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ORIGINAL ARTICLE

Design and synthesis of 5-methylpyrazine-2-carbohydrazide derivatives: A new anti-tubercular scaffold

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Abstract A simple synthetic methodology was employed for synthesis of series of 5-methylpyrazine-2-carbohydrazide derivatives (PM series). *In vitro* anti-tubercular activity was evaluated against *Mycobacterium tuberculosis* ($H_{37}Rv$) in Middle brook 7H-9 broth medium. Amongst synthesized compounds, seven compounds showed remarkable anti-tubercular activity. The 2-D QSAR illustrates the design PM series of compounds as potential anti-tubercular scaffolds that can be further optimized to improve the activity.

1. Introduction

Tuberculosis is one of the world's great public health threats due to resistance to existing drugs and simultaneous presence of HIV infections. Thus, tuberculosis keeps challenging medicinal chemists to develop new compounds. There are two main strategies for the development of new agents against tuberculosis. The first one requires extraordinary molecular diversity, and the second one is using clinically active and their chemical modification. This second approach is easy and accessible through newly developed computational techniques. The latter strategy rekindled our interest toward pyrazinamide (PZA), which is one of the frontline agents prescribed for the treatment of multidrug resistant tuberculosis (MTB). Recently a proposed mechanism of action has been reported to be the inhibition of the eukaryotic-like fatty acid synthetase I (FASI) of MTB. PZA is considered to be a prodrug of pyrazinoic acid (POA), which is believed to be the active inhibitor of MTB. Activation of PZA to POA was regulated by an enzyme pyrazinamidase present in all PZA-sensitive strains of MTB (World Health Organization Geneva, Switzerland, 2000; Cynamon et al., 1992, 1991; Trnka et al., 1964).

There are two main reasons for selection of pyrazine-2-carbohydrazide as a lead scaffold in this study that are simplicity of synthetic methodology and search on new compounds related to 'Pyrazine derivatives' which are known for their anti-tubercular activity (Fig. 1a) Sriram et al., 2006. The structural requirement for inhibition of FASI and anti-mycobacterial activity suggests the presence of a pyrazine ring with an acyl moiety (Zimhony et al., 2007).

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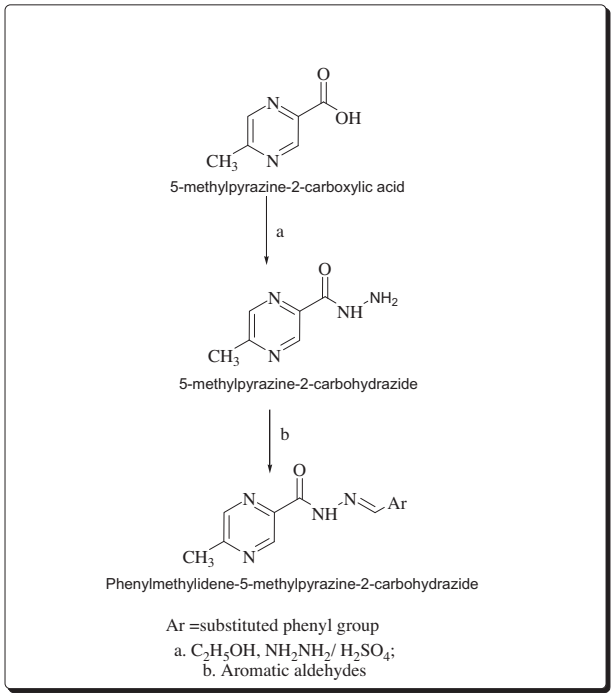

Scheme 1 PM series.

In the present study, pyrazinoic acid hydrazides (Fig. 1b) (Miniyar and Bhat, 1999) which are active against *Mycobacterium tuberculosis*, an attempt has been made to condense various substituted aromatic aldehydes to explore the possibilities of their activity against *M. tuberculosis* (Vergara, 2009).

