Role of Phenothiazines and Structurally Similar Compounds of Plant Origin in the Fight against Infections by Drug Resistant Bacteria

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Abstract: Phenothiazines have their primary effects on the plasma membranes of prokaryotes and eukaryotes. Among the components of the prokaryotic plasma membrane affected are efflux pumps, their energy sources and energy providing enzymes, such as ATPase, and genes that regulate and code for the permeability aspect of a bacterium. The response of multidrug and extensively drug resistant tuberculosis to phenothiazines shows an alternative therapy for the treatment of these dreaded diseases, which are claiming more and more lives every year throughout the world. Many phenothiazines have shown synergistic activity with several antibiotics thereby lowering the doses of antibiotics administered to patients suffering from specific bacterial infections. Trimeprazine is synergistic with trimethoprim. Flupenthixol (Fp) has been found to be synergistic with penicillin and chlorpromazine (CPZ); in addition, some antibiotics are also synergistic. Along with the antibacterial action described in this review, many phenothiazines possess plasmid curing activities, which render the bacterial carrier of the plasmid sensitive to antibiotics. Thus, simultaneous applications of a phenothiazine like TZ would not only act
as an additional antibacterial agent but also would help to eliminate drug resistant plasmid from the infectious bacterial cells.

**Keywords:** phenothiazines; antimicrobial activities; efflux pumps; quorum sensing; reversal of antibiotic resistance

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1. **Introduction**

Antibiotics have been found to be one of humankind’s most imperative weapons in combating microbial infections. Although there are highly effective antibiotics to cure nearly all major infectious diseases, such health benefits have come under threat, not only because many of these possess toxicity but also due to emergence of antibiotic-resistant bacteria. Therefore, the medicines required to cure major diseases threaten to erode the medical advances of recent decades. New antibacterial molecules and new therapeutic approaches are needed to overcome multi drug resistant (MDR) and extreme drug resistant (XDR) states in severe infectious diseases [1–4]. Thus, there is an indispensable need to explore newer molecules with lesser degrees of resistance [5]. Since the 1970s, several groups of workers independently undertook a systematic study to determine antimicrobial action of drugs belonging to various pharmacological classes not recognized as antimicrobials. This resulted in the accumulation of a large amount of evidence on many types of drugs possessing moderate to powerful antimicrobial action. All such drugs with antimicrobial activity are collectively termed as non-antibiotics (Kristiansen [6]). After the discovery by Paul Ehrlich [7] of the antimicrobial action of methylene blue, the search for drugs with antimicrobial property began. Ultimately the neuroleptic phenothiazine chlorpromazine (CPZ) was synthesized in 1950s. With global use of chlorpromazine, reports showed that patients receiving chlorpromazine had a lower incidence of bacterial infections [8]. After this, there was a boom in search for drugs, such as, antihistamines, anti-inflammatory agents, antihypertensives, cardiovascular drugs, antipsychotics and neuroleptics with possibilities of potent antimicrobial properties [9]. However, the antihistaminic and antipsychotic agents have been studied most extensively for their antimicrobial action both *in vitro* and *in vivo* [10–13].

2. **Antimicrobial Action of Phenothiazines**

Phenothiazines proved to be a unique class of compounds with prominent antibacterial activity against most of the pathogenic bacteria (Table 1). The MIC values of CPZ (chlorpromazine); Pr (promazine); Pz (promethazine); Pc, (prochlorperazine); Md (methdilazine); Fz (fluphenazine); Tm (trimeprazine); Tf (trifluoperazine); Tp (triflupromazine); Tz,(thioridazine); and Fp (flupenthixol). CPZ, Pr, Md, Fz, Tm, Tf, and Fp with respect to most of the Gram positive bacteria were from 10 µg/mL level, a few organisms could be inhibited by Md and Fz at 2 to 5 µg/mL level. The compound Tf was highly active against Gram positive bacteria as several of them revealed MIC as low as 2 µg/mL. Among Gram negative organisms, vibrios were most sensitive to many of the phenothiazines. However, several strains of *Salmonella* spp. and *Shigella* spp. exhibit greater sensitivity than others of the same genera. Klebsiellae, pseudomonads and acenetobacters were highly
resistant to almost all of these drugs. Many of these phenothiazines were bacteriostatic, while some others were able to kill a pathogen within 6 to 18 h.

Table 1. Antibacterial activity of synthetic phenothiazines by in vitro screening.

| Phenothiazine | MIC µg/mL | Bacteriostatic/ Bactericidal |
|--------------|-----------|-------------------------------|
|              | Gram +ve | Gram –ve |                      |
| CPZ          | 10–50    | 25–100 | Bacteriostatic for Gm –ve, Bactericidal for Gm +ve |
| Pr           | 10–50    | 10–100 | Bacteriostatic |
| Pz           | 50–200   | 100–200 | Bacteriostatic |
| Pc           | 25–100   | 50–400 | Bacteriostatic |
| Md           | 10–100   | 25–200 | Bactericidal |
| Fz           | 10–100   | 10–100 | Bactericidal |
| Tm           | 10–100   | 10–100 | Bactericidal |
| Tf           | 10–100   | 25–200 | Bactericidal |
| Tp           | 2–50     | 2–100  | Bactericidal |
| Tz           | 32–64    | 100–800 | Bacteriostatic for Gm –ve, Bactericidal for Gm +ve |
| Fp           | 50–800   | 10–100 | Bacteriostatic |

Please note: The bactericidal effect can be reached with multiple of the MICs. CPZ, chlorpromazine; Pr, promazine; Pz, promethazine; Pc, prochlorperazine; Md, methdilazine; Fz, fluphenazine; Tm, trimeprazine; Tf, trifluoperazine; Tp, triflupromazine; Tz, thioridazine; Fp, flupenthixol.

