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Synthesis and antitubercular activity of quaternized promazine and promethazine derivatives

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Abstract—Quaternized chlorpromazine, triflupromazine, and promethazine derivatives were synthesized and examined as antitubercular agents against both actively growing and non-replicating *Mycobacterium tuberculosis* H37Rv. Impressively, several compounds inhibited non-replicating *M. tuberculosis* at concentrations equal to or double their MICs against the actively growing strain. All active compounds were non-toxic toward Vero cells (IC_{50} > 128 μM). *N*-Allylchlorpromazinium bromide was only weakly antitubercular, but replacing allyl with benzyl or substituted benzyl improved potency. An electron-withdrawing substituent on the phenothiazine ring was also essential. Branching at the carbon chain decreased antitubercular activity. The optimum antitubercular structures possessed *N*-(4- or 3-chlorobenzyl) substitution on triflupromazine.

Tuberculosis (TB), the disease caused by *Mycobacterium tuberculosis (Mtb)*, infects approximately two billion people. The World Health Organization estimates that about two million people die each year from TB due to the lack of and inability to afford proper health care.[1] Overcrowding and ill-nourishment of poor people living in large cities leads to a high incidence of the disease due to the ease at which the infection can be transferred.[2] This situation contributes to the accelerated speed at which TB spreads in underdeveloped countries. There is also an alarming increase in cases of TB caused by multidrug-resistant strains of *M. tuberculosis* (*Mtb*), due in part to inadequate drug therapy as a result of incorrectly selected medications or suboptimal drug dosing.[3] Thus, there is a need for new drugs targeting enzymes essential to mycobacterial survival. One such target is type II NADH-menaquinone dehydrogenase (ndh-2). By inhibiting ndh-2, the electron transport chain in *Mtb* becomes blocked and shuts down. Ndh-2 is the only NADH dehydrogenase enzyme expressed in *Mtb* and is thus vital to its survival.[4] Ndh-2 is also found in a number of other bacteria such as *Staphylococcus aureus* and *Enterococcus faecalis* but is not expressed in humans.[5]

Humans rely only on type I NADH dehydrogenase (ndh-1) and thus minimal toxicity in humans is predicted with ndh-2 inhibitors. In the 1950s and 1960s psychiatrists noticed a more pronounced inhibition of *Mtb* occurring in TB-infected, schizophrenic patients taking higher doses of an antipsychotic drug, chlorpromazine, as opposed to patients who were taking lower doses of this drug.[6] Later, Weinstein et al. discovered that *N*-(benzyl)-chlorpromazinium inhibited *Mtb* in vitro at an even lower concentration than chlorpromazine itself.[4] This was the first quaternized promazine derivative (QPD) discovered to be an antibacterial agent against *Mtb*. Chlorpromazine and its QPD were both found to selectively inhibit ndh-2; there was no inhibition of ndh-1.[4,7] Other phenothiazines were also described to have antitubercular activity.[8,9] In order to establish a preliminary SAR for QPDs, we decided to synthesize

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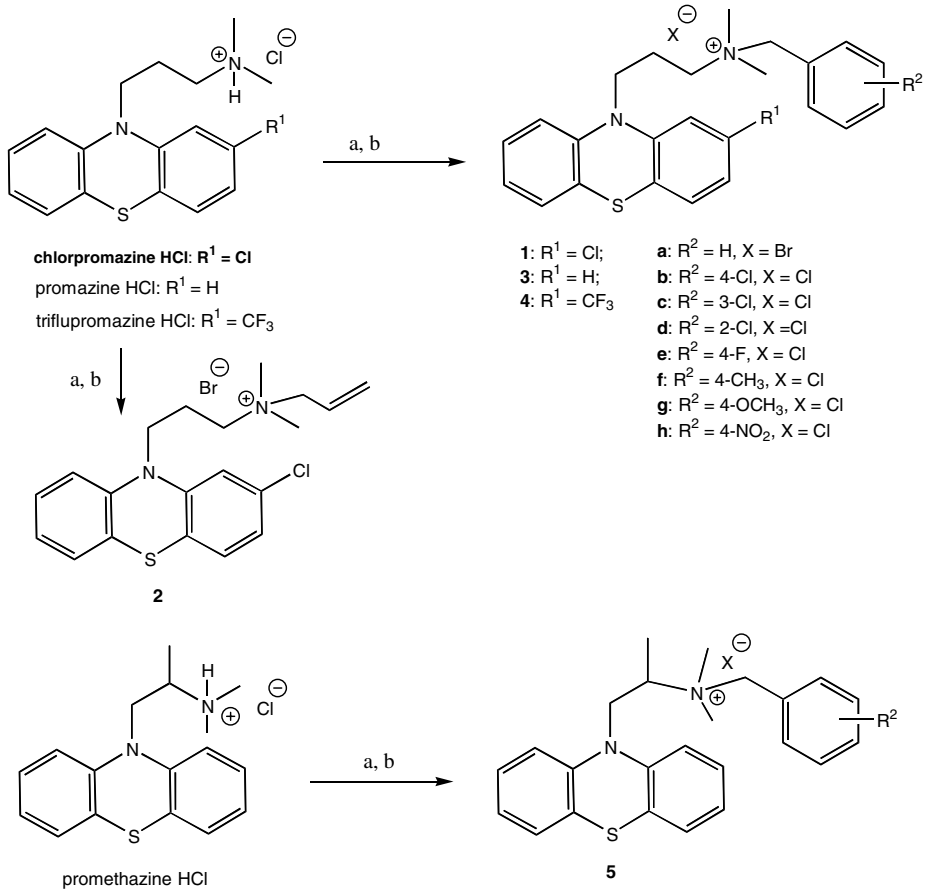

Scheme 1. Reagents: (a) potassium carbonate, water, ethyl acetate; (b) benzyl or allyl halide, acetone.

quaternized derivatives of promazine, chlorpromazine, and trifluprom azine, and to measure their MICs against both actively growing and non-replicating *Mtb*.

