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Synthesis and antifungal properties of α-methoxy and α-hydroxyl substituted 4-thiatetradecanoic acids

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Abstract

4-Thiatetradecanoic acid exhibited weak antifungal activities against *Candida albicans* (ATCC 60193), *Cryptococcus neoformans* (ATCC 66031), and *Aspergillus niger* (ATCC 16404) (MIC = 4.8–12.7 mM). It has been demonstrated that α-methoxylation efficiently blocks β-oxidation and significantly improve the antifungal activities of fatty acids. We examined whether antifungal activity of 4-thiatetradecanoic acid can be improved by α-substitution. The unprecedented (±)-2-hydroxy-4-thiatetradecanoic acid was synthesized in four steps (20% overall yield), while the (±)-2-methoxy-4-thiatetradecanoic acid was synthesized in five steps (14% overall yield) starting from 1-decanethiol. The key step in the synthesis was the hydrolysis of a trimethylsilyloxynitrile. In general, the novel (±)-2-methoxy-4-thiatetradecanoic acid displayed significantly higher antifungal activities against *C. albicans* (ATCC 60193), *C. neoformans* (ATCC 66031), and *A. niger* (ATCC 16404) (MIC = 0.8–1.2 mM), when compared with 4-thiatetradecanoic acid. In the case of *C. neoformans* the (±)-2-hydroxy-4-thiatetradecanoic acid was more fungitoxic (MIC = 0.17 mM) than the α-methoxylated analog, but not as effective against *A. niger* (MIC = 5.5 mM). The enhanced fungitoxicity of the (±)-2-methoxy-4-thiatetradecanoic acid, as compared to decylthiopropionic acid, might be the result of a longer half-life in the cells due to a blocked β-oxidation pathway which results in more time to exert its toxic effects. Thus, these novel fatty acids may have applications as probes to study fatty acid metabolic routes in human cells.

Keywords: Antifungal; Decylthiopropionic acid; Methoxylated fatty acids; Synthesis; Thia fatty acids

1. Introduction

Thia fatty acids with the sulfur atom at the 3 and 4 positions have been of considerable interest for their effects on the metabolism of fatty acids (Dyroy et al., 2006; Skrede et al., 1997). The 3-thia fatty acids have been reported to decrease the serum concentration of triacylglycerols and cholesterol and to induce in hepatocytes peroxisomal β-oxidation more efficiently than normal fatty acids (Hovik et al., 1990; Skrede et al., 1995). In particular, tetradecylthioacetic acid is also known to have beneficial antioxidant properties, to lower hyperglycemia, and displays a positive effect on insulin levels (Dyroy et al., 2006). On the other hand, 4-thia fatty acids inhibit β-oxidation (or it occurs rather slowly) in peroxisomes and mitochondria thereby inducing hepatic lipidosis when administered to rats, i.e., the hepatic levels of triacylglycerols are increased (Hvattum et al., 1992). The difference between the 3- and 4-thia fatty

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acids can be ascribed as to the position of the sulfur atom in the fatty acyl chain; the 3-thia fatty acids cannot be β-oxidized and are rather metabolized by ω-oxidation (Gudbrandsen et al., 2005). On the other hand, the 4-thia fatty acids can be β-oxidized once, but the resulting metabolites (alkylthioacrylyl-CoA) accumulate in the mitochondria thereby inhibiting fatty acid oxidation (Hvattum et al., 1992).

Despite much of the above studies on the metabolism of thia fatty acids in humans nothing is known as to their antifungal properties. However, earlier work by Gordon and collaborators (Kishore et al., 1993) indicated that the myristoyl-CoA:protein *N*-myristoyltransferase (NMT) of the yeast *Saccharomyces cerevisiae*, an enzyme that catalyzes the transfer of myristic acid ($C_{14:0}$) from myristoyl-CoA to the α-amino nitrogen of glycine, has a greater affinity for 4-thiatetradecanoic acid than for the 3-thiatetradecanoic acid (Kishore et al., 1993). Moreover, when the authors compared human NMT versus *S. cerevisiae* NMT they found that the 4-thiatetradecanoic acid has a greater affinity for the *S. cerevisiae* NMT than for the human NMT. On the other hand, the 3-thiatetradecanoic acid had almost the same affinity for both types of NMTs (Kishore et al., 1993).

