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Racemic and optically active 2-methoxy-4-oxatetradecanoic acids: novel synthetic fatty acids with selective antifungal properties

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Abstract

The unprecedented (±)-2-methoxy-4-oxatetradecanoic acid and the optically pure (*S*)-2-methoxy-4-oxatetradecanoic acid were synthesized in six steps and in 11–14% overall yields starting with either 1,2-*O*-isopropylidene-*rac*-glycerol or 1,2-*O*-isopropylidene-(*S*)-glycerol. The key step in the synthesis was the selective monosilylation of a dibutylstannylene intermediate. The title compounds displayed selective fungitoxicity in the range of 0.08–0.22 mM against *Cryptococcus neoformans* ATCC 66031 and *Aspergillus niger* ATCC 16404, but no significant activity against *C. albicans* ATCC 14053 and ATCC 60193 (>2.6 mM). Albeit being good substrates for *N*-myristoyltransferases (NMTs), the racemic and the *S*-enantiomer of the oxygenated 2-methoxylated compounds showed no significant difference in antifungal activity. This finding suggests an alternative mechanism of fungitoxicity other than NMT inhibition.

Keywords: 2-Methoxy-4-oxatetradecanoic acid; *Cryptococcus neoformans*; *N*-myristoyltransferases; Fatty acids

1. Introduction

The antifungal properties of fatty acids and their mechanism of action have been previously reported (Garg and Muller, 1993; Sjogren et al., 2003). For example, a study of the cytotoxicity of several short-chain fatty acids on three strains of *Candida albicans* revealed that capric acid (10:0) caused the greatest reduction of the infectivity titers ($\geq$6.75 $\log_{10}$CFU) at 37 °C as compared to the control at 10 mM (Bergsson et al., 2001). Transmission electron microscopy (TEM) studies on *C. albicans* showed disorganization of the cytoplasm due to a disrupted or disintegrated plasma membrane caused by a hydrostatic turgor pressure within the cell. A similar mechanism of action was proposed for (*Z*)-9-heptadecenoic acid (17:1), which inhibits the growth and germination of the fungi *Phytophthora infestans* and *Idriella bolleyi* (Avis and Bélanger, 2001). The mechanism of inhibition of

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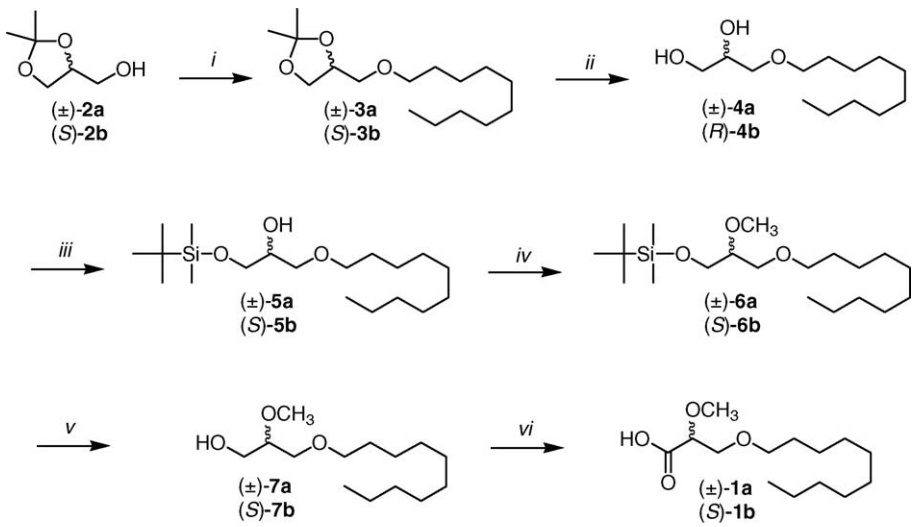
the (*Z*)-9-heptadecenoic acid (17:1) was explained in terms of membrane disruption resulting in the release of intracellular electrolytes and of proteins and eventually cytoplasmic disintegration of mycelia.