Table 1. QPD antitubercular activity

Compound (R^2)	MIC (SD) in μM versus *M. tuberculosis* that is	
	actively growing	non-replicating
1a (H)	7.33(0.4)	11.1(2.4)
1b (4-Cl)	6.06(1.9)	6.7(0.9)
1c (3-Cl)	4.5(1.3)	6.7(0.4)
1d (2-Cl)	5.6(1.8)	7.6(0.3)
1e (4-F)	7.6(0.2)	13.7(0.9)
1f (4-CH_3)	4.7(1.0)	7.5(0.3)
1g (4-OCH_3)	8.5(1.3)	11.9(1.2)
1h (4-NO_2)	12.3(4.0)	27.5(1.6)
2	30.6	105.9
3b (4-Cl)	9.31(2.2)	15.4(3.2)
3c (3-Cl)	7.5(0.3)	13.0(0.6)
3d (2-Cl)	14.3	24.6
3e (4-F)	14.6	32.9
3f (4-CH_3)	9.9(2.0)	15.0(0.2)
4b (4-Cl)	3.81(0.1)	6.1(0.4)
4c (3-Cl)	3.8(0.1)	5.8(0.9)
4d (2-Cl)	7.3(0.3)	7.5(0.2)
4e (4-F)	6.4(2.2)	10.0(1.5)
4f (4-CH_3)	6.8(0.2)	6.9(0.6)
5a (H)	31.6	99.7
5f (4-CH_3)	12.1	34.7
5g (4-OCH_3)	14.4	105.2
5h (4-NO_2)	20.7	>128.0

The syntheses of the quaternized promazine and promethazine derivatives were based on the procedure described by Kahn et al. (Scheme 1).[10]

1.00 gram of promazine, chlorpromazine, or trifluprom azine hydrochloride was dissolved in water and then potassium carbonate was added to raise the pH to 10. The aqueous mixture was subsequently extracted with ethyl acetate. The combined organic extracts were dried with sodium sulfate, gravity filtered, and the solvent was removed in vacuo. The products obtained from these reactions were viscous oils. The viscous oils were dissolved in acetone and reacted with 1 equiv of each alkyl halide. The reaction vials were sealed and left to run overnight. Ethyl ether was then used to precipitate our final products out of solution. Those QPDs creating gums were triturated with hexanes. The purified products of these reactions were generally white crystalline solids, with the exception of **1h** and **5h**, which were slightly yellow. Yields ranged from 40% to 94%. ^{1}H NMR, ^{13}C NMR, and HMBC spectra, along with elemental analysis, confirmed the structures and purity of our final products.

The minimum inhibitory concentration (MIC) of QPDs against actively growing *M. tuberculosis* H_{37}Rv was determined using the microplate alamar blue assay (MABA)[11,12] and was defined as the lowest concentration effecting a reduction in fluorescence of $\geqslant$90% relative to drug-free controls. Compounds for

which an initial screening revealed an MIC of 10 μM or less were subsequently tested in triplicate. Activity against non-replicating *M. tuberculosis* was determined by a 10-day anaerobic exposure of a low oxygen-adapted culture containing the *Vibrio harveyii* luciferase gene to QPDs. After a subsequent 28-h aerobic "recovery", the ability to make a luminescent signal was determined following the addition of the *n*-decanal substrate.[13,14] The LORA MIC was defined as the lowest concentration effecting a reduction of $\geqslant$90% luminescence relative to drug-free controls. The activities of three compounds (**1c**, **3c**, and **4c**) were confirmed for both MABA and LORA using a colony-forming unit determination by subculturing from the microplate onto drug-free 7H11 agar. MICs for actively growing *M. tuberculosis* were 1.9-, 2.1-, and 2.9-fold higher than the MABA MICs, and 1.7-, 1.9-, and 2.1-fold higher than the corresponding LORA MICs.

The in vitro cytotoxicity for VERO cells was determined for all compounds with a MABA MIC of less than 10 μM using a dye reduction assay following 3 days exposure to test compounds as previously described.[12] The IC_{50} of all compounds was >128 μM (except for **3b** which was >64 μM, the highest concentration tested).

From the MICs in Table 1, *N*-benzyl substitution in QPDs is a requirement for significant antitubercular activity (**1a** vs. **2**). Alkyl chain branching (**5a**, **5f**–**h**) decreases potency. Three of the QPDs having MICs *both* <4 μM against actively growing *Mtb* and <8 μM against non-replicating *Mtb* possess *N*-(4- or 3-chlorobenzyl) groups and electron-withdrawing substituents on the phenothiazine ring (**1c**, **4b**–**c**). Based on this MIC data and the lack of in vitro mammalian cell toxicity, we will attempt to improve these leads by studying the antitubercular potency of other electron-withdrawing substituents on the phenothiazine ring and various halogen substitutions on the benzyl group. If these improved leads also lack mammalian cell toxicity, animal studies will be warranted.

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