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Design and synthesis of novel anti-tuberculosis agents from the celecoxib pharmacophore

Santosh B. Salunke [a,†], Abul K. Azad [b,c,†], Naval P. Kapuriya [a,d], Joan-Miquel Balada-Llasat [e], Preeti Pancholi [e], Larry S. Schlesinger [b,c,*], Ching-Shih Chen [a,f,*]

[a] Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, United States
[b] Center for Microbial Interface Biology, The Ohio State University, 460 W 12th Avenue, Columbus, OH 43210, United States
[c] Department of Microbial Infection and Immunity, The Ohio State University College of Medicine, Wexner Medical Center, 460 W 12th Avenue, Columbus, OH 43210, United States
[d] Division of Pharmaceutical and Organic Chemistry, M. & N. Virani Science College, Saurashtra University, Rajkot 360005, Gujarat, India
[e] Department of Pathology, The Ohio State University, 410 W. 10th Avenue, Columbus, OH 43210, United States
[f] Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan

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ABSTRACT

The identification of compounds with anti-mycobacterial activity within classes of molecules that have been developed for other purposes is a fruitful approach for the development of anti-tuberculosis (TB) agents. In this study we used the scaffold of celecoxib which exhibits several activities against different pathogens, for the design and focused synthesis of a library of 64 compounds. For the primary screen, we used a bioluminescence-based method by constructing a luciferase-expressing reporter *M.tb* strain which contains the entire bacterial Lux operon cloned in a mycobacterial integrative expression vector. Through the screening of this library, we identified 6 hit compounds with high in vitro anti-mycobacterial activity (IC_{50} ~0.18–0.48 μM). In particular, compounds **41**, **51** and **53** were capable of inhibiting *M.tb* as effectively as the anti-TB drug isoniazid (INH) at 5 μM over a 72-h period, as analyzed by both bioluminescence- and colony forming unit (CFU)-based assays. All hit compounds also showed anti-*M.tb* activities against several multi-drug-resistant (MDR) strains. Most of the hit compounds showed no cytotoxicity for human macrophages at concentrations as high as 40 μM, setting the stage for further optimization and development of these anti-TB hit compounds both ex vivo and in vivo.

1. Introduction

TB is one of the leading human infectious diseases caused by the bacterium *Mycobacterium tuberculosis* (*M.tb*).[1,2] It remains a major world-wide public health problem, especially in third-world countries. According to the World Health Organization (WHO), TB incidence and prevalence were estimated to be 8.7 and 12 million cases, respectively, in 2011.[3] Approximately, 1.4 million people died from TB in the same year.[3] Most importantly, one-third of the world population has latent TB infection and 10% of those infected people will develop active TB at some point during their life time. Furthermore, multi drug-resistant (MDR) TB has become increasingly prevalent, and extremely drug-resistant (XDR) and totally drug-resistant (TDR) forms of TB are also emerging,[4,5] posing a major threat to progress in global TB control. Only 1% of patients with MDR-TB are estimated to be on appropriate drug treatment, with predicted poor treatment outcomes.[6] The existence of chronic diseases such as diabetes is known to increase the risk of developing active TB by 3-fold.[2] All of these situations together highlight an urgent need of new drugs with novel mechanisms of action, especially for the effective management of MDR-, XDR- and TDR TB.

The anti-TB therapies available today are inadequate to address many inherent and emerging treatment challenges. These include the need for multiple drugs to be taken simultaneously and for a long duration (thus poor compliance), significant toxicities and reduced efficacy based on the emergence of single-, multi-, and extensively-drug resistance.[7,8] There has been some progress over the past decade in the acceleration of TB drug discovery and evaluation, particularly for regimens to shorten the duration of

Abbreviations: M.tb, *Mycobacterium tuberculosis*; TB, tuberculosis; INH, isoniazid; IC_{50}, 50% inhibitory concentration; CFU, colony forming unit; MIC, minimum inhibitory concentration; MDR, multi drug-resistant; XDR, extremely drug-resistant; TDR, totally drug-resistant; PDK-1, phosphoinositide-dependent kinase-1; ATP, adenosine triphosphate; WHO, World Health Organization.

* Corresponding authors. Tel.: +1 (614) 292 0918 (L.S.S.); tel.: +1 (614) 688 4008; fax: +1 (614) 688 8556 (C.-S.C.).
E-mail addresses: larry.schlesinger@osumc.edu (L.S. Schlesinger), chen.844@osu.edu (C.-S. Chen).
† These authors contributed equally to this work.

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treatment and reduce the likelihood of the development of resistance. At present, at least 15 compounds are in preclinical development and clinical trials for TB.[9,10] Among them, four existing TB drugs are redeveloped or repurposed and six new chemical compounds are specifically developed for TB.[9,11–13] Despite this progress, the global drug TB pipeline is still grossly insufficient to address the unmet treatment needs. Therefore, new drug candidates and targets are essential to raise the probability of developing novel, short course and safe regimens applicable to drug-susceptible and drug-resistant TB. One strategy to accomplish this goal is to identify compounds with anti-mycobacterial activity within classes of molecules that have been developed for other purposes.

