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Novel amide and sulphonamide derivatives of 6-(piperazin-1-yl) phenanthridine as potent *Mycobacterium tuberculosis* H37Rv inhibitors

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ABSTRACT

A series of thirty three novel 6-(piperazin-1-yl)phenanthridine amide and sulphonamide analogues were synthesized, characterized and screened for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) H37Rv strain. These compounds exhibited minimum inhibitory concentration (MIC) between 1.56 and ≥50 μg/mL. Out of these derivatives, few compounds **6l**, **6r**, **7b**, **7f**, **7g** and **7k** exhibited moderate activity (MIC = 6.25 μg/mL) and compounds **6b**, **6e**, **6k**, **6n**, **7h**, **7i** and **7n** displayed good activity (MIC = 3.13 μg/mL), whereas compounds **6m, 6s** and **7d** exhibited excellent anti-tubercular activity (MIC = 1.56 μg/mL). In addition, MTT assay was accomplished on the active analogues of the series against mouse macrophage (RAW 264.7) cells to evaluate the toxicity profile of the newly synthesized compounds and selectivity index of the compounds was determined. Additionally, compounds **6b** and **7d** were docked to the ATPase domain of *M. tuberculosis* GyrB protein to know the interaction profile and structures of compounds **6b** and **7d** were further substantiated through single crystal XRD.

1. Introduction

Tuberculosis (TB) is a contagious disease caused by tubercular bacilli. In spite of several efforts by scientists across the world, this ancient scourge cannot be curtailed. It remains as one of the major public health concerns after the human immunodeficiency virus. Worldwide in 2013, 9 million people fell ill with TB, including 1.1 million cases among people living with HIV. 1.5 million died from TB, including 0.36 million who were HIV-positive. An estimated 0.48 million people developed multidrug-resistant TB (MDR-TB) and there were an estimated 0.21 million deaths from MDR-TB. Around 9.0% of MDR-TB cases have extensively drug-resistant TB (XDR-TB). Around 100 countries have XDR-TB cases. More than 50 companies are involved in the development of new TB drugs. Since last four decades there are no new anti TB drugs except for Bedaquiline and Delamanid which became the first novel TB drugs approved. These two are used only for the treatment of pulmonary MDR-TB patients in serious or life-threatening conditions [1,2]. TB drug discovery and development has not progressed to the extent necessary to annihilate the illness completely. Hence, it is extremely essential to examine new compounds to cure against drug resistant forms of this mortal disease along with minimal side effects. Focus should also be on developing compounds which not only reduce the cost, but also the treatment period.

Phenanthridine derivatives are significant core moieties found in a variety of natural products, important class of alkaloids and other synthetic vital molecules with a wide range of biological activities and applications, including antibacterial [3,4], anticancer [5,6], antimalarial [7], anti-HIV [8], anti-HCV agents [9], antiplasmodial [10] and in particular as antitubercular agents [11–15].

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Recently, Cappoen et al. reported 1,2,3,4,8,9,10,11-Octahydrobenzo [*j*]phenanthridine-7,12-diones as new leads against *Mycobacterium tuberculosis* [11]. Our group also recently reported synthesis and evaluation of anti-tubercular activity of 6-(4-substitutedpiperazin-1-yl) phenanthridine analogues as anti-TB agents [12].

Lipophilicity of compounds plays an important role in the biological activities which they display. Attaching an alkyl/aryl amide and sulphonamides enhances the lipophilicity of compounds [16,17]. Consequently, alkyl/aryl amide and alkyl/aryl sulphonamide derivatives are known to exhibit wide range of pharmacological activities and physiochemical properties. Indeed, these alkyl/aryl amide and alkyl/aryl sulphonamide analogues display enhanced antibacterial and anti-TB activity [17–22].

In the quest for developing novel anti-TB agents we recently reported two compounds with sulphonamide moiety [13]. Interestingly out of the two compounds, one of them exhibited excellent anti-TB activity (MIC = 1.56 μg/mL) and this prompted us to explore further structure activity relationship (SAR) of the compounds and hence prepared this series of compounds based on sulphonamides. Since carboxamides are analogous to sulphonamides, we also decided to synthesise various amide derivatives and study their SAR. Hence, we designed new (substituted) (4-(phenanthridin-6-yl)piperazin-1-yl)methanone and 6-(4-((4- substituted)sulfonyl) piperazin-1-yl)phenanthridine derivatives expecting increased biological activity to combat this fatal disease. Design strategy to achieve title compounds [11–15,17–22] is depicted in Fig. 1.

