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Original article

Synthesis and bio-evaluation of phenothiazine derivatives as new anti-tuberculosis agents

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ABSTRACT

Two series of phenothiazine derivatives were designed and synthesized. All compounds were tested for anti-tuberculosis activities against *Mycobacterium tuberculosis* $H_{37}R_V$. In comparison with mother compound of chlorpromazine, compound **6e** shows promising anti-tuberculosis activity and much less mammalian cell cytotoxicity, compound **6e** merits to be further explored as new anti-tuberculosis agents.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease that seriously threatens human health. Moreover, in the past decade worldwide efforts have been made to treat TB due to the fast increasing population of TB, the emergence of drug-resistant TB, and the worldwide HIV and TB co-infection [1,2]. In 2012, it is estimated that 8.6 million people developed the disease of TB, among them 13% were also proved to be HIV-positive. The situation becomes more serious since an estimated of 450,000 people in 2012 developed multi-drug resistant TB (MDR-TB) and an estimated 170,000 died of MDR-TB [3].

Currently, regimens for the treatment of TB which the bacteria are antibiotic susceptible must contain multiple first-line anti-tuberculosis drugs such as isoniazid, rifampicin, pyrazinamide and ethambutol [3]. Whereas, the treatment of multi-drug resistant TB is more complicated, consists of what are called second-line drugs which are more expensive than first-line drugs and have more adverse effects [4]. Generally, the treatment procedure can take up to two years, and one third of MDR-TB patients will unfortunately eventually die of this disease [5]. Therefore, it is urgent to develop novel anti-tuberculosis agents with high efficacy and low toxicity, particularly with different mode of actions compared to current existed anti-TB drugs.

Phenothiazines are in clinical use as an effective anti-psychotic for the treatment of psychosis for about 60 years. Interestingly, phenothiazines were also reported of in vitro activities of anti-tumor [6], anti-bacterial [7], anti-plasmid [8] and anti-tuberculosis [9,10]. Previous reports have demonstrated in vitro and in vivo activity of some known phenothiazine derivatives, such as promethazine, chlorpromazine (CPZ), trifluoperazine and thioridazine (TZ) (Fig. 1) against drug-susceptible and drug-resistant TB bacteria [11,12]. There are also confirmed reports of a TB patient cured with CPZ [13]. However, significant side effects especially extrapyramidal motor symptoms (EPMS) which comprises Hyperkinetic-dystonic syndrome, Parkinson syndrome and Tardive dyskinesia constrained the application of phenothiazine derivatives in clinic [14]. Unfortunately, these psychotic related side effects is likely associated with or even a necessary condition for anti-psychotic efficacy [14,15]. Moreover, the anti-tuberculosis MIC_{90} of CPZ analogs ranges from 0.9 mg/L to 32 mg/L, which exceeds the maximum safe serum level (0.5 mg/L) acceptable for the patient with psychotic [9]. Thus, it is necessary to eliminate the anti-psychotic efficacy and EPMS in order to develop this series of compounds as new anti-tuberculosis drugs (Fig. 2).

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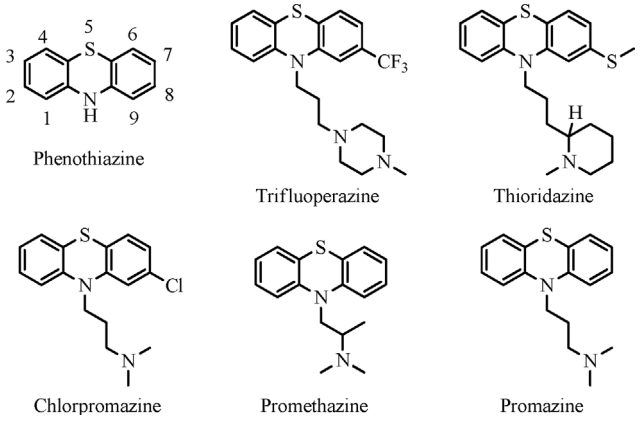

Fig. 1. 10*H*-phenothiazine and phenothiazines.