Our studies showed that 4-thiatetradecanoic acid has weak antifungal activities against *Candida albicans* (ATCC 60193), *Cryptococcus neoformans* (ATCC 66031), and *Aspergillus niger* (ATCC 16404) (MIC = 4.8–12.7 mM). We have previously shown that α-methoxylation of tetradecanoic acid can significantly improve the antifungal activities of tetradecanoic acid (Carballeira et al., 2005). We investigated whether α-substitution of 4-thiatetradecanoic acid can improve the antifungal activities of 4-thiatetradecanoic acid by inhibition of β-oxidation or fungi NMT.

It was our expectation that the α-methoxy substitution would essentially block the only β-oxidation pathway left for the 4-thia fatty acid thus allowing for only ω-oxidation to be the most likely catabolic pathway for this novel fatty acid, which could render the α-methoxy 4-thia fatty acid metabolically similar to a 3-thia fatty acid and thus become a good tool for further lipid metabolic studies. Furthermore, based on our previous findings (Carballeira et al., 2005), it was expected that the α-methoxy-4-thia fatty acid to be a better antifungal compound than the parent 4-thia fatty acid. Therefore, in this work we present the first total synthesis for a 2-methoxy-4-thia fatty acid, namely the (±)-2-methoxy-4-thiatetradecanoic acid (**1**), and report on its antifungal properties and compare it to other analogs of similar chain lengths.

2. Materials and methods

2.1. Instrumentation

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker DPX-300 spectrometer. ^{1}H NMR chemical shifts are reported with respect to internal $(CH_3)_4Si$ (77.0 ppm), ^{13}C NMR chemical shifts are reported in parts per million relative to $CDCl_3$ (77.0 ppm). GC–MS analyses were recorded at 70 eV using a Hewlett Packard 5972A MS equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane crosslinked with 5% phenyl methylpolysiloxane. High-resolution mass spectral data was performed at the Emory University Mass Spectrometry Center on a thermo LTQ-FTMS using flow injection analysis.

2.2. 2-(2-Thiadodecanoyl)-1,3-dioxolane (*2*)

Into a two necked 100 mL round-bottomed flask equipped with a magnetic stirrer and a condenser was added NaH (0.54 g, 23.0 mmol) and diluted with THF (10 mL). The solution was kept under argon. The temperature was lowered to 0 °C and the 1-decanethiol (4.6 mL, 23.0 mmol) was slowly added to the solution. The reaction mixture was warmed to room temperature in a period of 35 min, then cooled to 0 °C and the 2-(2-bromomethyl)-1,3-dioxolane (1.5 mL, 15.0 mmol) was added. The reaction was refluxed for 5 h and extracted with ether (2× 15 mL), dried over Na_2SO_4, and filtered. The solvent was removed in vacuo affording 3.59 g of 2-(2-thiadodecanoyl)-1,3-dioxolane (**2**) (92% yield). ^{1}H NMR ($CDCl_3$) δ 5.04 (1H, t, J = 4.6 Hz, H-2), 4.00 (2H, AA′ BB′, –OCH_2–), 3.88 (2H, AA′ BB′, –OCH_2–), 2.70 (2H, d, J = 4.6 Hz, H-1′), 2.60 (2H, t, J = 7.4 Hz, H-3′), 1.57 (2H, quintet, J = 7.4 Hz, H-4′), 1.35 (2H, m, H-5), 1.25 (12H, brs, –CH_2–), 0.86 (3H, t, J = 7.0 Hz, –CH_3–); ^{13}C NMR ($CDCl_3$) δ 104.3 (d, C-2), 65.2 (t), 35.4 (t), 32.9 (t), 31.9 (t), 29.6 (t), 29.51 (t), 29.47 (t), 29.3 (t), 29.2 (t), 28.8 (t), 22.6 (t), 14.1 (q, CH_3); GC–MS (70 eV) *m/z* (relative intensity) 260 (M^+, 2), 187 (1), 87 (1), 86 (1), 83 (1), 75 (1), 74 (3), 73 (100), 69 (2), 67 (1), 61 (3), 60 (1), 59 (1), 57 (2), 56 (1), 55 (5); HRMS (ESI) Calcd for $C_{14}H_{27}O_2S$ $[M-H]^+$ 259.1726, found 259.1724.

