RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

International Microbiology (2015) 18:1-12
doi:10.2436/20.1501.01.229. ISSN (print): 1139-6709. e-ISSN: 1618-1095
www.im.microbios.org

Phenothiazines as a solution for multidrug resistant tuberculosis: From the origin to present

Jette E. Kristiansen,[1¶] Sujata G. Dastidar,[2¶] Shauroseni Palchoudhuri,[2] Debalina Sinha Roy,[2] Sukhen Das,[3] Oliver Hendricks,[4] Jørn B. Christensen[5*]

[1]Memphys Centre for Biomembrane Physics, Department of Physics and Chemistry, University of Southern Denmark, Odense, Denmark. [2]Department of Microbiology, Herbicure Healthcare Bio-Herbal Research Foundation, Saraldighi (E), Boral, Kolkata, India. [3]Department of Physics, Jadavpur University, Kolkata, India.
[4]King Christian X Hospital for Rheumatic Diseases, University of Southern Denmark, Grasten, Denmark.
[5]Department of Chemistry, University of Copenhagen, Copenhagen, Denmark

Received 16 January 2015 · Accepted 6 March 2015

Summary. Historically, multiplicity of actions in synthetic compounds is a rule rather than exception. The science of non-antibiotics evolved in this background. From the antimalarial and antitrypanosomial dye methylene blue, chemically similar compounds, the phenothiazines, were developed. The phenothiazines were first recognised for their antipsychotic properties, but soon after their antimicrobial functions came to be known and then such compounds were designated as non-antibiotics. The emergence of highly drug-resistant bacteria had initiated an urgent need to search for novel affordable compounds. Several phenothiazines awakened the interest among scientists to determine their antimycobacterial activity. Chlorpromazine, trifluoperazine, methdilazine and thioridazine were found to have distinct antitubercular action. Thioridazine took the lead as researchers repeatedly claimed its potentiality. Although thioridazine is known for its central nervous system and cardiotoxic side-effects, extensive and repeated in vitro and in vivo studies by several research groups revealed that a very small dose of thioridazine is required to kill tubercle bacilli inside macrophages in the lungs, where the bacteria try to remain and multiply silently. Such a small dose is devoid of its adverse side-effects. Recent studies have shown that the (–) thioridazine is a more active antimicrobial agent and devoid of the toxic side effects normally encountered. This review describes the possibilities of bringing down thioridazine and its (–) form to be combined with other antitubercular drugs to treat infections by drug-resistant strains of *Mycobacterium tuberculosis* and try to eradicate this deadly disease. [**Int Microbiol** 2015; 18(1):1-12]

Keywords: *Mycobacterium tuberculosis* · phenotiazines · thioridazine · tuberculosis

Introduction

The origin of phenothiazine dates back to 1883, when German chemist August Heinrich Bernthsen was carrying out analyses to determine the chemical constituents of two dyes, Lauth's violet and methylene blue. The dye methylene blue, whose chemical nucleus is phenothiazine, had been produced even earlier by another German, Heinrich Caro [11]. The dye industry originated in 1856 with William Perkin's preparation of the first synthetic dye aniline purple. The increasing demand of organic chemicals such as dyes promoted the development of synthetic organic chemistry. It was soon realised that knowing structure-activity relations of organic compounds was essential for the synthesis and manufacture of new dyes. Bernthsen synthesised the phenothiazine named as methylene blue on this background [11].

*Corresponding author: J.B. Christensen
Department of Chemistry, University of Copenhagen
Copenhagen, Denmark
E-mail: jbc@chem.ku.dk

¶These authors contributed equally to the work.

One of the first major applications of phenothiazines beyond their use in dye industry was made in 1934 by entomologists in the US Department of Agriculture. They prepared dozens of synthetic organic sulphur compounds, among which, the most toxic compound for mosquito larvae was a phenothiazine, thiodiphenylamine [12]. Another important veterinary use of a synthetic phenothiazine was its application as an anthelmintic due to its effectiveness against swine ascaris [31]. The third action of phenothiazine to be known was its antimalarial activity reported in the extensive studies by Paul Guttmann and Paul Ehrlich [29] on methylene blue, which basically is a phenothiazine. In this connection, note that, although Ehrlich was not involved directly with the diagnosis or therapy of tuberculosis, he was instrumental in the successful staining of tubercle bacilli.

Robert Koch started his investigation staining and identifying the infective organism of tuberculosis from the grey tubercles in the lungs of animals that had died from tuberculosis. On March 24, 1882, Koch reported, in a lecture in Berlin, his success in staining and cultivating tubercle bacilli from infected lung tissues [34]. Ehrlich, who was overwhelmed with the discovery, obtained a pure culture from Koch and experimented with his various stains. He used a shorter staining time and applied nitric acid and alcohol to decolourise the surrounding tissues. By accident, he learned the benefit of heating the slide where the staining was carried out. In May 1882, Ehrlich published a detailed description of his staining technique [23]. Ehrlich's improvement of the staining technique was soon acknowledged by Koch [50]. However, the first demonstration of a bacterium from a human infection had been made in 1875. Later Ziehl and Nielsen improved Ehrlich's method and stained with carbol fuchsin so that the bacilli became red and very prominently visible [59]. The work by Guttman and Ehrlich was confirmed much later by Fourneau et al. [25], who synthesised many compounds by modifying the main molecular structure of the phenothiazine methylene blue.

Syntheses and characterization of phenothiazines

Halpern and Ducrot [30] synthesized many phenothiazine-amine derivatives among which fenethazine was found to have a long lasting antihistaminic property. Charpentier et al. [14] described the synthesis of promethazine, which surpassed the action of fenethazine in its antihistaminic action; this was later used to treat motion sickness.

Subsequently, many more phenothiazines were synthesised to determine other effects. Bovet et al. [11] declared that their new phenothiazine compound diethazine had effects on sympathetic and parasympathetic nerve functions in dogs and rabbits. Macht and Hoffmaster [39] were the first to report on the use of conditioned reflex tests in animals to identify an effect of synthetic antihistamines on the central nervous system. Winter and Flataker [58] described the central antihistaminic effect of phenothiazines in animals with the help of the method of Macht and Mora [40]. They believed that the antihistaminic action of phenothiazines achieved their depressant effect by central action. The effect of phenothiazines could be nullified by caffeine to a large extent. Hence, they thought that cerebral cortex was the probable site of phenothiazine's central action. Their observations awoke the interest to synthesise many more phenothiazine compounds.

