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Synthesis and antitubercular activity of phenothiazines with reduced binding to dopamine and serotonin receptors

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Abstract—Analogs of the psychotropic phenothiazines were synthesized and examined as antitubercular agents against *Mycobacterium tuberculosis* H37Rv. The compounds were subsequently counter-screened for binding to the dopaminergic-receptor subtypes D1, D2, D3 and the serotonergic-receptor subtypes 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C}. The most active compounds showed MICs from 2 to 4 μg/mL and had overall reduced binding to the dopamine and serotonin receptors compared to chlorpromazine and trifluoperazine.

Approximately one-third of the world's people are infected with *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB). The World Health Organization estimates there were 9 million new cases of TB and 1.7 million TB deaths in 2005, primarily in Africa and Asia.[1] Currently 3.4% of disease cases involve multi-drug-resistant TB (MDR-TB) resistant to both isoniazid and rifampin.[1] Even more alarming are recent reports of extensively drug-resistant TB (XDR-TB)[2], which is resistant to all of the first-line one-half of the second-line TB antibiotics. Thus, new TB drugs that can treat MDR-TB or shorten the duration of the treatment regimen are urgently needed.

Phenothiazines have been reported as having antitubercular activity for many years, and the phenothiazine drug chlorpromazine (CPZ) is reported to have been successfully used to treat a TB patient (Fig. 1). [3] Naturally, since the phenothiazines tested against *Mtb* are psychotropic drugs, there has been resistance to using them to treat TB because of the cognitive side effects.[4] CPZ, thioridazine, and trifluoperazine (TPZ) are known to exert their psychotropic effects by binding to an array of postsynaptic receptors, including the dopamine receptors,[5] serotonergic-receptors,[6] histaminergic-receptors, α1/α2-receptors, and muscarinic M1/M2-receptors.[7] Although phenothiazines have a complex pharmacological profile, the dopamine and serotonin receptors are primarily responsible for the psychotropic effects.[5] Recently, the molecular target of CPZ and TPZ in *Mtb* was shown to be the type-2 NADH dehydrogenase (NDH-2) encoded by the *ndh* gene.[8] It was demonstrated that TPZ binds directly to NDH-2 as a noncompetitive inhibitor with respect to NADH and is uncompetitive with respect to the primary quinone substrate, ubiquinone Q2.[9] Identification of the target of these drugs in *Mtb* indicates that it may be possible to separate antitubercular activity from psychotropic activity by increasing the selectivity of the compounds or reducing their ability to cross the blood–brain barrier.

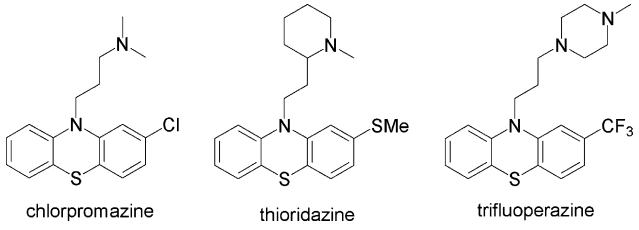

Figure 1. Psychotropic phenothiazine drugs with antitubercular activity.

Our initial approach to this problem was to generate a focused library of phenothiazines that would be screened for in vitro growth inhibition against *Mtb*,

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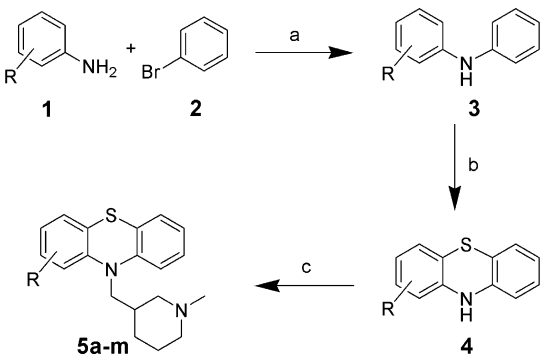
and then to counter-screen them for binding to a panel of dopaminergic and serotonergic receptors. Since no structural relationship between *Mtb* NDH-2 and the postsynaptic neurological receptors is evident in humans, our hypothesis is that it should be possible to create phenothiazines with enhanced selectivity toward *Mtb* growth inhibition and reduced binding to the dopaminergic and serotonergic receptors.

