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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.

Keywords

antimycobacterial; alkynoic; InhA; sulfur

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Additional information

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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 patients 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 (C₇₀-C₉₀) 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 C16-C24 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., 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 saprophytic 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 μ M and 4 μ M, respectively (compared to 36 μ M 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 to 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) Mycobacterium 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 (CH₂Cl₂) 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 CDCl₃ on a Bruker APX 400-MHz NMR spectrometer. ¹H and ¹³C signals assignments were based on previously reported data (Bengsh et al., 1986; Gunstone et al., 1976) and ¹H-COSY and ¹³C-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 purchased from EM Science (Gibbstown, NJ). Reactions were monitored by TLC on aluminum plates of silica gel 60 F 254 (EM Science, Gibbstown, NJ); and visualized using short-wavelength ultraviolet light followed by 10% sulfuric acid in ethanol.

2.2. General method for the carboxylation of terminal alkynes

To a solution of 1-alkyne (1.70 mmol) in dry THF (7 mL) at -23°C was added *n*-butyllithium (2.5 M solution in hexanes, 0.69 mL, 1.73 mmol) under nitrogen. The reaction mixture was stirred at -23°C for 1 h and then warmed to 0°C. A stream of carbon dioxide

was bubbled into the suspension for 1 h. The residue was poured into ether/10% aqueous hydrochloric acid solution and extracted with diethyl ether. The combined organic phases were dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate/glacial acetic acid 75/25/1) to give 2-alkynoic acids.

2.3. Preparation of 2,4-hexadecadiynoic acid 2

2.3.1. 1,3-Pentadecadiyne 1—To a solution of 1,4-bis(trimethylsilyl)-1,3-butadiyne (505 mg, 2.60 mmol) in dry THF (3.6 mL) at -78 °C was added, under nitrogen, methyllithium (1.5 M as complex with lithium bromide; 1.7 mL, 2.6 mmol). The solution was stirred at rt for 3.5 h, and then cooled to -78 °C. A solution of 1-bromoundecane (487 mg, 2.07 mmol) in dry THF (1.8 mL) and dry HMPA (7.2 mL) was added. The black reaction mixture was stirred 1 h at -78 °C and then overnight at rt, poured into water, and extracted with hexane. The combined organic phases were dried (Na₂SO₄), filtered, and evaporated to dryness under reduced pressure. A slurry of potassium fluoride dihydrate (523 mg, 5.56 mmol) in dimethylformamide (DMF, 11 mL) was added to the residue. The resulting dark solution was stirred at rt for 4 h, poured into 3 N aqueous hydrochloric acid, and extracted with hexane. The combined organic phases were washed with 3 N aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and saturated brine, dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by filtration through a small pad of silica gel (elution with hexane). The filtrate was evaporated to dryness under reduced pressure; yield 57%: ¹H NMR δ 0.86 (3H, t, *J* = 6.7 Hz, ω-CH₃), 1.25 (16H, m, (CH₂)₈), 1.50 (2H, m, CH₂C≡C), 1.87 (1H, t, *J* = 2.6 Hz, C≡CH), 2.22 (2H, m, CH₂C≡C); ¹³C NMR δ 14.0 (C-15), 19.2 (C-5), 22.7 (C-14), 28.2 (C-6), 28.8 (C-7), 29.1 (C-8), 29.3 (C-12), 29.5 (C-9), 29.6 (C-10 and 11), 31.9 (C-13), 64.3, 65.7, 80.0 and 82.8 (C-1 to 4).

2.3.2. 2,4-Hexadecadiynoic acid 2—Carboxylation was performed as described in section 2.2: yield 73%, mp 62.5–63.0 °C (lit. (Wailles, 1959) mp 63.0–64.5 °C), ¹H NMR δ 0.86 (3H, t, *J* = 6.6 Hz, ω-CH₃), 1.24 (16H, m, (CH₂)₈), 1.55 (2H, m, CH₂C≡C), 2.33 (2H, t, *J* = 6.9 Hz, CH₂C≡C), 10.7 (1H, br s, CO₂H); ¹³C NMR δ 14.0 (C-16), 19.5 (C-6), 22.7 (C-15), 27.7 (C-7), 28.8 (C-8), 29.0 (C-9), 29.3 (C-13), 29.4 (C-10), 29.6 (C-11 and 12), 31.9 (C-14), 63.6 and 64.6 (C-4 and 5), 74.6 (C-2), 89.6 (C-3), 157.3 (C-1).

2.4 Preparation of 2,5- alkanediynoic acids

2.4.1. Preparation of 1-alkyne 3—A suspension of lithium acetylide ethylenediamine complex (412 mg, 4.5 mmol) in dry dimethyl sulfoxide (2.2 mL) was stirred for 10 min at room temperature under nitrogen. After cooling to 8 °C, 1-bromoalkane (4.1 mmol) was added dropwise (if the bromoalkane was a solid, it was solubilized first in THF). The reaction mixture was stirred at room temperature overnight, and then cooled to 0 °C. Water (1 mL) was added very slowly. The suspension was stirred for 10 min at room temperature, poured into water (60 mL), and extracted with hexane. The combined organic phases were dried over anhydrous sodium sulfate, filtered, evaporated to dryness under vacuum, and purified by filtration through a small pad of silica gel (elution with hexane) to give **3** (Smith and Beumel, 1974):

1-Hexadecyne (3d, 64%): ^1H NMR δ 0.86 (3H, t, J = 6.2 Hz, ω -CH₃), 1.24 (22H, m, (CH₂)₁₁), 1.49 (2H, m, CH₂CC $\equiv$ C); 1.89 (1H, t, J = 2.6 Hz, C $\equiv$ CH), 2.14 (2H, td, J = 2.6 Hz, J = 6.9 Hz, CH₂C $\equiv$ C); ^{13}C NMR δ 14.1 (C-16), 18.4 (C-3), 22.7 (C-15), 28.6 (C-4), 28.8 (C-5), 29.1 (C-6), 29.4 (C-13), 29.6 (C-7), 29.7 (C-8 to 12), 32.0 (C-14), 68.0 (C-1), 84.6 (C-2).

1-Heptadecyne (3e 90%): ^1H NMR δ 0.86 (3H, t, J = 6.6 Hz, ω -CH₃), 1.24 (24H, m, (CH₂)₁₂), 1.51 (2H, m, CH₂CC $\equiv$ C); 1.91 (1H, t, J = 2.6 Hz, C $\equiv$ CH), 2.13 (2H, td, J = 2.6 Hz, J = 6.9 Hz, CH₂C $\equiv$ C); ^{13}C NMR δ 14.1 (C-17), 18.4 (C-3), 22.7 (C-16), 28.5 (C-4), 28.8 (C-5), 29.1 (C-6), 29.4 (C-14), 29.7 (C-7 to 13), 31.9 (C-15), 68.0 (C-1), 84.8 (C-2).

1-Nonadecyne (3f, 90%): ^1H NMR δ 0.87 (3H, t, J = 6.7 Hz, ω -CH₃), 1.25 (28H, m, (CH₂)₁₄), 1.51 (2H, m, CH₂CC $\equiv$ C); 1.88 (1H, t, J = 2.6 Hz, C $\equiv$ CH), 2.15 (2H, td, J = 2.6 Hz, J = 6.9 Hz, CH₂C $\equiv$ C); ^{13}C NMR δ 14.1 (C-19), 18.4 (C-3), 22.7 (C-18), 28.6 (C-4), 28.8 (C-5), 29.2 (C-6), 29.4 (C-16), 29.6 (C-7), 29.7 (C-8 to 15), 32.0 (C-17), 68.0 (C-1), 84.5 (C-2).

2.4.2 Preparation of 1,4-alkadiyne 4—To a solution of ethylmagnesium bromide [6.6 mmol; prepared from ethyl bromide (500 μL , 6.6 mmol) and magnesium (160 mg, 6.6 mmol) in dry THF (7.5 mL)] was added 1-alkyne (6.0 mmol), under nitrogen at rt. The resulting solution was heated at 45 °C for 2 h and then cooled to –10 °C. Cuprous iodide (19 mg, 0.1 mmol) was added and the suspension was stirred for 20 min. Propargyl bromide (550 μL , 6.10 mmol) was then added dropwise at –10 °C. The suspension was allowed to slowly warm to 20 °C, stirred overnight at rt, quenched with saturated aqueous ammonium chloride solution, and extracted with hexane. The organic phases were dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by chromatography (elution with heptane).

1,4-Pentadecadiyne (4a, 76%): ^1H NMR δ 0.86 (3H, t, J = 7.1 Hz, ω -CH₃), 1.24 (14H, m, (CH₂)₇), 1.46 (2H, m), 2.03 (1H, t, J = 2.6 Hz, C $\equiv$ CH), 2.13 (2H, tt, J = 2.3 Hz, J = 7.1 Hz, CH₂C $\equiv$ C), 3.13 (2H, m, C $\equiv$ CCH₂C $\equiv$ C); ^{13}C NMR δ 9.6 (C-3), 14.1 (C-15), 18.7 (C-6), 22.7 (C-14), 28.7 (C-7), 28.9 (C-8), 29.1 (C-9), 29.3 (C-12), 29.5 (C-11), 29.6 (C-10), 31.9 (C-13), 68.4 (C-1), 72.9 (C-4), 78.9 (C-5), 81.4 (C-2).

1,4-Heptadecadiyne (4b, 70%): ^1H NMR δ 0.85 (3H, t, J = 7.0 Hz, ω -CH₃), 1.24 (18H, m, (CH₂)₉), 1.45 (2H, m, CH₂C-C $\equiv$ C), 2.02 (1H, t, J = 2.6 Hz, C $\equiv$ CH), 2.12 (2H, m, J = 7.0 Hz, CH₂C $\equiv$ C), 3.11 (2H, m, C $\equiv$ CCH₂C $\equiv$ C); ^{13}C NMR δ 9.5 (C-3), 14.1 (C-17), 18.7 (C-6), 22.7 (C-16), 28.7 (C-7), 28.9 (C-8), 29.1 (C-9), 29.4 (C-14), 29.5 (C-13), 29.6 (C-10 to 12), 31.9 (C-15), 68.3 (C-1), 72.9 (C-4), 78.9 (C-5), 81.3 (C-2).

1,4-Octadecadiyne (4c, 80%): ^1H NMR δ 0.86 (3H, t, J = 7.0 Hz, ω -CH₃), 1.24 (20H, m, (CH₂)₁₀), 1.46 (2H, m, CH₂C-C $\equiv$ C), 2.03 (1H, t, J = 2.7 Hz, C $\equiv$ CH), 2.13 (2H, tt, J = 2.3 Hz, J = 7.1 Hz, CH₂C $\equiv$ C), 3.13 (2H, m, C $\equiv$ CCH₂C $\equiv$ C); ^{13}C NMR δ 9.6 (C-3), 14.1 (C-18), 18.7 (C-6), 22.7 (C-17), 28.7 (C-7), 28.9 (C-8), 29.1 (C-9), 29.3 (C-15), 29.5 (C-14), 29.6 (C-10 to 13), 31.9 (C-16), 68.3 (C-1), 72.9 (C-4), 79.0 (C-5), 81.3 (C-2).

1,4-Nonadecadiyne (4d, 78%): ^1H NMR δ 0.84 (3H, t, $J = 7.0$ Hz, $\omega\text{-CH}_3$), 1.22 (22H, m, $(\text{CH}_2)_{11}$), 1.43 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 2.00 (1H, t, $J = 2.6$ Hz, $\text{C}\equiv\text{CH}$), 2.11 (2H, tt, $J = 2.3$ Hz, $J = 7.0$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.09 (2H, m, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$); ^{13}C NMR δ 9.5 (C-3), 14.1 (C-19), 18.6 (C-6), 22.7 (C-18), 28.6 (C-7), 28.9 (C-8), 29.1 (C-9), 29.4 (C-16), 29.5 (C-15), 29.6 (C-10 to 14), 31.9 (C-17), 68.3 (C-1), 72.9 (C-4), 78.8 (C-5), 81.2 (C-2).

1,4-Eicosadiyne (4e, 63%): ^1H NMR δ 0.85 (3H, t, $J = 7.0$ Hz, $\omega\text{-CH}_3$), 1.23 (24H, m, $(\text{CH}_2)_{12}$), 1.46 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 2.03 (1H, t, $J = 2.7$ Hz, $\text{C}\equiv\text{CH}$), 2.13 (2H, tt, $J = 2.4$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.12 (2H, tt, $J = 2.4$ Hz, $J = 2.7$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$); ^{13}C NMR δ 9.6 (C-3), 14.1 (C-20), 18.7 (C-6), 22.7 (C-19), 28.7 (C-7), 28.9 (C-8), 29.1 (C-9), 29.4 (C-17), 29.5 (C-16), 29.6 (C-10), 29.7 (C-11 to 15), 31.9 (C-18), 68.3 (C-1), 72.9 (C-4), 79.0 (C-5), 81.3 (C-2).