The anti-tuberculosis activity of phenothiazine skeleton has not yet been properly explored. However, comparison of SARs of anti-tuberculosis and anti-psychotic obtained from limited phenothiazine derivatives suggests that anti-TB activity is mainly from the scaffold of phenothiazine, since there is no obvious SAR trend can be concluded from the side chain and substituents of related phenothiazine derivatives [16,17]. It is revealed that the terminal amine group in the 10-side chain as well as C-2 substitution determines the optimal neuroleptic activity on central nervous system [18].

With the clues of marginal anti-tuberculosis activity of phenothiazine compounds, we proposed to discover new anti-TB drugs by investigation of the relationship between the structure of phenothiazine derivatives and their anti-tuberculosis activities and further designed new compounds based on the information.

2. Experimental

2.1. Inhibitor design

It is known that the side chain with an amino group is a necessity for anti-psychotic activity of phenothiazine derivatives [18,19], but it may not be necessary for anti-tuberculosis activity. We first explored the possibility to change the side chain, including removing the amino group. Then, further modification on the tricyclic ring and the side chain will be carried out. For series 1, we replaced 10-substituents with different non-basic substituents to eliminate the corresponding side effects. In addition, based on structure transformation of CPZ, we also did slightly modification on the phenothiazine core to decrease the conjugation system or replace phenothiazine with thioxanthenes and 9H-thioxanthene.

For series 2, we are aware of structure similarity of the basic moiety of 10-substituted side chain between CPZ and TM207. TMC207 was approved by the FDA with a MIC_{90} of 0.06 μg/mL in 2012 [20]. In mouse models of TB infection, TMC207 exceeded the anti-tuberculosis activities of WHO recommended combination of rifampin, isoniazid and pyrazinamide [21]. Given the highly potency of TMC207 as anti-tuberculosis agents, we extracted part of the moiety from TMC207 to replace the 10-substituted side chain of CPZ and designed 3-(dimethylamino)-1-(3-fluorophenyl)-1-(9*H*-thioxanthen-9-yl) propan-1-ol as depicted (Series 2) in Fig. 3.

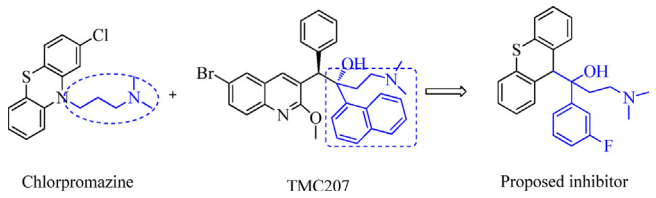

Fig. 3. The design strategy of series 2.

2.2. Chemistry

The ^1H NMR, ^{13}C NMR, HMBC, HMQC, H-HCOSY, DEPT were recorded on Mercury-300 and Mercury-400 spectrometer. Chemical shifts are reported as δ values with *tetra*methylsilane (TMS), employed as the internal standard. HR-ESIMS data were measured on Micromas AutoSpec Ultima-TOF spectrometer.

2.2.1. Series 1

N-Alkyl derivatives **2**, **4**, **5** in Series 1 were synthesized using phenothiazine as the starting material (Scheme 1). Compounds **2** and **4** was obtained through reported procedure [22]. Methylation of compound **4** with MeI/NaH gave 10-(3-methoxypropyl)-10*H*-phenothiazine **5** in the yield of 79.6%. The synthesis of **6a–d** was achieved via the combination of phenothiazine with various reagents. Treatment of phenothiazine with NaH and 1-bromobutane or (3-bromopropoxy) benzene provided **6a** and **6b** in the yields of 56.4% and 63.1% respectively. Treatment of phenothiazine with 3-(1,3-dioxoisoindolin-2-yl)propanoyl chloride or benzoyl chloride in pyridine at 50 °C afforded **6c** and **6d** in the yields of 95.0% and 81.0%. Compound **6d** was prepared through the treatment of phenothiazine with iodobenzene via coupling reaction in 87.3% yield.

