Synthesis and antituberculosis activity of new fatty acid amides

Caroline Da Ros Montes D'Oca [a], Tatiane Coelho [b], Tamara Germani Marinho [a], Carolina Rosa Lopes Hack [a], Rodrigo da Costa Duarte [a], Pedro Almeida da Silva [b,†], Marcelo Gonçalves Montes D'Oca [a,*,†]

[a] Laboratório Kolbe de Síntese Orgânica, Escola de Química e Alimentos, Universidade Federal do Rio Grande, Rio Grande, RS, Brazil
[b] Laboratório de Micobacteriologia, Faculdade de Medicina, Universidade Federal do Rio Grande, Rio Grande, RS, Brazil

ABSTRACT

This work reports the synthesis of new fatty acid amides from C16:0, 18:0, 18:1, 18:1 (OH), and 18:2 fatty acids families with cyclic and acyclic amines and demonstrate for the first time the activity of these compounds as antituberculosis agents against Mycobacterium tuberculosis $H_{37}Rv$, M. tuberculosis rifampicin resistance (ATCC 35338), and M. tuberculosis isoniazid resistance (ATCC 35822). The fatty acid amides derivate from ricinoleic acid were the most potent one among a series of tested compounds, with a MIC 6.25 µg/mL for resistance strains.

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Mycobacterium tuberculosis causes more human deaths than any other single infectious organism with an estimated eight million new tuberculosis (TB) cases and two million fatalities each year.[1] Although currently available treatment regimens can cure most cases susceptible to drugs, factors such as long lead times of therapy, the need for multidrug, resistance emergence, co-infection with HIV and persistent strains, requires the urgent need to develop new drugs against TB.[2,3] In particular, strains resistant to two most important drugs, isoniazid (INH) and rifampicin (RMP), can cause serious repercussions in the epidemiology and disease control.[4] INH is a prodrug that requires processing by the bacterial catalase–peroxidase to become active. Once activated, it inhibits the biosynthesis of mycolic acids, which are essential components of the mycobacterial cell wall. This drug is bactericidal against metabolically active bacilli and bacteriostatic against resting bacilli. Resistance to INH is mostly associated with mutations or deletions in *kat*G; other mutations related with INH resistance occur in the coding region of *inh*A gene (or its promoter),[3] however, some INH resistant strains do not present any alteration in these loci. For these strains, it was been postulated that INH resistance could be due to mutations in an unknown genes, permeability decrease or efflux increase.[5] A great variety of studies have been performed to resolve the resistance and toxicity of INH, wherein amphiphilic prodrugs are of interest because they are prone to penetrate into bacterial cells.[6]

The fatty acids amides have been considered as a new family of biologically lipids by different biochemical and pharmacological studies,[7] because it have industrial applications[8] and important biological activities.[9] Research about these compounds has been shown to be involved in many organ systems, such immune, energy balance, food intake, metabolic homeostasis, cardiovascular, depressive effects, fertility, pain, and neuroprotection.[10–12] The fatty acid amides may also have a role in cancer biology because of its effects on cell proliferation.[13,14] The identification of two biologically active fatty acid amides, *N*-arachidonylethanolamide (anandamide)[15] and *cis*-oleamide (*c*-OA),[16] the best-known and best-understood members of a much larger family (Fig. 1), stimulated researches for isolation, characterization, and synthesis of
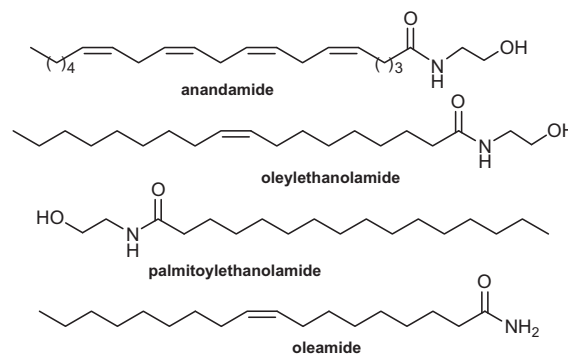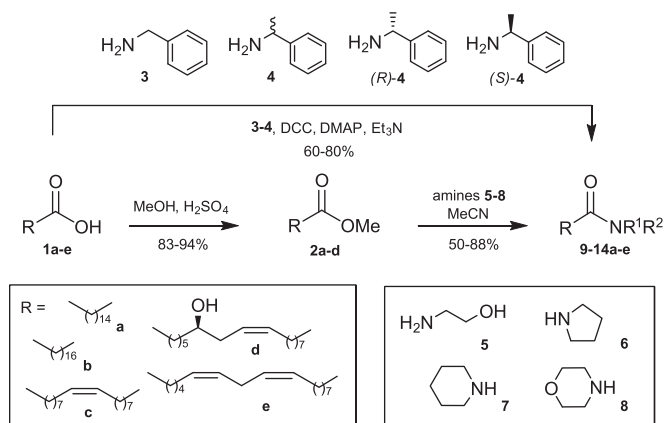