2.3. 3-Thiatetradecanal (*3*)

Into a 100 mL round-bottomed flask equipped with a magnetic stirrer was added the dioxolane **2** (2.0 g, 8.0 mmol) diluted with 20 mL of a solution of

water–acetone (1:1). Amberlyst 15® (excess) was added to the solution which was stirred at 60 °C for 24 h. The reaction mixture was filtered, extracted with ethylacetate (2× 20 mL) and dried over Na_2SO_4. The solvent was removed in vacuo affording 1.05 g (60% yield) of 3-thiatetradecanal (**3**). ^{1}H NMR ($CDCl_3$) δ 9.45 (1H, t, J = 3.5 Hz, CHO), 3.17 (2H, d, J = 3.5 Hz, H-2), 2.42 (2H, t, J = 7.4 Hz, H-4), 1.55 (2H, quintet, J = 7.4 Hz, H-5), 1.34 (2H, m, H-6), 1.25 (12H, brs, –CH_2–), 0.87 (3H, t, J = 6.9 Hz, –CH_3); ^{13}C NMR ($CDCl_3$) δ 193.9 (d, C-1), 41.4 (t), 31.8 (t), 31.6 (t), 30.9 (t), 29.5 (t), 29.4 (t), 29.2 (t), 29.1 (t), 28.6 (t), 22.6 (t), 14.1 (q, CH_3); GC–MS (70 eV) *m/z* (relative intensity) 216 (M^+, 16), 188 (6), 187 (44), 173 (25), 171 (3), 143 (4), 129 (18), 115 (7), 112 (3), 101 (16), 99 (3), 97 (23), 96 (4), 95 (3), 89 (5), 88 (5), 87 (28), 85 (5), 84 (8), 83 (57), 82 (7), 81 (6), 77 (3), 76 (6), 75 (7), 71 (10), 70 (14), 69 (54), 68 (8), 67 (12), 65 (2), 63 (4), 62 (4), 61 (90), 60 (26), 57 (32), 56 (18), 55 (100).

2.4. (±)-2-Trimethylsilyloxy-4-thiatetradecanonitrile (*4*)

Into a 100 mL round-bottomed flask equipped with a magnetic stirrer was added the 3-thiatetradecanal (**3**) (1.48 g, 7.0 mmol) diluted in dichloromethane (10 mL). The temperature was lowered to 0 °C and trimethylsilyl cyanide (1.19 mL, 9.0 mmol) was added. After 1 min three drops of Et_3N were added and the solution was stirred at 0 °C for 3 h. The solution was extracted with ether (2× 15 mL), dried over Na_2SO_4 and the solvent removed in vacuo. The reaction afforded 1.64 g (74% yield) of nitrile **4** as a viscous oil, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 4.46 (1H, t, J = 6.7 Hz, H-2), 2.84 (2H, d, J = 6.7 Hz, H-3), 2.64 (2H, t, J = 7.4 Hz, H-5), 1.58 (2H, m, H-6), 1.37 (2H, m), 1.26 (12H, brs, –CH_2–), 0.87 (3H, t, J = 6.4 Hz, –CH_3), 0.2 (9H, s, –Si(CH_3)$_3$); ^{13}C NMR ($CDCl_3$) δ 119.3 (d, C-2), 62.8 (t), 37.5 (t), 33.3 (t), 31.9 (t), 29.6 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 28.7 (t), 22.7 (t),14.1 (q),−0.38 (q); GC–MS (70 eV) *m/z* (relative intensity) 315 (M^+, 4), 300 (3), 189 (5), 188 (13), 187 (100), 144 (2), 133 (6), 117 (4), 97 (13), 87 (3), 85 (4), 84 (5), 83 (29), 81 (2), 75 (9), 73 (22), 71 (6), 70 (2), 69 (17), 61 (30), 59 (4), 57 (13), 55 (22).

