Antifungal free fatty acids: A Review

Carolina H. Pohl, Johan L.F. Kock and Vuyisile S. Thibane

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, 9300, South Africa

Fatty acids are known to possess antibacterial, antimalarial and antifungal activity. The development of resistance of microbes, including fungi and yeasts, towards antimicrobial agents already in use, necessitates the search for alternative antimicrobials, including fatty acids and their derivatives (e.g. methylated and hydroxyl fatty acids). Although fatty acids may not be as effective as chemical fungicides, they pose less environmental risks. They are not only biodegradable, but exhibit a high degree of specificity. In addition, fatty acids are accepted food additives and importantly, pathogenic fungi are less likely to become resistant to antifungal fatty acids. The most important target of antifungal fatty acids is the cell membrane. They cause an increase in membrane fluidity, which will result in leakage of the intracellular components and cell death. Other targets include protein synthesis, which may be inhibited by myristic acid analogues, fatty acid metabolism as well as topoisomerase activity which may be inhibited by amongst others acetylenic fatty acids.

Keywords: fatty acid; antifungal mechanism

1. Introduction

Fatty acids are organic acids characterized by the presence of a carboxyl group (-COOH) at one end and a methyl group ($-CH_3$) at the other end. Fatty acids are ubiquitous in nature and as such they belong to a physiologically important class of molecule involved in cell energy storage (e.g. adipose tissues), membrane structure (phospholipid bilayer) and in various signalling pathways [1]. Fatty acids vary in length and degree of saturation, with naturally occurring fatty acids having a chain length of 4 to 28 carbons which may be saturated or unsaturated [2]. Saturated fatty acids are straight chains and consist of a carbon chain with single bonds, while unsaturated fatty acids contain one or more double carbon-carbon bonds (C=C) which introduces fixed bends into the carbon chain.

Fatty acids in the form of phospholipids are important components of the lipid bi-layer of the cell membrane of all cells. The cell membrane has an essential general role of maintaining cell order and integrity and a number of disease-control mechanisms involve compounds that directly (by partitioning into the membrane and inducing disorder) or indirectly (by inhibiting fatty acid biosynthetic pathways) target the phospholipids of the cell membrane [3]. This is also true for certain fatty acids or their derivatives. Suksamrarn and co-workers [4] showed that scleropyric acid, a naturally occurring C18 fatty acid, has antiplasmodial activity against *Plasmodium falciparum*. Recent research has also shown that 2-hexadecynoic acid, a 2-alkynoic fatty acid, has antibacterial activity against *Mycobacterium tuberculosis* [5] and that linoleic acid (18:2), a polyunsaturated fatty acid, has antifungal activity against several plant pathogenic fungi [1].

The use of fatty acids as antifungal agents offers some advantages. Liu and co-workers [1] proposed that antifungal fatty acids can replace chemicals in use to control plant diseases worldwide, which negatively impact the environment by affecting non-target organisms. Avis [3] also showed that antifungal compounds targeting fungal membranes are prone to pathogen resistance that will shorten their effective life span, but the synergism of these compounds with antifungal fatty acids could provide prolonged usage [6]. An example of this was demonstrated by Ells and co-workers [7] who found that arachidonic acid (20:4), a polyunsaturated fatty acid, may increase antifungal susceptibility of biofilms formed by two closely related *Candida* species and could thus result in the reduction of the dose of the antimycotic agent required to inhibit biofilm formation. In addition, the trend towards green consumerism also drives the search for natural, environmentally friendly compounds such as fatty acids, as food preservatives [8]. This review will aim at identifying antifungal free fatty acids that may combat fungal infections affecting plants, humans and food sources as well as identifying their possible mode of action.

2. Interaction of fatty acids with cell membranes

The fungal membrane has the fundamental role of maintaining cell order and integrity and hence antifungal treatment mostly target the fungal membrane [3]. Avis and Bélanger [9] determined the general mechanism with which antifungal fatty acids directly interacts with the fungal cell membrane. The antifungal fatty acids naturally insert themselves into the lipid bi-layer of the fungal membranes and physically disturb the membrane, resulting in increased fluidity of the membrane. These elevations in membrane fluidity will cause a generalized disorganization of the cell membrane that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder and eventually cell disintegration.