Figure 1. Structures of biologically active fatty acid amides.

Scheme 1. Synthesis of the fatty acid amides **9–14a–e**.

novel fatty acid amides with important pharmacological proprieties, such as palmitoylethanolamide,[17] oleylethanolamide,[18] and macamides.[19] Studies of the structure–activity relationships of the analogs anandamide and oleamide revealed the importance of their fatty acid moieties for different biological activity.[20,21]

In the present study, several new fatty acid amides, derivates from saturated and unsaturated fatty acid chains with cyclic and acyclic amines were synthesized. The antituberculosis activity of this new biologically lipids family was tested for the first time in vitro against *M. tuberculosis* H_{37}Rv, which is susceptible to RMP and INH, *M. tuberculosis* rifampicin resistance (RMPr, ATCC 35338), and *M. tuberculosis* isoniazid resistance (INHr, ATCC 35822).

The synthesis of the fatty acid amides **9–14a–e** is summarized in Scheme 1. The amides **9a–e**, **10a–e**, and **12e** were synthesized by the reaction of the respective fatty acid **1a–e** (0.3 mmol) with amines **3**, **4** (0.3 mmol), triethylamine (0.3 mmol), catalytic amount of (dimethylamino)pyridine (DMAP), and dicyclohexyl carbodiimide (DCC, 0.3 mmol) in CH_2Cl_2 added drop-to-drop to the above reaction mixture and stirred at room temperature for 24 h. The solid dicyclohexylurea formed was removed by filtration and the filtrated was dried under reduced pressure. The amides **11–14a–d**[22] were synthesized from fatty acid methyl esters (FAMEs) **2a–d** obtained from esterification of the respective fatty acid **1a–d**. The fatty acid methyl esters (0.3 mmol) aminolisys reaction was realized in the presence of the amines **5–8** (1.8 mmol) and acetonitrile for 24 h. The progress of the reactions was monitored by silica gel TLC. The raw products were purified by chromatography column on silica gel (*n*-hexane/ethyl acetate, 7:3) and analyzed by NMR ^{1}H, ^{13}C, IR, and ESI-MS/MS.

The antimycobacterial activity of the compounds was tested and the minimum inhibitory concentration (MIC) was determined using REMA (Resazurin Microtitre Assay) method.[2] Rifampicin and isoniazid (Sigma Chemical Co.) were used as reference drugs. The bacterial suspensions were prepared in sterile water containing 3 mm glass beads. The suspensions were homogenized by vortex agitation and the turbidity was adjusted in agreement with tube one of the scale of McFarland (3.2×10^6 colony-forming units/mL). The inoculums was prepared by diluting the bacterial suspension 1:20 in Middlebrook 7H9 medium (4.7 g Middlebrook 7H9 base; Difco, Becton Dickinson). Briefly, the assay is performed in 96-well microplates using 7H9 OADC medium enriched with 10% (v/v) oleic acid–dextrose–albumin–catalase (BBL) and with fatty acid amides dissolved in dimethyl sulfoxide (DMSO). MIC determination was carried out by twofold serial dilutions of the compounds (range 100.0–3.2 μg/mL) dispensed into each well of a 96-well microtitre plate. The microplate was incubated at 37 °C for 7 days. After this period, it was added 30 μL of resazurin in each

