2,6-Hexadecadiynoic Acid and 2,6-Nonadecadiynoic Acid: Novel Synthesized Acetylenic Fatty Acids as Potent Antifungal Agents

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ABSTRACT: The hitherto unknown 2,6-hexadecadiynoic acid, 2,6-nonadecadiynoic acid, and 2,9-hexadecadiynoic acid were synthesized in two steps and in 11–18% overall yields starting from either 1,5-hexadiyne or 1,8-nonadiyne. Among all the compounds 2,6-hexadecadiynoic acid displayed the best overall antifungal activity against both the fluconazole-resistant *Candida albicans* strains ATCC 14053 and ATCC 60193, with a minimum inhibitory concentration (MIC of 11 μM), and against *Cryptococcus neoformans* ATCC 66031 (MIC < 5.7 μM). 2,9-Hexadecadiynoic acid did not display any significant cytotoxicity against the fluconazole-resistant *C. albicans* strains, but it showed fungitoxicity against *C. neoformans* ATCC 66031 with a MIC value of < 5.8 μM. Other FA, such as 2-hexadecynoic acid, 5-hexadecynoic acid, 9-hexadecynoic acid, and 6-nonadecynoic acid were also synthesized and their antifungal activities compared with those of the novel acetylenic FA. 2-Hexadecynoic acid, a known antifungal FA, exhibited the best antifungal activity (MIC = 9.4 μM) against the fluconazole-resistant *C. albicans* ATCC 14053 strain, but it showed a MIC value of only 100 μM against *C. albicans* ATCC 60193. 2,6-Hexadecadiynoic acid and 2-hexadecynoic acid also displayed a MIC of 140–145 μM toward *Mycobacterium tuberculosis* $H_{37}Rv$ in Middlebrook 7H12 medium. In conclusion, 2,6-hexadecadiynoic acid exhibited the best fungitoxicity profile compared with other analogues. This diynoic FA has the potential to be further evaluated for use in topical antifungal formulations.

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The 2-alkynoic FA have been known to be fungitoxic (1–2). The fungal activity of these compounds depends on the FA chain length and pH of the medium (1–2). The optimal chain lengths (between 8 and 16 carbons) have been established for the 2-alkynoic FA to exert maximum fungistatic effects (2). Among the 2-alkynoic FA 2-hexadecynoic acid has received the most attention for its antifungal, antimicrobial, and cytotoxic properties (3–5). The bioactivity of 2-hexadecynoic acid has been ascribed to its ability to inhibit the elongation of saturated and unsaturated FA as well as its potential to inhibit the FA acylation process, particularly triglyceride synthesis (4–5).

Other isomeric alkynoic FA, in particular the 6-alkynoic FA, are also fungistatic. For example, when an unsaturation is introduced between C-6 and C-7 in a fatty acyl chain, the FA displays good fungitoxicity. It is shown that 6-hexadecynoic acid is a good substrate for the enzyme myristoyl-CoA:protein *N*-myristoyltransferase from *Saccharomyces cerevisiae*, an important enzyme for fungal growth and a good target for antifungal therapy (6). Furthermore, 6-nonadecynoic acid, recently isolated from the roots of *Pentagonia gigantifolia*, is reported to be fungistatic against some fungal strains of fluconazole-resistant *Candida albicans* (7) as well as against *Cryptococcus neoformans* (8). *C. albicans* and *C. neoformans* are pathogenic fungi of concern in immunocompromised patients. 6-Alkenoic FA have also been evaluated. For example, 6-hexadecenoic acid is antimicrobial to gram-positive bacteria (MIC of 10–20 μg/mL) and blocks the adherence of *C. albicans* to porcine stratum corneum (9).

With this information at hand regarding the antifungal activities of 2-alkynoic and 6-alkynoic FA, we envisaged the possibility of combining in a single molecule the C-2 and C-6 ynoic functionalities by synthesizing 2,6-diynoic FA and evaluating their antifungal properties. It was expected that novel 2,6-diynoic FA would have a better antifungal profile than either of its parent 2-alkynoic or 6-alkynoic FA. The synthesis of 2,6-octadecadiynoic acid as a precursor to furan-containing FA has been previously reported by Lie Ken Jie *et al.* (10–11). However, to the best of our knowledge, the FA 2,6-hexadecadiynoic acid (**3a**) and 2,6-nonadecadiynoic acid (**3c**) are novel. We describe the synthesis of the dimethylene-interrupted diynoic FA **3a** and **3c**, as well as the synthesis of 2,9-hexadecadiynoic acid (**3b**). Comparative antifungal activities of the compounds indicate that **3a** has the best fungitoxicity profile among the studied compounds and the known 2-hexadecynoic acid.