1,4-Docosadiyne (4f, 68%): ^1H NMR δ 0.86 (3H, t, $J = 7.0$ Hz, $\omega\text{-CH}_3$), 1.23 (28H, m, $(\text{CH}_2)_{14}$), 1.46 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 2.03 (1H, t, $J = 2.6$ Hz, $\text{C}\equiv\text{CH}$), 2.13 (2H, tt, $J = 2.4$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.12 (2H, tt, $J = 2.4$ Hz, $J = 2.6$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$); ^{13}C NMR δ 9.6 (C-3), 14.1 (C-22), 18.7 (C-6), 22.7 (C-21), 28.7 (C-7), 28.9 (C-8), 29.1 (C-9), 29.4 (C-19), 29.5 (C-18), 29.6 (C-10), 29.7 (C-11 to 17), 31.9 (C-20), 68.3 (C-1), 72.9 (C-4), 79.0 (C-5), 81.3 (C-2).

2.4.3. 2,5-Alkanediynoic acids 5—To a solution of **4** (0.9 mmol) in dry THF (36 mL) at -78°C was added *n*-butyllithium (2.5 M in hexanes, 360 μL , 0.9 mmol) dropwise. The mixture was stirred at -78°C for 30 min. Carbon dioxide gas was bubbled into the reaction mixture until the solvents had evaporated. To the residue was added diethyl ether/10% aqueous hydrochloric acid solution. The aqueous phase was extracted with diethyl ether. The combined organic phases were dried (Na_2SO_4), filtered, and evaporated to dryness under reduced pressure. Purification began with column chromatography (elution with hexane/ethyl acetate/ acetic acid 4/1/0.1), followed by two recrystallizations from hexane and lyophilisation from cyclohexane to afford a beige powder.

2,5-Hexadecadiynoic acid (5a, 62%): mp $42.0\text{--}42.5^\circ\text{C}$, ^1H NMR δ 0.86 (3H, t, $J = 6.9$ Hz, $\omega\text{-CH}_3$), 1.23 (14H, m, $(\text{CH}_2)_7$), 1.46 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 2.13 (2H, tt, $J = 2.0$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, t, $J = 2.0$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 10.3 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-16), 18.6 (C-7), 22.7 (C-15), 28.5 (C-8), 28.8 (C-9), 29.1 (C-10), 29.3 (C-13), 29.5 (C-12), 29.6 (C-11), 31.9 (C-14), 70.2 (C-2), 72.0 (C-5), 82.8 (C-6), 86.3 (C-3), 158.0 (C-1). ESI-MS exact mass for $\text{C}_{16}\text{H}_{24}\text{O}_2$ 248.1776, found $[\text{M-H}]^-$ 247.1816.

2,5-Octadecadiynoic acid (5b, 42%): mp 54.0°C (lit.(Christie and Holman, 1967) mp $53.0\text{--}54.0^\circ\text{C}$), ^1H NMR δ 0.85 (3H, t, $J = 7.1$ Hz, $\omega\text{-CH}_3$), 1.24 (18H, m, $(\text{CH}_2)_9$), 1.46 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 2.12 (2H, tt, $J = 2.1$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, t, $J = 2.1$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 9.7 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-18), 18.6 (C-7), 22.7 (C-17), 28.5 (C-8), 28.8 (C-9), 29.1 (C-10), 29.4 (C-15), 29.5 (C-14), 29.6 (C-11 to 13), 31.9 (C-16), 70.1 (C-2), 71.9 (C-5), 82.9 (C-6), 86.3 (C-3), 158.0 (C-1). ESI-MS exact mass for $\text{C}_{18}\text{H}_{28}\text{O}_2$ 276.2089, found $[\text{M-H}]^-$ 275.2089.

2,5-Nonadecadiynoic acid (5c, 60%): mp 56.0–56.5 °C, ^1H NMR δ 0.86 (3H, t, $J = 7.1$ Hz, $\omega\text{-CH}_3$), 1.24 (20H, m, $(\text{CH}_2)_{10}$), 1.46 (2H, m, $\text{CH}_2\text{C-C}\equiv\text{C}$), 2.13 (2H, tt, $J = 2.3$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, t, $J = 2.3$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 8.0 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-19), 18.6 (C-7), 22.7 (C-18), 28.5 (C-8), 28.8 (C-9), 29.1 (C-10), 29.4 (C-16), 29.5 (C-15), 29.6 (C-11 to 14), 31.9 (C-17), 70.2 (C-2), 71.8 (C-5), 82.9 (C-6), 86.2 (C-3), 156.8 (C-1). ESI-MS exact mass for $\text{C}_{19}\text{H}_{30}\text{O}_2$ 290.2246, found $[\text{M-H}]^-$ 289.1693.

2,5-Eicosadiynoic acid (5d, 43%): mp 60.0–60.5 °C, ^1H NMR δ 0.86 (3H, t, $J = 7.0$ Hz, $\omega\text{-CH}_3$), 1.23 (22H, m, $(\text{CH}_2)_{11}$), 1.46 (2H, m, $\text{CH}_2\text{C-C}\equiv\text{C}$), 2.12 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, m, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 9.4 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-20), 18.6 (C-7), 22.7 (C-19), 28.5 (C-8), 28.9 (C-9), 29.1 (C-10), 29.4 (C-17), 29.5 (C-16), 29.6 (C-11), 29.7 (C-12 to 15), 31.9 (C-18), 70.2 (C-2), 72.0 (C-5), 82.8 (C-6), 86.1 (C-3), 157.6 (C-1). ESI-MS exact mass for $\text{C}_{20}\text{H}_{32}\text{O}_2$ 304.2402, found $[\text{M-H}]^-$ 303.2347.

2,5-Heneicosadiynoic acid (5e, 42%): mp 61.0–62.0 °C, ^1H NMR δ 0.86 (3H, t, $J = 7.1$ Hz, $\omega\text{-CH}_3$), 1.23 (24H, m, $(\text{CH}_2)_{12}$), 1.46 (2H, m, $\text{CH}_2\text{C-C}\equiv\text{C}$), 2.12 (2H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, t, $J = 2.4$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 7.5 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-21), 18.6 (C-7), 22.7 (C-20), 28.5 (C-8), 28.9 (C-9), 29.1 (C-10), 29.4 (C-18), 29.5 (C-17), 29.6 (C-11), 29.7 (C-12 to 16), 31.9 (C-19), 70.2 (C-2), 71.9 (C-5), 82.8 (C-6), 86.1 (C-3), 157.3 (C-1). ESI-MS exact mass for $\text{C}_{21}\text{H}_{34}\text{O}_2$ 318.2559, found $[\text{M-H}]^-$ 317.2481.

2,5-Tricosadiynoic acid (5f, 30%): mp 66.0–67.0 °C, ^1H NMR δ 0.86 (3H, t, $J = 7.0$ Hz, $\omega\text{-CH}_3$), 1.23 (28H, m, $(\text{CH}_2)_{14}$), 1.46 (2H, m, $\text{CH}_2\text{C-C}\equiv\text{C}$), 2.13 (2H, tt, $J = 2.3$ Hz, $J = 7.1$ Hz, $\text{CH}_2\text{C}\equiv\text{C}$), 3.30 (2H, t, $J = 2.3$ Hz, $\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{C}$), 9.5 (1H, br s, CO_2H); ^{13}C NMR δ 10.2 (C-4), 14.1 (C-23), 18.6 (C-7), 22.7 (C-22), 28.5 (C-8), 28.9 (C-9), 29.1 (C-10), 29.4 (C-20), 29.5 (C-19), 29.6 (C-11), 29.7 (C-12 to 18), 31.9 (C-21), 70.0 (C-2), 73.3 (C-5), 82.6 (C-6), 85.91 (C-3), 157.3 (C-1). ESI-MS exact mass for $\text{C}_{23}\text{H}_{38}\text{O}_2$ 346.2872, found $[\text{M-H}]^-$ 345.2729.

2.5. Preparation of 2,10-nonadecadiynoic acid

2.5.1. 1-Bromo-7-hexadecyne 6—Phosphorus tribromide (170 μL , 1.79 mmol) was added dropwise to a solution of 7-hexadecyn-1-ol (1.00 g, 4.19 mmol) in dry ether (10 mL). The reaction mixture was refluxed for 2.5 h and stirred at rt for 18 h, then poured over crushed ice and extracted with ether. The ether phases were combined, dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure, and purified by chromatography (elution with hexane) to give **6** (708 mg, 56%): ^1H NMR δ 0.83 (3H, t, $J = 6.6$ Hz, $\omega\text{-CH}_3$), 1.23 (10H, m, $(\text{CH}_2)_5$), 1.39 (8H, m, $(\text{CH}_2)_4$), 1.81 (2H, m, $\text{CH}_2\text{C-Br}$), 2.09 (4H, m, $\text{CH}_2\text{C}\equiv\text{C}$), 3.34 (2H, t, $J = 6.8$ Hz, CH_2Br); ^{13}C NMR δ 13.9 (C-16), 18.7 (C-6 and 9), 22.6 (C-15), 27.6 (C-3), 27.8 (C-4), 28.8 (C-5 and 10), 29.3 (C-11 to 13), 31.7 (C-14), 32.6 (C-1), 33.3 (C-2), 79.6 (C-7), 80.3 (C-8).

2.5.2. 1,9-Octadecadiyne 7—A suspension of lithium acetylide ethylenediamine complex (247 mg, 2.70 mmol) in dry DMSO (4 mL) was stirred for 10 min at room temperature under nitrogen. After the mixture was cooled to 8 °C, 1-bromo-7-hexadecyne (708 mg, 2.35 mmol) was added dropwise. The reaction mixture was stirred at rt overnight

and cooled to 0 °C. Water (1 mL) was added very slowly. The suspension was stirred for 10 min at rt, then poured into water (60 mL), and extracted with hexane. The combined organic phases were dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by chromatography (elution with hexane) to give 1,9-octadecadiyne (423 mg, 73%): ¹H NMR δ 0.82 (3H, t, *J* = 6.7 Hz, ω-CH₃), 1.21 (8H, m, (CH₂)₄), 1.35 (12H, m, (CH₂)₆), 1.86 (1H, t, *J* = 2.6 Hz, C≡CH), 2.10 (6H, m, CH₂C≡C); ¹³C NMR δ 14.0 (C-18), 18.3 (C-3), 18.7 (C-8 and 11), 22.6 (C-17), 28.2 (C-5 and 6), 28.4 (C-4), 28.8 and 29.0 (C-7 and 12), 29.1 (C-13 to 15), 31.8 (C-16), 68.0 (C-1), 79.9 (C-9), 80.2 (C-10), 84.4 (C-2).

2.5.3. 2,10-Nonadecadiynoic acid 8—To a solution of **7** (423 mg, 1.70 mmol) in dry THF (7 mL) at –23 °C was added *n*-butyllithium (2.5 M solution in hexanes, 0.69 mL, 1.73 mmol) under nitrogen. The reaction mixture was stirred at –23 °C for 1 h and then warmed to 0 °C. A stream of carbon dioxide was bubbled into the suspension until THF was evaporated. The residue was poured into ether/10% aqueous hydrochloric acid solution and extracted with diethyl ether. The combined organic phases were dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate/glacial acetic acid 75/25/1) to give 2,10-nonadecadiynoic acid (416 mg, 84%): mp 36.5–37.0 °C, ¹H NMR δ 0.85 (3H, t, *J* = 6.6 Hz, ω-CH₃), 1.24 (8H, m, (CH₂)₄), 1.39 (10H, m, (CH₂)₅), 1.57 (2H, m, CH₂C-C≡C), 2.11 (4H, m, CH₂C≡CCH₂), 2.33 (2H, t, *J* = 7.0 Hz, CH₂C≡C), 9.7 (1H, br s, CO₂H); ¹³C NMR δ 14.0 (C-19), 18.7 (C-4, 9 and 12), 22.6 (C-18), 27.3 (C-5), 28.1 (C-7), 28.3 (C-6), 28.8 (C-8 and 13), 29.1 (C-14 to 16), 31.8 (C-17), 72.7 (C-2), 79.9 (C-10), 80.5 (C-11), 92.5 (C-3), 158.1 (C-1). ESI-MS exact mass for C₁₉H₃₀O₂ 290.2246, found [M-H][–] 289.2043.

2.6. Preparation of 2,16-heptadecadiynoic acid 10

2.6.1. 1,15-Hexadecadiyne 9—The hexadecadiyne was prepared as described for 1,9-octadecadiyne **7**. Yield 66%: ¹H NMR δ 1.25 (12H, m, (CH₂)₆); 1.36 (4H, m, CH₂C-C≡C); 1.49 (4H, m, CH₂C-C≡C); 1.91 (1H, t, *J* = 2.6 Hz, C≡CH); 2.16 (4H, td, *J* = 2.6 Hz, *J* = 7.2 Hz, CH₂-C≡C); ¹³C NMR δ 18.4 (C-3 and 14), 28.5 (C-4 and 13), 28.7 (C-5 and 12), 29.1 (C-6 and 11), 29.5 and 29.6 (C-8, 9, 10 and 11), 68.0 (C-1 and 16), 84.8 (C-2 and 15).

