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Original article

Synthesis, crystal structure and biological evaluation of substituted quinazolinone benzoates as novel antituberculosis agents targeting acetohydroxyacid synthase

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ABSTRACT

Acetohydroxyacid synthase (AHAS) catalyzes the first essential biosynthetic step of branched-chain amino acids and is a biologically safe target against *Mycobacterium tuberculosis* (MTB). In our previous research, we used virtual screening to identify some novel AHAS inhibitors as potent antituberculosis agents. In this study, we synthesized twenty-four additional quinazolinone benzoates and explored their antitubercular activity. Five of these compounds displayed significant MTB-AHAS inhibition and their IC_{50} values were determined to be in the range of 6.50 μM–12.08 μM. Importantly, these compounds also exhibited strong in vitro activity (MICs in the range of 2.5–10 mg/L) and intracellular activity against clinically isolated extensively drug-resistant strains of *M. tuberculosis*. Taken together, these results indicated that the quinazolinone benzoate compounds should be regarded as promising lead compounds for the development of potent antituberculosis drugs with a novel mode of action.

1. Introduction

Mycobacterium tuberculosis (MTB) infection has become an increasing health threat due to the worldwide emergence of multidrug-resistant MTB (MDR-MTB) and extensively drug-resistant MTB (XDR-MTB). According to the 2013 global tuberculosis report released by the World Health Organization, 450,000 new cases of MDR-TB were estimated to have occurred in 2012 [1]. Drug-resistance in pathogens is generally caused by target gene mutation, resulting in clinical drugs, such as rifampin and isoniazid, no longer being effective (Fig. 1A and B). It is therefore an urgent task to discover and design novel antitubercular drugs that work on different biological targets and take effect by new inhibitory mechanisms [2].

Acetohydroxyacid synthase (AHAS, EC 2.2.1.6, also referred to as acetolactate synthase, ALS) is the first enzyme in the biosynthetic pathway of branched-chain amino acids (BCAAs), and is a biologically safe target for several families of commercial herbicides [3–12]. In 1998, Grandoni et al. showed that the commercial AHAS inhibitor sulfometuron methyl inhibited MTB in a mouse model using a daily dosage of 500 mg/kg, suggesting that inhibitors of the BCAA biosynthetic pathway could be potential antituberculosis agents [13]. In 2005, Yoon et al. expressed and characterized the MTB-AHAS catalytic subunit and found that sulfonylurea herbicides were good inhibitors of this enzyme [14]. Nevertheless, subsequent research on sulfunoylureas has been limited, and AHAS inhibition has not been measured [15–17]. The best minimum inhibitory concentration (MIC) data reported was 10 mg/L for sulfometuron methyl (SMM, Fig. 1C), based on the reported method [16,17]. It was not until recently that we identified some novel bacterial AHAS

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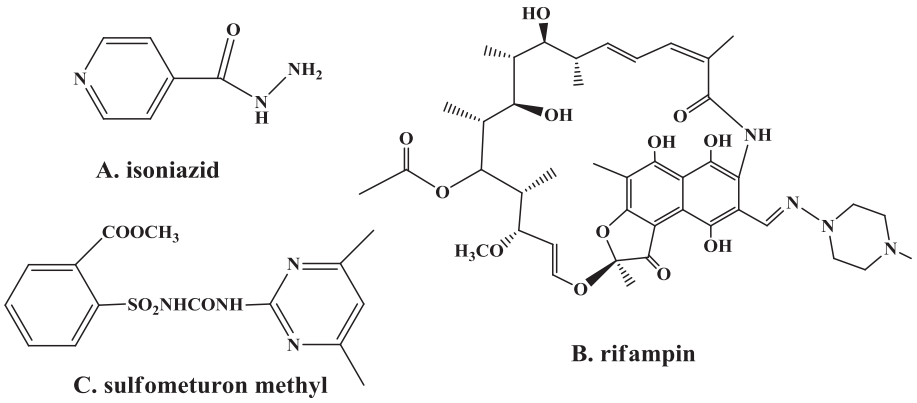


Fig. 1. Structures of isoniazid (A), rifampin (B) and sulfometuron methyl (C).

