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Parallel synthesis and antileishmanial activity of ether-linked phospholipids

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ABSTRACT

The synthesis and antileishmanial activity of 18 edelfosine analogues are described. Compounds were obtained in parallel combining solid phase and solution phase synthesis. The most active analogue is characterized by the octadecyl group in position 2 of the glycerol chain. Considering that this substitution determines the loss of antitumor activity, a different mechanism of antileishmanial action can be hypothesized.

Leishmaniasis is an infectious disease caused by kinetoplastid protozoans of the genera *Leishmania*, affecting 12 million people around the world with an annual fatality rate of approximately 50,000 people.[1] The currently available drugs are Pentostam (sodium stibogluconate), Glucantime (meglumine antimonate), both are antimonial salts; Amphotericin B, a macrolide antibiotic,[2] and alkylphosphocholines.[3] These drugs suffer from one or more of the following adverse effects, rather high costs and drug resistant parasites.[2] Therefore, new safe and effective drugs are needed.

Alkyl ether phospholipids (AEPs) are metabolically stable analogues of 2-lysophosphatidylcholine that display antitumor and antiparasitic activities.[4] Edelfosine (ET-18-OCH_3) is the most important member of this family and it has demonstrated antiparasitic activities against two strains of *L. donovani*.[5] The first site of interaction of phospholipid analogues in both tumor cells and protozoa is the cell membrane.[6] The primary site of damage in edelfosine-treated *L. donovani* amastigotes appears to be the flagellar pocket and the adjacent cytoplasm.[7] Most of the research on parasites has been focused on miltefosine, an alkylphospholipid analogue (Fig. 1). Miltefosine has been registered after successful Phase IV clinical trials under the trade name Impavido® for the oral treatment of visceral leishmaniasis.[8] Miltefosine has a long half-life (100–200 h) in humans and a low therapeutic ratio, characteristics that could encourage development of resistance; moreover, it is not suitable for pregnant women because it has been shown to be teratogenic in animals[9] and it did not give satisfactory results when administered to HIV-coinfected patients.[10] Moreover, although potential antitumor activity of miltefosine and other phospholipid analogues was demonstrated, it is still not known how these drugs exert their antiprotozoal activity.[11,12] Recently, fluorescent analogues of miltefosine have been prepared and confocal microscopy showed high concentration and homogeneous intracellular distribution of the fluorescence in *L. donovani* promastigotes.[13] Based on these observations, it is possible to suppose a multitarget leishmanicidal mechanism of miltefosine. Structural modifications have been introduced in the lipid portion and the head group of miltefosine which have led to derivatives with potent activity, reduced cytotoxicity, and haemolytic activity.[14–16]

Although several edelfosine derivatives have been synthesized for more than 40 years,[17] very few compounds have been tested against *L. donovani*. We report here the parallel synthesis and the antileshmanial activities of 18 analogues of edelfosine, obtained by a solid phase and solution phase synthetic approach (Table 1).

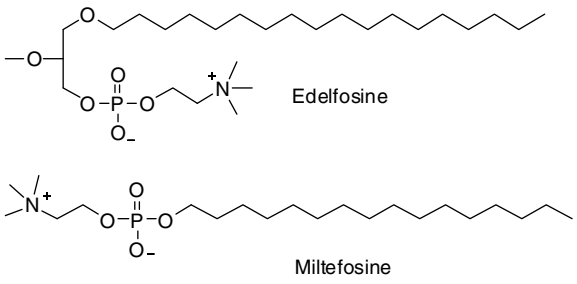

Figure 1. Alkyl phospholipids.

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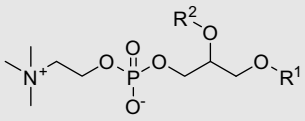
Table 1
Compounds synthesized



R^1				
R^2				
	—[a]	**1**	**2**	**3**
	4	**5**	**6**	**7**
	—[a]	**8**	**9**	**10**
	11	**12**	**13**	**14**
$C_{18}H_{37}$	**15**	**16**	**17**	**18**

[a] Not obtained in sufficient amount and/or purity.