Sterols, especially the fungal sterol, ergosterol, tend to buffer such induced elevations in membrane fluidity, therefore fungal membranes with low sterol content are sensitive and unable to cope with such excessive elevations in membrane fluidity [10,11]. However, important to note that this proposed general mechanism for the antifungal activity of fatty acids is not always supported by experimental results and that other, as yet undiscovered actions may also contribute to the antifungal activity of fatty acids [8]. The chemical composition of fatty acids as well as the pH of the medium plays an important role in the ability of these compounds to inhibit fungi [12,13].

2.1 Saturated fatty acids

Antifungal free fatty acids can be saturated or unsaturated and in general the antifungal efficiency of fatty acids increases with an increase in chain length [2]. Hydrophobic groups of saturated fatty acids play an important role in bioactivity [14]. However, the increase of hydrophobicity with longer chain length may reduce their solubility in aqueous systems [15] and hydrophobic groups may be prevented from reaching sufficient concentrations to interact with acyl chains of membrane phospholipids [16]. Branen and co-workers [14] reported that dodecanoic (lauric) acid (12:0) has the best balance between hydrophobic and hydrophilic groups. Table 1 lists different saturated fatty acids of varying chain length with activity against fungi, including plant and human pathogens and spoilage organisms.

Table 1 Saturated fatty acids with antifungal activity.

Fatty acid	Fungus	Reference
Butanoic (butyric) acid (4:0)	*Alternaria solani*	[1]
	Cucumerinum lagenarium	[1]
	Fusarium oxysporum	[1]
	Myrothecium verrucaria	[11]
	Trichoderma viride	[11]
Pentanoic acid (5:0)	*Myrothecium verrucaria*	[12]
	Trichoderma viride	[12]
Hexanoic (caproic) acid (6:0)	*Botrytis cinerea*	[17]
	Cucumerinum lagenarium	[1]
	Fusarium oxysporum	[1]
	Myrothecium verrucaria	[12]
	Trichoderma viride	[12]
Heptanoic acid (7:0)	*Myrothecium verrucaria*	[12]
	Trichoderma viride	[12]
Octanoic (caprylic) acid (8:0)	*Alernaria solani*	[1]
	Cucumerinum lagenarium	[1]
	Fusarium oxysporum	[1]
	Kluyveromyces marxianus	[18]
	Myrothecium verrucaria	[12]
	Saccharomyces cerevisiae	[18,19]
	Trichoderma viride	[12]
Nonanoic (pelargonic) acid (9:0)	*Crinipellis pernicosa*	[20]
	Microsporum gypseum	[21]
	Moniliophthora roreri	[20]
	Myrothecium verrucaria	[12]
	Trichoderma viride	[12]

Decanoic (capric) acid (10:0)	*Alternaria solani*	[1]
	Aspergillus nidulans	[22]
	Aspergillus fumigatus	[22]
	Candida albicans	[23,24]
	Cucumerinum lagenarium	[1]
	Fusarium oxysporum	[1]
	Kluyveromyces marxianus	[18, 22]
	Microsporum gypseum	[21]
	Myrothecium verrucaria	[12]
	Penicillium commune	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]
	Saccharomyces cerevisiae	[18,19,25]
	Trichoderma viride	[12]
Undecanoic acid (11:0)	*Myrothecium verrucaria*	[12]
	Saccharomyces cerevisiae	[11]
	Trichoderma viride	[12]
	Trichophyton rubrum	[26]
Dodecanoic (lauric) acid (12:0)	*Aspergillus niger*	[8,27]
	Blumeria graminis	[28]
	Candida albicans	[23, 24,29]
	Colletotrichum gloeosporioides	[30]
	Cucumerinum lagenarium	[1]
	Fusarium avenaceum	[13]
	Fusarium oxysporum	[1,13]
	Myrothecium verrucaria	[12]
	Pythium ultimum	[28]
	Rhizoctonia solani	[28]
	Saccharomyces cerevisiae	[11]
Tetradecanoic (myristic) acid (14:0)	*Alternaria solani*	[1]
	Aspergillus niger	[8,31]
	Candida albicans	[23]
	Emericella nidulans	[8]
	Fusarium oxysporum	[1]
	Penicillium glabrum	[8]
	Penicillium italicum	[8]
Palmitic acid (16:0)	*Alernaria solani*	[1]
	Aspergillus niger	[8]
	Aspergillus terreus	[8]
	Cucumerinum lagenarium	[1]
	Emericella nidulans	[8]
	Fusarium oxysporum	[1]

2.2 Unsaturated fatty acids

Unsaturated fatty acids contain fixed bend C=C bonds and will therefore occupy a greater cross section when inserted into the membrane [9]. They are proposed to have increased fungicidal activity due to their increased motional freedom inside the membrane. It must however be noted that increased oxidative stress caused by incorporation of polyunsaturated lipids in the membrane, may contribute to the antifungal activity of these fatty acids [32]. Table 2 lists unsaturated free fatty acids with antifungal activity.