EXPERIMENTAL PROCEDURES

Instrumentation. ^1H and ^{13}C NMR spectra were recorded on either a Bruker DPX-300 or a Bruker DRX-500 spectrometer. ^1H NMR chemical shifts are reported with respect to internal

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Abbreviations: AMB, amphotericin B; FLC, fluconazole; LORA, low oxygen recovery assay; MABA, microplate alamar blue assay; MIC, minimum inhibitory concentration; NCCLS, National Committee for Clinical Laboratory Standards; SDA, Sabouraud dextrose agar; SDB, Sabourand dextrose broth.

Me_4Si, and chemical shifts are given in parts per million (ppm) relative to $CDCl_3$ (77.0 ppm). Mass spectral data were acquired on a GC–MS (Hewlett-Packard 5972A MS ChemStation) instrument at 70 eV, equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethylsiloxane crosslinked with 5% phenyl methylpolysiloxane. Infrared spectra were recorded on a Nicolet 600 FT-IR spectrophotometer.

Microorganisms. C. albicans ATCC 60193, *C. albicans* ATCC 14053, and *C. neoformans* ATCC 66031 were obtained from American Type Culture Collection (Manassas, VA). Stock cultures were kept on Sabouraud dextrose agar (SDA; Becton-Dickinson and Co., Sparks, MD). Subcultures were prepared on SDA at 35–37°C. Suspension cultures were prepared by inoculation of single colonies in 7 mL of normal saline solution. Prior to preparation of susceptibility assays, yeast cells were resuspended in normal saline to yield a transmittance of 73–75% at 530 nm, which provided an equivalent concentration of 10^6 cells/mL. The medium was Sabourand dextrose broth (SDB; Becton Dickinson and Co.).

Chemicals and antifungal agents. Amphotericin B (AMB) was purchased from Acros Organics (Geel, Belgium), and was kept as a 5-mM stock in DMSO at 0°C and used within 1 wk of preparation. Fluconazole (FLC) was purchased from Medisa Inc. (New York, NY), or was provided from Vera Laboratories Ltd. (Hyderabad, India), and was kept as a 20-mM stock solution at 0°C. Working dilutions were made in SDB medium. Higher concentrations of compounds were used for those with weak antifungal activities. The final maximum concentration of DMSO in the assays was 5% (vol/vol). DMSO was not inhibitory to the organisms tested.

Susceptibility testing. Microdilutions for control experiments with *C. albicans* and *C. neoformans* were performed according to the modified method of the National Committee for Clinical Laboratory Standards (NCCLS) as described by Galgiani (12) and according to the more recent NCCLS M27-A microdilution methods as described previously (13–14). Dilutions were prepared in 0.1 mL of SDB; the inocula were 10^4 cells of either *C. albicans* or *C. neoformans*. The tubes were incubated for 24–48 h at 36 ± 1°C, and turbidity was read visually. MIC were calculated in comparison with growth control as the lowest concentration that showed inhibition for AMB, FLC, and the test compounds.

General procedure for the monoalkylation of the diynes. To a stirred solution of the diyne (8.3–12.8 mmol) in dry THF (20–25 mL), *n*-Buli (2.5 M, 7.5–11.5 mmol) in dry hexane (3.0–5.0 mL) was added dropwise while keeping the temperature at –78°C. After 45 min, hexamethylphosphoramide (HMPA) (3.0–5.0 mL) and the bromoalkane (7.7–11.5 mmol) were added dropwise to the reaction mixture while maintaining the temperature at –78°C. After 24 h, the reaction mixture was worked up by pouring into a large volume of water, and extracting with diethyl ether (2 × 20 mL). The organic layer was washed with brine (1 × 20 mL) before drying ($MgSO_4$). Filtration, rotoevaporation of the solvent, and fractional distillation afforded the monoalkylated diynes **2a**–**2c** in 21–47% yields after purification by Kugel-Rohr distillation (60–100°C and 3 mm Hg) of the impurities.

