Synthesis and Antifungal Activities of Myristic Acid Analogs

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Summary

Myristic acid analogs that are putative inhibitors of *N*-myristoyltransferase were tested *in vitro* for activity against yeasts (*Saccharomyces cerevisiae, Candida albicans, Cryptococcus neoformans*) and filamentous fungi (*Aspergillus niger*). Several (±)-2-halotetradecanoic acids including (±)-2-bromotetradecanoic acid (**14c**) exhibited potent activity against *C. albicans* (MIC = 39 μM), *C. neoformans* (MIC = 20 μM), *S. cerevisiae* (MIC = 10 μM), and *A. niger* (MIC < 42 μM) in RPMI 1640 media. Improved synthetic methods have been developed for the synthesis of 12-fluorododecanoic acid (**12a**) and 12-chlorododecanoic acid (**12c**). Three novel fatty acids, 12-chloro-4-oxadodecanoic acid (**8a**), 12-phenoxydodecanoic acid (**12i**), and 11-(4-iodophenoxy)-undecanoic acid (**13d**) were also synthesized and tested.

Introduction

Myristoyl CoA:protein *N*-myristoyltransferase (NMT) is an attractive target for antiviral and antifungal therapy. Protein *N*-myristoylation refers to the co-translational linkage of myristic acid (C14:0), via an amide bond, to the NH_2-terminal glycine (Gly) residues of a variety of fungal and viral proteins. This reaction is catalyzed by NMT (E.C. 2.3.1.97). These fungal myristoylated proteins include ADP ribosylation factor (Arf1p. Arf2p) in *Candida albicans, Saccharomyces cerevisiae,* and *Cryptococcus neoformans*[1]. Inhibitors of protein synthesis, such as myristic acid analogs, block protein *N*-myristoylation[2]. The cellular incorporation of myristyl analogs that have physicochemical properties different from those of myristic acid (**1**) could perturb fungal protein function. The observation that fungal growth can be inhibited by perturbation of the myristoylation process suggests that the enzyme responsible for this protein modification could provide a suitable target for therapeutic agents[1]. *Aspergillus niger* contains saturated fatty acids such as myristic acid (**1**)[3], and it would therefore be expected to be sensitive to the incorporation of myristic acid analogs. A variety of myristic acid analogs that possess different physicochemical properties, containing halogen, azide, oxygen, sulfur, and aromatic substituents have been reported. Accordingly, 10-phenyldecanoic acid (**2**), 12-[2-(1-methylimidazolyl)]dodecanoic acid (**3**), and 12-[1-(1,2,3-triazolyl)]dodecanoic acid (**4**), have been designed to serve as alternative substrates for NMT (Figure 1)[2–5]. The hetereocyclic derivatives **3**, **4** were good substrates for NMT, but were quite toxic at low concentrations < 4–100 μM[5].

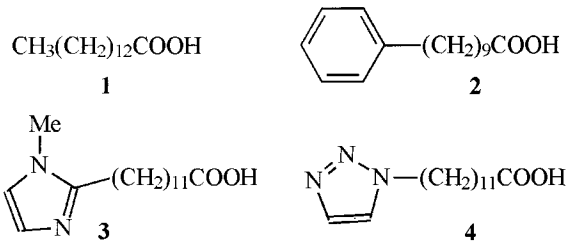

Figure 1. Structures of some fatty acid analogs having an aromatic or heteroaromatic ring.

In vitro antifungal structure-activity relationships for a variety of myristic acid analogs including some novel compounds are described. This broad family of compounds permitted us to examine the effect of physicochemical properties, such as partition coefficient and acidity upon antifungal activity.

Chemistry

The compounds that were investigated can be categorized into five groups:

a) 4-Oxa and 9-oxa analogs:	$R(CH_2)_mO(CH_2)_nCOOH$ R = Cl, n = 2, m = 8 (**8a**), R = Me, n = 2, m = 9 (**8b**), R = Me, n = 7, m = 4 (**8c**)
b) 12-Substituted dodecanoic acids:	$R(CH_2)_{11}COOH$ R = F (**12a**), I (**12b**), Cl (**12c**), Br (**12d**), MeO (**12e**), EtS (**12f**), Ph (**12g**), N_3 (**12h**), PhO (**12i**).
c) 11-Substituted undecanoic acids:	$R(CH_2)_{10}COOH$ R = MeO (**13a**), EtS (**13b**), Br (**13c**), 4-I-PhO (**13d**), PhO (**13e**).
d) 2-Substituted tetradecanoic acids:	$CH_3(CH_2)_{11}CH(R)COOH$ R = F (**14a**), OH (**14b**), Br (**14c**), I (**14d**), Cl (**14e**).
e) 2-Bromotetradecanoate esters:	$CH_3(CH_2)_{11}CH(Br)COOR$ R = Me (**15a**), Et (**15b**), *i*-Pr (**15c**).

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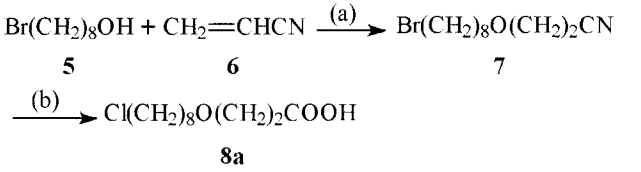
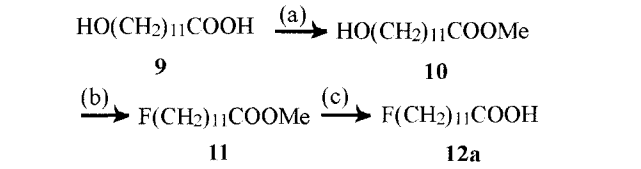
Improved synthetic methods have been developed for the synthesis of 12-fluorododecanoic acid (**12a**) and 12-chlorododecanoic acid (**12c**). 12-Chloro-4-oxadodecanoic acid (**8a**), 12-phenoxydodecanoic acid (**12i**), and 11-(4-iodophenoxy)undecanoic acid (**13d**) are new fatty acids (see Table 1).

12-Chloro-4-oxadodecanoic acid (**8a**) was prepared by cyanoethylation of 8-bromooctanol (**5**) followed by hydrolysis of the cyano group to the carboxyl group as illustrated in Scheme 1.



Scheme 1. Reagents and conditions: (a) NaH, 55–60 °C 3 h; (b) HCl, CH_3COOH, 25 °C, 2 h.

12-Fluorododecanoic acid (**12a**) was prepared previously using a tedious method in six steps from ethyl 11-bromoundecanoate[6]. In the present study, 12-fluorododecanoic acid (**12a**) was prepared using a three step reaction that involved the elaboration of 12-hydroxydodecanoic acid (**9**) to the methyl ester derivative **10** by reaction with trimethylsilyl chloride in methanol[7]. Subsequent reaction with diethylaminosulfur trifluoride (DAST), to replace the hydroxyl group by a fluorine substituent[8], and then acid hydrolysis of the methyl ester **11** yielded 12-fluorododecanoic acid (**12a,** 65%) (Scheme 2).

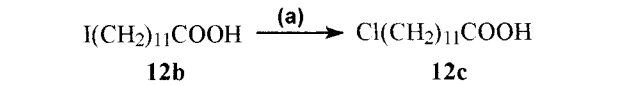


Scheme 2. Reagents and conditions: (a) $(CH_3)_3SiCl$, CH_3OH, 25 °C, 16 h; (b) DAST, benzene, 25 °C, 2 h; (c) HCl, dioxane, reflux, 2 h.