Table 2 Unsaturated fatty acids with antifungal activity.

Fatty acid	Fungus	Reference
Butenoic acid (4:1)	*Myrothecium verrucaria*	[12]
Hexenoic acid (6:1)	*Myrothecium verrucaria*	[12]
Heptenoic acid (7:1)	*Myrothecium verrucaria*	[12]
	Trichoderma viride	[12]
Octenoic acid (8:1)	*Aspergillus niger*	[12]
	Myrothecium verrucaria	[12]
	Trichoderma viride	[12]
Nonenoic acid (9:1)	*Myrothecium verrucaria*	[12]
	Trichoderma viride	[12]
Decenoic acid (10:1)	*Myrothecium verrucaria*	[12]
Undecenoic acid (11:1)	*Myrothecium verrucaria*	[12]
	Saccharomyces cerevisiae	[11]
Dodecenoic acid (12:1)	*Myrothecium verrucaria*	[12]
Myristoleic acid (14:1)	*Candida albicans*	[23]
Palmitoleic acid (16:1)	*Candida albicans*	[23]
Heptadecenoic acid (17:1)	*Botrytis cinerea*	[9]
	Cladosporium cucumerinum	[9,10,33]
	Fusarium oxysporum	[10]
	Phytophthora infestans	[9]
	Pythium aphanidermatum	[9]
	Sphaerotheca fuliginea	[33,34]
Oleic acid (18:1)	*Crinipellis pernicosa*	[35]
	Pythium ultimum	[35]
Linoleic acid (18:2)	*Alternaria solani*	[1]
	Candida albicans	[23]
	Crinipellis pernicosa	[35]
	Fusarium oxysporum	[1]
	Pyrenophora avanae	[35]
	Pythium ultimum	[35]
	Rhizoctonia solani	[35]
Linolenic acid (18:3)	*Crinipellis pernicosa*	[35]
	Pyrenophora avanae	[35]
	Pythium ultimum	[35]
	Rhizoctonia solani	[35]
Stearidonic acid (18:4)	*Candida albicans*	[32]
	Candida dubliniensis	[32]
Eicosapentaenoic acid (20:5)	*Candida dubliniensis*	[32]
Docosapentaenoic acid (22:5)	*Candida albicans*	[32]
	Candida dubliniensis	[32]

2.3 Methylated fatty acids

Methylated fatty acids are polyunsaturated fatty acids characterized by the presence of a methyl substituent on the carbon chain. 6-Methylheptadecenoic acid (6-Me 17:1) is produced by the yeast, *Pseudozyma flocculosa,* and is fungitoxic against powdery mildew, *Sphaerotheca fuliginea* [33,34]. In addition, 12-methyltetradecanoic (12-Me 14:0) acid has been shown to inhibit appressorium formation in the rice pathogen, *Magnaporthe oryzae* [36]. 6-Methylheptadecenoic acid is partitioned into the fungal membrane and induces disorder due to its bulkiness caused by the *cis* double bond as well as methyl branch which occupies a large cross-section in the fungal membrane [33], however, the specific action of (12-Me 14:0) is still unknown.

2.4 Oxylipins

Oxylipins are products of oxygenase-catalyzed reactions of fatty acids [37]. Plant oxylipins represents a vast diverse family of secondary metabolites, believed to occur in all higher plants. They originate from oxidation and further conversion of predominantly 18:2 and 18:3. Interestingly, it was found that the most antifungal oxylipins were also regulators of plant defence responses [38].

