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New class of methyl tetrazole based hybrid of (*Z*)-5-benzylidene-2-(piperazin-1-yl)thiazol-4(%H)-one as potent antitubercular agents

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ABSTRACT

In search of potential therapeutics for tuberculosis, we describe here the synthesis and in vitro antitubercular activity of a novel series of thiazolone piperazine tetrazole derivatives. Among all the synthesized derivatives, four compounds (**10**, **14**, **20** and **33**) exhibited more potent activity (MIC = 3.08, 3.01, 2.62 and 2.51 μM) than ethambutol (MIC = 9.78 μM) and pyrazinamide (MIC = 101.53 μM) against *Mycobacterium tuberculosis*. Furthermore, they displayed no toxicity against Vero cells (C1008) and mouse bone marrow derived macrophages (MBMDMϕ). These investigated analogues have emerged as possible lead molecule to enlarge the scope of the study.

Tuberculosis (TB) is a greatly insidious disease caused by *Mycobacterium tuberculosis* and continues to be a major threat to global public health.[1] TB is characterized by tubercle lesion in the lungs and also affects the skin, lymph, nodes, brain and almost every other body organ.[2] According to the World Health Organization (WHO) report, 8.6 million people were infected with TB in 2012, of which 450,000 suffers from multidrug-resistant pulmonary TB.[3] TB is also proclaimed to be a global health emergency due to increase in secondary infections in immunocompromised patients [such as those infected with human immunodeficiency virus (HIV)][4] and the appearance of resistant strains of *M. tuberculosis* [multidrug resistant (MDR) and extensively drug resistant (XDR) TB strains].[5–8] Nowadays, drugs available for the treatment of TB are incapable to cure the patients due to several limitations such as, MDR, XDR and other side effects. The current treatment routine for drug susceptible *M. tuberculosis* requires the daily administration of first-line drugs which include ethambutol, isoniazid, pyrazinamide and rifampin for two months followed by an additional four months of daily administration of isoniazid and rifampin.[9] However several side effects such as inconsistent treatment, patient noncompliance and the no availability of these drugs during this period have contributed to the failure to achieve a cure.[10,11] Despite the severe global impact of TB, it has been a neglected disease and no new drugs have been developed specifically against mycobacteria since the 1960s.[12,13] Although a number of classes of compounds have been reported with a consequence on *M. tuberculosis*, treatment failure is too often a fact.[14] Therefore, the urgency and the growing need for the new class of chemical compounds are well accepted. Today's, an attractive concept of molecular hybridization for infectious disease is becoming popular as an emerging structural modification tool to design new molecules with improved bioactivity.

In this perspective, the imperative privileged scaffolds such as rhodanine, thiazolidine-2, 4-dione- and pseudothiohydantoin-based compounds are regularly used as a powerful device for medicinal chemist[15] and have a broad substrate scope for the synthesis of various heterocyclic moieties with wide range of pharmacological activities (Fig. 1) such as antimalarial,[16] antimicrobial,[17,18] antiviral, anti-diabetic,[19] and anticonvulsant effects.[20] However rhodanine and thiazolidine-2, 4-dione-based compounds inhibit the biosynthetic pathway of deoxythymidine-diphosphate-rhamnose, which is the providing cofactor for rhamnose incorporation into mycobacterial cell wall (Fig. 2).[21,22] In most lessons, we have reported the antimalarial and antimicrobial activities of rhodanine and their derivatives.[23,24]

Previously, Bogatcheva et al. synthesized a library of ethambutol inspired diamines based analogues and reported their activity against *M. tuberculosis*.[25] Earlier several groups reported that piperazine substituents displayed broad range of biological activities like antimalarial,[26] antimicrobial,[27] anticonvulsant and antiarrhythmic activities.[28,29]

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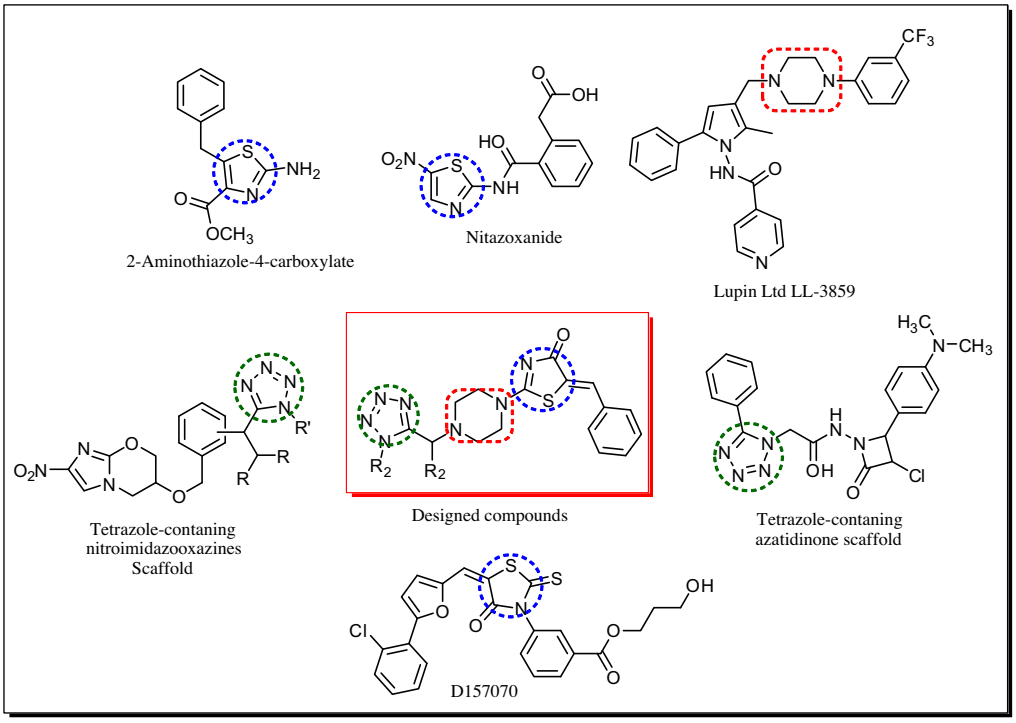