The mechanism by which the phenothiazines act on bacterial cells in vitro has been studied by several researchers during the past few years. In 1979, Kristiansen [14] observed that CPZ was bacteriostatic to S. aureus at low level, but as the doses of CPZ were increased, it produced bactericidal action on the same organism. It was shown further that CPZ was involved in bacterial haemolysins, as the erythrocytic membranes of animals were altered in such way that haemolysis of the membrane was affected. Therefore, at low concentrations CPZ possibly interfered with the transport of potassium through the bacterial membrane much in the same way as it occurs in mammalian tissue [14]. In 1986, Galeazzi et al [15] observed that CPZ was a competent cell permeabilizer and was capable of conducting microbial peroxidase and peroxidase like reactions. Whenever studied, CPZ increases the permeability of the bacterium to antibiotics, as evident from the items presented in this herein review.

In 1991, Amaral and Lorian [16] observed that when E. coli was grown at the sub-MIC level of CPZ, the cells became elongated and filament-like in 5 h, but reverted to rod-like shape after 24 h. It was found that the electrophoretic pattern of proteins extracted from the cell envelopes of all forms of CPZ treated cells was distinctly different from those of both the untreated cells of E. coli.

In 2000, Amaral et al [17] observed that CPZ failed to produce any inhibitory effect on cell proliferation of Salmonella that were allowed to remain in the sub-inhibitory state of agglutinability with the specific O antibody. Thus, the resistance to CPZ was dependent upon changes induced by CPZ in the cell wall. It was postulated that CPZ probably was able to bind with 55 KDa protein in the cell wall and interfered with the recognition of O antigen by the specific antibody.
According to Radhakrisnan et al [18], phenothiazine thioridazine (TZ) proved to be a unique drug, as it could induce complete destruction of different Gram positive bacteria within a span of only two hours; however, with respect to all the different Gram negative organisms it was observed that although there was a gradual decrease in the number of viable cells after addition of Tz in a highly multiplying state of the organisms, the cells remained viable up to 18 h, revealing the bacteriostatic nature of Tz on such bacteria. It was suggested that the drug was possibly able to penetrate quite easily the peptidoglycan layer of the cell wall of Gram positive bacteria, but was unable to have any negative effect on the components of the outer membrane of Gram negative cell envelope such as lipoprotein or the lipopolysaccharide.

Since there is no specific drug to cure the sleeping sickness caused by Trypanosoma brucci, Page and Lagando [19] investigated the action of Tz on the pellicular membrane complex of the infective bloodstream form of the parasite. Although Tz could induce rapid changes in cell shape but failed to affect structural integrity of the microtubular complex. However, the drug was successful in damaging both the nuclear and the cytoplasmic membranes. In this way like CPZ Tz was also found to have action on cell envelopes.

Investigations on the structure activity relationships of the phenothiazines containing halogen atoms showed that their antimicrobial properties were possibly linked to the methyl-thio substituent at position 10 and a halogen moiety at position 2 of the basic phenothiazine ring [20]. As the thioxanthen skeleton is similar to phenothiazine except for the absence of a tertiary nitrogen atom at position 9, the presence of a trifluoromethyl group at position 2 of the tricyclic ring may be possible for rendering the antibacterial property to Fp, producing a structure similar to the anti-inflammatory antibacterial agent diclofenac sodium [10]. All these studies revealed that both CPZ and Tz have different kinds of action on the cell envelopes of both Gram positive and Gram negative bacteria.

To evaluate the efficacy of phenothiazines in animal systems, a series of studies were conducted with a Swiss strain of male white mice weighing 18–20 g each were taken (Table 2). The naturally mouse virulent bacterium Salmonella enterica serovar Typhimurium NCTC 11 and NCTC 74 obtained from London served as the challenge strains. Both these strains were simultaneously sensitive to many antibiotics and the phenothiazines. Virulence of strains was significantly increased with repeated mouse passages and the median lethal dose (MLD or LD50) was determined following standard technique [21]. Protective capacity of each phentiazine was determined by injecting a definite dose of the drug followed by challenge with 50 LD50 dose of the virulent salmonella to groups of mice. Toxicity levels of the compound were determined at the same time. In a separate experiment, the actual bacterial load in various organs was determined in treated and untreated mice. While evaluating the effects of phenothiazines in challenged mice it was noted that Pr was the best drug since it could offer protection at the level of 2–8 µg/mouse, and Tm was the next in order. However, Pr was much less toxic than Tm since the latter produced severe convulsion followed subsequently by death when the doses were greater than 16 µg/mouse. The drugs Md, Tf, Tp and Fp were much less toxic and offered statistically significant protection at the levels of 15–30 µg/mouse. Since the in vitro MIC of Tz in Salmonella enterica 74 was 500 µg/mL 200 µg/mouse was required to protect the challenged mice. Higher amounts of Tz also produced convulsion in animals (Table 2).
Table 2. Anti-salmonella activity of phenothiazines in vivo.

| Phenothiazine | Drug (µg/g) per mouse |
|---------------|-----------------------|
|               | Toxic dose | Protective dose |
| Pr            | >64        | 2–8             |
| Md            | >320       | 15–30           |
| Fz            | >120       | 30–60           |
| Tm            | >16        | 4–8             |
| Tf            | >60        | 15–30           |
| Tf            | >60        | 15–30           |
| Tz            | >500       | 200             |
| Fp            | >60        | 15–30           |

Pr, promazine; Md, methdilazine; Fz, fluphenazine; Tm, trimeprazine; Tf, trifluoperazine; Tp, triflupromazine; Tz, thioridazine; Fp, flupenthixol.

