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ORIGINAL ARTICLE

Synthesis and molecular modeling studies of novel pyrrole analogs as antimycobacterial agents

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Abstract In the present investigation, a series of 4-(4-pyrrol-1-yl/2,5-dimethyl-4-pyrrol-1-yl) benzoic acid hydrazide analogs, some derived oxadiazoles and azines have been synthesized in good yields and structures of these compounds were established by IR, ^{1}H NMR, ^{13}C NMR, mass spectral and elemental analysis. The newly synthesized title compounds were evaluated for their antimicrobial as well as antimycobacterial activities. Among the tested compounds, **6j** and **9c** displayed promising anti-tubercular activity. Further, some compounds were also assessed for their cytotoxic activity (IC_{50}) against mammalian Vero cell lines and A_{549} (lung adenocarcinoma) cell lines using the MTT assay method. The results revealed that these compounds exhibit anti-tubercular activity at non-cytotoxic concentrations. The docking of inhibitors into InhA using Sybyl-X 2.0 software revealed the vital interactions and binding conformation of the inhibitors.

1. Introduction

Tuberculosis (TB) is a deadly disease caused by mycobacteria of the "tuberculosis complex", including primarily *Mycobacterium tuberculosis*, but also *Mycobacterium bovis* and *Mycobacterium africanum* (Wolinsky, 1992; Sensi and Grass, 1996). TB has re-emerged as one of the leading causes of death worldwide (nearly 3 million deaths annually) in the last decade (Bloom and Murray, 1992). There will be an estimation of 1.3 million multi/extensively drug resistant TB (M/XDR-TB) cases that need to be treated between 2010 and 2015 (WHO, 2010).

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Due to this increased microbial resistance, new classes of antimicrobial agents with novel mechanisms are today's need to fight against the multidrug-resistant infections.

One of the most effective first-line anti-TB drugs is isoniazid. Many isoniazid analogs have been synthesized and tested as antimycobacterials. In a critical review published recently, the existence of more than 3000 compounds based on the INH core was reported, about 66% of them being hydrazones (Ballell et al., 2005; Janin, 2007).

M. tuberculosis contains unique signature fatty acids, the mycolic acids, that are unusually long chain α-alkyl, β-hydroxy fatty acids of 60–90 carbons (Takayama et al., 2005). The antitubercular drugs isoniazid (isonicotinic acid hydrazide (INH)) and ethionamide have been shown to target the synthesis of these mycolic acids, which are central constituents of the mycobacterial cell wall. Among the enzymes involved in FAS-II, the NADH-dependent enoyl-ACP reductase encoded by the *Mycobacterium* gene *InhA* is a key catalyst in mycolic acid biosynthesis. Studies over the years have established that InhA is the primary molecular target of INH (Banerjee et al., 1994), the drug for the past 40 years has been, and continues to be, the frontline agent for the treatment of tuberculosis.

Molecular modeling and pharmacokinetic studies have confirmed that the introduction of the 1,3,4-oxadiazole ring to the inhibitors can change their flexibility, polarity as well as metabolic stability, and 1,3,4-oxadiazole scaffold can also act as acceptors of hydrogen bond formation, which makes it possible to be used as an isosteric substituent for amide or ester groups (Guimaraes et al., 2005; Joshi et al., 2013a,b). Several phthalazine derivatives have been reported to possess antitumor (Loh et al., 2005; Sung Kim et al., 2004), antihypertensive (Demirayak et al., 2004; Watanabe et al., 1998), anticonvulsant (Kornet and Shackleford, 1999; Nassar, 1997), antidiabetic (Madhavan et al., 2001; Lenz et al., 2002), antimicrobial (Cardia et al., 2003), antimycobacterial (Sriram et al., 2010), and anti-inflammatory activities (Napoletano et al., 2000; Chakraborti et al., 2003). Nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which are part of many natural products, fine chemicals, and biologically active pharmaceuticals. This prompted us to explore the biological activity of such molecules through structural modifications. In the present study, synthesis, molecular modeling and evaluation of potential InhA inhibitors having pyrrole as the core structure are attempted. Other chemotherapeutically-active groups into the designed structures are introduced, with a hope to impart synergism to the target compounds (Fig. 1). Surflex-Docking was applied to study the interactions between 36 pyrrole-shiffbases, -oxadiazoles, -phthalazines/pyridazines and InhA. These developed models can help in understanding the SAR of the pyrrole derivatives and can also serve as a valuable guide for the design of novel inhibitors with robust potency (Fig. 2).

