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Original article

Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters

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ABSTRACT

A total of 51 novel benzimidazoles were synthesized by a 4-step reaction starting from basic compound 4-fluoro-3-nitrobenzoic acid under relatively mild reaction conditions. The structure of the novel benzimidazoles was confirmed by mass spectra as well as ^{1}H NMR spectroscopic data. Out of the 51 novel synthesized compounds, 42 of them were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* H_{37}Rv strain using BacTiter-Glo™ Microbial Cell Viability (BTG) method. Results of activity screened using Alamar Blue method was also provided for comparison purposes. Two of the novel benzimidazoles synthesized showed moderately good activity with IC_{50} of less than 15 μM. Compound **5g**, *ethyl 2-(4-(trifluoromethyl)phenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate*, was found to be the most active with IC_{50} of 11.52 μM.

1. Introduction

Tuberculosis (TB) is the oldest documented infectious disease. It is a chronic necrotizing bacterial infection with wide variety of manifestations caused by *Mycobacterium tuberculosis*, which has plagued humans throughout recorded and archeological history [1]. The primary site of infection is the lungs, followed by dissemination *via* the circulatory and lymphatic system to secondary sites including the bones, joints, liver and spleen.

In 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases of TB, 1.1 million (range, 0.9–1.2 million) deaths from TB among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) death from HIV-associated TB [2]. The introduction of the first line drugs like streptomycin, para-aminosalicylic acid and isoniazid for treatment some 50 years ago has witnessed in a remarkable decline in TB cases all over the world. The active TB is currently treated with a four first-line drug regimen comprising mainly isoniazid, rifampicin, pyrazinamide and ethambutol for a period of at least 6 months [3,4]. However, it did not take long for *M. tuberculosis* to find its way around these compounds, and since the mid of 1980s, the disease has been undergoing a resurgence driven by variety of changes in social, medical and economic factors as well as *M. tuberculosis*' resistance to the above-mentioned drugs itself. Despite extensive research in the last 40 years, no new anti-TB drugs have been introduced into the market by means of passing actual clinical trials [5]. Although a new TB drug, Bedaquiline, was released in USA [6] in December 2012, the trial was based on paradoxical surrogate measure to gain "fast track" approval by USFDA. However, decisions making under such time pressure may lead to unanticipated safety problems as shown by the higher chances of death by patients taking this drug, indicating treatment failure [7].

The benzimidazole nucleus is of significant importance in medicinal chemistry research and many benzimidazole-containing compounds exhibit important biological properties such as antiviral [8], anti-inflammatory [9] and anti-HIV [10]. In the light of the affinity they display toward a variety of enzymes and protein receptors, medicinal chemists thus classify them as "*privileged substructures*" for drug design [11].

Recently, there have been reported work done on utilizing benzimidazole derivatives to counter TB with relatively good results [12,13]; thus further reinforcing our belief that benzimidazole could potentially be a lead compound in our effort to discover new potent anti-TB agents.

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In the present paper, we wish to report the synthesis and antimycobacterial activity of novel 2-substituted benzimidazole derivatives.

2. Results and discussion

2.1. Chemistry

The procedure to synthesize benzimidazole derivatives was adopted and modified from literature [14]. Our synthetic study into novel benzimidazoles started with 4-fluoro-3-nitro benzoic acid which was esterified in the presence of catalytic sulfuric acid in ethanol by refluxing for 8 h to afford the ethyl ester **1** in 75% yield. The ethylbenzoate **1** was then treated with various amines (see Experimental section) and DIPEA in dry dichloromethane at room temperature yielded amino compound **2**, which was reduced to the amine **3** using ammonium formate and 10% Pd/C for 3 h to give 60% yield. The structure of 3-amino ethylbenzoate **3** was confirmed by chromatographic analysis.

The phenylenediamine **3** was then refluxed with various substituted bisulfite adduct of aromatic aldehydes [15] in DMF overnight to afford benzimidazole derivatives **5**–**7** in moderate to good yields (38–90%). The structure of the novel benzimidazoles was confirmed by spectroscopic analysis and further unambiguously ascertained by single X-ray crystallographic analysis [16–19]. Among the literature reports available for the synthesis of benzimidazoles by the reaction of phenylenediamine with acid chloride [20], aldehyde [21] and acid [22], we found that access into benzimidazole derivatives *via* this metabisulfite route is efficient, environmental friendly and afforded good to excellent yield of the benzimidazoles.