Several tetradecanoic acid (14:0) analogues have been studied in vitro for their ability to inhibit the enzyme *N*-myristoyltransferase (NMT) (Langer et al., 1992). Many fungal species synthesize a variety of *N*-myristoyl proteins using NMT as catalyst (Lodge et al., 1994; Weinberg et al., 1995). Incorporation of these analogues into the fungi competes with 14:0 for the binding to the NMT and myristoylation of fungi proteins. This event perturbs fungal protein function such as protein folding, resulting in fungal growth inhibition. An example of a tetradecanoic acid analogue is the (±)-2-bromotetradecanoic acid with MICs between 0.01 and 0.04 mM against *C. albicans*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae* and *Aspergillus niger* (Parang et al., 1996). Another interesting tetradecanoic acid analogue is the 4-oxatetradecanoic acid, which is fungicidal to *C. neoformans*, producing a 10,000-fold reduction in viable cell number 1 h after administration (Langer et al., 1992). Metabolic labeling studies confirmed that the 4-oxatetradecanoic acid is a substrate for *C. neoformans N*-myristoyltransferase, despite the fact that its antifungal effect could also be due to a perturbation of the fungal lipid metabolism.

Just recently, we isolated and synthesized the novel 2-methoxytetradecanoic acid and determined that it is antifungal against *C. albicans*, *C. neoformans* and *A. niger* at concentrations of 0.11 mM (Carballeira et al., 2004). The mechanism of fungal cytotoxicity for the 2-methoxytetradecanoic acid is not known, but it is proposed that this methoxylated fatty acid inhibits *N*-myristoyltransferase. In fact, preliminary molecular modeling computations on a partial model of the enzyme sc-NMT (performed by Dr. Amiram Goldblum of the Hebrew University of Jerusalem) showed the binding of the (*S*)-2-methoxytetradecanoic acid to the NMT active site. However, optically pure 2-methoxylated fatty acids need to be synthesized in order to test this hypothesis.

With the above methoxylated fatty acid antifungal properties at hand, we envisaged the synthesis of the hitherto unknown (±)-2-methoxy-4-oxatetradecanoic acid (**1a**). This novel fatty acid may exhibit the synergic antifungal properties of the 4-oxatetradecanoic acid and the 2-methoxytetradecanoic acid present in a single molecule. The added 4-oxa functionality will definitely affect the biophysical properties of the methoxy-



i) NaH, 1-bromodecane, THF; *ii*) Amberlyst 15, MeOH; *iii*) Bu_2SnO, Et_3N, CH_2Cl_2, t-$BuMe_2SiCl$; *iv*) NaH, MeI, THF; *v*) TBAF, THF; *vi*) PDC, DMF.

Scheme 1. Synthesis of (±)- and (*S*)-2-methoxy-4-oxatetradecanoic acids.

substituted fatty acids making their hydrophobicity comparable to the C_{10}–C_{12} fatty acids (Langer et al., 1992). The synthetic approach allowed the synthesis of (±)-**1a** and both of its enantiomers, since the synthesis could start with either the 1,2-*O*-isopropylidene-(*S*)-glycerol or the 1,2-*O*-isopropylidene-(*R*)-glycerol (Scheme 1). As mentioned above, optically pure 2-methoxylated fatty acids are desirable substrates for mechanistic studies of fungitoxicity of 2-substituted myristic acid analogues and correlation with chirality. In addition, the six-step synthesis that we present herein will facilitate the construction of other short-chain analogues of this novel series of fatty acids, which could display interesting biological properties.

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, while ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded on a Bruker DRX-500 spectrometer. ^{1}H NMR chemical shifts are reported with respect to internal $(CH_3)_4Si$, ^{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 ChemStation equipped with a 30 m × 0.25 mm special performance capillary column (HP-5MS) of polymethyl siloxane crosslinked with 5% phenyl methylpolysiloxane. IR spectra were recorded on a Nicolet 600 FT-IR spectrophotometer (Thermo-Nicolet, Madison, WI, USA).

2.2. 4-Decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (*3a*)