The whole genome sequencing of many human pathogens, has allowed for the identification of literally thousands of new essential bacterial drug targets, but has failed to bring a robust number of novel antibacterial drugs to the market place.[14–16] A strong prevailing consensus has been the idea that there is value in reassessing antibacterial target space for previously unexplored or under-explored targets amenable to an approach based on repurposing eukaryotic pharmacophores. One such directed class of targets is eukaryotic protein kinases that have not been studied extensively in anti-infective drug discovery. Even though protein kinases are much less common in bacterial metabolism, an ambitious attempt was made to screen a large library of pyridopyrimidine compounds for antibacterial activities.[17] That study yielded a new mechanistic class of antibiotics that target biotin carboxylase, a bacterial enzyme involved in fatty acid biosynthesis with an ATP-binding site very similar to that of eukaryotic protein kinases. Importantly, sufficient structural differences exist within the ATP-binding site of biotin carboxylase to allow for the identification of molecules with considerable specificity for the bacterial enzymes relative to host protein kinases.[18] It is now estimated that about 20–30% of drug candidates, in general, currently under clinical development are protein kinase inhibitors. As part of our research program on the development of anti-cancer agents, we have used the scaffold of celecoxib to develop many protein kinase inhibitors, including those of phosphoinositide-dependent protein kinase-1 (PDK-1)/Akt signaling (OSU-03012)[19–26] and p21-activated protein kinases (PAKs).[27] Among them, OSU-03012 has been shown to exhibit antifungal activity,[28] and antibacterial activity against the intracellular bacterial pathogens *Francisella tularensis* and *Salmonella typhimurium* in human macrophages, in part, through autophagy induction.[29–31] Based on these findings, we successfully identified several compounds from the celecoxib-derived focused compound library established in our laboratories with promising anti-bacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA).[32] Here, we report a systematic evaluation and identification of a series of new anti-mycobacterial agents sharing celecoxib's core structure.

2. Results and discussion

2.1. Determination of a chemical scaffold from celecoxib derivatives for the synthesis of focused compounds for screening against mycobacteria (*M. smegmatis* and *M.tb*)

We initially screened 5 compounds from our celecoxib-derived compound library, including OSU-03012, and compounds **A**–**D** (Fig. 1A), against the fast-growing mycobacterial species *M. smegmatis* in broth culture by the CFU assay. Compound **A** was found to significantly inhibit the growth of *M. smegmatis* compared to the other four compounds (Fig. 1). These five compounds were next tested against *M.tb* in broth culture. Again, compound **A** was the only one showing a significant inhibitory effect (approximately 1 log reduction of growth) of *M.tb* ($^{*}p$ <0.05) (Fig. 1B). Consequently, we focused our attention on using compound **A** as the scaffold for the focused synthesis of the second-generation

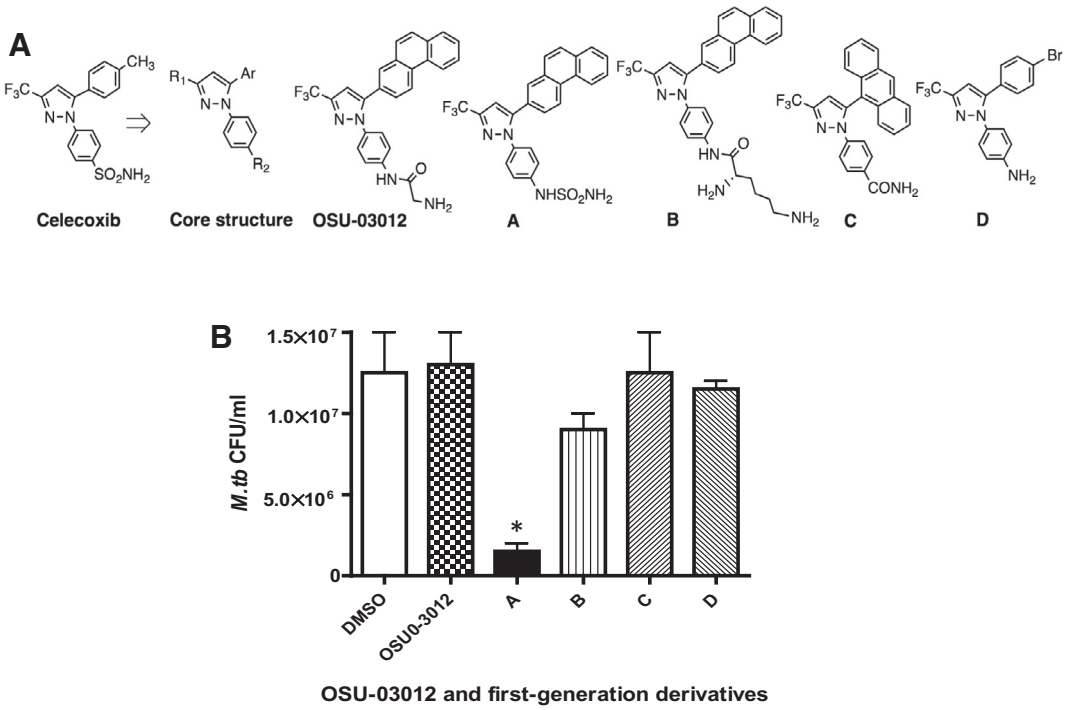

Figure 1. (A) Structures of OSU-03012 and compounds A–D. (B) CFU-based screening of OSU-03012 and compounds A–D for their antibacterial activities against *M.tb*. Each of the test compounds (20 μg/ml) was added to log phase (OD_{600} ~0.6) culture of *M.tb* $H_{37}R_v$ and incubated for 48 h. The size of the initial inoculum (day 0) was ~5×10^6 bacteria/ml after 100-fold dilution of the culture. The treated culture was serially diluted by 10-fold and spread onto agar medium for bacterial growth. *M.tb* colonies were enumerated and expressed in CFUs/ml. Data bars shown are the means ± SEM ($n = 3$). DMSO was used as the vehicle control. $^{*}P$ <0.05.