The method of Finkelstein was used to synthesize 12-iodododecanoic acid (**12b**) and (±)-2-iodotetradecanoic acid (**14d**) by reaction of the corresponding bromo analog (**12d, 14c**) with sodium iodide in acetone[9].

The selective introduction of a chlorine substituent at the terminal methyl position of fatty acids is a challenging synthetic reaction. It was found that addition of concentrated nitric acid to a mixture of an alkyl iodide and hydrochloric acid resulted in an exothermic reaction that was accompanied by expulsion of elemental iodine to yield the corresponding the alkyl chloride as the major product[10]. This methodology was used for the successful preparation of 12-chlorododecanoic acid (**12c**) (Scheme 3) and (±)-2-chlorotetradecanoic acid (**14e**).

11-Phenoxyundecanoic acid (**13e**) was prepared by reaction of phenol with 11-bromoundecanoic acid (**13c**) in aqueous sodium hydroxide[11]. In this study, a similar method was



Scheme 3. Reagents and conditions: (a) HNO_3, HCl, 15–20 °C, 2 h.

used for the synthesis of 11-phenoxyundecanoic acid (**13e,** 68%), and for the previously unknown 12-phenoxydodecanoic acid (**12i,** 96%) and 11-(4-iodophenoxy)undecanoic acid (**13d,** 43%) in ethanol-potassium hydroxide. 12-Phenyldodecanoic acid (**12g**, 49%) was prepared using the four step reaction sequence reported by Goodman *et al.*[12]. An alternate method reported by Eisenhut *et al.*[13] was employed for the preparation of 10-phenyldecanoic acid (**2**, 49%) and 12-phenyldodecanoic acid (**12g**, 23%).

(±)-Methyl 2-bromotetradecanoate (**15a,** 92%), (±)-ethyl 2-bromotetradecanoate (**15b,** 72%), and (±)-isopropyl 2-bromotetradecanoate (**15c,** 92%) were prepared by esterification of (±)-2-bromotetradecanoic acid (**14c**) with trimethylsilyl chloride in either MeOH, EtOH, or i-PrOH, respectively.[9]

The calculated pK_a values for most myristic acid analogs investigated was 4.91, except for the 2-substituted and the 4-oxa-analogs. For example, (±)-2-bromotetradecanoic acid (**14c**) had a calculated pK_a of 3.06, whereas 4-oxatetradecanoic acid (**8b**) had a calculated pK_a value of 4.37. The calculated partition (log P = 3.3–7.3) and distribution (log $D_{7.0}$ = 0.7–4.8) coefficients for the fatty acids investigated (see Table 1) extended over a broad range.

Results and Discussion

A group of myristic acid analogs were investigated to develop antifungal structure-activity relationships, and to determine the effect of physicochemical properties such as partition coefficient and acidity upon antifungal activity. A large difference in anti-fungal activities was observed for the myristoyl analogs investigated.

The *in vitro* antifungal test results for the fatty acids (**1**, **2**, **8a–c**, **12–14**) and myristate esters **15a–c** are presented in Table 1. The 4-oxa (**8b**) and 9-oxa (**8c**) derivatives were active against *C. neoformans* (MIC = 0.04–0.17 mM) and exhibited moderate activity against *S. cerevisiae* (0.34 mM) and *A. niger* (0.34–0.68 mM). In contrast, 12-chloro-4-oxatetradecanoic acid (**8a**) was an inactive antifungal agent. Studies by Kishore *et al.*[14,15] suggest that a key feature for the NMT binding site is the *omega*-terminus of the fatty acid which interacts with a conical sensing device that detects both length and steric size. Interatomic distances between the carbonyl (CO) moiety and the *omega*-atom measured after AM1 calculations showed interatomic distances of 14.99 Å for **8a**, relative to 16.01 and 16.03 Å for **8b** and **8c**, respectively. The decreased antifungal activity exhibited by **8a**, relative to **8b** and **8c**, may be due to the shorter interatomic distance in **8a** and the larger size of the of the *omega*-chlorine atom relative to the *omega*-methyl present in **8b** and **8c**.

12-Substituted analogs **12a–h** were active against *C. neoformans* (0.04–0.18 mM), but they exhibited weak activity against *C. Albicans* (1.9 to 5 mM). Compounds **12b** and **12d** were active against *A. niger* at a concentration < 0.04 mM.

Phenyl-substituted derivatives generally exhibited weak antifungal activity. For example, 10-phenyldecanoic acid (**2**) was equally active against *C. neoformans* and *S. cerevisiae* (MIC = 0.16 mM), but it was only weakly active against *A. niger* and *C. albicans*. 12-Phenyldodecanoic acid (**12g**) was found to be a potent antifungal agent only against *C. neoformans* (MIC = 0.04 mM).

Table 1. Structures, physicochemical properties and antifungal activity (MIC values, mM)[a] against *Candida albicans*, *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, and *Aspergillus niger* for fatty acids and esters.

No.	Structure	log P[b]	log $D_{7.0}$[c]	pK_a[d]	*C. albicans* ATCC 14053	*C. neoformans* KF-33	*S. cerevisiae* PLM 454	*A. niger* PLM 1140
1	$Me(CH_2)_{12}COOH$	5.97	3.9	4.91	>5	>5	>5	2.7
2	$Ph(CH_2)_9COOH$	5.56	3.5	4.91	2.5	0.16	0.16	1.3
8a	$Cl(CH_2)_8O(CH_2)_2COOH$	3.29	0.7	4.37	>5	>5	5.3	0.8
8b	$Me(CH_2)_9O(CH_2)_2COOH$	4.45	1.8	4.37	1.4	0.17	0.34	0.34
8c	$Me(CH_2)_4O(CH_2)_7COOH$	3.88	1.8	4.91	0.23	0.04	0.34	0.68
12a	$F(CH_2)_{11}COOH$	4.29	2.2	4.91	2.9	0.18	0.72	1.4
12b	$I(CH_2)_{11}COOH$	5.34	3.3	4.91	1.9	0.12	1.2	< 0.03
12c	$Cl(CH_2)_{11}COOH$	4.82	2.7	4.91	2.7	0.09	0.17	0.17
12d	$Br(CH_2)_{11}COOH$	5.03	2.9	4.91	2.2	0.14	0.18	<0.04
12e	$MeO(CH_2)_{11}COOH$	3.88	1.8	4.91	2.7	0.09	0.3	0.09
12f	$EtS(CH_2)_{11}COOH$	5.47	3.4	4.91	>5	0.08	3.1	2.4
12g	$Ph(CH_2)_{11}COOH$	6.59	4.5	4.91	>5	0.04	1.1	2.3
12h	$N_3(CH_2)_{11}COOH$	4.88	2.8	4.91	1.3	0.16	0.3	0.3
12i	$PhO(CH_2)_{11}COOH$	6.14	4.1	4.91	>5	>5	>5	5.3
13a	$MeO(CH_2)_{10}COOH$	3.36	1.3	4.91	>5	0.36	0.72	1.4
13b	$EtS(CH_2)_{10}COOH$	4.95	2.9	4.91	5.1	0.08	0.63	1.3
13c	$Br(CH_2)_{10}COOH$	4.51	2.4	4.91	1.2	0.08	0.08	0.3
13d	4-I-$PhO(CH_2)_{10}COOH$	6.89	4.8	4.91	>5	>5	1.5	3.1
13e	$PhO(CH_2)_{10}COOH$	5.62	3.6	4.91	>5	0.14	1.1	2.2
14a	$Me(CH_2)_{11}CH(F)COOH$	6.20	2.4	2.83	>5	5.1	1.3	5.1
14b	$Me(CH_2)_{11}CH(OH)COOH$	5.17	2.1	4.01	>5	5.1	>5	2.6
14c	$Me(CH_2)_{11}CH(Br)COOH$	6.93	3.0	3.06	0.04	0.02	0.01	<0.04
14d	$Me(CH_2)_{11}CH(I)COOH$	7.25	3.4	3.30	0.1	0.03	0.01	0.1
14e	$Me(CH_2)_{11}CH(Cl)COOH$	6.72	2.8	2.99	0.59	0.04	0.04	0.59
15a	$Me(CH_2)_{11}CH(Br)COOMe$	7.29	7.2	–	>5	1.9	>5	0.49
15b	$Me(CH_2)_{11}CH(Br)COOEt$	7.81	7.7	–	>5	3.7	>5	0.93
15c	$Me(CH_2)_{11}CH(Br)COOPr\text{-}i$	8.33	8.2	–	>5	>5	>5	>5
Amphotericin B					0.0002	0.0004	0.0009	0.003
Acetone					>5	>5	>5	>5
Methanol					>5	>5	>5	>5
Fluconazole					0.007	ND[e]	0.03	ND
DMSO					12.5% *v/v*	6.3% *v/v*	6.3% *v/v*	6.3% *v/v*