2. Chemistry

In this report, we chalked out for the synthesis of 6-(4-substitutedpiperazin-1-yl) phenanthridine derivatives starting from 9-fluorenone as outlined in Scheme 1. We synthesized compound **5** according to our reported protocol with slight modification [12]. 9-Fluorenone upon treatment with hydroxylamine hydrochloride and sodium acetate afforded *N*-hydroxy-9*H*-fluoren-9-imine (**2**). Further heating **2** with polyphosphoric acid and phosphorus pentoxide gave phenanthridin-6(5*H*)-one (**3**). 6-Chlorophenanthridine (**4**) was synthesized by refluxing **3** with phosphorus oxychloride and *N*,*N*-dimethylaniline. Cardinal synthon 6-(piperazin-1-yl)phenanthridine (**5**) was prepared by irradiating a mixture of 6-chlorophenanthridine, anhydrous piperazine, triethylamine (TEA) and *N*,*N*-dimethylformamide (DMF) under microwave conditions.

Initially, we tried monitoring the reaction conditions with various bases and solvents as summarised in Table 1. We set off our investigation for the synthesis of **6b**, under various reaction conditions. As a model reaction, compound (**5**) was treated with benzoic acid and various amide coupling reagents were employed to get desired compound (entry 1–11). Initially, we employed TEA as base, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and 1-hydroxybenzotriazole (HOBt) as amide coupling reagents in the presence of dichloromethane (DCM) as solvent at 0 °C to rt. Resultant mixture was stirred for 8 h to yield **6b** in about 58% (entry 1). Changing the solvent to DMF under similar reaction conditions yielded **6b** in about 52%. Alternatively, changed amide coupling reagents such as 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[*4,5-b*]pyridinium 3-oxid hexafluorophosphate (HATU), (Benzotriazol-1-yloxy) tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and base as *N*,*N*-diisopropylethylamine (DIPEA) under similar condition yields are 38–42% (entry 3–5). Next we hope to improve the yields, 1-Propanephosphonic anhydride (T_3P) was employed to alleviate the yield (entry 6–11). Initially, the reaction was carried out with various bases and solvents (entry 6–7) to give the desired compound in moderate yield. The reaction was further optimised with TEA and DCM at various intervals of time (entry 8–11), to give **6b** in good yield. Found entries 9–10 potently improved the yield and actuate reaction period 6–8 h and reputable technique. Having the optimised reaction conditions handy, a series of **19** compounds **6a**–**s** was synthesized in good yield, thus validating the scope of the present protocol.

Further, compound **5** was reacted with various sulfonyl chlorides, using TEA as base and DCM as solvent at 0 °C to rt for 1–2 h to yield **7a**–**n** in good yields. All the title compounds were purified by column chromatography. Both analytical and spectral data (^{1}H NMR, ^{13}C NMR, FTIR, Elemental analysis and MS) of all the synthesized compounds were confirmed prior to their evaluation of antimycobacterial activity.

In general, nucleophilic aromatic substitution at 6-chlorophenanthridine was confirmed by FTIR analysis of key

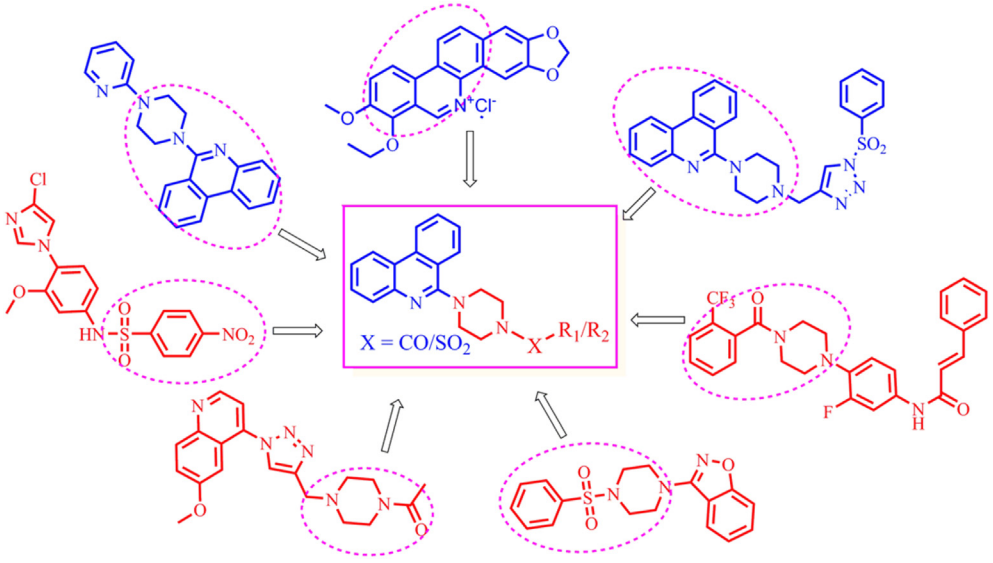

Fig. 1. Design strategy to achieve title compounds.