Scheme 1. Synthesis of compounds **2**, **4**, **5**. Reagents and conditions: (a) NaH, THF, room temperature 1 h, 80.4%; (b) KOH, glycol/H_2O, reflux 16 h, 82.0%; (c) H_2SO_4, EtOH, reflux 6 h, 99.4%; (d) $LiAlH_4$, THF, 5 h, 88.4%; (e) MeI, NaH, THF, 2 h, 79.6%; (f) for **6a** (56.4%) and **6b** (63.1%): NaH, room temperature 2 h; for **6c** (95.0%) and **6d** (81.0%): pyridine, 50 °C, 5 h; for **6e**: $Pd_2(dba)_3$, DPPF, NaOBu, toluene, 90 °C, 16 h, 87.3%.

The design of compounds **9a** and **9b** are aimed to slightly modify the phenothiazine skeleton. Treatment 2(3*H*)-benzothiazolone with 1-bromopentane in NaOEt/EtOH under reflux afforded the intermediate **7** in 86.5% yield. Followed by hydrolysis in KOH/EtOH under reflux for 2 h, intermediate **8** was then prepared in 98.0% yield. The synthesis of compound **9a** and **9b** was accomplished by condensation of **8** and cyclohexane-1,3-dione or Tetronic Acid in 76.7% and 93.1% yields respectively. 9*H*-thioxanthene analogs **11**, **12a–12b** were synthesized as described in Scheme 2. The reduction of 9*H*-thioxanthen-9-one with sodium

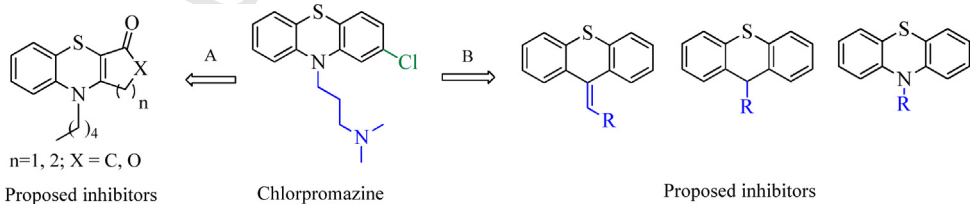

Fig. 2. The design strategy of series 1.

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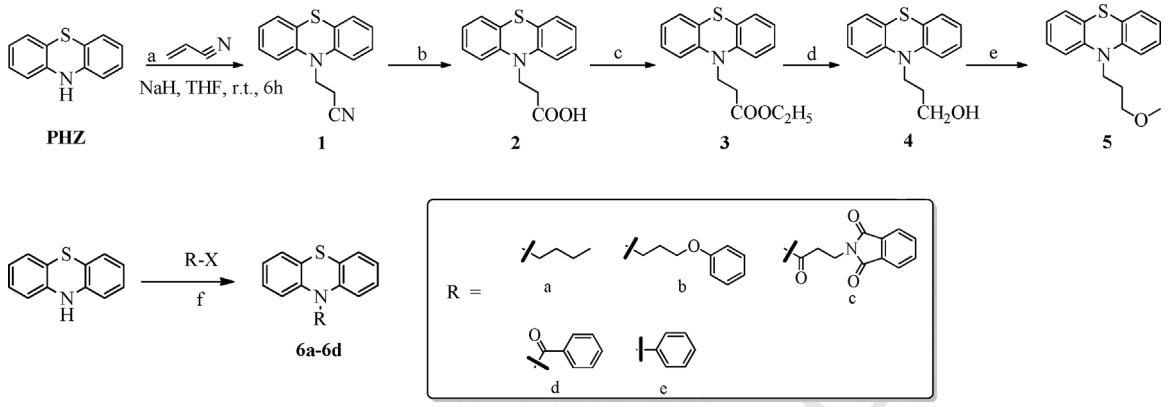
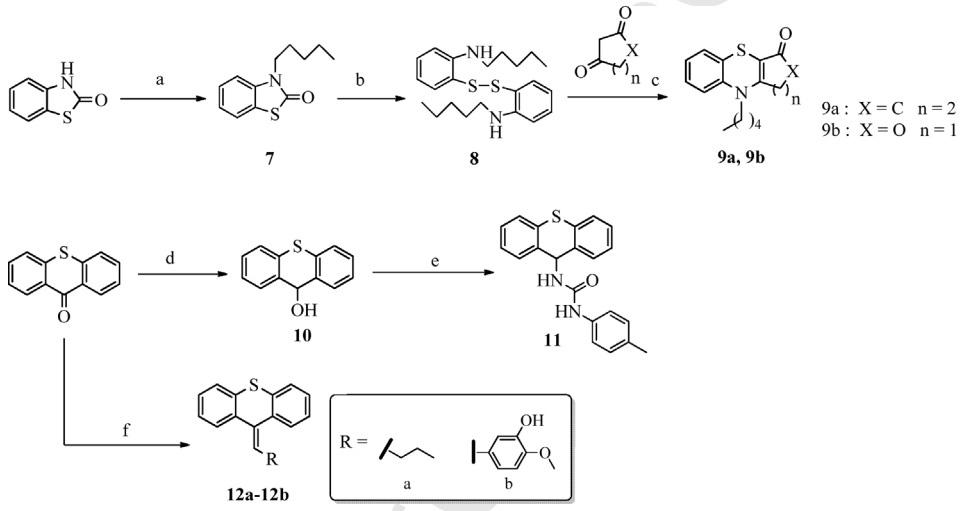


