European Journal of Medicinal Chemistry 79 (2014) 57–65

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Facile synthesis of benzonitrile/nicotinonitrile based *s*-triazines as new potential antimycobacterial agents

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ARTICLE INFO

Article history:
Received 1 November 2013
Received in revised form
28 March 2014
Accepted 31 March 2014
Available online 1 April 2014

Keywords:
s-Triazine
Thiazolidin-4-one
Palladium-catalyzed
Antimycobacterial activity

ABSTRACT

A common strategy to synthesize 4/6-(4-(4-methylpiperazin-1-yl)-6-(4-(4-oxo-2-phenylthiazolidin-3-yl)phenyl)-1,3,5-triazin-2-yloxy)benzonitriles/nicotinonitriles was developed by applying an efficient palladium-catalyzed C–C Suzuki coupling. Moreover, the synthesized compounds were also tested for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$ using BACTEC MGIT and Lowenstein–Jensen MIC methods. Several compounds displayed profound antimycobacterial activity in combination with low toxicity towards mammalian cells. The best results were observed amongst the nicotinonitrile substituted *s*-triazine analogs and it could be a potential starting point to develop new lead compounds in the fight against *M. tuberculosis* $H_{37}Rv$. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis.

1. Introduction

In order to minimize the rapid multidrug-resistance in pathogenic microbes, there is an urgent need to discover and develop new drugs that acts through a novel mechanism of action [1]. In the existing circumstances, Tuberculosis (TB) is a serious global public health problem and a leading cause of death in most of the developing regions of the world. According to the recent WHO global TB report, 1.4 million people have been died from TB, including almost one million deaths among HIV-negative individuals and 0.43 million HIV-positive people in 2011 [2a]. Current control efforts are severely hampered due to *Mycobacterium tuberculosis* (MTB) being a leading opportunistic infection in patients with acquired immune deficiency syndrome and the spreading of multidrug-resistant tuberculosis (MDR-TB) [2b]. Prevention of drug resistance depends on appropriate treatment of TB patients with combination drug regimens and early detection of resistance followed by tailored treatment with second line antimycobacterial agents. These resistant strains are alarming because only a few effective drugs available for the treatment of MDR-TB [3].

Since last two decades, several heterocyclic compounds from the series of *s*-triazine and thiazolidin-4-one have been synthesized, and their pharmacological activity has consequently been investigated. It has been reported that, *s*-triazine ring skeleton possess a broad spectrum of biological and pharmaceutical activities [4–6]. Moreover, thiazolidin-4-one derivatives are also reported to have important biological activities such as anti-inflammatory [7a], antituberculosis [7b], anticancer [8a,7b], anti-tumor [8c], anti-HIV [8d], antibacterial [9a], antifungal [9b], anti-oxidant [9c], antiviral [9d], anticonvulsant [10a], diuretics [10b], nematicidal [10c], antihistaminic [10d] activity etc. Recently, Pathak et al. have synthesized substituted thiazolidin-4-one derivatives (Fig. 1a) and checked for their biological activity. They reported that most of the synthesized compounds possessed significant antimycobacterial activity (0.35–1 μg/mL) against *M. tuberculosis* $H_{37}Rv$ [11]. In our previous study, we introduced various piperazine and piperidine derivatives to the *s*-triazine core (Fig. 1b) and identified the compounds with remarkable antimycobacterial activity [12]. In our continuous effort towards synthesis of new heterocyclic bioactive agents, two series of thiazolidin-4-one fused *s*-triazines were synthesized by applying an efficient palladium catalyzed C–C Suzuki coupling using catalyst system $Pd(OAC)_2$, Xphos and K_3PO_4 as a base in toluene solvent. Synthesized analogs were further tested for antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ to observe the variation in biological activity. All the active compounds were further analyzed for their cytotoxicity against mammalian cells from the VERO line.

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http://dx.doi.org/10.1016/j.ejmech.2014.03.085
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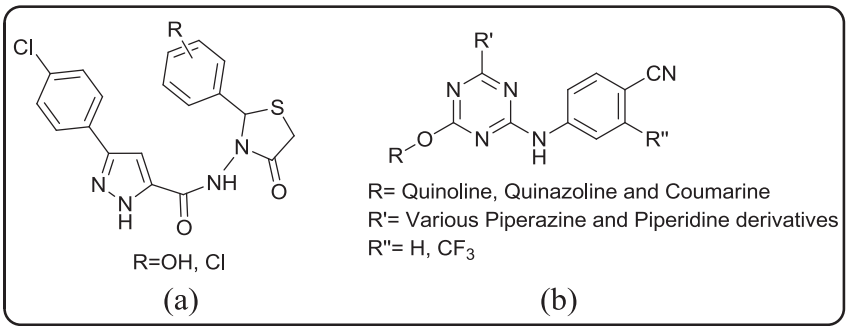

Fig. 1. Lead compounds among *s*-triazine and thiazolidin-4-one derivatives with antimycobacterial activity.