[a] The result is the average of three separate experiments. [b] Partition coefficient of the fatty acid calculated using the PrologP 5.1 program. [c] Distribution coefficient of the fatty acid at pH 7.0 calculated using the PrologD prediction program. [d] Calculated dissociation constant. [e] ND = Not determined.

Table 2. *In vitro* cytotoxicity of some fatty acids in the KB cell line.

Compound	TD_{50} (mM)[a]
2	0.18
12d	0.17
12e	0.19
13b	0.19
14c	0.15
Adriamycin[b]	0.00002

[a] The concentration of the test compound that was cytotoxic to 50% of the cells. The value is the average of three separate experiments.
[b] Adriamycin as the reference drug.

The antifungal activity of the 2-substituted tetradecanoic acids **14a–e** against *C. neoformans*, *A. niger*, *C. albicans*, and *S. cerevisiae* were also determined. Compounds **14c–e** were active against all four fungi. The 2-bromo analog **14c** was the most potent antifungal fatty acid tested with antifungal MICs of 0.02 mM, 0.01 mM, 0.04 mM, and < 0.04 mM, respectively, against *C. neoformans*, *S. cerevisiae*, *C. albicans*, and *A. Niger*.

The KB cell line was used for *in vitro* cytotoxicity assessment. Toxicity (TD_{50} in mM) was determined for selected compounds (Table 2).

Log P values were calculated using the PrologP 5.1 program[16]. To confirm the validity of the log P calculation, the experimental data were compared with acquired data for some fatty acids. For example, the experimental value of 6.1[17] reported for myristic acid (**1**) is in good agreement with

the calculated log P value of 5.97. The distribution coefficient (D) considers the partitioning of all ionic species of a compound (Table 1). In order to calculate the log $D_{7.0}$ value for a test compound, both the logarithms of the partition coefficient (log P) and the dissociation constant(s) (pK_a) are needed. To perform these calculations, the test compound structure is drawn graphically, after which the computational program PrologD automatically calculates these parameters by activating the PrologP and PrologD modules of PALLAS[16].

The pKalc 3.1 program module[16] was used to calculate pK_a values, and the validity of these data was established by comparing the experimental values of a few fatty acids with their calculated pK_a value. For example, the experimental pK_a value of 5.0 for myristic acid (**1**)[18] is comparable with the calculated pK_a value of 4.9. As would be anticipated, the calculated pK_a value for 12-chloro-4-oxadodecanoic acid (**8a**) and 4-oxatetradecanoic acid (**8b**) was lower (4.37) than that of myristic acid (**1**) (4.91) due to the electronegative effect of the 4-oxa moiety.

Since a large difference in the pK_a values of 2-substituted fatty acids is often observed, a more precise calculation was required for these analogs (**14a–e**). The pK_a value of the 2-substituted myristic acid analogs (**14a–e**) listed in Table 1 was calculated by subtracting the substituent pK_a value (ΔpK_a) for a substituent attached to the α-carbon of an aliphatic acid[19] from the pK_a value for myristic acid (**1**, pK_a = 4.91). The validity of this method was confirmed by comparing experimental and calculated pK_a values for 2-halo- and 2-hydroxy- derivatives of acetic acid[20] where the magnitude of the pK_a value correlates with the electronegativity of C-2 substituent of the acid.

The most active antifungal agent in this group of compounds was (±)-2-bromotetradecanoic acid (**14c**), which exhibited potent activity against *C. albicans*, *C. neoformans*, *S. cerevisiae* and *A. niger* (Table 1) in RPMI 1640 growth medium. It has been reported that (±)-2-bromotetradecanoic acid (**14c**) does not exhibit antifungal activity against *A. niger* at pH 4.0 and pH 5.6 in Sabouraud dextrose agar after 5 days at 28 °C[21]. In this study the culture medium RPMI 1640 (pH = 7) was used to test **14c** against *A. niger* (MIC < 0.04 mM). At pH = 7, the concentration of the ionized form is 8700-fold more than the concentration of unionized form of this carboxylic acid, whereas this is only 347-fold larger at pH = 5.6. Therefore the difference in antifungal activity between reported and current studies may be due to differences in pH of the culture media used in these experiments. Other 2-substituted analogs such as (±)-2-fluorotetradecanoic acid (**14a**) and (±)-2-hydroxydodecanoic acid (**14b**) exhibited very weak antifungal activity. These results would be expected if the mechanism of action involved fungal peptide alkylation at C-2 of the myristoyl analog, since bromine is a much better leaving group than fluorine or hydroxyl substituents. This alkylation mechanism could be confirmed in part by testing (±)-2-iodotetradecanoic acid (**14d**) and (±)-2-chlorotetradecanoic acid (**14e**). Since iodine is a better leaving group than bromine, and chlorine is a less effective leaving group than bromine, one would predict that (±)-2-iodotetradecanoic acid (**14d**) would be a more potent antifungal agent than the 2-bromo analog **14c**, whereas (±)-2-chlorotetradecanoic acid (**14e**) should be a less active antifungal agent than the 2-bromo analog **14c**. The antifungal activities of the 2-iodo- (**14d**) and 2-chloro- (**14e**) substituted analogs were not significantly different than the (±)-2-bromotetradecanoic acid (**14c**) which suggests that factors other than displacement of the C-2 halogen substitutent are determinants of antifungal activity. The close proximity of the C-2 halogen substituent or the 4-oxa moiety to the carboxylic acid group increases the ability of the COOH to undergo dissociation (due to the electronegative effect of halogen or oxygen). The pK_a of the carboxylic acid moiety could be an important factor in myristoylation of fungal peptides, since the weakly active antifungal 2-substituted fatty acids **14a** (pK_a = 2.83) and **14b** (pK_a = 4.01) have a lower or higher calculated pKa than the more active antifungal analogs **14c-e** (pK_a range = 2.99–3.30) (Table 1). Binding of the fatty acid to NMT is one of the first steps in myristoylation of a peptide that requires an active carboxylic acid moiety. Three alkyl esters of (±)-2-bromotetradecanoic acid (**14c**) were synthesized to determine the role of the carboxylic acid moiety with respect to antifungal activity. The differences in lipophilicity and the size of the alkyl ester substituent present in (±)-methyl 2-bromotetradecanoate (**15a**), (±)-ethyl 2-bromotetradecanoate (**15b**) and (±)-isopropyl 2-bromotetradecanoate (**15c**) could provide a correlation of antifungal activity with physicochemical properties. All three esters exhibited low antifungal activity against *C. neoformans*, *A. niger*, *C. albicans* and *S. cerevisiae*, which suggests that the carboxylic acid substituent is required for antifungal activity and that inhibition of myristoylation is the major mechanism involved. The observation that myristic acid (**1**) was devoid of antifungal activity is in agreement with the fact that myristic acid analogs inhibit NMT myristoylation to elicit their antifungal activity by competing with myristic acid for a binding site(s) on NMT. It was of interest to ascertain whether the activity exhibited by myristoyl analogs in the coupled *in vitro* enzyme assay such as *S. cerevisiae* NMT[14] could accurately forecast antifungal activity. Introduction of an heteroatom such as oxygen or sulfur into the myristoyl moiety results in loss of a site for hydrophobic interaction and creates a potential hydrogen bond acceptor.[14] A comprehensive description of the *in vitro* NMT assay, provided in earlier publications, showed that at 0.5 mM (±)-2-bromotetradecanoic acid (**14c**) reduced NMT activity by 86%. However, (±)-2-fluorotetradecanoic acid (**14a**), (±)-2-hydroxytetradecanoic acid (**14b**), and myristic acid (**1**) provided only 27%, 53%, and 27% inhibition, respectively[2]. This is in agreement with our data that (±)-2-bromotetradecanoic acid (**14c**) is a more potent antifungal agent than (±)-2-fluorotetradecanoic acid (**14a**) and (±)-2-hydroxytetradecanoic acid (**14b**).