It is known that phenothiazines are concentrated by macrophages almost up to 100-fold of its original amount in a medium in which macrophages are maintained in the laboratory [22,23]. These increases of intracellular concentration take place in the lysosome [13,22,23] resulting in reaching the bactericidal level of the compound [22–24]. According to Amaral et al [17] a phenothiazine may promote loss of 55 KDa virulence protein and hence there is a great possibility that viable cells of salmonella lose their virulence inside the phagolysosome. Although a very large number of viable cells of S. enterica are retrieved from untreated animals 18h after challenge, there was always statistically significant reduction in the number of viable cells recovered from treated animals. From such data, however, a definite conclusion cannot be made regarding loss of virulence proteins in the phagocytosed salmonellae until the exact mechanism is unveiled and determined. Nevertheless, it is now known that a phenothiazine such as TZ affects the activity of genes that play a role in the survival of the Gram-negative bacterium [24,25]. The main genes affected by exposure to a phenothiazine such as thioridazine are those that code for plasma membrane based proteins that regulate the permeability of the cell envelope [25].

3. Antimicrobial Action Phenothiazine-Like Compounds from Plants

In a study of determination of antimicrobial potentiality of different plant extracts Dastidar et al. [26] observed that a prenylflavonone labeled as YS06 procured from the root of Sophora plant was active both in vitro and in vivo (Table 3). The in vitro MIC values were between 25 and 200 µg/mL level of the pure compound; it was bactericidal and could ably protect mice infected with S. enterica at doses of 40–80 µg/mouse. Such a phenomenon was further confirmed by determining reduction in the number of viable cells in mice receiving both prenylflavonone and the challenge when compared to the set of animals that were given the challenge only. Subsequently an isoflavonoid compound (YS19) derived from the same plant revealed that this was a bacteriostatic agent and could inhibit bacterial growth at 25–200 µg/mL level and successfully protected mice at an amount of 30–60 µg/mouse [27]. Subsequent animal experiments showed that much like YS06, YS19 could also reduce number of viable salmonellae in spleen, liver and heart blood of mice receiving both the agent and organism.
Table 3. Antibacterial action of plant derived compounds.

| Compound of plant origin          | MIC (µg/mL) | Type of action | Animal protection dose / mouse |
|-----------------------------------|-------------|----------------|-------------------------------|
| Prenylflavonone YS06              | 25–100      | 25–100         | Bactericidal                  | 40–80 µg |
| Isoflavonoid YS19                 | 25–200      | 25–200         | Bacteristatic                 | 30–60 µg |
| *Mesua ferrea* flower extract     | 50–100      | 50–100         | Bactericidal                  | 50–100 µg |
| Flavonone from *Butea frondosa* bark | 50–200    | 50–200         | Bacteristatic                 | 50–200 µg |

Mazumder et al. [28] observed that the flower extract of *M. ferrea* possessed potent *in vitro* bactericidal action on salmonellae, and that the extract was able to offer significant protection to mice challenged with virulent salmonellae. In 2008, Mishra et al. isolated a flavonone from the bark of *Butea frondosa* and detected powerful antibacterial action both *in vitro* and *in vivo*. Many other antimicrobial compounds have been isolated [29–33]. Thus microorganisms are not the only source of antibacterial agents like antibiotics, but various other studies further strengthen the possibilities of procuring and securing from many types of natural sources.

4. Special Aspects and Activities of Phenothiazines

The majority of medicinal compounds in use today owe their origin to a given phenothiazine [34]. This is not surprising since these compounds have activities on the plasma membrane of bacteria [35–37], protozoa [38], eukaryotes [39]; in short, all living cells. The following sections discuss specific aspects of phenothiazine activities inasmuch as these activities have potential for the development of new medicinal compounds for therapy of infections and cancers. The reader is encouraged to visit reference 32 for a comprehensive presentation of the evolution of phenothiazines as antimicrobial agents.

*Phenothiazines and the Plasma Membrane*

In general, phenothiazines are electron donors and bind by charge transfer complexes (CTC) formation to target molecules when an electron is supposed to go from the highest filled molecular orbital to the lowest empty orbital of the acceptor molecule on the target. When the phenothiazine acts as an electron donor at the surface of the plasma membrane of the cell or within the lipid bilayer of the plasma membrane, then the electron transfer on the outside will result in depolarization of the membrane. Because this depolarization reduces the activity of the plasma membrane (conductivity, etc.), the phenothiazine has been referred to as a membrane-stabilizing agent. However, when the phenothazine acts as an electron donor on the cytoplasmic side of the plasma membrane, hyperpolarization results and membrane-linked processes are inhibited. If the biological activity is actually due to charge transfer complex formation, we expect pharmacological activity from electron donation by the phenothiazine (there are some exceptions to this rule: CPZ- sulfon- or, sulphoxydes and methylene blue, where due to the asymmetric distribution of charge distribution main cause for ineffective activity). In general, one may say that the activity of the phenothiazine on the medial side of the plasma membrane is dependent upon a very high concentration of the compound. These concentrations are clinically irrelevant since they cannot be safely achieved in the patient but can be readily achieved *in vitro*. Therapeutically, a phenothiazine such as CPZ is administered at far lower...
concentrations that limit the activity of the agent to the surface of the plasma membrane (i.e., electron acceptor). It should be noted that a variety of agents can obviate the surface activity of CPZ such as caffeine [40]. In vitro caffeine forms precipitates with CPZ therefore reducing the neuroleptic effects of the agent. At the level of the plasma membrane they can disperse CPZ from its binding sites of the neuron hence patients who are managed with CPZ must take care not drink excessively caffeine rich liquids such as tea and coffee.

The main mechanism of action of most phenothiazines that have a variety of effects on the activity of the plasma membrane involves the inhibition of calcium binding to calcium dependent enzymes [41]. However, because of the differentiation of cells, the constituents on the surface of the plasma membrane determine whether a specific phenothiazine will have activity on that given cell type [42]. This means that various members of the phenothiazine group may present with specific activities; e.g., neuroleptics chlorpromazine and flupentazine and the phenothiazine derived antihistamines methdilazine and trimeprazine the tranquilliser promethazine. Nevertheless, although the major mechanisms may differ, whenever studied, most phenothiazines have activity against bacteria albeit at in vitro concentrations which are clinically irrelevant.

