Bioorganic & Medicinal Chemistry 20 (2012) 4614–4624

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Chemoenzymatic synthesis and biological evaluation of 2- and 3-hydroxypyridine derivatives against *Leishmania mexicana*

Guadalupe García Liñares [a], Gonzalo Parraud [a], Carlos Labriola [b], Alicia Baldessari [a,*]

[a] Laboratorio de Biocatálisis, Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, piso 3, C1428EGA Buenos Aires, Argentina
[b] Instituto de Investigaciones Bioquímicas, Av. Patricias Argentinas, 435C1405BWE Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 24 April 2012
Revised 8 June 2012
Accepted 15 June 2012
Available online 23 June 2012

Keywords:
Hydroxypyridine derivatives
Lipase-catalyzed
Synthesis
Leishmaniasis

ABSTRACT

A series of hydroxyalkyl and acyloxyalkyl derivatives of 2- and 3-hydroxypyridine was synthesized and their biological activity was evaluated as growth inhibitors of protozoan *Leishmania mexicana*. Thirty novel compounds were obtained through a chemoenzymatic methodology in two reaction steps. The influence of various reaction parameters in the enzymatic step, such as enzyme source, acylating agent/substrate ratio, enzyme/substrate ratio, solvent and temperature, was studied. Some of the evaluated compounds showed a remarkable activity as *Leishmania mexicana* growth inhibitors, obtaining the best results with the acetylated derivatives. The advantages showed by the enzymatic methodology, such as mild reaction conditions and low environmental impact, make the biocatalysis a convenient way to prepare these derivatives of substituted pyridines with application as potential antiparasitic agents.

1. Introduction

Leishmaniasis is considered by World Health Organization to be one of the most serious diseases worldwide caused by protozoan parasites.[1] The leishmaniasis are a complex of clinical diseases produced by at least 17 species and some mutants of the protozoan *Leishmania*.[2,3] The spectrum of disease comprises three major syndromes: cutaneous, mucocutaneous and visceral leishmaniasis. *Leishmania mexicana* is one of the species that produce cutaneous leishmaniasis.[4] Twelve million people suffer from human leishmaniasis worldwide with an incidence of two million cases per year and 350 million individuals at risk focused at tropical and subtropical regions.[5–7] Moreover, the incidence of leishmania and HIV coinfection has been increased worldwide due to occurrence of visceral leishmaniasis in urban areas.[8]

Control of this disease remains a problem because effective vaccines are not available up until now and the chemotherapy is still deficient, using old drugs associated to long-term treatments, parasitic resistance, and different drug sensitivity. The chemotherapy for leishmaniasis has been based for over 60 years on the use of pentavalent antimonial drugs: meglumine antimoniate (Glucantime)[9] (**1**) or sodium stibogluconate (Pentostam) (**2**) are still the first-choice drugs for Leishmaniasis (Fig. 1). However, these drugs are very unsatisfactory due to their frequent toxic effects and the growing rates of resistance to them in several parts of the world.[10] Second line drugs include pentamidine (**3**) and amphotericin B (**4**), which are mainly employed in resistant cases when the antimonials fail.[11] The disadvantages of these drugs include their high cost, the need for long-term treatment, the lack of an oral formulation, the loss of effectiveness due to parasites resistance and serious toxic effects.[10,12,13] Recently, the oral drug miltefosine (**5**)[14] was approved for the treatment of human visceral *Leishmania* infections, but a WHO report indicates that it is teratogenic.[15,16] Two interesting drugs under clinical trials are the antibiotic paromomycin (**6**)[2,17] and sitamaquine (**7**)[18] (Fig. 1).

Extensive studies have been made in the last few years of new and diverse molecules used as possible antileishmanial drugs. For example, arylquinuclidine[19] derivatives, developed as cholesterol-lowering agents, were potent in vitro growth inhibitors of *Leishmania amazonensis* and 1,3,4-thiadiazolium-2-aminide[20]exhibited significant activity against *Leishmania amazonensis*. Some tetrahydro-1-benzazepine derivatives were found to have good activity against *Leishmania chagasi* promastigotes.[21] The activity of hydroxyurea was also tested against *Leishmania mexicana*, showing to be a good candidate for drug therapy for leishmaniasis because it induces parasite death when it is used at concentrations ranging from 10 to 100 μg/mL.[22] A series of 1,3,5-triazines and pyrimidines derivatives were synthesized and screened for their in vitro antileishmanial activity against *Leishmania donovani* and some compounds have shown more than 90% inhibition against promastigotes.[23] However, the problems of drug resistance and the side effects of the chemotherapies used at present, have not been solved. Consequently, the development

* Corresponding author. Tel.: +54 11 4576 3300x262; fax: +54 11 4576 3385.
E-mail address: alib@qo.fcen.uba.ar (A. Baldessari).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.06.028

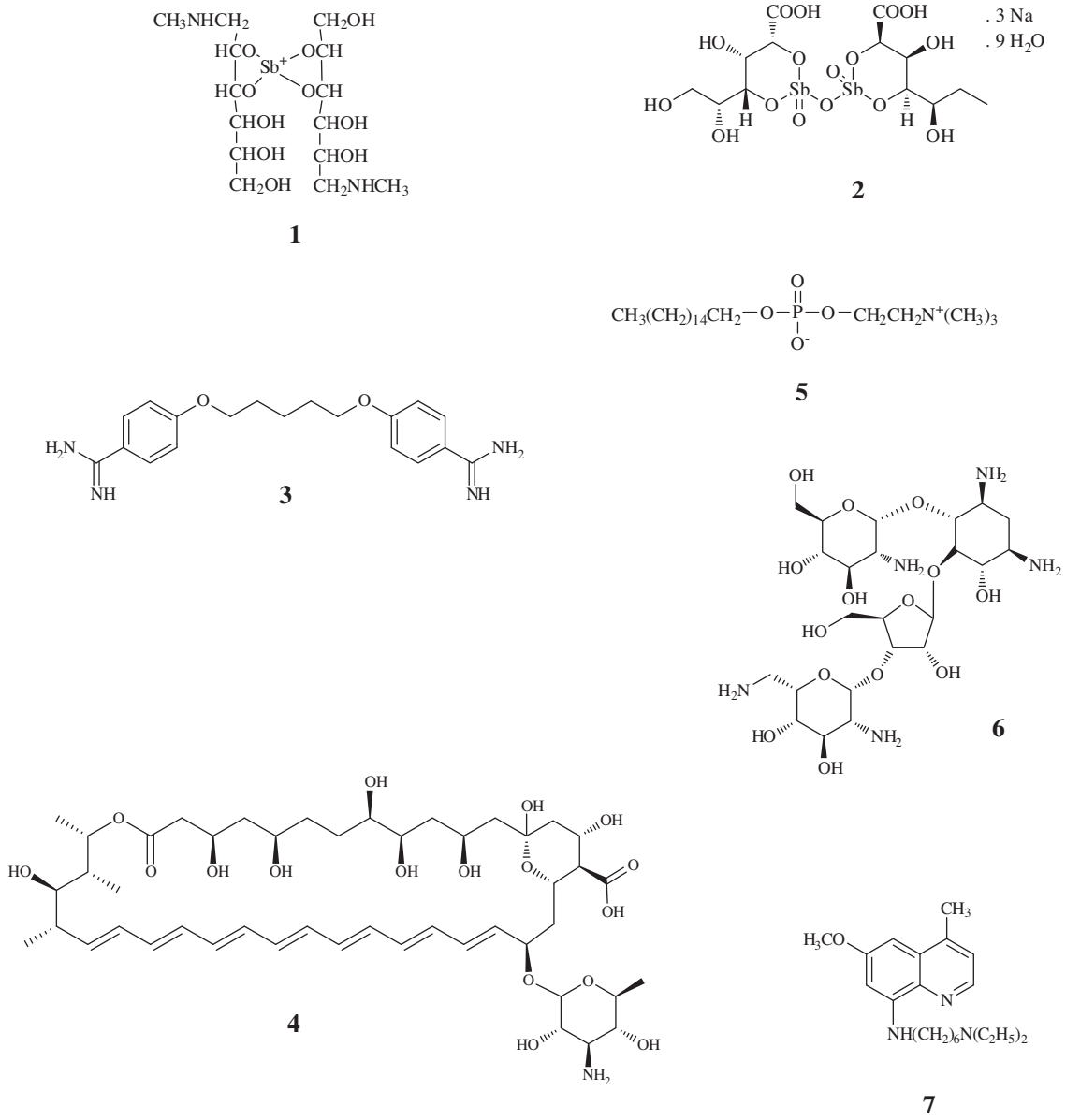


Figure 1. Current drugs clinically employed for the treatment of leishmaniasis.

of novel, safe and affordable compounds with potent anti-leishmanial activity is urgently needed.

On the other hand, it is known that the use of enzymes and whole cells of microorganisms in synthesis of pharmaceuticals derivatives is increasing in the last years.[24–26] It is recognized that enzymes are capable of accepting a wide array of substrates, and catalyze enantio-, chemo- and regioselective reactions. As a result, biocatalysts allow carrying out different chemical transformations without the need for tedious protection and deprotection steps, especially in compounds with several functional groups. Over the last years, biocatalysis in non-aqueous media has been widely used for several synthetic reactions such as esterification, transesterification, aminolysis, polymerization, etc.[27] Enzymes are also well-known by their high enantioselective behavior and this property has formed the basis for the widespread use of enzymes for the synthesis of enantiomerically pure compounds.[28]

Studies carried out in our laboratory on the esterification and transesterification of multiple substrates have shown that lipases are useful in the synthesis of pharmaceuticals and biologically active compounds.[29–33]

In the present study, we report the synthesis of a series of 2- and 3-hydroxypyridine derivatives **8a–n** and **9a–p** (Fig. 2).

The chemoenzymatic strategy allowed us to obtain these novel compounds which were biologically evaluated against promastigote *Leishmania mexicana*.

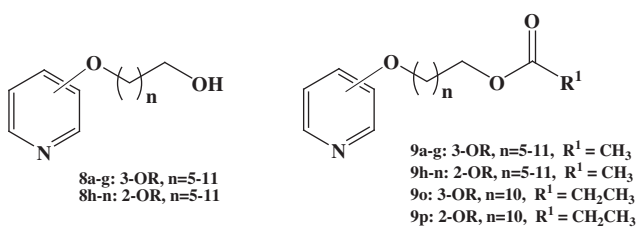


Figure 2. Structures of 2-hydroxy- and 3-hydroxypyridine derivatives.

2. Results and discussion

2.1. Rationale

Studies of features of the biochemistry and physiology of *Leishmania* spp. have recognized several potential molecular targets for the design of new drugs. The chemotherapeutic search is based on metabolic differences between the pathogenic parasite and mammals hosts. Thus, the selective inhibition of key biosynthetic pathway for parasite survival would not have any toxic effects for the host. A valid molecular target for antileishmania compounds is the enzyme dihydrofolatereductase (DHFR).[2] DHFR catalyses the reduction of dihydrofolate to tetrahydrofolate. Tetrahydrofolate is methylated to form methylene tetrahydrofolate, which is a vital cofactor to convert deoxyuridine monophosphate into thymidine monophosphate. DHFR constitutes an interesting target for drug design, because DHFR inhibition prevents biosynthesis of thymidine, leading to cell death.[34] In addition, the leishmanial DHFR and the corresponding human enzyme show structural differences.[35] A selective inhibition of leishmanial DHFR would lead to growth impairing of the parasite because these microorganisms do not have a mechanism of transport of this cofactor from the host.

