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Research paper

Synthesis and SAR evaluation of novel thioridazine derivatives active against drug-resistant tuberculosis

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ABSTRACT

The neuroleptic drug thioridazine has been recently repositioned as possible anti-tubercular drug. Thioridazine showed anti-tubercular activity against drug resistant mycobacteria but it is endowed with adverse side effects. A small library of thioridazine derivatives has been designed through the replacement of the piperidine and phenothiazine moieties, with the aim to improve the anti-tubercular activity and to reduce the cytotoxic effects. Among the resulting compounds, the indole derivative **12e** showed an antimycobacterial activity significantly better than thioridazine and a cytotoxicity 15-fold lower.

1. Introduction

According to the recent World Health Organization annual report, tuberculosis (TB) remains one of the deadliest communicable infections [1]. Nearly one third of the worldwide population is latently infected with *Mycobacterium tuberculosis* (MTB), the etiological agent of tuberculosis in humans, and almost 9 million people develop active TB infections per annum. In addition, 14.8% of global TB patients are co-infected with HIV and can be credited as one of the most common causes of death among AIDS patients [2,3]. This global scenario is due to many causes including the lack of rapid diagnostic tools, the non-compliance of hospitalised patients to the 6–12 months multidrug therapy and institutions lacking the proper drug regimens to treat all the people infected [4]. As a consequence of these transgressions, and after half a century of little to no innovation in the field, MTB have developed multi-drug resistant (MDR) [5–7], extensively-drug resistant (XDR) [8] and totally-drug resistant (TDR) [9] strains, which are resistant to almost all the known available drugs. In 2012, the quinoline derivative bedaquiline [10,11] became the first new drug launched in the market in the last 40 years, since the discovery of rifampin. Currently a number of lead molecules are in clinical trials, such as the diamine SQ109 [12], the fluoroquinolone gatifloxacin [13] and the linezolid [14]. However, the conventional therapeutic approach potentially exacerbates the incidence of new MDR-TB strains and therefore it is inevitable that MTB will evolve resistance against these novel drugs [15,16].

Conventional drug discovery approaches need the identification of a specific target for the development and optimization of a specific molecule. However, it is well known that singular mutations of the targets active site could result in the nullification of drug activity [17]. The current treatment of TB involves the administration of several drugs simultaneously, this reduces the

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incidence of resistant MTB strains by avoiding single point mutations resistances against singular treatments. However, several side effects and poor patient compliance are associated with the present multiple therapy. A potentially successful approach to defeat TB is to discover a drug capable of inhibiting multiple MTB targets simultaneously whilst also retaining activity against MDR and latent TB with an ultimate objective of shortening the current TB regimens.

Thioridazine (TZ) **1**, a long established neuroleptic drug, has been recently repositioned as anti-tubercular drug finding application in the treatment of MDR-TB [18,19]. TZ is currently used in therapy as a third line anti-tubercular drug due to the side-effects on the central nervous system and cardiovascular system which restrict its clinical use [20]. Despite the mechanism of action of TZ having not been fully elucidated, recent studies showed that it inhibits efflux pumps in mycobacteria and alters the cell-envelope permeability of MTB [21–23]. Furthermore, TZ **1** is able to affects the physiology of alveolar macrophages, enhancing the retention of potassium ions and promoting the acidification of phagolysosomal vacuole [24], finally leading to the degradation of intramacrophagic MTB.

Despite the chemistry and structure-activity relationship (SAR) properties of TZ, and related neuroleptic drugs, having been widely investigated in the past, to the best of our knowledge no drug derivatization and optimization studies have been carried out on TZ analogues as inhibitors of MTB.

Herein, we report the synthesis, biological evaluation and SAR studies of a narrow library of novel TZ derivatives. In particular, we aimed at the design and identification of novel TZ analogues with improved activity against TB and MDR-TB strains as well as reduced cytotoxic effects. Three series of derivatives were planned in order to explore the chemical space around the TZ nucleus, as shown in Fig. 1. In the first series, the *N*-methyl substituent on the piperidine ring was removed or replaced with different alkyl groups to evaluate its importance for anti-tubercular activity. In the second series, the piperidine ring of TZ was replaced with different aliphatic heterocyles, keeping fixed the distance between the piperidine nitrogen and the phenothiazine ring.

The role of the thiomethyl group attached to the phenothiazine ring was also investigated in this series. Finally, in the third series the phenothiazine core, which is responsible for the main side effects on the nervous system, was replaced with different heteroaromatic rings, with the aim to reduce the toxicity of the molecule.

