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New INH–pyrazole analogs: Design, synthesis and evaluation of antitubercular and antibacterial activity

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ABSTRACT

With the aim of developing promising antitubercular and antibacterial leads, we have designed and synthesized a new series of isonicotinohydrazide based pyrazole derivatives (**5a**–**r**). All new derivatives (**4a**–**b** and **5a**–**r**) were screened for in vitro antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB) strain. Four compounds **5j**, **5k**, **5l** and **4b** emerged as promising antitubercular agents with MIC of ⩽4.9 μM which is much lower than the MIC of the first line antitubercular drug, ethambutol. The 3-chlorophenyl substituent at position-3 of the pyrazole ring enhanced the antiTB activity of the molecules. Three derivatives **5b**, **5k** and **4b** exhibited promising antibacterial activity against the tested bacterial strains. The active molecules were nontoxic to normal Vero cells and showed high selectivity index (>160). The structure and antitubercular activity relationship was further supported by in silico molecular docking study of the active compounds against enoyl acyl carrier protein reductase (InhA) enzyme of *M. tuberculosis*.

Tuberculosis (TB) is the most dangerous of various microbial diseases, which causes ill health and mortality of millions of people each year. It stands second after HIV-AIDS among the various death causing diseases. The world health organization (WHO) report estimates that there were 9 million new TB cases and 1.3 million deaths in the year 2013.[1] The WHO has recommended a standard strategy for the treatment of TB by a program called DOTS (Directly Observed Treatment, Short-course), which includes six month regimen of four first-line drugs: isoniazid, rifampicin, ethambutol and pyrazinamide.[2] Despite the availability of strong and powerful antitubercular drugs, tuberculosis is still a leading threat for human life; the recent emergence of multi drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) further cause serious challenges in TB control. It is also clear from the WHO data that the mortality rate and spread of the disease by TB infection is very high in Human Immunodeficiency Virus (HIV) infected people.[3] Thus there is an apparent need for the discovery of fast acting TB drugs which cause fewer side effects and eliminate the infection in short treatment period.

In recent days, development of new drugs by molecular modification of a lead compound or already existing drug with an established activity has become an active area of research in medicinal chemistry. Such a molecular modification can possibly results in enhancement of the activity and sometimes may also help in reducing the toxicity of the molecule. This approach of molecular modification has become a promising strategy to design and develop potent antiTB agents. Isoniazid (INH) is one of the most studied antiTB drugs. It is a potent first-line antiTB drug; a prodrug triggered via oxidation that forms an adduct with NAD (+) to inhibit NADH dependent targets of *Mycobacterium tuberculosis* (MTB) bacillus, such as the enoyl-acyl carrier protein reductase (InhA).[4–6] But the major drawback of INH is its failure against the treatment of MDR-TB, especially among patients infected with HIV. The recent studies indicate that the incorporation of lipophilic moieties into the framework of INH can increase permeation of the drug into the tissues of the mammalian host and into the waxy cell wall of the bacterium.[7–9] The functionalization of the INH is possible via its reactive hydrazide group which can easily react with carbonyl compounds to form hydrazones or undergo cyclization reactions. LL-3858 (**I**) (Fig. 1) is one such isoniazid derivative (developed by Lupin Limited) which shows activity against both drug sensitive and multidrug resistant TB[10,11] and is in the initial stages of phase II clinical trial for the treatment of tuberculosis.[12] Also, a few other hydrazone derivatives of INH (**II**–**IV**) showed promising anti-TB activity.[13,14]

In view of these facts, we envisaged to design new INH hydrazone analogs which could possibly mimic the INH–metabolite–NAD (+) adduct in inhibiting InhA. Accordingly, we have designed a new class of INH derivatives containing active 3,4-disubstituted pyrazole unit; the pyrazole unit as a replacement for the

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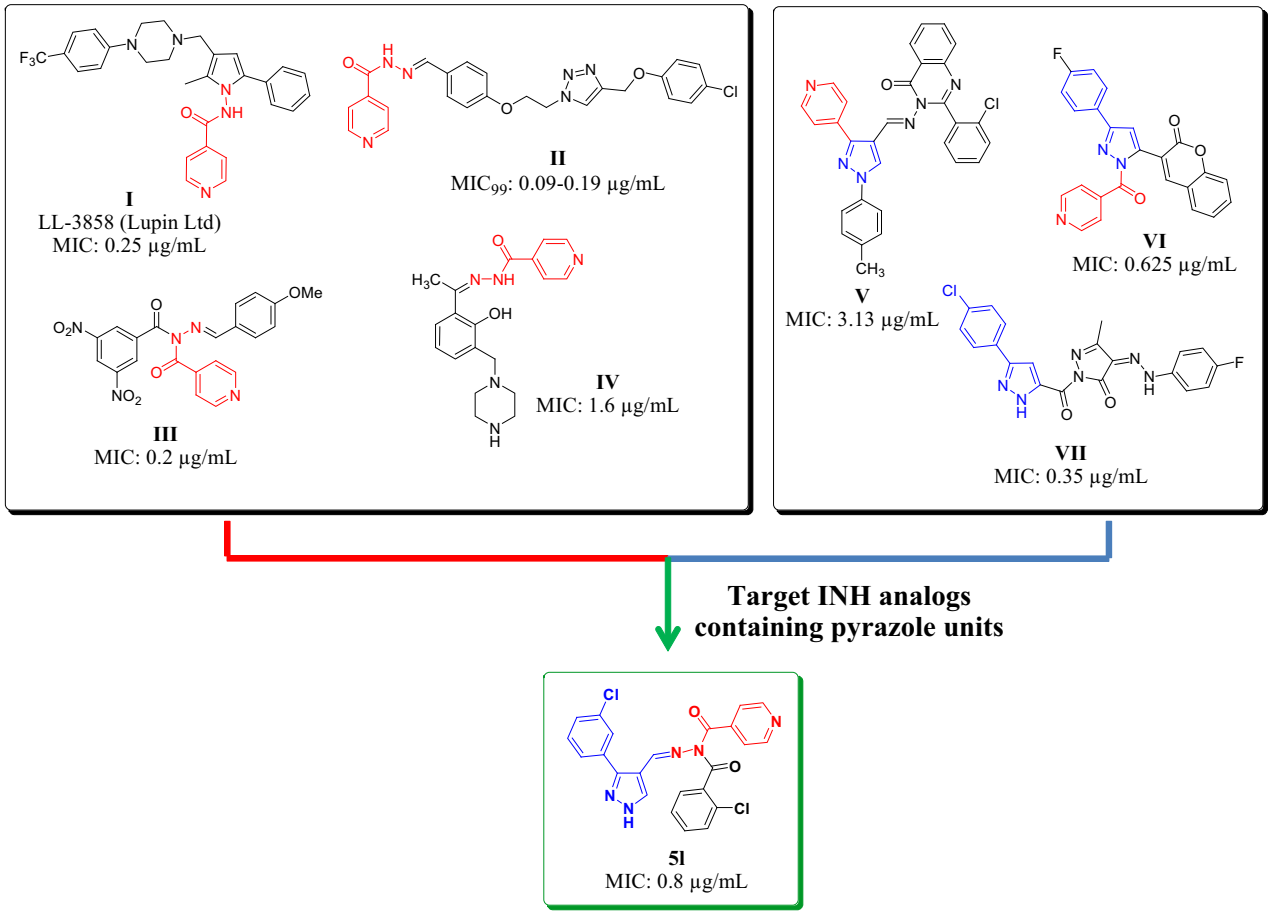