(±)-2-Bromotetradecanoic acid (**14c**), (±)-2-iodotetradecanoic acid (**14d**), and (±)-2-chlorotetradecanoic acid (**14e**) have calculated log P values between 6.7–7.3 (log $D_{7.0}$ between 2.8 and 3.4) (Table 1). There appears to be a relationship between antifungal activity against all fungi tested and the calculated log P for these 2-halo myristic acid analogs where the more lipophilic 2-Br and 2-I analogs are more potent than the less lipophilic 2-F and 2-OH derivatives. These data suggest that there is an optimal partition coefficient range for maximum antifungal activity. Based on previous *in vitro* NMT assay data, the observation that the

activities of NMT acyl-CoA substrates diminish with increasing polarity[5], is in agreement with our data for 2-substituted fatty acids.

Some oxa-substituted analogs such as 11-methoxyundecanoic acid (**13a**) and the thio-substituted analogs (**12f, 13b**) were only weak antifungals. This is in agreement with *in vitro* NMT assay data which showed that they are poor NMT substrates[14]. Interatomic distances between the carbonyl and *omega*-moieties, measured after AM1 calculations, were 14.83, 18.16, and 17.77 Å for **13a**, **12f**, and **13b**, respectively relative to myristic acid (16.63 Å).

4-Oxatetradecanoic acid (**8b**), which is incorporated into *C. neoformans* Arf, is reported to be fungicidal at 0.3 mM. Langer *et al.* reported that 4-oxatetradecanoic acid (**8b**) produced an approximately 10–100-fold reduction in the viability of *C. albicans* and *S. cerevisiae* at a 0.3-0.5 mM concentration[1]. The oxa- (**8b, 8c, 12e, 13a**) and thia- (**12f, 13b**) myristic acid analogs generally exhibited medium antifungal activity. The 4-oxa analog (**8b**) exhibited potent activity against *C. neoformans* (MIC = 0.17 mM) that is comparable to reported data. Myristic acid analogs with an oxygen atom at position 9 in the fatty acid backbone are good NMT substrates. Detailed kinetic studies have shown that when 9-oxatetradecanoic acid (**8c**) is added to the coupled *in vitro* assay system, the calculated K_m of the peptide increases only about 2-fold compared to myristic acid (**1**), but the rate of acylpeptide formation is slightly higher, producing a peptide catalytic efficiency 70% of that for myristic acid (**1**)[22]. The *in vitro* anti-fungal data acquired indicate that 9-oxatetradecanoic acid (**8c**) is an relatively active agent only against *C. neoformans* (MIC = 0.04 mM) (Table 1).

An additional study was carried out to assess whether antifungal activity correlates with the presence and position of an aromatic ring or a heteroatom. It has been suggested that the conversion of 10-phenyldecanoic acid (**2**) to its acyl-CoA derivative produces a 10-fold increase in peptide catalytic efficiency relative to the nonanoic or undecanoic acid derivatives. The K_m for 10-phenyldecanoyl-CoA (1.2 μM) is comparable to that of myristoyl-CoA (3.8 μM)[14]. The antifungal activity exhibited by myristic acid analogs is not affected significantly by oxygen substitution in the chain or by placement of an iodine substituent at the *para*-position of a phenyl ring (**2, 12g, 12i, 13e, 13d**). Kishore *et al.*[14] proposed that compounds having a terminal phenyl moiety should not fit into the postulated conical receptor, and therefore should exhibit weaker activity than the analogs having terminal methyl groups. Our *in vitro* antifungal data for **12g, 12i, 13d, 13e** confirm this postulation, since these compounds generally exhibited weaker antifungal activity than **2** (Table 1). This may be due to the enzymes inability to accommodate longer fatty acids at the conical binding site. Accordingly, interatomic distances (AM1 calculations) between the carbonyl and *omega*-moieties showed that **12g** (17.59 Å), **13e** (17.47 Å), **12i** (18.80 Å) and **13d** (19.42 Å) are longer than the corresponding interatomic distance for **2** (15.11 Å).

Other halogenated analogs such as 11-bromoundecanoic acid (**13c**), 12-iodododecanoic acid (**12b**), 12-bromododecanoic acid (**12d**), and 12-chlorododecanoic acid (**12c**) were active against *C. neoformans* and *S. cerevisiae*. 12-Fluorododecanoic acid (**12a**) was a much less active antifungal agent compared to the iodo (**12b**), chloro (**12c**), and bromo (**12d**), analogs against *A. niger* (Table 1). A plausible explanation for the differences in their relative potency may be due to their affinity for the binding site of NMT. It has been suggested that specific amino acid residues present in NMT bind to specific parts of the fatty acid backbone and that the fatty acyl CoA may be rigidly bound by NMT. It is thought that the acyl-CoA binding site possesses a complex sensor that measures the distance between the carboxyl and the omega end of the fatty acid, and the steric volume at the omega terminus[14].

It has been reported that myristic acid analogs readily traverse the cell membrane and act as substrates for NMT. For some cellular *N*-myristoylproteins, incorporation of these myristic acid analogs leads to analog-specific and -dependent redistribution from membrane to cytosolic fractions. This dual level of selectivity (selective incorporation and selective perturbation of function) probably accounts for their lack of cellular toxicity[5]. *In vitro* cytotoxicity data acquired using a KB cell line are in agreement with the reported data since none of the myristic acid analogs tested (**2, 12d, 12e, 13b, 14c**) exhibited cytotoxicity (TD_{50} = 0.15-0.19 mM). In the case of (±)-2-bromotetradecanoic acid (**14c**), the TD_{50} concentration in the KB cell line is 4–12 fold higher (Table 2) than its antifungal MIC values in RPMI 1640 growth medium (Table 1).