2.6.2. 2,16-Heptadecadiynoic acid 10—The heptadecadiynoic acid was prepared as described for 2,10-nonadecadiynoic acid **8**. Yield 15%: ¹H NMR δ 1.25 (16H, m, (CH₂)₈); 1.50 (2H, m, H-14); 1.55 (2H, m, CH₂C-C≡C); 1.91 (1H, t, *J* = 2.5 Hz, C≡CH); 2.16 (4H, td, *J* = 2.5 Hz, *J* = 7.1 Hz, CH₂-C≡CH), 2.32 (2H, t, *J* = 7.1 Hz, CH₂-C≡C-CO), 9.1 (1H, brs, CO₂H); ¹³C NMR δ 18.4 (C-15), 18.7 (C-4), 27.3 (C-5), 28.5 (C-14), 28.7 (C-13), 28.8 (C-6), 29.0 (C-7), 29.1 (C-12), 29.3 (C-8), 29.4 (C-9), 29.5 (C-10 and 11), 68.0 (C-17), 72.5 (C-2), 84.8 (C-16), 92.8 (C-3), 158.1 (C-1). ESI-MS exact mass for C₁₇H₂₆O₂ 262.1933, found [M-H][–] 261.1855.

2.7. Preparation of thio and sulfinyl analogs

2.7.1 General procedure for the coupling of an alkyl thiol with an alkyl dibromide—A suspension of alkane 1-thiol (2.6 mmol), 1,ω-dibromoalkane (3.0 mmol) and potassium hydroxide (181 mg, 3.20 mmol) in ethanol (25 mL) was refluxed for 6 h,

cooled to rt, and acidified with 1 N aqueous hydrochloric acid. Ethanol was evaporated under reduced pressure, the residue was poured into H₂O-CH₂Cl₂ (1/1) and extracted with CH₂Cl₂. The organic phases were combined, dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate 99/1) to give:

1-Bromo-12-(ethylthio)-dodecane (11a, 64%): ¹H NMR δ 1.19 (3H, t, *J* = 7.4 Hz, CH₃C-S), 1.22 (16H, m, (CH₂)₈), 1.52 (2H, m, CH₂C-S), 1.80 (2H, m, CH₂C-Br), 2.46 (4H, m, CH₂S), 3.35 (2H, t, *J* = 6.8 Hz, CH₂Br).

1-Bromo-10-(hexylthio)-decane (11b, 71%): ¹H NMR δ 0.84 (3H, t, *J* = 6.6 Hz, ω-CH₃), 1.25 (18H, m, (CH₂)₉), 1.53 (4H, m, CH₂C-S), 1.81 (2H, m, CH₂C-Br), 2.45 (4H, t, *J* = 7.5 Hz, CH₂S), 3.31 (2H, t, *J* = 6.8 Hz, CH₂Br).

1-Bromo-12-(hexylthio)-dodecane (11c, 46%): ¹H NMR δ 0.81 (3H, t, *J* = 6.7 Hz, ω-CH₃), 1.20 (22H, m, (CH₂)₁₁), 1.49 (4H, m, CH₂C-S), 1.77 (2H, m, CH₂C-Br), 2.41 (4H, m, CH₂S), 3.31 (2H, t, *J* = 6.8 Hz, CH₂Br).

2.7.2. Thio-1-alkynes 12—The alkynes were prepared as described for 1,9-octadecadiyne.

14-(Ethylthio)-1-tetradecyne (12a, 83%): ¹H NMR δ 1.18 (3H, t, *J* = 7.5 Hz, ω-CH₃), 1.20 (16H, m, (CH₂)₈), 1.51 (4H, CH₂C-S + CH₂C-C≡C), 1.86 (1H, t, *J* = 2.6 Hz, C≡CH), 2.11 (2H, td, *J* = 2.6 Hz, *J* = 6.8 Hz, CH₂C≡C), 2.44 (2H, t, *J* = 7.3 Hz, CH₂S), 2.46 (2H, qd, *J* = 7.5 Hz, CH₂S); ¹³C NMR δ 14.7 (C-2'), 18.4 (C-3), 25.8 (C-1'), 28.4 (C-4), 28.7 (C-5), 28.9 (C-12), 29.0 (C-6), 29.2 (C-11), 29.5 (C-7 to 10), 29.6 (C-14), 31.7 (C-13), 68.0 (C-1), 84.5 (C-2).

12-(Hexylthio)-1-dodecyne (12b, 96%): ¹H NMR δ 0.81 (3H, t, *J* = 6.9 Hz, ω-CH₃), 1.22 (18H, m, (CH₂)₉), 1.46 (6H, m, CH₂C-C≡C and CH₂C-S), 1.85 (1H, t, *J* = 2.6 Hz, C≡CH), 2.09 (2H, td, *J* = 2.6 Hz, *J* = 6.8 Hz, CH₂C-C≡C), 2.42 (4H, t, *J* = 7.5 Hz, CH₂S); ¹³C NMR δ 13.9 (C-6'), 18.3 (C-3), 22.4 (C-5'), 28.4 (C-5), 28.5 (C-4), 28.6 (C-3'), 28.8 (C-6), 29.0 (C-10), 29.1 (C-7), 29.3 (C-8 and 9), 29.6 (C-12 and 1'), 31.3 (C-4'), 32.1 (C-11 and 2'), 67.9 (C-1), 84.4 (C-2).

14-(Hexylthio)-1-tetradecyne (12c, 58%): ¹H NMR δ 0.86 (3H, t, *J* = 6.8 Hz, ω-CH₃), 1.24 (22H, m, (CH₂)₁₁), 1.52 (6H, m, CH₂C-C≡C and CH₂C-S), 1.89 (1H, t, *J* = 2.5 Hz, C≡CH), 2.14 (2H, td, *J* = 2.5 Hz, *J* = 6.9 Hz, CH₂C≡C), 2.46 (4H, t, *J* = 6.9 Hz, CH₂S); ¹³C NMR δ 14.0 (C-6'), 18.4 (C-3), 22.5 (C-5'), 28.5 (C-5), 28.6 (C-4), 28.7 (C-3'), 28.9 (C-6), 29.1 (C-12), 29.2 (C-7), 29.5 (C-8 to 11), 29.7 (C-14 and 1'), 31.5 (C-4'), 32.2 (C-13 and 2'), 68.0 (C-1), 84.7 (C-2).

2.7.3. (Alkylthio)alk-2-ynoic acids 13—The acids were prepared as described in the general method for carboxylation of terminal alkynes.

15-(Ethylthio)pentadec-2-ynoic acid (13a, 69%): mp 48.5–49.0 °C, ^1H NMR δ 1.21 (3H, t, J = 7.5 Hz, ω -CH₃), 1.23 (16H, m, (CH₂)₈), 1.55 (4H, CH₂C-S and CH₂C $\equiv$ C), 2.32 (2H, t, J = 6.8 Hz, CH₂C $\equiv$ C), 2.48 (4H, m, CH₂S), 10.6 (1H, br s, CO₂H); ^{13}C NMR δ 14.7 (C-2'), 18.7 (C-4), 25.9 (C-1'), 27.3 (C-5), 28.7 (C-6), 28.9 (C-7 and 13), 29.2 (C-12), 29.3 (C-8), 29.4 (C-9 to 11), 29.6 (C-15), 31.6 (C-14), 72.6 (C-2), 92.6 (C-3), 158.0 (C-1). ESI-MS exact mass for C₁₇H₃₀O₂S 298.1967, found [M-H][−] 297.1888.

13-(Hexylthio)tridec-2-ynoic acid (13b, 66%): mp 45.0–45.5 °C, ^1H NMR δ 0.86 (3H, t, J = 6.8 Hz, ω -CH₃), 1.26 (18H, m, (CH₂)₉), 1.55 (6H, m, CH₂C-C $\equiv$ C and CH₂C-S), 2.32 (2H, t, J = 7.0 Hz, CH₂C $\equiv$ C), 2.47 (4H, t, J = 7.0 Hz, CH₂S), 9.3 (1H, br s, CO₂H); ^{13}C NMR δ 14.0 (C-6'), 18.7 (C-4), 22.5 (C-5'), 27.4 (C-5), 28.6 (C-3'), 28.8 (C-6), 28.9 (C-7 and 11), 29.2 (C-10), 29.3 (C-8), 29.4 (C-9), 29.7 (C-13 and 1'), 31.4 (C-4'), 32.2 (C-12 and 2'), 72.6 (C-2), 92.6 (C-3), 157.9 (C-1). ESI-MS exact mass for C₁₉H₃₄O₂S 326.2280, found [M-H][−] 325.2207.

15-(Hexylthio)pentadec-2-ynoic acid (13c, 56%): ^1H NMR δ 0.86 (3H, t, J = 6.6 Hz, ω -CH₃), 1.24 (22H, m, (CH₂)₁₁), 1.55 (6H, m, CH₂C-C $\equiv$ C and CH₂C-S), 2.32 (2H, t, J = 7.0 Hz, CH₂C $\equiv$ C), 2.47 (4H, m, CH₂S), 10.2 (1H, br s, CO₂H); ^{13}C NMR δ 14.0 (C-6'), 18.7 (C-4), 22.5 (C-5'), 27.4 (C-5), 28.0 (C-3'), 28.8 (C-6), 28.9 (C-7 and 13), 29.2 (C-12), 29.4 (C-8), 29.5 (C-9 to 11), 29.8 (C-15 and 1'), 31.4 (C-4'), 32.2 (C-14 and 2'), 72.6 (C-2), 92.6 (C-3), 158.0 (C-1). ESI-MS exact mass for C₂₁H₃₈O₂S 354.2593, found [M-H][−] 353.2407.

2.7.4. (Alkylsulfinyl)alk-2-ynoic acids 14—A solution of **13** (0.13 mmol) and hydrogen peroxide (30%, 0.5 mL) in acetone (20 mL) was stirred overnight at rt, concentrated, and the residue was purified by flash chromatography (elution with hexane/ethyl acetate/glacial acetic acid 1/4/0.05) to give:

15-(Ethylsulfinyl)pentadec-2-ynoic acid (14a, 88%): mp 54.0–55.0 °C, ^1H NMR δ 1.23 (16H, m, (CH₂)₈), 1.31 (3H, t, J = 7.5 Hz, ω -CH₃), 1.53 (2H, m, CH₂C-C $\equiv$ C), 1.72 (2H, m, CH₂C-S), 2.27 (2H, t, J = 6.8 Hz, CH₂C $\equiv$ C), 2.6–2.8 (4H, m, CH₂SO), 11.2 (1H, br s, CO₂H); ^{13}C NMR δ 6.8 (C-2'), 18.6 (C-4), 22.5 (C-14), 27.4 (C-5), 28.4 (C-13), 28.6 (C-6 and 12), 28.9 (C-7), 29.1 (C-8 to 11), 44.9 (C-1'), 50.8 (C-15), 72.8 (C-2), 89.3 (C-3), 155.7 (C-1). ESI-MS exact mass for C₁₇H₃₀O₃S 314.1916, found [M-H][−] 313.1870.

13-(Pentylsulfinyl)tridec-2-ynoic acid (14b, 67%): mp 55.0–56.0 °C, ^1H NMR δ 0.85 (3H, t, J = 6.4 Hz, ω -CH₃), 1.25 (18H, m, (CH₂)₉), 1.49 (2H, m, CH₂C-C $\equiv$ C), 1.72 (4H, m, CH₂C-S), 2.27 (2H, t, J = 6.9 Hz, CH₂C $\equiv$ C), 2.6–2.9 (4H, m, CH₂SO), 9.1 (1H, br s, CO₂H); ^{13}C NMR δ 13.9 (C-6'), 18.7 (C-4), 22.4 and 22.6 (C-12, 2' and 5'), 27.4 (C-5), 28.4 (C-3'), 28.6 (C-11), 28.7 (C-6 and 10), 29.0 (C-7), 29.1 (C-8 and 9), 31.3 (C-4'), 51.7 (C-13 and 1'), 71.4 (C-2), 89.3 (C-3), 155.7 (C-1). ESI-MS exact mass for C₁₉H₃₄O₃S 342.2229, found [M-H][−] 341.2079.