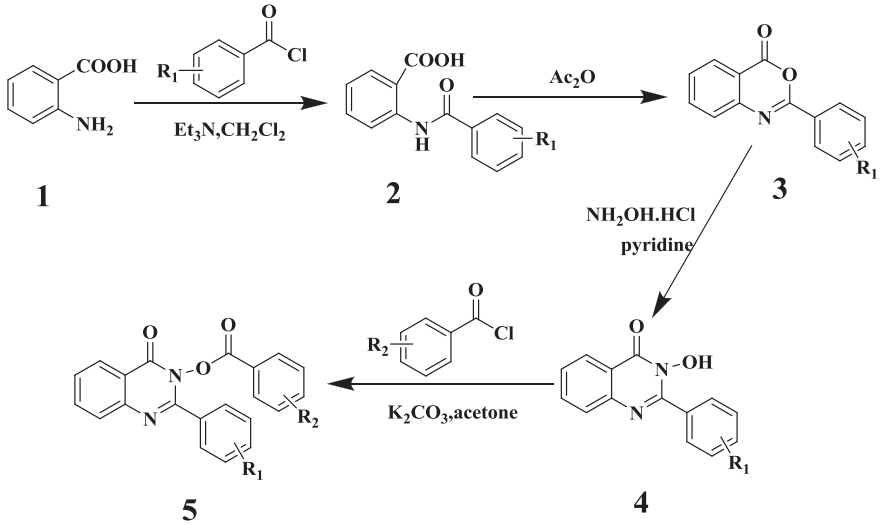
inhibitors via virtual screening based on the MTB-AHAS homology model [18]. The best identified compound inhibited *Escherichia coli* AHAS II significantly and had MIC values for pathogens as low as 2.5 mg/L, which is much better than known sulfonylurea inhibitors. Herein, as a follow-up study, we report a series of new substituted quinazolinone benzoates based on the lead compound and determine both their MTB-AHAS inhibition and antitubercular activities in a cell-based assay.

2. Results and discussion

2.1. Synthesis of the target compounds

The chemical synthesis route for the target compounds is listed in Scheme 1. Quinazolinone benzoate derivatives were synthesized using 2-aminobenzoic acid **1** as a starting material, which reacted with substituted benzoyl chloride in a dichloromethane/triethylamine mixture at room temperature to yield substituted benzoylanthranilic acid **2** [19]. Compound **2** was then refluxed in acetic anhydride to produce compound **3** [20], which further reacted with hydroxylamine hydrochloride under reflux in pyridine to obtain compound **4** [21]. Upon reaction with another substituted benzoyl chloride and potassium carbonate in dried acetone under reflux, target compounds **5a**–**5x** were finally produced [20]. Although most of the intermediates and the final title compounds were easy to synthesize and purify, compounds **4a**–**e** were difficult to isolate from the reaction mixture. The yields in other steps were generally moderate to high (53–93%); however for the synthesis of compound **4**, the yields were very low (9–15%). Most of the chemical intermediates **2a**–**e**, **3a**–**3** and **4a**–**e** have been reported previously [22]. Consequently, we only confirmed their structures by ^{1}H NMR here. The structures of all the target compounds **5a**–**5x** were characterized by melting point determination, ^{1}H NMR, ^{13}C NMR, IR, elemental analysis or ESI-MS.

Several quinazolinone benzoate derivatives were selected for crystallization; however, only **5k** was successfully recrystallized from ethyl acetate/ethanol to give colorless crystals suitable for single-crystal X-ray diffraction. The crystal structure of **5k** is shown in Fig. 2. The linkage of the C16–O2–N2 atom reveals that the quinazolinone benzoate was successfully synthesized. The whole molecule adopts a twisted shape. The quinazolinone and the two phenyl rings are in different planes.



R_1=H, 3-F, 3-CH_3, 3-OCH_3, 2-CH_3
R_2=3-CF_3, 3-Cl, 4-CH_3, 3-CH_3, 3-OCH_3
H, 3-F, 4-Cl, 2-CH_3, 3-NO_2

Scheme 1. Synthesis route of the target quinazolinone benzoate compounds.