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Synthesis and biological activity of alkynoic acids derivatives against mycobacteria

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ABSTRACT

2-Alkynoic acids have bactericidal activity against *Mycobacterium smegmatis* but their activity fall sharply as the length of the carbon chain increased. In this study, derivatives of 2-alkynoic acids were synthesized and tested against fast- and slow-growing mycobacteria. Their activity was first evaluated in *M. smegmatis* against their parental 2-alkynoic acids, as well as isoniazid, a first-line antituberculosis drug. The introduction of additional unsaturation or heteroatoms into the carbon chain enhanced the antimycobacterial activity of longer chain alkynoic acids (more than 19 carbons long). In contrast, although the modification of the carboxylic group did not improve the antimycobacterial activity, it significantly reduced the toxicity of the compounds against eukaryotic cells. Importantly, 4-(alkylthio) but-2-ynoic acids, had better bactericidal activity than the parental 2-alkynoic acids and on a par with isoniazid against the slow-grower *Mycobacterium bovis* BCG. These compounds had also low toxicity against eukaryotic cells, suggesting that they could be potential therapeutic agents against other types of topical mycobacterial infections causing skin diseases including *Mycobacterium abscessus, Mycobacterium ulcerans,* and *Mycobacterium leprae*. Moreover, they provide a possible scaffold for future drug development.

1. Introduction

Mycobacteria are genus of bacteria that cause a myriad of debilitating infections in humans. Once thought to be on the way to eradication, tuberculosis (TB) is still the world's number two killer from a single infectious agent. TB, a disease caused by the bacillus *Mycobacterium tuberculosis*, infects one third of the world's population. In 2013, 9 million new TB cases were reported and 1.5 million people die of the disease (WHO, 2014). Short-course chemotherapy for drug-susceptible TB requires six months and patient non-compliance often leads to the development of multidrug-resistant (MDR) and extensively drug-resistant (XDR)-TB, which impedes TB control (WHO, 2014). Patients infected with drug-resistant TB can still be cured but the treatment becomes long (2 years), expensive, and toxic for the patient (Torun et al., 2005). In the past 10 years, new drugs have been developed for the treatment of TB that are in different phases of clinical trials, but more lead compounds need to be identified in order to sterilize TB infection, reduce TB chemotherapy duration and prevent TB relapse (Zumla et al., 2014). Mycobacteria cause other human infections in addition to TB. Leprosy, the age old skin disfiguring plague, is a disease caused by *Mycobacterium leprae* that has yet to be eradicated. *Mycobacterium ulcerans* causes terrible skin lesions and is very difficult to treat. *Mycobacterium abscessus* is a growing problem causing skin infections and is totally resistant to all known drugs (Nessar et al., 2012). Clearly new drugs are needed to combat these mycobacterial infections.

Mycobacteria have a thick and complex cell wall that acts as a protective barrier against many antibacterial agents. The major constituents of the cell wall are mycolic acids, which are long chain (C_{70}–C_{90}) α-branched β-hydroxy fatty acids. Mycobacterial fatty acid biosynthesis is very peculiar as it uses both the eukaryotic fatty acid synthase system I (FASI) to synthesize fatty acids up to C_{16}–C_{24} in length (Bloch, 1977; Kikuchi et al., 1992; Peterson and Bloch, 1977) and the prokaryotic fatty acid synthase system II (FASII) to elongate these fatty acids to mycolic acids (Fig. 1). Isoniazid (INH), a first-line antituberculosis drug, inhibits mycolic acid biosynthesis (Takayama et al., 1975; Takayama et al., 1972) by targeting InhA (Vilchèze et al., 2006), the NADH-dependent enoyl-ACP reductase (Quemard et al., 1995) of FASII (Marrakchi et al.,

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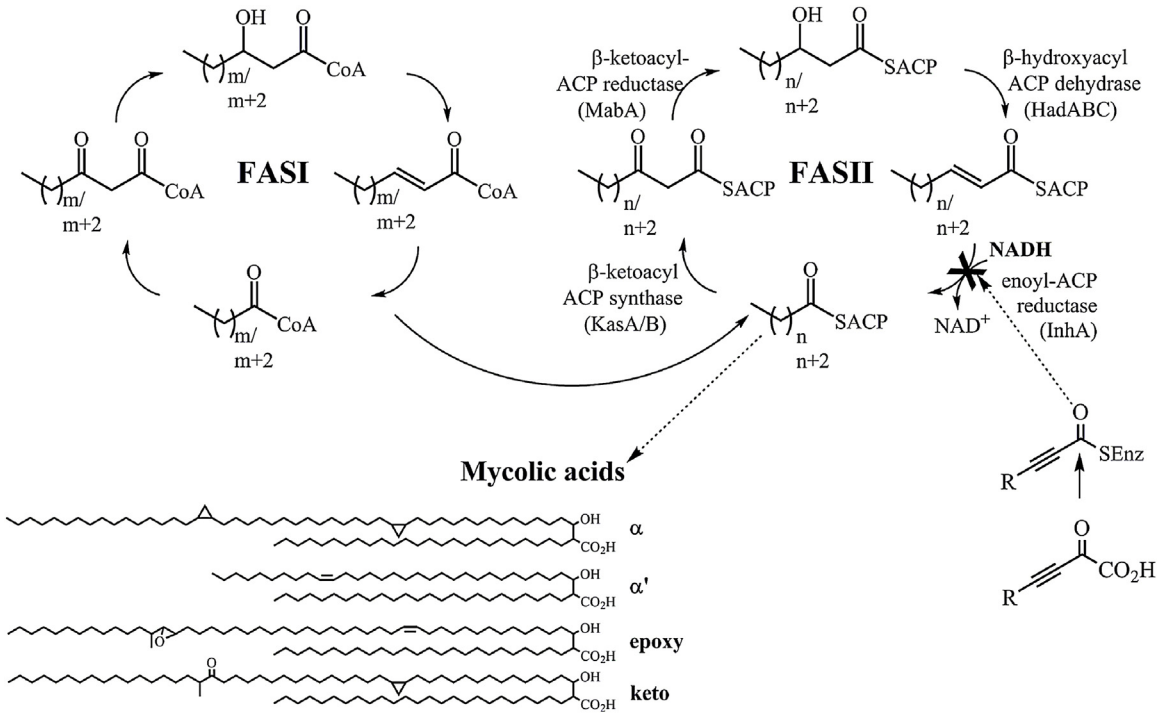

