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Short communication

Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents

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ABSTRACT

A new library of phenothiazine and 1,3,4-thiadiazole hybrid derivatives (**5a**–**u**) was designed based on the molecular hybridization approach and the molecules were synthesized in excellent yields using a facile single-step chloro-amine coupling reaction between 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanones and 2-amino-5-subsituted-1,3,4-thiadiazoles. The compounds were evaluated for their *in vitro* inhibition activity against *Mycobacterium tuberculosis* H37Rv (*MTB*). Compounds **5g** and **5n** were emerged as the most active compounds of the series with MIC of 0.8 μg/mL (~1.9 μM). Also, compounds **5a**, **5b**, **5c**, **5e**, **5l** and **5m** (MIC = 1.6 μg/mL), and compounds **5j**, **5k** and **5o** (MIC = 3.125 μg/mL) showed significant inhibition activity. The structure-activity relationship demonstrated that an alkyl (methyl/n-propyl) or substituted (4-methyl/4-Cl/4-F) phenyl groups on the 1,3,4-thiadiazole ring enhance the inhibition activity of the compounds. The cytotoxicity study revealed that none of the active molecules are toxic to a normal Vero cell line thus proving the lack of general cellular toxicity. Further, the active molecules were subjected to molecular docking studies with target enzymes InhA and CYP121.

1. Introduction

Tuberculosis (TB) is a contagious disease caused by the infection with bacteria *Mycobacterium tuberculosis*. It causes a massive amount of human deaths despite the availability of more than 20 antiTB drugs and the Bacille Calmette Guerin (BCG) vaccine [1]. Further, the traditional anti-TB agents have limited efficacy against the new forms of TB, extensively drug-resistant TB (XDRTB) and multidrug resistant TB (MDRTB). Hence there is an emergent requirement for the development of newer fast acting antiTB drugs which have a safer toxic profile. In this direction, several molecular design strategies are being employed in order to identify potent chemical entities [2]. The molecular hybridization approach which involves the hybridization of two active pharmacophores into a single molecular framework has become one of most promising approaches to develop potent antiTB agents because many such hybrid derivatives possessed improved efficiency and efficacy, when compared to the parent compounds [3–5]. In our previous studies on imidazo[2,1-*b*] [1,3,4]thiadazole (ITD) based hybrid molecules, we have incorporated substituted benzimidazole or 1,2,3-triazole moiety at position-5 of the ITD ring and evaluated the antitubercular activity of the hybrid molecules. Interestingly, most of the hybrid compounds exhibited significant inhibition activity against *Mtb* H37Rv strain and a safe toxicity profile towards a normal cell line [6,7]. These results and some literature reports on significant antitubercular activity of phenothiazine and 1,3,4-thiadiazole derivatives prompted us to design new phenothiazine-thiadiazole hybrids for the development of potent molecules. The *in vitro* antitubercular activity of phenothiazines is well-known for many years [8–10]. In clinical trials, thioridazine (I) (Fig. 1) is being used in combination with Linezolid and Moxifloxacin as front-line drug in combinatorial therapeutic approaches for the treatment of *Mtb* infection [11]. Chlorpromazine (II) is effective against virulent *Mtb* strain H37Rv in cultured human macrophage model of infection and it is described as synergistic with both INH and RIF [12]. A recent report demonstrated the significant antitubercular activity of a series of 4,5-dihydro-1*H*-phenothiazine containing pyrazolo[3,4-*d*]pyrimidines (III). One of the derivatives was more potent (with MIC_{MABA} value of 0.025 μg/mL) than the standard drug Isoniazid [13]. Further, several N-substituted

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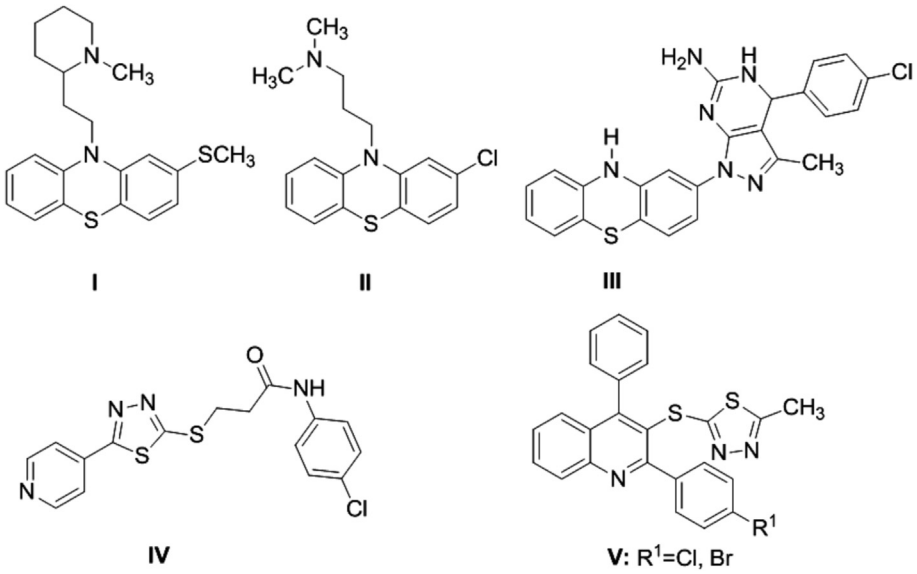