Hydroxy fatty acids carry a hydroxyl group on the carbon chain. The hydroxyl group gives fatty acids special properties, such as higher viscosity and reactivity when compared with other fatty acids [39]. Hydroxy fatty acids show wide range of antifungal activity [22] although different result were obtained regarding the importance of the position of the hydroxyl group in the activity against specific fungi [22,39]. Sjögren and co-workers [22] proposed that hydroxyl fatty acids readily partition into the lipid bilayers of fungal membrane and increase membrane permeability and the release of intracellular electrolytes and proteins. Table 3 lists the hydroxy fatty acids found to have antifungal activity.

Similarly, a mixture of 9-OH 18:2, 13-OH 18:2, 7,10-diOH 18:1, 9,10,13 triOH 18:1 and 9,12,13 triOH 18:1 produced by *Pseudomonas* 42A2, from 18:2, was antifungal against *Arthroderma uncinatum*, *Macrophonia phaesolina*, *Penicillium funiculosum*, *Trichophyton mentagrophytes* and *Verticillium dhaliae* [48]. In addition to the hydroxy fatty acids, the intermediate lipoxygenase products, 9-hydroperoxy octadecadienoic acid (9-HPODE) and 13-hydroperoxy octadecadienoic acid (13-HPODE) and the hydroperoxides produced from linolenic acid (18:3) [9-hydroperoxy octadecatrienoic acid (9-HPOTE), 13-hydroperoxy octadecatrienoic acid (13-HPOTE)] could inhibit growth of the rice blast fungus, *Magnaporthe grisea* [40] as well as *Cladosporium herbarium* and *Phytophthora parasitica* [38]. 13-hydroperoxy octadecatrienoic acid could also inhibit the growth of *Alternaria brassicae* and *Leptosphaeria maculans* [37]. The antifungal mechanism(s) of these oxylipins are not known.

Table 3 Hydroxy fatty acids with antifungal activity.

Fatty acid	Fungus	Reference
2-OH 12:0	*Aspergillus fumigatus*	[22]
	Aspergillus nidulans	[22]
	Kluyveromyces marxianus	[22]
	Penicillium commune	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]
3-OH 10:0	*Aspergillus fumigatus*	[22]
	Aspergillus nidulans	[22]
	Kluyveromyces marxianus	[22]
	Penicillium commune	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]
3-OH 11:0	*Aspergillus fumigatus*	[22]
	Aspergillus nidulans	[22]
	Kluyveromyces marxianus	[22]
	Penicillium commune	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]
3-OH-12:0	*Aspergillus fumigatus*	[22]
	Aspergillus nidulans	[22]
	Kluyveromyces marxianus	[22]
	Penicillium commune	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]
3-OH 14:0	*Kluyveromyces marxianus*	[22]
	Penicillium roqueforti	[22]
	Pichia anomala	[22]
	Rhodotorula mucilaginosa	[22]

9-OH 18:2	*Magnaporthe grisea*	[40]
	Botrytis cinerea	[38]
	Cladosporium herbarium	[38]
	Colletotrichum acutatum	[41]
	Colletotrichum fragariae	[41]
	Colletotrichum gloeosporioides	[41]
	Phomopsis obscurans	[41]
	Phomopsis viticola	[41]
9-OH 18:3	*Botrytis cinerea*	[38]
	Cladosporium herbarium	[38]
	Phytophthora parasitica	[38]
13-OH 18:2	*Botrytis cinerea*	[38]
	Cladosporium herbarium	[38]
	Magnaporthe grisea	[40]
13-OH 18:3	*Alternaria brassicae*	[37]
	Botrytis cinerea	[38]
	Cladosporium herbarium	[38]
	Leptosphaeria maculans	[37]
	Phytophthora parasitica	[38]
7,10-diOH 18:1	*Candida albicans*	[42]
9,10-diOH 18:1	*Alternaria brassicae*	[37]
	Sclerotinia sclerotiorum	[37]
12,13-diOH 18:1	*Alternaria brassicae*	[37]
	Leptosphaeria maculans	[37]
	Sclerotinia sclerotiorum	[37]
7,8,9-triOH 16:1	*Phythophthora capsici*	[43]
7,10,12-triOH 18:1	*Magnaporthe grisea*	[44]
8,11,12-triOH 18:1	*Phythophthora capsici*	[43]
9,10,11-triOH 18:1	*Phythophthora capsici*	[43]
9,12,13-triOH 18:1	*Blumeria graminis*	[45]
	Ceratocystis fimbriata	[46]
12,13,17-triOH 18:1	*Botrytis cinerea*	[39]
	Erysiphe graminis	[39]
	Phytophthora infestans	[39]
	Puccinia recondite	[39]
2-OH-4-thia 14:0	*Cryptococcus neoformans*	[47]