2.7.4. 4-(Alkylthio)but-2-ynoic acids 16—A suspension of alkane 1-thiol (2.25 mmol), 3-bromo-1-propyne (200 μL , 2.24 mmol) and potassium hydroxide (134 mg, 2.40 mmol) in ethanol (21 mL) was refluxed for 6 h, cooled to rt, and acidified with 1 N aqueous hydrochloric acid. Ethanol was evaporated under reduced pressure, the residue was poured

into H₂O-CH₂Cl₂, and extracted with CH₂Cl₂. The organic phases were combined, dried (Na₂SO₄), filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in THF (6 mL) and cooled to -23°C. *n*-Butyllithium (2.5 M in hexanes, 0.60 mL, 1.50 mmol) was slowly added. The reaction mixture was stirred at -23°C for 1 h and then warmed to 0 °C. A stream of carbon dioxide was bubbled into the suspension until THF was evaporated. The residue was poured into ether/10% aqueous hydrochloric acid solution. The aqueous phase was extracted three times with ether. The organic phases were pooled, dried (Na₂SO₄), filtered, evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate/glacial acetic acid 85/15/1) to give:

4-(Dodecylthio)but-2-ynoic acid (16a, 52%): mp 47.0–47.5 °C, ¹H NMR δ 0.88 (3H, t, *J* = 7.0 Hz, ω-CH₃), 1.26 (16H, m, (CH₂)₈), 1.40 (2H, m), 1.62 (2H, m, CH₂C-S), 2.69 (2H, t, *J* = 7.4 Hz, CH₂S), 3.37 (2H, s, S-CH₂C≡C), 7.9 (1H, br s, CO₂H); ¹³C NMR δ 14.1 (C-12'), 19.1 (C-4), 22.7 (C-11'), 28.7 (C-3'), 28.8 (C-4'), 29.2 (C-1'), 29.3 (C-9'), 29.4, 29.5 and 29.6 (C-5' to 8'), 31.9 (C-10'), 32.0 (C-2'), 74.0 (C-2), 87.3 (C-3), 157.4 (C-1). ESI-MS exact mass for C₁₆H₂₈O₂S 284.1810, found [M-H]⁻ 283.1599.

4-(Tetradecylthio)but-2-ynoic acid (16b, 48%): mp 54.0–54.5 °C, ¹H NMR δ 0.88 (3H, t, *J* = 7.2 Hz, ω-CH₃), 1.26 (20H, m, (CH₂)₁₀), 1.40 (2H, m), 1.62 (2H, m, CH₂C-S), 2.69 (2H, t, *J* = 7.2 Hz, CH₂S), 3.37 (2H, s, S-CH₂C≡C), 9.2 (1H, br s, CO₂H); ¹³C NMR δ 14.1 (C-14'), 19.1 (C-4), 22.7 (C-13'), 28.7 (C-3'), 28.8 (C-4'), 29.2 (C-1'), 29.3 (C-11'), 29.5, 29.6 and 29.7 (C-5' to 10'), 31.9 (C-12'), 32.0 (C-2'), 74.1 (C-2), 86.6 (C-3), 156.7 (C-1). ESI-MS exact mass for C₁₈H₃₂O₂S 312.2123, found [M-H]⁻ 311.2046.

2.8. Preparation of N'-(hexadec-2-ynoyl)isonicotinohydrazide 17

To a solution of 2-hexadecynoic acid (66 mg, 0.24 mmol) in dry THF (6.6 mL) was added diisopropylamine (55 mL, 0.31 mmol) and isobutyl chloroformate (50 mL, 0.38 mmol) under nitrogen. The solution was stirred overnight at rt, filtered through Celite, concentrated, and redissolved into dry THF (2 mL). Isoniazid (120 mg, 0.87 mmol) was added and the suspension was stirred at rt under nitrogen for two days. The reaction mixture was poured into water and extracted with CH₂Cl₂. The combined organic phases were combined, dried (Na₂SO₄), evaporated to dryness under reduced pressure, and purified by preparative TLC (elution with hexane/ethyl acetate 4/6) to give **17** (68 mg, 70%): ¹H NMR δ 0.84 (3H, t, *J* = 6.7 Hz, ω-CH₃), 1.21 (20H, m, (CH₂)₁₀), 1.51 (2H, m, CH₂C-C≡C), 2.26 (2H, t, *J* = 7.0 Hz, CH₂C≡C), 7.67 and 8.66 (4H, 2 br s, pyridine ring), 9.6 (2H, br s, NH); ¹³C NMR δ 13.9 (C-16), 18.7 (C-4), 22.6 (C-15), 27.5 (C-5), 28.8 (C-6), 29.0 (C-13), 29.3 (C-7), 29.4 (C-12), 29.6 (C-8 to 11), 31.8 (C-14), 72.8 (C-3), 92.7 (C-2), 121.4 (C-3'), 138.7 (C-2'), 149.9 (C-4'), 163.0 and 175.5 (C-1 and 1'). ESI-MS exact mass for C₂₂H₃₃N₃O₂ 371.2573, found [M+H]⁺ 372.2673.

2.9. Preparation of 3-(octadec-2-ynoyloxy)propyl pyrazinoate and isonicotinate

2.9.1. 3-Bromopropyl octadec-2-ynoate 18—A mixture of 2-octadecynoic acid (280 mg, 1.00 mmol), 1,3-dibromopropane (0.15 mL, 1.47 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.15 mL, 1.0 mmol) in benzene (2 mL) was refluxed for 15 h, cooled to rt, and poured into hexane/water (1/1, 50 mL). The aqueous phase was

extracted with CH₂Cl₂. The combined organic phases were dried (Na₂SO₄), evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate 97/3) to give **18** (83%): ¹H NMR δ 0.85 (3H, t, *J* = 7.0 Hz, ω-CH₃), 1.23 (24H, m, (CH₂)₁₂), 1.55 (2H, m, CH₂C-C≡C), 2.19 (2H, qt, *J* = 6.3 Hz, CH₂C-Br), 2.30 (2H, t, *J* = 7.2 Hz, CH₂C≡C), 3.46 (2H, t, *J* = 6.5 Hz, CH₂Br), 4.27 (2H, t, *J* = 6.0 Hz, CH₂OCO).

2.9.2. 3-(Octadec-2-ynoyloxy)propyl pyrazine-2-carboxylate 19a—The esterification reaction was done as described for **18** using 2-pyrazinecarboxylic acid (48 mg, 0.39 mmol) and 3'-bromopropyl-2-octadecynoate (140 mg, 0.35 mmol) to give **19a** (69%): mp 46.5–47°C, ¹H NMR δ 0.84 (3H, t, *J* = 7.1 Hz, ω-CH₃), 1.21 (24H, m, (CH₂)₁₂), 1.53 (2H, m, CH₂C-C≡C), 2.19 (2H, qt, *J* = 6.3 Hz, CH₂C-O), 2.28 (2H, t, *J* = 7.2 Hz, CH₂C≡C), 4.30 (2H, t, *J* = 6.1 Hz, CH₂O), 4.53 (2H, t, *J* = 6.3 Hz, CH₂O), 8.70 and 8.74 (2H, br s, H-5' and 6'), 9.28 (1H, br s, H-3'): ¹³C NMR δ 14.1 (C-18), 18.6 (C-4), 22.6 (C-17), 27.4 (C-5), 27.7 (C-2'), 28.8 (C-6), 29.0 (C-7), 29.3 (C-8 and 15), 29.6 (C-9 to 14), 31.9 (C-16), 62.1 and 62.8 (C-1' and 3'), 72.8 (C-2), 92.3 (C-3), 143.2, 144.4, 146.3 and 147.7 (pyrazine ring), 153.6 and 163.8 (CO₂). ESI-MS exact mass for C₂₆H₄₀N₂O₄ 444.2988, found [M+H]⁺ 445.2948.

2.9.3. 3-(Octadec-2-ynoyloxy)propyl isonicotinate 19b—The esterification reaction was done as described above using isonicotinic acid (58 mg, 0.47 mmol) and 3'-bromopropyl-2-octadecynoate (162 mg, 0.40 mmol) to give **19b** (64%): mp 34.0°C, ¹H NMR δ 0.84 (3H, t, *J* = 7.0 Hz, ω-CH₃), 1.21 (24H, m, (CH₂)₁₂), 1.53 (2H, m, CH₂C-C≡C), 2.14 (2H, qt, *J* = 6.2 Hz, CH₂C-O), 2.28 (2H, t, *J* = 7.2 Hz, CH₂C≡C), 4.30 (2H, t, *J* = 6.2 Hz, CH₂OC(O)C≡C), 4.44 (2H, t, *J* = 6.0 Hz, CH₂OCO), 7.84 and 8.76 (4H, 2 br s, pyridine ring); ¹³C NMR δ 14.1 (C-18), 18.6 (C-4), 22.6 (C-17), 27.4 (C-5), 27.7 (C-2'), 28.8 (C-6), 29.0 (C-7), 29.3 (C-8 and 15), 29.6 (C-7 to 14), 31.9 (C-16), 62.1 and 62.3 (C-1' and 3'), 72.7 (C-2), 92.3 (C-3), 123.0 (C-2'' and 6''), 137.4 (C-1''), 150.2 (C-3'' and 5''), 153.6 and 164.8 (CO₂). ESI-MS exact mass for C₂₇H₄₁NO₄ 443.3036, found [M+H]⁺ 444.2992.

2.10. Preparation of 2-hydroxyethyl alk-2-ynoates

A mixture of 2-alkynoic acid (1.00 mmol), 2-bromoethanol (140 μL, 2 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (300 μL, 2.0 mmol) in benzene (5 mL) was refluxed for 15 h, cooled to rt, and poured into hexane/water (1/1, 50 mL). The aqueous phase was extracted with CH₂Cl₂. The combined organic phases were dried (Na₂SO₄), evaporated to dryness under reduced pressure, and purified by flash chromatography (elution with hexane/ethyl acetate 7/3) to give **20**.

2-Hydroxyethyl hexadec-2-ynoate (20a, 77%)—¹H NMR δ 0.86 (3H, t, *J* = 6.7 Hz, ω-CH₃); 1.24 (18H, m, (CH₂)₉); 1.37 (2H, m, CH₂C-C-C≡C), 1.57 (2H, m, CH₂C-C≡C); 2.33 (2H, t, *J* = 7.2 Hz, CH₂C≡C); 3.84 (2H, m, CH₂COH), 4.27 (2H, m, CH₂OH); ¹³C NMR δ 14.1 (C-16'); 18.7 (C-4'); 22.6 (C-15'); 27.5 (C-5'); 28.8 (C-6'); 29.0 (C-7'); 29.4 (C-8' and 13'); 29.6 (C-9' to 12'); 31.9 (C-14'); 60.3 (C-2); 66.9 (C-1); 72.9 (C-2'); 89.9 (C-3'); 154.2 (C-1'). ESI-MS exact mass for C₁₈H₃₂O₃ 296.2351, found [M+H]⁺ 297.2579.

2-Hydroxyethyl octadec-2-ynoate (20b, 54%)— ^1H NMR δ 0.85 (3H, t, J = 6.1 Hz, ω -CH₃); 1.23 (22H, m, (CH₂)₁₁); 1.37 (2H, m, CH₂C-C-C $\equiv$ C), 1.56 (2H, m, CH₂C-C $\equiv$ C); 2.31 (2H, t, J = 6.7 Hz, CH₂C $\equiv$ C); 3.84 (2H, m, CH₂COH), 4.26 (2H, m, CH₂OH); ^{13}C NMR δ 14.1 (C-18'); 18.7 (C-4'); 22.7 (C-17'); 27.4 (C-5'); 28.8 (C-6'); 29.0 (C-7'); 29.4 (C-8' and 15'); 29.7 (C-9' to 14'); 31.9 (C-16'); 60.8 (C-2); 67.1 (C-1); 72.7 (C-2'); 90.7 (C-3'); 153.9 (C-1'). ESI-MS exact mass for C₂₀H₃₆O₃ 324.2664, found [M+H]⁺ 325.2854.

2.11. Preparation of 2,3-dihydroxypropyl alk-2-ynoate 21

To a solution of 2-alkynoic acid (0.46 mmol) in dry CH₂Cl₂ (4 mL) were added under nitrogen (*R*)-(-)-isopropylidene glycerol (50 μL , 0.40 mmol), 4-(dimethylamino)pyridine (DMAP, 4 mg, 0.03 mmol), and *N,N'*-dicyclohexylcarbodiimide (DCC, 134 mg, 0.65 mmol). The suspension was stirred for 15 h at rt, filtered, and purified by column chromatography (elution with hexane/ethyl acetate 95/5) to give the protected ester. The protected ester (0.12 mmol) in solution in dry CH₂Cl₂ (4 mL) was stirred with *p*-toluenesulfonic acid (14 mg, 0.07 mmol) under nitrogen. After 20 h, triethylamine (10 μL , 0.07 mmol) was added, and the reaction mixture was washed with saturated sodium bicarbonate solution, dried (Na₂SO₄), filtered, and purified by flash chromatography (elution with hexane/ethyl acetate 3/2) to give the monoacylglycerol **21**.

