Bioorganic & Medicinal Chemistry Letters 26 (2016) 1776–1783

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis, biological evaluation and molecular docking study of some novel indole and pyridine based 1,3,4-oxadiazole derivatives as potential antitubercular agents

N. C. Desai [a,*], Hardik Somani [a], Amit Trivedi [a], Kandarp Bhatt [a], Laxman Nawale [b], Vijay M. Khedkar [b], Prakash C. Jha [c], Dhiman Sarkar [b]

[a] Division of Medicinal Chemistry, Department of Chemistry (DST-FIST Sponsored), Mahatma Gandhi Campus, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364 002, Gujarat, India
[b] Combi Chem Bio Resource Centre, National Chemical Laboratory, Pune 411 008, India
[c] School of Chemical Sciences, Central University of Gujarat, Sector-30, Gandhinagar 382 030, Gujarat, India

ARTICLE INFO

Article history:
Received 24 September 2015
Revised 10 February 2016
Accepted 16 February 2016
Available online 16 February 2016

Keywords:
Indole
1,3,4-Oxadiazole
Pyridine
Antitubercular activity
Molecular docking
Cytotoxicity

ABSTRACT

A series of indole and pyridine based 1,3,4-oxadiazole derivatives **5a–t** were synthesized and evaluated for their in vitro antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$ (MTB) and *Mycobacterium bovis* BCG both in active and dormant state. Compounds **5b**, **5e**, **5g** and **5q** exhibited very good antitubercular activity. All the newly synthesized compounds **5a–t** were further evaluated for antiproliferative activity against HeLa, A549 and PANC-1 cell lines using modified MTT assay and found to be noncytotoxic. On the basis of cytotoxicity and MIC values against *Mycobacterium bovis* BCG, selectivity index (SI) of most active compounds **5b**, **5e**, **5g** and **5q** was calculated (SI = GI_{50}/MIC) in active and dormant state. Compounds **5b**, **5e** and **5g** demonstrated SI values $\geqslant 10$ against all three cell lines and were found to safe for advance screening. Compounds **5a–t** were further screened for their antibacterial activity against four bacteria strains to assess their selectivity towards MTB. In addition, the molecular docking studies revealed the binding modes of these compounds in active site of enoyl reductase (InhA), which in turn helped to establish a structural basis of inhibition of mycobacteria. The potency, low cytotoxicity and selectivity of these compounds make them valid lead compounds for further optimization.

Tuberculosis (TB) remains a major global health problem being the second leading cause of death from infectious diseases worldwide, after the human immunodeficiency virus (HIV). Conventional treatments fail either because of poor patient compliance to the drug regimen or due to the emergence of multidrug-resistant tuberculosis (MDR-TB). *Mycobacterium tuberculosis* (MTB) infection is difficult to treat, requiring 6–9 months of chemotherapy with a cocktail of four antibiotics isoniazid, rifampin, pyrazinamide and ethambutol. In addition to toxic side effects, the lengthy treatment regime results in poor patient compliance and thus drug resistant strains are beginning to emerge.[1] In recent years, the emergence of MDR and XDR (extensively drug-resistant) tuberculosis strains has amplified the incidences of TB. In addition, TB is a co-infection of HIV-AIDS (Acquired Immune Deficiency Syndrome) and accounts for 26% of AIDS related death worldwide.[2–6] In 2013, an estimated 9 million people were affected by *M. tuberculosis* and 1.5 million died from the disease, including 360,000 deaths among HIV-positive patients.[7] To address these problems, it has become inevitable to design, synthesize and develop effective anti-mycobacterial agents are compulsory.

The indole moiety is probably the most widely spread nitrogen heterocycle in nature. It is an essential part of the amino acid tryptophan and the neurotransmitter serotonin, and the indole scaffold is also found in numerous naturally occurring plant based alkaloids. The biological importance of indole heterocycles and their pharmacological and medical potential have made them extremely attractive and rewarding research targets and these qualities have motivated countless researchers to study their synthesis and pharmacological properties.[8] The biological activities of indoles cover a wide spectrum, including anticancer,[9,10] antimicrobial,[10] anti-inflammatory,[11] antimalarial,[12] cytotoxic[12] and antitubercular[13] activities.