Among the activities reported for phenothiazines are those that affect the activity of efflux pumps of bacteria, mycobacteria and cancer cells that express a multi-drug resistant phenotype [43]. Efflux pumps extrude noxious agents that penetrate into the cell and therefore afford protection from those agents. To the bacterium or cancer cell, antibiotics and anticancer agents are noxious agents that must be expelled prior to reaching their intended targets. Although all living cells have these efflux pumps at a basal level, they can be rapidly over-expressed when the concentration of an agent is increased [44–48]. Moreover, other proteins that regulate permeability of the cell envelope such as porins, are down-regulated [45,46].

With respect to bacteria, serial exposure to increasing concentrations of an antibiotic results in progressive increases in resistance to the given antibiotic. Serial exposure of pansusceptible Mycobacterium tuberculosis to progressive increases of isoniazid (INH) increases resistance to the drug [49]. Similar exposure of antibiotic susceptible Escherichia coli to increasing concentrations of tetracycline promote progressive increases of resistance to the antibiotic that is accompanied by increased expression of genes that regulate and code for various efflux pumps of the organism [50]. If at any one point during the latter study the last concentration of tetracycline is serially maintained, further increases in the expression of efflux pump genes takes place and accompanied with accumulation of mutations in genes that code for proteins sensitive to beta-lactams, streptomycin and gyrase A. As prolonged exposure to a constant concentration of tetracycline, the expression of efflux pump genes is reduced to base-line levels [51,52]. These results have been interpreted to indicate that the organism follows the 2nd law of thermodynamics inasmuch as the energy needed for maintenance of an over-expressed efflux pump system is great, and given the unchanging environment containing a high level of the noxious agent (antibiotic), it can conserve energy by activating a mutator gene that promotes mutations in essential proteins, as predicted by Chopra et al. [53]. In all studies so far conducted, including those involving other Gram-negatives [25] and Gram-positives [54–56] and mycobacteria [49,57–59] phenothiazines such as chlorpromazine and thioridazine reverse the antibiotic induced resistance.
The mechanism by which a phenothiazine reverses efflux pump mediated resistance to an antibiotic appears to be indirect. Firstly, depending on the environmental pH, the source of energy that drives the efflux pump differs. At pH lower than 7, the phenothiazine does not inhibit the efflux of a noxious agent whereas at pH above 7, inhibition of efflux results from exposure to a concentration of the phenothiazine that is devoid of antibacterial activity [60]. These results are interpreted to indicate the possibility that the phenothiazine inhibits the generation of hydronium ions from the hydrolysis of ATP by ATP synthase activity, and therefore, the maintenance of the proton motive force is affected. Because at low pH of the environment, the hydronium ions that are bound at the surface of the cell envelop [61,62] create the proton motive force, the needed energy for efflux is independent of metabolism and therefore not affected by then phenothiazine. Lastly, phenothiazines are well known inhibitors of the proton motive force at pH ca. 7 [63,64], therefore the interpretation of the pH dependent effects of the phenothiazine on the efflux pump of bacteria receives support.

5. Therapy of MDR/XDR/TDR TB

Since the 1950s, as a consequence of extensive use of chlorpromazine for therapy of psychosis, sporadic reports appeared suggesting that this neuroleptic could cure a pulmonary tuberculosis infection [8]. However, it was the advent of multi-drug resistance world-wide during the late 1980’s that the use of chlorpromazine for therapy of tuberculosis was seriously considered and immediately dismissed due to the severe toxicity produced by this neuroleptic. Moreover, the concentrations of chlorpromazine needed were in the range of 15 to 30 mg/L, and this was far greater than that which could be safely achieved in the patient (maximum plasma concentration clinically achieved safely is ca. 0.5 mg/L). Nevertheless, interest in chlorpromazine as an anti-tubercular drug continued and when Crowle and his group [65] showed that clinically relevant concentrations of chlorpromazine in the medium could promote the killing of intracellular *Mycobacterium tuberculosis* [65], interest in this agent was increased. Soon thereafter, the milder neuroleptic thioridazine was shown to have activity against all encountered antibiotic resistant strains of *Mycobacterium tuberculosis* (mono-resistant; multi-drug resistant and extensively drug resistant strains of *Mycobacterium tuberculosis*) [66]. Later studies demonstrated that thioridazine promoted the killing of intracellular multi-drug resistant [67] and extensively drug resistant strains [68] of *Mycobacterium tuberculosis* and could cure the mouse infected with antibiotic susceptible [69] and multi-drug resistant [70] strains of *Mycobacterium tuberculosis*.

These studies laid down the foundation for the first demonstration that thioridazine in combination with antibiotics to which the *Mycobacterium tuberculosis* was initially resistant, could cure rather quickly, patients infected with extensively drug resistant *Mycobacterium tuberculosis* [71]. The mechanism by which these cures have been achieved involves the activation of lysosomal hydrolases resulting from the inhibition of potassium efflux from this organelle [72–74] and by inhibition of the source of energy needed for adequate function of the efflux pump system that afforded a multi-drug resistant phenotype of the infecting organism [72–74]. It should also be mentioned that the use of thioridazine as monotherapy of the extensively drug resistant tuberculosis patient results in rapid improvement in the quality of life in that the patients regain their appetite, put on weight, night sweats are reduced and even obviated, and because of the neuroleptic activity of thioridazine, stress that results from this infection is markedly reduced [75]. These latter studies have been expanded by
Utwadia et al. [76], and as a result, thioridazine has been recommended for use as a “salvage drug” for therapy of the extensively drug resistant TB patient [75].