To a mixture of NaH (0.88 g, 36 mmol) in dry THF (15 ml) at 0 °C under argon was added dropwise, 1,2-*O*-isopropylidene-*rac*-glycerol (3.6 ml, 29 mmol). The resulting mixture was stirred at room temperature for 30 min, and then refluxed for 1 h. The solution was cooled to 0 °C and 1-bromodecane (3.8 ml, 18 mmol) was added dropwise. The reaction mixture was refluxed for 24 h. Then, the reaction was cooled to room temperature, washed with water (2 × 15 ml), extracted with ether (2 × 15 ml) and dried with Na_2SO_4, affording 3.2 g (65% yield) of 4-decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (**3a**) as a colorless oil after purification using silica gel column chromatography and eluting with hexane/ether (9:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 4.26 (1H, m, 4-H), 4.06 (1H, dd, J = 8.2 Hz, J = 6.5 Hz), 3.73 (1H, dd, J = 8.1 Hz, J = 6.3 Hz), 3.54–3.40 (4H, m, –OCH_2–), 1.60 (2H, m), 1.43 (3H, s, –CH_3), 1.37 (3H, s, –CH_3), 1.30 (14H, brs, –CH_2–), 0.87 (3H, t, J = 6.9 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 109.3 (s, C-2), 74.7 (d, C-4), 71.9 (t), 71.8 (t), 66.9 (t), 31.9 (t), 30.3 (t), 29.58 (t), 29.55 (t), 29.4 (t), 29.3 (t), 26.7 (t), 26.0 (t), 25.4 (t), 22.7 (t), 14.1 [q, CH_3]; GC–MS (70 eV) *m/z* (relative intensity) 272 (M^+, 1), 258 (3), 257 (19), 213 (1), 169 (1), 138 (1), 115 (1), 103 (1), 102(5), 101 (100), 99 (3), 97 (1), 87 (5), 86 (3), 85 (11), 84 (1), 83 (6), 81 (1), 75 (1), 73 (11), 72 (6), 71 (11), 70 (2), 69 (5), 68 (1), 67 (1), 61 (4), 60 (1), 59 (9), 58 (3), 57 (38), 56 (4), 55 (14), 54 (1), 53 (1); analysis calculated for $C_{16}H_{32}O_3$: C, 70.54; H, 11.84; found: C, 70.87; H, 12.25.

2.3. 3-Decyloxypropane-1,2-diol (*4a*)

Into a 50-ml round-bottomed flask containing 4-decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (1.2 g, 4.4 mmol) was added 20 ml of MeOH and excess of Amberlyst® 15 (wet) ion-exchange resin. The reaction was stirred at room temperature for 24 h, filtered and extracted with ethylacetate (2 × 25 ml). The organic extract was dried using Na_2SO_4. The solvent was evaporated in vacuo affording 0.78 g (74% yield) of 3-decyloxypropane-1,2-diol (**4a**) as a white solid, mp = 29–31 °C, after purification using silica gel column chromatography and eluting with hexane/ether (9:1); IR (neat) ν_{max} 3374, 2923, 2853, 1468, 1380, 1123 and 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.85 (1H, m), 3.70 (1H, dd, J = 11.4 Hz, J = 3.8 Hz), 3.63 (1H, dd, J = 11.4 Hz, J = 5.4 Hz), 3.55–3.43 (4H, m, –OCH_2–), 2.80 (2H, s, –OH), 1.60 (2H, m), 1.30 (14H, brs, –CH_2–), 0.83 (3H, t, J = 6.9 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 72.4 (d, C-2), 71.8 (t), 70.4 (t), 64.2 (t, C-1), 31.8 (t), 29.5 (t), 29.4 (t), 29.3 (t), 29.2 (t), 29.1 (t), 26.0 (t), 22.6 (t), 14.0 (q); GC–MS (70 eV) *m/z* (relative intensity) 232 (M^+, 1), 201.2 (2), 172 (1), 171 (6), 170 (1), 169 (1), 158 (1), 157 (1), 142 (1), 141(4), 140 (6), 139 (1), 138 (2), 118 (1), 117 (6), 116 (2), 112 (2), 111 (3), 110 (1), 109 (1), 105 (1), 101 (1), 100 (1), 99 (9), 98 (2), 97 (10), 96 (2), 95 (1), 93

(6), 89 (1), 87 (2), 86 (3), 85 (50), 84 (5), 83 (21), 82 (5), 81 (2), 77 (1), 75 (8), 74 (10), 73 (2), 72 (3), 71 (53), 70 (9), 69 (16), 68 (4), 67 (3), 62 (1), 61 (17), 60 (3), 59 (1), 58 (8), 57 (100), 56 (14), 55 (34), 54 (3), 53 (3); analysis calculated for $C_{13}H_{28}O_3$: C, 67.20; H, 12.14; found: C, 67.01; H, 12.45.