In 1949, Henri Laborit, a French navy neurosurgeon, reported on the use of antihistamines to combat circulatory shock during and after surgery. Laborit started using the phenothiazine compound promethazine as potentiator for general anaesthesia and also to facilitate artificial hibernation in surgery. Based on Laborit's work, the French chemical company Rhône-Poulenc decided to synthesize many more such chemicals, which led to the synthesis of more than 4000 different phenothiazines. In 1951, Laborit received samples including chlorpromazine labelled as 4560RP from Rhône-Poulenc for his clinical and pharmacological studies to evaluate them as potentiators of anaesthesia in his surgical practice. Although he found these compounds useful for artificial hibernation, Laborit emphasised on the undeniable complexity of chlorpromazine. Historically, the major impetus to move chlorpromazine into psychiatry did not come directly from Rhône-Poulenc. In fact, the studies carried out by Laborit and his co-workers were more responsible for the first trials of chlorpromazine by French psychiatrists, including Sigwald and Bouthier [51], and Delay and Deniker [18], each team working independently in 1952. Thus, within a year chlorpromazine became world's best choice for psychiatry.

Although the credit of having been the first miracle drug for psychiatry is given to chlorpromazine, in 1899 the Italian physician Pietro Bodoni [9] had demonstrated that methylene blue had sedative effect in a variety of psychotic conditions in his patients. Bodoni further suggested that methylene blue could be given to psychiatric patients on a regular basis [9]. However, when chlorpromazine came on the market, only a very few papers referred to the fact that chlorpromazine was a successor of methylene blue and also that all synthesised phenothiazines and related compounds were antimicrobial and

neuroleptic, the so-called "narcobiotics." Rhône-Poulenc was busy synthesizing various phenothiazines and distributing them to be tested in order to determine which of their compounds should be the best neuroleptic for patients. Several researchers found that one particular compound labelled as 3277 RP had antitubercular properties in vitro [37]. Such an antitubercular activity of chlorpromazine was obvious among different clinicians engaged in the treatment of psychoses and severe neuroses [27]. However, this unique function of chlorpromazine was not considered for further development to call it and label it as an antimycobacterial drug due to the serious side-effects produced by its prolonged administration. Meanwhile isoniazid had come on the market in 1952 and its success for treating patients suffering from *Mycobacterium tuberculosis*, coupled with information about other effective drugs, including streptomycin and rifampicin, lessened the chances of chlorpromazine being considered as an antimycobacterial agent [16]. Nevertheless, studies from different parts of the world reported on the antimycobacterial properties of several phenothiazines, including chlorpromazine.

Reports of antimycobacterial properties in phenothiazines

Phenothiazines as a class of easily available compounds have been repeatedly reported to have a moderate to powerful effect against many clinical strains of Gram-positive and Gram-negative bacteria both in vitro and in vivo [17]. Soon after the results of astounding success in treating neuroleptic patients with chlorpromazine became known, reports on its action against tuberculosis started appearing in scientific journals. One such important work was by Popper and Lorian [46] in 1959, where the in vitro minimum inhibitory concentration (MIC) of chlorpromazine against *M. tuberculosis* was found to be as low as 25 μg/ml (Table 1). Subsequently, in 1961 Bourdon [10] again proved that chlorpromazine had anti-tubercular action in vitro. Those studies, however, did not create interests in the development of chlorpromazine as a possible antitubercular agent because of the terrible side-effects observed in patients receiving chlorpromazine in routine therapy.

While studying the action of a few phenothiazines on different types of bacteria, Molnar et al. [42] described that the growth of *M. tuberculosis*, *M. bovis* and *M. butyricum* was inhibited by chlorpromazine practically at an identical concentration. The MICs for *M. tuberculosis* were 10 μg/ml for chlorpromazine and levomepromazine, 20 μg/ml for diethazine and promethazine, whilst chlorpromazine sulfoxyde was ineffective even at 100 μg/ml. Both chlorpromazine and promethazine exerted a measurable bactericidal activity on *M. tuberculosis* at 50 μg/ml; total destruction of the organism and partial loss of acid fastness of the cells could be observed at 300 μg/ml of chlorpromazine. In 1986, Kristiansen and Vergmann [35] made an intensive research on the antibacterial effect of various phenothiazine and thioxanthene derivatives on different species of mycobacteria in vitro (Table 1). They observed that the MICs of levomepromazine-maleate and chlorpromazine against mycobacterial strains were 25 μg/ml and 12.5 μg/ml, respectively. However, all stereo-isomers of clopenthixol were demonstrated to be twice as potent as chlorpromazine, but equally potent as chlorprothixen. Again, following the same procedure, the stereo-isomeric compounds of flupenthixol were shown to be more efficient than those of clopenthixol and chlorprothixene.

All these observations suggested that the stereo-isomeric analogue of thioxanthene derivatives should have significant antibacterial activity against the slow-growing mycobacteria. This in vitro study also revealed that these compounds were effective with other resistant mycobacterial strains, including *M. avium* and *M. intracellulare,* within the same concentration range as mentioned earlier. Subsequently, chlorpromazine was tested by Crowle et al. [16] for its ability to inhibit the replication of *M. tuberculosis* and *M. avium* in cultured normal human macrophages, as determined by counts of viable bacteria 0, 4, and 7 days after bacterial infection of the macrophages. Chlorpromazine was able to inhibit the intracellular bacteria at concentrations ranging from 0.23 μg/ml to 3.6 μg/ml, and was more effective intracellularly than extracellularly (Table 1).

According to Ratnakar and Murthy [47], trifluoperazine, a known calmodulin antagonist, completely inhibits the growth of mycobacteria. In a synthetic medium containing 0.2% Tween 80, the MIC of this drug ranged from 5 to 8 μg/ml for the human pathogenic strain *M. tuberculosis* $H_{37}R_V$, and *M. tuberculosis* was resistant to isoniazid. When added to a growing culture of *M. tuberculosis* $H_{37}R_V$ on the 10th day (mid exponential phase), trifluoperazine at the level of 50 μg/ml further arrested growth of this organism. Those authors [47] also observed that trifluoperazine, at a concentration of 50 μg/ml, when added to the cells along with the labeled precursors, inhibited the incorporation of ^{14}C acetate into lipids (63%) and uptake of ^{14}C glycine (74%) and ^{3}H thymidine (52%) by whole cells of *M. tuberculosis* $H_{37}R_V$, after 6 h of exposure. However, after 48 h, the inhibition was 87%, 97% and 74% respectively, in comparison with the labelled compounds as mentioned above. They further reported that when the drug

Table 1. Distribution pattern of minimum inhibitory concentration (MIC) of different phenothiazines on clinical isolates of *Mycobacterium tuberculosis*