The pharmacology of the existing phenothiazine drugs makes it clear that substitutions around the phenothiazine ring can lead to pronounced effects on their biological activities.[7] First, a diphenylamine intermediate was synthesized by coupling a substituted aniline with bromobenzene using a Buchwald palladium coupling[10] (Scheme 1). The diphenylamines were then cyclized through reaction with sulfur and catalytic iodide[11] under microwave irradiation, using previously described conditions,[12] to give substituted 10*H*-phenothiazines in 10%–50% yields, with the remaining material recovered as unreacted starting material. These phenothiazines were then alkylated with a common basic side chain to permit direct comparison of effects of the substituted ring substituents. This route allowed us to synthesize 10 substituted phenothiazine derivatives along with three compounds, **5a, 5b,** and **5c,** made from commercially available rings (Table 1).

The existing phenothiazine drugs all have a basic side chain at the 10-position, so we maintained the distal nitrogen and added diverse substitutions with both an alkyl and a benzyl linker (Scheme 2). The unsubstituted 10*H*-phenothiazine rings were first alkylated with a bromo- or iodo-alkane, then converted to alkyl iodides that were poised for nucleophilic attack by a primary or a secondary amine diversity reagent. Amines were chosen to encompass aliphatic, cyclic aliphatic, aromatic, and heteroaromatic groups at this position. Also, this set of amine starting materials gave products consisting of both secondary and tertiary amines. Overall, yields ranged from 20% to 80%, and thus were acceptable for our initial screening efforts.

There are precedents for some classes of compounds to have greater activity when the minimal active units are linked together to form a dimer or 'bis' compound. This phenomenon has been observed for quinoline antimalarials[13] and acridine antiprion[14] compounds. To explore this possibility for the phenothiazines, we synthesized two bis-phenothiazines using an alkyl and an aromatic linking group (Scheme 3).



Scheme 1. Reagents and conditions: (a) Pd_2dba_3, X-phos, K_3PO_4, toluene, reflux, 20 h. (b) Sulfur (2 equiv), I_2 (cat), H_2O, μW, 190 °C, 20 min. (c) 3-Chloromethyl-1-methylpiperazine, LHMSD (1 M in hexanes), toluene, 80 °C, 15 min.

The microplate alamar blue assay (MABA) was used to assess the antitubercular activity of the phenothiazines, measured as the Minimum Inhibitory Concentration (MIC) against *Mtb* strain H37Rv (Table 1).[15] The unsubstituted phenothiazine **5a** was significantly less potent than the analog with the 2-CF_3 substitution found in TPZ. The two compounds with phenyl substitutions, **5d** and **5e**, were the most potent, with MICs of 4.5 and 2.1 μg/mL, respectively. The increased activity of the phenyl substituted phenothiazine rings suggests that there may be space for additional steric interaction in the receptor binding pocket in this region. It is also notable that replacement of the phenothiazine ring with a phenoxazine ring (compound **5b**) and introduction of a heteroatom into the ring system (compounds **5i** and **5j**) led to significant losses of activity.

The compounds with substitutions to the side chain generally led to a loss in activity relative to TFP, with the exception of compounds **9f** and **9h**, with MICs of 4.6 μg/mL and 4.2 μg/mL, respectively. These compounds are the only two with side chains that are both secondary amines and are expected to be protonated at the pH of the assay, 7.4. In general, the compounds with the benzyl substitution (**12a–k**) and bulkier side chains (**12c, 12d, 12j–k**) were less active. Interestingly, the two bis-phenothiazines, **13** and **14**, were both potent compounds, with MICs of 2.3 μg/mL and 2.0 μg/mL, respectively.