*2.4. 1-(tert-Butyldimethylsilanyloxy)-3-decyloxypropan-2-ol (**5a**)*

To a solution of 3-decyloxypropane-1,2-diol (0.41 g, 1.7 mmol), dibutyltin oxide (0.44 g, 1.7 mmol) and triethylamine (0.3 ml, 2.1 mmol) in dichloromethane (20 ml) was added dropwise *tert*-butyldimethylsilyl chloride (0.26 g, 1.7 mmol) in dichloromethane. The reaction mixture was stirred at room temperature for 90 min, extracted with ether (2×25 ml), dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified using silica gel column chromatography and eluting with hexane/ether (9:1), affording 0.46 g (75% yield) of 1-(*tert*-butyldimethylsilanyloxy)-3-decyloxypropan-2-ol (**5a**) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.80 (1H, m, H-2), 3.63 (2H, m), 3.48–3.43 (4H, m, $-OCH_2-$), 2.47 (1H, d, $J = 5.0$ Hz, $-OH$), 1.60 (2H, m), 1.25 (14H, brs, $-CH_2-$), 0.85 (9H, s, $C(CH_3)_3$), 0.83 (3H, t, $J = 6.9$ Hz, $-CH_3$), 0.07 (6H, s CH_3–Si); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 71.6 (d, C-2), 71.4 (t), 70.6 (t), 64.0 (t, C-1), 31.9 (t), 29.63 (t), 29.56 (t), 29.5 (t), 29.3 (t), 26.4 (t), 26.1 (t), 25.9 (q), 22.6 (t), 18.3 (s), 14.1 (q, CH_3), -5.4 (q, CH_3–Si); GC–MS (70 eV) *m/z* (relative intensity) 346 (M^+, 1), 290 (1), 271 (1), 289 (5), 231 (1), 207 (1), 175 (3), 173 (1), 159 (1), 151 (1), 150 (2), 149 (14), 135 (1), 134 (2), 133 (21), 132 (11), 131 (100), 129 (1), 119 (3), 118 (1), 117 (10), 116 (1), 115 (4), 107 (1), 106 (1), 105 (15), 104 (1), 103 (6), 102 (1), 101 (12), 99 (3), 97 (2), 91 (1), 90 (1), 89 (8), 88 (1), 86 (1), 85 (18), 84 (1), 83 (4), 81 (1), 77 (4), 76 (2), 75 (30), 74 (2), 73 (19), 72 (1), 71 (19), 70 (2), 69 (6), 68 (1), 67 (1), 61 (1), 60 (1), 59 (6), 58 (4), 57 (41), 56 (5), 55 (13), 54 (1), 53 (1); analysis calculated for $C_{19}H_{42}SiO_3$: C, 65.84; H, 12.21; found: C, 65.75; H, 12.46.

*2.5. tert-Butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (**6a**)*

To a solution of NaH (0.02 g, 0.83 mmol) in THF at 0 °C under argon was added 1-(*tert*-butyldimethylsilanyloxy)-3-decyloxypropan-2-ol (0.16 g, 0.46 mmol), the reaction was stirred for 5 min, then methyl iodide (0.04 ml, 0.65 mmol) was added dropwise. The reaction was stirred at room temperature for 2 h. The reaction mixture was extracted with ether (2×25 ml) and dried over Na_2SO_4, which after evaporation in vacuo afforded 0.12 g (70% yield) of *tert*-butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (**6a**) as a colorless oil after purification using silica gel column chromatography and eluting with hexane/ether (9:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.68 (2H, m), 3.53 (1H, dd, $J = 10.2$ Hz, $J = 4.0$ Hz), 3.46 (3H, s, $-OCH_3$), 3.45 (2H, m), 3.37 (2H, m, $-OCH_2-$), 1.55 (2H, m), 1.25 (14H, brs, $-CH_2-$), 0.85 (9H, s, $C(CH_3)_3$), 0.83 (3H, t, $J = 6.9$ Hz, $-CH_3$), 0.07 (6H, s CH_3–Si); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 81.0 (d, C-2), 71.7 (t), 70.1 (t), 62.4 (t, C-1), 58.1 (q, OCH_3), 31.9 (t), 29.62 (t), 29.60 (t), 29.56 (t), 29.3 (t), 26.1 (t), 25.9 (t), 25.9 (q), 22.7 (t), 18.3 (s), 14.1 (q), -5.4 (q, CH_3–Si), -5.5 (q, CH_3–Si); GC–MS (70 eV) *m/z* (relative intensity) 360 (M^+,1), 305 (1), 304 (3), 303 (13), 273 (1), 272 (3), 271 (12), 243 (1), 215 (1), 197 (1), 189 (1), 173 (1), 171 (1), 165 (1), 164 (2), 163 (13), 149 (1), 148 (3), 147 (26), 145 (5), 141 (1), 134 (1), 133 (7), 132 (11), 131 (100), 129 (4), 121 (1), 120 (3), 119 (32), 118 (2), 117 (15), 116 (2), 115 (5), 109 (1), 107 (1), 105 (5), 103 (1), 102 (1), 101 (9), 100 (1), 99 (7), 97 (3), 95 (2), 91 (7), 90 (5), 89 (65), 87 (1), 86 (2), 85 (35), 83 (7), 81 (2), 77 (2), 76 (1), 75 (19), 74 (4), 73 (31), 72 (3), 71 (38), 70 (2), 69 (9), 68 (1), 67 (2), 61 (2), 60 (1), 59 (12), 58 (10), 57 (77), 56 (6), 55 (19), 54 (1); analysis calculated for $C_{20}H_{44}SiO_3$–$C_6H_{14}Si$: C, 68.25; H, 12.27; found: C, 68.47; H, 12.60.