Most known DHFR inhibitors contain a heterocyclic aromatic ring. For example, pyrimidines or triazines with amino substituents at the 2- and 4-positions of general formula **10**–**12** inhibited DHFR activity (Fig. 3).[36–38] Some of these compounds were potent inhibitors of DHFR activity but the maximum activity against parasite cell not always correlate with the more potent enzymatic inhibitors. It was reported that long chain derivatives (8–12 carbon atoms) were the most potent cell growth inhibitors.[37]

On the other hand, there are several papers reporting biologically active compounds which exhibit a pyridine ring in their structure. Different substituted pyridines showed diverse biological activities, such as topoisomerase I or II inhibition,[39,40] anticancer activity,[41–43] antimicrobial activity,[44] cyclooxygenase-2 inhibition,[45] kinase inhibition,[46] antiviral activity,[47] etc. Little has been reported about new compounds that show to be very effective as DHFR and parasite growth inhibitors. Considering the above mentioned work and taking into account that pyridine derivatives can be DHFR inhibitors, we have decided to prepare new compounds containing a pyridine ring in place of aminopyrimidine ring substituted with alkyloxy groups of different chain length.

2.2. Synthesis

In order to obtain the compounds of interest, a chemoenzymatic methodology was applied, involving two steps: the first chemical and the second enzymatic.

The preparation of this new family of hydroxypyridine derivatives is illustrated in Scheme 1.

2.2.1. Synthesis of pyridinyloxyalkanols

The first reaction involved a nucleophilic substitution between the corresponding hydroxypyridine and various lineal haloalkanols with alkyl chains containing from six to twelve carbon atoms. The reaction was performed in a suspension of potassium hydroxide in dimethyl sulfoxide via a modified Williamson procedure.[48] Pyridinyloxyalkanols of general formula **8** were obtained in good to very good yields. In a second lipase catalyzed step, compounds **8** were treated with esters and, through a transesterification reaction, compounds of general formula **9** were obtained.

2.2.2. Enzymatic synthesis of pyridinyloxyakyl esters

2.2.2.1. Optimal conditions. With the aim of achieving the optimal conditions for the enzymatic reaction we studied the behavior of various lipases and some reaction parameters. In every case 12-(pyridin-3-yloxy)-dodecan-1-ol (**8g**) was used as substrate.

To begin, several commercial lipases in different solvents were evaluated in the acetylation reaction: *Candida antarctica* lipase B (CAL B), *Candida rugosa* lipase (CRL), Lipozyme, lipase from the fungus *Rizhomucor miehei* (LIP) and *Carica papaya* lipase (CPL). Reactions were carried out at 30 °C using an enzyme: substrate ratio of 10 and ethyl acetate as acylating agent and solvent. As it can be seen from Table 1 (entries 1–5), all the enzymes were active achieving **9g** in total conversion at different reaction time. CAL B gave the most satisfactory results, affording **9g** in 1 h of reaction at 30 °C. LIP and CRL were also active but showed a lower performance. CPL was effective at 55 °C showing total conversion after 96 h.

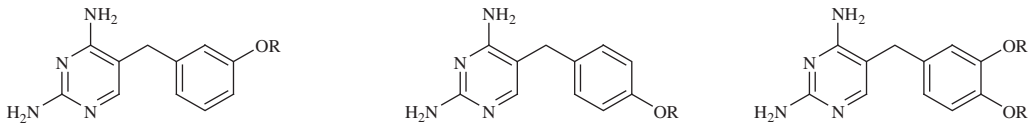
Then, we performed several experiments changing reaction parameters such as enzyme/substrate ratio (E/S) and substrate concentration. Table 1 (entries 6–11 and 12–15) show the results obtained for a CAL B catalyzed reaction at 30 °C at different E/S ratio and substrate concentration, respectively.

It has been observed that working at E/S: 0.1, total conversion was obtained at 7 h of reaction. This is a remarkably low value for CAL B catalyzed reactions compared with previous work in which it was reported that an E/S: 2 or higher was necessary to achieve goods results.[29]

As we had obtained good results with ethyl acetate as acylating agent in previous work,[30–32] we tried ethyl esters (ethyl acetate and ethyl propionate) as acylating agents. In order to get more convenient reaction conditions, in the case of acetylation we also tested activated esters such as vinyl and isopropenyl acetates. Some enzymatic acetylations need the use of these activated esters due to low reactivity of the substrate in the presence of the lipase.[33] But pyridinyloxyalkanols showed to be excellent substrates for the biocatalytic acetylation with CAL B and ethyl acetate.

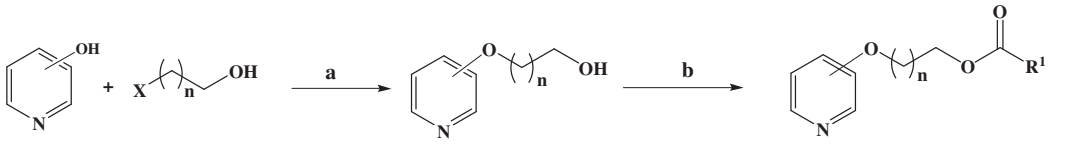
Considering that it is more convenient to work with substrate at the highest possible concentration, we studied the enzymatic transesterification at substrate concentrations between 0.04 and 0.4 M. It can be observed (Table 1, entry 13) that the optimum concentration for the reaction of 8 g was found to be 0.08 M. Total conversion was achieved at 7 h of reaction. Table 1 (entries 14–15) shows that a higher substrate concentration significantly increases reaction time.

Taking into account the studies previously mentioned, we have chosen as standard conditions for the enzymatic reaction: CAL B as



10 11 12

Figure 3. Benzyl-2,4-diaminopyrimidines as inhibitors of leishmanial DHFR activity.



13a: 3-OH
13b: 2-OH

14a: X = Cl, n=5
14b: X = Br, n=6
14c: X = Br, n=7
14d: X = Br, n=8
14e: X = Br, n=9
14f: X = Br, n=10
14g: X = Br, n=11

8a: 3-OR, n=5
8b: 3-OR, n=6
8c: 3-OR, n=7
8d: 3-OR, n=8
8e: 3-OR, n=9
8f: 3-OR, n=10
8g: 3-OR, n=11
8h: 2-OR, n=5
8i: 2-OR, n=6
8j: 2-OR, n=7
8k: 2-OR, n=8
8l: 2-OR, n=9
8m: 2-OR, n=10
8n: 2-OR, n=11

9a: 3-OR, n=5, $R^1 = CH_3$
9b: 3-OR, n=6, $R^1 = CH_3$
9c: 3-OR, n=7, $R^1 = CH_3$
9d: 3-OR, n=8, $R^1 = CH_3$
9e: 3-OR, n=9, $R^1 = CH_3$
9f: 3-OR, n=10, $R^1 = CH_3$
9g: 3-OR, n=11, $R^1 = CH_3$
9h: 2-OR, n=5, $R^1 = CH_3$
9i: 2-OR, n=6, $R^1 = CH_3$
9j: 2-OR, n=7, $R^1 = CH_3$
9k: 2-OR, n=8, $R^1 = CH_3$
9l: 2-OR, n=9, $R^1 = CH_3$
9m: 2-OR, n=10, $R^1 = CH_3$
9n: 2-OR, n=11, $R^1 = CH_3$
9o: 3-OR, n=10, $R^1 = CH_2CH_3$
9p: 2-OR, n=10, $R^1 = CH_2CH_3$

Scheme 1. Chemoenzymatic synthesis of 2-hydroxy- and 3-hydroxypyridine derivatives.

Table 1
Optimization of reaction parameters for lipase-catalyzed preparation of 12-(pyridin-3-yloxy)-dodecyl acetate (**9g**)

Entry	Enzyme	Temperature (°C)	E/S	Substrate concentration (M)	*t* (h) (total conversion)
Lipase					
1	CAL B	30	10	0.04	1
2	LIP	30	10	0.04	24
3	CRL	30	10	0.04	96
4	CPL	55	10	0.04	144
5	CPL	55	15	0.04	96
E/S					
6	CAL B	30	2	0.04	1
7	CAL B	30	1	0.04	1
8	CAL B	30	0.5	0.04	2
9	CAL B	30	0.2	0.04	4
10	CAL B	30	0.1	0.04	7
11	CAL B	30	0.02	0.04	96
Substrate concentration					
12	CAL B	30	0.1	0.04	7
13	CAL B	30	0.1	0.08	7
14	CAL B	30	0.1	0.2	24
15	CAL B	30	0.1	0.4	72

Acylating agent and solvent: ethyl acetate.

biocatalyst, temperature: 30 °C, an E/S ratio of 0.1 and substrate concentration: 0.08 M. These conditions were optimized for the acetylation reaction of **8g** with ethyl acetate.

2.2.2.2. Acylation of pyridinyloxyalkanols. Using the standard conditions described in the previous section, we applied the enzymatic strategy in the preparation of the acetyl derivatives of the pyridinyloxyalkanols **9a–n**.

Accordingly, the compounds **8a–n**, obtained in the first step of the synthesis, were dissolved in ethyl acetate, that acted both as acylating agent and solvent. The enzyme was then added and the suspension was shaken at 30 °C for 1 hour (Scheme 1). The lipase afforded each acetyl derivative (**9a–n**) in excellent yield. All products were completely identified by spectroscopic methods.

2.2.3. Synthetic results

Table 2 shows that pyridinyloxyalkanols of general formula **8** were obtained in good to very good yields.

These yields were not influenced by the length of the chain of haloalkanol. With the purpose of increasing the product yield, we have tried the same procedure but using tetrahydropyranyl ethers a protecting groups for the hydroxyl. In this case, the products were obtained in similar yields but with the disadvantage of adding two additional steps to the synthetic way.

Moreover, it was observed that reaction yields with 2-hydroxypyridine were lower than with the 3-hydroxy isomer. The keto-enol tautomerism in 2-hydroxypyridine could explain its lower reactivity. All compounds **8** were fully characterized by using NMR and IR spectroscopy and high resolution mass spectrometry.

With regard to the enzyme-catalyzed acylation, acetate derivatives of pyridinyloxyalkanols were obtained in almost quantitative yield (90–97% of isolated compound), in a slightly higher yield in the case of the 3-hydroxy derivatives. Yield increased as the alkyl chain length increased. Two propionate derivatives **9o** and **9p** were also prepared using ethyl propionate as acylating agent, which showed to be not so efficient as ethyl acetate. For the same substrate **8f**, the propionyl derivative **9o** was obtained only in 60% yield, 37% lower than the 97% yield in **9f**. In the case of the 2-hydroxy derivative, the decrease in yield for the propionylation of **8m** was even more marked, affording **9p** in only 45% yield.