The pyridine motif is among the most common *N*-hetero aromatics incorporated into the structure of various therapeutic agents. Many naturally occurring and synthetic compounds

* Corresponding author. Tel./fax: +91 278 2439852.
E-mail address: dnisheeth@rediffmail.com (N.C. Desai).

http://dx.doi.org/10.1016/j.bmcl.2016.02.043
0960-894X/

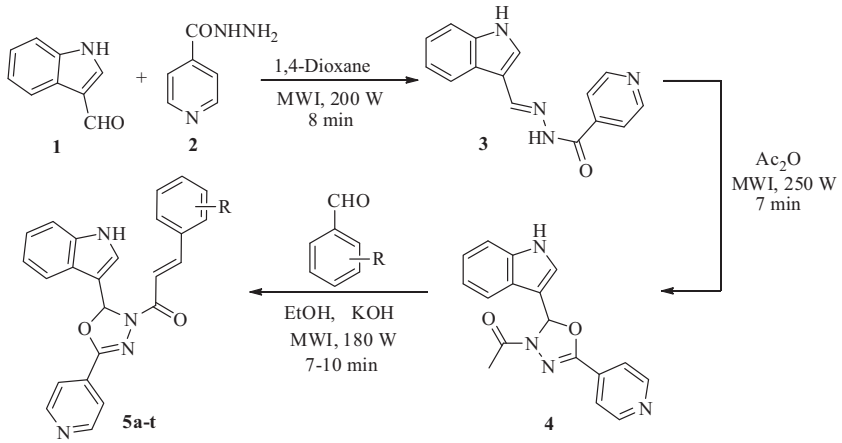
bearing pyridine scaffold possess interesting biological properties. Compounds containing pyridine ring display a broad spectrum of biological activities, including anticancer,[14] antimicrobial,[15] anticonvulsant,[16] antibacterial,[17] anti-inflammatory,[18] antitumor[19] and antiviral[20] activities. On the other hand, compounds containing 1,3,4-oxadiazole ring display a broad spectrum of biological activities, including anticancer,[21] antimicrobial and cytotoxic,[22] anticonvulsant,[23] antiepileptic,[24] antitubercular[25] and anti-allergic[26] activities. 1,3,4-Oxadiazole is a good bioisostere of amide and ester functional groups and is reported to contribute substantially to pharmacological activity by participating in hydrogen bonding interactions with various receptors.[27] On the basis of the principle of combination of active structural moieties, it is reported that substitution by oxadiazoles at the 3-position of the indole nucleus enhances the biological activities.[28,29]

In an effort to prepare better therapeutic agents for the treatment of tuberculosis and in continuation to our previous work,[30–34] amalgamation of three biologically versatile heterocyclic scaffolds like indole, pyridine and 1,3,4-oxadiazole in single molecular platform was undertaken. To establish structure activity relationship and for the development of new antitubercular agents, the synthesized compounds were screened for their in vitro antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$ and *Mycobacterium bovis* BCG. Furthermore, molecular docking studies helped in revealing the mode of action of these compounds through their interactions with the active site of the *Mycobacterium tuberculosis* enoyl reductase (InhA) enzyme.