2,3-Dihydroxypropyl hexadec-2-ynoate (21a, 81%)— ^1H NMR δ 0.86 (3H, t, J = 7.0 Hz, ω -CH₃), 1.24 (18H, m, (CH₂)₁₁), 1.34 (2H, m), 1.56 (2H, m, CH₂C-C $\equiv$ C), 2.33 (2H, t, J = 7.1 Hz, CH₂C $\equiv$ C), 2.52 (1H, br s, OH), 2.87 (1H, br s, OH), 3.59 (1H, dd, J = 5.8 Hz, J = 11.5 Hz, CH₂OH), 3.63 (1H, dd, J = 3.7 Hz, J = 11.5 Hz, CH₂OH), 3.99 (1H, m, CHOH), 4.17 (1H, dd, J = 6.3 Hz, J = 11.5 Hz, CH₂OCO), 4.32 (1H, dd, J = 4.6 Hz, J = 11.5 Hz, CH₂OCO); ^{13}C NMR δ 14.1 (C-16'); 18.7 (C-4'); 22.6 (C-15'); 27.5 (C-5'); 28.8 (C-6'); 29.0 (C-7'); 29.4 (C-13'); 29.6 (C-8' to 12'); 31.8 (C-14'); 63.5 (C-3), 66.1 (C-1), 69.9 (C-2), 72.8 (C-2'); 92.7 (C-3'); 154.2 (C-1'). ESI-MS exact mass for C₁₉H₃₄O₄ 326.2457, found [M+H]⁺ 327.2636.

2,3-Dihydroxypropyl octadec-2-ynoate (21b, 91 %)—mp 52.0–52.5°C, ^1H NMR δ 0.85 (3H, t, J = 7.0 Hz, ω -CH₃), 1.23 (22H, m, (CH₂)₁₁), 1.36 (2H, m), 1.55 (2H, m, CH₂C-C $\equiv$ C), 2.30 (2H, t, J = 7.1 Hz, CH₂C $\equiv$ C), 2.52 (1H, br s, OH), 2.87 (1H, br s, OH), 3.59 (1H, dd, J = 5.8 Hz, J = 11.5 Hz, CH₂OH), 3.69 (1H, dd, J = 3.7 Hz, J = 11.5 Hz, CH₂OH), 3.94 (1H, m, CHOH), 4.17 (1H, dd, J = 6.3 Hz, J = 11.5 Hz, CH₂OCO), 4.23 (1H, dd, J = 4.6 Hz, J = 11.5 Hz, CH₂OCO); ^{13}C NMR δ 14.1 (C-18'), 18.7 (C-4'), 22.7 (C-17'), 27.4 (C-5'), 28.9 (C-6'), 29.0 (C-7'), 29.4 (C-8' and 15'), 29.6 (C-9' to 14'), 31.9 (C-16'), 63.2 (C-3), 66.3 (C-1), 69.8 (C-2), 72.6 (C-2'), 91.1 (C-3'), 153.9 (C-1'). ESI-MS exact mass for C₂₁H₃₈O₄ 354.2770, found [M+H]⁺ 355.2892.

2.14. Bacterial strains and media

The *M. smegmatis* and *M. bovis* BCG Pasteur strains were obtained from the laboratory stocks. The *M. smegmatis* strains were grown at 37°C in Mueller-Hinton broth (Difco) supplemented with 0.05% (v/v) Tween 80. The *M. bovis* BCG strains were grown at 37°C in Sauton media (Connell, 1994). The solid media used was Middlebrook 7H10 (Difco) supplemented with 0.2% (v/v) glycerol and 10% (v/v) OADC enrichment (Difco).

2.15. Minimum inhibitory concentration (MIC) determination

The alkynoic acid derivatives were dissolved in DMSO. *M. smegmatis* cultures were grown in Mueller-Hinton media and *M. bovis* BCG cultures were grown in Sauton media as described above. MICs were determined on solid media and liquid media. For MICs determined on plates, cultures were grown to an $OD_{600nm} \approx 1$ and ten-fold serial dilutions were plated on plates containing different concentrations of compound tested. The MIC₉₉ was determined as the concentration of compound that reduced the number of colony forming unit/ml by 99%. For determination of MIC in liquid media, cultures were grown to an $OD_{600nm} \approx 0.7$ and diluted 1/500. Serial two-fold dilutions of each drug were prepared directly in a sterile 96-well plate using 0.1 ml media and 0.1 ml of diluted culture was added to each well. The last row of the 96-well plate was used as a growth (no compound) control. The plates were incubated at 37 °C for 2 (*M. smegmatis*) or 10 (*M. bovis*) days and read in a Perkin Elmer Victor³V 1420 multi label counter. The MIC was determined as the concentration of compound that inhibited 99% of the growth.

2.16. Analysis of fatty acid methyl esters (FAMES) and mycolic acid methyl esters (MAMES) by thin layer chromatography (TLC)

Early log-phase mycobacterial cultures (10 ml, $OD_{600nm} \approx 0.5$) were treated with the compounds at the indicated concentrations for 1 hr (*M. smegmatis*) or 24 h (*M. bovis*) at 37 °C before labeling with [$1-^{14}C$]- acetate (10 μ Ci) for 1 hr (*M. smegmatis*) or 6 h (*M. bovis*) at 37 °C. The cultures were spun. The cell pellets were washed once with water, resuspended in distilled water (1 ml) and treated with a 40% solution of tetrabutylammonium hydroxide (1 ml) at 100 °C for 20 h. The suspensions were cooled, methyl chloride (2 ml) and methyl iodide (0.1 ml) were added, and the mixtures were mixed for 1 h at 25 °C. The organic phases were washed once with an aqueous solution of 3N hydrochloric acid, once with water, dried over anhydrous sodium sulfate, filtered, evaporated under nitrogen, and resuspended in methylene chloride (0.25 ml). The resulting solutions contain both FAMES and MAMES. An aliquot (5 μ l) of each solution was spotted onto a silica gel 60 F254 thin-layer chromatography (TLC) plate. The TLC plate was eluted 3 times with hexane/ethyl acetate (95/5, v/v). FAMES and MAMES were detected by autoradiography after exposure for 48 h at -80 °C.

2.17. Determination of the critical micelle concentration (CMC)

CMCs were obtained by serial-dilution of the fatty acids in phosphate-buffered saline containing 2.5 μ M of Rhodamine 6G. For each dilution, the maximum absorption wavelength was determined using a Perkin Elmer spectrophotometer. Once the fatty acid forms a micelle, the wavelength of maximum absorption of the dye incorporated into the micelle shifts (Courtney et al., 1986). The CMCs were determined as the highest concentration of fatty acid that did not allow incorporation of the dye.

2.18. Cytotoxicity assay

African green monkey kidney cells were seeded in 96-well plates and cultured in DMEM containing 10% fetal bovine serum. Increasing concentrations of alkynoic acid derivatives (from 1 μ g/ml up to 5 mg/ml) and DMSO (from 0.002 to 5 %, v/v) were added to the wells

and the plates were incubated at 37°C for 3 days. The viability was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann, 1983). Plates were read at 590 nm in a Perkin Elmer Victor³V 1420 multi label counter.

3. Results and Discussion

3.1. Chemistry

3.1.2. Introduction of additional unsaturation or heteroatom in the carbon chain

—Our strategy to obtain a series of derivatives of 2-alkynoic acids with a modified carbon chain focused on preparing intermediates with a terminal bromide that could be transformed into a terminal alkyne by displacement with lithium acetylide ethylene diamine (EDA) complex at room temperature in DMSO (Smith and Beumel, 1974). Treatment of the terminal alkyne with *n*-butyllithium in tetrahydrofuran (THF) at –23 °C followed by carboxylation using dry ice and subsequent acidification afforded the terminal 2-ynoic acid (Camps et al., 1992).

3.1.2.1. Alkadiynoic acids: 2,4-Hexadecadiynoic acid **2** was prepared from 1,4-bis(trimethylsilyl)-1,3-butadiyne by monodesilylation with methylolithium as a complex with lithium bromide at –78°C, followed by coupling with 1-bromoundecane in hexamethylphosphoramide (HMPA) and THF, and a second desilylation using potassium fluoride dihydrate to give 1,3-pentadecadiyne **1** in 57% yield (Scheme 1.1) (Xu et al., 1991). Carboxylation and acidification as described above gave 2,4-hexadecadiynoic acid **2** in 73% yield.

2,5-Alkadiynoic acids **5** (Scheme 1.2) were obtained by coupling of an alkynylmagnesium bromide with propargyl bromide in the presence of cuprous iodide at –10°C in THF (Millar and Underhill, 1986). The condensation reaction occurred in moderate to good yield (63% to 80%). The carboxylation of 1,4-alkadiynes using the usual procedure led only to traces of 2,5-alkadiynoic acids. At 0°C, the addition of *n*-butyllithium under nitrogen formed a brown mixture. To limit the formation of by-products, the carboxylation of **4** was done at a lower temperature (–78°C), the reaction mixture was diluted 10 times, and the reaction time was shortened. After two recrystallizations, the acids **5** were obtained in 30% to 62% yield. The proton and carbon NMR corresponded to published data (Gunstone et al., 1976) (for CH₂ between the two triple bonds: $\delta_H = 3.$, $\delta_C = 10.2$). Another method was also tested to prepare 1,4-alkadiynes, which consisted in the condensation of acetylide to a 2-alkyne bearing a terminal leaving group (tosylate) in the presence of cuprous bromide but this reaction occurred with a very low yield (36%) (Brandsma, 1971).

Alkadiynoic acids with more than two methylene groups between the two unsaturations were prepared by treatment of 1-bromoalkynes or 1, ω -dibromo-alkanes with lithium acetylide, ethylenediamine complex followed by carboxylation of the lithioalkyne as described above (Scheme 1.3 and 1.4). 1-Bromo-7-hexadecyne **6** was obtained from commercially available 7-hexadecyn-1-ol using phosphorus tribromide in ether (Reijnders et al., 1979). Carboxylation of the alkyne **7** afforded 2,10-nonadecadiynoic acid **8** in 84% yield, while mono-carboxylation of the dialkyne **9** gave 2,16-heptadiynoic acid **10** in 15% yield.

3.1.2.2. Thio-2-alkynoic acids: Thioalkynoic acids were prepared according to the method published by Pascal et al (Pascal and Ziering, 1986). The sulfur atom was placed 1, 10, or 12 methylene groups away from the triple bond at the position 2 based on the availability of starting materials. Condensation of alkyl thiols and dibromoalkanes in the presence of potassium hydroxide afforded 1-bromothioethers **11** in moderate yields. Introduction of the triple bond and the carboxylic group were done as described above to yield thia-2-alkynoic acids **13**. Oxidation of **13** with hydrogen peroxide in acetone at room temperature led to the corresponding sulfoxides **14** in moderate yield (Scheme 2.1).

5-Thia-2-alkynoic acids **16** were obtained using propargyl bromide for the condensation reaction instead of dibromoalkanes. As with the preparation of 2,5-alkadiynoic acids, the carboxylation of the terminal alkyne occurred with production of unidentified side products that lowered the yield (about 50%) (Scheme 2.2). The compounds were characterized by their typical NMR signal for the methylene group at position 4 (singlet, $\delta_{\text{H}} = 3.37$, $\delta_{\text{C}} = 19.1$).

3.1.3. Head group modification of 2-alkynoic acids—The carboxylic group of 2-alkynoic acids was esterified with polar alcohols or with the antituberculosis drugs isoniazid or pyrazinamide or their analogs.

The coupling of 2-HA with INH (isonicotinic hydrazide) was achieved by activating the carboxylic group with isobutyl chloroformate and diisopropylamine in THF and then reacting the resulting mixed anhydride with isoniazid in suspension in THF. The coupled product **17** was obtained in 70% yield (Scheme 3.1). 2-OA was coupled to pyrazinoic acid or isonicotinic acid by introducing a three-carbon spacer between the two acids. 2-OA was acylated with 1,3-dibromopropane in benzene, using DBU as a base, in 83% yield (Ono et al., 1978). A second acid (pyrazinoic or isonicotinic acid) was then coupled to the resulting bromoester **18**, using the same procedure, to lead to the diester **19** in 60–70% yield (Scheme 3.2). The order of coupling was found to be important. When the first coupling reaction involved isonicotinic acid and 1,3-dibromopropane, the resulting 3'-bromopropyl isonicotinoyl was obtained in only 36% yield.

2-Hydroxyethyl 2'-alkynoates **20** were prepared by condensation of 2-alkynoic acids with bromoethanol using DBU as a base as described above. The yields varied from 54% to 77% (Scheme 3.3).

1-(2'-Alkynoyl)-*sn*-glycerol **21** were obtained from commercially available (*R*)-(-)-isopropylidene glycerol by acylation of the free primary hydroxyl group with 2-alkynoic acid in presence of 1,3-dicyclohexylcarbodiimide (DCC) and catalytic 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 followed by careful deprotection of the acetonide **26** using *p*-TsOH in CH_2Cl_2 . (Scheme 3.4).