Phenothiazine	MIC (μg/ml)	Method used	Reference
Chlorpromazine	25	ADT[a]	[46]
Chlorpromazine	12	ADT	[10]
Chlorpromazine	10	ADT	[42]
Levomepromazine	10		
Promethazine	20		
Diethazine	20		
Chlorpromazine sulfoxide	>100		
Chlorpromazine	12.5	ADT	[35]
Levomepromazine	25		
Trans (E)-flupenthixol	6.25		
Cis (Z)-flupenthixol	6.25		
Trans (E)-clopenthixol	12.5		
Cis (Z)-clopenthixol	6.25		
Trans (E)-chlorprothixen	6.25		
Cis (Z)-chlorprothixen	12.5		
Chlorpromazine	0.9	Mac[b]	[16]
Trifluoperazine	5–8	ADT	[47]
Methildiazine	5–12.5	ADT	[13]
Thioridazine	8-32	Bactec[c]	[4]
Chlorpromazine	4–32	Bactec[c]	[4]
Trifluoperazine	8–32	ADT	[26]
Chlorpromazine*	10	Bactec[c]	[44]
Chlorpromazine**	20–30		
Thioridazine*	15		
Thioridazine**	20–30		
Thioridazine	4	ADT	[54]
Trifluoperazine	2.5–7.5	ADT	[3]
SILA 421	2–16	ADT	[52]
Thioridazine	2–16		
Thioridazine S -enantiomer	4–16		
Thioridazine R -enantiomer	4–16		
Chlorpromazine	1–16		
Promazine	16 to >32		

[a]ADT, Agar Diffusion Test.
[b]Mac, macrophage.
[c]Bactec, generation of $^{14}CO_2$ in Bactec 460 System, 12B vials.
*MIC observed against sensitive strains of *M. tuberculosis*.
**MIC observed against multidrug resistant strains of *M. tuberculosis*.

was added to cells taking up and metabolizing the labelled precursors at a later point 3 h for ^{14}C acetate and ^{3}H thymidine and 12 h for ^{14}C glycine, trifluoperazine inhibited the uptake of all the precursors up to 24 h. It was suggested that this phenothiazine would have multiple sites of action and acted probably by affecting the synthesis of lipids, proteins and DNA.

Dastidar, Chakrabarty and their collaborators had worked on the detection and determination of antimicrobial action of

various categories of pharmacological agents since 1976 [17]. During their search they were able to detect moderate to powerful activity against grampositive and gramnegative bacteria in several antihistaminic phenothiazines. One such potent compound was methdilazine. In an intensive study, Chakrabarty et al. [13] employed 14 different reference strains of the genus *Mycobacterium* and screened them for their in vitro action against methdilazine along with two known antitubercular agents, streptomycin and rifampicin. The regular medium for determining in vitro activity was Kirchner's liquid medium, while the results were subsequently confirmed with the help of Lowenstein–Jensen medium. The MIC of methdilazine varied from 5 μg/ml to 12.5 μg/ml with respect to six test strains of *M. tuberculosis*; but most of the other strains of *Mycobacterium* were inhibited at 12.5–15.0 μg/ml of the drug, only except *M. gordonae*, whose MIC was as low as 5.0 μg/ml. Both streptomycin and rifampicin were highly effective on these strains, with MIC values ranging from 1.0 μg/ml to 2.0 μg/ml in respect of all the tested mycobacterial strains.

In 1996, Amaral and Kristiansen [4] observed that both chlorpromazine and thioridazine, another antipsychotic phenothiazine, could inhibit the respiration of clinical isolates of multiple drug-resistant *M. tuberculosis*. All these isolates were resistant to isoniazid and at least to another or even three of the other antitubercular drugs listed as first-line drugs, including streptomycin, rifampicin, ethambutol, and pyrazinamide. The authors emphasised that thioridazine, being much less toxic compared to chlorpromazine, might have a greater potential for its usage in freshly diagnosed patients of tuberculosis before determining their antibiotic sensitivity profile (Table 1).

Gadre and co-workers [26], while determining the in vitro action of trifluoperazine on clinical isolates of drug sensitive and resistant *M. tuberculosis*, observed that the MIC of trifluoperazine ranged from 4 μg/ml to 8 μg/ml among most of the test strains. However, the levels of MIC of trifluoperazine were between 4 μg/ml to16 μg/ml in strains that were resistant to one or two common antitubercular agents. Gadre et al. [26] further suggested that trifluoperazine, being a known calmodulin antagonist, might inhibit the growth of tubercle bacilli, which have been shown to contain calmodulin-like protein inside their cells (Table 1).

Ordway and her collaborators [44] became interested in determining the intracellular killing capacity of thioridazine versus chlorpromazine against sensitive as well as drug-resistant *M. tuberculosis* with the help of BACTEC 460-TB method (Table 1). They observed that both chlorpromazine and thioridazine killed intracellular antibiotic sensitive and resistant *M. tuberculosis* at concentrations, in the medium, well below those present in the plasma of patients treated with these agents for psychosis. Such concentrations in vitro were not toxic to macrophages, nor did they have any effect on the in vitro cellular immune processes. The authors further mentioned that as the phenothiazines are known to be concentrated by macrophages that phagocytose and have in situ activity against mycobacteria, these agents could be considered for use as adjuvants for the management of freshly diagnosed tuberculosis in patients from populations with a high prevalence of multidrug-resistant tuberculosis. Furthermore, chlorpromazine, thioridazine, and promethazine were shown to enhance the activity of rifampicin and streptomycin when used in combinations at concentrations that are minimally effective when employed separately against clinical strains of *M. tuberculosis* resistant to two or more antibiotics (poly-drug-resistant *M. tuberculosis*). The phenothiazines had no effect on the activity of isoniazid against test strains of poly-drug-resistant *M. tuberculosis*. Since the toxic side effects due to systemic administration of thioridazine in patients were much lower than those recorded after chronic administration of chlorpromazine, Ordway et al. [44] postulated that thioridazine could be considered as a suitable anti-tubercular drug and could be given to recently diagnosed patients suffering from pulmonary tuberculosis.

Van Ingen et al. [54] carried out a detailed in vitro experiment to find out the effect of thioridazine on 25 strains of various species of *Mycobacterium* including 8 strains of *M. tuberculosis*. Susceptibility to thioridazine was tested at concentrations ranging from 1 to 128 μg/ml in Middlebrook 7H10 medium, where sufficient growth of rapid growers was noted after 4 days and that of slow growers was between 8 and 11 days.

The MIC of thioridazine with respect to all the 8 strains of *M. tuberculosis* (including extremely-drug-resistant, multidrug-resistant and susceptible mycobacteria) was 4 μg/ml (Table 1), whereas the MIC of thioridazine ranged from 16 μg/ml to 32 μg/ml among most of the other mycobacteria. The level of activity of thioridazine against *M. abscessus* was maximum (64 μg/ml). This particular organism is known to cause severe disease in humans, but whether the concentration effect of thioridazine is achieved within macrophages for infection due to such a bacterium is yet to be ascertained [54]. However, with respect to *M. kansasii* type 1 and *M. xenopi* the MIC of thioridazine was 2 μg/ml and 8 μg/ml respectively.

