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Synthesis and biological evaluation of a new series of *N*-acyldiamines as potential antibacterial and antifungal agents

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ABSTRACT

In continuation of our efforts to find new antimicrobial compounds, series of fatty *N*-acyldiamines were prepared from fatty methyl esters and 1,2-ethylenediamine, 1,3-propanediamine or 1,4-butanediamine. The synthesized compounds were screened for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and for their antifungal activity against four species of Candida (*C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis*). Compounds **5a** (*N*-(2-aminoethyl)dodecanamide), **5b** (*N*-(2-aminoethyl)tetracanamide) and **6d** (*N*-(3-aminopropyl)oleamide) were the most active against Gram-positive bacteria, with MIC values ranging from 1 to 16 μg/mL and were evaluated for their activity against 21 clinical isolates of methicillin-resistant *S. aureus*. All the compounds exhibited good to moderate antifungal activity. Compared to chloramphenicol, compound **6b** displayed a similar activity (MIC_{50} = 16 μg/mL). A positive correlation could be established between lipophilicity and biological activity.

There is no doubt about the success of antimicrobial therapy throughout the world, but the indiscriminate use of antibiotics for the prevention and treatment of diseases has accelerated the emergence of drug-resistant strains. Antimicrobial resistance has become one of the most serious health threats, resulting in an urgent need to find new antimicrobial compounds.

The antimicrobial properties of fatty acids and their derivatives are well known.[1] Free fatty acids are potent enzyme inhibitors, and unsaturated fatty acids are usually more active than the saturated ones.[2,3] The polyunsaturated linoleic acid displays antifungal activity against several plant pathogenic fungi[4] and arachidonic acid may increase antifungal susceptibility of biofilms formed by *Candida* species[5] to antibacterial compounds. Fatty amides derived from anthranilic acid have been shown to exhibit Gram-positive growth[6] and unsaturated fatty esters are active against oral microorganisms.[7]

We showed in a previous work that *N*-acyldiamines inhibited the growth of several strains of Gram-positive and Gram-negative bacterial strains.[8] It is believed that these compounds act as non-ionic surfactants: the hydrophobic portion of the molecule is able to penetrate the lipid bilayer structure of the bacterial membrane, disturbing its permeability and causing the cell death.

In continuation to our ongoing efforts to find new antimicrobial,[9–11] the present study reports the preparation and in vitro evaluation of the antimicrobial activities against Gram-positive and Gram-negative bacteria and fungi of a series of *N*-acyldiamines synthesized by the reaction of long chain saturated fatty acids (12–16 carbons) or oleic acid with 1,2-ethylendiamine, 1,3-propanediamine and 1,4-butanediamine.

The synthesis of the compounds was performed according to literature procedure.[8] Briefly, the methyl fatty esters were treated with an excess of the diamines (1,2-ethylenediamine, 1,3-propanediamine, 1,4-butanediamine in methanol under reflux (Scheme 1), leading to the expected amides in good yields. All the compounds were purified by recrystallization from methanol, except compounds **5d, 6d** and **7d** which were purified by column chromatography on silica gel. All the compounds were characterized by IR and NMR spectroscopy. The spectroscopic data (Supplementary data) were in full agreement with those expected.

The antibacterial activity of the synthesized compounds was assessed in vitro against two Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 12228), two Gram-negative bacteria (*Escherichia coli* ATCC 11229 and *Pseudomonas aeruginosa* ATCC 27853) and 21 clinical isolates of MRSA obtained from the culture collection at the Laboratory of Bacterial Physiology and Molecular Genetics, Federal