6. Concluding Remarks

Antipsychotics block D2 receptors in the dopamine pathway of brain such that dopamine released in this pathway has a lesser effect. The tricyclic compound phenothiazines are used as antidepressant and anxiolytic and antipsychotic agents. They accumulate in the brain provoking blockade of dopamine receptors inasmuch as excess release of dopamine in the mesolimbic pathway has been linked to psychotic experiences. High potency antipsychotic drug like haloperidol can be applied in doses of a few milligrams causing sleepiness and a calming effect in patients within minutes, while low potency antipsychotics like CPZ or TZ require doses of several hundred milligrams to produce the same action. These have a much greater anticholinergic and antihistaminic actions that can counteract dopamine related side effects. Most of the antimicrobial phenothiazines are of this order.

Phenothiazines have their primary effects on the plasma membranes of prokaryotes and eukaryotes. Among the components of the prokaryotic plasma membrane affected are efflux pumps, their energy sources, energy providing enzymes, such as ATPase and genes that regulate and code for the permeability aspect of a bacterium. The response of multidrug and extensively drug resistant tuberculosis to phenothiazines shows an alternative therapy for treatment of these dreaded disease that is claiming more and more lives every year throughout the world. Many phenothiazines have shown synergistic activity with several antibiotics thereby lowering the doses of antibiotics administered to patients suffering from specific bacterial infections. Trimeprazine is synergistic with trimethoprim [77]. Fp has been found to be synergistic with penicillin [78] and CPZ plus some antibiotics are also synergistic [16].

Along with antibacterial action described in this review, many phenothiazines possess plasmid curing activities, which render the bacterial carrier of the plasmid sensitive to antibiotics [55,77–81]. Thus simultaneous applications of a phenothiazine like TZ would not only act as an additional antibacterial agent but also would help to eliminate drug resistant plasmid from the infectious bacterial cells.

References and Notes

1. Falagas, M.E.; Bliziotis, I.A. Pandrug-resistant Gram-negative bacteria: The dawn of the post-antibiotic era. *Int. J. Antimicrob. Agents* 2007, 29, 630–636.
2. Amaral, L.; Udwadia, Z.F.; van Soolingen, D. A cheap and effective anti-Mdr/Xdr/Tdr Tb drug is already available. *Biochem. Pharmacol.* J. 2012, doi:10.4172/2167-0501.1000e137.
3. Amaral, L.; Molnar, J. Why and how the old neuroleptic thioridazine cures the XDR-TB patient. *Pharmaceuticals* 2012, 5, 1021–1031.
4. Amaral, L.; Udwadia, Z.; Abbate, E.; van Soolingen, D. The added effect of Thioridazine in treatment of resistant TB. *Int. J. Tuberc. Lung Dis.* 2012, 16, 1706–1708.
5. Chopra, I.; Schofield, C.; Everett, M.; O’Neill, A.; Miller, K.; Wilcox, M.; Frère, J.-M.; Dawson, M.; Czaplewski, L.; Urleb, U.; et al. Treatment of health-careassociated infections caused by Gram-negative bacteria: A consensus statement. *Lancet Infect. Dis.* 2008, 8, 133–139.
6. Kristiansen, J.E. The antimicrobial activity of non-antibiotics. Report from a congress on the antimicrobial effect of drugs other than antibiotics on bacteria, viruses, protozoa, and other organisms. *APMIS Suppl.* 1992, 30, 7–14.

7. Guttmann, P.; Ehrlich, P. Ueber die wirkung des methylenblau bei malaria. *Berliner Klinische Wochenschrift* 1891, 39, 953–956.

8. Kristiansen, J.E.; Amaral, L. The potential management of resistant infections with non-antibiotics. *J. Antimicrob. Chemother.* 1997, 40, 319–327.

9. Dastidar, S.G.; Saha, P.K.; Sanyamat, B.; Chakrabarty, A.N. Antibacterial activities of ambodryl and benadryl. *J. Appl. Bact.* 1976, 41, 209–214.

10. Annadurai, S.; Basu, S.; Ray, S.; Dastidar, S.G.; Chakrabarty, A.N. Antimicrobial activity of the antiflammatory agent diclofenac sodium. *Indian J. Exp. Biol.* 1998, 36, 86–90.

11. Kumar, K.A.; Ganguly, K.; Mazumdar, K.; Dutta, N.K.; Dastidar, S.G.; Chakrabarty, A.N. Amlodipine: A cardiovascular drug with powerful antimicrobial property. *Acta Microbiol. Pol.* 2003, 52, 285–292.

12. Mazumdar, K.; Ganguly, K.; Kumar, K.A.; Dutta, N.K.; Chakrabarty, A.N.; Dastidar, S.G. Antimicrobial potentiality of a new non-antibiotic: The cardiovascular drug oxyfedrine hydrochloride. *Microbiol. Res.* 2003, 158, 259–264.

13. Dasgupta, A.; Dastidar, S.G.; Shiratki, Y.; Motohashi, N. Antibacterial Activity of Artificial Phenothiazines and Isoflavones from Plants. In *Bioactive Heterocycles VI*; Springer: Berlin/Heidelberg, Germany, 2008; Volume 15, pp. 67–132.

14. Kristiansen, J.E. Experiments to illustrate the effect of chlorpromazine on the permeability of the bacterial cell wall. *Acta Pathol. Microbiol. Scand. B* 1979, 87, 317–319.

15. Galeazzi, L.; Turchetti, G.; Grilli, G.; Groppa, G.; Giunta, S. Chlorpromazine as permeabilizer and reagent for detection of microbial peroxidase and peroxidaselike activities. *Appl. Environ. Microbiol.* 1986, 52, 1433–1435.

16. Amaral, L.; Lorian, V. Effects of chlorpromazine on the cell envelope proteins of *Echerichia coli*. *Antimicr. Agents Chemother.* 1991, 35, 1923–1924.

17. Amaral, L.; Kristiansen, J.E.; Thomsen, V.F.; Markowich, B. The effect of chlorpromazine on the outer cell wall constituents of *Salmonella typhimurium* ensuring resistance to the drug. *Int. J. Antimicr. Agents* 2000, 14, 225–229.