Fig. 1. Representative phenothiazine and 1,3,4-thiadiazole based antitubercular agents.

phenothiazine derivatives demonstrated promising antitubercular activity [14–18]. On the other hand, 1,3,4-thiadiazole derivatives are a important class of heterocyclic compounds in medicinal chemistry research [19,20] which exhibit wide variety of biological effects including anti-tubercular [21], anticancer [22], antibacterial [23] and anti-fungal [24,25] activities. Also, 1,3,4-thiadiazole ring is the core structural unit in several marketed drugs such as Acetazolamide, Methazolamide, sulfamethizole, Cefazedone, Cefazolin, Ceftezole etc. In addition, a few literature reports revealed promising antitubercular activity of 1,3,4-thiadiazoles [26]. For example, 3-heteroarylthioquinoline derivatives [27] of 1,3,4-thiadiazole demonstrated MIC of ~3.5 μM against *Mtb* (IV) whereas a pyridinyl-thiadiazole derivative (V) exhibited MIC of 0.07 μM [21] (Fig. 1). In view of these facts on promising antimycobacterial activity of 1,3,4-thiadiazole and phenothiazine derivatives, we envisaged the incorporation of these two molecular units in to a single molecular framework and synthesized a new series of hybrid derivatives (**5a**–**u**).

2. Results and discussion

2.1. Chemistry

The target phenothiazine-thiadiazole hybrids were synthesized according to the synthetic route presented in Scheme 1. 2-Subsituted-1-(2-chloro-10*H*-phenothiazin-10-yl) ethanones (**2a**-**c**) were synthesized by the reaction of substituted phenothiazines (**1a**-**c**) with chloroacetyl chloride under reflux conditions [15,28]. 5-Methyl-2-amino-1,3,4-thiadiazole (**4a**) was synthesized by reacting thiosemicarbazide with acetyl chloride (**3a**) using a reported procedure [6]. Other 5-substituted-1,3,4-thiadiazole-2-amines (**4b**–**g**) were synthesized by treating the corresponding carboxylic acid (**3b**-**g**) with thiosemicarbazide in the presence of phosphorous oxychloride. The target compounds, 1-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-2-(10*H*-phenothiazin-10-yl)ethanone derivatives (**5a**–**u**) were synthesized by the reaction between compounds **4a**–**g** and **2a**-**c** in ethanol under reflux conditions. The plausible reaction mechanism for the formation of final derivatives (**5a**–**u**) is shown in Scheme 2. The structure of the target molecules (**5a**–**u**) was confirmed by spectral (^{1}H NMR, ^{13}C NMR, ESI-MS and FTIR) and elemental analysis. For instance, the ^{1}H NMR spectrum of compound **5a** showed a broad singlet with one proton at δ 8.16 ppm due to the imine (C═NH) proton and another singlet at δ 2.20 ppm due to methyl protons of the 1,3,4- thiadiazole ring. The singlet at δ 4.74 ppm corresponds to the CH_2 group. The broad NH peak at δ 8.16 ppm was disappeared upon D_2O exchange. Also, its mass spectrum showed the molecular ion peak at *m*/*z* 355.1, which corresponds to M+1 peak of the molecule and is in agreement with its molecular formula $C_{17}H_{14}N_4OS_2$. The IR spectrum of compound **5a** showed an absorption peak at 3314 cm^{-1} due to N–H stretching (C═NH). Further, the signals at 1696 and 1601 cm^{-1} correspond to C═O and C═N stretching respectively. The spectral and elemental analysis data of all target compounds are given in the experimental part and some representative spectra are given in the Supplementary information. The physical data of compounds (**5a**–**u**) are tabulated in Table 1.

2.2. In vitro antimycobacterial activity

All the target derivatives (**5a**–**u**) were screened against *Mtb* H37Rv (ATCC27294) using MABA method [29] and their antimycobacterial activity was evaluated in terms of minimum inhibitory concentration (MIC) values. The MIC values in μg/mL of **5a**–**u** along with those of standard drugs for comparison are presented in Fig. 2. The MIC values of the compounds are in the range of 0.8–50 μg/mL. Interestingly, eleven compounds of the series showed significant inhibitory activity (MIC ≤ 3.125 μg/mL) among which eight compounds exhibited MIC ≤ 1.6 μg/mL (Fig. 3). The MIC values of these compounds are comparable with those of the standard drugs ethambutol (EMB) and ciprofloxacin (INN). Compounds **5g** and **5n** which contain 4-methylphenyl and *n*-propyl groups respectively on the thiadiazole ring emerged as the most potent leads with a MIC of 0.8 μg/mL and are more potent than standard drugs EMB and INN. The nature of the substituents on the phenothiazine (R^1) and 1,3,4-thiadiazole (R^2) rings affected the activity of the molecules. The presence of a methyl (compounds **5a**–**c**) or *n*-propyl (compounds **5m**–**o**) group on the 1,3,4-thiadiazole ring substantially increased the antitubercular activity regardless of the nature of the substituent at R^1 (H, Cl or CF_3). Among derivatives which contain an unsubstituted phenothiazine ring (R^1 = H), those with a 4-methylphenyl (**5g**) or 4-fluorophenyl (**5j**) substituent at R^2 displayed substantial activity. In the case of