Properties of selected phenothiazines

A tentative epidemiological cut-off of thioridazine was obtained by Angeby et al. [8] in 51 strains of sensitive *M. tuberculosis* and 67 strains of multidrug-resistant/extremely-drug resistant *M. tuberculosis* with the help of MIC determination using Middlebrook 7HI0 medium. Finally, a cut off value of 16 mg/l was proposed by the authors [8]. Although such a concentration was clinically not achievable in serum, thioridazine was found to become concentrated intracellularly and an amount of only 0.1 mg/l was able to kill *M. tuberculosis* cells residing inside cells [8]. The MIC value >16 mg/l was found in 6% of multidrug and extremely-drug-resistant strains for which the authors stated that resistance mechanism against thioridazine had already been present in the drug-resistant clinical strains of *M. tuberculosis*.

The calmodulin antagonist trifluoperazine was studied in detail by Advani et al. [3] for its activity towards *M. tuberculosis*, since such compounds are known to have multiple sites of action, including lipid synthesis, DNA processing, protein synthesis and respiration. Sensitive wild type, as well as multidrug-resistant strains of *M. tuberculosis*, were treated with trifluoperazine under different growth conditions of stress, including low pH, starvation, presence of nitric oxide and inactivated THP-1 infectious model. Perturbation in growth kinetics of tubercle bacilli at different concentrations of trifluoperazine was checked to determine the MIC of trifluoperazine per active as also dominant bacterial cells; they observed that trifluoperazine was able to significantly reduce the actively replicating as well as non-replicating cells of *M. tuberculosis*, thereby producing inhibition in growth of multidrug-resistant tuberculosis bacilli (Table 1). Since trifluoperazine showed enhanced action against intracellular bacilli, the authors postulated that this phenothiazine could also get accumulated in macrophages. Furthermore, the concentration required to produce such a phenomenon was non-toxic to macrophages [3].

Organo-silicon compounds are known efflux pump inhibitors and have anti-tubercular activity. One such compound, SILA 421, had the same pathway as the other efflux pump inhibitors of *M. tuberculosis*. Furthermore SILA 421 was found to modify the mdr-1 efflux pumps of *M. tuberculosis* and could enhance the killing action of *M. tuberculosis* by macrophage. Simons et al. carried out a comparative study to find out the efficacy of SILA 421 versus several known anti-tubercular phenothiazines using 21 clinical isolates of sensitive and resistant *M. tuberculosis* strains. SILA 421 was found to be equally as in vitro as the other well known efflux pump inhibitor thioridazine [52] (Table 1).

Methdilazine was assessed in a thoroughly designed in vivo experiment using Swiss albino mice. In that test, $> 9 \times 10^9$ viable cells of *M. tuberculosis* $H_{37}Rv$ 102 were given as the challenge to two groups of mice, one of which received methdilazine daily. After six weeks, all the animals in both groups were sacrificed and bacterial load was determined individually from liver, spleen, intestine and lung of each animal. The results showed that the protective capacity of methdilazine was statistically significant. Further studies on the action of methdilazine were carried out by Dutta et al. in 2009 [20]. They administered methdilazine singly and in combination with either streptomycin or isoniazid to separate groups of mice infected with *M. tuberculosis* $H_{37}Rv$ 102. Maintaining proper control groups throughout the entire period of investigation, those authors found that, although the combination of methdilazine and streptomycin did not result in a significant synergism, methdilazine was highly significantly synergistic with isoniazid [20].

Apart from methdilazine, the only other phenothiazine that has been studied in detail in animal systems is thioridazine. In 2007, Martins and her colleagues [41] tried to evaluate the effectiveness of thioridazine at different dose levels in BALB/c mice that had been infected intraperitoneally with a high dose of *M. tuberculosis* ATCC $H_{37}Rv$, thirty days prior to initiation of the treatment. A group of mice receiving no drug remained as the control. The dosages of thioridazine, calculated on the basis of human application, ranged from 0.05 to 0.5 mg/day. The group of animals that received 0.5 mg of thioridazine every day revealed a reduction of the colony forming units counts (CFU) in the lungs within 30 days. The therapeutic schedule was continued and after 300 days the number of mycobacteria in the lungs was found to be distinctly low. With the help of the same mouse model, van Sooligen et al. [55] determined the action of thioridazine in separate groups of animals infected with susceptible and multidrug-resistant *M. tuberculosis*. After a two-month period of oral administration of 32 and 70 mg/kg of thioridazine to two separate groups of animals, the CFU in the lungs were reduced significantly. Moreover, when thioridazine was added to a regimen containing rifampicin, isoniazid and pyrazinamide for infection with susceptible bacilli, a statistically significant synergistic effect ($P < 0.01$) was achieved. In a thoroughly designed pharmacokinetic study Dutta et al. [54] tried to determine the tuberculocidal activity of thioridazine in guinea pigs. The animals were aerosol infected with *M. tuber-*

culosis and single drug treatment with thioridazine was initiated 4 weeks later. The human equivalent dose of thioridazine was determined to be 5 mg/kg, which was given for 5 days per week. The initial bacterial burden in the lungs reduced after treatment with thioridazine; however, such a reduction was less than what was observed in animals treated with isoniazid. The tolerance limit of thioridazine in guinea pigs was found to be 40 mg/kg [21].

For assessing the activity of thioridazine singly and in combination with standard anti-tubercular drugs, single dose and steady state pharmacokinetic study [22] were performed in BALB/c mice to establish human equivalent doses of thioridazine. In order to determine the bactericidal activity of thioridazine against *M. tuberculosis*, three separate experiments were carried out including a dose ranging study of thioridazine monotherapy and effectiveness of human equivalent doses of thioridazine with or without isoniazid/or rifampicin. Therapeutic efficacy was assessed by the deviation in mycobacterial load in the lungs of test animals. The human-equivalent dose of thioridazine was found to be 25 mg/kg, which was very well tolerated by test mice [22]. Furthermore thioridazine was found to accumulate at higher concentrations in lung tissue compared to their amount in the serum. The authors also observed that thioridazine was not only synergistic with isoniazid, but prevented emergence of isoniazid resistant mutants in lung tissues of mice [22].

Multidrug resistance in *Mycobacterium tuberculosis*

Tuberculosis is a highly potent communicable disease worldwide. The causative organism, *M. tuberculosis,* is airborne and is frequently transmitted from person to person particularly among people of lower income group suffering from malnutrition and immunodeficiency. According to the World Health Organisation (WHO), in 2012 an estimated 8.6 million new cases was reported and 1.3 million died from the disease; of those, 320,000 were HIV positive. The distribution pattern of cases worldwide showed 29% in South-East Asia, 27% in Africa, 19% in Western Pacific regions. Note that India and China accounted for 26% and 12% of total cases respectively. The treatment regime recommended by the WHO is known as Directly Observed Therapy, Short-course (DOTS), in which age-old drugs such as isoniazid, rifampicin, ethambutol, and pyrazinamide are administered simultaneously for the first two months after presumptive diagnosis. Such a schedule is followed by a prolonged therapy with isoniazid and rifampicin for subsequent 4–7 months depending on the severity of the infection. But this does not guarantee a complete cure from the disease state. Moreover, more virulent forms, designated as multidrug-resistant and extremely drug-resistant tuberculosis and their frequent association with HIV has further provoked the crisis of the infection.