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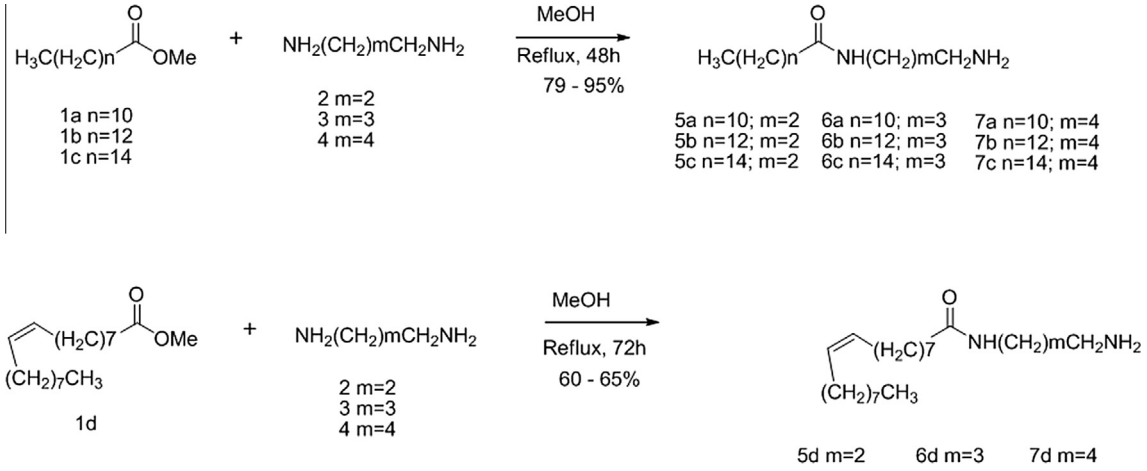


Scheme 1. Synthesis of *N*-acyldiamines **5a–d**, **6a–d** and **7a–d**.

University of Juiz de Fora. The minimal inhibitory concentration (MIC) was determined by the broth dilution method in Mueller Hinton medium (DIFCO Laboratories, Detroit, MI, USA), according to the CLSI.[12] Chloramphenicol and vancomycin were used as reference antimicrobials considering the bacterial groups evaluated (Table 1).

For the determination of the bactericidal concentrations of the compounds, selected aliquots (two dilutions below the MIC and two dilutions higher than the MIC) were transferred with the aid of a sterile swab to a Petri dish containing Mueller Hinton Agar (MHA, Himedia Laboratories, Mumbai, India AMH). The plates were incubated under aerobic conditions at 37 °C for 24 h. The minimum bactericidal concentration (MBC) was determined considering the solution of the lower concentration of tested compound which resulted in no microbial growth after culture in MHA. If bacterial growth was observed at all concentrations studied, the compound was classified as bacteriostatic.[13,14]

The antifungal activities of the synthesized compounds were evaluated against four strains of Candida (*Candida albicans* ATCC 18804, *Candida tropicalis* ATCC 750, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 22019). Antifungal susceptibility testing was performed using the broth microdilution method according to CLSI guidelines for antifungigram (CLSI) document M27-A3.[15] Itraconazole was used as reference antifungal agent.

The MIC values are shown in Table 2. With the exception of compounds **5c** and **6b**, all of the *N*-acyldiamines showed good to moderate activity against at least one the bacteria tested. In general they were more active against Gram-positive bacteria than against Gram-negative strains. Amides **6c** and **7c**, both carrying a saturated hexadecanoyl moiety were active against two of the

Table 1
Minimum inhibitory concentrations (MIC, μg/mL)

	Gram-positive		Gram-negative	
Compounds	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
5a	16	16	8	8
5b	8	16	64	1024
5c	>1024	>1024	>1024	>1024
5d	>1024	8	>1024	>1024
6a	256	32	128	64
6b	>1024	1024	>1024	1024
6c	>1024	4	32	>1024
6d	2	1	4	>1024
7a	64	16	64	32
7b	16	16	256	1024
7c	512	16	1024	>1024
7d	16	4	8	>1024
Chloramphenicol	16	32	16	32
Vancomycin	0.5	—	—	—

Table 2
Minimal bactericidal concentrations (μg/mL)

Compounds	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
5a	16 μg/mL	16 μg/mL	16 μg/mL	16 μg/mL
5b	128 μg/mL	**	**	**
5c	—	—	—	—
5d	—	**	—	—
6a	**	32 μg/mL	512 μg/mL	256 μg/mL
6b	—	**	—	**
6c	—	64 μg/mL	**	—
6d	128 μg/mL	16 μg/mL	**	—
7a	512 μg/mL	16 μg/mL	64 μg/mL	32 μg/mL
7b	1024 μg/mL	32 μg/mL	1024 μg/mL	1024 μg/mL
7c	**	128 μg/mL	1024 μg/mL	—
7d	**	128 μg/mL	**	**
Chloramphenicol	**	128 μg/mL	**	**