Other oxylipins, that have antifungal activity, are 12-oxophytodienoic acid, 9-keto 18:2, 9-keto 18:3 and 13-keto 18:3, 9,10-epoxy 18:1 (against *Cladosporium herbarium* and *Phytophthora parasitica*), 9-oxo 18:2, 13-oxo 18:2 (against *Botrytis cinerea*, *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Phomopsis obsrurans* and *Phomopsis viticola*), 12-keto-7,8,9-triOH 16:1 (against *Phythophthora capsici*), 9,10-epoxy 18:1 (against *Leptosphaeria maculans*) and 12,13-epoxy 18:1 (against *Cladosporium herbarium* and *Sclerotinia sclerotiorum*), etherolenic acid (against *Alternaria brassicae* and *Leptosphaeria maculans*) and etheroleic acid (against *Blumeria graminis*) [37,38,41, 43, 45]. However, many oxylipins are chemically unstable and prone to degradation in the presence of biomolecules [38] or may be metabolised by several enzymes [49,50]. This may explain the transient nature of the growth inhibitory effect that has been reported [37].

3. Inhibition of *N*-myristoyltransferase

The enzyme myristoyl-CoA; protein *N*-myristoyltransferase (NMT) recognises chain length rather than hydrophobicity of fatty acids and attaches tetradecanoic (myristic) acid (14:0) to the amino-terminal glycine of eukaryotic proteins, leading to an irreversible protein modification [51,52]. *N*-myristoyltransferase is essential for growth and survival of several fungi and yeasts [53] with many of the fungal species synthesizing a variety of *N*-myristoyl proteins using NMT as a catalyst. Several myristic acid analogues have been shown to compete with 14:0 for binding to NMT, resulting in disturbance of protein function [31]. Table 4 lists myristic acid analogues proposed to inhibit fungal NMT.

Table 4 Myristic acid analogues with antifungal activity.

Fatty acid	Fungus	Reference
2-bromo 14:0	*Aspergillus niger*	[54]
	Candida albicans	[54]
	Cryptococcus neoformans	[54]
	Saccharomyces cerevisiae	[54]
3-oxa 14:0	*Cryptococcus neoformans*	[51]
4-oxa 14:0	*Cryptococcus neoformans*	[51]
5-oxa 14:0	*Cryptococcus neoformans*	[51]
6-oxa 14:0	*Cryptococcus neoformans*	[51]
2-methoxy 14:0	*Aspergillus niger*	[55]
	Candida albicans	[55]
	Cryptococcus neoformans	[55]

For the oxatetradecanoic acids, the position of the oxygen for methyl substitution is critical in determining their antifungal activity, with the order of potency being 4-oxatatradecanoic acid > 5-oxatatradecanoic acid > 3-oxatetradienoic acid ~ 6-oxatatradecanoic acid [51]. A synthetic antifungal fatty acid, 2-methoxy-4-oxa 14:0, which combines the functionalities of 2-methoxy 14:0 and 4-oxa 14:0, and with activity against *Apergillus niger*, *Cryptococcus neoformans* and fluconazole sensitive strain of *Candida albicans*, was synthesised by Carballeira and co-workers [31]. Although this fatty acid is capable of binding NMT, there was no correlation between antifungal activity and chirality of this fatty acid, suggesting an alternative unknown mechanism, possibly membrane disruption [31]. Another possible mechanism is inhibition of β-oxidation, since it is known that α-methoxylation of fatty acids inhibit β-oxidation [47].

4. Inhibition of fatty acid metabolism

One of the best known antifungal fatty acid is probably 10-undecenoic acid, also known as undecylenic acid. It is the active ingredient in over the counter topical antifungal preparations [56] and has been succesful in treating dermatomycosis caused by *Trichophyton rubrum*, *Epidermophyton inguinale* and *Microsporum audouini* [57] onychomycosis caused by *Trichophyton rubrum* [58] as well as tinea pedis caused by *Trichophyton mentagrophytes* and *Trichophyton rubrum* [59]. In addition, it was found to inhibit hyphal formation in *Candida albicans* [60] and to prevent overgrowth of this pathogenic yeast in the human host [61]. The mechanism involved is speculated to be inhibition of enzymes involved in lipid synthesis [60].