2. Results and discussion

2.1. Chemistry

The synthesis of PM series compounds involved a three-step process, in the first step 5-methylpyrazinoic acid was concerted into 5-methylethylpyrazinoate in the presence of ethanol and catalytic amount of Conc. H_2SO_4. The obtained 5-methylethylpyrazinoate was converted into 5-methylpyrazinoic acid hydrazide by using hydrazine hydrate (99%). Finally, the 5-methylpyrazinoic acid hydrazide was condensed with different substituted aromatic aldehydes in the presence of ethanol yielding various substituted Phenylmethylidene-5-methylpyrazine-2-carbohydrazide derivatives (see Scheme 1) (Table 1).

2.2. Biological activity

The anti-TB activity of PM series against *M. tuberculosis* H37RV strain was performed by the Middlebrooke 7H-9 method. Compound PM 14 (5-methyl-N′-{[4-dimethylamino) phenyl] methylidene} pyrazine-2-carbohydrazide) was found to be more promising against *M. tuberculosis* amongst the compounds tested at concentration 10–50 μg/mL, whereas compounds PM 5–7, 11–13 (Table 2) were moderately active between 25 and 50 μg/mL concentration as compared with the standard anti-TB agents and the −log MIC activity was found in the range of 1.011–1.274. The most active compound PM 14 showed maximum activity 1.409 and was found to be more sensitive than INH (1.137) and PZA (1.115) standard

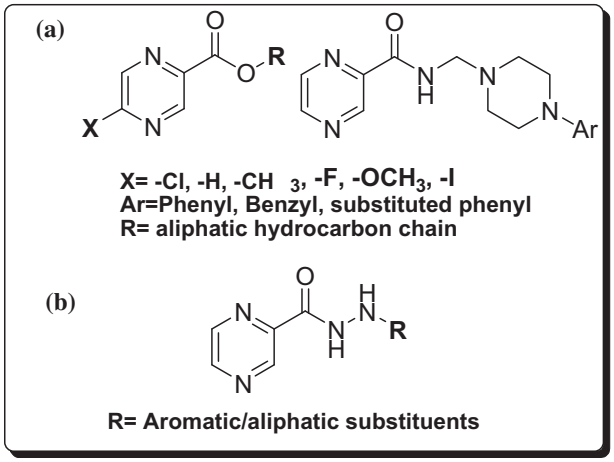

Figure 1 Pyrazine derivatives reported for anti-tubercular activity.

anti-TB agents. It is clearly observed that 5-methyl substitution has a certain role in the activity result.

2.3. Acute toxicity studies

The lethal dose (LD50) value of PM 14 anti-TB compounds from the PM series was determined in albino rats as per OECD (Organization of Economic and Co-operation Development) guidelines. As there were no signs of mortality or any clinical abnormality, the compounds were categorized under GHS (Globally Harmonized System) Category 5, >2000–5000 mg/kg body weight, with LD50 cut-off at 2500 mg/kg body weight.

2.4. 2D QSAR study

The 2-D QSAR experiment was performed using the software, TSAR 3.3, Accelerys, USA on the synthesized compounds. Multiple linear regression analysis was performed on the present series keeping biological activity as dependant variables. Various regression equations were generated, but the equation, which showed good correlation between the physicochemical property and their biological activity, was selected.

The 2-D QSAR experiment (Fig. 2) illustrates the design of PM series of compounds as potential anti-tubercular scaffolds that can be further optimized to improve the activity.

The best QSAR equation that shows statistically significance parameter is shown below.

$$\log BA = -0.50514621(\text{Inertia Moment 3 Length whole molecule}) + 0.24788234(\text{Kier Chi 0 atoms Index whole molecule}) - 0.94991529$$

R	r^2	r^2cv	F	s
0.8500	0.7225	0.6390	14.32	0.1428

The above equation revealed that the following two parameters contributed for the potential anti-TB activity. Kier chi indices are the connectivity indices, which define the position of the substituents on the aromatic ring system present in the molecule. Consider the examples of most active molecules

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