only, H37Rv.gfp only and broth only as controls. Data are from one representative experiment of three independent experiments. INH, isoniazid; TZ, thioridazine.
The compounds tested in this study have retained their antimycobacterial properties in the absence of neuroleptic effects, positioning these compounds favourably for consideration as candidates for further development and application against the more common drug-susceptible forms of TB. The concentrations at which mycobacterial inhibition occurs compare well with published data for other well-described bactericidal phenothiazines, but are above average for other antimycobacterials. The primary objective of the modifications to the phenothiazine core was to retain mycobacterial activity whilst abolishing neuroleptic activity. Although we successfully achieved separation of neuroleptic and mycobacterial activity, this resulted in reduction of efficacy, evident in higher MIC values. Despite the higher than average MIC values, cytotoxicity of PTZ3, PTZ4, PTZ31 and PTZ32, tested against cultured primary BMDMs, was negligible (data not shown). Based on the structures of the four phenothiazines, we have observed that modification at the C-2 position influences MIC values. This may provide a potential strategy for future modifications to attempt to regain the efficacy of the parent compound. For lead compounds, a more thorough approach, however, would be to utilize standard medicinal chemistry techniques to modify the core structure to improve the medicinal properties of the compounds. This is an iterative process, which will only conclude when either a viable drug candidate has been established or all possible modifications have been exhausted as a means to lower the MIC values.

Our data also confirmed that the phenothiazine derivatives synthesized can access intracellular bacilli and inhibit replication. Previous studies have argued that the clinical concentrations at which phenothiazines are bactericidal in culture are unattainable and that the accumulation of drugs within macrophages which phenothiazines are bactericidal in culture are unattainable. Previous studies have argued that the clinical concentrations at which phenothiazines are bactericidal in culture are unattainable and that the accumulation of drugs within macrophages which phenothiazines are bactericidal in culture are unattainable.

The mechanism(s) by which the non-neuroleptic phenothiazines position these compounds favourably for consideration as candidates for further development and application against the more common drug-susceptible forms of TB. The concentrations at which mycobacterial inhibition occurs compare well with published data for other well-described bactericidal phenothiazines, but are above average for other antimycobacterials. The primary objective of the modifications to the phenothiazine core was to retain mycobacterial activity whilst abolishing neuroleptic activity. Although we successfully achieved separation of neuroleptic and mycobacterial activity, this resulted in reduction of efficacy, evident in higher MIC values. Despite the higher than average MIC values, cytotoxicity of PTZ3, PTZ4, PTZ31 and PTZ32, tested against cultured primary BMDMs, was negligible (data not shown). Based on the structures of the four phenothiazines, we have observed that modification at the C-2 position influences MIC values. This may provide a potential strategy for future modifications to attempt to regain the efficacy of the parent compound. For lead compounds, a more thorough approach, however, would be to utilize standard medicinal chemistry techniques to modify the core structure to improve the medicinal properties of the compounds. This is an iterative process, which will only conclude when either a viable drug candidate has been established or all possible modifications have been exhausted as a means to lower the MIC values.

Our data also confirmed that the phenothiazine derivatives synthesized can access intracellular bacilli and inhibit replication. Previous studies have argued that the clinical concentrations at which phenothiazines are bactericidal in culture are unattainable and that the accumulation of drugs within macrophages significantly increases the minimum effective concentration. However, the separation of neuroleptic and antimycobacterial properties makes the application of higher therapeutic doses to achieve mycobacterial inhibition unnecessary. The mechanism(s) by which the non-neuroleptic phenothiazines reported here inhibit M. tuberculosis replication remains unknown. It may indeed be similar to those of other phenothiazines that inhibit Ca$^{2+}$ and K$^+$ transport and promote acidification of the phagolysosome, thereby transforming non-killing macrophages into effector killing cells. A key survival strategy of phagocytosed M. tuberculosis is the inhibition of phagosome maturation within macrophages. Central to this mechanism of bacterial control is the acidification of the phagosomal lumen, which is Ca$^{2+}$ and K$^+$ dependent.

Regardless of the mechanism of killing, the lack of neuroleptic effects may support administration of the phenothiazine derivatives at higher concentrations to obtain increased clinical serum concentrations and thereby improve their inhibitory potential, including the possibility of shortening treatment duration. However, clinical therapeutic translation of the findings reported here will require substantial additional studies, including in vivo toxicity and efficacy verification.

To date, effort has primarily been focused on the use of phenothiazines as therapy against resistant forms of TB, with considerable success. The successful application of thioridazine as therapy against extensively drug-resistant TB exemplifies the significance of phenothiazines for advocacy on a broader scale. Nonetheless, well-known side effects remain a considerable concern in clinical application. This study has yielded a novel subclass of phenothiazines that constitutes a new chemical entity with favourable attributes as a potential antimycobacterial drug and holds promise for wider application as anti-TB therapy.
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Transparency declarations

None to declare.

Supplementary data

Additional information on the synthesis of the modified phenothiazines and Figures S1 to S16 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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