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Design, synthesis and antimycobacterial evaluation of 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine hybrid analogues

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ABSTRACT

A series of twenty six new 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine analogues were synthesized by seven steps and evaluated for their anti-tubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Among the tested compounds, **7j**, **7p**, and **7r** exhibited moderate activity (MIC = 6.25 μg/mL) and compounds **7a**, **7f**, **7g**, **7n** and **7v** exhibited good activity (MIC = 3.125 μg/mL), while **7h** displayed excellent activity (MIC = 1.56 μg/mL) by inhibiting 99% growth of *M. tuberculosis* $H_{37}Rv$ strain. In addition, all the active compounds were subjected to cytotoxic studies against mouse macrophage (RAW264.7) cell lines and the selectivity index values for most of the compounds is >**10** indicating suitability of compounds in an endeavour to attain lead molecule for further drug development.

1. Introduction

Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent. In 2012, 8.6 million people fell ill with TB and 1.3 million died from TB. Over 95% of TB deaths occur in low and middle income countries, and it is among the top three causes of death for women aged 15 to 44. People falling ill with TB each year is declining, although very slowly. An estimated 22 million lives saved through use of Directly Observed Therapy Shortcourse and the Stop TB strategy recommended by World Health Organization [1]. Detection of Multi-Drug resistant TB (MDR-TB) patients is increasing every year as a result of scanty treatment and once TB organisms acquire resistance they can spread from person to person in the same way as drug-sensitive TB. The biggest increases were in India, South Africa and Ukraine. Also, emergence of extensively drug-resistant TB (XDR-TB) and Rifampicin-resistant TB has still deteriorated the TB treatment by not responding to the standard six month treatment with first-line anti-TB drugs and can take two years or more to treat with drugs that are less effective, more toxic and more expensive [2]. Efforts have been steered to manipulate the biological processes of existing drugs for effective development of new chemical entities against TB as a rational approach.

Ascribable to complexity and toxicity of the current TB drug regimens and emergence of various forms of drug resistant TB warranted the scientific community to focus on exploring novel chemotherapeutical agents. As TB is an inevitable disease among HIV patients, it is identified that current TB drugs (Rifabutin, Rifampicin and Rifapentine) interact with the antiretroviral drugs [3] taken by HIV positive people; hence it is essential to scrutinize cost effective, less toxic chemical entities preferably with new biochemical pathways to shorten treatment time, and to interrupt drug–drug interaction.

2-Aminothiazoles are well acknowledged potential therapeutic agents viz. antiviral, NPY_5 antagonists, PGE_2 inhibitors, anticancer, opiod receptor agonists, antimycobacterial, anti-inflammatory, antiprion etc., [4–11]. The wide applicability of thiazole framework in drug discovery is attributed to the use of inexpensive starting materials and adoption of simple synthetic strategy to wangle diverse molecules. In particular, nitazoxanide (NTZ) and its active metabolite tizoxanide (TIZ) are known to inhibit replicative and non-replicative *Mycobacterium tuberculosis* (MTB) [12]. Some of the drugs based on aminothiazoles moiety are depicted in Fig. 1.

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In addition, Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) and the National Institutes of Health, Molecular Libraries Screening Centres Network disclosed encouraging results from an MTB whole-cell high-throughput assay and identified aminothiazoles as an important core which is depicted in Fig. 2 [9,13–15]. Few research groups synthesized various 2-(2-hydrazinyl)thiazole derivatives and reported good activity against MTB strain $H_{37}Rv$ [16–18]. Similarly, Makam et al., reported 2-(2-hydrazinyl)thiazole derivatives which are structurally similar to thiolactomycin using rational hybrid approach with good anti-TB activity [19]. Sridhar et al., synthesized N^1-hydroxy-*N*-(4*H*,5*H*-naphtho[1,2-*d*]thiazol-2-yl)methanimidamide analogues which inhibit MTB methionine aminopeptidases [20]. Ranjith et al., synthesized (2-aminothiazol-4-yl)methyl 3-(2-cyanoethyl)benzoate and found to exhibit good anti-TB activity than isoniazid against MTB $H_{37}Rv$, *Mycobacterium Smegmatis* (ATCC 19420), *Mycobacterium Fortuitum* (ATCC 19542) and MDR-TB strains [21]. Meissner et al., synthesized 3-bromo-*N*-(4-(pyridin-2-yl)thiazol-2-yl)benzamide derivatives which inhibit MTB $H_{37}Rv$ strain at 0.008 μg/mL [9]. Very recently, Pieroni et al., synthesized *N*-substituted-2-aminothiazole derivatives and clearly briefed the structure activity relationship emphasizing on the mandatory of aminomethyl group at second position of thiazole [22].

MTB cells wrap a protective sheath on the cell wall composed of complex carbohydrates, proteins and glycolipids [23] making most of the drugs very complicated to penetrate through this thick, non-permeable, hydrophobic barrier which defies current treatment. This being one of the greatest challenges put forward to the scientists while designing a drug. Piperazine linkers have made vast impact on drug discovery process as they have ability to increase the lipophilicity of the molecule to a greater extent. Bogatcheva et al., synthesized various diamine linked moieties and reported good *in vitro* and *in vivo* anti-tubercular activity against MTB $H_{37}Rv$ strain [24]. Huang et al., synthesized 2-methyl benzothiazoles which inhibited the growth of MTB $H_{37}Rv$ strain at 1.4 μM [25].