It is known that 3-thia fatty acids and 4-thia fatty acids inhibit β-oxidation in mammals and Carballeira and co-workers [47] indicated that 4-thia 14:0 has weak antifungal activity against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus niger*. In an attempt to improve the antifungal activity of these fatty acids, they synthesised the α-methoxylated, 2-methoxy-4-thia 14:0, and could increase the antifungal activity significantly. This may be due to the inhibition of β-oxidation, resulting in an increased half life of the fatty acid. This would allow the fatty acid to be incorporated more efficiently into the membrane. This increase in antifungal activity by methoxylation was studied further with the synthesis of 4-methoxy 10:0 [62]. This novel fatty acid increased the antifungal activity of 10:0 against a fluconazole resistant *Candida albicans* strain and *Cryptococcus neoformans* 17-fold and also exhibited antifungal activity against *Aspergillus brasiliensis* and *Issatchenkia orientalis*, especially in the (R) configuration [63], although the mechanism of action is still unknown.

Acetylenic fatty acids are characterized by the presence of a carbon triplet bond (C≡C). As found for plant derived oxylipins, acetylenic acids produced by certain plants upon fungal attack could also be seen as defence mechanisms [64]. Several acetylenic fatty acids from marine sponges have also demonstrated antifungal activity. These include the C31 acetylenic acids, corticatic acids A-C (against *Mortierella ramaniana*) [65] and a C14 acetylenic acid [CH_3-CH_2-CH=CH-C≡C-CH=CH-C≡C-$(CH_2)_3$-COOH] (against *Candida albicans*) [66]. The 2-alkynoic fatty acids, particularly those with chain lengths from eight to 12 carbon atoms, were found to be antifungal against *Aspergillus niger*, *Myrothecium verrucaria* and *Trichoderma viride* [11]. It has been found that the 2-alkynoic acids (especially 2-hexadecynoic acid) can inhibit fatty acid elongation and acylation during triglyceride synthesis [67,68]. Several natural 6-acetylenic acid were found to inhibit fungal growth. 6-Octadecynoic acid and 6-nonadecynoic acid were found to inhibit some strains of *Candida albicans, Trichophyton mentagrophytes* and *Trichophyton rubrum* [69-71]. 6-Nonadecynoic acid could also inhibit *Cryptococcus neoformans* [70]. In addition, 6-hexadecynoic acid, 6-heptadecynoic acid, 6-octadecynoic acid as well as 6-nonadecynoic acid were also active against *Candida krusei* [71]. The possible mechanism for the inhibitory activity of these two compounds is inhibition of sphingolipid synthesis [69], although 6-hexadecynoic acid was found to be a substrate for *Saccharomyces cerevisiae* NMT, due to the physical configuration of the bend introduced by the triple bond [72], suggesting that other structurally similar 6-acetylenic acid

may also possibly be substrates for this enzyme. This antifungal activity prompted Carballeira and co-workers [68] to study 2,6- hexaecadiynoic acid and 2,6-nonadecadiyenoic acid. They found that both of these fatty acids had increased activity against *Candida albicans* and *Cryptococcus neoformans* compared to the parent compounds 2-hexadeynoic acid and 6-hexadecynoic acid. Yoon and co-workers [73] also demonstrated that 9,11,13-octadecatriynoic acids have antifungal activity against the plant pathogenic fungi, *Fusarium oxysporum*, *Magnaporthe oryzae*, *Phytophthora capsici*, *Phytophthora infestans*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Another group of fatty acids with potential antifungal activity is the cyclopropane fatty acids [5]. A C17 cyclopentane fatty acid, from *Pseudomonas* has actvity against *Pythium ultimum* [74] and majusculoic acid, a bromated cyclopentane fatty acid, is effective against *Candida albicans* and *Candida glabrata* [75]. Although the presice antifungal mechanisms of these fatty acids are unknown, certain cyclopentane fatty acids can inhibit fatty acid desaturase enzymes in animals [76,77].