In the present work, the alkylglycerol portion of edelfosine was modified, while the phosphocholine head group was kept constant. Substituents have been selected from the pool of commercially available starting materials with the aim of modifying the pharmacokinetics of edelfosine. The octadecyl chain (R^1) was substituted with aromatic groups (benzyl, benzotriazolmethyl, 3,5-dimethylisoxazolmethyl) and the small allyl group; the methyl (R^2) of edelfosine was replaced with long or short aliphatic chains (C_4H_9 or $C_{18}H_{37}$) or aromatic groups (phenyl, naphthylmethyl, or benzyl).

The first part of the synthetic scheme adopted (Scheme 1) is characterized by a solid phase synthesis of dialkylglycerols **22**. Solid phase has been adopted in order to increase the selectivity of the reaction of symmetrical 2-alkyloxy-1,3-propandiols **19** to give dialkylglycerols.[18] Thus, commercially available or easily obtainable 2-alkyloxy-1,3-propandiols **19** have been anchored to Merriffield's support functionalized by Ellman's dihydropyranyl linker using pyridinium *p*-toluenesulfonate (PPTS) as catalyst.[19,20] The primary alcohols **20** were then alkylated on solid phase under Williamson's conditions using various commercially available alkylbromides or chlorides. Dialkylglycerols **21** were then cleaved from the polymeric support using PPTS in dichloroethane/*n*-butanol. Alkylation of primary alcohols on solid phase was optimized in parallel (Table 2) using several solvents (DMF, DMSO, dimethylacetamide (DMA), *tert*-butanol (*t*-ButOH), Pyridine) and several bases (NaH, *t*-ButOK, Cs_2CO_3), and optimal reaction conditions were achieved using DMA as solvent and *t*-ButOK as base[21] (Table 2).

In the second part of our synthetic scheme (Scheme 1), the crude alcohols **22** were reacted in parallel in solution using known procedures to give the corresponding phosphocholines (**1–18**).[22]

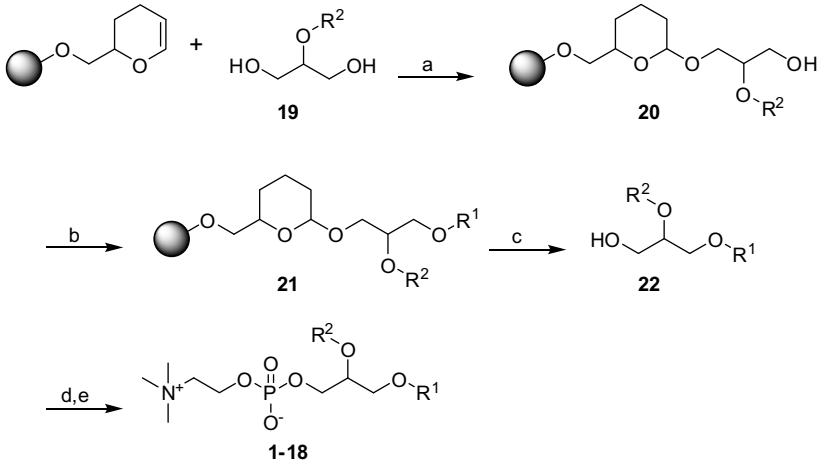
The final products were purified by silica gel column chromatography and analyzed by ^{1}H NMR and mass spectrometry.[23] In Table 3, the yields of the final products are reported.

The in vitro activity of these products against axenic amastigote forms of *L. donovani* was determined using a previously described assay.[24] All compounds presented IC_{50} values higher than miltefosine, the reference compound, which shows a similar IC_{50} to edelfosine. Compound **17** characterized by the *O*-octadecyl chain in R^2 and the benzotriazolyl substituent in R^1 is the most active compound with an IC_{50} of 0.66 μM, a value very similar to the one for miltefosine. Analogues (**15**, **16**, and **18**) having the C_{18} alkyl chain in R^2, but different substituents in R^1, showed higher IC_{50} values (6.75, 1.6, and 1.3 μM, respectively). By changing the R^2 substituent with the shorter butyl alkyl chain (**11**–**14**), the activity is almost completely abolished. The introduction of the phenyl substituent (**8**–**10**) in R^2 greatly reduced the activity. The naphthylmethyl and the benzyl substituents showed intermediate