2.5. (±)-2-Hydroxy-4-thiatetradecanoic acid (*5*)

Into a 50 mL round-bottomed flask equipped with a magnetic stirrer was added the nitrile **4** (0.60 g, 2.0 mmol) diluted in 5.0 mL of concentrated HCl. The solution was stirred overnight at room temperature followed by 2 h at 65 °C. The reaction mixture was washed with water, extracted with ether (2× 10 mL), and dried over Na_2SO_4. The solvent was removed in vacuo affording 0.26 g (50% yield) of (±)-2-hydroxy-4-thiatetradecanoic acid (**5**) as a white solid. mp 84–86 °C; ^{1}H NMR ($CDCl_3$) δ 4.40 (1H, dd, J = 4.2 Hz, J = 6.3 Hz, H-2), 3.05 (1H, dd, J = 4.2 Hz, J = 14.2 Hz, H-3), 2.90 (1H, dd, J = 6.3 Hz, J = 14.2 Hz, H-3), 2.55 (2H, t, J = 7.2 Hz, H-5), 1.58 (2H, quintet, J = 7.2 Hz, H-6), 1.35 (2H, m), 1.25 (12H, brs, –CH_2–), 0.87 (3H, t, J = 5.9 Hz, –CH_3); ^{13}C NMR ($CDCl_3$) δ 176.6 (s, C-1), 69.8 (d), 36.2 (t), 32.9 (t), 31.9 (t), 29.5 (t), 29.3 (t), 29.2 (t), 28.8 (t), 22.7 (t), 14.1 (q); HRMS (ESI) Calcd for $C_{13}H_{25}O_3S$ $[M-H]^+$ 261.1535, found 261.1527.

2.6. Methyl (±)-2-hydroxy-4-thiatetradecanoate (*5a*)

Was prepared from **5** to confirm GC purity by means of the reaction of the acid with methanolic HCl followed by purification using column chromatography on Si gel eluting with hexane/ether (9:1). GC–MS (70 eV) *m/z* (relative intensity) 276 (M^+, 11), 258 (4), 226 (2), 217 (8), 198 (2), 189 (5), 188 (13), 187 (100), 184 (5), 173 (10), 171 (7), 146 (3), 135 (2), 119 (5), 118 (8), 103 (7), 97 (28), 95 (3), 89 (12), 87 (18), 83 (67), 71 (16), 69 (55), 67 (11), 61 (90), 59 (18), 57 (40), 56 (12), 55 (97).

2.7. (±)-2-Methoxy-4-thiatetradecanoic acid (*1*)

Into a 50 mL round-bottomed flask was placed NaH (0.13 g, 5.0 mmol) diluted in THF (5 mL). Then 2-hydroxy-4-thiatetradecanoic acid (**5**) (0.20 g, 0.76 mmol) in 5 mL of THF, was added to the reaction mixture. The reaction was stirred for 20 min, then cooled to 0 °C and iodomethane (0.5 mL, 6.0 mmol) was slowly added. The reaction was stirred for 4 h at room temperature, and then HCl(conc) was added to make the solution acidic. The reaction mixture was extracted with ether (2× 10 mL), dried over Na_2SO_4, and the solvent was removed in vacuo. The crude product was purified using silica gel column chromatography and eluting first with hexane/EtOAc (9:1) to remove the impurities, followed by hexane/EtOAc (7:3) to obtain 0.15 g (70% yield) of the (±)-2-methoxy-4-thiatetradecanoic acid (**1**) as a viscous oil. ^{1}H NMR ($CDCl_3$) δ 3.98 (1H, dd, J = 4.4 Hz, J = 6.9 Hz, H-2), 3.48 (3H, s, –OCH_3), 2.95 (1H, dd, J = 4.4 Hz, J = 14.0 Hz, H-3), 2.85 (1H, dd, J = 6.9 Hz, J = 14.0 Hz, H-3), 2.59 (2H, t, J = 7.4 Hz, H-5), 1.55 (2H, quintet, J = 7.4 Hz, H-6), 1.34 (2H, m), 1.26 (12H, brs, –CH_2–), 0.87 (3H, t, J = 6.4 Hz, –CH_3); ^{13}C NMR