NMT displays a high degree of specificity for some fatty acyl-CoA's *in vitro*. There was a poor correlation between activity in the coupled *in vitro* NMT assay system and the antifungal effect for some of the analogs examined. The lack of such a correlation emphasizes the need to compare the substrate specificities of the NMT employed with the corresponding fungal enzyme. This information, together with an assessment of the mechanism of analog uptake into the fungi and the pathways of their subsequent metabolic processing, should allow one to develop systems that will more accurately predict the ability of different classes of myristic acid analogs to inhibit fungi replication[5].

In conclusion, an optimal pK_a value between 2.9–3.3, and a log *P* value between 6.7–7.3, are required for potent broad spectrum antifungal activity against *C. albicans, C. neoformans, S. cerevisiae*, and *A. niger*. The steric volume of substituents is likely another important factor that influences the binding affinity of the myristic acid analog to the NMT binding site. The data acquired can be used to determine differences between fungi with respect to the NMT-fatty acyl-CoA interaction. Each type of NMT must have specific binding sites for interaction with inhibitors, since some fatty acids such as **13a**, **13b**, and **13e** lack antifungal activity against *C. albicans*, but were active against *C. neoformans*. The activities of myristic acid analogs in which one or more methylene group(s) was replaced by oxygen, sulfur, and/or an aromatic ring indicate that fungal NMT exhibits a surprising selectivity related to the nature and position of the heteroatom and the position of the aromatic groups. These observations suggest that modulation of NMT activity may represent a potentially useful strategy for treating fungal infections. The utility of myristic acid analogs as therapeutic agents will be enhanced by a clearer understanding of their uptake, metabolism and incorporation.

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Experimental

Melting points were determined with a Büchi capillary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) were determined using Me_4Si as internal standard (^{1}H NMR) on a Bruker AM-300 spectrometer. Chemical shifts are given in ppm downfield from tetramethylsilane as an internal standard (^{1}H NMR). The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks. High resolution mass spectra were determined on an AEI MS 50 spectrometer equipped with a Mass Spectrometry Services MASPEC data system. Silica gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica gel. Microanalyses were performed by the Chemistry department, University of Alberta using an EA1108-Elemental Analyzer, Carlo Erba Instruments and were within ± 0.4% of theoretical values for all elements listed, unless otherwise indicated. Physicochemical properties were estimated using the PALLAS computational program. The *pK*alc 3.1 module estimates acidity/basicity and calculates pK_a values, even for substances that have low water solubility or are unstable in water[16].The PrologP module was used to calculate the log *P* values for myristic acid analogs and myristate esters in an octanol/water system based on their chemical structure[16]. The PrologD 2.0 module was used to predict the logarithm of the distribution coefficient, log *D* from the compound structure[16]. Interatomic distances were measured after AM1 calculations using the Hyperchem Release 4.0 computational program.

4-Oxatetradecanoic acid (**8b**)[11], 9-oxatetradecanoic acid (**8c**)[5], 12-methoxydodecanoic acid (**12e**)[4], 12-thioethyldodecanoic acid (**12f**)[4], 12-azidododecanoic acid (**12i**)[23], 11-methoxyundecanoic acid (**13a**)[4], 11-thioethylundecanoic acid (**13b**)[4], and (±)-2-fluorotetradecanoic acid (**14a**)[2] were synthesized using literature procedures. Myristic acid (**1**), 11-bromoundecanoic acid (**13c**), 12-bromododecanoic acid (**12d**), (±)-2-hydroxytetradecanoic acid (**14b**), and (±)-2-bromotetradecanoic acid (**14c**) were purchased from the Aldrich Chemical Co. All other reagents and chemicals were obtained from Aldrich unless noted otherwise. RPMI 1640 media, obtained from the GIBCO BRL Co., was buffered with 0.165 M MOPS and sodium hydroxide to adjust the pH to 7.0 at 25 C. *Aspergillus niger PLM 1140, Candida albicans ATCC 14053, Cryptococcus neoformans KF-33,* and *Saccharomyces cervisiae PLM 454* were used in the anti-fungal screens in RPMI 1640 growth medium.

12-Bromo-4-oxadodecanonitrile (**7**)

Sodium hydride (5.92 mg, 0.25 mmol, 97%) was added to a solution of 8-bromo-1-octanol (**5**, 1.00 g, 0.82 ml, 4.78 mmol), and the mixture was stirred for 0.5 h at room temperature. Acrylonitrile (**6**, 0.80 ml, 12.05 mmol) was added slowly to this mixture during 5 min at 55–60 °C. After stirring for 3 h at 55–60 °C, and then for 15 h at room temperature, ether (24 ml) was added. The organic phase was washed with water (8 ml), brine (8 ml) and dried (Na_2SO_4). The residue, which consisted of one major product, was purified by silica gel column chromatography using hexane-ethyl acetate, 80:20 (*v/v*) as eluent to yield 12-bromo-4-oxadodecanonitrile (**7**) as a liquid (1.06 g, 84.5%) which was used immediately for the synthesis of 12-chloro-4-oxadodecanoic acid (**8a**).– ^{1}H NMR ($CDCl_3$): δ 1.22–1.48 (br m, 8H, methylene envelope), 1.54 (m, *J* = 6.4 Hz, *J* = 6.5 Hz, 4H, OCH_2CH_2), 1.83 (quint, *J* = 6.5 Hz, *J* = 7.2 Hz, 2H, CH_2CH_2Br), 2.67 (t, *J* = 6.3 Hz, 2H, CH_2CN), 3.48 (t, *J* = 6.5 Hz, 2H, CH_2Br), 3.54 (t, *J* = 6.4 Hz, 2H, CH_2O), 3.61 (t, *J* = 6.3 Hz, 2H, CH_2O).– ^{13}C NMR ($CDCl_3$) δ 65.11 (CH_2O), 71.24 (CH_2O), 117.79 (*C*N).

12-Chloro-4-oxadodecanoic acid (**8a**)

A solution of the nitrile (**7**, 1.06 g, 4.05 mmol) in acetic acid (5 ml) and 37% (*w/v*) hydrochloric acid (15 ml) was refluxed for 2 h. After removal of the solvent *in vacuo*, water (50 ml), and then ethyl acetate (150 ml) were added to the residue. The organic phase was washed with water (50 ml), brine (50 ml), and dried (Na_2SO_4). The residual oil obtained was purified by Kugelrohr distillation to afford **8a** (0.723 g, 76%).– ^{1}H NMR ($CDCl_3$): δ 1.22-1.59 (br m, 10H, methylene envelope), 1.69 (quint, *J*= 7.0 Hz, *J*= 6.5 Hz, 2H, CH_2), 2.55 (t, *J* = 6.5 Hz, 3H, CH_2CO), 3.38 (t, *J* = 6.5 Hz, 2H, CH_2O), 3.45 (t, *J* = 6.5 Hz, 2H, CH_2Cl), 3.63 (t, *J* = 6.5 Hz, 2H, CH_2O), 11.05 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 34.74 (CH_2CO), 44.92 (CH_2Cl), 65.54 (CH_2O), 71.04 (CH_2O), 177.32 (*C*OO).– $C_{11}H_{21}ClO_3$, Anal. C, H.