With this collective information and encouraged by our recent anti-TB results emphasizing on molecular hybridization approach [26–28] we drew a synthetic stratagem (Fig. 2) to fit all these imperative pharmacophoric groups into one distinct scaffold and synthesized 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine analogues.

2. Chemistry

We synthesized 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(3-(4-substitutedpiperazin-1-yl)alkyl)piperazine analogues as sketched in Fig. 3. We adopted reported procedure with slight modification to prepare 4-(4-(2-chloroethyl)phenyl)-2-substitutedthiazole (**3**) [29], then **3** was coupled with anhydrous piperazine in *N,N*-dimethylformamide (DMF) at 100 °C for 3 h to give 1-(4-(2-substitutedthiazol-4-yl)phenethyl)piperazine (**4**). Compound 4-(4-(4-(2-substitutedthiazol-4-yl)phenethyl)piperazin-1-yl)alkan-1-ol (**5**) was obtained by coupling **4** with bromoalkanols in the presence of triethylamine (TEA) and DMF at 100 °C for 3 h; further heating **5** at 120 °C with 40% hydrobromic acid for 2 h fetched 1-(4-(2-substitutedthiazol-4-yl)phenethyl)-4-(n-bromoalkyl)piperazine (**6**). Subsequently various substituted piperazines were coupled with **6** at 120 °C for 3 h using TEA and DMF to achieve title compounds (**7a**–**z**). All the title compounds displayed multiplet in the range 2.75–2.95 ppm and 3.45–3.65 ppm corresponding to piperazine ($-CH_2-$) protons. Characteristic aromatic proton (C-5) of thiazole ring resonated in the range 6.50–6.75 ppm. Both analytical and spectral data (^1H NMR, ^{13}C NMR, IR and HRMS) of all the synthesized compounds were confirmed and employed further for their antimycobacterial evaluation.

3. Results and discussion

3.1. Antimycobacterial activity

All the synthesized compounds were tested for their ability to inhibit the growth of MTB $H_{37}R_V$ strain by Microplate Alamar Blue Assay (MABA). Isoniazid and Rifampicin were used as the positive drug standard. The *in vitro* antimycobacterial test results of title compounds are tabulated in Table 1 as minimum inhibitory concentration (MIC) and the activity ranges between 1.56 and >6.25 μg/mL. Compounds with MIC ≤6.25 μg/mL were further subjected to cytotoxicity studies. Amongst the series, compounds **7j**, **7p** and **7r** inhibit 99% growth of MTB $H_{37}Rv$ strain at a concentration 6.25 μg/mL whereas compounds **7a**, **7f**, **7g**, **7n** and **7v** inhibit 99% growth of MTB $H_{37}Rv$ strain at a concentration 3.125 μg/mL. Compound **7h** emerged as the most promising candidate by inhibiting 99% growth of MTB $H_{37}Rv$ strain at a concentration 1.56 μg/mL.

Among the synthesized compounds electron releasing groups like phenyl and benzhydryl piperazine with propyl and butyl linker respectively, bearing *N*-methylthiazol-2-amine backbone were found to be inactive (**7c** and **7i**, MIC = 50 μg/mL). Next, keeping propyl linker intact we synthesized 2-methylthiazole moiety bearing phenyl piperazine (**7e**) which exhibited 2 fold increase in the activity (MIC = 25 μg/mL). Subsequently an alkyl chain was increased to fetch compound **7b** which exhibited similar activity

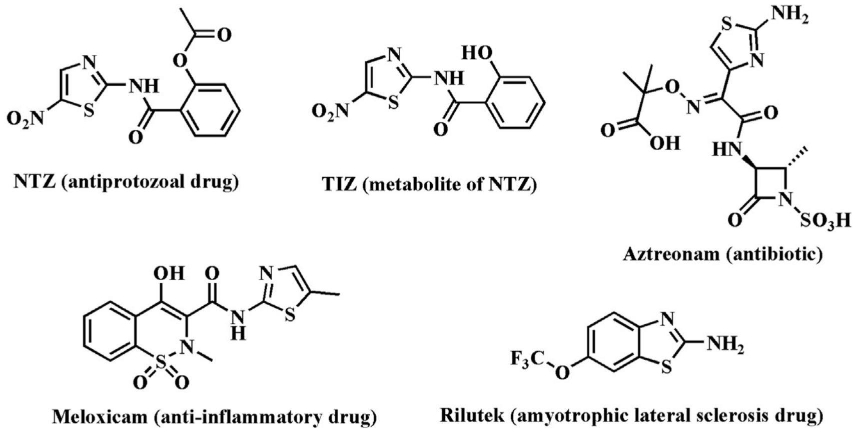
NTZ (antiprotozoal drug)
TIZ (metabolite of NTZ)
Aztreonam (antibiotic)
Meloxicam (anti-inflammatory drug)
Rilutek (amyotrophic lateral sclerosis drug)

Fig. 1. Drugs currently in use based on 2-aminothiazole skeleton.