*2.6. 3-Decyloxy-2-methoxypropan-1-ol (**7a**)*

To a solution of *tert*-butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (0.11 g, 0.31 mmol) in THF (10 ml) at 0 °C under argon was added tetrabutylammonium fluoride (0.09 ml, 0.31 mmol). The reaction was stirred for 24 h and extracted with ether (2×25 ml), dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude product was purified using silica gel column chromatography and eluting with hexane/ether (9:1) followed by ether to obtain the 3-decyloxy-2-methoxypropan-1-ol (**7a**) (0.06 g, 74% yield) as a colorless oil. IR (neat) ν_{max} 3430,

2922, 2853, 1464, 1377, 1194, 1115 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.75 (1H, m), 3.65 (1H, m), 3.55 (2H, m, $-OCH_2-$), 3.45 (3H, s, $-OCH_3$), 3.43 (3H, m, $-OCH_2-$ and-OCH-), 2.20 (1H, s, –OH), 1.55 (2H, m), 1.25 (14H, brs, $-CH_2-$), 0.83 (3H, t, $J = 6.9$ Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 81.0 (d, C-2), 71.9 (t), 70.6 (t), 62.7 (t, C-1), 57.7 (q, $-OCH_3$), 31.9 (t), 29.56 (t), 29.54 (t), 29.4 (t), 29.3 (t), 26.0 (t), 22.7 (t), 14.1 (q); GC–MS (70 eV) *m/z* (relative intensity) 246 (M^+,1), 201 (1), 169 (1), 157 (2), 156 (1), 141 (1), 138 (1), 107 (1), 99 (2), 97 (2), 96 (1), 95 (1), 89 (1), 88 (1), 86 (1), 85 (10), 84 (1), 83 (4), 82 (1), 81 (1), 77 (1), 76 (1), 75 (34), 74 (9), 72 (1), 71 (12), 70 (2), 69 (5), 68 (1), 67 (1), 61 (1), 60 (1), 59 (32), 58 (100), 57 (26), 56 (4), 55 (13), 54 (1); analysis calculated for $C_{14}H_{30}O_3$: C, 68.25; H, 12.27; found: C, 68.15; H, 12.47.