Table 1
Fatty acid amides antituberculosis activity

Entry	R	NR^{1}R^2	Fatty acid amides	MIC^a (μg/mL)		
				H37Rv	35338^b	35822^c
1			(*R*)-**10a**	>100	>100	>100
2			(*S*)-**10a**	>100	>100	>100
3			**11a**	>100	>100	>100
4			**12a**	25	25	25
5			**13a**	25	25	25
6			(*R*)-**10b**	>100	>100	>100
7			(*S*)-**10b**	>100	>100	>100
8			**9c**	(−)	(−)	100
9			(*R*)-**10c**	(−)	50	12.5
10			(*S*)-**10c**	(−)	(−)	(−)
11			**10c**	(−)	100	(−)
12			**11c**	25	25	12.5
13			**12c**	25	12.5	6.25
14			**13c**	50	25	12.5
15			**14c**	25	12.5	6.25
16			(*R*)-**9d**	25	12.5	12.5
17			(*R*,*R*)-**10d**	100	25	12.5
18			(*R*,*S*)-**10d**	100	50	100
19			(*R*)-**12d**	12.5	6.25	6.25
20			(*R*)-**14d**	50	12.5	6.25
21			(*R*)-**10e**	100	25	12.5
22			(*S*)-**10e**	50	12.5	6.25
23			**12e**	12.5	12.5	12.5

^{a} MIC INH: 0.02 μg/mL; RMP: 1 μg/mL.
^{b} RMPr, mutation on rpoB His-526-Tir gen.
^{c} INHr, mutation on katG gen.

well and incubated for 2 more days at 37 °C. The reading was done from the oxi-reduction of the resazurin, noticing change of color when cellular growth was taking place. The activity of the tested compounds is provided in Table 1.

Concerning the fatty acid moiety of the tested compounds, MIC results indicate that introduction of alkene chains increase the activity. For the unsaturated fatty acid chains was observed a degree MIC of >100 μg/mL to 50, 25, and 12.5 μg/mL, comparing compounds (*R*)-palmitoylmethylbenzylamide (**10a**) and (*R*)-oleylmethylbenzyla-

mide (**10c**), palmitoylethanolamide (**11a**), and oleylethanolamide (**11c**). Regarding the polar moiety (R^1) of tested compounds, the lower MIC was found when used cyclic amines. The best results, also in connection with a lipophilic factor, were found when unsaturated chains were combined with cyclic amines, in special pyrrolidine and morpholine (compounds **12c**, **14c**, **12d**, **14d**, and **12e**). The chiral compounds also demonstrated influence about antituberculosis activity. In assay with racemic oleylmethylbenzylamide (**10c**), MIC 100 μg/mL was obtained for RMPr and any activity for H_{37}Rv and INHr. The compound (*S*)-**10c** was not active against *M. tuberculsis*, but its enantiomer (*R*)-**10c** inhibited bacterial growth for RMPr and INHr in MIC 50 and 12.5 μg/mL, respectively. This fact suggests that, to **10c** probably the presence of (*S*)-enantiomer inhibits the action of (*R*)-enantiomer. For the compounds linoleylmethylbenzylamide (*R*)- and (*S*)-**10e** the lowest MIC were obtained for (*S*)-enantiomer. In this case, probably the presence of polyunsaturated chain exerts greater influence over (*S*)-isomer.

Finally, amides derivates from ricinoleic acid were tested. Ricinoleic acid (C18:1, OH) or 12-hydroxy-9-*cis*-octadecenoic acid is the major constituent (80–90%) of castor oil (*Ricinus comunnis*)[23] and is an uncommon fatty acid which contains both a double bond and a hydroxyl group in chain. Hydroxyl groups are rare in plant oils and afford these oils some interesting chemical properties. The fatty acid amides **9d**, **10d**, **12d**, and **14d** derived from ricinoleic acid showed interesting results against tuberculosis strains. The compound ricinoleylpyrrolidilamide, (*R,R*)-**12d**, showed the best antitubercular activity, with MIC 12.5 and 6.25 μg/mL for resistance strains.

Concluding, this study demonstrated for the first time, the fatty acid amides activity as *M. tuberculosis* inhibitors. The compound (*R,R*)-**12d** showed the best inhibitory activity, include for *M. tuberculosis* RMPr and INHr strains, presenting a MIC similar to pyrazinamide, ethambutol, and ofloxacin.[3] Taking into account our preliminary results on the evaluated families compounds, our efforts are now focused on the understanding of the antimicrobial activity of fatty acid chains, however, the fact that there were no significant differences in antimicrobial activity against strains sensitive and resistant to permit infer the absence of cross-resistance with rifampicin and isoniazid, which is strongly positive for TB control-resistant. Efforts are also undertaken towards elaboration of new fatty acid compounds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.149.

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