3.2. Biology

3.2.1. 2-Alkynoic acid derivatives inhibit the growth of fast- and slow-grower, drug-susceptible mycobacteria—The 2-alkynoic acid derivatives were tested against the fast-grower *M. smegmatis* mc²155 (Snapper et al., 1990) and the slow-grower *M. bovis*

BCG Pasteur. Modification of the carbon chain of the 2-alkynoic acids with either additional unsaturations or heteroatoms did not improve the MIC of the compounds against *M. smegmatis*, although the derivatives with a carbon chain of more than 19 carbons had better MIC than their corresponding parents. We had previously demonstrated that, in *M. smegmatis*, 2-HA was a pro-drug. 2-HA was metabolized into 3-HA and 3-ketohexadecanoic acid, which inhibited fatty acid degradation and fatty acid biosynthesis, respectively. It was the combined inhibition of fatty acid biosynthesis and fatty acid degradation that rendered 2-HA bactericidal in *M. smegmatis* (Morbidoni et al., 2006). It is possible that the modification of the carbon chain at the 5-position impede the isomerisation of the triple bond from the 2 to the 3 position, limiting the formation of the 3-alkynoic acid required for the antimycobacterial activity of 2-alkynoic acid. The mono (**20a**, **20b**) and dihydroxyl (**21a**, **21b**) esters (monoacyl glycerol) were as potent against *M. smegmatis* as their parental compounds (Table 1). The esterification of the 2-alkynoic acids would allow for a better penetration of the compounds. Once inside the cells, an esterase could release the 2-alkynoic acid which could then inhibit *M. smegmatis* as previously described. Coupling of 2-OA with either pyrazinoic acid or isonicotinic acid had opposite results. Pyrazinoic acid and isonicotinic acid are analogs of two first-line antitubercular compounds: pyrazinamide and isoniazid. Both compounds are pro-drugs that need to be activated by specific enzymes to be bactericidal. Pyrazinamide is activated by the pyrazinamidase PncA to form pyrazinoic acid (POA), the active form of the drug (Scorpio and Zhang, 1996). Pyrazinoic acid esters have been shown to have antimycobacterial activity (Cynamon et al., 1995) so we hypothesized that an esterase could cleave the ester **19b** releasing both 2-OA and POA inside *M. smegmatis*. Compound **19b** had an MIC of 11 μ M, suggesting that although the coupling of the two compounds was not synergistic since the MIC was only 3 times higher than for 2-OA alone, the compound could be cleaved in situ. Isoniazid is activated by the catalase peroxidase KatG (Zhang et al., 1992) into an isonicotinoyl radical, which reacts with NAD⁺ to form an INH-NAD adduct (Rozwarski et al., 1998; Wilming and Johnsson, 1999) that inhibits InhA (Lei et al., 2000). Direct coupling of 2-HA with isoniazid led to **17** which had no activity against *M. smegmatis*. The most likely explanation for the lack of activity of the isoniazid derivative is that this compound is not recognized by KatG and therefore the compound is not cleaved and activated. Furthermore, the coupling of 2-OA with isonicotinic acid with a 3-carbon spacer in between (compound **19a**) led to a compound with a very high MIC (90 μ M) suggesting that either the compound did not get cleaved (unlikely considering the activity of **19b**) or that the release of isonicotinic acid was antagonistic to the activity of 2-OA.

3.2.2. Differential effects of 2-alkynoic acids derivatives on fast- and slow-growing mycobacteria—The 2-alkynoic acid derivatives were more active against *M. bovis* BCG than against *M. smegmatis* with most of the compounds having MIC less than 10 μ M (Table 1). Interestingly, modification of the carbon chain did not increase the MIC as observed for *M. smegmatis*. Instead, the compounds were as or more potent (**16a/b**) than the corresponding 2-alkynoic acids. There was a reproducible decrease in MIC for compounds with longer-carbon chain in *M. bovis* that was not observed in *M. smegmatis* (**5b** versus **5c/d**, **13b** versus **13c**, **16a** versus **16b**). Even 2-eicosynoic acid (2-EA) had some activity in *M. bovis* while being totally inactive in *M. smegmatis*. This increase susceptibility of *M.*

bovis to longer-chain fatty acids might be attributed to the ability of its FASI and FASII systems to synthesize longer-chain fatty acids than *M. smegmatis*. Interestingly, the pyrazinoate ester of 2-OA was still active against *M. bovis* with an MIC of 6 μ M. Considering that the MIC of *n*-propyl pyrazinoate is about 0.48 mM at neutral pH against *M. tuberculosis* (Zimhony et al., 2007) and that the MIC of pyrazinoate esters is considerably lower (MICs from 1 to 100 μ M) when tested in acidic pH (Cynamon et al., 1995), this compound may be a potent inhibitor of *M. tuberculosis* growth in vitro under acidic conditions.

Next, the 2-alkynoic acid derivatives were tested in liquid cultures (Fig. 2A). While 100 μ M of the derivatives had limited if any effect on the growth of *M. smegmatis*, a bactericidal activity was observed for 2-HA, 2-OA, **16a/b** and **20b**. The 4-thio-2-alkynoic acid compounds were as active as 2-HA or 2-OA and 10 times more bactericidal than INH. This trend was confirmed in *M. bovis* where 20 μ M of **16a** and **16b** were more potent than the parent 2-alkynoic acids or INH, resulting in a 4-log decrease in colony-forming units within 3 days (Fig. 2B). Although *M. smegmatis* is not a virulent strain, it has been used successfully to identify new drugs that are active against *M. tuberculosis*. Bedaquiline, which has been recently approved by the FDA for the treatment of MDR-TB, was first identified in a whole cell screen assay against *M. smegmatis* (Andries et al., 2005). In drug screening, *M. smegmatis* has the advantage of being non-virulent and growing with a doubling time of 3 h instead of 22 h for *M. tuberculosis* and *M. bovis*. On the other hand, *M. smegmatis* genome is 6.99 Mbp versus 4.41 Mbp for the *M. tuberculosis* genome. The difference in genome sizes might lead to the identification of compounds active against *M. smegmatis* but not *M. tuberculosis* or rejection of compounds inactive against *M. smegmatis* that might have potential against *M. tuberculosis*. In this present work, most of our derivatives had no or limited activity against *M. smegmatis* based on their MIC data, yet their MIC was up to 160 times lower (compound **16b**) against the slow-grower *M. bovis* BCG. *M. smegmatis* may have additional detoxification pathway or efflux pumps that may have played a role in the lack of activity of these compounds. One set of derivatives, the 4-(alkylthio)but-2-ynoic acids, were as potent against *M. smegmatis* as *M. bovis* in liquid cultures. Although *M. bovis* infection affects mostly the bovine population, cases have been reported in humans (Muller et al., 2013). Disseminated BCG disease has been observed in infants receiving BCG vaccination and resulted in high mortality rate (Talbot et al., 1997). In addition, a rare complication from the treatment of superficial bladder cancer with intravesical *M. bovis* BCG is the development of disseminated BCG disease (Srivastava et al., 2011). *M. bovis* is inherently resistant to pyrazinamide due to a genetic mutation in *pncA* (Scorpio and Zhang, 1996), which renders treatment of such an infection lengthier as pyrazinamide is a key sterilizing drug in the TB drug regimen chemotherapy. Any novel compound or scaffold for which new drugs could be designed would improve drug regimen and have the capability to shorten chemotherapy for mycobacterial diseases.

3.2.3. 2-Alkynoic acid derivatives inhibit mycolic acid biosynthesis in

mycobacteria—2-Alkynoic acids were designed as inhibitors of the 2-enoyl-ACP reductase of the FASII system. Therefore inhibition by the 2-alkynoic acid should result in loss of mycolic acids, the FASII end product (Fig. 1). When *M. smegmatis* was treated with

2-HA or 2-OA, the inhibition of FASII was observed as expected but the inhibition of the FASI system in a dose-dependent manner was also observed (Morbidoni et al., 2006). To test whether the modified 2-alkynoic acids still targeted the fatty acid biosynthesis system, an early log phase culture of *M. smegmatis* was treated with derivatives **5c**, **5d**, **8**, **10**, **13a**, **13b**, **13c**, **16a**, **16b**, **20a**, **20b**, **21a**, **21b**, or INH at 100 μ M for 1 hour and then labeled with [$1\text{-}^{14}\text{C}$]-acetate for 1 h. (Fig. 3A). Mycolic acid biosynthesis was inhibited in *M. smegmatis* treated with INH, 2-OA, **5d**, **13a**, **16a/b**, and **21b** and fatty acid biosynthesis was inhibited only in *M. smegmatis* treated with **16b**. In *M. bovis*, inhibition of both fatty acid and mycolic acid biosyntheses was observed only with **16a** and **16b** (Fig. 3B). Longer-chain dialkynoic acids (C19 and C20) failed to inhibit mycolic acid biosynthesis at the concentration tested (20 μ M) in *M. bovis*. This data indicates that these derivatives inhibit predominantly FASII and therefore suggests that their main mechanism of action is similar to their parent compounds.

3.2.4. 2-Alkynoic acid derivatives inhibit the growth of INH-resistant *M. smegmatis* and *M. bovis* BCG strains—

To confirm that the derivatives inhibit FASII by targeting InhA, they were tested against *M. smegmatis* strains that were resistant to INH due to either overexpression of *inhA* (Larsen et al., 2002) or a Ser94Ala mutation in *inhA*. The Ser94Ala mutation was first identified in an INH-resistant *M. smegmatis* strain mc²651 (Banerjee et al., 1994). Since then, this mutation has been identified in INH-resistant *M. tuberculosis* clinical isolates and has been shown to be sufficient to cause INH resistance in *M. tuberculosis* (Vilchèze et al., 2006). All the compounds tested were found to be as active against mc²651 as against the wild-type *M. smegmatis* strain (Table 2). At the most, there was a 2-fold increase in the MIC for some of the compounds. mc²2389, the *M. smegmatis* strain overexpressing *inhA*, was more resistant to the 2-alkynoic acids and their hydroxyl ester derivatives (up to 7 fold). The derivatives with a modification in the carbon chain had MICs that were no more than 3-fold higher than the wild-type *M. smegmatis* strain. The Ser94Ala mutation was shown to disrupt the hydrogen bonding network and reduce the binding of the INH-NAD adduct to InhA (Vilchèze et al., 2006). In *M. smegmatis*, this mutation confers a 10-fold increase in the MIC of INH (from 36 μ M in mc²155 to 0.36 mM in mc²651). The lack of resistance observed in mc²651 might imply that the Ser94Ala mutation is not located near the binding site of the fatty acid in InhA and therefore does not alter significantly the binding of the 2-alkynoyl derivatives and InhA inhibition or that InhA is not the target of these compounds. The fact that mc²2389 was resistant to the majority of the compounds imply that they indeed inhibit InhA but not as strongly as INH. The differences in susceptibility of the *inhA* overexpressing strain suggest that the two families of compounds, the hydroxyl ester derivatives and the modified carbon chain derivatives, target other enzymes in addition to InhA with the former being a better InhA inhibitor.

The compounds were also tested against mc²2421, a spontaneous INH-resistant *M. bovis* strain carrying a D389Y mutation in *katG*. Surprisingly, some of the derivatives with a modification in the carbon chain (**5c**, **5d**) were 2- to 4-fold less active against the INH-resistant strain, although the 2-alkynoic acids and their hydroxyl esters derivatives were as potent against this strain as against *M. bovis* BCG.

3.2.5. Critical micelle concentration of 2-alkynoic acid derivatives—Since a possible relationship between critical micelle concentration (CMC) and biological activities had been previously postulated (Courtney et al., 1986), the CMCs for the 2-alkynoic acid derivatives were measured (Table 3). All the compounds tested had CMC above the MIC for *M. bovis* suggesting that the effect on *Mtb* was not due to a detergent-like function of the fatty acids which would result in membrane damage. However, with the exception of **16a**, the CMC was well below the MIC of the compounds against *M. smegmatis* indicating that a general effect on the membrane might contribute to the antimycobacterial activity of these compounds against *M. smegmatis*.

3.2.6. Activity of 2-alkynoic acid derivatives against Vero cells—The cytotoxicity of these analogs was tested against African green monkey cells (Table 4). Some of the derivatives were 5 to 10 times less toxic than their parent compounds. Compounds **16a** and **16b** had EC₅₀ values 200 times greater than their MIC in *M. bovis* BCG. While the high toxicity of 2-HA had been previously reported (Sanabria-Rios et al., 2014), the introduction of an additional unsaturation or a sulfur atom in the carbon chain drastically reduced the toxicity of 2-HA as did the esterification of 2-HA with hydroxyl esters. Surprisingly, the same modification applied to 2-OA did not significantly improved the toxicity of the fatty acids.

4. Conclusion

Two sets of 2-alkynoic acid derivatives were synthesized and tested against fast- and slow-grower mycobacteria. Esterification of the carboxylic group with hydroxyl ester slightly improved the antimycobacterial activity against *M. smegmatis* and *M. bovis* but more importantly significantly reduced the toxicity of the compounds. The best derivatives were the 4-(alkylthio)but-2-ynoic acids **16** with lower toxicity and high bactericidal activity against *M. bovis* BCG. The positioning of the sulfur atom at the 4 position was important as the other thioderivatives were not as potent antimycobacterials. Although these derivatives were designed to inhibit the enoyl-ACP reductase InhA of the FASII system, they in fact inhibit both fatty acid synthases FASI and FASII. This had been seen previously when testing other inhibitors identified through enzymatic cell assay targeting InhA. Although the compounds did inhibit InhA, they also inhibited both FASI and FASII (Vilcheze et al., 2011). Multi-target compounds have the advantage of reducing or eliminating the possibility of resistance. Considering the ease at which *M. tuberculosis* can become mono-, multi-, or extensively resistant to drugs, compounds that cannot generate resistant mutants would be a crucial asset for the TB pharmacopeia.