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

To a solution of 3-decyloxy-2-methoxy-propan-1-ol (0.10 g, 0.42 mmol) in DMF (0.8 ml) was added a solution of pyridinium dichromate (0.80 g, 2.1 mmol) in DMF (1 ml). The reaction mixture was stirred for 24 h at room temperature under argon, extracted with ethylacetate (2 × 25 mL), dried over Na_2SO_4 and the solvent evaporated in vacuo affording 0.08 g (75% yield) of (±)-2-methoxy-4-oxatetradecanoic acid (**1a**) as a colorless oil after purification using silica gel column chromatography and eluting with hexane/ether (9:1); IR (neat) ν_{max} 3500–2500, 2922, 2853, 1740, 1598, 1456, 1375, 1199, 1114 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.95 (1H, dd, $J = 5.1$ Hz, $J = 2.9$ Hz, 2-H), 3.81 (1H, dd, $J = 10.5$ Hz, $J = 2.9$ Hz, 3-H), 3.73 (1H, dd, $J = 10.5$ Hz, $J = 5.1$ Hz, 3-H), 3.45 (3H, s, $-OCH_3$), 3.43 (2H, brt, $J = 8.1$ Hz, 5-H), 1.55 (2H, m), 1.25 (14H, brs, $-CH_2-$), 0.83 (3H, t, $J = 6.9$ Hz, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 173.7 (s, C-1), 80.0 (d, C-2), 72.1 (t), 70.4 (t), 58.7 (q, $-OCH_3$), 31.8 (t), 29.5 (t), 29.4 (t), 29.3 (t), 25.9 (t), 22.6 (t), 14.0 (q, $-CH_3$); GC–MS (70eV) *m/z* (relative intensity) 260 (M^+, 1), 230 (1), 172 (1), 171 (7), 141 (1), 139 (1), 121 (2), 112 (1), 111 (1), 104 (1), 102 (1), 99 (6), 98 (1), 97 (6), 96 (1), 95 (1), 93 (2), 92 (1), 91 (4), 90 (100), 86 (2), 85 (32), 84 (2), 83 (17), 82 (2), 81 (2), 79 (1), 76 (1), 75 (20), 74 (2), 73 (4), 72 (8), 71 (38), 70 (5), 69 (13), 68 (2), 67 (3), 61 (8), 60 (1), 59 (9), 58 (43), 57 (73), 56 (10), 55 (34), 54 (2), 53 (3); analysis calculated for $C_{14}H_{28}O_4$: C, 64.58; H, 10.84; found: C, 64.46; H, 11.04.

2.8. (*S*)-4-Decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (*3b*)

To a mixture of NaH (0.55 g, 23 mmol) in dry THF (15 ml) at 0 °C under argon was added dropwise 1,2-*O*-isopropylidene-(*S*)-glycerol (1.40 ml, 11 mmol). The resulting mixture was stirred at room temperature for 30 min, and then refluxed for 1 h. The solution was cooled to 0 °C and 1-bromodecane (2.35 ml, 11.3 mmol) was added dropwise. The mixture was refluxed for 24 h. Then, the reaction mixture was cooled to room temperature, washed with water (2 × 15 ml), extracted with ether (2 × 15 ml) and dried over Na_2SO_4, the organic solvent was evaporated, affording 1.9 g (63% yield) of (*S*)-4-decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (**3b**) as a colorless oil after purification using using silica gel column chromatography and eluting with hexane/ether (9:1) with spectral data identical to the one reported in Section 2.2.

2.9. (*R*)-3-Decyloxypropane-1,2-diol (*4b*)

To a 50-ml round-bottomed flask containing (*S*)-4-decyloxymethyl-2,2-dimethyl-[1,3]-dioxolane (1.2 g, 4.4 mmol) was added 20 ml of MeOH and excess of Amberlyst®15 (wet) ion-exchange resin. The reaction was stirred at room temperature for 24 h. Then, the reaction was filtered and extracted with ethyl acetate (2 × 25 ml). The organic extract was dried using Na_2SO_4 and the solvent evaporated in vacuo affording 0.72 g (70% yield) of (*R*)-3-decyloxypropane-1,2-diol (**4b**) ($[\alpha]_{28}^{D} = -4.0$; $c = 0.03$, $CHCl_3$) as a white solid (mp = 38–39 °C) after purification using silica gel column chromatography and eluting with hexane/ether (9:1) and with nuclear magnetic resonance spectral data identical to the one reported herein in Section 2.3.

2.10. (*S*)-1-(tert-Butyldimethylsilanyloxy)-3-decyloxy-propan-2-ol (*5b*)

To a solution of (*R*)-3-decyloxypropane-1,2-diol (0.44 g, 1.77 mmol), dibutyltin oxide (0.47 g, 1.77 mmol) and triethylamine (0.34 ml, 2.12 mmol) in dichloromethane was added dropwise, *tert*-butyldimethylsilyl chloride (0.29 g, 1.7 mmol) in

dichloromethane. The reaction mixture was stirred at room temperature for 90 min, and then extracted with ether (2 × 25 ml), dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude was purified using silica gel column chromatography and eluting with hexane/ether (9:1), affording 0.49 g (75% yield) of (*S*)-1-(*tert*-butyldimethylsilanyloxy)-3-decyloxy-propan-2-ol (**5b**) ($[\alpha]_{28}^{D} = -1.5$; $c = 0.014$, $CHCl_3$) as a colorless oil with spectral data identical to the one reported herein in Section 2.4.