Table 2
Pyridinyloxyalkanols (**8**) and their acylated derivatives (**9**)

		n	Pyridinyloxyalkanol (**8**) % yield	Pyridinyloxyalkanol acetate (**9**) % yield[a]
a	3-OH	5	66	90
b	3-OH	6	70	92
c	3-OH	7	62	90
d	3-OH	8	65	95
e	3-OH	9	65	95
f	3-OH	10	68	97
g	3-OH	11	72	99
h	2-OH	5	52	90
i	2-OH	6	55	90
j	2-OH	7	55	90
k	2-OH	8	60	94
l	2-OH	9	58	95
m	2-OH	10	50	95
n	2-OH	11	51	95
o	3-OH	10	—	60
p	2-OH	10	—	45

Reaction conditions as described in experimental.
[a] **9o** and **9p**: propionate.

The enzymatic acylation offered a good alternative to prepare ester derivatives of the pyridinyloxyalkanols. Although, the synthesis of esters performed by chemical methods is not difficult, it has the disadvantage of using acetic anhydride and pyridine or dimethylaminopyridine as reagents.[49] The enzymatic approach showed interesting advantages. The reaction was simple, it was performed at room temperature and the products were obtained in excellent yield by simple filtration and solvent evaporation. Moreover, ethyl esters used as nucleophile and solvent, are economic and less toxic than most of acylating agents commonly used in traditional synthetic procedures. The lipase is biodegradable and consequently more friendly to the environment than chemical catalysts. In addition, as the enzyme is insoluble in the reaction medium, it is easily removed by filtration and can be re-used. In the acetylation reaction of pyridinyloxyalkanols, CAL B kept 82% of activity after six reaction cycles.

2.3. Biological evaluation

The effect of different concentrations of the new pyridinyloxyalkanols (**8a–n**) and their acylated derivatives (**9a–p**) on promastigote form of *Leishmania mexicana* growth was studied during 10 days. Geneticin, a well-known antiparasitic agent, was used as a standard inhibitor at a concentration of 100 μg/mL. The evaluation was carried out by reading parasites in a Neubauer chamber every two day for ten days. A remarkable inhibitory effect on *Leishmania mexicana* growth was observed in the case of **8g**, **9g** and **9e.** The rest of the tested compounds were inactive even at a concentration of 50 μg/mL. For the lowest dosage of 10 μg/mL, 25% inhibition was detected after 6 days of incubation of **8g** and **9g**. With 20 μg/mL, 50% inhibition was observed after 1 day of exposure and a maximum of 95% and 80%, respectively was reached after 10 days. Figures 4–6 show the growth curve of promastigote *Leishmania mexicana* in the presence of compounds **8g**, **9g** and **9e**.

The three compounds showed to be the most potent against *L. mexicana* with IC_{50} = 16, 12 and 20 μg/mL, respectively, better than the standard inhibitor geneticin (IC_{50} = 50 μg/mL). Compound **9d** resulted to be moderately effective. The acetylated product **9g**, with 14 carbon atom in the side chain is the most potent, indicating that the presence of the acetyl group through the alcohol esterification increased the inhibitory activity exhibited by **8g**. Compound **9g** has twelve carbon atoms in the alcohol moiety and two atoms in the acyl chain. With the aim to test the activity of a compound with the same chain length but containing eleven carbon atoms in the alcohol moiety and three carbon atoms in the acyl chain, we prepared **9o**. This compound showed no inhibitory activity on *Leishmania mexicana* growth, indicating that this fine structural difference can influence biological activity.

3. Conclusions

This work describes a chemoenzymatic strategy for the synthesis of thirty novel pyridinyloxyalkanols and acylated derivatives, which were completely spectroscopically characterized. Lipases from different sources exhibited different performance as catalysts in the second step. CAL B lipase gave the best results. The advantages presented by this methodology are: mild reaction conditions, economy and low environmental impact. The acylation reaction with CAL-B was of great interest due to a very low E/S ratio allowed to obtain the desired products at 7 h of reaction with a 100% conversion. This result constitutes an additional advantage of this method.

All of the compounds were biological evaluated against promastigote *Leishmania mexicana*. Products **8g**, **9g** and **9e** showed to be very effective as inhibitors of parasite growth with IC_{50} = 16, 12 y 20 μg/mL. These products can be useful as potential drugs for chemotherapy of leishmaniasis. In order to establish a more complete structure-activity relationship, studies of DHFR inhibition are currently under progress.

4. Experimental

4.1. General

Chemicals were commercially available and used without further purification. Solvents were distilled before use. Dichloromethane was distilled from phosphorus pentoxide. Lipase from *Candida rugosa* (CRL) (905 U/mg solid) was purchased from Sigma Chemical Co.; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g) and Lipozyme RM 1 M (LIP) (7800 U/g) were generous gifts of Novozymes Spain; *Carica papaya* lipase (CPL) is the remaining solid fraction of papaya latex, after wash off of proteases using distilled water. CPL is a naturally immobilized enzyme and was a generous gift of Dr. Georgina Sandoval, CIATEJ, México. All enzymes were used 'straight from the bottle'. Enzyme/substrate ratio (E/S): enzyme amount in mg/substrate amount in mg. Enzymatic reactions were carried out on Innova 4000 digital incubator shaker, New Brunswick Scientific Co. at 33 and 55 °C and 200 rpm. To monitor the reaction progress aliquots were withdrawn and analyzed by

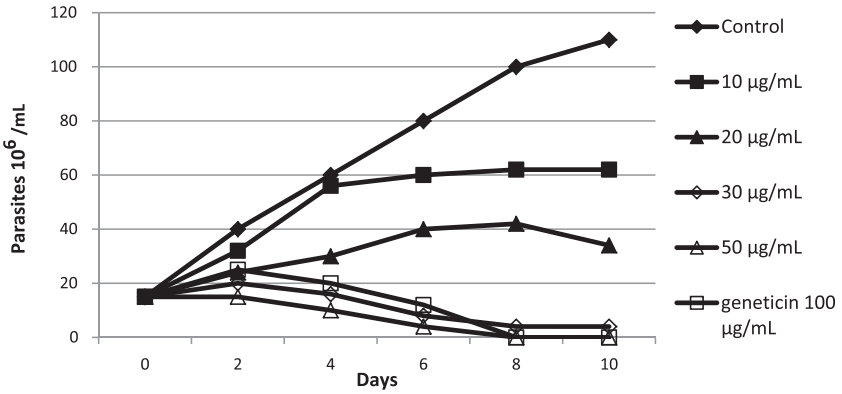


Figure 4. Effect of **8g** concentration on *Leishmania mexicana* growth rate.

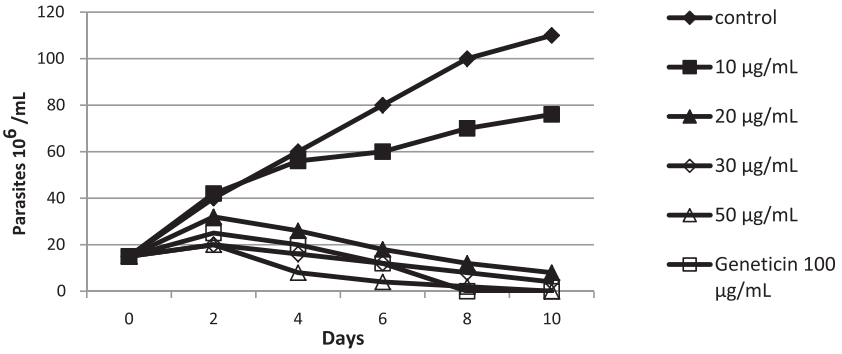


Figure 5. Effect of **9g** concentration on *Leishmania mexicana* growth rate.

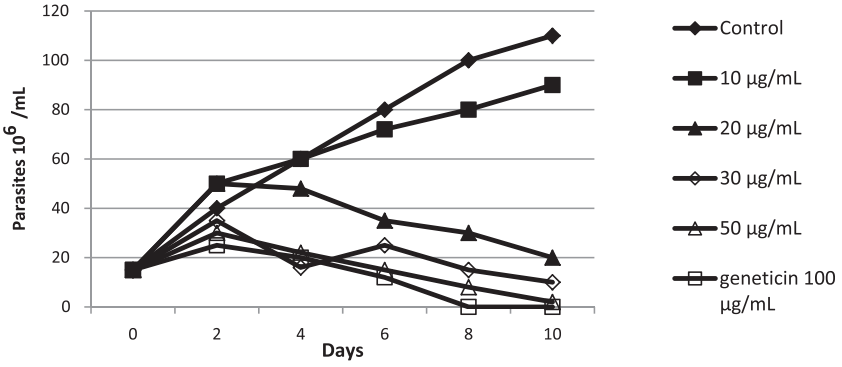


Figure 6. Effect of **9e** concentration on *Leishmania mexicana* growth rate.

TLC performed on commercial 0.2 mm aluminum-coated silica gel plates (F254) and visualized by 254 nm UV or immersion in an aqueous solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.04 M), $Ce(SO_4)_2$ (0.003 M) in concentrated H_2SO_4 (10%). Melting points were measured in a Fisher Johns apparatus and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ as solvent using a Bruker AC-200 spectrometer operating at 200.13 and 50.32 MHz for ^1H and ^{13}C, respectively, and a Bruker AM-500 NMR instrument operating at 500.14 and 125.76 MHz for ^1H and ^{13}C respectively. The ^1H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at $\delta = 7.26$ ppm. Coupling constants are reported in Hz. ^{13}C NMR spectra were fully decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at $\delta = 77.0$ ppm. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quadruplet; qn, quintet; dd, double doublet, etc. IR spectra were recorded with a Nicolet Magna 550 spectrometer. High Resolution Mass Spectrometry was recorded with Thermo Scientic EM/DSQ II—DIP. The results were within ±0.02% of the theoretical values.

4.2. Synthesis of pyridinyloxyalkanols. General procedure

To a solution of 2-hydroxy or 3-hydroxypyridine (200 mg, 2 mmol) in dimethyl sulfoxide (5.0 mL) was added potassium hydroxide (450 mg, 8 mmol). The mixture was stirred at room temperature for 30 min. Then, the corresponding alkylhalide (2 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between saturated solution ammonium chloride (50 mL) and methylene chloride (50 mL). The aqueous phase was extracted with methylene

chloride (2 × 50 mL). The combined organic layers were washed with saturated solution of sodium chloride (5 × 50 mL), dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography (silica gel) employing mixtures of hexane/EtOAc as eluent (7:3–2:3).