However there are numerous infections by mycobacteria that are topical skin infection. Notably, *M. ulcerans* is a slowly growing mycobacterium that causes buruli ulcers. Another mycobacterium, *M. abscessus*, is an emerging pathogen often found contaminating medical devices, or in immunosuppressed patients or immunocompetent patients that have gotten tattoos or pedicure (De Groote and Huitt, 2006). *M. abscessus* is a fast-growing pathogen for which we have no drugs (Nessar et al., 2012). Considering that long-chain fatty acids might be more suited for topical treatment, experiments are underway to test if these alkynoic acid derivatives are active against these pathogens that grow in the skin.

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References

- Andries K, Verhasselt P, Guillemont J, Gohlmann HW, Neefs JM, Winkler H, Van Gestel J, Timmerman P, Zhu M, Lee E, Williams P, de Chaffoy D, Huitric E, Hoffner S, Cambau E, Truffot-Pernot C, Lounis N, Jarlier V. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science*. 2005; 307:223–227. [PubMed: 15591164]
- Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs WR Jr. *inhA*, a gene encoding a target for isoniazid and ethionamide in *Mycobacterium tuberculosis*. *Science*. 1994; 263:227–230. [PubMed: 8284673]
- Bengsh E, Perly B, Deleuze C, Valero A. A general rule for the assignment of the carbon-13 NMR peaks in fatty acid chains. *J. Magnetic Resonance*. 1986; 68:1–13.
- Bloch K. Control mechanisms for fatty acid synthesis in *Mycobacterium smegmatis*. *Adv. Enzymol. Relat. Areas Mol. Biol.* 1977; 45:1–84. [PubMed: 21523]
- Brandsma, L. Preparative acetylenic chemistry. New York: Elsevier, Amsterdam; 1971.
- Camps F, Hospital S, Rosell G, Delgado A, Guerrero A. Synthesis of biosynthetic inhibitors of the sex pheromone of *Spodoptera littoralis*. Part II: Acetylenic and cyclopropane fatty acids. *Chem. Phys. Lipids*. 1992; 61:157–167. [PubMed: 1511488]
- Christie WW, Holman RT. Synthesis and characterization of the complete series of methylene-interrupted cis,cis-octadecadienoic acids. *Chem. Phys. Lipids*. 1967; 1:407–423.
- Connell ND. *Mycobacterium*: isolation, maintenance, transformation, and mutant selection. *Methods Cell Biol.* 1994; 45:107–125. [PubMed: 7707982]
- Courtney HS, Simpson WA, Beachey EH. Relationship of critical micelle concentrations of bacterial lipoteichoic acids to biological activities. *Infect. Immun.* 1986; 51:414–418. [PubMed: 3943894]
- Cynamon MH, Gimi R, Gyenes F, Sharpe CA, Bergmann KE, Han HJ, Gregor LB, Rapolu R, Luciano G, Welch JT. Pyrazinoid acid esters with broad spectrum in vitro antimycobacterial activity. *J. Med. Chem.* 1995; 38:3902–3907. [PubMed: 7562923]
- De Groote MA, Huitt G. Infections due to rapidly growing mycobacteria. *Clinical Infectious Diseases*. 2006; 42:1756–1763. [PubMed: 16705584]
- Gunstone FD, Pollard MR, Scrimgeour CM, Gilman NW, Holland BC. Fatty acids. Part 48. ¹³C nuclear magnetic resonance studies of acetylenic fatty acids. *Chem. Phys. Lipids*. 1976; 17:1–13. [PubMed: 975444]
- Kikuchi S, Rainwater DL, Kolattukudy PE. Purification and characterization of an unusually large fatty acid synthase from *Mycobacterium tuberculosis* var. bovis BCG. *Arch. Biochem. Biophys.* 1992; 295:318–326. [PubMed: 1586161]
- Kondo E, Kanai K. The relationship between the chemical structure of fatty acids and their mycobactericidal activity. *Jpn J. Med. Sci. Biol.* 1977; 30:171–178. [PubMed: 409867]
- Larsen MH, Vilcheze C, Kremer L, Besra GS, Parsons L, Salfinger M, Heifets L, Hazbon MH, Alland D, Sacchettini JC, Jacobs WR Jr. Overexpression of *inhA*, but not *kasA*, confers resistance to isoniazid and ethionamide in *Mycobacterium smegmatis*, *M. bovis BCG* and *M. tuberculosis*. *Mol. Microbiol.* 2002; 46:453–466. [PubMed: 12406221]
- Lei B, Wei CJ, Tu SC. Action mechanism of antitubercular isoniazid. Activation by *Mycobacterium tuberculosis* KatG, isolation, and characterization of *inhA* inhibitor. *J Biol Chem*. 2000; 275:2520–2526. [PubMed: 10644708]
- Manjunatha UH, SP SR, Kondreddi RR, Noble CG, Camacho LR, Tan BH, Ng SH, Ng PS, Ma NL, Lakshminarayana SB, Herve M, Barnes SW, Yu W, Kuhen K, Blasco F, Beer D, Walker JR, Tonge PJ, Glynn R, Smith PW, Diagana TT. Direct inhibitors of *InhA* are active against *Mycobacterium tuberculosis*. *Sci. Transl. Med.* 2015; 7:269ra263.

- Marrakchi H, Laneelle G, Quemard A. InhA, a target of the antituberculous drug isoniazid, is involved in a mycobacterial fatty acid elongation system, FAS-II. *Microbiology*. 2000; 146(Pt 2):289–296. [PubMed: 10708367]
- Millar JG, Underhill EW. Short synthesis of 1,3Z, 6Z, 9Z-tetraene hydrocarbons. *Lepidopteron sex attractants*. *Can. J. Chem.* 1986; 64:2427–2430.
- Morbidoni HR, Vilchèze C, Kremer L, Bittman R, Sacchettini JC, Jacobs JWR. Dual Inhibition of Mycobacterial Fatty Acid Biosynthesis and Degradation by 2-Alkynoic Acids. *Chem. Biol.* 2006; 13:297–307. [PubMed: 16638535]
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983; 65:55–63. [PubMed: 6606682]
- Muller B, Durr S, Alonso S, Hattendorf J, Laisse CJ, Parsons SD, van Helden PD, Zinsstag J. Zoonotic *Mycobacterium bovis*-induced tuberculosis in humans. *Emerg. Infect. Dis.* 2013; 19:899–908.
- Nessar R, Cambau E, Reytrat JM, Murray A, Gicquel B. *Mycobacterium abscessus* a new antibiotic nightmare. *J. Antimicrob. Chemother.* 2012; 67:810–818. [PubMed: 22290346]
- Ono N, Yamada T, Saito T, Tanaka K, Kaji A. A convenient procedure for esterification of carboxylic acids. *Bull. Chem. Soc. Jpn.* 1978; 51:2401–2404.
- Pascal RA Jr, Ziering DL. Synthesis of heteroatom-substituted analogues of stearic acid. *J. Lipid Res.* 1986; 27:221–224. [PubMed: 3958625]
- Peterson DO, Bloch K. *Mycobacterium smegmatis* fatty acid synthetase. Long chain transacylase chain length specificity. *J. Biol. Chem.* 1977; 252:5735–5739. [PubMed: 885878]
- Quemard A, Sacchettini JC, Dessen A, Vilcheze C, Bittman R, Jacobs WR Jr, Blanchard JS. Enzymatic characterization of the target for isoniazid in *Mycobacterium tuberculosis*. *Biochemistry*. 1995; 34:8235–8241. [PubMed: 7599116]
- Reijnders PJ, Fransen HR, Buck HM. Synthesis of (E,E)-1,5-cycloalkadienes. Part II. *Rec. Trav. Chim. Pays Bas.* 1979; 98:511–514.
- Rozwarski DA, Grant GA, Barton DH, Jacobs WR Jr, Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. *Science*. 1998; 279:98–102. [PubMed: 9417034]
- Saito H, Tomioka H, Yoneyama T. Growth of group IV mycobacteria on medium containing various saturated and unsaturated fatty acids. *Antimicrob. Agents Chemother.* 1984; 26:164–169. [PubMed: 6486760]
- Sanabria-Rios DJ, Rivera-Torres Y, Maldonado-Dominguez G, Dominguez I, Rios C, Diaz D, Rodriguez JW, Altieri-Rivera JS, Rios-Olivares E, Cintron G, Montano N, Carballeira NM. Antibacterial activity of 2-alkynoic fatty acids against multidrug-resistant bacteria. *Chem. Phys. Lipids*. 2014; 178:84–91. [PubMed: 24365283]
- Scorpio A, Zhang Y. Mutations in *pncA*, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nat. Med.* 1996; 2:662–667. [PubMed: 8640557]
- Smith WN, Beumel OF. Preparation of alkynes and dialkynes by reaction of monohalo- and dihaloalkanes with lithium acetylenide-ethylene diamine complex. *Synthesis*. 1974; 06:441–442.
- Snapper SB, Melton RE, Mustafa S, Kieser T, Jacobs WR Jr. Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. *Mol. Microbiol.* 1990; 4:1911–1919. [PubMed: 2082148]
- Srivastava A, Ostrander J, Martin S, Walter N. *Mycobacterium bovis* infection of total hip arthroplasty after intravesicular bacille Calmette-Guerin therapy. *Am. J. Orthop. (Belle Mead NJ)*. 2011; 40:E226–E228. [PubMed: 22263218]
- Takayama K, Schnoes HK, Armstrong EL, Boyle RW. Site of inhibitory action of isoniazid in the synthesis of mycolic acids in *Mycobacterium tuberculosis*. *J. Lipid Res.* 1975; 16:308–317. [PubMed: 806645]
- Takayama K, Wang L, David HL. Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 1972; 2:29–35. [PubMed: 4208567]
- Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated bacille Calmette-Guerin disease after vaccination: case report and review. *Clin. Infect. Dis.* 1997; 24:1139–1146. [PubMed: 9195072]

- Torun T, Gungor G, Ozmen I, Bolukbasi Y, Maden E, Bicakci B, Atac G, Sevim T, Tahaoglu K. Side effects associated with the treatment of multidrug-resistant tuberculosis. *Int. J. Tuberc. Lung Dis.* 2005; 9:1373–1377. [PubMed: 16468160]
- Vilcheze C, Baughn AD, Tufariello J, Leung LW, Kuo M, Basler CF, Alland D, Sacchettini JC, Freundlich JS, Jacobs WR Jr. Novel inhibitors of InhA efficiently kill *Mycobacterium tuberculosis* under aerobic and anaerobic conditions. *Antimicrob. Agents Chemother.* 2011; 55:3889–3898. [PubMed: 21628538]
- Vilcheze C, Bittman R. An efficient asymmetric synthesis of diacylglycerols. *J. Lipid Res.* 1994; 35:734–738. [PubMed: 8006527]
- Vilcheze C, Morbidoni HR, Weisbrod TR, Iwamoto H, Kuo M, Sacchettini JC, Jacobs WR Jr. Inactivation of the *inhA*-encoded fatty acid synthase II (FASII) enoyl-acyl carrier protein reductase induces accumulation of the FASII end products and cell lysis of *Mycobacterium smegmatis*. *J. Bacteriol.* 2000; 182:4059–4067. [PubMed: 10869086]
- Vilchèze C, Wang F, Arai M, Hazbon MH, Colangeli R, Kremer L, Weisbrod TR, Alland D, Sacchettini JC, Jacobs WR Jr. Transfer of a point mutation in *Mycobacterium tuberculosis inhA* resolves the target of isoniazid. *Nat. Med.* 2006; 12:1027–1029. [PubMed: 16906155]
- Wailes PC. The synthesis of the stereoisomeric hexadeca-2,4-dienoic acids and their isobutylamides. *Aus. J. Chem.* 1959; 12:173–189.
- WHO. Global tuberculosis report 2014. 2014. http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf?ua=1.
- Wilming M, Johnsson K. Spontaneous formation of the bioactive form of the tuberculosis drug isoniazid. *Angew Chem Int Ed Engl.* 1999; 38:2588–2590. [PubMed: 10508348]
- Xu Z, Byun HS, Bittman R. Synthesis of photopolymerizable long-chain conjugated diacetylenic acids and alcohols from butadiyne synthons. *J. Org. Chem.* 1991; 56:7183–7186.
- Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature.* 1992; 358:591–593. [PubMed: 1501713]
- Zimhony O, Vilcheze C, Arai M, Welch JT, Jacobs WR Jr. Pyrazinoic acid and its n-propyl ester inhibit fatty acid synthase type I in replicating tubercle bacilli. *Antimicrob. Agents Chemother.* 2007; 51:752–754. [PubMed: 17101678]
- Zumla AI, Gillespie SH, Hoelscher M, Philips PP, Cole ST, Abubakar I, McHugh TD, Schito M, Maeurer M, Nunn AJ. New antituberculosis drugs, regimens, and adjunct therapies: needs, advances, and future prospects. *Lancet Infect. Dis.* 2014; 14:327–340. [PubMed: 24670627]

Highlights

- 2-Alkynoic acids with modified carbon chain or carboxylic group were synthesized.
- Synthesis of these analogs was straightforward and could be easily scaled-up.
- Compounds were active against fast- and slow-grower mycobacteria.
- The best inhibitor had a sulfur atom at the 4-position in the carbon chain.
- Modifications of 2-alkynoic acids decrease their toxicity against eukaryotic cells.