** Bacteriostatic.

Table 3
Minimum bactericidal activity (μg/mL) of compounds **5a**, **5b** and **6d** against MRSA

Clinical isolates of MRSA	**5a**	**5b**	**6d**	Chloramphenicol	Vancomycin
1	1024	—[a]	16	64	1
2	256	512	16	4	1
3	1024	512	16	4	0.5
4	256	512	512	8	1
5	32	512	16	4	1
6	32	512	64	8	1
7	32	—[a]	16	64	1
8	16	—[a]	16	64	0.5
9	32	—[a]	16	8	1
10	32	512	8	64	1
11	>1024	—[a]	16	64	1
12	>1024	—[a]	16	64	1
13	32	—[a]	64	64	1
14	>1024	128	1024	64	1
15	1024	64	16	32	1
16	1024	—[a]	8	32	1
17	256	128	16	4	0.5
18	32	128	8	8	1
19	128	—[a]	4	128	0.5
20	256	—[a]	1024	4	0.5
21	>1024	128	512	8	0.5

[a] Bacteriostatic.

Table 4
MIC_{50} and MIC_{90}

Compounds	MIC (μg/mL)		
	50%	90%	Range
5a	32	>1024	16–>1024
5b	32	256	32–256
6d	16	512	4–1024
Chloramphenicol	16	32	8–128

Table 5
Antifungal activity of *N*-acylated diamines (MIC μg/mL)

Compounds	*C. albicans* ATCC 18804	*C. tropicalis* ATCC 750	*C. glabrata* ATCC 90030	*C. parapisilosis* ATCC 22019
5a	128	16	16	16
5b	4	16	16	32
5c	4	16	8	4
5d	128	128	64	128
6a	256	128	32	64
6b	32	32	64	16
6c	64	16	8	8
6d	**2**	**4**	**4**	**4**
7a	256	128	1024	256
7b	16	64	32	128
7c	8	64	32	128
7d	**2**	**4**	**2**	**1**
Itraconazole	0.5	>512	256	>512

Bold values are the highest MIC.

bacteria strain tested. This was expected: considering the cell wall complexity of Gram-negative bacteria, it is expected to be less sensitive to amphiphilic compounds than Gram-positive bacteria. The three dodecanoic acid derivatives **5a**, **6a** and **7a** were active against the four bacteria tested, with MIC values ranging from 8 to 256 μg/mL. The amide **5a** exhibited a higher growth inhibitory potency when compared to the positive control chloramphenicol. The best MIC values, ranging were obtained for the *N*-oleylamine **6d**, against *S. aureus*, *S. epidermidis* and *E. coli* (2 μg/mL, 1 μg/mL and 4 μg/mL, respectively).

These results show that, if lipophilicity is important for the activity, the late one decreases with the increase of the carbon chain, as evidenced by the loss of activity observed comparing the MIC values obtained for the tetradecanoyl series (compounds **5a**, **6a**, **7a**) to those obtained for the hexadecanoyl series (compounds **5c**, **6c** and **7c**).

Although oleic acid has a longer carbon chain (18 carbon atoms) its derivatives **6d** and **7d** showed high antibacterial activities against Gram-positive bacteria and against *E. coli*, with MIC values ranging from 1 to 16 μg/mL. Thus, it seems that not only the size of the lipophilic chain is important, but also the presence of insaturations. Others works described the higher potency of unsaturated fatty acids versus saturated acid.

Regarding to the diamine moiety, it appears from the comparison of the MIC values obtained for compounds **5**–**7a**, or for compounds **5**–**7b**, that ethylenediamine provides a better antibacterial activity than 1,3-propylenediamine or 1,4-butylamine. This could not be observed for the oleyl series (compounds **5**–**7d**) in which the highest MIC is of the 1,3-propanediamine derivative **7d**.