The ^{1}H NMR spectrum of benzimidazole **5g** showed a singlet at δ 1.43 ppm due to the methyl group. The *N*-methylene protons from the piperazine appeared as a triplet at δ 2.33 ppm while the *O*-methylene showed a triplet at 3.55 ppm. Similar ^{1}H patterns were obtained for other substituted benzimidazoles derivatives. Proton NMR assignment for **5g** was shown as representation for the other compounds in the series (Fig. 1). The ^{13}C NMR spectrum of **5g** which resonated at δ 150.81 and 167.36 ppm is assigned to imine (C=N) and ester carbonyl carbon respectively (Tables 1 and 2).

The mechanism for the formation of the novel benzimidazole derivatives is proposed and summarized in Scheme 1.

2.2. Pharmacology

A total of 51 novel benzimidazole derivatives were synthesized and 42 of them were then analyzed for their antimycobacterial activities against *M. tuberculosis* $H_{37}R_V$ (MTB-H_{37}Rv). Another 9 compounds were obtained in gel-like form. As there might be ambiguity over the trace amount of solvent effect and the consistency of the weight from gel-like compounds, we omitted those compounds from further biological activity testing. Results are shown in Table 3.

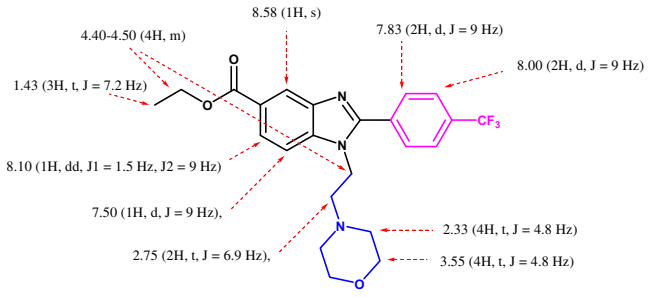

Fig. 1. ^{1}H and ^{13}C NMR chemical shifts of titled compound.

In vitro antimycobacterial activity of the compounds was evaluated against *M. tuberculosis* $H_{37}R_V$ (MTB-H_{37}Rv) in a HTS (High Throughput Screening) assay adapted from the microdilution AlamarBlue (AB) broth method as reported by Collins and Franzblau [23]. For comparison, an alternative method for end-point detection was assessed using the Promega reagent BacTiter-Glo™ Microbial Cell Viability (BTG). The BTG assay is a quantitative ATP assay for bacteria using luciferase production as an end-point detection point. Data was analyzed using the IDBS Activity Base software and the dose response result was analyzed using a four parameter logistic fit to the data (Excel Fit equation 205) with the maximum and minimum locked at 100 and 0. From these curves, EC_{90} and EC_{50} values were calculated. As references, six standard drugs used for TB treatment (Amikacin, Cycloserine, Ethambutol, Isoniazid, Pyrimethamine and Rifampicin) were also evaluated in the assays.

Comparing the three series of substitution at the 2-position of the benzimidazole core, it can be concluded that 4-(2-aminoethyl) morpholine gave the best activity. This could be due to the fact that heterocyclic moiety, such as morpholine group are biologically active. It also showed that electron donating groups somehow give rise to better antimycobacterial activities, which is consistent with those reported in literature [24,25]. Thus, a further modification of the 4-substitution on the sodium bisulfite adduct was carried out to further improve the activity. We synthesized compounds with a wide range of substitution including compounds with electron-donating as well as electron-withdrawing groups. Generally, we found that electron withdrawing group substituents at 4-position in the phenyl ring is important for good activities as shown by **5g**, **5b**, **5e** and **5p**. Of all 42 compounds which have been tested, compound **5g**, *ethyl 2-(4-(trifluoromethyl)phenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate* was found to be the most active with IC_{50} of 11.52 μM, IC_{90} of 16.53 μM and MIC of 50 μM respectively. It was followed closely by **5b**, *ethyl 2-(4-bromophenyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazole-5-carboxylate*, with IC_{50} of 12.54 μM, IC_{90} of 14.48 μM and MIC of 50 μM respectively.

Both these compounds are more active than the standard drugs cycloserine and pyrimethamine. However none of the compounds screened in the present derivatives are found to be more potent than other used standard drugs. This clearly showed that the presence of electron donating group substitution at 4-position of the phenyl ring, caused marked improvement in antimycobacterial activity.

All the compounds were also tested for cytotoxicity (IC_{50}) in VERO cells at concentrations of 62.5 μg/mL. After 72 h of exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 nonradioactive cell proliferation assay according to the manufacturer's protocol. All of the active compounds were found to be non-toxic till 62.5 μg/mL.

Encourage by the positive results we have reported here, further modification on the 2-susbstituted position on the benzimidazole core as well as 4-position on the bisulfite adducts as currently in progress in our laboratory.

3. Experimental

3.1. Chemistry

All chemicals were supplied by Sigma–Aldrich (U.S.A) and Merck Chemicals (Germany). Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the