2.11. (S)-tert-Butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (6b)

To a solution of NaH (0.019 g, 0.78 mmol) in THF at 0 °C under argon was added (*S*)-1-(*tert*-butyldimethylsilanyloxy)-3-decyloxy-propan-2-ol (0.18 g, 0.53 mmol), the reaction was stirred for 5 min, and then CH_3I (0.05 ml, 0.80 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, extracted with ether (2 × 25 ml), dried over Na_2SO_4 and the solvent evaporated in vacuo affording 0.11 g (60% yield) of (*S*)-*tert*-butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (**6b**) as a colorless oil, purified using silica gel column chromatography and eluting with hexane/ether (9:1), and with spectral data identical to the one reported herein in Section 2.5.

2.12. (R)-3-Decyloxy-2-methoxy-propan-1-ol (7b)

To a solution of (*S*)-*tert*-butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (0.11 g, 0.31 mmol) in THF (10 ml) at 0 °C under argon was added TBAF (0.1 ml, 0.34 mmol) and the reaction mixture was stirred for 24 h. The reaction mixture was extracted with ether (2 × 25 ml), dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude product was purified using silica gel column chromatography and eluting with hexane/ether (9:1) and ether, which afforded the (*R*)-3-decyloxy-2-methoxypropan-1-ol (0.057 g, 74% yield) as a colorless oil with spectral data identical to the one reported herein in Section 2.6.

2.13. (S)-2-Methoxy- 4-oxatetradecanoic acid (1b)

To a solution of (*R*)-3-decyloxy-2-methoxypropan-1-ol (0.18 g, 0.74 mmol) in DMF (1 ml) was added a solution of pyridinium dichromate (1.4 g, 3.7 mmol) in DMF (3 ml). The reaction mixture was stirred overnight at room temperature under argon. Then, it was extracted with ethylacetate (2 × 25 ml), dried over Na_2SO_4 and the solvent evaporated in vacuo affording 0.15 g (75% yield) of (*S*)-2-methoxy-4-oxatetradecanoic acid (**1b**) ($[\alpha]_{28}^{D} = -7.5$; $c = 0.015$, $CHCl_3$) as a colorless oil, purified using silica gel column chromatography and eluting with hexane/ether (9:1), with spectral data identical to the one reported herein in Section 2.7.

2.14. Microorganisms

C. albicans ATCC 14053, *C. albicans* ATCC 60193, *A. niger* ATCC 16404 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 make a transmittance of 73–75% at 530 nm. This transmittance provides a concentration of 10^6 cells/ml for *C. albicans* 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).

2.15. Chemicals and antifungal agents

Amphotericin B (AMB) was purchased from Acros, NJ, 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., NY, 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.

2.16. 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 (1993) and by the more recent NCCLS M27-A microdilution methods as described previously Carballeira et al. (2004) and 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 six-step synthesis for the (±)-2-methoxy-4-oxatetradecanoic acid (**1a**) was accomplished as outlined in Scheme 1. This synthesis started with commercially available 1,2-*O*-isopropylidene-*rac*-glycerol that was coupled with 1-bromodecane under basic conditions using NaH in dry THF affording the decyloxymethyldioxolane **3a** in a 65% yield. The acetal in dioxolane **3a** was removed by treatment with acidic Amberlyst®15 (wet) ion-exchange resin in methanol for 24 h, which afforded 3-decyloxypropane-1,2-diol (**4a**) in a 74% yield. Selective monosilylation of diol **4a** was achieved by forming the corresponding dibutylstannylene intermediate (David and Hanessian, 1985) with dibutyltin oxide in triethylamine, followed by quenching of the intermediate with *tert*-butyldimethylsilyl chloride in dichloromethane, which afforded selectively in a 75% yield the 1-(*tert*-butyldimethylsilanyloxy)-3-decyloxypropan-2-ol (**5a**). Methylation of **5a** was accomplished by the reaction with methyl iodide and sodium hydride as base in tetrahydrofuran, which readily afforded *tert*-butyl-(3-decyloxy-2-methoxypropoxy)dimethylsilane (**6a**) in a 70% yield. Deprotection of **6a** was accomplished using the standard procedure of tetrabutylammonium fluoride in tetrahydrofuran at 0 °C under argon that resulted in the formation of 3-decyloxy-2-methoxypropan-1-ol (**7a**) in a 74% yield. The final step in the synthesis was the oxidation of the alcohol in **7a** with pyridinium dichromate in DMF, which afforded the target molecule (±)-2-methoxy-4-oxatetradecanoic acid (**1a**) in a 75% yield. The overall yield for this six-step synthesis was 14%. The (*S*)-2-methoxy-4-oxatetradecanoic acid (**1b**) was also synthesized (11% overall yield) as described above, but using 1,2-*O*-isopropylidene-(*S*)-glycerol as the starting material (Scheme 1). Nuclear magnetic resonance spectroscopy, infrared spectroscopy and mass spectrometry confirmed the structures (±)-**1a** and (*S*)-**1b**.