4.2.1. 6-(Pyridin-3-yloxy)-hexan-1-ol (8a)

Yield 66% of pure compound as a colorless oil. R_f 0.39 (EtOAc); IR (KBr, cm^{-1}) 2927, 2852, 1635, 1457, 1312, 1220, 1044, 772, 669; ^1H NMR ($CDCl_3$) δ 1.44–1.50 (m, 4H, H-3, H-4), 1.60 (qn, J = 6.8 Hz, 2H, H-2), 1.80 (qn, J = 6.7 Hz, 2H, H-5), 3.65 (t, J = 6.4 Hz, 2H, H-1), 3.99 (t, J = 6.4 Hz, 2H, H-6), 7.20 (m, 2 H, H-4′, H-5′); 8.17 (dd, J_1 = 2.2 Hz, J_2 = 3.9 Hz, 1 H, H-2′); 8.27 (dd, J_1 = 1.1 Hz, J_2 = 2.3 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.5 (C-3), 25.7 (C-4), 29.0 (C-5), 32.6 (C-2), 62.5 (C-1); 68.1 (C-6); 121.1 (C-4′); 123.8 (C-5′); 137.8 (C-2′); 141.7 (C-6′); 155.5 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{11}H_{17}NNaO_2$ 218.1157. Found: $C_{11}H_{17}NNaO_2$ 218.1152.

4.2.2. 7-(Pyridin-3-yloxy)-heptan-1-ol (8b)

Yield 70% of pure compound as a colorless oil. R_f 0.41 (EtOAc); IR (KBr, cm^{-1}) 2920, 2852, 1629, 1460, 1312, 1225, 1050, 770, 665; ^1H NMR ($CDCl_3$) δ 1.36–1.40 (m, 6H, H-3, H-4, H-5), 1.57 (m, 2H, H-2), 1.80 (qn, J = 6.7 Hz, 2H, H-6), 3.64 (t, J = 6.4 Hz, 2H, H-1), 4.00 (t, J = 6.4 Hz, 2H, H-7), 7.23 (s, 2 H, H-4′, H-5′); 8.20 (s, 1 H, H-2′); 8.30 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.5 (C-3), 25.8 (C-4, C-6), 29.1 (C-5), 32.1 (C-2), 62.6 (C-1); 68.2 (C-7); 121.3 (C-4′); 124.0 (C-5′); 137.6 (C-2′); 141.5 (C-6′); 155.3 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{12}H_{19}NNaO_2$ 232.1313. Found: $C_{12}H_{19}NNaO_2$ 232.1308.

4.2.3. 8-(Pyridin-3-yloxy)-octan-1-ol (8c)

Yield 62% of pure compound as a colorless oil. R_f 0.45 (EtOAc); IR (KBr, cm^{-1}) 2930, 2850, 1640, 1450, 1309, 1220, 1045, 776, 670; ^1H NMR ($CDCl_3$) δ 1.36–1.38 (m, 6H, H-3, H-4, H-5), 1.46 (m, 2H, H-6), 1.57 (m, 2H, H-2), 1.80 (m, 2H, H-7), 3.64 (t, J = 6.6 Hz, 2H, H-1), 4.01 (t, J = 6.5 Hz, 2H, H-8), 7.26 (s, 2 H, H-4′, H-5′); 8.21 (s, 1 H, H-2′); 8.31 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.6 (C-3), 25.8 (C-7), 29.0 (C-4), 29.2 (C-5), 29.3 (C-6), 32.7 (C-2), 62.9 (C-1); 68.5 (C-8); 122.3 (C-4′); 124.2 (C-5′); 136.8 (C-2′); 140.8 (C-6′); 155.5 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{13}H_{21}NNaO_2$ 246.1470. Found: $C_{13}H_{21}NNaO_2$ 246.1466

4.2.4. 9-(Pyridin-3-yloxy)-nonan-1-ol (8d)

Yield 65% of pure compound as a colorless oil. R_f 0.46 (EtOAc); IR (KBr, cm^{-1}) 2900, 2840, 1620, 1465, 1300, 1210, 1040, 765, 659; ^1H NMR ($CDCl_3$) δ 1.30–1.34 (m, 8H, H-3, H-4, H-5, H-6), 1.46 (m, 2H, H-7), 1.56 (m, 2H, H-2), 1.81 (m, 2H, H-8), 3.64 (t, J = 6.6 Hz, 2H, H-1), 4.04 (t, J = 6.5 Hz, 2H, H-9), 7. 40 (s, 2 H, H-4′, H-5′); 8.25 (s, 1 H, H-2′); 8.33 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 25.8 (C-8), 28.9 (C-4), 29.1 (C-5), 29.2 (C-6), 29.4 (C-7), 32.7 (C-2), 63.0 (C-1); 69.0 (C-9); 124.4 (C-4′); 124.9 (C-5′); 134.7 (C-2′); 140.0 (C-6′); 156.1 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{14}H_{23}NNaO_2$ 260.1626. Found: $C_{14}H_{23}NNaO_2$ 260.1621.

4.2.5. 10-(pyridin-3-yloxy)-decan-1-ol (8e)

Yield 65% of pure compound as a colorless oil. R_f 0.47 (EtOAc); IR (KBr, cm^{-1}) 2895, 2850, 1625, 1450, 1310, 1230, 10504, 762, 660; ^1H NMR ($CDCl_3$) δ 1.30–1.33 (m, 10H, H-3, H-4, H-5, H-6, H-7), 1.44 (m, 2H, H-8), 1.55 (m, 2H, H-2), 1.79 (m, 2H, H-9), 3.63 (t, J = 6.6 Hz, 2H, H-1), 4.00 (t, J = 6.5 Hz, 2H, H-10), 7.27 (t, J = 2.7 Hz, 2 H, H-4′, H-5′); 8.21 (t, J = 2.8 Hz, 1 H, H-2′); 8.30 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 25.9 (C-9), 29.0–29.3 (C-4, C-5, C-6, C-7, C-8), 32.8 (C-2), 62.9 (C-1); 68.6 (C-10); 122.4 (C-4′); 124.2 (C-5′); 136.6 (C-2′); 140.6 (C-6′); 155.6 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{15}H_{25}NNaO_2$ 274.1783. Found: $C_{15}H_{25}NNaO_2$ 274.1778.

4.2.6. 11-(Pyridin-3-yloxy)-undecan-1-ol (8f)

Yield 68% of white solid mp 49–50 °C. R_f 0.50 (EtOAc); IR (KBr, cm^{-1}) 2922, 2849, 1638, 1459, 1309, 1215, 1040, 776, 664; ^1H NMR ($CDCl_3$) δ 1.29–1.33 (m, 12H, H-3, H-4, H-5, H-6, H-7, H-8), 1.46 (qn, J = 7.3 Hz, 2H, H-9), 1.56 (qn, J = 7.0 Hz, 2H, H-2), 1.79 (qn, J = 7.1 Hz, 2H, H-10), 3.63 (t, J = 6.6 Hz, 2H, H-1), 4.00 (t, J = 6.5 Hz, 2H, H-11), 7.21 (m, 2 H, H-4′, H-5′); 8.19 (d, J = 2.9 Hz, 1 H, H-2′); 8.30 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 25.9 (C-10), 29.1–29.5 (C-4, C-5, C-6, C-7, C-8, C-9), 32.8 (C-2), 63.0 (C-1); 68.4 (C-11); 121.5 (C-4′); 124.0 (C-5′); 137.6 (C-2′); 141.5 (C-6′); 155.4 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{16}H_{27}NNaO_2$ 288.1939. Found: $C_{16}H_{27}NNaO_2$ 288.1934.

4.2.7. 12-(Pyridin-3-yloxy)-dodecan-1-ol (8g)

Yield 72% of white solid mp 37–38 °C. R_f 0.52 (EtOAc); IR (KBr, cm^{-1}) 2900, 2850, 1642, 1447, 1310, 1209, 1040, 770, 658; ^1H NMR ($CDCl_3$) δ 1.25–1.33 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.45 (qn, J = 7.4 Hz, 2H, H-10), 1.56 (qn, J = 7.1 Hz, 2H, H-2), 1.80 (qn, J = 7.1 Hz, 2H, H-11), 3.64 (t, J = 6.6 Hz, 2H, H-1), 4.02 (t, J = 6.5 Hz, 2H, H-12), 7.31 (s, 2 H, H-4′, H-5′); 8.22 (s, 1 H, H-2′); 8.31 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 25.8 (C-11), 29.0–29.5 (C-4, C-5, C-6, C-7, C-8, C-9, C-10), 32.8 (C-2), 63.0 (C-1); 68.7 (C-12); 123.1 (C-4′); 124.4 (C-5′); 136.0 (C-2′); 140.1 (C-6′); 155.8 (C-3′); HRMS: $[M+Na]^+$ Calcd $C_{17}H_{29}NNaO_2$ 302.2096. Found: $C_{17}H_{29}NNaO_2$ 302.2091.

4.2.8. 6-(Pyridin-2-yloxy)-hexan-1-ol (8h)

Yield 52% of pure compound as a colorless oil. R_f 0.12 (EtOAc); IR (KBr, cm^{-1}) 3022, 2922, 1615, 1555, 1444, 1282, 1190, 1024, 758, 649; ^1H NMR ($CDCl_3$) δ 1.31–1.37 (m, 4H, H-3, H-4), 1.51 (qn, J = 6.4 Hz, 2H, H-2), 1.70 (qn, J = 6.8 Hz, 2H, H-5), 3.56 (t, J = 6.2 Hz, 2H, H-1), 3.87 (t, J = 7.2 Hz, 2H, H-6), 6.13 (dt, J_1 = 1.3 Hz, J_2 = 6.7 Hz, 1 H, H-3′); 6.50 (d, J = 9.0 Hz, 1 H, H-5′); 7.23 (dd, J_1 = 1.4 Hz, J_2 = 7.0 Hz, 1 H, H-2′); 7.28 (m, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.1 (C-3), 26.0 (C-4), 29.1, (C-5), 32.3 (C-2), 49.5 (C-1); 62.2 (C-6); 106.1 (C-3′); 120.8 (C-5′); 137.4 (C-4′); 139.3 (C-6′); 162.6 (C-2′); HRMS: $[M+H]^+$ Calcd $C_{11}H_{18}NO_2$ 196.1338. Found: $C_{11}H_{18}NO_2$ 196.1333.

4.2.9. 7-(Pyridin-2-yloxy)-heptan-1-ol (8i)

Yield 55% of pure compound as a colorless oil. R_f 0.15 (EtOAc); IR (KBr, cm^{-1}) 3025, 2952, 1625, 1550, 1450, 1302, 1220, 1040, 770, 660; ^1H NMR ($CDCl_3$) δ 1.33 (m, 6H, H-3, H-4, H-5), 1.53 (m, 2H, H-2), 1.74 (m, 2H, H-6), 3.62 (t, J = 6.7 Hz, 2H, H-1), 3.93 (t, J = 7.0 Hz, 2H, H-7), 6.21 (t, J = 6.1 Hz, 1 H, H-3′); 6.65 (d, J = 9.0 Hz, 1 H, H-5′); 7.30 (m, 1 H, H-4′); 7.37 (m, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.6 (C-3), 26.5 (C-6), 29.1 (C-4, C-5), 32.6 (C-2), 50.1 (C-1); 62.9 (C-7); 106.5 (C-3′); 120.9 (C-5′); 137.5 (C-4′); 139.6 (C-6′); 162.3 (C-2′); HRMS: $[M+Na]^+$ Calcd $C_{12}H_{19}NNaO_2$ 232.1313. Found: $C_{12}H_{19}NNaO_2$ 232.1310.