Methyl 12-hydroxydodecanoate (**10**)

Chlorotrimethylsilane (0.81 g, 0.95 ml, 7.45 mmol) was added to a solution of 12-hydroxydodecanoic acid (**9,** 0.50 g, 2.31 mmol) in dry methanol (12.0 ml) under a nitrogen atmosphere and the reaction mixture was stirred at room temperature for 16 h. Methanol was removed *in vacuo* and the residue obtained was recrystallized from petroleum ether to afford **10** (0.475 g, 89.2 %) which was used immediately for the synthesis of methyl 12-fluorododecanoate (**11**); mp 31–32 °C.– ^{1}H NMR ($CDCl_3$): δ 1.17–1.3 (br m, 14H, methylene envelope), 1.36–1.56 (br m, 4H, CH_2CH_2OH, CH_2CH_2COOMe), 2.27 (t, *J* = 7.7 Hz, 2H, CH_2COOMe), 2.3 (s, 1H, OH), 3.60 (t, *J* = 6.5 Hz, 2H, CH_2OH), 3.63 (s, 3H, OCH_3).– ^{13}C NMR ($CDCl_3$) δ 51.36 (OCH_3), 62.94 (CH_2OH), 174.32 (*C*OO).

Methyl 12-fluorododecanoate (**11**)

Diethylaminosulfur trifluoride (DAST) (0.61 g, 0.5 ml, 3.78 mmol) was added to a solution of methyl 12-hydroxydodecanoate (**10**, 0.23 g, 1 mmol) in 20 ml anhydrous benzene and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was mixed with 5% sodium bicarbonate (20 ml) and extracted with ethyl acetate (3 × 50 ml). The organic phase was separated, washed with water, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue, which consisted of one major product, was purified by silica gel column chromatography using hexane-ethyl acetate, 70:30 (*v/v*) as eluent to yield methyl 12-fluorododecanoate (**11**) as a liquid (0.2 g, 86.2 %) which was used immediately for the synthesis of 12-fluorododecanoic acid (**12a**).– ^{1}H NMR ($CDCl_3$): δ 1.17–1.37 (br m, 14H, methylene envelope), 1.47–1.71 (br m, 4H, CH_2CH_2F, CH_2CH_2COOMe), 2.23 (t, *J* = 7.5 Hz, 2H, CH_2COOMe), 3.58 (s, 3H, OCH_3), 4.34 (dt, $J_{H,F}$ = 47.5 Hz, 2H, $J_{CH2,CH2}$ = 6.3 Hz, CH_2F).–^{13}C NMR ($CDCl_3$) δ 33.93 (CH_2CO), 51.20 (OCH_3), 83.95 (d, $J_{C,F}$ = 163.39 Hz, CH_2F), 174.08 (*C*OO).

12-Fluorododecanoic acid (**12a**)

A mixture of methyl 12-fluorododecanoate (**11**, 0.20 g, 0.86 mmol) in dioxane (0.27 ml) and 37% (*w/v*) hydrochloric acid (0.45 ml) was refluxed for 2 h. After cooling to room temperature, the mixture was extracted with chloroform (3 × 1 ml) and the combined chloroform solutions were extracted with 10% (*w/v*) aqueous potassium hydroxide solution (5 ml). The combined alkaline solutions were washed with diethyl ether (3 × 1 ml) and acidified with concentrated HCl. The carboxylic acid which separated was taken up in chloroform (10 ml) and the chloroform solution was dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue obtained, which consisted of one major product, was purified by silica gel column chromatography using hexane-ethyl acetate, 80:20 (*v/v*) as eluent. Recrystallization from petroleum ether yielded 12-fluorododecanoic acid (**12a,** 0.122 g, 65%); mp 59–60 °C (*lit.*[8] 59.5–61 °C).– ^{1}H NMR ($CDCl_3$): δ 1.10–1.40 (br m, 14H, methylene envelope) 1.46–1.80 (br m, 4H, CH_2CH_2F, CH_2CH_2COOH), 2.28 (t, *J* = 7.3 Hz, 2H, CH_2COOH), 4.36 (dt, $J_{H,F}$ = 47.4 Hz, $J_{CH2,CH2}$ = 6.3 Hz, 2H, CH_2F), 10.00 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 33.96 (CH_2CO), 84.17 (d, $J_{C,F}$ = 164.73 Hz, CH_2F), 179.77 (*C*OO).– ^{19}F NMR ($CDCl_3$) δ 56.79 (dddd, J_{gem} = 47.4 Hz, J_{vic} = 24.4 Hz, 1F, CH_2F).

12-Iodododecanoic acid (**12b**)

A mixture of 12-bromododecanoic acid (**12d**, 0.25 g, 0.9 mmol) and sodium iodide (0.403 g, 2.7 mmol) in dry acetone (7.0 ml) was stirred for 18 h at room temperature. The solvent was removed *in vacuo*, the residue obtained was dissolved in water (60 ml) and extracted with chloroform (3 × 60 ml). The combined extracts were dried (Na_2SO_4), and the solvent was removed *in vacuo*. The residue obtained was recrystallized from petroleum ether to afford **12b** (0.276 g, 95%); mp 62–63 °C (*lit.*[24] 61–62.5 °C).– ^{1}H NMR ($CDCl_3$): δ 1.14–1.44 (br m, 14H, methylene envelope), 1.54 (quint, *J* = 7.7 Hz, *J* = 7.1 Hz, 2H, CH_2CH_2COOH), 1.74 (quint, *J* = 7.1 Hz, *J* = 6.6 Hz, 2H, CH_2CH_2I), 2.36 (t, *J* = 7.1 Hz, 2H, CH_2CO), 3.20 (t, *J* = 7.1 Hz, 2H, CH_2I), 10.3 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 7.19 (CH_2I), 34.05 (CH_2CO), 180.17 (*C*OO).– $C_{12}H_{23}IO_2$, Anal. C, H.

12-Chlorododecanoic acid (**12c**)

Nitric acid (70% *w/v*, 2.2 ml) was added to a mixture of hydrochloric acid (37% *w/v*, 1.5 ml) and 12-iododododecanoic acid (**12b**, 0.20 g, 0.61 mmol) at 15–20 °C. The reaction was allowed to proceed at 15–20 °C with stirring for 2 h, the precipitated elemental iodine was filtered off and the filtrate was poured onto ice (1.0 g). The aqueous layer was extracted with methylene chloride (3 × 3 ml), the methylene chloride extract was washed consecutively with water, a 2% (*w/v*) aqueous $Na_2S_2O_3$ to remove iodine and with water. The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo* and the residue obtained, which consisted of one major product, was purified by silica gel column chromatography using hexane-ethyl acetate, 80:20 (*v/v*) as eluent to yield **12c** (0.096 g, 66%); (mp 42.5–43 °C) (*lit.*[25] 43–44 °C).– ^{1}H NMR ($CDCl_3$): δ 1.18–1.46 (br m, 14H, methylene envelope), 1.52 (quint, 2H, CH_2), 1.73 (quint, 2H, CH_2), 2.32 (t, *J* = 7.5 Hz, 2H, CH_2CO), 3.50 (t, *J* = 6.9 Hz, 2H, CH_2Cl), 10.94 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 34.02 (CH_2CO), 45.15 (CH_2Cl), 180.10 (*C*OO).– $C_{12}H_{23}ClO_2.1/4\ H_2O$, Anal. C, H.