Compounds **5a**, **5b** and **6d** evaluated for their antibacterial activity against 21 clinical isolates of methicillin-resistant *S. aureus* (MRSA) isolated from patients of the University Hospital Juiz de Fora, between the years 2005 and 2010. The tests were performed using the broth dilution method as described above to determine the minimum bactericidal activity of the compounds (Table 3). The activities are also presented in terms of inhibitory potencies (minimum inhibitory concentration at which 50% or 90% of strains tested were inhibited) and variation of MICs (Table 4).

The results show that the compounds **5a**, **5b** and **6d** were active against almost all of the MRSA isolates. The oleyl derivative **6d** showed bactericidal activity against all the tested clinical samples, with MIC values ranging from 4 to 1024 μg/mL. Furthermore this compound presented the same antibacterial potency in vitro than chloramphenicol, with MIC_{50} = 16 μg/mL.

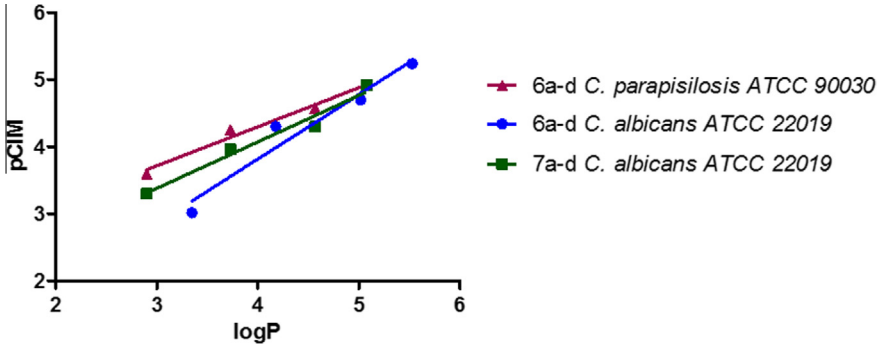
From the results shown in Table 5, it is possible to observe that the *N*-acyldiamines displayed significant antifungal activity, and in most cases the MIC values were lower than the values found for the reference drug. The most active compounds were the oleyl derivatives **6d** and **7d**. Free fatty acids are known to have antifungal properties, as the aliphatic chain can insert into the cell membrane and disturb it. The higher antifungal activity of unsaturated fatty acids is due to the greater cross section they occupy in the membrane and possibly to the increased oxidative stress they cause.

In an attempt to correlate the lipophilicity to the antifungal activity of the tested compounds, the MIC values expressed as mmol L^{-1} were converted to pMIC and log*P* was calculated using ChemDraw Ultra (CambridgeSoft). P is an important physicochemical parameter that reflects the ability of the compound to penetrate biological membranes, has a great influence on the bioavailability of the drug, and is often correlated with its biological activity. The coefficient is expressed as log*P* and can be determined experimentally or calculated. The data were subjected to linear regression analysis using Origin 8.0 software (Scheme 2). A high collinearity, indicated by the coefficient of pearson (r >0.9), was found for two series of *N*-acyldiamines **6a**–**d** and **7a**–**d**, indicating that in these series, the antifungal activity depends on lipophilicity of compounds.

6a–**d** *C. parapisilosis* ATCC 22019: pMIC = 0.5816log(*P*) + 1.9702 r = 0.97

6a–**d** *C. albicans* ATCC 18804: pMIC = 0.69404log(*P*) + 1.29526 r = 0.95

7a–**d** *C. albicans* ATCC 18804: pMIC = 0.95094log(*P*) − 0.02844 r = 0.92



Scheme 2. Linear regression analysis for antifungal activity.

The *N*-acyldiamines were synthesized in good yields from fatty acids and showed good in vitro antibacterial and antifungal activities against ATCC isolates of bacteria (Gram-positive and Gram-negative), clinical isolates of MRSA and fungus. *N*-(2-aminoethyl)dodecanamide **5a** showed the best activity against the ATCC bacteria tested, and *N*-(3-aminopropyl)oleamide **6d** was the most active against MRSA clinical isolates. Compound **6d** and *N*-(4-aminobutyl)oleamide **7d** where the most active against *Candida* strains, and it was possible to establish a correlation between their lipophilicity and antifungal activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.08.047.

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