4.2.10. 8-(Pyridin-2-yloxy)-octan-1-ol (8j)

Yield 55% of pure compound as a colorless oil. R_f 0.17 (EtOAc); IR (KBr, cm^{-1}) 3022, 2932, 1615, 1545, 1420, 1300, 1210, 1030, 770, 654; ^1H NMR ($CDCl_3$) δ 1.28–1.31 (m, 8H, H-3, H-4, H-5, H-6), 1.53 (m, 2H, H-2), 1.74 (m, 2H, H-7), 3.61 (t, J = 6.6 Hz, 2H, H-1), 3.92 (t, J = 7.3 Hz, 2H, H-8), 6.21 (t, J = 6.2 Hz, 1 H, H-3′); 6.68 (d, J = 8.9 Hz, 1 H, H-5′); 7.27 (d, J = 5.7 Hz, 1 H, H-4′); 7.35 (t, J = 7.5 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.6 (C-3), 26.5 (C-7), 29.0 (C-4), 29.1 (C-5), 29.2 (C-6), 32.6 (C-2), 50.4 (C-1); 62.9 (C-8); 106.8 (C-3′); 120.9 (C-5′); 137.6 (C-4′); 139.9 (C-6′); 162.7 (C-2′); HRMS: $[M+Na]^+$ Calcd $C_{13}H_{21}NNaO_2$ 246.1470. Found: $C_{13}H_{21}NNaO_2$ 246.1465.

4.2.11. 9-(Pyridin-2-yloxy)-nonan-1-ol (8k)

Yield 60% of pure compound as a colorless oil. R_f 0.20 (EtOAc); IR (KBr, cm^{-1}) 3020, 2930, 1620, 1540, 1425, 1310, 1200, 1042, 777, 648; ^1H NMR ($CDCl_3$) δ 1.28 (m, 10H, H-3, H-4, H-5, H-6, H-7), 1.50 (m, 2H, H-2), 1.71 (m, 2H, H-8), 3.59 (t, J = 6.5 Hz, 2H, H-1), 3.89 (t, J = 6.6 Hz, 2H, H-9), 6.15 (t, J = 6.6 Hz, 1 H, H-3′); 6.56 (d, J = 9.0 Hz, 1 H, H-5′); 7.29 (m, 2 H, H-4′, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.6 (C-3), 26.5 (C-8), 29.0 (C-4), 29.2 (C-5, C-6), 29.3 (C-7), 32.6 (C-2), 49.9 (C-1); 62.8 (C-9); 106.1 (C-3′); 120.9 (C-5′); 137.5 (C-4′); 139.3 (C-6′); 162.6 (C-2′); HRMS: $[M+H]^+$ Calcd $C_{14}H_{24}NO_2$ 238.1807. Found: $C_{14}H_{24}NO_2$ 238.1802.

4.2.12. 10-(Pyridin-2-yloxy)-decan-1-ol (8l)

Yield 58% of pure compound as a colorless oil. R_f 0.26 (EtOAc); IR (KBr, cm^{-1}) 3002, 2912, 1635, 1515, 1400, 1320, 1215, 1035, 780, 664; ^1H NMR ($CDCl_3$) δ 1.27–1.32 (m, 12H, H-3, H-4, H-5, H-6, H-7, H-8), 1.55 (m, 2H, H-2), 1.74 (m, 2H, H-9), 3.63 (t, J = 6.9 Hz, 2H, H-1), 3.92 (t, J = 7.0 Hz, 2H, H-10), 6.18 (t, J = 6.5 Hz, 1 H, H-3′); 6.62 (d, J_1 = 5.8 Hz,1 H, H-5′); 7.27 (d, J_1 = 5.6 Hz, 1 H, H-4′); 7.33 (t, J = 6.4 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.6 (C-3), 26.5 (C-9), 29.1–29–3 (C-4, C-5, C-6, C-7, C-8), 32.7 (C-2), 50.1 (C-1); 63.0 (C-10); 106.3 (C-3′); 121.0 (C-5′); 137.6 (C-4′); 139.5 (C-6′); 162.7 (C-2′); HRMS: $[M+H]^+$ Calcd $C_{15}H_{26}NO_2$ 252.1964. Found: $C_{15}H_{26}NO_2$ 252.1959.

4.2.13. 11-(Pyridin-2-yloxy)-undecan-1-ol (8m)

Yield 50% of white solid mp 35–36 °C. R_f 0.39 (EtOAc); IR (KBr, cm^{-1}) 3012, 2912, 1615, 1540, 1412, 1320, 1205, 1040, 770, 664; ^1H NMR ($CDCl_3$) δ 1.26–1.31 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.55 (qn, J = 7.0 Hz, 2H, H-2), 1.73 (qn, J = 6.8 Hz, 2H, H-10), 3.62 (t, J = 6.6 Hz, 2H, H-1), 3.90 (t, J = 7.5 Hz, 2H, H-11), 6.14 (dt, J_1 = 1.3 Hz, J_2 = 6.6 Hz, 1 H, H-3′); 6.56 (d, J = 9.1 Hz,1 H, H-5′); 7.24 (dd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.30 (ddd, J_1 = 2.2 Hz, J_2 = 6.7 Hz, J_3 = 9.0 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 26.6 (C-10), 29.1–29–4 (C-4, C-5, C-6, C-7, C-8, C-9), 32.7 (C-2), 49.9 (C-1); 63.0 (C-11); 105.9 (C-3′); 121.1 (C-5′); 137.5 (C-4′); 139.2 (C-6′); 162.6 (C-2′); HRMS: $[M+Na]^+$ Calcd $C_{16}H_{27}NO_2$ 288.1939. Found: $C_{16}H_{27}NO_2$ 288.1934.

4.2.14. 12-(Pyridin-2-yloxy)-dodecan-1-ol (8n)

Yield 51% of white solid mp 58–59 °C. R_f 0.56 (EtOAc); IR (KBr, cm^{-1}) 2992, 2889, 1608, 1515, 1395, 1294, 1200, 1030, 769, 644; ^1H NMR ($CDCl_3$) δ 1.26–1.33 (m, 16H, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10), 1.57 (m, 2H, H-2), 1.74 (m, 2H, H-11), 3.64 (t, J = 6.7 Hz, 2H, H-1), 3.92 (t, J = 6.5 Hz, 2H, H-12), 6.16 (dt, J_1 = 1.4 Hz, J_2 = 6.7 Hz, 1 H, H-3′); 6.57 (dd, J_1 = 0.6 Hz, J_2 = 9.2 Hz, 1 H, H-5′); 7.25 (dd, J_1 = 1.9 Hz, J_2 = 6.6 Hz, 1 H, H-4′); 7.31 (ddd, J_1 = 2.2 Hz, J_2 = 6.7 Hz, J_2 = 9.0 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 25.7 (C-3), 26.6 (C-11), 29.2–29–5 (C-4, C-5, C-6, C-7, C-8, C-9, C-10), 32.8 (C-2), 49.9 (C-1); 63.0 (C-12); 105.9 (C-3′); 121.1 (C-5′); 137.5 (C-4′); 139.2 (C-6′); 162.6 (C-2′); HRMS: $[M+Na]^+$ Calcd $C_{17}H_{29}NNaO_2$ 302.2096. Found: $C_{17}H_{29}NNaO_2$ 302.2091.

4.3. Synthesis of pyridinyloxyalkyl esters. General procedure

To a solution of the corresponding pyridinyloxyalkanol (50 mg) in ethyl acetate or ethyl propionate (2.5 mL) was added CAL-B lipase (5 mg). The mixture was shaken at 200 rpm and 30 °C. Once the reaction was finished, the enzyme was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography (silica gel) employing mixtures of hexane/EtOAc as eluent (9:1–3:2).

4.3.1. 6-(Pyridin-3-yloxy)-hexyl acetate (9a)

Yield 90% of pure compound as a colorless oil. R_f 0.59 (EtOAc); IR (KBr, cm^{-1}) 3440, 2930, 2860, 1732, 1655, 1580, 1465, 1370, 1240, 1045, 771; ^1H NMR ($CDCl_3$) δ 1.44 (m, 2H, H-3), 1.51 (m, 2H, H-4), 1.67 (qn, J = 7.0 Hz, 2H, H-2), 1.82 (qn, J = 6.9 Hz, 2H, H-5), 2.06 (s, 1H, CH_3COO–), 4.02 (t, J = 6.4 Hz, 2H, H-1), 4.08 (t, J = 6.7 Hz, 2H, H-6), 7.27 (m, 2 H, H-4′, H-5′); 8.21 (m, 1 H, H-2′); 8.32 (m, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3COO–), 25.6 (C-3, C-4), 28.5 (C-5), 29.0 (C-2), 64.4 (C-1); 68.2 (C-6); 122.4 (C-4′); 124.3 (C-5′); 136.5 (C-2′); 140.7 (C-6′); 155.5 (C-3′), 171.3 (CH_3COO–); HRMS: $[M+H]^+$ Calcd $C_{13}H_{20}NO_3$ 238.1443. Found: $C_{13}H_{20}NO_3$ 238.1438.

4.3.2. 7-(Pyridin-3-yloxy)-heptyl acetate (9b)

Yield 92% of pure compound as a colorless oil. R_f 0.60 (EtOAc); IR (KBr, cm^{-1}) 3442, 2930, 2858, 1736, 1652, 1570, 1475, 1360, 1239, 1040, 770; ^1H NMR ($CDCl_3$) δ 1.36 (m, 6H, H-3, H-4, H-5), 1.62 (m, 2H, H-2), 1.85 (qn, J = 6.7 Hz, 2H, H-6), 2.04 (s, 1H, CH_3COO–), 4.04 (q, J = 6.7 Hz, 4H, H-1, H-7), 7.30 (s, 2 H, H-4′, H-5′); 8.23 (s, 1 H, H-2′); 8.33 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3COO–), 25.9 (C-3), 28.6 (C-6), 29.1 (C-4, C-5), 29.4 (C-2), 64.6 (C-1); 68.4 (C-7); 121.4 (C-4′, C-5′); 137.6 (C-2′); 141.5 (C-6′); 155.6 (C-3′), 171.2 (CH_3COO–); HRMS: $[M+H]^+$ Calcd $C_{14}H_{22}NO_3$ 252.1600. Found: $C_{14}H_{22}NO_3$ 252.1596.

