Tuberculosis xxx (2016) 1–6

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Characterization of 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone as a novel inhibitor of methionine aminopeptidases from *Mycobacterium tuberculosis*

Sarah F. John [a], Emmanuel Aniemeke [a], Ngan P. Ha [b], Curtis R. Chong [c, d], Peihua Gu [e], Jiangbing Zhou [e], Ying Zhang [e], Edward Graviss [b], Jun O. Liu [f, **], Omonike A. Olaleye [a, *]

[a] Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX 77004, USA
[b] Department of Pathology and Genomic Medicine, The Methodist Hospital Research Institute, Houston, TX 77030, USA
[c] Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
[d] Department of Medicine, Brigham and Women's Hospital, Boston, MA 02115, USA
[e] Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA
[f] Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

Keywords:
HIV
TB
Co-infection
Drug screening
Drug interactions
Drug discovery

A B S T R A C T

Mycobacterium tuberculosis (*Mtb*) and the Human Immunodeficiency Virus (HIV) pose a major public health threat. The 2015 World Health Organization (WHO) report estimates that one in three HIV deaths is due to *Mtb*, the causative agent of Tuberculosis (TB). The lethal synergy between these two pathogens leads to a decline in the immune function of infected individuals as well as a rise in morbidity and mortality rates. The deadly interaction between TB and HIV, along with the heightened emergence of drug resistance, drug-drug interactions, reduced drug efficacy and increased drug toxicity, has made the therapeutic management of co-infected individuals a major challenge. Hence, the development of new drug targets and/or new drug leads are imperative for the effective therapeutic management of co-infected patients. Here, we report the characterization of 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (311), a known inhibitor of HIV-1 replication and transcription as a new inhibitor of methionine aminopeptidases (MetAPs) from *Mycobacterium tuberculosis*: *Mt*MetAP1a and *Mt*MetAP1c. MetAP is a metalloprotease that removes the N-terminal methionine during protein synthesis. The essential role of MetAP in microbes makes it a promising chemotherapeutic target. We demonstrated that 311 is a potent and selective inhibitor of MtMetAP1a and MtMetAP1c. Furthermore, we found that 311 is active against replicating and aged non-growing *Mtb* at low micromolar concentrations. These results suggest that 311 is a promising lead for the development of novel class of therapeutic agents with dual inhibition of TB and HIV for the treatment of TB-HIV co-infection.

1. Introduction

Mycobacterium tuberculosis (*Mtb*) and Human Immunodeficiency Virus (HIV) are both leading causes of mortality globally [1]. The synergistic interaction between *Mtb* and HIV in co-infected individuals has heightened the need for the development of novel chemotherapeutic agents. Unlike other opportunistic infections, *Mtb* the etiological agent of Tuberculosis (TB) remains a major public health threat, affecting an estimated 9.6 million individuals in 2014 [1]. TB is the leading cause of death in individuals affected with HIV, and a significant rise in active TB is seen with individuals who are HIV positive [2,3].

The chemotherapeutic management of TB-HIV co-infection poses a major challenge for patients because of the increased pill burden, toxic effects, and drug-drug interactions [4–7]. The current recommended World Health Organization treatment regimen for individuals affected by *Mycobacterium tuberculosis* is DOT (Directly

* Corresponding author. College of Pharmacy and Health Sciences, Texas Southern University, GH 125, Houston, TX 77004, USA.
** Corresponding author. Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Room 516 Hunterian Building, 725 N. Wolfe Street, Baltimore, MD 21205, USA.
E-mail addresses: joliu@jhu.edu (J.O. Liu), olaleyeoa@tsu.edu (O.A. Olaleye).

http://dx.doi.org/10.1016/j.tube.2016.09.025
1472-9792/

Observed Treatment). The DOT standard regimen requires patients to take isoniazid, a rifamycin (rifampin or rifabutin), pyrazinamide, and ethambutol for two months followed by four to seven months of isoniazid and a rifamycin [8]. HIV treatment regimen requires use of multidrug antiretroviral therapy (ARV). This regimen requires the use of at least three drugs from the five available ARV drug classes [9]. However, because of the increased pill burden placed on these co-infected individuals, noncompliance of the treatment regimen can be a problem [10]. Furthermore, the rifamycin class is an important component for the DOT regimen because of its ability to perturb the growth of slow growing and intracellular *Mtb*, but it is also a potent inducer of hepatic cytochrome P (CYP) 450 and uridine diphosphate gluconyltransferase (UGT) 1A1 enzymes [9,10]. For example, rifampin, is an inducer of CYP3A4 which increases the clearance of the antiretroviral drugs [9,10]. This class also interacts with several antiretroviral drug classes such as protease inhibitors, non-nucleoside reverse transcriptase inhibitors, and CCR5 receptor antagonist, Maraviroc [9].

The emergence of drug-resistant *Mtb* strains is also curbing the effectiveness of current therapies, which has increased cause for concern and new challenges for treatment of both pathogens. A global rise in the incidence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and totally drug-resistant (TDR) strains of *Mtb* in TB-HIV co-infected individuals has further underscored the importance of developing anti-mycobacterials with novel targets [4,11]. While recent studies have provided important insights to the development of new anti-bacterial and anti-viral drugs; discovery and development of a novel class of TB-HIV co-infection inhibitors that are efficacious and selective with improved pharmacologic profiles is vital. Therefore, identification of anti-tuberculosis agents with novel mechanisms of action that also have anti-HIV activity may help address the issue of resistance, reduction in pill burden, reduction in the cost of treatment, and possibly increase patient compliance.