5. Inhibition of topoisomerase I

Topoisomerase I is responsible for relaxing DNA supercoils that result during replication and translation [78]. Although it is known that fatty acids can inhibit mammalian topoisomerase I [5,79], very little research has been conducted on the ability of fatty acids to inhibit fungal topoisomerases. Sanabria Rios [80] studied the potential of antifungal acetylenic acids (2,6-hexadecynoic acid, 2-hexadecynoic acid, 6-hexadecynoic acid) to inhibit human topoisomerase I in comparison to 16:0. However, the results for topoisomerase I inhibition did not correlate with the antifungal activity of these fatty acids. We agree with this author that the topoisomerase I inhibitory assays should however be performed with purified fungal topoisomerase in order to provide insight into the possible inhibitory effect of fatty acids on fungal topoisomerases.

6. Conclusions

In conclusion we can see that fatty acids and their derivatives hold great potential as environmentally friendly antifungal agents or leads for novel antifungal drugs. The knowledge about the different mechanisms of action indicate that the cell membrane is a major target for these compounds, but that specific enzymes and metabolic pathways are also targets for these compounds. It is also clear that specific fatty acids (such as certain acetylenic fatty acids) may be active in several pathways. The current knowledge about the cellular targets of antifungal fatty acids is summarised in figure 1.

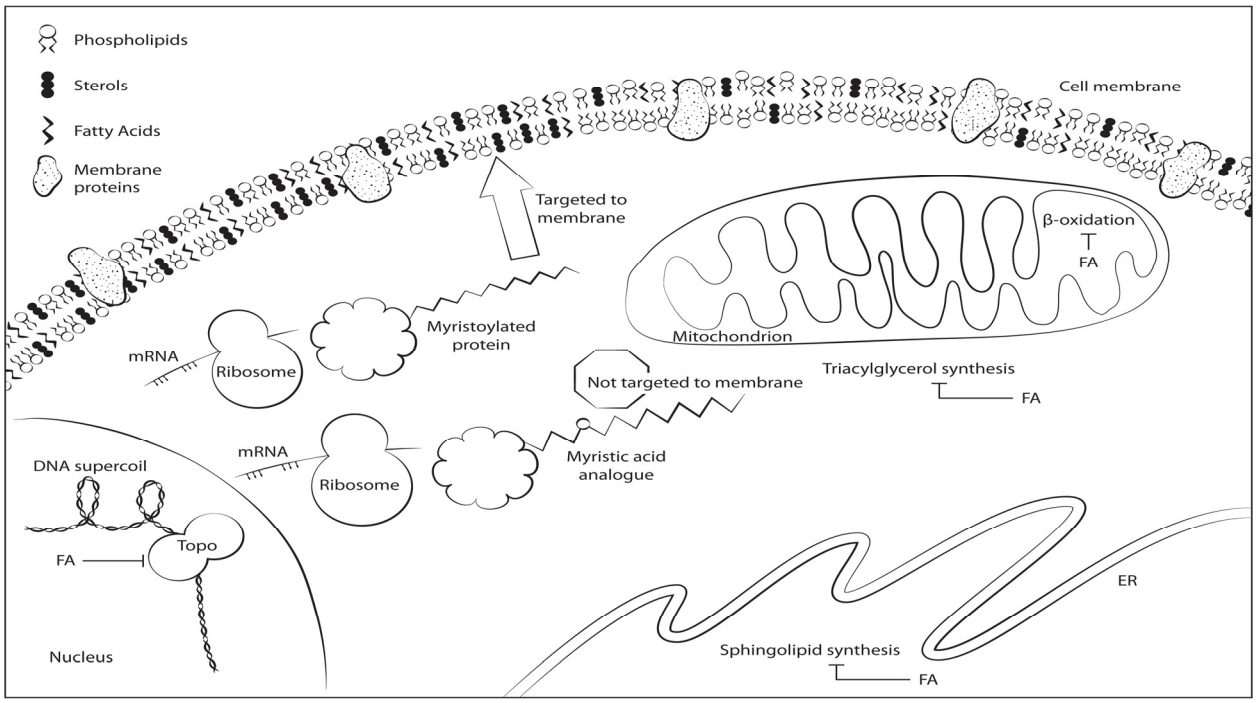

Fig. 1 Antifungal mechanisms of free fatty acids. They may disrupt the cell membrane, especially in cells with low sterol content (as indicated in the right hand section of the cell membrane); they may inhibit myrisoylation of proteins and subsequent targeting of these proteins to the cell membrane; they may inhibit b-oxidation, triacylglycerol synthesis and sphingolipid synthesis and they may inhibit topoisomerase activity.

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