(i) 1,8-Pentadecadiyne (***2b***). 1,8-Pentadecadiyne (**2b**) was obtained in a 21% yield as a viscous oil from the reaction of 1.22 mL of 1,8-nonadiyne (1.00 g, 8.32 mmol) and 1.16 mL of 1-bromohexane (1.37 g, 8.32 mmol) according to the general procedure described above. IR (neat) ν_{max} 3311, 2932, 2859, 2118, 1463, 1332, 1192, 1100, 629 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.19 (2H, *dt*, *J* = 2.6 and 6.9 Hz, H-10), 2.13 (4H, m, H-3, H-7), 1.93 (1H, *t*, *J* = 2.6 Hz, H-1), 1.57–1.24 (14H, m, CH_2), 0.88 (3H, *t*, *J* = 7.0 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 84.53 (*s*, C-2), 80.48 (*s*), 79.84 (*s*), 68.15 (*d*, C-1), 31.36 (*t*, C-13), 29.10 (*t*), 28.61 (*t*), 28.53 (*t*), 28.04 (*t*), 27.95 (*t*), 22.56 (*t*, C-14), 18.73 (*t*), 18.69 (*t*), 18.33 (*t*), 14.04 (*q*, C-15); GC–MS *m/z* (relative intensity) M^+ 204 (0.1), 189 (1), 175 (4), 161 (4), 147 (14), 133 (28), 119 (32), 107 (11), 105 (54), 95 (13), 91 ($C_7H_7^+$, 100), 81 (30), 79 (61), 67 (51), 55 (31).

(*ii*) *1,5-Pentadecadiyne* (***2a***). 1,5-Pentadecadiyne (**2a**) was obtained in a 40% yield as a viscous oil from the reaction of 2.5 mL of 1,5-hexadiyne (1.00 g, 12.80 mmol) and 2.2 mL of 1-bromononane (2.38 g, 11.50 mmol) according to the general procedure described above. IR (neat) ν_{max} 3313, 2926, 2855, 2122, 1466, 1338, 1257, 635 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.38 (4H, *m*, H-3, H-4), 2.14 (2H, *brt*, *J* = 7.0 Hz, H-7), 2.00 (1H, *t*, *J* = 2.0 Hz, H-1), 1.52–1.27 (14H, *m*, CH_2), 0.88 (3H, *t*, *J* = 6.9 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 83.11 (*s*, C-2), 81.61 (*s*), 78.09 (*s*), 68.94 (*d*, C-1), 31.89 (*t*, C-13), 29.50 (*t*), 29.28 (*t*), 29.16 (*t*), 28.97 (*t*), 28.83 (*t*), 22.67 (*t*, C-14), 19.18 (*t*), 18.93 (*t*), 18.71 (*t*), 14.09 (*q*, C-15); GC–MS *m/z* (relative intensity) M^+ 204 (0.1), 161 (1), 147 (3), 133 (13), 119 (19), 109 (6), 105 (31), 95 (16), 91 ($C_7H_7^+$, 100), 81 (22), 79 (31), 67 (32), 65 (20), 55 (29).

(*iii*) *1,5-Octadecadiyne* (***2c***). 1,5-Octadecadiyne (**2c**) was obtained as a viscous oil in a 47% yield from the reaction of 2.5 mL of 1,5-hexadiyne (2.00 g, 12.80 mmol) and 1.8 mL of 1-bromododecane (1.87 g, 7.70 mmol) according to the general procedure described above. IR (neat) ν_{max} 3313, 2925, 2854, 2122, 1466, 1338, 1257, 635 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.38 (4H, *m*, H-3, H-4), 2.14 (2H, *brt*, *J* = 6.9 Hz, H-7), 2.00 (1H, *m*, H-1), 1.55–1.26 (20H, *m*, CH_2), 0.88 (3H, *t*, *J* = 6.8 Hz, H-18); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 83.11 (*s*, C-2), 81.62 (*s*), 78.09 (*s*), 68.93 (*d*, C-1), 31.92 (*t*, C-16), 29.67 (*t*), 29.64 (*t*), 29.63 (*t*), 29.55 (*t*), 29.35 (*t*), 29.16 (*t*), 28.97 (*t*), 28.83 (*t*), 22.68 (*t*, C-17), 19.18 (*t*), 18.93 (*t*), 18.71 (*t*), 14.10 (*q*, C-18); GC–MS *m/z* (relative intensity) M^+ 246 (0.1), 175 (1), 161 (2), 147 (8), 133 (27), 119 (27), 105 (38), 95 (16), 91 ($C_7H_7^+$, 100), 81 (20), 79 (35), 67 (28), 65 (24), 55 (29).