Mycobacterium tuberculosis resides within the pulmonary cavities where the supply of oxygen, pH and nutrition are quantitatively very low. The bacterium causes the host to have an active immune response during the infection, which results in the release of highly reactive oxygen and nitrogen species that may at first sight seem toxic to the mycobacterium bacilli. But the organism has developed resistance mechanisms to counteract that crisis. This resistance accounts for the success of *M. tuberculosis* as an intracellular pathogen. Most antibiotics that are used for the treatment of tuberculosis are only active against growing mycobacteria, but not against the dormant pathogens. The correlation between antibiotic activity and bacterial growth state in streptomycin-dependent *M. tuberculosis* has been reported. The main reasons for such a resistance are the change in bacterial metabolic pathway or a difference in physiological state that is described as phenotypic resistance. Due to this phenotypic resistance, the dormant bacilli effectively escape the immune system to the current line drugs, although genetic resistance in some organisms contributes to the resistance to tuberculosis chemotherapy.

The term multidrug-resistant is applied to those isolates of *M. tuberculosis* that are resistant to isoniazid and rifampicin, while extremely-drug-resistant isolates are often resistant to isoniazid, rifampicin, streptomycin, any flouroquinolone and any of the injectible anti-tuberculosis drugs like amikacin/kanamycin/capreomycin. The involvement of multidrug-resistant or extremely-drug-resistant *M. tuberculosis* strain in an infection is often due to misuse or overuse of the scheduled drugs or the failure of prolonged therapy that is required to be continued for months for the complete cure of the infection. Multidrug-resistant/extremely-drug-resistant strains may also arise if the treatment schedule is allowed for more than 12 months as in the case of a new severe infection of tuberculosis. Since many drugs are prescribed and given simultaneously for such cases, sufficient pressure is applied to the causative agent to select multidrug-resistant mutants. Such a situation could not occur by a single mode of antibacterial action, as different drugs have different target sites. A single-point mutation for resistance to any type of agents may influence the occurrence of resistance to the same class of compounds.

However, anti-tubercular drugs are very varied as far as their mode of action is concerned. Therefore, structurally similar drugs may have the same mutated target. Thus, there is an urgent need to use less targeted therapeutics in terms of microbial anatomy. Multi-site target attack is rather less common in actual practice. But, usage of such drugs may result in damaging the internal microbiota of individuals, particularly elderly patients who might easily be infected by opportunistic microorganisms [59]. Thus there is a crucial necessity to explore novel therapeutics with improved activities such as enhanced function against multidrug-resistant and extremely-drug-resistant tuberculosis, rapid mycobactericidal mechanism, and ability to fast penetration of host cells followed by bactericidal effect in the intracellular environment.

According to DiMasi et al. [19], the cost of a new drug discovery may exceed 800 million USD for a novel chemical entity and hence there is insufficient economic incentive for a pharmaceutical industry to develop novel drugs against infectious diseases endemic in developing and underdeveloped countries. Under such circumstances, the advancement of existing chemical compounds with well-documented antimicrobial potentiality should be explored in order to obtain affordable drugs to the great multitude of patients worldwide suffering from virulent multidrug-resistant infections including tuberculosis.

During the past sixty years phenothiazines have turned out to be a very important class of compounds owing to their remarkable biological and pharmacological properties. Several studies revealed that they were the first antipsychotics and are still the best choice for psychic patients. They are not only antibacterial or antifungal, but have many other properties, such as antiviral, anticancer, antimalarial, antifilarial, trypanocidal, antihistaminic, analgesic, anti-inflammatory activities. Various studies have further revealed that some phenothiazines are capable of inducing drug resistances in bacteria [43,45]. Phenothiazines are capable of exerting activities on biological systems via the interaction of multicyclic ring system like π to π interaction or interaction in DNA, or via the lipophilic character leading to penetration through biological membranes to reach the site of action [45]. The detailed account of numerous studies presented in this review indicates that several phenothiazines act both in vitro and in vivo against sensitive as well as multidrug-resistant (MDR) tubercle bacilli. Such active phenothiazines include chlorpromazine, methdilazine, trifluoperazine and thioridazine. Many researchers have tested chlorpromazine for its potent antibacterial and antimalarial properties. This compound, however, could not be considered for therapy of bacterial infections due to its frequent serious side-effects [5]. But in the early 1980s, when multidrug-resistant tuberculosis surfaced in several developed countries, the search for new anti-tuberculosis compounds became a social necessity. At this time there was a resurgence of the anti-tubercular activity of chlorpromazine. This activity was ensured at such high concentrations that were far beyond those clinically achievable in serum, although it was known that phenothiazines had a great affinity for lung tissue which is ≤100 μg/g wet tissue [28]. Kristiansen and Vergmann [35] had reported that all of their test mycobacteria were inhibited at ≤25 μg/ml of chlorpromazine. Heterogenous populations of both actively living and latent tuberculosis lesions are known to be present in individuals infected with tuberculosis.

Several studies [3,13] indicate the action of both trifluoperazine and methdilazine that could kill both the above populations which implied that the target pathways are common to both the multiplying and dormant forms of the organism. Multidrug therapy replaced monotherapy in tuberculosis infections since the early 1960s as this was considered to be the main cause for the development of drug resistance. Various approaches should be used in testing the antimicrobial susceptibility against *M. tuberculosis* so that further experiments with drug combinations can be made in vivo. The synergistic action of phenothiazines with a number of anti-tubercular agents has already been reported [20,22]. Both trifluoperazine and methdilazine tend to cause accumulation and retention of antimycobacterial drugs in macrophages. Thus there should be approaches for developing more effective and less harmful derivatives of such drugs that can be used as an alternative to trigger the function of the compound.

Although chlorpromazine was found to kill *M. tuberculosis* in newly phagocytosed human macrophages [4,44], due to the side-effects on the central nervous system, the use of the compound as an anti-tubercular drug remained restricted. Chlorpromazine was largely replaced by the neuroleptic drug thioridazine, which is less toxic and is much more effective in in vitro systems against all forms of antibiotic resistant strains of *M. tuberculosis* and also it has fewer side effects than chlorpromazine [4]. Thus the interest in thioridazine began; this compound promotes the killing of multidrug-resistant *M. tuberculosis* by macrophages, at a much lower concentration used in the therapy of psychosis, in the experimental medium [44].