4.3.3. 8-(Pyridin-3-yloxy)-octyl acetate (9c)

Yield 90% of pure compound as a colorless oil. R_f 0.65 (EtOAc); IR (KBr, cm^{-1}) 3441, 2932, 2856, 1732, 1650, 1572, 1471, 1352, 1230, 1039, 770; ^1H NMR ($CDCl_3$) δ 1.38 (m, 6H, H-3, H-4, H-5), 1.49 (m, 2H, H-6), 1.65 (qn, J = 7.6 Hz, 2H, H-2), 1.85 (qn, J = 7.7 Hz, 2H, H-7), 2.07 (s, 1H, CH_3COO–), 4.08 (t, J = 6.7 Hz, 4H, H-1, H-8), 7.48 (t, J = 7.7 Hz, 2 H, H-4′, H-5′); 8.30 (s, 1 H, H-2′); 8.37 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3COO–), 25.8 (C-3, C-7), 28.5 (C-4), 28.9 (C-5), 29.0 (C-6), 29.1 (C-2), 64.5 (C-1); 68.7 (C-8); 123.4 (C-4′); 124.5 (C-5′); 135.7 (C-2′); 139.9 (C-6′); 155.8 (C-3′), 171.2 (CH_3COO–); HRMS: $[M+H]^+$ Calcd $C_{15}H_{23}NNaO_3$ 288.1576. Found: $C_{15}H_{23}NNaO_3$ 288.1571.

4.3.4. 9-(Pyridin-3-yloxy)-nonyl acetate (9d)

Yield 95% of pure compound as a colorless oil. R_f 0.66 (EtOAc); IR (KBr, cm^{-1}) 3439, 2933, 2845, 1722, 1650, 1560, 1449, 1342, 1229, 1010, 769; ^1H NMR ($CDCl_3$) δ 1.32 (m, 8H, H-3, H-4, H-5, H-6), 1.46 (m, 2H, H-7), 1.61 (m, 2H, H-2), 1.79 (m, 2H, H-8), 2.04 (s, 1H, CH_3COO–), 4.00 (t, J = 6.6 Hz, 2H, H-1), 4.04 (t, J = 6.6 Hz, 2H, H-9), 7. 22 (s, 2 H, H-4′, H-5′); 8.21 (s, 1 H, H-2′); 8.31 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3COO–), 25.8 (C-3, C-8), 28.5 (C-4), 29.1 (C-5, C-6), 29.4 (C-7), 29.7 (C-2), 64.6 (C-1); 68.4 (C-9); 121.6 (C-4′); 124.0 (C-5′); 137.4 (C-2′); 141.3 (C-6′); 155.4 (C-3′), 171.2 (CH_3COO–); HRMS: $[M+H]^+$ Calcd $C_{16}H_{26}NO_3$ 280.1913. Found: $C_{16}H_{26}NO_3$ 280.1908.

4.3.5. 10-(Pyridin-3-yloxy)-decyl acetate (9e)

Yield 95% of pure compound as a colorless oil. R_f 0.70 (EtOAc); IR (KBr, cm^{-1}) 3445, 2927, 2850, 1732, 1635, 1550, 1479, 1379, 1202, 1012, 768; ^1H NMR ($CDCl_3$) δ 1.30–1.33 (m, 10H, H-3, H-4, H-5, H-6, H-7), 1.45 (qn, J = 7.3 Hz, 2H, H-8), 1.61 (qn, J = 7.2 Hz, 2H, H-2), 1.79 (qn, J = 7.1 Hz, 2H, H-9), 2.04 (s, 1H, CH_3COO–), 4.00 (t, J = 6.7 Hz, 2H, H-1), 4.04 (t, J = 6.8 Hz, 2H, H-10), 7.24 (s, 2 H, H-4′, H-5′); 8.21 (s, 1 H, H-2′); 8.31 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 (CH_3COO–), 25.8 (C-3), 25.9 (C-9), 28.6 (C-4), 29.0 (C-5), 29.1 (C-6), 29.2 (C-7), 29.4 (C-8, C-2), 64.6 (C-1); 68.5 (C-10); 122.0 (C-4′); 124.1 (C-5′); 137.0 (C-2′); 141.0 (C-6′); 155.5 (C-3′); 171.2 (CH_3COO–); HRMS: $[M+Na]^+$ Calcd $C_{17}H_{27}NNaO_3$ 316.1889. Found: $C_{17}H_{27}NNaO_3$ 316.1883.

4.3.6. 11-(Pyridin-3-yloxy)-undecyl acetate (9f)

Yield 97% of pure compound as a colorless oil. R_f 0.75 (EtOAc); IR (KBr, cm^{-1}) 3430, 2915, 2839, 1726, 1655, 1560, 1456, 1359, 1239, 1040, 772; ^1H NMR ($CDCl_3$) δ 1.29 (m, 14H, H-3, H-4, H-5,

H-6, H-7, H-8, H-9), 1.61 (qn, J = 6.9 Hz, 2H, H-2), 1.79 (qn, J = 6.9 Hz, 2H, H-10), 2.04 (s, 1H, $\underline{C}H_3COO$–), 3.99 (t, J = 6.5 Hz, 2H, H-1), 4.04 (t, J = 6.7 Hz, 2H, H-11), 7.19 (m, 2 H, H-4′, H-5′); 8.19 (dd, J_1 = 2.0 Hz, J_2 = 6.6 Hz, 1 H, H-2′); 8.30 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$–), 25.9 (C-3, C-10), 28.6 (C-4), 29.1–29.5 (C-5, C-6, C-7, C-8, C-9, C-2), 64.6 (C-1); 68.3 (C-11); 121.1 (C-4′); 123.7 (C-5′); 137.8 (C-2′); 141.7 (C-6′); 155.5 (C-3′), 171.2 ($\underline{C}H_3COO$–); HRMS: $[M+Na]^+$ Calcd $C_{18}H_{29}NNaO_3$ 330.2045. Found: $C_{18}H_{29}NNaO_3$ 330.2040.

4.3.7. 12-(Pyridin-3-yloxy)-dodecyl acetate (9g)

Yield 99% of pure compound as a colorless oil. R_f 0.80 (EtOAc); IR (KBr, cm^{-1}) 3432, 2932, 2860, 1736, 1655, 1571, 1462, 1365, 1240, 1036, 760; ^{1}H NMR ($CDCl_3$) δ 1.24–1.32 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.45 (qn, J = 7.2 Hz, 2H, H-10), 1.61 (qn, J = 7.0 Hz, 2H, H-2), 1.79 (qn, J = 7.0 Hz, 2H, H-11), 2.03 (s, 1H, $\underline{C}H_3COO$–), 4.01 (t, J = 6.6 Hz, 2H, H-1), 4.04 (t, J = 6.7 Hz, 2H, H-12), 7.26 (s, 2 H, H-4′, H-5′); 8.21 (s, 1 H, H-2′); 8.31 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$–), 25.9 (C-3, C-11), 28.6–29–5 (C-2, C-4, C-5, C-6, C-7, C-8, C-9, C-10), 64.6 (C-1); 68.6 (C-12); 122.4 (C-4′); 124.2 (C-5′); 136.7 (C-2′); 140.7 (C-6′); 155.6 (C-3′), 171.2 ($\underline{C}H_3COO$–); HRMS: $[M+Na]^+$ Calcd $C_{19}H_{31}NNaO_3$ 344.2202. Found: $C_{19}H_{31}NNaO_3$ 344.2197.

4.3.8. 6-(Pyridin-2-yloxy)-hexyl acetate (9h)

Yield 90% of pure compound as a colorless oil. R_f 0.50 (EtOAc); IR (KBr, cm^{-1}) 3440, 2932, 2858, 1735, 1655, 1580, 1540, 1465, 1367, 1240, 1045, 771; ^{1}H NMR ($CDCl_3$) δ 1.37 (m, 4H, H-3, H-4), 1.61 (qn, J = 6.2 Hz, 2H, H-2), 1.75 (qn, J = 6.7 Hz, 2H, H-5), 2.03 (s, 1H, $\underline{C}H_3COO$–), 3.93 (t, J = 7.4 Hz, 2H, H-1), 4.03 (t, J = 6.6 Hz, 2H, H-6), 6.21 (t, J = 6.5 Hz, 1 H, H-3′); 6.64 (d, J = 9.1 Hz, 1 H, H-5′); 7.26 (dd, J_1 = 2.0, Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.34 (ddd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, J_3 = 9.0 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 20.8 ($\underline{C}H_3COO$), 25.5 (C-3), 26.2 (C-4), 28.4 (C-5), 29.1 (C-2), 50.0 (C-1); 64.3 (C-6); 106.7 (C-3′); 120.9 (C-5′); 137.4 (C-4′); 139.7 (C-6′); 162.9 (C-2′); 171.3 ($CH_3\underline{C}OO$); HRMS: $[M+H]^+$ Calcd $C_{13}H_{20}NO_3$ 238.1443. Found: $C_{13}H_{20}NO_3$ 238.1440.

4.3.9. 7-(Pyridin-2-yloxy)-heptyl acetate (9i)

Yield 90% of pure compound as a colorless oil. R_f 0.58 (EtOAc); IR (KBr, cm^{-1}) 3445, 2930, 2860, 1738, 1650, 1570, 1520, 1475, 1369, 1242, 1040, 772; ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 6H, H-3, H-4, H-5), 1.58 (m, 2H, H-2), 1.73 (m, 2H, H-6), 2.04 (s, 1H, $\underline{C}H_3COO$–), 3.91 (t, J = 7.4 Hz, 2H, H-1), 4.03 (t, J = 6.7 Hz, 2H, H-7), 6.15 (dt, J_1 = 1.0 Hz, J_2 = 4.6 Hz, 1 H, H-3′); 6.57 (d, J = 9.1 Hz, 1 H, H-5′); 7.23 (m, 1 H, H-4′); 7.32 (m, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.8 (C-3), 26.5 (C-6), 28.5 (C-4), 29.1 (C-5), 29.2 (C-2), 50.1 (C-1); 64.6 (C-7); 106.4 (C-3′); 120.9 (C-5′); 137.5 (C-4′); 139.5 (C-6′); 162.8 (C-2′); 171.2 ($CH_3\underline{C}OO$); HRMS: $[M+H]^+$ Calcd $C_{14}H_{22}NO_3$ 252.1600. Found: $C_{14}H_{22}NO_3$ 252.1597.

4.3.10. 8-(Pyridin-2-yloxy)-octyl acetate (9j)

Yield 90% of pure compound as a colorless oil. R_f 0.62 (EtOAc); IR (KBr, cm^{-1}) 3442, 2928, 2862, 1740, 1652, 1572, 1538, 1470, 1360, 1240, 1042, 770; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 8H, H-3, H-4, H-5, H-6), 1.58 (m, 2H, H-2), 1.74 (m, 2H, H-7), 2.04 (s, 1H, $\underline{C}H_3COO$–), 3.92 (t, J = 7.4 Hz, 2H, H-1), 4.03 (t, J = 6.7 Hz, 2H, H-8), 6.18 (t, J = 6.7 Hz, 1 H, H-3′); 6.61 (d, J = 8.9 Hz, 1 H, H-5′); 7.25 (m, 1 H, H-4′); 7.34 (m, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.8 (C-3), 26.5 (C-7), 28.5 (C-4), 29.0 (C-5,C-6), 29.2 (C-2), 50.1 (C-1); 64.5 (C-8); 106.3 (C-3′); 121.0 (C-5′); 137.5 (C-4′); 139.5 (C-6′); 164.4 (C-2′); 172.2 ($CH_3\underline{C}OO$); HRMS: $[M+H]^+$ Calcd $C_{15}H_{23}NNaO_3$ 288.1576. Found: $C_{15}H_{23}NNaO_3$ 288.1572.