12-Phenoxydodecanoic acid (**12i**)

Phenol (0.17 g, 1.81 mmol) was dissolved in ethanol (19 ml) and potassium hydroxide (0.23 g, 4.10 mmol) was added. 12-Bromododecanoic acid (**12d**, 0.50 g, 1.79 mmol) was dissolved in ethanol (10 ml) and added to the first solution under a nitrogen atmosphere. The reaction mixture was refluxed under nitrogen for 4 h, acidified with a solution of 37% (*w/v*) hydrochloric acid (10 ml) in water (100 ml) and the solvents were removed *in vacuo*. The residue was dissolved in water (50 ml), extracted with chloroform (3 × 60 ml), the organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue obtained was recrystallized from petroleum ether-ethyl acetate to give crystals which consisted of one major product that was purified by silica gel column chromatography using hexane-ethyl acetate, 80:20 (*v/v*) as eluent to yield 12-phenoxydodecanoic acid (**12i**) which was recrystallized from hexane (0.50 g, 95.5 %); mp 76–77 °C.– ^{1}H NMR ($CDCl_3$): δ 1.66 (quint, *J* = 7.5 Hz, *J* = 7.2 Hz, 2H, CH_2CH_2CO), 1.81 (quint, *J* = 7.0 Hz, *J* = 6.5 Hz, 2H, CH_2CH_2O), 1.22–1.54 (br m, 14H, methylene envelope), 2.37 (t, *J* = 7.5 Hz, 2H, CH_2CO), 3.88 (t, *J* = 6.5 Hz, 2H, CH_2O), 6.86–7.00 (m, 3H, aromatic), 7.23–7.60 (m, 2H, aromatic), 10.30 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 34.05 (CH_2CO), 67.83 (CH_2O), 114.48 (aromatic C), 120.40 (aromatic C), 129.33 (aromatic C), 159.10 (aromatic C), 180.22 (*C*OO).– $C_{18}H_{28}O_3.1/2\ H_2O$, Anal. C, H.

11-(4-Iodophenoxy)undecanoic acid (**13d**)

The same procedure used for the synthesis of **12i**, but using 4-iodophenol in the place of phenol and 11-bromoundecanoic acid (**13c**) in place of 12-bromododecanoic acid (**12d**) afforded **13d** (0.147 g, 43.2%); mp 90–91 °C.– ^{1}H NMR ($CDCl_3$): δ 1.22–1.50 (br m, 12H, methylene envelope), 1.63 (quint, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H, CH_2), 1.76 (quint, *J* = 7.2 Hz, *J* = 6.6 Hz, 2H, CH_2), 2.35 (t, *J* = 7.2 Hz, 2H, CH_2CO), 3.90 (t, *J* = 7.2 Hz, 2H, CH_2O), 6.66 (m, *J ortho* = 8.8 Hz, *Jmeta* = 3.5 Hz, 2H, aromatic), 7.53 (m, *Jortho* = 8.8 Hz, *Jmeta* = 3.5 Hz, 2H, aromatic), 9.25 (1H, br s, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 33.99 (CH_2CO), 68.11 (CH_2O), 82.39 (aromatic C), 116.96 (aromatic C), 138.15 (aromatic C), 159.03 (aromatic C), 179.85 (*C*OO).– $C_{17}H_{25}IO_3$, Anal. H: Calcd. for C, 50.51; found, 50.99.

(±)-2-Iodotetradecanoic acid (**14d**)

A mixture of (±)-2-bromotetradecanoic acid (**14c**, 0.50 g, 1.63 mmol) and sodium iodide (0.729 g, 4.86 mmol) in dry acetone (12.7 ml) was stirred for 18 h at room temperature. The solvent was evaporated *in vacuo*, the residue was dissolved in water (60 ml) and extracted with chloroform (3 × 60 ml). The combined extracts were dried (Na_2SO_4), filtered, and the solvent was removed *in vacuo*. The residue was recrystallized from petroleum ether to afford **14d** (0.48 g, 83.3%); mp 47–48 °C.– ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.0 Hz, 3H, CH_3), 1.2–1.5 (br m, 20H, methylene envelope), 1.92–2.4 (m, 2H, $CH_2CHICOOH$), 4.31 (t, *J* = 7.5 Hz, 2H, *CH*I), 9.3 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 14.10 (CH_3), 20.13 (*C*HI), 35.81 (CH_2CHI), 177.35 (*C*OOH).– MS (70 eV) *m/z* (%) = 354 (3) [M^+], 227 (57) [$M^+ - I^\bullet$], 209 (22) [$M^+ - H_2O - I^\bullet$], 57 (89) [$C_4H_9^+$], 55 (100) [$C_4H_7^+$].

(±)-2-Chlorotetradecanoic acid (**14e**)

Nitric acid (70% *w/v*, 4.11 ml) was added to a mixture of 37% (*w/v*) hydrochloric acid (2.8 ml) and (±)-2-iodotetradecanoic acid (**14d**) (0.411 g, 1.16 mmol) at 15–20 °C and the mixture was stirred at this temperature for 2 h. The precipitated solid, which was filtered off, consisted of one major product that was purified by silica gel column chromatography using hexane-ethyl acetate (70:30, *v/v*) as eluent to yield **14e** (0.087 g, 28.5%); mp 43–44 °C (*lit.*[26] mp 41–42 °C).– ^{1}H NMR ($CDCl_3$): δ 0.90 (t, *J* = 7.0 Hz, 3H, CH_3), 1.2–1.6 (br m, 20H, methylene envelope), 1.90–2.16 (m, 2H, $CH_2CHClCOOH$), 4.33 (dd, *J* = 6.0 Hz, *J* = 8.1 Hz, 2H, *CH*Cl), 10.0 (br s, 1H, COO*H*).– ^{13}C NMR ($CDCl_3$) δ 14.09 (CH_3), 34.77 (CH_2CHCl), 57.09 (*C*HCl), 174.99 (*C*OOH).

(±)-2-Bromotetradecanoate Esters: General Synthesis

Chlorotrimethylsilane (0.47 g, 0.52 ml, 4.32 mmol) was added to a solution of (±)-2-bromotetradecanoic acid (**14c**, 0.392 g, 1.28 mmol) in dry alcohol (6.62 ml) under a nitrogen atmosphere and the mixture was stirred at room temperature for 16 h. The solvent was removed under reduced pressure and the residue obtained consisted of one major product that was purified by silica gel column chromatography using hexane-ethyl acetate (70:30, *v/v*) as eluent to yield the ester product as a liquid.

(±)-Methyl 2-bromotetradecanoate (**15a**)

The method described above was carried out using dry methanol to yield **15a** (0.377 g, 92%) as an oil.– ^{1}H NMR ($CDCl_3$): δ 0.85 (t, *J* = 7.0 Hz, 3H, CH_3), 1.16–1.50 (br m, 20H, methylene envelope), 1.88–2.12 (br m, 2H, CH_2CHBr), 3.75 (s, 3H, OCH_3), 4.18 (t, *J* = 7.3 Hz, 1H, *CH*Br).– ^{13}C NMR ($CDCl_3$) δ 14.03 (CH_3), 34.88 (CH_2CHBr), 45.64 (*C*HBr), 52.73 (OCH_3), 170.27 (*C*OO).– MS (70 eV) *m/z* (%) = 321 (10) [M^+], 241 (64) [$M^+ - Br^\bullet$], 87 (100) [$C_4H_7O_2^+$], 55 (62) [$C_4H_7^+$].