All these compounds were then counter-screened for binding to three dopamine and three serotonin receptors at 10 μM using a radioligand displacement assay (Table 1).[16] This assay allows us to assess the selectivity toward *Mtb* growth inhibition versus binding to the postsynaptic receptors. The control compounds, CPZ and TFP, both demonstrated near maximum binding to all the receptors except the 5-HT_{1A} receptor at 10 μM concentration. The ring-substitution analogs (**5a–m**) retained high binding to most of the receptors with some loss in affinities to the D1, 5-HT_{1A}, and 5-HT_{2C} receptors. The most potent of this series, **5d** and **5e**, had little tendency to bind to the serotonin receptors, but had only a moderate reduction in affinity for the dopamine receptors. Notably, the two compounds with the greatest overall reduction in binding to these receptors were the phenoxazine compound **5b** and the 3-morpholino substituted compound **5m**, which were also inactive against *Mtb*. The compounds with an alkyl linker and diversity at the pendant nitrogen (**9a–k**) retained a binding profile similar to that of the control drugs, with the exception of **9k**, containing a large diphenyl ether substitution on the nitrogen. These data seem to indicate that large groups in this position could reduce much of the binding to the dopamine and serotonin receptors. The

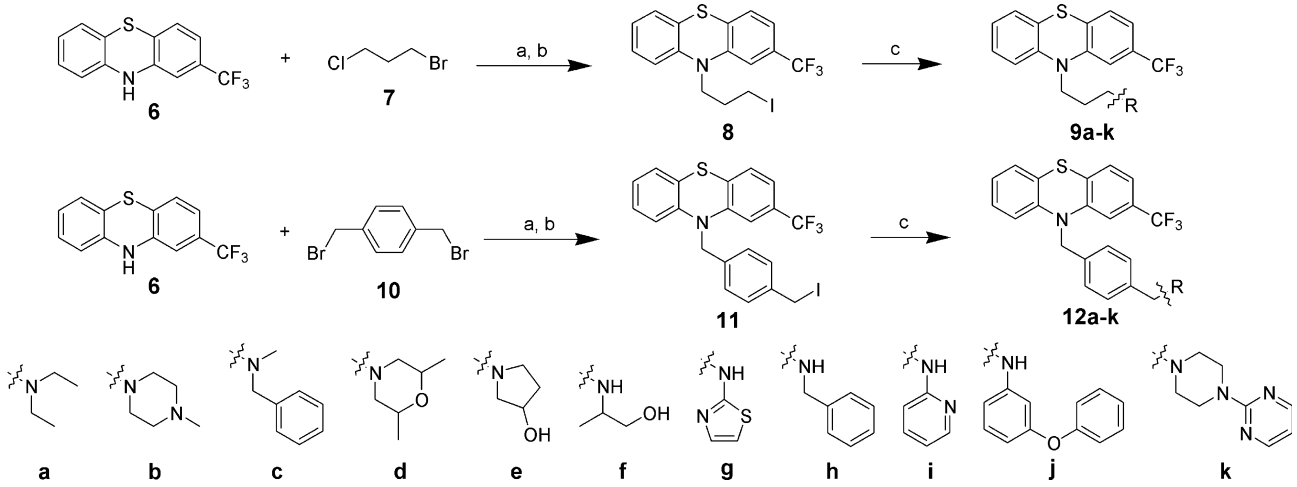
Table 1. Phenothiazine antitubercular activity and percentage of ligand displacement for dopamine and serotonin receptors

Compound	Ring substitution	MABA MIC (μg/mL)	D1[a](%)	D2[a](%)	D3[a](%)	5-HT_{1A}[a](%)	5-HT_{2A}[a](%)	5-HT_{2C}[a](%)
CPZ HCl	2-Cl	6–12	96	96	96	67	99	97
TFP HCl	2-CF_3	6–12	99	98	95	44	99	86
5a	H	17	4	88	64	0	84	29
5b	Phenoxazine	>20	4	57	26	0	24	12
5c	2-CF_3	7.2	6	87	59	5	75	56
5d	2-Ph	4.5	22	97	80	1	44	14
5e	3-Ph	2.1	83	96	97	0	92	58
5f	3-F	10.8	35	87	70	7	99	89
5g	3-Cl	14.3	37	94	87	0	95	67
5h	3-SMe	11.6	NT	NT	NT	NT	NT	NT
5i	1-(N)[b]	>10	20	86	59	19	85	47
5j	3-(N)[b]	>20	NT	NT	NT	NT	NT	NT
5k	3-Me	7.2	23	93	63	0	80	26
5l	3-OMe	>20	8	75	54	0	71	41
5m	3-Morpholino	>20	2	59	1	0	16	7
9a	2-CF_3	15	97	100	100	63	100	94
9b (TFP)	2-CF_3	7.6	96	100	99	76	99	91
9c	2-CF_3	>20	91	99	95	31	98	68
9d	2-CF_3	>20	98	99	99	35	98	30
9e	2-CF_3	11	95	74	85	62	96	95
9f	2-CF_3	4.6	96	100	98	58	100	91
9g	2-CF_3	14	96	95	98	54	98	81
9h	2-CF_3	4.2	96	97	100	61	99	85
9i	2-CF_3	20	96	85	100	45	98	93
9j	2-CF_3	>20	0	0	52	0	25	5
9k	2-CF_3	16	96	50	78	94	95	85
12a	2-CF_3	>10	95	97	97	23	95	57
12b	2-CF_3	15	0	14	0	0	0	0
12c	2-CF_3	>20	1	66	20	4	0	0
12d	2-CF_3	>20	44	75	77	0	75	7
12e	2-CF_3	8.4	95	79	88	0	90	65
12f	2-CF_3	6.4	98	93	97	24	97	59
12g	2-CF_3	>10	0	11	0	0	0	0
12h	2-CF_3	>20	0	13	0	0	0	0
12i	2-CF_3	>10	97	90	86	0	94	53
12j	2-CF_3	>20	5	26	6	0	31	0
12k	2-CF_3	>20	60	72	88	10	60	0
13	2-CF_3	2.3	56	48	94	3	94	83
14	2-CF_3	2.0	0	40	51	0	0	0