4.3.11. 9-(Pyridin-2-yloxy)-nonyl acetate (9k)

Yield 94% of pure compound as a colorless oil. R_f 0.65 (EtOAc); IR (KBr, cm^{-1}) 3435, 2928, 2852, 1728, 1640, 1560, 1515, 1469, 1389, 1212, 1032, 770; ^{1}H NMR ($CDCl_3$) δ 1.30–1.32 (m, 10H, H-3, H-4, H-5, H-6, H-7), 1.61 (qn, J = 6.9 Hz, 2H, H-2), 1.75 (qn, J = 6.8 Hz, 2H, H-8), 2.05 (s, 1H, $\underline{C}H_3COO$–), 3.94 (t, J = 7.0 Hz, 2H, H-1), 4.05 (t, J = 6.8 Hz, 2H, H-9), 6.21 (t, J = 6.5 Hz, 1 H, H-3′); 6.68 (d, J = 9.0 Hz,1 H, H-5′); 7.28 (dd, J_1 = 0.9 Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.35 (ddd, J_1 = 1.0 Hz, J_2 = 6.7 Hz, J_3 = 8.9 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.8 (C-3), 26.6 (C-8), 28.5 (C-4), 29.1 (C-5, C-6), 29.2 (C-7), 29.3 (C-2), 50.2 (C-1); 64.6 (C-9); 106.5 (C-3′); 120.9 (C-5′); 137.5 (C-4′); 139.6 (C-6′); 162.6 (C-2′); 171.2 ($CH_3\underline{C}OO$); HRMS: $[M+Na]^+$ Calcd $C_{16}H_{25}NNaO_3$ 302.1732. Found: $C_{16}H_{25}NNaO_3$ 302.1727.

4.3.12. 10-(Pyridin-2-yloxy)-decyl acetate (9l)

Yield 95% of pure compound as a colorless oil. R_f 0.70 (EtOAc); IR (KBr, cm^{-1}); ^{1}H NMR ($CDCl_3$) δ 1.26–1.31 (m, 12H, H-3, H-4, H-5, H-6, H-7, H-8), 1.59 (qn, J = 7.1 Hz, 2H, H-2), 1.73 (qn, J = 7.4 Hz, 2H, H-9), 3.90 (t, J = 7.0 Hz, 2H, H-1), 2.03 (s, 1H, $\underline{C}H_3COO$–), 3.90 (t, J = 7.0 Hz, 2H, H-1), 4.03 (t, J = 6.9 Hz, 2H, H-10), 6.14 (dt, J_1 = 1.4, J_2 = 6.7 Hz, 1 H, H-3′); 6.56 (d, J = 9.1 Hz,1 H, H-5′); 7.24 (dd, J_1 = 1.6 Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.28 (ddd, J_1 = 2.1 Hz, J_2 = 6.7 Hz, J_2 = 8.9 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.8 (C-3), 26.6 (C-9), 28.5 (C-4), 29.1 (C-5, C-6), 29.2 (C-7), 29.3 (C-8, C-2), 49.9 (C-1); 64.6 (C-10); 105.9 (C-3′); 121.0 (C-5′); 137.5 (C-4′); 139.2 (C-6′); 162.6 (C-2′), 171.2 ($CH_3\underline{C}OO$); HRMS: $[M+H]^+$ Calcd $C_{17}H_{28}NO_3$ 294.2069. Found: $C_{17}H_{28}NO_3$ 294.2064.

4.3.13. 11-(Pyridin-2-yloxy)-undecyl acetate (9m)

Yield 95% of pure compound as a colorless oil. R_f 0.71 (EtOAc); IR (KBr, cm^{-1}) 3440, 2925, 2850, 1736, 1655, 1580, 1530, 1466, 1366, 1241, 1045, 772; ^{1}H NMR ($CDCl_3$) δ 1.29–1.31 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.60 (qn, J = 7.1 Hz, 2H, H-2), 1.73 (qn, J = 7.3 Hz, 2H, H-10), 2.04 (s, 1 H, C$\underline{H}_3$COO), 3.91 (t, J = 7.5 Hz, 2H, H-1), 4.04 (t, J = 6.8 Hz, 2H, H-11), 6.15 (dt, J_1 = 1.2 Hz, J_2 = 6.7 Hz, 1 H, H-3′); 6.56 (d, J = 9.0 Hz, 1 H, H-5′); 7.24 (dd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.30 (ddd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, J_3 = 9.0 Hz, 1 H, H-6′);13C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.9 (C-3), 26.6 (C-10), 28.6–29.2 (C-4, C-5, C-6, C-7, C-8, C-9), 29.4 (C-2), 49.9 (C-1); 64.6 (C-11); 105.9 (C-3′); 121.1 (C-5′); 137.5 (C-4′); 139.2 (C-6′); 162.6 (C-2′); 171.3 ($CH_3\underline{C}OO$); HRMS: $[M+Na]^+$ Calcd $C_{18}H_{29}NNaO_3$ 330.2045. Found: $C_{18}H_{29}NNaO_3$ 332.2040.

4.3.14. 12-(Pyridin-2-yloxy)-dodecyl acetate (9n)

Yield 95% of pure compound as a colorless oil: R_f 0.75 (EtOAc); IR (KBr, cm^{-1}) 3437, 2942, 2858, 1736, 1651, 1570, 1520, 1465, 1369, 1242, 1040, 770; ^{1}H NMR ($CDCl_3$) δ 1.24–1.31 (m, 16H, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10), 1.60 (qn, J = 7.0 Hz, 2H, H-2), 1.73 (qn, J = 7.1 Hz, 2H, H-11), 2.03 (s, 1 H, C$\underline{H}_3$COO), 3.92 (t, J = 7.0 Hz, 2H, H-1), 4.03 (t, J = 6.8 Hz, 2H, H-12), 6.18 (t, J = 6.3 Hz, 1 H, H-3′); 6.63 (d, J = 8.9 Hz, 1 H, H-5′); 7.31 (dd, J_1 = 1.5 Hz, J_2 = 7.3 Hz, 1 H, H-4′); 7.34 (ddd, J_1 = 1.8 Hz, J_2 = 6.8 Hz, J_3 = 8.8 Hz, 1 H, H-6′);13C NMR ($CDCl_3$) δ 21.0 ($\underline{C}H_3COO$), 25.9 (C-3), 26.6 (C-11), 28.6–29.2 (C-4, C-5, C-6, C-7, C-8, C-9), 29.4 (C-2,, C-11), 50.1 (C-1); 64.6 (C-12); 106.3 (C-3′); 120.9 (C-5′); 137.6 (C-4′); 139.5 (C-6′); 162.6 (C-2′); 171.2 ($CH_3\underline{C}OO$); HRMS: $[M+H]^+$ Calcd $C_{19}H_{32}NO_3$ 322.2382. Found: $C_{19}H_{32}NO_3$ 322.2377.

4.3.15. 11-(Pyridin-3-yloxy)-undecyl propionate (9o)

Yield 60% of pure compound as a colorless oil. R_f 0.74 (EtOAc); IR (KBr, cm^{-1}) 3429, 2932, 2857, 1735, 1656, 1580, 1462, 1369,

1240, 1040, 770; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 7.6 Hz, 3H, C$\underline{H}_3$CH$_2$COO), 1.29 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.61 (qn, J = 6.6 Hz, 2H, H-2), 1.79 (qn, J = 6.7 Hz, 2H, H-10), 2.31 (q, 2H, CH$_3$C$\underline{H}_2$COO-), 3.99 (t, J = 6.2 Hz, 2H, H-1), 4.04 (t, J = 6.6 Hz, 2H, H-11), 7.19 (s, 1 H, H-4′), 7.20 (s, 1 H, H-5′); 8.20 (m, 1 H, H-2′); 8.30 (s, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 9.2 (C$\underline{H}_3$CH$_2$COO-), 25.9 (C-3, C-10), 27.7 (CH$_3$C$\underline{H}_2$COO-), 28.6 (C-4), 29.1–29.3 (C-5, C-6, C-7, C-8), 29.5 (C-2, C-9), 64.5 (C-1); 68.3 (C-11); 121.2 (C-4′); 137.8 (C-2′); 141.7 (C-5′); 155.5 (C-6′), 171.2 (CH$_3$CH$_2$C$\underline{O}$O-); HRMS: [M+Na]$^+$ Calcd $C_{19}H_{31}NNaO_2$ 344.2202. Found: $C_{19}H_{31}NNaO_2$ 344.2197.

4.3.16. 11-(Pyridin-2-yloxy)-undecyl propionate (9p)

Yield 45% of pure compound as a colorless oil. R_f 0.54 (EtOAc); IR (KBr, cm^{-1}) 3438, 2930, 2858, 1736, 1657, 1579, 1569, 1466, 1367, 1242, 1043, 773; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J = 7.6 Hz, 3 H, C$\underline{H}_3$CH$_2$COO), 1.26–1.31 (m, 14H, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 1.60 (qn, J = 7.0 Hz, 2H, H-2), 1.73 (qn, J = 7.3 Hz, 2H, H-10), 2.32 (q, J = 7.6 Hz, 2H, CH$_3$C$\underline{H}_2$COO-), 3.91 (t, J = 7.4 Hz, 2H, H-1), 4.05 (t, J = 6.8 Hz, 2H, H-11), 6.15 (dt, J_1 = 1.0 Hz, J_2 = 6.7 Hz, 1 H, H-3′); 6.56 (d, J_1 = 9.0 Hz, 1 H, H-5′); 7.24 (dd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, 1 H, H-4′); 7.31 (ddd, J_1 = 2.0 Hz, J_2 = 6.7 Hz, J_3 = 9.0 Hz, 1 H, H-6′); ^{13}C NMR ($CDCl_3$) δ 9.2 (C$\underline{H}_3$CH$_2$COO-), 25.9 (C-3), 26.6 (C-10), 27.6 (CH$_3$C$\underline{H}_2$COO-), 28.6 (C-4), 29.1–29.3 (C-5, C-6, C-7, C-8), 29.4 (C-2, C-9), 50.0 (C-1); 64.5 (C-11); 105.9 (C-3′); 121.1 (C-5′); 137.5 (C-4′); 139.3 (C-6′); 162.7 (C-2′), 174.6 (CH$_3$CH$_2$C$\underline{O}$O-); HRMS: [M+Na]$^+$ Calcd $C_{19}H_{31}NNaO_3$ 344.2202. Found: $C_{19}H_{31}NNaO_3$ 344.2197.