In the murine model, thioridazine has been found to be effective against both antibiotic susceptible and multidrug-resistant tuberculosis infection [41,55]. It has been effective

also against ten out of twelve cases of extremely-drug-resistant tuberculosis patients [6]. Therefore, the therapy of extremely-drug-resistant tuberculosis may be conducted on a compassionate basis with thioridazine plus standard therapeutic regimen.

Chlorpromazine has been found to affect the bacterial efflux pump; therefore, it reduces the resistance to antibiotics in bacterial strains [56]. The result was found to be similar in the case of phenothiazines. They could inhibit Nor A efflux pump of *Staphylococcus aureus* and the QAC efflux pump of plasmid-carrying multidrug-resistant *Staphylococcus aureus* [33]. The *Acr AB* efflux pump of *E.coli* was found to be inhibited by both phenothiazines, chlorpromazine and thioridazine [57]. These phenothiazines could also inhibit the main efflux pump of *M. smegmatis* and the efflux pump of *M. avium* [48]. Since it affects the survival genes of *M. tuberculosis*, the effect of thioridazine may presumably be universal. Phenothiazines act by inhibiting the activity of calcium-dependent ATPase, which leads to the acidification of phagolysosome and its subsequent activation of the hydrolases, resulting in the inhibition of replication of the bacterium [7]. Thioridazine inhibits the EmrE-encoded efflux pump in *M. tuberculosis* [49]. Further, thioridazine treated cell cause the expression of *Rv3065* gene, which encodes the multidrug transport integral membrane protein EmrE, and that of another putative efflux pump gene *Rv1634*. When *Rv3065* homologue is deleted in *M. smegmatis*, it has been seen that there is an increased susceptibility to a number of drugs including fluoroquinolones, ethidium bromide, and acriflavin [49].

During the macrophage infection by *M. tuberculosis*, the action of several efflux pumps and their regulators induce the tolerance of thioridazine inside the mycobacterial cell [2]. The accumulation of mutations in isoniazid targets is caused by the overexpression of the efflux pumps and the treatment with thioridazine, chlorpromazine and verapamil can reduce the resistance of *M. tuberculosis* to isoniazid [38]. Efflux pump inhibitors enhance the killing of intracellular *M. tuberculosis* by non-killing macrophages. Thioridazine is able to inhibit the expression of efflux pumps in *M. tuberculosis* in such a manner that the anti-mycobacterial drugs fail to arrive at their intended targets in the cell. Thioridazine can also inhibit the activity of existing efflux pumps that are responsible for the multidrug-resistant phenotype of *M. tuberculosis*. Thus, for preventing the emergence of multidrug-resistance tuberculosis thioridazine should be used for developing new therapeutic strategies [38].

Kristiansen and her co-workers probed on various properties of phenothiazines including inhibiting potassium efflux, antimicrobial potentialities and reversal of drug resistances among pathogenic bacteria [36]. They reported that antimicrobial action of phenothiazines and central nervous system activity of the same compounds are not related [36]. In their studies they further observed that phenothiazines containing an exocyclic double bond, termed as thioxanthenes, had a more potent antimicrobial activity [36]. However, such thioxanthenes acted more intensely on the central nervous system than the actual phenothiazines [24,36]. Therefore, there is a possibility to separate central nervous system and antimicrobial functions by synthesising isomeric forms of the same-compound with an additional halogen moiety, which is known to potentiate antimicrobial action [36].

Classical phenothiazine compounds having different stereoisomeric forms (+/–) have distinct differences in producing antimicrobial action [32,36,53]. In 2013, Christensen et al. [15] made a detailed comparative analysis on the in vitro and in vivo efficacies of the (+) and (–) enantiomers of thioridazine along with its racemic form. Their study yielded a significant antibacterial action in all the forms of thioridazine, indicating of further the levorotatory (–) form to be superior in terms of both its in vitro and in vivo efficacies [15]. Since the racemic form of thioridazine containing both (+) and (–) is known to possess very potent anti-mycobacterial action [36] the (–) thioridazine with no central nervous system activity should lead to a breakthrough in the treatment of tuberculosis, particularly synergistic action of thioridazine with isoniazid [22] should also be further investigated with this form *i*n vitro and in vivo. Thus all the recent studies presented here have established the addition of thioridazine to the existing combined regimen for the treatment of multidrug-resistant and extremely-drug-resistant *M. tuberculosis*.

One hundred years ago Paul Ehrlich had been treating patients suffering from trypanosomiasis and malaria with the help of methylene blue, the first ever compound of phenothiazines. Since the late 1940s the entire class of phenothiazines has evolved not only as neuroleptics but also as antimicrobials. The potentiality of thioridazine as a highly suitable candidate for multidrug-resistant/extremely-drug-resistant tuberculosis has been repeatedly proved in Argentina, where patients dying from infection by extremely drug-resistant tuberculosis were cured with a combination of linezolid, moxifloxacin and thioridazine [1]. In this way this review offers a new horizon for successful therapy of patients suffering from multidrug-resistant/extremely-drug-resistant tuberculosis with thioridazine, preferably its levorotatory form, as a drug additional to the list of conventional therapeutic regimen.

Conclusion

Tuberculosis is one of the most serious human infections throughout the world. One of the foremost reasons for its prevalence both in developed and developing countries is the lack of and inability to afford proper healthcare for such a serious disease. Overcrowding and malnutrition of poor communities in large cities is a major cause of the high incidence of tuberculosis particularly due to the easy transmission. The accelerated speed of the spread of tuberculosis during the last few decades and the higher incidence of infection by multidrug-resistant and extremely-drug-resistant *M. tuberculosis* have led to an alarming situation in all the continents. Although the antimycobacterial property of chlorpromazine became known soon after its widespread use as a potent antipsychotic agent, chlorpromazine failed to receive attention of the clinicians not only due to the toxic side effects but also due to the discovery of many pharmacologically active antitubercular compounds. In course of time, attention to chlorpromazine was replaced by the cogener thioridazine which continuously received attention of researchers for its antimycobacterial potentialities. Thioridazine is not only active against tubercle bacilli but also against a series of grampositive and gramnegative bacteria. The understanding of the concept of non-antibiotics is primarily centered on recognition of antimicrobial properties in commercially available pharmacologically diverse compounds. Following this pathway chlorpromazine, trifluoperazine, methdilazine and thioridazine have evolved as potent antimycobacterial drugs. Although many phenothiazines have been retracted from the market for their cardiotoxicity, the studies presented above clearly prove that the requirement of the amount of thioridazine for treating tuberculosis in BALB/c mice is rather small due to its unique property of being concentrated inside macrophages that host tubercle bacilli. The report by Dutta et al. [22] on thioridazine on the well validated murine model used for preclinical screening of antitubercular drug claimed that the human equivalent dose of thioridazine was well tolerated by test animals and that thioridazine was found to accumulate at high concentrations in lung tissues compared to serum levels. Moreover, a very recent study has revealed that QT prolongation effect was centered on racemate and (+) thioridazine while such an action was much less in (–) thioridazine. Thus for the treatment of the multidrug-resistant/extremely-drug-resistant tuberculosis a new avenue will open up by synthesising and initiating clinical trials with (–) thioridazine to facilitate an orchestrated response against this deadly pathogen.