Synthesis of the target compounds **5a–t** was achieved through the pathway illustrated in Scheme 1. Indole-3-carbaldehyde (**1**) was taken as starting material and reacted with isoniazid (**2**) to afford *N*′-((1*H*-indol-3-yl)methylene)isonicotinohydrazide (**3**), which on cyclization with acetic anhydride yielded intermediate 1-(2-(1*H*-indol-3-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl) ethanone (**4**). The intermediate obtained was heated with an appropriately substituted aldehyde derivatives in ethanol (99.9%) furnished the desired compounds **5a–t**. The structures of all these newly synthesized compounds **5a–t** were confirmed by IR, NMR, mass spectral and C, H, N elemental analyses and were in full agreement with proposed structures. Formation of final compounds **5a–t** were confirmed by characteristic IR spectrum absorption bands in the range of 3370–3420 cm^{-1} and 1660–1680 cm^{-1} corresponding to —NH stretching of indole and >C=O respectively. Singlets at δ 6.50–6.70 and 10.10–10.40 ppm in ^1H NMR correspond to protons of 1,3,4-oxadiazole and —NH of indole respectively. The aromatic ring protons and β-unsaturated ketone were observed in the range of δ 6.90–8.10 ppm. Characteristic peaks at δ 167.0–167.5 ppm in ^{13}C NMR confirmed the presence of >C=O in α,β-unsaturated ketone. The mass spectrum of **5a–t** revealed that observed molecular ion peaks were in agreement with molecular weight of respective compounds.

In a standard primary screen, all the newly synthesized compounds **5a–t** were evaluated for their in vitro antitubercular activity against *M. tuberculosis* $H_{37}Ra$ and *M. bovis* BCG at concentrations of 30, 10 and 3 μg/mL using an established XTT Reduction Menadione assay (XRMA) and NR (Nitrate reductase) assay, respectively.[34,35] Compounds showing 90% inhibition of bacilli at or lower than 30 μg/ml were selected for further dose response curve. The drugs in clinical use, rifampicin and isoniazid were used as reference. The results are reported in Table 1. In general, the newly synthesized compounds showed excellent selectivity towards *M. bovis* BCG compared to *M. tuberculosis* $H_{37}Ra$. The antitubercular activity results suggested that none of the compounds showed any significant activity against *M. tuberculosis* $H_{37}Ra$. Compounds **5b**, **5e**, **5g** and **5q** substituted with 2-OH, 2-NO_2, 3-NO_2 and 2,4-$(Cl)_2$ functional groups displayed excellent MICs ranging from 0.94 to 5.17 μg/mL against *M. bovis* BCG. Compound **5b** came out as the most active compound against active *M. bovis* BCG with MIC of 0.94 μg/mL while, compound **5e** was most active against dormant *M. bovis* BCG with MIC of 0.85 μg/mL. Compounds **5g** (active state MIC: 2.5 μg/mL, dormant state MIC: 2.37 μg/mL) and **5q** (active state MIC: 5.17 μg/mL, dormant state MIC: 4.97 μg/mL) were approximately 2–6-fold less active than the most active compounds **5b** and **5e**. From the standpoint of structure activity relationship, it was observed that the antitubercular activity was significantly affected by the substitution pattern at phenyl ring of chalcone moiety. It was observed that presence of —OH and —NO_2 functional groups at 2nd position of phenyl ring was favorable for enhanced antitubercular activity while any alteration in this substitution pattern witnessed a substantial decrease in antitubercular potency.

After identifying a good number of active antitubercular leads, cytotoxicity of all the newly synthesized compounds **5a–t** were tested against three human cancer cell lines, HeLa (human cervical cancer cell line), A549 (human lung adenocarcinoma cell line) and PANC-1 (human pancreas carcinoma cell line) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay[36–38] with paclitaxel as a positive control. The cytotoxicity results presented in Table 2 are expressed in terms of GI_{90} and



R = H, 2-OH, 3-OH, 4-OH, 2-NO_2, 3-NO_2, 4-NO_2, 4-CH_3, 4-OCH_3, 2-F, 3-F, 4-F, 2-Cl, 3-Cl, 4-Cl, 2,3-$(Cl)_2$, 2,4-$(Cl)_2$, 2,6-$(Cl)_2$, 3-Br, 4-Br

Scheme 1. Synthetic route for the preparation of title compounds **5a–t**.