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Design, synthesis and evaluation of indole-2-carboxamides with pan anti-mycobacterial activity

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ABSTRACT

Current treatment regimens for non-tuberculous mycobacteria (NTM) and tuberculosis (TB) generally require long duration of therapy with multiple drugs, some of which are broad spectrum antibiotics. Despite some advances in antimicrobial compounds, there remains a need in therapy for antibiotics with specific mycobacterial targets. It has been shown that MmpL3 is an essential transporter required for the translocation of mycolic acids to the mycobacterial cell envelope. Here, we synthesized a series of indole-2-carboxamides that inhibit MmpL3 and have potent pan-activity against mycobacterial species. The compounds were tested against several fast and slow-growing *Mycobacterium* species, including *M. abscessus*, *M. massiliense, M. bolletii, M. chelonae, M. tuberculosis, M. avium*, *M. xenopi* and *M. smegmatis.* The target of these indole-based compounds makes them selective for mycobacteria, while showing no clinically relevant bactericidal activity against *S. aureus* or *P. aeruginosa*. These compounds were tested against THP-1, a human-cell line, and showed minimal *in vitro* cytotoxicity and good selectivity indices. The data shown and discussed suggest that lead indole-2-carboxamides are strong contenders for further preclinical testing as NTM therapeutics.

1. Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental pathogens that can cause a wide variety of infections, such as: progressive pulmonary disease, skin and soft tissue infections, lymphadenitis, and disseminated disease.[1,2] The most common human pathogens are the species *M. avium* complex (MAC) and *M. kansasii*, however other clinically relevant species include the *M. abscessus complex (MABSC, including the subspecies M. abscessus, M. massiliense and M. bolletii)* and *M. fortuitum.*[3] NTM commonly cause pulmonary disease in older, immunocompromised patients who have underlying lung disease. The precise frequency of NTM disease is unknown because reporting for NTM is not mandatory in the United States and many other countries.[4,5] Furthermore, current incidence and prevalence data are likely underestimated due to the indolent nature of NTM pulmonary disease, the most common form of NTM infections. A growing number of studies suggest that the number of NTM infections and mortality rates continue to increase.[4,6] Growing prevalence of NTM isolates between 1997 (9.1/100,000) and 2003 (14.1/100,000) has been observed in Ontario, Canada.[7] More recently, Taiwan has reported increasing incidence of NTM disease, 10.2 cases per 100,000 persons in 2008 compared to 2.7 cases per 100,000 persons in 2000.[8,9] In addition, estimates from 1999 to 2010 suggest the number of immediate NTM-related deaths in the United States rose significantly and are expected to increase over the next few years, given the increasing median age in North America.[10] Although NTM are opportunistic pathogens, they represent a matter of significant concern to health practitioners because of increasing incidence and mortality rates. As the number of patients with immune-compromising conditions continues to grow, both opportunistic and primary cases of NTM infection present a significant challenge for current antibiotic therapy.

In contrast to the insidious nature of NTM infections, *M. tuberculosis* is the cause of highly transmittable disease and infects about one-third of the world's population.[11] In 2015, tuberculosis (TB) became the leading infectious disease killer in the world and

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causes illness in approximately 9.6 million people, killing 1.5 million each year.[11] The global incidence of multi-drug-resistant tuberculosis (MDR-TB) remains unchanged at 3.3%, causing growing concern for healthcare professionals.[11] MDR-TB is classified as resistant to both rifampicin and isoniazid, two critical antibiotics used in standard treatment. This form of TB goes largely undetected, as the estimated number of cases of MDR-TB in 2014 was 480,000 people and the number of these patients who were started on appropriate treatment was only about 111,000.[11] Current treatment against mycobacterial infections are inadequate and this may be due, at least in part, to the absence of timely diagnostics and a paucity of narrow spectrum antimycobacterial antibiotics. The current treatment of NTM recommended by the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) include regimens of multiple antibiotics, potentially including macrolides, aminoglycosides, fluoroquinolones, oxazolidinones, tigecycline, carbapenems, cephalosporins, sulfonamides, ethambutol, and rifampicin.[3] Many of these recommendations are made on the basis of preliminary *in vitro* efficacy studies due to the lack of a standardized animal model.[3] Depending on the organism and site of infection, two to four of these agents are often used in combination for a duration of at least 12 months after the first negative culture.[3] The use of multiple antibiotics for prolonged periods of time is challenging due to common complications such as drug interactions and noncompliance.[3]

Problems surrounding current anti-TB therapy are similar to that of NTM. The treatment of drug-susceptible TB continues to revolve around the same four-antibiotic regimen (isoniazid, rifampicin, ethambutol, and pyrazinamide) introduced more than 40 years ago.[11] Although the treatment of TB has been extensively studied, therapeutic regimens are still somewhat lacking in that: 1) they require the use of multiple antibiotics and 2) the duration of therapy is long. These are both factors contributing to non-compliance and treatment failure, which can lead to the emergence of MDR-TB and extensively drug-resistant tuberculosis (XDR-TB). The treatment of MDR-TB/XDR-TB is more difficult and requires multiple broad-spectrum antibiotics, which exposes patients to complications like *Clostridium difficile* infections and other resistant microorganisms.[12] Clinical practice guidelines for the treatment of drug-resistant TB are currently under development by the ATS and IDSA.[13] Depending on the susceptibilities of particular drug-resistant TB strains, second-line anti-TB drugs are typically used for a duration of 2 years and treatment success rates range from 30 to 80%, underscoring the need for newer anti-mycobacterial drugs and treatment regimens that maximize efficacy and shorten duration of treatment.[14–21]