Competing interests. The authors Oliver Hendricks, Jørn B. Christensen and Jette E. Kristiansen own shares in NOA-SIC. The company holds Patent US 8,623,864, EP 168 940 5A1 & WO 2005 046694 A1 concerning "Thioridazine and Derivatives Thereof for Reversing Anti-Microbial Drug-Resistance".

References

1. Abbate E, Vescovo M, Natiello M, Cufre M, Garcia A, Gonzalez Montaner P, Ambroggi M, Ritacco V, van Soolingen D (2012) Successful alternative treatment of extensively drug resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin, and thioridazine. J Antimicrob Chemother 67:473-477
2. Adams KN, Takaki K, Connolly LE, Weidenhoft H, Winglee K, Humbert O, Edelstein PH, Cosma CL, Ramakrishnan L (2011) Drug tolerance in replicating mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145:39-53
3. Advani MJ, Siddique I, Sharma P, Reddy H (2012) Activity of trifluoperazine against replicating, non-replicating and drug resistant *M. tuberculosis*. PLoS ONE 7:1-9
4. Amaral L , Kristiansen JE, Abebe LS, Millett W (1996) Inhibition of the respiration of multidrug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis. J Antimicrob Chemother 38:1049-1053
5. Amaral L,Viveiros M, Kristiansen JE (2001) Phenothiazines: potential alternatives for the management of antibiotic resistant infections of tuberculosis and malaria in developing countries. Trop Med Int Health 6:1016-1022
6. Amaral L, Boeree MJ, Gillespie SH, Udwadia ZF, van Soolingen D (2010) Thioridazine cures extensively drug resistant tuberculosis (XDR-TB) and the need for global trial is now! Int J Antimicrob Agents 35:524-526
7. Amaral L, Martins M, Viveiros M (2007) Enhanced killing of intracellular multidrug-resistant Mycobacterium tuberculosis by compounds that affect the activity of efflux pumps. J Antimicrob Chemother 59:1237-1246
8. Angeby K, Juréen P, Chryssanthou E, Schön T (2012) Tentative susceptibility testing breakpoint for the neuroleptic drug thioridazine, a treatment option for multi- and extensively drug resistant tuberculosis. Int J Mycobacteriol 1:177-179
9. Bodoni P (1899) Dell'azione sedative del blue di metilene in varie forme di psicosi. Clinica Medica Italiana 24:217-222
10. Bourdon JL (1961) Contribution to the study of the antibiotic properties of chlorpromazine or 4560 RP. Ann Inst Pasteur 101:876-386
11. Bovet D, Fournel J, Charpentier P (1947) Pharmacodynamic properties of diethylaminoethylphenothiazine hydrochloride Therapie (Paris) 2:115-119
12. Campbell FL, Sullivan WN, Smith LE, Haller HL (1934) Insecticidal tests of synthetic organic compounds—chiefly tests of sulphur compounds against culicine mosquito larvae. J Econ Entomol 27:1176-1186
13. Chakrabarty AN, Bhattacharya CP, Dastidar SG (1993) Antimycobacterial activity of methdilazine(Md), an antimicrobic phenothiazine. APMIS 101:449-454
14. Charpentier P, Guillot P, Jacob R, Gaudecho J, Buisson P (1952) Recherches sur les di-methylamino-propyl-*N*-phenothiazine. C.R. Acad Sc 235: 59-60
15. Christensen JB, Hendricks O, Chaki S, Mukherjee S, Das A, Pal TK, Dastidar SG Kristiansen JE (2013) A comparative analysis of in vitro and in vivo efficacies of the enantiomers of thioridazine and its racemate. PLoS ONE 8:1-6