Scheme 1. Synthesis of compounds **2**, **4**, **5**. Reagents and conditions: (a) NaH, THF, r.t. 1 h, 80.4%; (b) KOH, glycol/H_2O, reflux 16 h, 82.0%; (c) H_2SO_4, EtOH, reflux 6 h, 99.4%; (d)



Scheme 2. Reagents and conditions: (a) NaOEt, 1-bromopentane, EtOH, reflux 4 h, 86.5%; (b) KOH, EtOH, reflux 2 h, 98.0%; (c) DMSO, 155 °C, 4 h; (d) sodium borohydride, EtOH, reflux 3 h, 94.4%; (e) acetic acid, r.t. 2 h, 95.9%; (f) Zn, $TiCl_4$, aldehyde (R-CHO), THF, reflux, **12a** (38.2%), **12b** (63.9%).

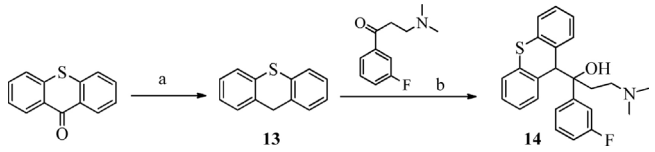
borohydride in EtOH gave the intermediate of **10** in 94.4% yield. Followed by nucleophilic substitution with 1-(*p*-tolyl) urea, compound **11** was afforded in 95.9% yield. Compounds **12a** and **12b** were synthesized from 9*H*-thioxanthen-9-one with 3-hydroxy-4-methoxybenzaldehyde via McMurry reaction in the yields of 38.2% and 63.9%, respectively.

Scheme 2. Reagents and conditions: (a) NaOEt, 1-bromopentane, EtOH, reflux, 4 h, 86.5%; (b) KOH, EtOH, reflux, 2 h, 98.0%; (c) DMSO, 155 °C, 4 h; (d) sodium borohydride, EtOH, reflux 3 h, 94.4%; (e) acetic acid, room temperature 2 h, 95.9%; (f) Zn, $TiCl_4$, aldehyde (R-CHO), THF, reflux, **12a** (38.2%), **12b** (63.9%).

2.3. Series 2

Series 2 was synthesized following Scheme 3. Treatment of 9*H*-thioxanthen-9-one with borane tetrahydrofuran complex afforded intermediate **13** in 93.5% yield. Activation of **13** with *n*-BuLi and then reacted with 3-(dimethylamino)-1-(3-fluorophenyl) propan-1-one to give compounds **14** in 40.2% yield.