General procedure for the carboxylation of the alkylated diynes. To a stirred solution of the alkylated diynes (**2a**–**2c**, 1.7–4.6 mmol) in dry THF (17–46 mL), *n*-Buli (2.5 M, 2.6–7.2 mmol) in dry hexane (1–3 mL) was added dropwise while keeping the temperature approximately at –78°C. After 45 min the reaction mixture was treated with dry CO_2 (by passing the CO_2 through sulfuric acid) and left stirring for 24 h. The reaction mixture was then worked up by pouring into a large volume of a saturated solution of ammonium chloride followed by extraction with diethyl ether (3 × 20 mL). Rotoevaporation of the solvent afforded the diynoic acids **3a**–**3c** in 28–52% yields

after purification by Kugel-Rohr distillation (60–100°C and 3 mm Hg) of the impurities. The higher yield (52%) was obtained for 2,9-hexadecadiynoic acid (**3b**).

*(i) 2,9-Hexadecadiynoic acid (**3b**).* 2,9-Hexadecadiynoic acid (**3b**) was obtained as an oil in a 52% yield from the reaction of 1,8-pentadecadiyne (0.35 g, 1.70 mmol) and excess dry CO_2 (by passing the CO_2 through sulfuric acid) according to the general procedure described above. IR (neat) ν_{max} 3400–2900 (*br*), 2932, 2859, 2238, 1688, 1462, 1410, 1332, 1279, 1076 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.36 (2H, *t*, *J* = 7.1 Hz, H-4), 2.14 (4H, *m*, H-8, H-11), 1.62–1.23 (14H, *m*, CH_2), 0.88 (3H, *t*, *J* = 7.0 Hz, H-16); ^{13}C NMR ($CDCl_3$,125 MHz) δ 157.08 (*s*, C-1), 92.01 (*s*, C-3), 81.24 (*s*), 80.71 (*s*), 72.69 (*s*, C-2), 31.35 (*t*, C-14), 29.08 (*t*), 28.54 (*t*), 28.43 (*t*), 27.96 (*t*), 26.98 (*t*), 22.56 (*t*, C-15), 18.72 (*t*), 18.69 (*t*), 18.55 (*t*), 14.04 (*q*, C-16).

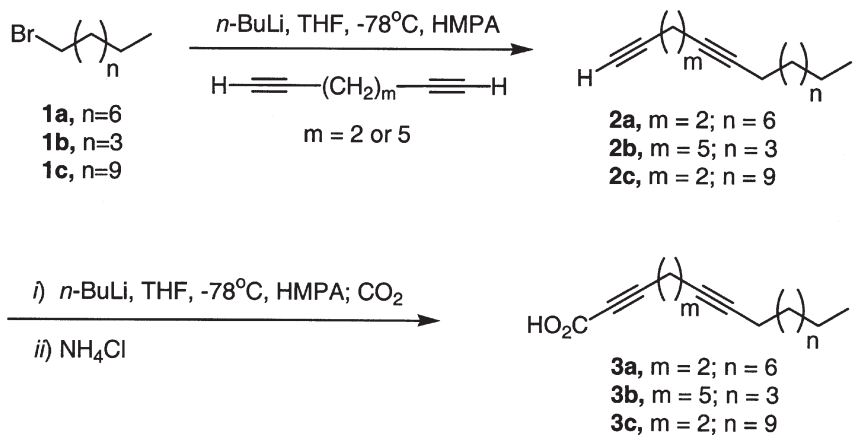
*(ii) 2,6-Hexadecadiynoic acid (**3a**).* 2,6-Hexadecadiynoic acid (**3a**) was obtained as a white solid in a 45% yield from the reaction of 1,5-pentadecadiyne (0.94 g, 4.60 mmol) and excess dry CO_2 (by passing the CO_2 through sulfuric acid) according to the general procedure described above. M.p. 48–50°C, IR (neat) ν_{max} 3400–2900 (*br*), 2953, 2915, 2848, 2246, 1674, 1469, 1425, 1300, 1276, 1075, 890, 608 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz,) δ 2.54 (2H, *brt*, *J* = 7.3 Hz, H-4), 2.44 (2H, *m*, H-5), 2.14 (2H, *tt*, *J* = 2.2 and 7.1 Hz, H-8), 1.47 (2H, *m*, H-9), 1.40–1.27 (12H, *m*, CH_2), 0.88 (3H, *t*, *J* = 6.9 Hz, H-16); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.58 (*s*, C-1), 90.10 (*s*, C-3), 82.44 (*s*), 77.10 (*s*), 73.14 (*s*, C-2), 31.83 (*t*, C-14), 29.48 (*t*), 29.29 (*t*), 29.15 (*t*), 28.86 (*t*), 28.83 (*t*), 22.67 (*t*, C-15), 19.51 (*t*, C-4), 18.67 (*t*, C-8), 17.94 (*t*, C-5), 14.08 (*q*, C-16). Anal. calcd. for $C_{16}H_{24}O_2$: C, 77.38%; H, 9.74%. Found: C, 76.17%; H, 10.19%.