Methionine aminopeptidase (MetAP) is a dinuclear metalloprotease involved in the highly conserved process of N-terminal methionine excision (NME), whereby the initiating methionine is removed from a nascent polypeptide chain [12,13]. The removal of N-terminal methionine is required for a large subset of cellular proteins to undergo important post-translational modification or maintain stability [14]. Furthermore, NME is conserved in both prokaryotes as well as eukaryotes [13,14]. In prokaryotes, the removal of a formyl group by peptide deformylase (PDF) serves as a prerequisite for the subsequent removal of the initiating methionine by MetAP [14]. Previous studies have suggested PDF as a potential target for new chemotherapeutic agents [15–19]. However mutations in the formyltransferase gene have been shown to render PDF nonessential [15–19]. The essentiality of MetAP makes it a promising target for the development of novel antibacterial agents [20,21]. MetAP has been studied as a potential target in rheumatic disease, various forms of cancer, and parasitic and fungal infections [22–30]. Methionine aminopeptidases are classified into two different types: MetAP1 and MetAP2. The latter contains a 60-amino acid insertion distinguishing it from MetAP1 [31]. Furthermore, the functionality and importance of MetAP has been shown in several organisms including *Escherichia coli, Salmonella typhimurium,* and *Mycobacterium tuberculosis* whereby the knockdown of the MetAP gene leads to lethality or decreased viability [32,33]. In *Saccharomyces cerevisiae*, the knockout of MetAP1 leads to slow growth while double knockout of MetAP1 and MetAP2 leads to inviability [34].

The essentiality of MetAP in prokaryotes makes it an attractive target for designing new anti-infective agents. In comparison to a prokaryotes such as *E. coli* that possess only one MetAP-coding gene (*Ec*MetAP1), *Mycobacterium tuberculosis* has two genes encoding type 1 MetAP: *Mt*MetAP1a and *Mt*MetAP1c, which share a 33% sequence similarity at the protein level. Both *Mycobacterium* isoforms have less than 48% and 30% similarity to hMetAP1 and hMetAP2, respectively (Fig. 1). Previously, we demonstrated that *Mt*MetAP1a is an essential enzyme, as knockdown of this gene led to decreased viability of *Mtb* and suggesting its promise as a novel pharmacological target [20].

Herein, we report the serendipitous identification of 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (311), as a novel inhibitor of *Mtb* MetAP from a high-throughput screen. Previously, 311 - an iron chelator [35], has been shown to have anticancer activity through inhibition of ribonucleotide reductase, decreasing cell proliferation [36] as well as antiplasmodial activity [37]. Moreover, Debebe et al. reported that 311 possessed anti-HIV activity through inhibition of HIV-1 transcription by deregulating and inhibiting CDK2 and CDK9 [38]. In this study, we discovered for the first time the potent and selective inhibitory activity of 311 against both *Mt*MetAP1a and *Mt*MetAP1c from *Mtb*. We also determined the effects of 311 against the human MetAP isoforms. We demonstrate that 311 exhibits anti-tubercular activity. Taken together, our observations raised the exciting possibility that 311 could serve as a lead for the development of a promising new chemotherapeutic agent for the treatment of TB-HIV co-infection. The optimization and development of such a new class of dual inhibitory agents could potentially reduce side effects, drug-drug interactions, and the pill burden among other benefits.

2. Materials and methods

2.1. Materials

Middlebrook 7H9, the *Mtb* culture medium was purchased from Becton Dickinson. The 175,000 small molecule compound library was provided by ASDI (Newark, DE). The MtMetAP1a and MtMetAP1c were generated by O.A. Olaleye at Johns Hopkins University School of Medicine and both HsMetAP1 and HsMetAP2 were generated in the J.O. Liu laboratory at the Department of Pharmacology at Johns Hopkins University School of Medicine. We prepared stocks solutions of our compounds in Dimethylsulfoxide (DMSO) purchased from Sigma Aldrich.

2.2. Primary high-throughput screening

A primary high-throughput screen was conducted using MAP-C2 microplate processor (Titertek Instruments, Inc., Huntsville, Alabama USA). As previously described [20], using a 175,000 small molecule compound library we identified 2-hydroxy-1- naphthaldehyde isonicotinoyl hydrazone as an inhibitor of *Mt*MetAP1c. Briefly, the 175,000 compounds at 30 μM were screened in a 384-well plates using a dipeptide chromogenic substrate, methionine-proline coupled to *p*-nitroaniline (Met-Pro-*p*NA) [39]. Each compound was dissolved in DMSO and stored at −20 °C until use. The total reaction volume was 50 μL, and each reaction contained 40 mM HEPES buffer (pH 7.5), 100 mM NaCl, 100 μg/mL BSA, 0.1 U/mL ProAP, 1.5 mM $CoCl_2$, 600 μM substrate (Met-Pro-*p*NA), and 252 nM *Mt*MetAP1c. The enzyme was preincubated with compounds for 20 min at room temperature followed by addition of 600 μM substrate. The reaction was incubated at room temperature for 30 min and monitored at 405 nm on a spectrophotometer. Compounds that showed greater than 30%–40% inhibition were chosen as "hits." The controls that were used were DMSO and drug free media.

Please cite this article in press as: John SF, et al., Characterization of 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone as a novel inhibitor of methionine aminopeptidases from *Mycobacterium tuberculosis*, Tuberculosis (2016), http://dx.doi.org/10.1016/j.tube.2016.09.025