Figure 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.

Figure 2.

The 4-(alkylthio)but-2-ynoic acids **16a** and **16b** are the most bactericidal 2-alkynoic acid analogs. (A) Exponentially growing cultures of *M. smegmatis* mc²155 ($OD_{600nm} \sim 1$) were diluted 1/50 and treated with 2-alkynoic acid derivatives for 24 h at 100 or 200 μ M. After 24h, the cultures were serial-diluted, plated on Middlebrook 7H10 plates, and the plates were incubated at 37°C for 3 days to determine colony-forming units (CFUs). (B) Exponentially growing cultures of *M. bovis* BCG ($OD_{600nm} \sim 1$) were diluted 1/50 and treated with 2-alkynoic acid derivatives at 20 μ M for 3 days. CFUs were determined by plating serial dilution and incubating the plates for 3 weeks at 37°C.

Figure 3.

2-Alkynoic acid derivatives inhibit mycolic acid biosynthesis in *M. smegmatis* and *M. bovis* BCG. **A.** Cultures of *M. smegmatis* mc²155 (**A**) or *M. bovis* BCG (**B**) were treated with 2-alkynoic acid derivatives or INH at 100 μ M (**A**) or 20 μ M (**B**) and then labeled with [1-¹⁴C]-acetate. Fatty acids were saponified and then methylated before being loaded onto the TLC (see Materials and Methods for details).

Scheme 1.
Synthesis of alkadiynoic acids.

1. Thio-2-alkynoic acids

2. 4-(Alkylthio)-2-butynoic acids

Scheme 2.
Synthesis of thio-2-alkynoic acids.

1. *N'*-(Hexadec-2-ynoyl)isonicotinohydrazide

2. 3-(Octadec-2-ynoyloxy)propyl pyrazinoate and isonicotinate

3. 2-Hydroxyethyl alk-2-ynoates

4. 1-(2'-Alkynoyl)-glycerol and tri-(2'-octadecynoyl)-glycerol

Scheme 3.
Synthesis of 2-alkynoyl derivatives.

Table 1

Minimum inhibitory concentration (MIC₉₉, µM) of 2-alkynoic acid derivatives against *M. smegmatis* mc²155 and *M. bovis* BCG Pasteur.

Compound	MIC mc ² 155	MIC BCG	Compound	MIC mc ² 155	MIC BCG
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ H (2-HA)	9.6	2.5	CH ₃ (CH ₂) ₅ S(CH ₂) ₁₀ C≡CCO ₂ H 13b	76.6	9.6
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ H (2-OA)	3.6	2.2	CH ₃ (CH ₂) ₅ S(CH ₂) ₁₂ C≡CCO ₂ H 13c	70.5	4.4
CH ₃ (CH ₂) ₁₅ C≡CCO ₂ H (2-NA)	34.0	10.6	CH ₃ CH ₂ SO(CH ₂) ₁₂ C≡CCO ₂ H 14a	> 200	nd
CH ₃ (CH ₂) ₁₆ C≡CCO ₂ H (2-EA)	> 200	40.5	CH ₃ (CH ₂) ₅ SO(CH ₂) ₁₀ C≡CCO ₂ H 14b	> 200	nd
CH ₃ (CH ₂) ₁₀ C≡CC≡CCO ₂ H 2	80.6	1.6	CH ₃ (CH ₂) ₁₁ SCH ₂ C≡CCO ₂ H 16a	43.9	1.1
CH ₃ (CH ₂) ₉ C≡CCH ₂ C≡CCO ₂ H 5a	100.7	nd	CH ₃ (CH ₂) ₁₃ SCH ₂ C≡CCO ₂ H 16b	80.0	0.5
CH ₃ (CH ₂) ₁₁ C≡CCH ₂ C≡CCO ₂ H 5b	90.5	22.6	CH ₃ (CH ₂) ₁₂ C≡CCONHNHCOInic 17	> 200	nd
CH ₃ (CH ₂) ₁₂ C≡CCH ₂ C≡CCO ₂ H 5c	86.1	5.4	CH ₃ (CH ₂) ₁₄ C≡CCO ₂ (CH ₂) ₃ OCOInic 19a	92.8	nd
CH ₃ (CH ₂) ₁₃ C≡CCH ₂ C≡CCO ₂ H 5d	82.2	5.1	CH ₃ (CH ₂) ₁₄ C≡CCO ₂ (CH ₂) ₃ OCOPyraz 19b	11.2	6.0
CH ₃ (CH ₂) ₁₄ C≡CCH ₂ C≡CCO ₂ H 5e	78.6	nd	CH ₃ (CH ₂) ₁₂ C≡CCO ₂ (CH ₂) ₂ OH 20a	6.8	1.0
CH ₃ (CH ₂) ₁₆ C≡CCH ₂ C≡CCO ₂ H 5f	>200	nd	CH ₃ (CH ₂) ₁₄ C≡CCO ₂ (CH ₂) ₂ OH 20b	2.3	1.9
CH ₃ (CH ₂) ₇ C≡C(CH ₂) ₆ C≡CCO ₂ H 8	68.9	5.4	CH ₃ (CH ₂) ₁₂ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21a	6.1	1.9
HC≡C(CH ₂) ₁₂ C≡CCO ₂ H 10	76.3	1.5	CH ₃ (CH ₂) ₁₄ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21b	3.0	0.9
CH ₃ CH ₂ S(CH ₂) ₁₂ C≡CCO ₂ H 13a	50.3	10.5			

nd: not done

Table 2

Minimum inhibitory concentration (MIC₉₉, μM) of 2-alkynoic acid derivatives against INH-resistant *M. smegmatis* and *M. bovis* BCG strains

Strain	mc ² 651 ^a	mc ² 2389 ^b	mc ² 2421 ^c
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ H (2-HA)	9.6	49.5	2.5
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ H (2-OA)	3.6	23.2	2.2
CH ₃ (CH ₂) ₁₅ C≡CCO ₂ H (2-NA)	34.0	63.7	10.6
CH ₃ (CH ₂) ₁₆ C≡CCO ₂ H (2-EA)	> 200	> 200	10.1
CH ₃ (CH ₂) ₁₀ C≡CC≡CCO ₂ H 2	80.6	100.7	2.5
CH ₃ (CH ₂) ₁₁ C≡CCH ₂ C≡CCO ₂ H 5b	181	> 200	22.6
CH ₃ (CH ₂) ₁₂ C≡CCH ₂ C≡CCO ₂ H 5c	172.3	172.3	21.5
CH ₃ (CH ₂) ₁₃ C≡CCH ₂ C≡CCO ₂ H 5d	> 200	> 200	20.5
CH ₃ (CH ₂) ₇ C≡C(CH ₂) ₆ C≡CCO ₂ H 8	> 200	> 200	2.7
HC≡C(CH ₂) ₁₂ C≡CCO ₂ H 10	152.6	47.7	1.2
CH ₃ CH ₂ S(CH ₂) ₁₂ C≡CCO ₂ H 13a	76.3	83.8	2.6
CH ₃ (CH ₂) ₅ S(CH ₂) ₁₀ C≡CCO ₂ H 13b	153.2	> 200	2.4
CH ₃ (CH ₂) ₅ S(CH ₂) ₁₂ C≡CCO ₂ H 13c	141.0	141.0	2.2
CH ₃ (CH ₂) ₁₁ SCH ₂ C≡CCO ₂ H 16a	46.3	92.5	4.6
CH ₃ (CH ₂) ₁₃ SCH ₂ C≡CCO ₂ H 16b	167.5	> 200	2.1
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ (CH ₂) ₂ OH 20a	13.6	42.3	1.0
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ (CH ₂) ₂ OH 20b	2.3	19.3	1.9
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21a	6.1	19.1	1.0
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21b	3.0	17.6	1.8

^a mc²651 (mc²155 *inhA* S94A) is an INH- and ETH-resistant *M. smegmatis* strain

^b mc²2389 (mc²155 pMV261::*inhA*) is an INH- and ETH-resistant *M. smegmatis* strain

^c mc²2421 (BCG *katG* D389Y) is an INH-resistant *M. bovis* BCG strain

Table 3Critical micelle concentration (CMC, μM) of 2-alkynoic acid derivatives

Compound	CMC
$\text{CH}_3(\text{CH}_2)_{12}\text{C}\equiv\text{CCO}_2\text{H}$ (2-HA)	80
$\text{CH}_3(\text{CH}_2)_{14}\text{C}\equiv\text{CCO}_2\text{H}$ (2-OA)	18
$\text{CH}_3(\text{CH}_2)_{11}\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{CCO}_2\text{H}$ 5b	18
$\text{CH}_3(\text{CH}_2)_{10}\text{C}\equiv\text{CC}\equiv\text{CCO}_2\text{H}$ 2	60
$\text{CH}_3(\text{CH}_2)_{12}\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{CCO}_2\text{H}$ 5c	35
$\text{CH}_3(\text{CH}_2)_{13}\text{C}\equiv\text{CCH}_2\text{C}\equiv\text{CCO}_2\text{H}$ 5d	25
$\text{CH}_3(\text{CH}_2)_7\text{C}\equiv\text{C}(\text{CH}_2)_6\text{C}\equiv\text{CCO}_2\text{H}$ 8	69
$\text{HC}\equiv\text{C}(\text{CH}_2)_{12}\text{C}\equiv\text{CCO}_2\text{H}$ 10	305
$\text{CH}_3\text{CH}_2\text{S}(\text{CH}_2)_{12}\text{C}\equiv\text{CCO}_2\text{H}$ 13a	134
$\text{CH}_3(\text{CH}_2)_5\text{S}(\text{CH}_2)_{10}\text{C}\equiv\text{CCO}_2\text{H}$ 13b	25
$\text{CH}_3(\text{CH}_2)_5\text{S}(\text{CH}_2)_{12}\text{C}\equiv\text{CCO}_2\text{H}$ 13c	42
$\text{CH}_3(\text{CH}_2)_{11}\text{SCH}_2\text{C}\equiv\text{CCO}_2\text{H}$ 16a	70
$\text{CH}_3(\text{CH}_2)_{13}\text{SCH}_2\text{C}\equiv\text{CCO}_2\text{H}$ 16b	3

Table 4Cytotoxicity (EC₅₀, μM) of 2-alkynoic acid derivatives

Compound	EC ₅₀	EC ₅₀ /MIC _{BCG}
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ H (2-HA)	54.5	22
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ H (2-OA)	79.5	32
CH ₃ (CH ₂) ₁₀ C≡CC≡CCO ₂ H 2	139.9	87
CH ₃ (CH ₂) ₁₁ C≡CCH ₂ C≡CCO ₂ H 5b	131.2	6
CH ₃ (CH ₂) ₁₂ C≡CCH ₂ C≡CCO ₂ H 5c	87.4	16
CH ₃ (CH ₂) ₁₃ C≡CCH ₂ C≡CCO ₂ H 5d	106.5	21
CH ₃ (CH ₂) ₇ C≡C(CH ₂) ₆ C≡CCO ₂ H 8	124.1	23
HC≡C(CH ₂) ₁₂ C≡CCO ₂ H 10	135.7	90
CH ₃ CH ₂ S(CH ₂) ₁₂ C≡CCO ₂ H 13a	434.9	41
CH ₃ (CH ₂) ₅ S(CH ₂) ₁₀ C≡CCO ₂ H 13b	160.1	17
CH ₃ (CH ₂) ₅ S(CH ₂) ₁₂ C≡CCO ₂ H 13c	118.7	27
CH ₃ (CH ₂) ₁₁ SCH ₂ C≡CCO ₂ H 16a	250.6	228
CH ₃ (CH ₂) ₁₃ SCH ₂ C≡CCO ₂ H 16b	92.7	185
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ (CH ₂) ₂ OH 20a	283.2	283
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ (CH ₂) ₂ OH 20b	97.1	51
CH ₃ (CH ₂) ₁₂ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21a	563.1	296
CH ₃ (CH ₂) ₁₄ C≡CCO ₂ CH ₂ CH(OH)CH ₂ OH 21b	88.5	98