2. Chemistry

Synthesis of scaffolds is depicted in Schemes 1 and 2. The key intermediate in the present study is the 4-pyrrol-1-yl benzoic acid hydrazide **4**, which was prepared by hydrazinolysis of the ethyl ester of 4-pyrrol-1-yl benzoate **3** with hydrazine hydrate. The reaction of **4** with different aldehydes in alcohol gave Schiff bases **5a–j**. Cyclization of **5a–j** with acetic anhydride afforded 1,3,4-oxadiazole derivatives **6a–j.** 4-Pyrrol-1-yl benzoic acid hydrazide **4** on condensation with different anhydrides afforded phthalazine and pyridazine derivatives **7a–h.** The compounds **9a–h** were synthesized by treating 4-(2,5-dimethylpyrrol-1-yl) benzoic acid hydrazide **8** with different anhydrides.

3. Biological activity

3.1. Antibacterial studies

Standard bacterial strains were procured from the American Type Culture Collection (ATCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The compounds **5a–j**, **6a–j**, **7a–h** and **9a–h** were evaluated for *in vitro* antimicrobial activities by the broth micro-dilution method (Goto et al., 1981; Villanova, 1985) against the following standard bacterial strains: *Staphylococcus aureus* (ATCC 11632), *Bacillus subtilis* (ATCC 60511), *Escherichia coli* (ATCC 10536) and *Vibrio cholera* (recultured). The MICs were determined for **5a–j**, **6a–j**, **7a–h** and **9a–h**; these results are summarized in Table 1.

3.2. Anti-tubercular studies

Anti-tubercular activities of all the compounds were assessed against *M. tuberculosis* $H_{37}Rv$ and MDR-TB using the Microplate Alamar Blue Assay (MABA) method (Franzblau et al., 1998) with the observed MICs given in Table 2. The method used is nontoxic, uses a thermally stable reagent and shows a good correlation with proportional and BACTEC radiometric methods.

4. Results and discussion

4.1. Chemistry

Synthetic strategies adopted to obtain the target compounds are depicted in Schemes 1 and 2. FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral data are in agreement with the proposed structures of all the synthesized compounds.

The hydrazones **5a–j** obtained from the hydrazide **4** showed carbonyl amide stretching in 1653–1636 cm^{-1} region and N–H bands in 3271–3192 cm^{-1} region. ^{1}H NMR and ^{13}C NMR data were also in agreement with the formation of hydrazones. ^{1}H NMR spectrum of **5g** showed two singlets at δ 8.44 and at δ 11.94, which were attributed to the N=CH and NH protons, respectively.

IR spectra of **6a–j** had different characteristics as they showed no NH stretching bands and only C=O bands in 1668–1662 cm^{-1} region which was attributed to the C=O stretching of the acetyl group. 1,3,4-oxadiazoline derivative **6g** obtained from the hydrazone **5g** gave a singlet at δ 7.14 in the ^{1}H NMR spectrum which was attributed to the O–CHR–N resonance of the oxadiazoline ring and a singlet at δ 2.31 was assigned for acetyl CH_3. The formation of the oxadiazolines was further supported by the ^{13}C NMR data of compound **6a–j.** While the N=CH carbon of compound **5g** in hydrazone structure was observed at δ 148.30 in its ^{13}C NMR spectra; the same carbon atom (OCHR–N) **6g** was observed at δ 83.79 after cyclization of the oxadiazoline ring.