Current drugs that are in the pharmaceutical pipeline for mycobacterial infections are largely being used against *M. tuberculosis* and not specifically against NTM. Despite the number of new drugs being studied in pre-clinical and clinical trials for TB, they are being studied in combination drug regimens with other broad-spectrum antibiotics. Furthermore, these drugs, including DC-159a,[22] SQ-641,[23] CPZEN-45,[24] BTZ043,[25] bedaquiline,[26] and pretomanid,[27] lack bactericidal activity against many NTM species. The development of narrow-spectrum anti-mycobacterial drugs could revolutionize the treatment of both TB and NTM.

Indole-2-carboxamides (IC) have been reported as novel antitubercular agents with activity against drug-resistant strains and in *in vivo* efficacy mouse models.[28–32] IC are bioisosteric isomers of published urea based *M. tuberculosis* inhibitors and have similar structure activity relationships.[33–35] Herein, we report the expansion of i) the chemical space for the published antitubercular IC compounds and ii) the spectrum of antimycobacterial activity. In addition, lead compounds have demonstrated a safe pharmacological profile and are inhibitors of the mycolic acid biosynthetic pathway in both TB and NTM strains. Specifically, they inhibit the translocation of trehalose-monomycolate (TMM) to the outer membrane, suggestive of Mycobacterial membrane protein large 3 (MmpL3) inhibition.

2. Results and discussion

2.1. Chemistry

Indole-2-carboxamide (IC) compounds were generated using published methods and is shown in Scheme 1.[30] Briefly, the reaction of the arylhydrazine with ethyl pyruvate in the presence of p-toluenesulfonic acid (pTsOH) afforded ethyl indole-2-carboxylate. After NaOH-mediated saponification of the ester, commercially available amines were coupled to indole-2-carboxylic acid using standard coupling conditions.

2.2. Anti-mycobacterial activity

Two IC series were evaluated for their anti-mycobacterial activity against various mycobacterial pathogens, including *M. abscessus, M. massiliense, M. bolletii, M. chelonae, M. tuberculosis, and M. smegmatis*. The first series is an unsubstituted indole (R = H, compounds **5**–**19**) and the second was as 4,6-dimethyl indole (R = CH_3, compounds **20**–**34**). A variety of commercially available bulky aliphatic and aromatic head groups were incorporated into final compounds that are consistent with published SAR for previously evaluated urea-based compounds and IC.[28,30,32–35]

Table 1 shows the minimum inhibitory concentration (MIC) values for the synthesized unsubstituted IC compounds. Overall, these compounds have limited pan-antimycobacterial activity as demonstrated by inhibition only from compounds **5** and **16**. Excluding *M. smegmatis,* compounds **5** and **16** show the best antimycobacterial activity with sub-μg/mL MIC values and compound **16** obtaining 2–4-fold increased activity over **5**. An adamantyl substitution in compound **5** confers activity, however the 3-ethyl adamantyl (**6**) or adamantyl with an ethyl or methyl linker (**7**, **8**) was not tolerated. Interestingly, changing the connection point from the 1-position (**5**) to the 2-position (**9**) on adamantine abolished NTM activity, however retained potent activity against *M. tuberculosis*. The methyl-linked adamantyl substitution on compound **8** elicits greater activity against NTM than the larger ethyl-linked compound **7**, suggesting an upper limit for linker bulk. Saturated cycloalkyl rings that are 8 carbons or smaller (**10**, **11**, **12**, and **13**) abolish all NTM activity. However, adding methyl components to smaller aliphatic rings can increase activity as seen with the isopinocampheyl substituted (**16**), which attains the highest pan-activity of the series.

The antitubercular SAR is much looser, as the constraints on for the bulky aliphatic group are less restrictive. A cyclooctyl (**10**) substituent maintains antimicrobial activity against *M. tuberculosis* with an MIC of 0.39 μg/mL, despite being absent for NTM species. However, smaller rings like cycloheptyl, cyclohexyl, and cyclopentyl (**11**, **12**, and **13**) are not tolerated for any species. There remains modest anti-TB activity with the 3-ethyl adamantyl (**6**) with an MIC of 1.25 μg/mL.

The second series evaluated for pan-antimycobacterial activity was the 4,6-dimethyl indole substitution (Table 2) and contributes to greater potency against the mycobacterial panel than with the unsubstituted indole compounds. The most potent pan-activity is seen with cyclooctyl (**25**) and cycloheptyl (**26**) head groups with MIC values ranging from 0.0039 to 0.625 μg/mL, depending on the species of *Mycobacterium*. The 4-methylcyclohexyl substituted compounds (**29** and **30**) also display significant anti-NTM and anti-TB activity with the exception of *M. smegmatis*. The pure trans isomer (**29**) and cis/trans mixture (**30**) achieved the same MIC