Fig. 1. 2-Alkynoic acids were designed as substrate inhibitors of the enoyl-ACP reductase InhA of the FASII system. Mycobacteria use both eukaryotic (FASI) and prokaryotic (FASII) fatty acid biosynthesis systems to synthesize fatty acids and mycolic acids. The major mycolic acids in *M. smegmatis* are the α,α' and epoxy while *M. bovis* BCG strain Pasteur produces α and keto-mycolates.

2000). Inhibition of InhA results in mycobacterial cell death (Vilcheze et al., 2000) which renders this enzyme an attractive target for drug development (Manjunatha et al., 2015). InhA reduces 2-alkenoyl-ACP to alkanoyl-ACP, the last step in fatty acid elongation (Quemard et al., 1995). We reasoned that long-chain 2-alkynoic acids would function as InhA substrate analogs (Fig. 1), and studied their effects in the saprophylatic fast-grower *Mycobacterium smegmatis* (Morbidoni et al., 2006). The most potent compounds were 2-hexadecynoic acid (2-HA) and 2-octadecynoic acid (2-OA), which had minimum inhibitory concentrations (MIC) against *M. smegmatis* of 10 μM and 4 μM, respectively (compared to 36 μM for INH). The activity of these compounds against *M. smegmatis* was dependent on the position of the triple bond and the chain length. Shifting the triple bond away from the carboxylic group significantly reduced the antimycobacterial activity. 2-Alkynoic acids with a short to moderate chain length (4–14 carbons) had no effect on the growth of *M. smegmatis* while the antimycobacterial activity of 2-alkynoic acids with a longer chain (19 to 25 carbons) quickly dropped with increased chain length. Fatty acids are known to possess antibacterial activities that vary with their chain length. Kondo and Kanai showed that *M. tuberculosis* and *Mycobacterium bovis* were most susceptible to tetradecanoic acid among saturated fatty acids and linolenic (*cis,cis,cis*-9,12,15-octadecatrienoic acid) and arachidonic (*cis,cis,cis,cis*-5,8,11,14-eicosatetraenoic acid) acids among olefinic acids (Kondo and Kanai, 1977). Subsequently, Saito et al. tested saturated and olefinic fatty acids against different rapidly growing mycobacteria and found that dodecanoic acid was the most toxic saturated fatty acid, and linolenic and eicosatrienoic acids were the most lethal olefinic fatty acids (Saito et al., 1984). It was concluded that the balance between the hydrophilicity due to the carboxylic group and the lipophilicity due to the long chain of these fatty acids played an essential role in their ability to penetrate the mycobacterial cell wall. Since 2-alkynoic acids competitively inhibit the InhA enzyme, a long-chain enoyl-ACP reductase, it was expected that the activity of 2-alkynoic acids against InhA would increase with the chain length. The fact that their activity peaked at C18 and then decreased sharply was ascribed to a reduced solubility in the culture media for fatty acids having more than 18 carbons. We hypothesized that 2-alkynoic acids with a modified carbon chain (introducing additional unsaturation, heteroatom) or head group (esterification with polar entities or antimycobacterial drugs) would have enhanced antimycobacterial activity. In this report, we describe the antimycobacterial activity of these new compounds against (i) *M. smegmatis,* a fast-growing, avirulent strain of mycobacteria and (ii) *M. bovis* BCG, a slow-growing, live attenuated strain of *M. bovis,* member of the *M. tuberculosis* complex.

2. Experimental

2.1. General procedures

Solvents were dried by distillation as follows and then stored over 3 Å molecular sieves: acetone over phosphorus pentoxide, dichloromethane (CH_2Cl_2) over calcium hydride, dimethylsulfoxide (DMSO) over calcium hydride, ether over lithium aluminum hydride, hexamethylphosphoramide (HMPA) over calcium hydride, tetrahydrofuran (THF) over lithium aluminum hydride, triethylamine over calcium hydride. Other solvents were ACS reagent grade and were used without further purification. All reagents were purchased from commercial sources. Proton and carbon nuclear magnetic resonance spectra were recorded in $CDCl_3$ on a Bruker APX 400-MHz NMR spectrometer. ^1H and ^{13}C signals assignments were based on previously reported data (Bengsh et al., 1986; Gunstone et al., 1976) and ^1H COSY and ^{13}C–^1H heteroatom shift correlation experiments done using the pulse program provided by Bruker. Melting points are uncorrected. Electrospray ionization (ESI) mass spectrometry analysis was performed on a Waters SynaptG2. Silica gel for flash chromatography was