Figure 1. Some representative isoniazid and pyrazole containing potent antiTB analogs. The target isoniazid analog **5l** exhibited the highest antiTB activity among the synthesized compounds.

five-member sugar of NAD (+) in the bioactive adduct. Further, some literature reports suggested that INH analogs containing pyrazole unit (**V**–**VII**) exhibit promising antitubercular activity.[15–17] So, we emphasized that introduction of the pyrazole unit may improve the inhibitory potential of the INH hydrazone analogs. In this direction, we synthesized a new class of INH analogs (**5a**–**r**) containing a pyrazole ring and evaluated their antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB) strain. Additionally, the compounds were screened against three common pathogenic bacterial strains as well.

The synthetic route of new isonicotinohydrazide based pyrazole derivatives (**5a**–**r**) is represented in Scheme 1. 3-Aryl-1*H*-pyrazole-4-carbaldehydes (**3a**–**b**) were synthesized by the Vilsmeier–Haack reaction of semicarbazones **2a**–**b**, which in turn were synthesized by the reaction of semicarbazide hydrochloride with substituted acetophenones (**1a**–**b**) in ethanol media.[18,19] The hydrazone scaffolds (**4a**–**b**) were synthesized in good yield by refluxing a mixture of **3a**–**b** and isonicotinohydrazide in the presence of catalytic amount of sulfuric acid.[20] The target molecules (**5a**–**r**) were synthesized by coupling intermediates **4a**–**b** with different aromatic acids using the typical acid–amine coupling agents, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole hydrate (HOBt) in presence of dimethylamino pyridine (DMAP) as the base at room temperature. The compounds were purified using appropriate methods like recrystallization and column chromatography. The structural details of the target compounds (**4a**–**b** and **5a**–**r**) are presented in Table 1.

All newly synthesized compounds were characterized using ^{1}H NMR, ^{13}C NMR, and mass spectral analysis followed by elemental analysis. In the ^{1}H NMR spectrum of **4a** a new singlet signal is appeared at δ 11.79 ppm which corresponds to the —NH of pyrazole ring whereas the —NH proton of isonicotinohydrazide appeared at δ 13.49 ppm. The signals due to the aromatic protons also are in agreement with the structure of **4a**. The ESI MS spectrum displayed a molecular ion peak at (*m*/*z*) 326.1 $(M+H)^+$ which is in agreement with its molecular formula ($C_{16}H_{12}ClN_5O$). The ^{1}H NMR spectrum of **5a** shows the pyrazole NH signal at δ 12.12 ppm and the signal due to the —NH of isonicotinohydrazide was absent. Also, the number of signals in the aromatic region is in well agreement with its structure. Furthermore, the ESI MS spectrum shows the molecular ion peak at (*m*/*z*) 448.2 $(M+H)^+$, which confirms its molecular formula ($C_{23}H_{15}ClFN_5O_2$). Similarly, the ^{13}C NMR spectral data also supports the structure of **5a**. The spectral and elemental data of all the target compounds and some representative spectra are given in the ESI.

All new compounds were screened against *M. tuberculosis* H37Rv (MTB) strain using the microplate alamar blue assay (MABA).[22,23] Isoniazid, ethambutol and pyrazinamide were used as the positive drug standards. The results of the in vitro antimycobacterial screening are given in Table 1. Four compounds namely **4b**, **5j**, **5k** and **5l** displayed significant inhibition activity with a MIC $\leqslant$ 4.9 μM. Among these, **5l** is the most potent molecule with a MIC of 1.7 μM which is comparable with the MIC value of standard drug INH, whereas the inhibition activity of compounds **4b**, **5j** and **5k** is much greater (in terms of MIC value) than that of standard drug, ethambutol. The 3-(chlorophenyl)-1*H*-pyrazole scaffolds, **4a** and **4b**, differ in their structure only in terms of the position of the chloro substituent on the phenyl ring. However,