Figure 1. Designing of thiazolone–piperazine–tetrazole based derivatives showing antitubercular activity.

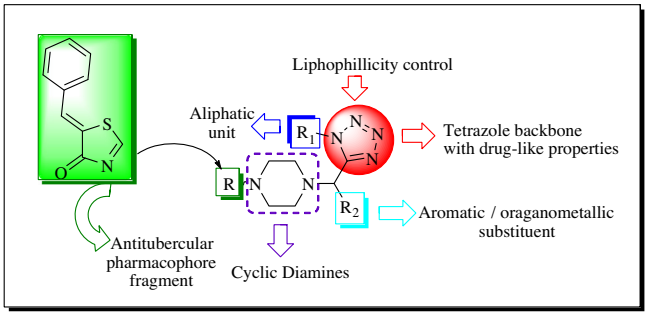

Figure 2. Illustration of the propose strategy for these compounds.

On the other hand, tetrazole represent an important class of heterocycles, which reveal a wide range of application in synthetic and medicinal chemistry.[30,31] They have the individuality to be resistant to biological degradation and therefore they are used as isosteric substituents of various functional groups. In fact, substituted tetrazoles have similar pK_a values than that of corresponding carboxylic acid and tetrazole analogs which increased lipophilicity that allows the easier crossing across the plasma membrane.

Based on this reason and as a part of our ongoing interest in the design and synthesis of new heterocycles towards anti-infective research programme,[32,33] we have synthesized new heterocycles based on Ugi multicomponent reaction via using piperazino linker.

Rhodanine is inexpensive commercially available reagents which make its use attractive as the starting material for the synthesis of these derivatives (**8**–**33**). One-pot methodology involves Knoevenagel condensation of benzaldehyde and rhodanine followed by the displacement of the thiocarbonyl sulphur with secondary amine (piperazine) in the same reaction mixture. The amine acts as the catalyst in the Knoevenagel condensation and work as nucleophiles in the second step.[34] To further optimize the piperazinyl thiazolone derivatives, we have synthesized a series of thiazolone piperazinyl–tetrazoles (**8**–**33**) via modified $TMSN_3$-Ugi MCR.[35] In this context, compound **4** was allowed to react at room temperature with various aldehydes (aromatic, heteroaromatic and organometallic), isocyanides and azidotrimethylsilane ($TMSN_3$) in methanol (dry) to form the desire thiazolone piperazinyl–tetrazole hybrids (Scheme 1), in good to excellent yield. The structures of the synthesized derivatives were substantiated by NMR, mass spectrometry (ESMS and HRMS) and IR spectroscopy. Purity of final compounds was determined with analytical HPLC.

Evaluation of antitubercular activity against *M. tuberculosis* $H_{37}Rv$ was carried out with a recommended protocol[36] using Middlebrook (MB) 7H10 agar medium. A 100 μL of serial two fold dilutions of the stock (1.0 mg/mL in DMSO, Dimethyl Sulphoxide) of test compounds and standard antitubercular drug {isoniazid (INH)} were incorporated in the medium (final volume, 2 mL/tube) supplemented with OADC (oleic acid, albumin fraction IV, dextrose and catalase). Compounds/drug containing tubes were kept in slanting position till the medium solidified. Culture of *M. tuberculosis* $H_{37}Rv$ grown on Lowenstein-Jensen (L-J) was harvested in N-saline containing 0.05% Tween-80. The culture was agitated with glass beads to make a single cell suspension. A working inoculum (2×10^7cfu/mL; 10 μL/tube) of mycobacterium was spread on the surface of the medium and the tubes were kept at 37 °C for 4 weeks for the appearance of colonies. Tubes containing no drug served as control. The minimum concentration of the drug (INH)/compounds that completely inhibited the growth of mycobacterium was recorded as Minimum Inhibitory Concentration (MIC) with respect to the used inoculum.

A total of 26 new thiazolone–tetrazole based hybrids (**8**–**33**) were screened for in vitro activity against *M. tuberculosis* $H_{37}Rv$. The results are depicted in Table 1. Among all the synthesized analogues, most of the compounds were found to be active with MIC values in the micromolar ranging from 2.51–23.9 μM. Four compounds **10**, **14**, **20** and **33** are 3.17, 3.24, 3.73 and 3.89 folds active than the antitubercular drug ethambutol (MIC = 9.78 μM). Compounds **16**, **28** and **29** were approximately equipotent as control; ethambutol (MIC = 9.78 μM) showing MIC values 10.8, 10.9 and 10.3 μM, respectively. Moreover, all the synthesized