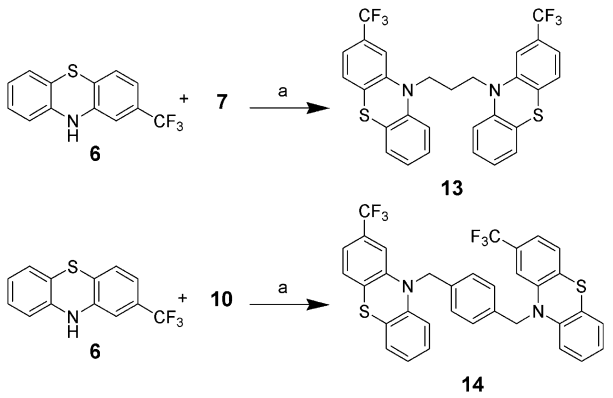
ND, Not tested.

[a] Value is the percent displacement of the radiolabeled ligand at 10 μM of test compound. The ligands used for each receptor are: D1—[^{3}H]SCH-23390; D2—[^{3}H]YM-09151-2; D3—[^{3}H]YM-09151-2; 5-HT_{1A}—[^{3}H]8-OH-DPAT; 5-HT_{2A}—[^{3}H]Ketanserin; 5-HT_{2C}—[^{3}H]Mesulergine.[16]

[b] Heteroatoms are located within the ring at the specified position.



Scheme 2. Reagents and conditions: (a) NaH (1.2 equiv), DMF, 80 °C, 2 h. (b) NaI (10 equiv), acetone, reflux, 48 h. (c) Amines (a–k) (2 equiv), K_3PO_4 (2 equiv), DMF, 80 °C, 2 h.



Scheme 3. Reagents and conditions: (a) NaH (2.2 equiv), DMF, 80 °C, 2 h.

compounds with the benzyl linkers and diversity at the pendant nitrogen had significantly reduced binding to both dopamine and serotonin receptors. In fact, compounds **12b, 12g,** and **12h** were almost devoid of any binding to these receptors, except for weak binding of the D2 receptor. Overall, these data indicate that an increase in the steric volume on the side chain reduces binding to these subtypes of the dopamine and serotonin receptors. The two bis-phenothiazine compounds have substantially reduced binding to the dopamine and serotonin receptors. Overall, these two compounds demonstrated the most significant increase in antitubercular activity and decrease in dopamine and serotonin receptor binding.

These data demonstrate that it is possible to increase the antitubercular activity and selectivity of phenothiazines, although further enhancements are warranted. The antitubercular activity is still in the low micromolar potency range, while binding to some of the dopamine and serotonin receptors can be estimated to be in the high nanomolar range. The pharmacokinetics and the ability to cross the blood–brain barrier will also have important effects on the relative activities of the compounds in vivo. These data do give an indication of the types of modifications that may further enhance the potency and selectivity to achieve an acceptable therapeutic window to warrant further development of phenothiazines as a new class of TB drugs.

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