18. Radhakrishnan, V.; Ganguly, K.; Ganguly, M.; Dastidar, S.G.; Chakrabarty, A.N. Potentiality of tricyclic compound thioridazine as an effective antibacterial and antiplasmid agent. *Indian J. Exp. Biol.* 1999, 37, 671–675.

19. Page, A.M.; Lagnado, J.R. Effects of phenothiazine neuroleptic drugs on the microtubular-membrane complex in bloodstream forms of *Trypanosoma brucei*. *Parasitology* 1995, 111, 493–504.

20. Bourlioux, P.; Moreaux, J.M.; Su, W.J.; Boureu, H. *In vitro* antimicrobial activity of 18 phenothiazine derivatives: Structure-activity relationship. *APMIS Suppl.* 1992, 30, 40–43.

21. Mitruka, B.M., Rawnsle, H.M., Vadehra, D.V., Eds. *Animal of Medical Research*; John Wiley & Sons, Inc.: NY, NY, USA, 1976; Volume 301, pp. 145–150.

22. Ordway, D.; Viveiros, M.; Leandro, C.; Arroz, M.J.; Amaral, L. Intracellular activity of clinical concentrations of phenothiazines including thioridazine against phagocyted *Staphylococcus aureus*. *Int. J. Antimicr. Agents* 2001, 20, 34–43.
23. Martins, M.; Dastidar, S.G.; Fanning, S.; Kristiansen, J.E.; Molnar, J.; Pagës, J.M.; Schelz, Z.; Spengler, G.; Viveiros, M.; Amaral, L. Potential role of non-antibiotics (helper compounds) in the treatment of multi-drug resistant Gram negative infections: Mechanisms for their direct and indirect activities. *Int. J. Antimicrob. Agents* **2008**, *31*, 198–208.

24. Dutta, N.K.; Mazumdar, K.; Dastidar, S.G.; Amaral, L. New Patentable use of an old neuroleptic compound thioridazine to combat against tuberculosis: Gene regulation perspective. *Recent Pat. Anti-Infect. Drug Discov.* **2011**, *6*, 128–138.

25. Spengler, G.; Rodrigues, L.; Martins, M.; McCusker, M.; Cerca, P.; Machado, L.; Costa, S.S.; Ntokoue, E.; Couto, I.; Viveiros, M.; *et al.* Genetic Response of *Salmonella enterica* serovar Typhimurium to Thioridazine rendering the organism resistant to the agent. *Int. J. Antimicrob. Agents* **2012**, *39*, 16–21.

26. Dastidar, S.G.; Mahapatra, S.K.; Ganguly, K.; Chakrabarty, A.N. Antimicrobial activity of prenylflavanones. *In Vivo* **2001**, *15*, 519–524.

27. Dastidar, S.G.; Manna, A.; Kumar, K.A.; Mazumdar, K.; Dutta, N.K.; Chakrabarty, A.N.; Motohashi, N.; Shirataki, Y. Studies on the antibacterial potentiality of isoflavones. *Int. J. Antimicrob. Agents* **2004**, *23*, 99–102.

28. Mazumder, R.; Dastidar, S.G.; Basu, S.P.; Mazumder, A. Effect of *Mesua ferrea* Linn. flower extract on *Salmonella*. *Indian J. Exp. Biol.* **2005**, *43*, 566–568.

29. Ramalhete, C.; Spengler, G.; Mulhovo, S.; Costa, S.; Couto, I.; Viveiros, M.; Ferreira, M.J.U.; Amaral, L. Inhibition of efflux pumps of methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis* resistant strains by triterpenoids from *Momordica balsamina*. *Int. J. Antimicrob. Agents* **2011**, *77*, 70–74.

30. Martins, A.; Vasas, A.; Viveiros, M.; Molnar, J.; Hohmann, J.; Amaral, L. Antibacterial properties of compounds isolated from *Carpobrotus edulis*. *Int. J. Antimicrob. Agents* **2011**, *37*, 438–444.

31. Ramalhete, C.; Spengler, G.; Serly, J.; Amaral, L.; Molnar, J.; Mulhovo, S.; Ferreira, M.J.U. Efflux modulators from *Momordica balsamina* L. in multidrug resistant bacterial strains. *Planta Med.* **2009**, *75*, 896–896.

32. Duarte, N.; Ferreira, M.J.; Martins, M.; Viveiros, M.; Amaral, L. Antibacterial activity of ergosterol peroxide against *Mycobacterium tuberculosis*: Dependence upon system and medium employed. *Phytother. Res.* **2007**, *21*, 601–604.

33. Lorenzi, V.; Muselli, A.; Bernardini, A.F.; Berti, L.; Pagës, J.M.; Amaral, L.; Bolla, J.M. *Helichrysum italicum* essential oil contains compounds that restore chloramphenicol activity on multi-drug resistant isolates from Gram-negative species. *Antimicrob. Agents Chemother.* **2008**, *53*, 2209–2211.

34. Wainwright, M.; Amaral, L.; Kristiansen, J.E. The Evolution of Antimycobacterial Agents from Non-Antibiotics. *Open J. Pharmacol.* **2012**, *2*, e1.

35. Pluta, K.; Morak-Mlodawska, B.; Jeleñ, M. Recent progress in biological activities of synthesized phenothiazines. *Eur. J. Med. Chem.* **2011**, *46*, 3179–3189.