Scheme 3. Reagents and conditions: (a) Borane tetrahydrofuran complex, THF, 0 °C to room temperature, 5 h, 93.5%; (d) n-BuLi, −40 °C to room temperature, 3 h, 40.2%.



Scheme 3. Reagents and conditions: (a) Borane tetrahydrofuran complex, THF, 0 °C to r.t., 5 h, 93.5%; (d) n-BuLi, −40 °C to r.t., 3 h, 40.2%.

3. Results and discussion

All of these synthesized compounds were screened for anti-TB activity. The protocol of the anti-TB activity against *M. tuberculosis* $H_{37}Rv$ strain with the Microplate Alamar Blue Assay (MABA) was described in previous research [23]. The cytotoxicity against HepG2 cell lines were carried out following the method of literature [24]. All of the compounds were evaluated with the maximum concentration of 32 μg/mL. The results of anti-TB studies and cell cytotoxicity were listed in Table 1.

Compound **6e** showed the most effective anti-tuberculosis activity with MIC_{90} value of 4 μg/mL against *M. tuberculosis* $H_{37}Rv$ strain, which is very impressive to play as a lead compound for further development. Moreover, compound **6e** did not show an obvious cytotoxicity at the concentration of 32 μg/mL, which enhances the potential to be further developed. In comparison, other compounds in series 1 with terminal alkyl (**6a**), hydroxyl (**4**), carboxyl (**2**), alkoxy (**5**) and phenoxy (**6b**) via an alkyl bridge show no anti-tuberculosis activity. And there were also no obvious

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Table 1
Anti-tuberculosis activity and cytotoxicity evaluations of synthesized compounds.

Compd.	MIC_{90} (μg/mL)	CC_{50} (μg/mL)
2	>32	>32
4	>32	24
5	>32	>32
6a	>32	>32
6b	>32	>32
6c	31	>32
6d	32	>32
6e	4	>32
9a	>32	>32
9b	>32	>32
11	31	>32
12a	>32	14
12b	29	15
14	7	12
CPZ HCl	22	8

Reference compound Rifampicin: MIC_{90} = 0.13 μg/mL.

improvement on the anti-tuberculosis activity when slightly modification on the phenothiazine core (**9a**, **9b**, **11**, **12a**) to discriminate the conjugation system or replace phenothiazine with thioxanthenes and 9*H*-thioxanthene. In addition, the weak anti-tuberculosis activity of compound **11** and **12b** also implicate that a non-basic aryl substituents at the 10-position of phenothiazine ring can also maintain anti-tuberculosis activity. In summary, compared to the mother drug CPZ, phenothiazine derived compounds without 2-substituent and with 10-non-basic substituents can still remain or further increase the anti-TB activity, such as **6e**. Importantly, these modifications are likely to eliminate the corresponding side effects such as anti-psychotic activity and EPMS.

In series 2, the representative compound **14** which adopts part of the moiety from TMC207 exhibits interesting anti-TB activity (7 μg/mL) as expected. However, compound **14** also show an obvious cyto-toxicity (Table 1). Thus, further development of this series of compounds was suspended.

4. Conclusion

Phenothiazine is known for its anti-psychotic pharmacological efficacy. Interestingly, these phenothiazine derivatives also show reasonable anti-TB activity but relative weak and with various psychotic related side effect. In this study, we designed two series phenothiazine derives compounds, and proved that 2-substituents and *N*, *N*-dimethyl amino terminal of Phenothiazine drugs which determine the optimal neuroleptic activity is not the essential elements to maintain the anti-TB activity. Our optimization also lead compound **6e** with increased the anti-TB activity (MIC_{90} = 4 μg/mL, CC_{50} > 32 μg/mL) compared to mother compound CPZ (MIC_{90} = 22 μg/mL, CC_{50} = 8 μg/mL), but much less toxic to mammalian cells. Compound **6e** has a new structure moiety different from current known anti-TB drugs and can be used as the lead for the further development of anti-TB agents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.03.027.

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