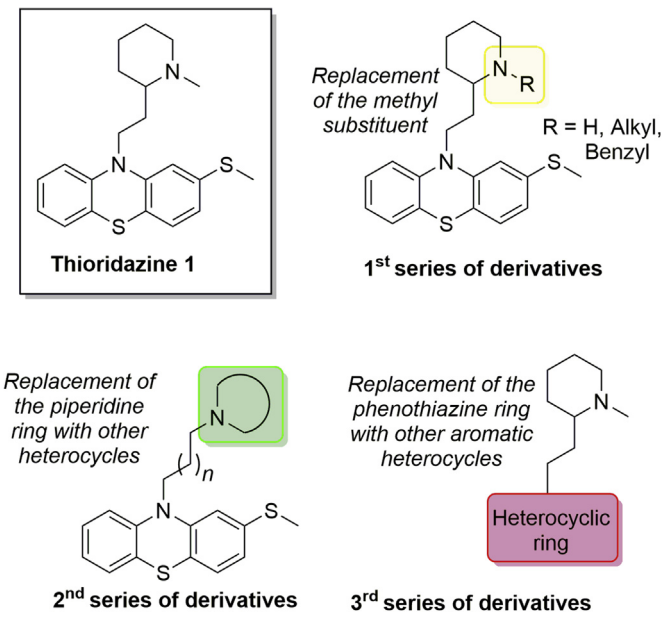


Fig. 1. General structures of the thioridazine analogues.

2. Results and discussion

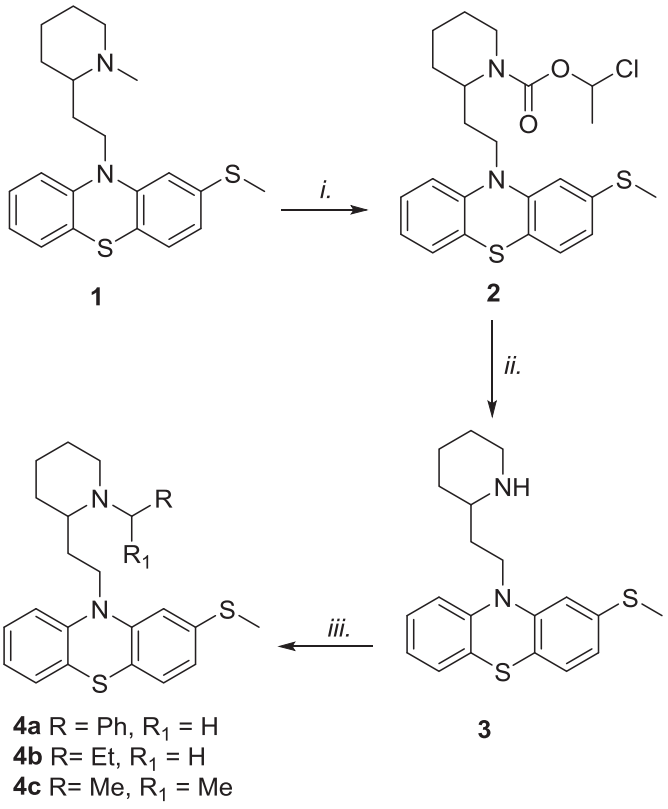
2.1. Chemistry

A series of N-substituted derivatives **4a-c** was first synthesised. TZ was successfully demethylated by treatment with 1-chloroethyl-chloroformate in refluxing DCE [25] followed by hydrolysis with MeOH under reflux, leading to derivative **3**. Reductive amination of **3** with different aldehydes/ketones led to the final *N*-alkyl-derivatives **4a-c** in good yields (62–68%). Scheme 1.

A second series of derivatives where the piperidine ring was replaced with different piperazine and thiomorpholine groups was then synthesised (Table 1). In addition, the thio-methyl substituent on the phenothiazine ring was removed or replaced with chlorine, to evaluate its importance for the anti-tubercular activity.

In particular, the chlorine substituent was chosen on the basis of similarity with chlorpromazine, a phenothiazine derivative closely related to TZ whose efflux pumps inhibitory activity is well known. In detail, the phenothiazines **5a-c** were first reacted with 1-bromo-3-chloropropane to yield the chloroderivatives **6a-c** which were in turn treated with different piperazines and with thiomorpholine to yield the desired products **7a-i**. The thio-methyl-phenothiazine **5a** was also reacted with 2-chloroacetyl chloride leading to **8**, which was in turn converted into derivatives **9a-b** by treatment with methylpiperazine or piperidine.

Finally, the third series of compounds bearing the ethyl-



Scheme 1. Synthesis of analogues **4a-c**. Reagents and conditions: *i.* 1-chloro-ethyl-chloroformate, DCE, Et_3N, reflux, 12 h; *ii.* MeOH, reflux, 12 h; *iii.* $NaBH(AcO)_3$, THF, AcOH, benzaldehyde for **4a**, or propionaldehyde for **4b**, or acetone for **4c**.