($CDCl_3$) δ 175.9 (s, C-1), 80.7 (d, C-2), 58.7 (q, OCH_3), 34.1 (t), 33.2 (t), 31.9 (t), 29.53 (t), 29.49 (t), 29.3 (t), 29.2 (t), 29.0 (t), 28.8 (t), 22.6 (t), 14.1 (q, CH_3); GC–MS (70 eV) *m/z* (relative intensity) 276 (M^+, 1), 248 (1), 247 (3), 246 (21), 231 (1), 189 (1), 188 (2), 187 (13), 185 (1), 176 (1), 175 (6), 174 (14), 173 (100), 171 (2), 161 (3), 143 (1), 140 (1), 129 (3), 120 (1), 119 (4), 112 (1), 111 (2), 107 (3), 106 (13), 105 (3), 101 (2), 97 (7), 96 (1), 91 (2), 90 (1), 89 (14), 88 (8), 87 (6), 85 (2), 84 (3), 83 (11), 82 (2), 81 (1), 79 (1), 75 (1), 73 (3), 71 (2), 70 (4), 69 (8), 68 (1), 67 (3), 61 (6), 60 (3), 59 (2), 57 (5), 56 (4), 55 (12). HRMS (ESI) Calcd for $C_{14}H_{27}O_3S$ $[M-H]^+$ 275.1675, found 275.1680.

2.8. 4-Thiatetradecanoic acid

Was obtained in a 75% yield from the reaction of 3-bromopropionic acid (1 g, 6 mmol) and decanethiol (2.4 mL, 12 mmol) using NaH in THF and following the procedure described by Skrede (Skrede et al., 1997).

2.9. Microorganisms

C. albicans ATCC 60193, *A. niger* ATCC 16404, and *C. neoformans* ATCC 66031 were obtained from American Type Culture Collection, Manassas, Virginia. 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 make a transmittance of 73–75% at 530 nm. This transmittance provides a concentration of 10^6 cells/mL for *C. ablicans* or *C. neoformans* and 10^6 spores/mL for *A. niger* in saline medium when compared to the control tube. The media was Sabourad dextrose broth (SDB; Becton Dickinson and Co., Sparks, MD).

2.10. Chemicals and antifungal agents

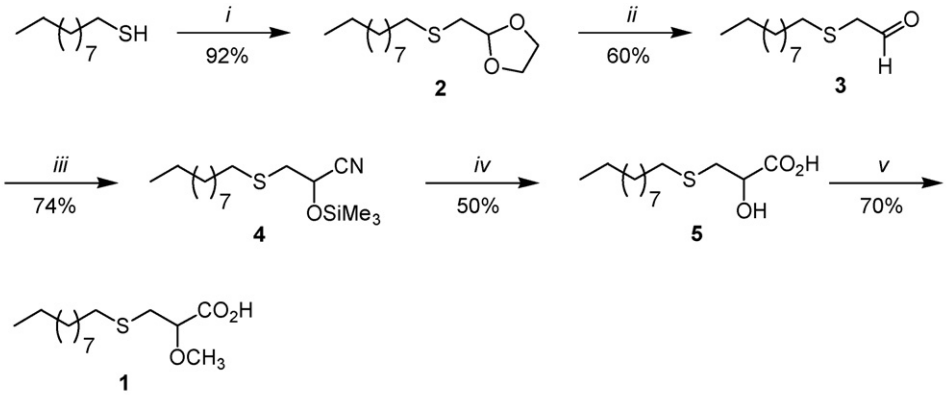
Amphotericin B (AMB) was purchased from Acros, New Jersey, USA and was kept as a 0.005 mM stock in DMSO at 0 °C and used during 1 week of preparation. Fluconazole (FLC) was purchased from Medisa Inc., New York, USA, or was provided from Vera Laboratories Ltd., Hyderabad, India, and was kept as a 0.02 mM stock solution at 0 °C. Working dilutions were made in SDB medium. The final maximum concentration of DMSO in the assays was 5% (v/v). DMSO was not inhibitory to the organisms in the concentrations tested.

2.11. Susceptibility testing

Microdilutions for control experiments with *C. albicans*, *A. niger*, and *C. neoformans* were the modified method of National Committee for Clinical Laboratory Standards (NCCLS) method as described by Galgiani (Galgiani, 1993) and by the more recent NCCLS M27-A microdilution methods as described previously (Carballeira et al., 2004; Nam et al., 2004). Dilutions were prepared in 0.1 mL of SDB; the inocula were either 10^4 *C. albicans* or *C. neoformans* cells or *A. niger* spores. The tubes were incubated for 24–28 h at 36 ± 1 °C, and turbidity was read visually. MICs were calculated in comparison to growth control as the lowest concentration that shows inhibition for AMB, FLC, and the test compounds.