36. Kristiansen, J.E.; Hendricks, O.; Delvin, T.; Butterworth, T.S.; Aagaard, L.; Christensen, J.B.; Flores, V.C.; Keyzer, H. Reversal of resistance in microorganisms by help of non-antibiotics. *J. Antimicrob. Chemother.* **2007**, *59*, 1271–1279.
37. Harris, F.; Chatfield, L.K.; Phoenix, D.A. Phenothiazinium based photosensitisers-photodynamic agents with a multiplicity of cellular targets and clinical applications. *Curr. Drug Targets* 2005, 6, 615–627.
38. Lala, A.K. Fluorescent and photoactivable probes in depth-dependent analysis of membranes. *Chem. Phys. Lipids* 2002, 116, 177–188.
39. Michalak, K.; Wesolowska, O.; Motohashi, N.; Molnar, J.; Hendrich, A.B. Interactions of phenothiazines with lipid bilayer and their role in multidrug resistance reversal. *Curr. Drug Targets* 2006, 7, 1095–1105.
40. Cheeseman, H.J.; Neal, M.J. Interaction of chlorpromazine with tea and coffee. *Br. J. Clin. Pharmacol.* 1981, 12, 165–169.
41. Weiss, B.; Prozialeck, W.; Cimino, M.; Barnette, M.S.; Wallace, T.L. Pharmacological regulation of calmodulin. *Ann. NY Acad. Sci.* 1980, 356, 319–345.
42. Davidoff, R.A. Antispasticity drugs: Mechanisms of action. *Ann. Neurol.* 1985, 17, 107–116.
43. Amaral, L.; Spengler, G.; Martins, A.; Armada, A.; Handzlik, J.; Kiec-Kononowicz, K.; Molnar, J. Inhibitors of bacterial efflux pumps that also inhibit efflux pumps of cancer cells. *Anticancer Res.* 2012, 32, 2947–2957.
44. Vila, J.; Martínez, J.L. Clinical impact of the over-expression of efflux pump in nonfermentative Gram-negative bacilli, development of efflux pump inhibitors. *Curr. Drug Targets* 2008, 9, 797–807.
45. Pagès, J.M.; Amaral, L. Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. *Biochim. Biophys. Acta* 2009, 1794, 826–833.
46. Amaral, L.; Fanning, S.; Pagès, J.M. Efflux pumps of gram-negative bacteria: Genetic responses to stress and the modulation of their activity by pH, inhibitors, and phenothiazines. *Adv. Enzymol. Relat. Areas Mol. Biol.* 2011, 77, 61–108.
47. Spengler, G.; Molnar, J.; Amaral, L. Thioridazine induces apoptosis of multidrug-resistant mouse lymphoma cells transfected with the human ABCB1 and inhibits the expression of P-glycoprotein. *Anticancer Res.* 2011, 31, 4201–4205.
48. Spengler, G.; Handzlik, J.; Ocsovszki, I.; Viveiros, M.; Kiec-Kononowicz, K.; Molnar, J.; Amaral, L. Modulation of multidrug efflux pump activity by new hydantoin derivatives on colon adenocarcinoma cells without inducing apoptosis. *Anticancer Res.* 2011, 31, 3285–3288.
49. Viveiros, M.; Portugal, I.; Bettencourt, R.; Victor, T.C.; Jordaan, A.M.; Leandro, C.; Ordway, D.; Amaral, L. Isoniazid-induced transient high-level resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 2002, 46, 2804–2810.
50. Viveiros, M.; Jesus, A.; Brito, M.; Leandro, C.; Martins, M.; Ordway, D.; Molnar, A.M.; Molnar, J.; Amaral, L. Inducement and reversal of tetracycline resistance in Escherichia coli K-12 and the expression of proton gradient dependent multidrug efflux pump genes. *Antimicrob. Agents Chemother.* 2005, 49, 3578–3582.
51. Martins, A.; Spengler, G.; Rodrigues, L.; Viveiros, M.; Ramos, J.; Martins, M.; Couto, I.; Fanning, S.; Pages, J.M.; Bolla, J.M.; et al. AcrAB mediated MDR phenotype is maintained after efflux pump genes and their regulators have restored wild type activities. *Int. J. Antimicrob. Agents* 2009, 34, 602–604.
52. Martins, A.; Spengler, G.; Molnar, J.; Amaral, L. Sequential responses of bacteria to noxious agents (antibiotics) leading to accumulation of mutations and permanent resistance. *Biochem. Pharmacol.* **2012**, *1*, doi:10.4172/2167-0501.1000104.

53. Chopra, I.; O'Neill, A.J.; Miller, K. The role of mutators in the emergence of antibiotic-resistant bacteria. *Drug Resist. Updat.* **2003**, *6*, 137–145.

54. Costa, S.S.; Falcão, C.; Viveiros, M.; Machado, D.; Martins, M.; Melo-Cristino, J.; Amaral, L.; Couto, I. Exploring the contribution of efflux on the resistance to fluoroquinolones in clinical isolates of *Staphylococcus aureus*. *BMC Microbiol.* **2011**, *11*, doi:10.1186/1471-2180-11-241.

55. Costa, S.S.; Ntokou, E.; Martins, A.; Viveiros, M.; Pournaras, S.; Couto, I.; Amaral, L. Identification of the plasmid-encoded qacA efflux pump gene in meticillin-resistant *Staphylococcus aureus* (MRSA) strain HPV107, a representative of the MRSA Iberian clone. *Int. J. Antimicrob. Agents* **2010**, *36*, 557–561.

56. Spengler, G.; Martins, A.; Schelz, Z.; Rodrigues, L.; Aagaard, L.; Martins, M.; Costa, S.S.; Couto, I.; Viveiros, M.; Fanning, S.; *et al.* Characterization of intrinsic efflux activity of *Enterococcus faecalis* ATCC29212 by a semi-automated ethidium bromide method. *In Vivo* **2009**, *23*, 81–87.

57. Viveiros, M.; Martins, M.; Rodrigues, L.; Machado, D.; Couto, I.; Ainsa, J.; Amaral, L. Inhibitors of mycobacterial efflux pumps as potential boosters for TB drugs. *Expert Rev. Anti-Infect. Ther.* **2012**, *10*, 983–998.