2. Results and discussion

2.1. Chemistry

The first step comprised formation of intermediate **2** by the nucleophilic displacement of one chlorine atom of *s*-triazine ring by *N*-methylpiperazine. The desired coupling agents **3a** and **3b** were synthesized by condensation of 4-hydroxybenzonitrile and 6-hydroxynicotinonitrile with intermediate **2** (Scheme 1). Synthesis of compound **4a**/**4b** involved the Suzuki cross-coupling reaction to facilitate the C–C bond formation between the *s*-triazine ring and phenyl ring. To optimize the reaction conditions for the synthesis of benzonitrile incorporated *s*-triazines, a series of ligands (Fig. 2), palladium sources, bases and solvents were screened for viability of coupling approach. The results of reaction optimization are summarized in Table 1.

To ensure the potency of the C–C coupling reaction, we have preliminary examined the coupling reaction with catalyst system $Pd(OAC)_2$, Xantphos L1, K_3PO_4 in DME at reflux temperature. The desired coupling product was obtained in 69% yield (Table 1, entry 1). The replacement of K_3PO_4 by K_2CO_3 decreased the yield drastically (entry 2). When the reaction was carried out in the presence of KO^tBu or Cs_2CO_3 instead of K_3PO_4, moderate yields of 57% and 63% were obtained respectively (entry 3, 4). Variations on the palladium source $Pd_2(dba)_3$, $[PdCl(allyl)]_2$ and $Pd(PPh_3)_4$ gave lower yields, even with longer reaction times (entries 5–7). However 1,4-dioxane gave almost similar yield to DME (entry 8). When switching to toluene, the product was obtained in higher yield (entries 9) as compared to the polar solvents (entry 10, 11). An assorted set of additional bidentate phosphine ligands such as BINAP L2, Dppf L4 or DPEphos L5 and sterically hindered monodentate ligand such as Xphos L3 were examined under these conditions to determine the efficacy of ligand in supporting a catalytic system for this transformation. As shown in entry (9, 11–15), among all the tested ligands (L1–L5), Xphos L3 proved to be the most effective with the yield of 82% (entry 13). Interestingly, increasing the catalyst loading from 1.5 mol % $Pd(OAC)_2$ to 3.0 mol % reduced the reaction time from 16 h to 13 h (entry 16). Finally, compound **3b** on Suzuki coupling with 4-aminophenylboronic acid pinacol ester with similar reaction conditions gave 86% yield (entry 17).

Finally, the Schiff base derivatives **6a**–**g**/**7a**–**g** were further synthesized by condensing the intermediates **4a**/**4b** with various aldehydes **5a**–**g**. Cyclization of these Schiff bases with thioglycolic acid, gave desired thiazolidin-4-one derivatives **8a**–**g**/**9a**–**g** (Scheme 2). The purity of the desired compounds was checked by TLC and elemental analysis. Spectral data IR, ^1H NMR, ^{13}C NMR and MS of the newly synthesized analogs **8a**–**g** and **9a**–**g** were in full agreement with their proposed structures.

2.2. Pharmacology

2.2.1. In vitro antimycobacterial activity

The investigation of *in vitro* antimycobacterial screening (Table 2) revealed that all the newly synthesized compounds showed moderate to good inhibition at 3.12–25 μg/mL against *M. tuberculosis* $H_{37}Rv$. The primary screening was conducted at a concentration of 6.25 μg/mL using BACTEC MGIT method only for the first selection of active compounds. The results observed from BACTEC MGIT method indicated that benzonitrile based *s*-triazine derivative **8f** and nicotinonitrile based derivative **9d**, **9f** and **9g** exhibited highest inhibition (99%) at a constant concentration level (6.25 μg/mL). These compounds were considered as most potent analogs against mycobacteria and were found to indicate equivalence antituberculosis potency as that of standard drug pyrazinamide. However, the results of secondary biological screening using Lowenstein-Jensen MIC method revealed that nicotinonitrile based *s*-triazine analog **9f** with hydroxy group to thiazolidin-4-one showed inhibition against *M. tuberculosis* $H_{37}Rv$ at 3.12 μg/mL MIC, equipotent to Ethambutol. In addition, two analogs **9d** with fluoro and **9g** with methoxy substituent to thiazolidin-4-one displayed 6.25 μg/mL of MIC. All the remaining derivatives were found to exhibit moderate to poor activity at MIC ranging from 12.5 to 100 μg/mL. From the above results it can be stated that nicotinonitrile based *s*-triazine derivatives were more active against *M. tuberculosis* $H_{37}Rv$ as compared with the benzonitrile derivatives. A close examination of the structures of the active compounds revealed that, their antimycobacterial activity is also bound to the nature of the substituent at the *para* position of the phenyl ring, linked to the thiazolidin-4-one ring. With respect to the cytotoxicity (IC_{50}) in VERO cells at different concentrations, most of the active compounds were found to be non-toxic ($IC_{50} > 50$ μg/mL).

3. Conclusion

In summary, we have developed an efficient catalytic system for the synthesis of various benzonitrile/nicotinonitrile and thiazolidin-4-one incorporated *s*-triazine analogs by applying palladium-catalyzed C–C Suzuki coupling using an efficient catalyst system $Pd(OAC)_2$, Xphos and K_3PO_4 as base in toluene. Several compounds have displayed remarkable *in vitro* antimycobacterial activity (MIC, 3.12–25 μg/mL) against *M. tuberculosis* $H_{37}Rv$ in combination with low toxicity towards mammalian cells. The bioassay results also reveals that, nicotinonitrile ring is essential for bioactivities in most of the cases. Finally, these compounds represent new structure scaffolds that could be further optimized for future development of more potent and selective antimycobacterial agents.