3. Results and discussion

A five-step synthesis for the (±)-2-methoxy-4-thiatetradecanoic acid (**1**) was accomplished as outlined in Scheme 1. This synthesis started with commercially available 1-decanethiol that was coupled with 2-(2-bromomethyl)-1,3-dioxolane under basic conditions using NaH in dry THF affording the 2-(2-thiadodecanoyl)-1,3-dioxolane (**2**) in a 92% yield. The acetal in dioxolane **2** was removed by treatment with acidic Amberlyst 15® (wet) ion-exchange resin in acetone–water (1:1) for 24 h, which afforded the 3-thiatetradecanal (**3**) in a 60% yield. The aldehyde **3** was then reacted with trimethylsilyl cyanide and triethylamine in dichloromethane at 0 °C affording the (±)-2-trimethylsilyloxy-4-thiatetradecanonitrile (**4**) in a 74% yield. Hydrolysis of the nitrile **4** was carried out in the presence of concentrated HCl by stirring for 24 h at room temperature followed by 2 h at 65 °C, which resulted in a 50% yield of the desired (±)-2-hydroxy-4-thiatetradecanoic acid (**5**). The hydrolysis of the nitrile **4** was the key step in this synthesis. The desired α-methoxylated 4-thia fatty acid **1** was then obtained in a 70% yield in the reaction of the α-hydroxy acid **5** with sodium hydride and iodomethane in tetrahydrofuran (THF) which only afforded methylation at the α-hydroxy group and not at the carboxylic acid. The overall yield for this five-step synthesis was 14%. Our developed synthetic methodology can be applied to synthesize other methoxylated 4-thia fatty acid analogs with different chain lengths by just changing the length of the starting 1-alkanethiol.



i) NaH, 2-(2-bromomethyl)-1,3-dioxolane, THF, 0°C, 5h; *ii)* Amberlyst, acetone-H_2O (1:1), 60°C, 24h; *iii)* trimethylsilyl cyanide/Et_3N, CH_2Cl_2, 0°C, 3h; *iv)* HCl (conc.), 65°C, 24h; *v)* NaH, CH_3I, THF, rt, 4h.

Scheme 1. Synthesis of (±)-2-hydroxy-4-thiatetradecanoic acid (**5**) and (±)-2-methoxy-4-thiatetradecanoic acid (**1**).

The fungitoxicity of the novel α-substituted 4-thia fatty acids **5** and **1**, as compared to 4-thiatetradecanoic acid and other analogs, is presented in Table 1. It is interesting to note that the 4-thiatetradecanoic acid is quite benign (MIC = 5–12 mM) since it is less toxic to the studied fungi than even myristic acid. As compared to the parent 4-thiatetradecanoic acid both α-hydroxylation and α-methoxylation increased the fungitoxicity of the 4-thia fatty acid. In general, α-methoxylation was more effective in increasing the antifungal activity (MIC = 0.8–1.2 mM) of the 4-thiatetradecanoic acid towards all the studied fungal strains (Table 1). However, there were some interesting differences between the strains. For example, in the case of *C. albicans* (ATCC 60193) α-hydroxylation and α-methoxylation were equally effective in increasing the antifungal activity (MIC = 1.0–1.2 mM) of the 4-thiatetradecanoic acid. However, in the case of *C. neoformans* (ATCC 66031) α-hydroxylation (MIC = 0.17 mM) was about sevenfold more effective than α-methoxylation (MIC = 1.24 mM) in increasing the fungitoxicity of the 4-thia fatty acid. In the case of *A. niger* (ATCC 16404) α-hydroxylation did not improve significantly the antifungal activity when compared to the parent analog. However, α-methoxylation enhanced the antifungal activity towards *A. niger* (MIC = 0.8 mM) approximately eightfold higher than that of 4-thiatetradecanoic acid (MIC = 6.3 mM).