(±)-Ethyl 2-bromotetradecanoate (**15b**)

The general synthetic method described above using dry ethanol afforded **15b** (0.307 g, 72%) as an oil.– ^{1}H NMR ($CDCl_3$): δ 0.87 (t, *J* = 6.8 Hz, 3H, CH_3), 1.18–1.52 (br m, 23H, methylene envelope, OCH_2CH_3), 1.88–2.14 (br m, 2H, CH_2CHBr), 4.21 (t, *J* = 7.3 Hz, 1H, *CH*Br), 4.21 (q, *J* = 7.3 Hz, 2H, OCH_2CH_3).– ^{13}C NMR ($CDCl_3$) δ 13.92 (CH_3), 14.06 (CH_3), 34.86 (CH_2CHBr), 46.15 (*C*HBr), 61.80 (OCH_3), 169.86 (*C*OO).– MS (70 eV) *m/z* (%) = 335 (11) [M^+], 255 (71) [$M^+ - Br^\bullet$], 237 (7) [$M^+ - H_2O - Br^\bullet$], 101 (54) [$C_5H_9O_2^+$], 69 (50) [$C_5H_9^+$], 55 (100) [$C_4H_7^+$].

(±)-Isopropyl 2-bromotetradecanoate (**15c**)

The same procedure using dry isopropyl alcohol gave **15c** (0.407 g, 92%) as an oil.– ^{1}H NMR ($CDCl_3$) δ 0.88 (t, *J* = 6.0 Hz, 3H, CH_3), 1.16–1.52 (br m, methylene envelope, 26H, $OCH(CH_3)_2$), 1.88–2.13 (br m, 2H, CH_2CHBr), 4.16 (t, *J* = 7.3 Hz, 3H, *CH*Br), 5.06 (sept, *J* = 6.0 Hz, 1H, $OCH(CH_3)_2$).– ^{13}C NMR ($CDCl_3$) δ 0.95 (CH_3); 14.05 (CH_3), 34.83 (CH_2CHBr), 46.56 (*C*HBr), 69.35 ($OCH(CH_3)_2$), 169.32 (*C*OO).– MS (70 eV) *m/z* (%) = 349 (2) [M^+], 306 (25) [M^+ – isopropyl$^\bullet$], 269 (21) [$M^+ - Br^\bullet$], 227 (65) [$M - Br^\bullet - C_3H_6^+$], 57 (100) [$C_4H_9^+$], 55 (89) [$C_4H_7^+$].

Toxicity Assays

In vitro KB cell cytotoxicity (TD_{50}, μg/ml) was determined for a few compounds by the tetrazolium salt (MTT) method which involves conversion of MTT to colored formazan, according to the Hansen *et al.* procedure[27].

In Vitro Antifungal Assay

The broth dilution method reported by Barchiesi *et al.* was used for determination of minimum inhibitory concentration (MIC values)[28].

References

[1] C. A. Langner, J. K. Lodge, S. J. Travis, J. E. Caldwell, L. Tianbao, Q. Li, M. L. Bryant, B. Devadas, G. W. Gokel, G. S. Kobayashi, J. I. Gordon, *J. Biol. Chem.* **1992**, *267*, 17159–17169.

[2] L. A. Paige, G. Zheng, A. D. Shawn, J. M. Cassady, R. L. Geahlen, *Biochem.* **1990**, *29*, 10566–10573.

[3] P. Chattopadhyay, S. K. Banaerjee, K. Sen, *Can. J. Micro.* **1985**, *31*, 352–355.

[4] J. I. Gordon, S. P. Adams, R. O. Heuckeroth, (Washington University) EP 0 415 902 A1 **1991** [Chem. Abstr. **1991** *115.*, P774y].

[5] B. Devadas, T. Lu, A. Katoh, N. S. Kishore, A. C. Wade, P. P. Mehta, D. A. Rudnick, M. L. Bryant, S. P. Adams, Q. Li, G. W. Gokel, J. I. Gordon, *J. Biol. Chem.* **1992**, *267*, 7224–7239.

[6] F. L. M. Pattison, W. C. Howell, A. J. McNamara, J. C. Schneider, J. F. Walker, *J. Org. Chem.* **1956**, *21*, 739–747.

[7] M. A. Brook, T. H. Chan, *Synthesis* **1983**, *13*, 201–203.

[8] M. Hudlicky, In *Org. React.*, John Wiley&Sons Inc., New York, **1988**, Chapter 3, *35*, 513–637.

[9] S. M. Peseckis, , I. Deichaite, M. D. Resh, *J. Biol. Chem.* **1993**, *268*, 5107–5114.

[10] N. V. Svetlakov, I. E. Moisak, I. G. Averko-Antonovich, *J. Org. Chem. USSR* **1969**, *5*, 971–972.

[11] J. Diekman, J. B. Thomson, C. Djerassi, *J. Org. Chem.* **1969**, *34*, 3147–3161.

[12] M. M. Goodman, G. Kirsch, F. F. Knapp, *J. Med. Chem.* **1984**, *27*, 390–397.

[13] M. Eisenhut, J. Liefhold, *Int. J. Rad. Appl. Instrum. A.* **1988**, *39*, 639–649.

[14] N. S. Kishore, T. Lu, L. J. Knoll, A. Katoh, D. A. Rudnick, P. P. Mehta, B. Devadas, M. Huhn, J. L. Atwood, S. P. Adams, G. W. Gokel, J. I. Gordon, *J. Biol. Chem.* **1991**, *266*, 8835–8855.

[15] N. S. Kishore, D. C. Wood, P. P. Mehta, A. C. Wade, T. Lu, G. W. Gokel, J. I. Gordon, *J. Biol. Chem.* **1993**, *268*, 4881–4902.

[16] PrologD 2.0 for Windows, *pK*alc 3.1 for Windows, PrologP 5.1 for Windows. **1995**, Compudrug Chemistry Ltd, Hollàn Ernö utca 5., H-1136 Budapest, Hungary.

[17] M. D'amboise, H. Toshihiko, *J. Liq. Chromatogr.* **1982**, *5*, 229–244.

[18] C. J. Drummond, F. Grieser, T. W. Healy, *J. Chem. Soc. Faraday Trans. 1*, **1989**, *85*, 521–535.

[19] D. D. Perrin, B. Dempsey, E. P. Serjeant, in *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, London **1981**, 27–52.

[20] Z Rapport, In *Handbook of Tables For Organic Compound Identification*, 3rd ed., Chemical Rubber Co., Clevcland, **1967**, 428–433.

[21] H. Gershon, M. W. McNeil, R. Parmegiani, P. A. Godfrey, J. M. Baricko, *Antimicrob. Agents and Chemother.* **1973**, *4*, 435–438.

[22] R. O. Heuckeroth, E. Jackson-Machelski, S. P. Adams, N. S. Kishore, M. Huhn, A. Katoh, T. Lu, G. W. Gokel, J. I. Gordon, *J. Lipid Res.* **1990**, *31*, 1121–1129.

[23] S . P. Adams, B. Devadas, J. I. Gordon, (Washington University) *EP* 0 480 901 A1 **1992** [Chem. Abstr. **1992** *,117*, P89841e].

[24] W. H. Kruizinga, R. M. Kellog,. *J. Am. Chem. Soc.* **1981**, *103*, 5183–5189.

[25] I. Bidd, D. W. Holdup, M. C. Whiting, *J. Chem. Soc. Perkin Trans.* **1987**, 2457–2458.

[26] Y. Ogata, T. Sugimoto, M. Inaishi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 255–256.

[27] M. B. Hansen, S. E. Nielsen, K. Berg, *J. Immunol. Methods* **1989**, *119*, 203–210.

[28] F. Barchiesi, A. L. Colombo, D. A. McGough, M. G. Rinaldi, *J. Clin. Micr.* **1994**, *32*, 2494–2500.

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