*(iii) 2,6-Nonadecadiynoic acid (**3c**).* 2,6-Nonadecadiynoic acid (**3c**) was obtained as a white solid in a 28% yield from the reaction of 1,5-octadecadiyne (0.89 g, 3.60 mmol) and excess dry CO_2 (by bubbling the CO_2 through sulfuric acid) according to the general procedure described above. M.p. 62–64°C, IR (neat) ν_{max} 3400–2900 (*br*), 2953, 2916, 2848, 2245, 1671, 1422, 1280, 912, 720 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 2.54 (2H, *brt*, *J* = 7.4 Hz, H-4), 2.44 (2H, *m*, H-5), 2.14 (2H, *tt*, *J* = 2.3 and 7.1 Hz, H-8), 1.47 (2H, *m*, H-9), 1.38–1.25 (18H, *m*, CH_2), 0.88 (3H, *t*, *J* = 6.9 Hz, H-19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.96 (*s*, C-1), 90.33 (*s*, C-3), 82.44 (*s*), 77.21 (*s*), 73.06 (*s*, C-2), 31.91 (*t*, C-17), 29.64 (*t*), 29.53 (*t*), 29.35 (*t*), 29.14 (*t*), 28.84 (*t*), 22.68 (*t*, C-18), 19.49 (*t*, C-4), 18.66 (*t*, C-8), 17.91 (*t*, C-5), 14.11 (*q*, C-19). Anal. calcd. for $C_{19}H_{30}O_2$: C, 78.57%; H, 10.41%. Found: C, 77.14%; H, 10.40%.

RESULTS AND DISCUSSION

Seven acetylenic FA were synthesized and evaluated for antifungal activities against *C. albicans* and *C. neoformans*. The acetylenic FA 2-hexadecynoic acid, 5-hexadecynoic acid, 9-hexadecynoic acid, and 6-nonadecynoic acid were synthesized according to the previously described procedures (1,8,15). Despite the fact that 2,6-octadecadiynoic acid has been previously synthesized (10–11), there are no reported syntheses for acids **3a**–**3c**. Therefore, a simple synthetic methodology for the preparation of novel FA **3a**–**3c** was developed based on two consecutive acetylide coupling reactions.

The synthesis of the dimethylene-interrupted 2,6-hexadecadiynoic acid (**3a**) and 2,6-nonadecadiynoic acid (**3c**) was conveniently accomplished using 1,5-hexadiyne (50% in pentane) as the starting material (Scheme 1). In the preparation of **3a**, 1,5-hexadiyne was coupled with 1-bromononane using *n*-BuLi in THF/HMPA at –78°C, which afforded 1,5-pentadecadiyne (**2a**) in a 40% yield. Likewise, for the synthesis of acid **3c**, 1,5-hexadiyne was coupled with 1-bromododecane using the same acetylide coupling conditions, which afforded 1,5-octadecadiyne (**2c**) in a 47% yield after final purification (Scheme 1). Coupling of the lithium acetylides of 1,5-pentadecadiyne (**2a**) or 1,5-octadecadiyne (**2c**) with carbon dioxide and subsequent protonation with ammonium chloride afforded 2,6-hexadecadiynoic acid (**3a**) or 2,6-nonadecadiynoic acid (**3c**) in 28–45% yields after final purification (Scheme 1). The overall yields for these two two-step syntheses ranged between 13 and 18%.