58. Machado, D.; Couto, I.; Perdigão, J.; Rodrigues, L.; Portugal, I.; Baptista, P.; Veigas, B.; Amaral, L.; Viveiros, M. Contribution of efflux to the emergence of isoniazid and multidrug resistance in *Mycobacterium tuberculosis*. *PLoS One* **2012**, *7*, e34538.

59. Rodrigues, L.; Machado, D.; Couto, I.; Amaral, L.; Viveiros, M. Contribution of efflux activity to isoniazid resistance in the *Mycobacterium tuberculosis* complex. *Infect. Genet. Evol.* **2012**, *12*, 695–700.

60. Amaral, L.; Cerca, P.; Spengler, G.; Machado, L.; Couto, I.; Viveiros, M.; Fanning, S.; Pagès, J.-M. Ethidium bromide efflux by salmonella: Modulation by metabolic energy, pH, ions and phenothiazines. *Int. J. Antimicrob. Agents* **2011**, *38*, 140–145.

61. Mulkidjianian, A.Y.; Cherepanov, D.A.; Heberle, J.; Junge, W. Proton transfer dynamics at membrane/water interface and mechanism of biological energy conversion. *Biochemistry (Moscow)* **2005**, *70*, 251–256.

62. Mulkidjianian, A.Y.; Heberle, J.; Cherepanov, D.A. Protons @ interfaces: Implications for biological energy conversion. *Biochim. Biophys. Acta* **2006**, *1757*, 913–930.

63. Varga, Z.G.; Armada, A.; Cerca, P.; Amaral, L.; Mior Ahmad Subki, M.A.; Savka, M.A.; Szegedi, E.; Kawase, M.; Motoshashi, N.; Molnár, J. Inhibition of quorum sensing and efflux pump system by trifluoromethyl ketone proton pump inhibitors. *In Vivo* **2012**, *26*, 277–285.

64. Ren, J.K.; Petőfi, S.; Molnár, J. Mechanisms of antimotility action of tricyclic compounds in *Proteus vulgaris*. *Acta Microbiol. Hung.* **1993**, *40*, 369–377.

65. Crowle, A.J.; Douvas, G.S.; May, M.H. Chlorpromazine: A drug potentially useful for treating mycobacteria infections. *Chemotherapy* **1992**, *38*, 410–419.
66. Amaral, L.; Kristiansen, J.E.; Abebe, L.S.; Millet, W. Inhibition of the respiration of multi-drug resistant clinical isolates of *Mycobacterium tuberculosis* by Thioridazine: Potential use for the initial therapy of freshly diagnosed tuberculosis. *J. Antimicrob. Chemother.* **1996**, *38*, 1049–1053.

67. Ordway, D.; Viveiros, M.; Leandro, C.; Amaral, L. Clinical concentrations of Thioridazine kill intracellular Multi-drug resistant *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* **2003**, *47*, 917–922.

68. Martins, M.; Viveiros, M.; Amaral, L. Sila Compound 421, an Inhibitor of efflux pumps of cancer cells, enhances the killing of intracellular XDRTB. *Int. J. Antimicrob. Agents* **2008**, *33*, 479–482.

69. Martins, M.; Viveiros, M.; Amaral, L. The curative activity of thioridazine on mice infected with *Mycobacterium tuberculosis*. *In Vivo* **2007**, *21*, 771–776.

70. van Soolingen, D.; Pando, R.H.; Orozco, H.; Aguilar, D.; Magis, C.; van Ingen, J.; Amaral, L.; Boeree, M. Thioridazine shows promising activity in a murine model of multi-drug resistant tuberculosis. *PLoS One* **2010**, *5*, e12640.

71. Abbate, E.; Vescovo, M.; Natiello, M.; Cufré, M.; García, A.; Gonzalez Montaner, P.; Ambroggi, M.; Ritacco, V.; van Soolingen, D. Successful alternative treatment of extensively drug-resistant tuberculosis in Argentina with a combination of linezolid, moxifloxacin and thioridazine. *J. Antimicrob. Chemother.* **2012**, *67*, 473–477.

72. Amaral, L. Totally Drug Resistant Tuberculosis can be Treated with Thioridazine in Combination with Antibiotics to which the Patient was Initially Resistant. *Biochem. Pharmacol.* **2012**, doi:10.4172/bcpc.1000e102.

73. Amaral, L.; Molnar, J. Potential therapy of multidrug-resistant and extremely drug-resistant tuberculosis with thioridazine. *In Vivo* **2012**, *26*, 231–236.

74. Radhakrishnan, V.; Ganguly, K.; Kawase, M.; Motohashi, N.; Molnár, J.; Viveiros, M.; Amaral, L. Synergistic interaction between proton pump inhibitors and resistance modifiers: Promoting effects of antibiotics and plasmid curing. *In Vivo* **2006**, *20*, 367–372.

75. Spengler, G.; Miczák, A.; Hajdú, E.; Kawase, M.; Amaral, L.; Molnár, J. Enhancement of plasmid curing by 9-aminoacridine and two phenothiazines in the presence of proton pump inhibitor 1-(2-benzoazoxazolyl)-3,3,3-trifluoro-2-propanone. *Int. J. Antimicrob. Agents* **2003**, *22*, 223–227.

76. Evdokimova, O.V.; Smirnov, I.V.; Artem'eva, N.A.; Rozhkova, E.A. Effect of promethazine hydrochloride (pipolphen) on the stability of R plasmid resistance in *Escherichia coli* (in Russian). *Antibiot Khimioter* **1997**, *42*, 8–11.
81. Molnár, J.; Gálfi, M.; Lózsa, A.; Nakamura, M.J. Inhibition of bacterial plasmid replication by stereoselective binding by tricyclic psychopharmacons. *Res. Commun. Chem. Pathol. Pharmacol.* **1984**, *43*, 235–249.

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