In Table 1 the previously reported antifungal activities of (±)-2-methoxy-4-oxatetradecanoic acid (Carballeira et al., 2005) have been given to compare the effect of 4-oxa substitution versus 4-thia substitution on antifungal activities. With respect to *C. neoformans* (ATCC 66031) and *A. niger* (ATCC 16404) the (±)-2-methoxy-4-oxatetradecanoic acid was a better antifungal fatty acid

Table 1
Antifungal activity (MIC values, mM)[a] against *Candida albicans* (SDB), *Cryptococcus neoformans* (SDB), and *Aspergillus niger* (SDB) at 35–37 °C after 24–48 h

Compound	*C. albicans* ATCC 60193	*C. neoformans* ATCC 66031	*A. niger* ATCC 16404
14:0[b]	3.80[c]	1.90	3.80
4-thia-14:0	12.66	4.75	6.33
2-OH-4-thia-14:0	1.04	0.17	5.53
2-OMe-4-thia-14:0	**1.24**	**1.24**	**0.83**
2-OMe-14:0[b]	0.11[c]	0.11	0.11
2-OMe-4-oxa-14:0[b]	3.50	0.08	0.22
FLC	>1.0	0.002	1.50
AMB	<0.0003	<0.0003	<0.0009
DMSO	>5	>5	>5

The bold values are for the title compound 1.
[a] The result is the average of three separate experiments. The upper limit of the standard error of the mean (S.E.M.) was ±10%.
[b] Antifungal data taken from Carballeira et al. (2005).
[c] These MIC values were measured against *C. albicans* 14053, also taken from Carballeira et al. (2005).

(MIC = 0.08–0.22 mM) than **1** (MIC = 0.8–1.2 mM). However, against *C. albicans* (ATCC 60193) the (±)-2-methoxy-4-thiatetradecanoic acid (**1**) was about threefold more fungitoxic (MIC = 1.24 mM). Overall, the (±)-2-methoxytetradecanoic acid continues to be the most effective antifungal fatty acid of the series (MIC = 0.11 mM) against the three fungal strains tested.

From our results it is clear that α-methoxylation increases the fungitoxicity of 4-thiatetradecanoic acid towards the studied fungal strains. The rationale behind the increased fungitoxicity of **1** versus the 4-thiatetradecanoic acid could be the result of several factors. There are clear differences in the way acid **1** is metabolized by the fungi as compared to 4-thiatetradecanoic acid. The presence of the additional α-methoxy group should introduce sufficient steric effect at the α-position to delay the β-oxidation of acid **1**, thus leaving ω-oxidation and sulfur oxidation as the most likely metabolic pathways for **1**. This steric blockade should increase the half-life of acid **1** in the cell as compared to the half-life of the 4-thiatetradecanoic acid, which can go through one cycle of β-oxidation. Since **1** is expected to have longer half-life in the fungal cells, it possibly gets incorporated more efficiently into the membrane phospholipids of the fungi and causes membrane disruption and eventual antifungal activities (Hvattum et al., 1992). Presumably, sulfur oxidation occurs more rapidly than methoxy hydroxylation (removal of the α-methoxy group) or ω-oxidation (Alterman et al., 1995), and metabolites such as the 2-methoxydecylsulfoxypropionic acid and 2-methoxycarboxymethylsulfoxypropionic acid could also be accumulating in the fungal cells (Hvattum et al., 1992). Alternatively fatty acid **1** or its oxidized metabolites may be inhibiting some key fatty acid metabolic enzymes in the fungal cells or the NMT enzyme of some of these fungi.

As seen in Table 1 there are also clear differences in antifungal activities between the different α-methoxylated myristic acid analogs, in particular between the 2-methoxy-4-oxatetradecanoic acid and **1**. This difference in antifungal activity could also be ascribed to differences in the pKa of the different substrates. It is evident that α-methoxylation increases the pKa of these acids, which could explain the difference between the α-methoxylated fatty acids and the non-methoxylated fatty acids. However, since 4-oxa substitution increases the acidity of myristic acid from 4.91 to 4.37 (Parang et al., 1996) and oxygen is more electronegative than sulfur, this difference in electronegativity could account for the better fungitoxicity of 2-methoxy-4-oxatetradecanoic acid towards *C. neoformans* and *A. niger*. In the case of *C. albicans*, **1** was a better antifungal agent than the corresponding 4-oxa analog. This is not surprising since the 4-oxatetradecanoic acid is known not to be effective against *C. albicans* (Langer et al., 1992).

Thus, in addition to interesting antifungal activities, α-methoxylated fatty acid **1** may be used as a probe to study fatty acid metabolic routes. Further mechanistic and metabolic studies are underway to determine the metabolism of **1**.

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