SCHEME 1

TABLE 1
Antifungal Activity Against *Candida albicans* (SDB) and *Cryptococcus neoformans* (SDB) at 35–37°C after 24–48 h[a]

	MIC (μM)		
Compound	*C. albicans* ATCC 14053	*C. albicans* ATCC 60193	*C. neoformans* ATCC 66031
2-Hexadecynoic	9.4	100.3	<6.3
5-Hexadecynoic	7,845	10,460	61.3
9-Hexadecynoic	8,260	11,014	10.8
6-Nonadecynoic	6,672	8,896	<4.3
2,6-Hexadecadiynoic (**3a**)	11.5	11.5	<5.7
2,9-Hexadecadiynoic (**3b**)	4,469	2,235	<5.8
2,6-Nonadecadiynoic (**3c**)	20.0	80.2	<5.0
FLC	>2,000	>1,000	<0.9
AMB	<0.3	<0.3	<0.3
DMSO	>5,000	>5,000	>5,000

[a]The results are the average of three separate experiments. SDB, Sabourand dextrose broth; FLC, fluconazole; AMB, amphotericin B.

The preparation of 2,9-hexadecadiynoic acid (**3b**) followed a similar synthetic strategy, with the exception that this synthesis started with the 1,8-nonadiyne. The diyne was coupled with 1-bromohexane using again *n*-BuLi in THF/HMPA at –78°C, which afforded 1,8-pentadecadiyne (**2b**) in a 21% yield after final purification. Final coupling of the lithium acetylide of 1,8-pentadecadiyne (**2b**) with carbon dioxide and subsequent protonation with ammonium chloride afforded the desired 2,9-hexadecadiynoic acid (**3b**) in a 52% yield after final purification (Scheme 1). The overall yield for this two-step synthesis was 11%.

The antifungal activity of the synthesized acetylenic FA against *Candida albicans* strains ATCC 14053 and ATCC 60193, and *Cryptococcus neoformans* ATCC 66031 in SDB were determined using a modified method of the National Committee for Clinical Laboratory Standards (NCCLS) as described by Galgiani and the more recent NCCLS M27-A microdilution methods as described previously (12–13) (Table 1). AMB and FLC were used as positive controls. Among the monoynoic FA, 2-hexadecynoic acid exhibited the best MIC values against FLC-resistant *C. albicans* ATCC 14053 and ATCC 60193 strains; these MIC values were 9.4 and 100 μM, respectively. However, as we have previously reported (8), 6-nonadecynoic acid showed the best MIC value (<4.3 μM) against *C. neoformans* ATCC 66031, but it was not very effective against the FLC-resistant *C. albicans* strains studied herein (8). Therefore, introduction of a triple bond at either C-2 or C-6 in the alkyl chain was effective in increasing the fungitoxicity of the FA. However, a triple bond at either C-5 or C-9 in the alkyl chain was not particularly effective in increasing the fungitoxicity of the FA.

Among the studied diynoic FA, 2,6-hexadecadiynoic acid (**3a**) displayed the best MIC values against both FLC-resistant *C. albicans* strains, ATCC 14053 and ATCC 60193, with MIC values of 11.5 μM. However, 2,6-nonadecadiynoic acid (**3c**) displayed the best MIC value (<5.0 μM) among the diynoic acids against *C. neoformans* ATCC 66031, but it displayed modest activity against the studied FLC-resistant *C. albicans* strains, with MIC values between 20 and 80 μM (Table 1). A comparison of the bioactivity of **3a** with that of the 2-hexadecynoic acid reveals that introduction of a second triple bond at C-6 increases the fungitoxicity of the acid toward *C. albicans* ATCC 60193 (MIC = 11.5 μM) approximately 9-fold compared with 2-hexadecynoic acid (MIC = 100 μM). Therefore, the diynoic acid **3a** has the potential of displaying a broader antifungal profile than the parent 2-hexadecynoic acid.