Table 2
Optimization of Williamson reaction on solid phase

R^2	Base	Solvent	Yields (%)	f/f_{max}[a] (%)
Naphthyl-1-methyl	NaH	DMSO	nd[b]	48[c]
Naphthyl-1-methyl	NaH	DMA	nd[b]	55[c]
Naphthyl-1-methyl	Cs_2CO_3	DMA	nd[b]	100[c]
Naphthyl-1-methyl	Cs_2CO_3	DMF	nd[b]	90[c]
Naphthyl-1-methyl	*t*-ButOK	DMA	91	77
Octadecyl	*t*-ButOK	DMA	82	100
Octadecyl	*t*-ButOK	*t*-ButOH	85	89
Octadecyl	*t*-ButOK	DMSO	15	100
Octadecyl	*t*-ButOK	DMF	76	80
Octadecyl	*t*-ButOK	Pyridine	70	65

[a] f/f_{max}, ratio of loads; *f*, mmoles of bound product per g of polimeric support after the reaction; f_{max}, mmol of bound product per g of polymeric support before the reaction.
[b] nd, not detectable.
[c] Calculated based on the recovery of starting material.



Scheme 1. Reagents and conditions: (a) PPTS, DCE; (b) R_1X, *t*-ButOK, DMA; (c) PPTS, *n*-ButOH, 1,2-dichloroethane; (d) 2-Chloro-1,3,2-dioxaphospholane-2-oxide, triethylamine; (e) trimethylamine.

Table 3
L. donovani inhibition assay results and yields for compounds **1–18**

Compound	*L. donovani*	
	Inhibition IC_{50} (μM)	Yields[a] (%)
1	4.15	22
2	>50	10
3	>50	15
4	26.7	33
5	13.8	13
6	33.1	15
7	>50	19
8	>50	20
9	28.2	17
10	>50	17
11	42.5	35
12	>50	27
13	>50	25
14	>50	28
15	6.75	36
16	1.60	27
17	0.66	19
18	1.30	20
Miltefosine	0.22–0.27	

[a] Yields calculated as the ratio of isolated product and polymer-bound diol **19**.

activities: compounds **5** and **1,** both characterized by the benzyl group in R^1, were the most active ones (IC_{50} of 13.8 and 4.15 μM, respectively).

Compound **17** was also tested in *L. donovani* infected macrophages and resulted (IC_{50} 0.78 μM), appreciably more active than the reference drug miltefosine (IC_{50} 1.74 μM).

From the results obtained, it is clear that an adequate level of lipophilicity is required in order to improve the antiparasitic activity. The most active compound obtained is characterized by the C_{18} chain at R^2. This is interesting since it is known that edelfosine analogues which have the C_{18} chain in this position do not show induction of apoptosis.[6] This indicates that the mechanism of antiparasitic action of these compounds is probably different from the antitumor mode of action which is based on induction of apoptosis. The good intracellular activity of compound **17** supports further analogue synthesis to identify new potential antileishmanial candidates.

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21. The resin (loading 1.0–1.5 mmol/g) was solvated under stirring at 60 °C in anhydrous DMA (2 ml/100–150 mg of resin). *t*-ButOK was then added (20 mmol/mmol of polymer-bound diol) and the mixture stirred vigorously at 60 °C for 10 min. The halogenide (1 equivalent) was then added and the resin was stirred at 60 °C for 12 h. Successively the mixture was diluted with H_2O/DMF 1:1, filtered, washed with H_2O (3 times), DMF (3 times), and finally with CH_2Cl_2 (3 times).
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23. Characterization of compound **17**: HPLC purity: >99% C18 reverse-phase column (4.0 × 250 mm), detection at 206 and 254 nm (diode array detector), eluent: MeOH–H_2O 9:1 v/v plus 10 mM H_3PO_4; flow rate of 1.2 ml min^{-1}, t_R 9.6 min; ^1H NMR ($CDCl_3$): δ 0.81 (t, 3H, J = 6.7 Hz), 1.19 (br s, 30H), 1.34–1.38 (m, 2H), 3.18 (s, 9H), 3.25–3.67 (m, 5H), 3.72–3.87 (m, 2H), 4.12 (m, 2H), 6.00 (m, 2H), 7.34–7.40 (m, 1H), 7.46–7.53 (m, 1H), 7.66–7.69 (m, 1H), 7.96–7.99 (m, 1H). Mass: $[M+H]^+$: *m/z* 641.5, $[M+Na^+]$: *m/z* 663.3.
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