4.4. Drug screening

Leishmania mexicana promastigotes were grown in a culture medium containing 33 g l^{-1} brain-heart infusion (Difco); 3 g l^{-1} tryptose (Difco); 3 g l^{-1} Na_2HPO_4; 0.4 g l^{-1} KCl; 0.3 g l^{-1} glucose. pH was about 7.5, without the need for adjustment. After sterilization (10 min at 121 °C) penicillin (100 IU mL^{-1}), streptomycin (100 μg/mL), haemin (20 μg/mL, added as 1 mL of a 2 g mL^{-1} solution in 0.1 N NaOH per litre of medium) and 20% v/v heat-inactivated (45 min at 57 °C) fetal calf serum were added. Cultures were performed at 28 °C in 600 mL cylindrical flasks with screw caps, containing 100 mL of medium. Shaking was performed manually, twice a day. All cultures were started with inocula taken from exponential growth parasite in the same medium. The susceptibilities of the promastigote form of *Leishmania* to synthetic compounds were tested by culturing them in cell-free medium at 28 °C. Growth experiments with promastigotes of *L. mexicana* were initiated with 15×10^6 parasites/mL, and the inhibitors were added at 10, 20, 30 and 50 μg/mL from concentrated stock solutions in DMSO at this moment.

A solution of 100 μg/mL of geneticin was used as a positive control. Parasite growth was determined each two days for 10 days. DMSO concentration in the cultures (0.1%) did not interfere with parasite growth or morphology.

Acknowledgments

We thank UBA X010, UBACYT 20020100100304, CONICET PIP 112-200801-00801/09 and ANPCyT PICT 2005-32735 for partial financial support. A.B., G.G.L. and C.L. are Research Members of CONICET (Argentina).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.06.028.

References and notes

1. World Health Organization, 1999. Tropical disease research: progress in international research, 1997–1998. World Health Organization, Geneva, Switzerland.
2. Garcia Liñares, G. E; Ravaschino, E. L; Rodriguez, J. B *Curr. Med. Chem.* **2006**, *13*, 335.
3. Zauli, R. C.; Yokoyama-Yasunaka, J. K. U.; Miguel, D. C.; Moura, A. S.; Pereira, L. I. A.; Da Silva, I. A.; Lemes, L. N. G.; Dorta, M. L.; De Oliveira, M. A. P.; Pitaluga, A. N.; Ishikawa, E. A. Y.; Rodrigues, J. C. F.; Traub-Cseko, Y. M.; Bijovsky, A.; Ribeiro-Dias, F.; Uliana, S. R. B. *Parasit. Vector* **2012**, *5*, 11.
4. Croft, S. L.; Yardley, V. *Curr. Pharm. Des.* **2002**, *8*, 319.
5. Hirst, S. I.; Stapley, L. A. *Parasitol. Today* **2000**, *16*, 1.
6. Marsden, P. D.; Jones, T. C. In *Human Parasitic Diseases Leishmaniasis*; Chang, K. P., Bray, R. S., Eds.; Elsevier Science Publishers: New York, 1985; p 183. Vol. 1.
7. Control of the leishmaniasis. Report of a WHO expert committee in World Health Organization Technical Report Series 1990, 793, 1.
8. Desjeux, P.; Alvar, J. *Ann. Trop. Med. Parasitol.* **2003**, *97*, 3.
9. Zauli-Nascimento, R. C.; Miguel, D. C.; Yokoyama-Yasunaka, J. K. U.; Pereira, L. I. A.; Pelli De Oliveira, M. A. P; Ribeiro-Dias, F.; Dorta, M. L; Uliana, S. R. B. *Trop. Med. Intern. Health* **2010**, *15*, 68.
10. Croft, S. L.; Sundar, S.; Fairlamb, A. H. *Clin. Microbiol. Rev.* **2006**, *19*, 111.
11. Croft, S. L.; Barrett, M. P.; Urbina, J. A. *Trends Parasitol.* **2005**, *21*, 508.
12. Urbina, J. A.; Docampo, R. *Trends Parasitol.* **2003**, *19*, 495.
13. Ouellette, M.; Drummelsmith, J.; Papadopoulou, B. *Drug Resist. Update.* **2004**, *7*, 257.
14. Sinderman, H.; Croft, S. L.; Engel, K. R.; Bommer, W.; Eibl, H. J.; Unger, C.; Engel, J. *Med. Microbiol. Immunol.* **2004**, *193*, 173.
15. Ganguly, N. K. *TDR News* **2002**, *98*, 2.
16. Pérez-Victoria, F. J.; Castanys, S.; Gamarro, F. *Antimicrob. Agents Chemother.* **2003**, *41*, 2397.
17. Cançado, J. R.; Brener, Z. In *Trypanosoma cruzi e Doença de Chagas*; Brener, Z., Andrade, Z., Eds.; Guanabara Koogan: Sao Paulo, 1979; p 362.
18. Schmuñis, G. A.; Szarfman, A.; Coarasa, L.; Guilleron, C.; Peralta, J. M. *Am. J. Trop. Med. Hyg.* **1980**, *29*, 170.
19. Fernandes Rodrigues, J. C.; Concepcion, J. L.; Rodrigues, C.; Caldera, A.; Urbina, J. A.; De Souza, W. *Antimicrob. Agents Chemother.* **2008**, *52*, 4098.
20. Rodrigues, R. F.; Charret, K. S.; da Silva, E. F.; Echevarria, A.; Amaral, V. F.; Leon, L. L.; Canto-Cavalheiro, M. M. *Antimicrob. Agents Chemother.* **2009**, *53*, 839.
21. Gómez-Ayala, S.; Castrillón, J. A.; Palma, A.; Leal, S. M.; Escobar, P.; Bahsas, A. *Bioorg. Med. Chem.* **2010**, *18*, 4721.
22. Martinez-Rojano, H.; Mancilla-Ramirez, J.; Quiñonez-Diaz, L.; Galindo-Sevilla, N. *Antimicrob. Agents Chemother.* **2008**, *52*, 3642.
23. Gupta, L.; Sunduru, N.; Verma, A.; Srivastava, S.; Gupta, S.; Goyal, N.; Chauhan, P. M. S. *Eur. J. Med. Chem.* **2010**, *45*, 2359.
24. Bommarius, A. S.; Riebel, B. R. *Biocatalysis, Fundamentals and Applications*; Wiley–VCH: Weinheim, 2004.
25. Buchholz, K.; Kasche, V.; Bornscheuer, U. T. *Biocatalysis and Enzyme Technology*; Wiley–VCH: Weinheim, 2005.
26. Saha, B. C.; Demirjian, D. C. *Applied biocatalysis in specialty chemicals and pharmaceuticals*; American Chemical Society (ACS) Press: Washington, DC, 2000.
27. Riva, S. In *Organic Synthesis with Enzymes in Non-Aqueous Media*; Carrea, G., Riva, S., Eds.; Wiley–VCH: Weinheim, 2008. Chapter 6.
28. Gotor, V.; Alfonso, I.; García-Urdiales, E. *Asymmetric Organic Synthesis with Enzymes*; Wiley-VCH: Weinheim, 2007.
29. Quintana, P. G.; Baldessari, A. *Steroids* **2009**, *74*, 1007.
30. Rustoy, E. M.; Baldessari, A. *Eur. J. Org. Chem.* **2005**, 4628.
31. Quintana, P. G.; Sandoval, G.; Baldessari, A. *Biocatal. Biotransform.* **2011**, *29*, 87.
32. Monsalve, L. N.; Roselli, S.; Bruno, M.; Baldessari, A. *Eur. J. Org. Chem.* **2005**, 2106.
33. Monsalve, L. N.; Roselli, S.; Bruno, M.; Baldessari, A. *J. Mol. Catal. B: Enzym.* **2009**, *57*, 40.
34. Blaney, J. M.; Hansch, C.; Silipo, C.; Vittoria, A. *Chem. Rev.* **1984**, *84*, 333.
35. Zuccotto, F.; Martin, A. C. R.; Laskowski, R. A.; Thornton, J. M.; Gilbert, I. H. *J. Comput. Aided Mol. Des.* **1998**, *12*, 241.
36. Sirawaraporn, W.; Sertsrivanich, R.; Booth, R. G.; Hansch, C.; Neal, R. A.; Santi, D. V. *Mol. Biochem. Parasitol.* **1988**, *31*, 79.
37. Chowdhury, S. F.; Villamor, V. B.; Guerrero, R. H.; Leal, L.; Brun, R.; Croft, S. L.; Goodman, J. M.; Maes, L.; Ruiz Perez, L. M.; Gonzalez Pocanowska, D.; Gilbert, I. H. *J. Med. Chem.* **1999**, *42*, 4300.
38. Pez, D.; Leal, I.; Zuccotto, F.; Boussard, C.; Brun, R.; Croft, S. L.; Yardley, V.; Ruiz Perez, L. M.; Gonzalez Pocanowska, D.; Gilbert, I. H. *Bioorg. Med. Chem.* **2003**, *11*, 4693.
39. Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, B. S.; Jeong, T. C.; Lee, C. S.; Lee, E. S. *Bioorg. Med. Chem.* **2007**, *15*, 4351.
40. Thapa, P.; Karki, R.; Thapa, U.; Jahng, Y.; Jung, M. J.; Nam, J. M.; Na, Y.; Kwon, Y.; Lee, E. S. *Bioorg. Med. Chem.* **2010**, *18*, 377.
41. Onnis, V.; Cocco, M. T.; Fadda, R.; Congiu, C. *Bioorg. Med. Chem.* **2009**, *17*, 6158.
42. Son, J. K.; Zhao, L. X.; Basnet, A.; Thapa, P.; Karki, R.; Na, Y.; Jahng, Y.; Jeong, T. C.; Jeong, B. S.; Lee, C. S.; Lee, E. S. *Eur. J. Med. Chem.* **2008**, *43*, 675.
43. Kovala-Demertzi, D.; Papageorgiou, A.; Papathanasis, L.; Alexandratos, A.; Dalezis, P.; Miller, J. R.; Demertzis, M. A. *Eur. J. Med. Chem.* **2009**, *44*, 1296.
44. Abdel-Megeed, M. F.; Badr, B. E.; Azaam, M. M.; El-Hiti, G. A. *Bioorg. Med. Chem.* **2012**, *20*, 2252.

45. Lu, X.; Zhang, H.; Li, X.; Chen, G.; Li, Q. S.; Luo, Y.; Ruan, B. F.; Chen, X. W.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 6827.
46. Kassis, P.; Brzeszcz, J.; Bénéteau, V.; Lozach, O.; Meijer, L.; Guével, R. L.; Guillouzo, C.; Lewiński, K.; Bourg, S.; Colliandre, L.; Routier, S.; Mérour, J. Y. *Eur. J. Med. Chem.* **2011**, *46*, 5416.
47. Schnute, M. E.; Cudahy, M. M.; Brideau, R. J.; Homa, F. L.; Hopkins, T. A.; Knechtel, M. L.; Oien, N. L.; Pitts, T. W.; Poorman, R. A.; Wathen, M. W.; Wieber, J. L. *J. Med. Chem.* **2005**, *48*, 5794.
48. García Liñares, G.; Gismondi, S.; Osa Codesido, N.; Moreno, S. N. J.; Docampo, R.; Rodriguez, J. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5068.
49. Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* **1993**, *58*, 142.