The acid 2,9-hexadecadiynoic acid (**3b**) was also studied, and it was not particularly effective against the FLC-resistant *C. albicans* strains studied herein, but it displayed fungitoxicity against *C. neoformans* ATCC 66031 with a MIC of < 5.8 μM. A comparison of the bioactivity of **3b** with that of the 9-hexadecynoic acid reveals that introduction of a second triple bond at C-2 increases the fungitoxicity of the parent 9-hexadecynoic acid against the three fungal strains studied, but the bioactivity of **3b** was still poor as compared with that of either **3a** or **3c**.

In order to determine the selectivity of the 2,6-hexadecadiynoic acid (**3a**) toward fungi (eukaryotic pathogens) vs. bacteria we chose to explore the antimycobacterial activity of both **3a** and 2-hexadecynoic acid toward *Mycobacterium tuberculosis* $H_{37}Rv$ following a procedure previously described (16). We decided on *M. tuberculosis* because there is a recent literature report indicating that 2-hexadecynoic acid can be toxic (MIC ~ 10–20 μM in Sauton's medium) to mycobacteria by the accumulation of two metabolites, specifically, 3-ketohexadecanoic acid, which blocks FA biosynthesis, and 3-hexadecynoic acid, an inhibitor of FA β-oxidation

TABLE 2
MIC of 2,6-Hexadecadiynoic Acid and 2-Hexadecynoic Acid Against *Mycobacterium tuberculosis* $H_{37}Rv$[a]

Compound	MIC (μM)	
	MABA	LORA
2,6-Hexadecadiynoic (**3a**)	145	>300
2-Hexadecynoic	141	>300
Rifampin	0.08	1.5

[a]MABA, microplate alamar blue assay; LORA, low oxygen recovery assay.

(17). 2-Hexadecynoic acid, therefore, can inhibit mycolic acid biosynthesis, FA biosynthesis, and FA degradation (β-oxidation) pathways of importance for mycobacteria (17).

Our antimycobacterial results are shown in Table 2. Two assays were used to study the antimycobacterial activity of the acetylenic FA: the microplate alamar blue assay (MABA) and the low oxygen recovery assay (LORA). The MABA is the "normal" MIC determination against replicating *M. tuberculosis* under aerobic conditions for 8 d. On the other hand, the LORA utilizes *M. tuberculosis* under anaerobic nonreplicating conditions for 11 d. As can be seen from Table 2, both acetylenic FA displayed similar MIC of around 140–145 μM (in Middlebrook 7H12 medium) against *M. tuberculosis* using the MABA assay, but also both acids were not inhibitory to nonreplicating and persistent *M. tuberculosis*, as determined by the LORA assay (16). From these experiments we can conclude that the additional Δ6 triple bond in **3a** does not help in increasing its antimycobacterial activity as compared with the 2-hexadecynoic acid, a fact that supports its postulated mechanism of action (17). However, it is evident that **3a** is a better antifungal agent than antimycobacterial agent due to its specificity toward fungal cells, because the additional Δ6 triple bond in **3a** makes a difference in the antifungal activity.

With respect to the toxicity of the 2-alkynoic acids it was recently demonstrated that 2-alkynoic acids could be used specifically against bacteria without toxicity to their host due to the fact that the FASI complex in microsomal systems is not inhibited by the 2-alkynoic acids (17). In addition, it was previously reported that despite the fact that 2-hexadecynoic acid inhibits the growth of HeLa cells, the simultaneous addition of palmitic acid to the culture medium reverses the growth inhibition observed in HeLa cells, thus implying that toxicity would not occur in animals consuming a normal diet (3).

Further experiments are required to determine the mechanism of antifungal activities by these novel 2,6-diynoic FA. One possible mechanism of action for the 2,6-diynoic FA is isomerization to a 2,3-allene (as the CoA derivative) and subsequent inhibition of the biosynthesis of the fungal FA as shown for the 2-hexadecynoic acid in intact animals (4). Likewise, also analogous to the suspected mechanism of action for 6-nonadecynoic acid, the 2,6-diynoic FA could also inhibit sphingolipid biosynthesis in these fungi (7). Both mechanisms of lipid biosynthesis inhibition could also be operative, thus explaining the enhanced activity of **3a**. Compound **3a** has the potential for further evaluation in topical formulations for the treatment of fungal infections, as an antifungal in wound dressings, or as a preservative in skin and hair care products.

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