The antifungal activities of acids (±)-**1a** and (*S*)-**1b** against *C. albicans* (SDB), *C. neoformans* (SDB) and *A. niger* (SDB), together with appropriate standards, are shown in Table 1. Acids (±)-**1a** and (*S*)-**1b** did not show any appreciable fungitoxicity against the two fluconazole resistant *C. albicans* ATCC 14053 and ATCC 60193. However, the compounds exhibited fungitoxicity against *C. neoformans* ATCC 66031 and *A. niger* ATCC 16404 with MIC values between 0.08 and 0.22 mM. These antifungal activities were comparable with MIC values previously reported by us for the (±)-2-methoxytetradecanoic acid (2-OMe-14:0) against the same strains of fungi (Carballeira et al., 2004). Particularly interesting is the observation

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 14053	*C. albicans* ATCC 60193	*C. neoformans* ATCC 66031	*A. niger* ATCC 16404
14:0	3.80 ± 0.01	–	1.90 ± 0.03	3.80 ± 0.04
2-OMe-14:0	0.11 ± 0.02	–	0.11 ± 0.004	0.11 ± 0.003
(±)-**1a**	2.60 ± 0.04	3.50 ± 0.04	0.08 ± 0.001	0.22 ± 0.005
(*S*)-**1b**	4.30 ± 0.03	4.80 ± 0.05	0.18 ± 0.006	0.18 ± 0.004
FLC	4.00 ± 0.05	>4.0	<0.0019	1.00 ± 0.01
AMB	<0.0002	<0.0002	<0.0002	<0.0002
DMSO	>5	>5	>5	>5

$P < 0.05$.
[a] The result is the average of three separate experiments.

that acid (±)-**1a** presented good fungitoxicity against *C. neoformans*, while neither (±)-**1a** nor (*S*)-**1b** showed fungitoxicity against *C. albicans* ATCC 14053, for which the 2-OMe-14:0 displayed a MIC of 0.11 mM. Therefore, the added 4-oxa functionality decreased the effect of the parent 2-OMe-14:0 against *C. albicans* ATCC 14053. This observation is similar to what was previously reported for the 4-oxatetradecanoic acid, where it was reported to be fungicidal to *C. neoformans*, but it had much less of an effect on the viability of *C. albicans* (Langer et al., 1992).

The mechanism of fungitoxicity of either (±)-**1a** or (*S*)-**1b** remains unknown. Molecular modeling studies indicate that these tetradecanoic acid analogues, (±)-**1a** or (*S*)-**1b,** fit into the active sites of *N*-myristoyltransferases (NMTs) and are substrates for the enzymes. In addition, the modeling studies show that (*S*)-**1b** is a better substrate for NMT than its *R* enantiomer. However, the antifungal data obtained for either (±)-**1a** or (*S*)-**1b** did not show any correlation between antifungal activity and chirality for the oxygenated 2-methoxylated acids. Therefore, this observation seems to point into the direction of the presence of an alternative mechanism for antifungal activities of these compounds. One possible mechanism could be that these acids are incorporated into the membranes of the fungi resulting in membrane disruption and eventual cytoplasmic disintegration of mycelia. In any instance, more studies are needed to fully understand the antifungal mechanism of this novel series of oxygenated fatty acids.

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