16. Crowle JA, Douvas GS, May MH (1992) Chlorpromazine: A drug potentially useful for treating mycobacterial infections. Chemotherapy 38:410-419
17. Dastidar SG, Kristiansen JE, Molnar J, Amaral L (2013) Role of phenothiazines and structurally similar compounds of plant origin in the fight against infections by drug resistant bacteria. Antibiotics 2:58-71
18. Delay J, Deniker P (1952) Réactions biologiques observées au cours du traitement par le chlorhydrate de diméthyl-amino-propyl-*N*-chlorophénothiazine (4560 R.P.). C.R. Congrés Médicine Alién Neurology France 50:514-518
19. DiMasi JA, Hansen RW, Grabowski HG (2003) The price of innovation: new estimates of drug development costs. J Health Econo 22:151-185
20. Dutta NK, Mazumdar K, Dasgupta A, Dastidar SG (2009) Activity of the phenothiazine methdilazine alone or in combination with isoniazid or streptomycin against *Mycobacterium tuberculosis* in mice. J Medical Microbiol 58:1667-1678
21. Dutta NK, Alsultan A, Peloquin CA, Karakousis PC (2013) Preliminary pharmacokinetic study of repeated doses of rifampin and rifapentine in guinea pigs. Antimicrob Agents Chemother 57: 1535-1537
22. Dutta NK, Pinn ML, KaraKousis PC (2014) Reduce emergence of isoniazid resistance with concurrent use of thioridazine against acute murine tuberculosis. Antimicrob Agents Chemother 58:4048-4053
23. Ehrlich P (1956) Modifikation der von Koch angegebenen Methode der Färbung von Tuberkelbazillen. Dtsch. Med. Wschr. In: Himmelweit F, Marquardt M, Dale H (eds) The collected papers of Paul Ehrlich, Vol 1. Histology, Biochemistry and Pathology, Pergamon Press, London, pp 311-313
24. Feinberg AP, Snyder SH (1975) Phenothiazine drugs: structure activity relationships explained by a confirmation that mimics dopamine. Proc Nat Acad Sc USA 72:1899-1903
25. Fourneau E,Trefouel J, Trefouel M, Bovet D, Benoit G (1931) Contribution à la chimiothérapie du paludisme essais sur les calfates. Ann Inst Pasteur 46: 514-541
26. Gadre DV, Talwar V (1999) In vitro susceptibility testing of *Mycobacterium tuberculosis* strains to trifluoperazine. J Chemother 11:203-206
27. Geiger H, Finkelstein BA (1954) Largactil in the treatment of tuberculosis. Schweiz Med Wschr 84:1063-1064
28. Gruenke LD, Craig JC, Klein FD, Nguyen TL, Hitzemann BA, Holaday JW, Loh HH, Braff L, Fischer A, Glick ID, et al. (1985) Determination of chlorpromazine and its major metabolites by GC/MS: application to biological fluids. Biomed Mass Spectrom 12:707-713
29. Guttmann P, Ehrlich P (1891) Über die Wirkung des Methylenblau bei Malaria. Berliner Klinische Wschr 28:953-956
30. Halpern BN, Ducroit R (1946) Recherches expérimentales sur une nouvelle série chimique des corps doués de propriétés antihistaminiques puissantes; les dérivés de la thiodiphénylamine (T.D.A). C.R. Soc Biology 140:361-363
31. Harwood PD (1946) Research on phenothiazine as an anthelmintic. Science Model 62:32-42
32. Hendricks O, Christensen JB, Kristiansen JE (2004) Antibakterielle Eigenschaften der Phenothiazine. Eine Behandlungsoption für die Zukunft? Chemother J 13:203-205
33. Kaatz GW, Moudgal W, Seo SM, Kristiansen JE (2003) Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in *Staphylococcus aureus*. Antimicrob Agents Ch 47:719-726
34. Koch R (1882) Die Ätiologie der Tuberkulose. Berliner Klinische Wschr 19:221-230
35. Kristiansen JE, Vergmann B (1986) The antibacterial effect of selected phenothiazines and thioxanthenes on slow growing mycobacteria. Acta Path Micro IM B 94:393-398
36. Kristiansen JE, Hendricks O, Delvin T, Butterworth TS, Aagaard L, Christiansen JB, Flores VC, Keyzer H (2007) Reversal of resistance in microorganisms by help of non-antibiotics. J Antimicrob Chemother 59:1271-1279
37. Levaditi C, Chaigneau-Erhard H, Henry-Eveno J (1951) L'antihistaminique 3277 RP (Phenergan) agit-il curativement dans la tuberculosis experimentale de la souris? C.R. Soc Biol 145:1454-1456
38. Machado D, Couto I, Perdigao J, Rodrigues L, Portugal I, Baptista P, Veigas B, Amaral L, Viveiros M (2012) Contribution of efflux to the emergence of isoniazid and multidrug resistance in *Mycobacterium tuberculosis*. PloS ONE 7:e34538.
39. Macht DI, Hoffmaster T (1948) Influence of benadryl and pyribehzassine on the neuromuscular system of rats. Federal Proceedings 7:242
40. Macht DI, Mora CF (1921) Effect of opium alkaloids on the behaviour of rats in the circular maze. J. Pharmacol Exp Therap 16:219-223
41. Martins M, Viveiros M, Kristiansen JE, Molnar J, Amaral L (2007) The curative activity of thioridazine on mice infected with *Mycobacterium tuberculosis*. In Vivo 21:771-776
42. Molnar J, Beladi I, Foldes I (1977) Studies on anti-tuberculotic action of some phenothiazine derivatives in vitro. Zentral Bakteriol (Orig A) 239:521-526
43. Mukherjee S, Chaki S, Barman S, Das S, Koley H, Dastidar SG (2012) Effective elimination of drug resistance genes in pathogenic *Pseudomonas aeruginosa* by antipsychotic agent thioridazine. Current Res Bacteriol 5:36-41
44. Ordway D, Viveiros M, Leandro C, Bettencourt R, Almeida J, Martins M, Kristiansen JE, Molnar J, Amaral L (2003) Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 47:917-922.
45. Pluta K, Morak-Milodawska B, Jelen M (2011) Recent progess in biological activities of synthesized phenothiazines. Europ J Med Chem 46:3179-3189
46. Popper M, Lorian V (1959) L'action de la chlorpromazine sur les microbes et le complexe bacteriostatique in vitro. Presse Med 67:212-213
47. Ratnakar P, Murthy PS (1993) Trifluoperazine inhibits the incorporation of labelled precursors into lipids, proteins and DNA of *Mycobacterium tuberculosis* H37Rv. FEMS Microb Lett 110:291-294
48. Rodrigues L, Sampaio D, Couto I, Machado D, Kern WV, Amaral L, Viveiros M (2009) The role of efflux pumps in macrolide resistance in *Mycobacterium avium* complex. Int J Antimicrob Agents 34:529-533
49. Rodrigues L, Wagner D, Viveiros M, Sampaio D, Couto I, Vavra M, Kern WV, Amaral L (2008) Thioridazine and chlorpromazine inhibition of ethidium bromide efflux in *Mycobacterium avium* and *Mycobacterium smegmatis*. J Antimicrob Chemother 61:1076-1082
50. Sakula A (1979) Robert Koch: Founder of the science of bacteriology and discoverer of the tubercle bacillus: A study of his life and work. Brit J Dis Chest 73:389-394
51. Sigwald J, Bouthier D (1953) Le chlorhydrate de chloro-3(dimethylamino–3-propyl)-10-phenothiazine en practique neuro-psychiatrique courante. Ann Medicine 54:150-182
52. Simons SO, Kristiansen JE, Hajos G, et al. (2013) Activity of the efflux pump inhibitor SILA 421 against drug-resistant tuberculosis. Int J Antimicrob Agents 41:488-489
53. Svendsen CN, Froimowitz M, Hrbek C, Campbell A, Kula N, Baldessarini RJ, Cohen BM, Babb S, Teicher MH, Bird ED (1988) Receptor affinity, neurochemistry and behavioral characteristics of the enantiomers of thioridazine: Evidence for different stereoselectives at D_1 and D_2 receptors in rat brain. Neuropharmacology 27:1117-1124.

54. van Ingen J, van der Laan T, Amaral L, Dekhuijzen R, Boeree MJ, van Soolingen D (2009) In vitro activity of thioridazine against mycobacteria. Internat J Antimicrob Agents 34:190-191
55. van Soolingen D, Hernandez-Pando R, Orozco H, Aguilar D, Magis-Escurra C, Amaral L, van Ingen J, Boeree MJ (2010) The antipsychotic thioridazine shows promising theraupetic activity in a mouse model of multidrug-resistant tuberculosis. PLoS ONE 5:e12640
56. Viveiros M, Leandro C, Amaral L (2003) Mycobacterial efflux pumps and chemotherapeutic implications. Int J Antimicrob Agents 22:274-278
57. Viveiros M, Dupont M, Rodrigues L, Couto I, Davin-Regli A, Martins M, Pages JM, Amaral L (2007) Antibiotic stress, genetic response and altered permeability of *E. coli*. PLoS ONE 2:365-372
58. Winter C, Flataker L (1951) The effect of antihistaminic drugs upon the performance of trained rats. J Pharmacol Exp Ther 101:56-162
59. Wainwright M, Amaral L, Kristiansen JE (2012) The evolution of anti-mycobacterial agents from non-antibiotics. Open J Pharmacol 2:1-11
60. Zeihl F (1882) Zur Färbung des Tuberkelbacillus. Dtsch Med Wshr 8: 451-453