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Design and synthesis of new adamantyl-substituted antileishmanial ether phospholipids

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ABSTRACT

A series of new 2-[3-(2-alkyloxy-ethyl)-adamantan-1-yl]-ethoxy substituted ether phospholipids was synthesized and their antileishmanial activity was evaluated against *Leishmania infantum* amastigotes. The majority of the new analogues were significantly less cytotoxic than miltefosine while, antiparasitic activity depended on the length of the 2-alkyloxy substituent. The most potent compounds were {2-[[[3-(2-hexyloxy-ethyl)-adamant-1-yl]-ethoxy]hydroxyphosphinyloxy]ethyl}-*N*,*N*,*N*-trimethyl-ammonium inner salt (**5b**) and {2-[[[3-(2-octyloxy-ethyl)-adamant-1-yl]-ethoxy]hydroxyphosphinyloxy]ethyl}-*N*,*N*,*N*-trimethyl-ammonium inner salt (**5c**).

Leishmaniasis, is a parasitic disease which constitutes a major public health problem especially in the tropical and subtropical regions of the world. The causative agent is the protozoan parasite of the genus Leishmania and depending on the species, the infection leads to a large spectrum of clinical manifestations including cutaneous, mucocutaneous, and visceral forms. It is estimated that it causes 70,000 deaths annually, a rate surpassed only by malaria among other parasitic diseases.[1] The parasite is transmitted by the bite of the infected phlebotomine sandfly and even though most forms of the disease are transmissible only among animals, human leishmaniasis is increasingly spreading throughout the world. It is currently endemic in 88 countries on five continents (Africa, Asia, Europe, and North and South America), and the population at risk reaches 350 million people. According to WHO, 12 million people are infected worldwide, with 2 million new cases per year.[2] The distinguishable forms of the disease include visceral leishmaniasis (VL*a*, or 'kala-azar'), mucocutaneous leishmaniasis (MCL, ulceration of the skin and hyper development of the mucous membranes), and cutaneous and diffuse cutaneous leishmaniasis (CL and diffuse CL) and, if untreated, can have devastating consequences. Establishment of the infection and progression of the disease are favored by the compromised immune system of patients, like those infected with HIV, and as a result, leishmania/HIV co-infection is now considered an extremely serious new disease with severe clinical, diagnostic, chemotherapeutic, epidemiological, and economic implications. To date, co-infection with leishmaniasis and HIV has been reported in 34 countries in Africa, Asia, Europe, and South America.[3] Chemotherapy is currently the only way to treat the various forms of leishmaniasis, since no vaccine is yet available, however, the arsenal of drugs against the disease is still limited. Today, first line antileishmanial drugs are pentavalent antimonials (sodium stibogluconate and meglumine antimonate),[4] which are slowly being replaced by liposomal amphotericin B,[5] pentamidine or the first oral drug against the visceral form of the disease, miltefosine.[6] However, all the aforementioned drugs have serious drawbacks such as toxicity, poor efficacy or high cost. In addition, the emergence of drug resistant parasites has complicated the current chemotherapeutic strategies and thus, the invention of more effective and less toxic drugs is highly desirable.[7] This has led many research groups including our to design and synthesize novel antileishmanial compounds. Miltefosine (hexadecylphosphocholine) constituted a major breakthrough in antileishmanial chemotherapy, since this compound is effective against both visceral and cutaneous leishmaniasis, displays good bioavailability, and is currently registered as an oral drug for the treatment of the disease in India (in 2002) and Colombia (in 2005).[8,9] Despite its advantages, miltefosine has a long half-life (100–200 h) in humans and a low therapeutic ratio, characteristics that could encourage development of resistance, especially in India where VL is an anthroponosis. Moreover, it is not suitable for pregnant women because it has been

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shown to cause teratogenesis in animals[10] and it did not give satisfactory results when administered to HIV-coinfected patients, since most of them relapsed.[11] The efficacy of miltefosine against other non-Indian VL and the efficacy against the wide range of CL syndromes in the new and the old World are also major issues that need to be addressed.[12] With the issues of resistance,[13] side effects and production cost still requiring special attention and the mechanism of action still requiring elucidation, the field of design and synthesis of novel antileishmanial phospholipid derivatives is an ongoing challenge, especially against the strain *Leishmania infantum*, which causes the AIDS-associated co-infection in Europe. In this context, we recently reported[14–16] the synthesis of series of ring-substituted ether phospholipids, incorporating 4-alkylidenecyclohexyloxylethyl, cyclohexylidene, cyclodecylidene cyclopentadecylidene or adamantylidene groups in the lipid portion linked to the phosphate polar head-group by an oligomethylene bridge of 5 or 11 carbons. In addition, we have varied the polar head-group, using *N,N,N*-trimethylammonium, *N*-methylpiperidino, or *N*-methylmorpholino moieties, or we introduced more rigid head groups, namely the *trans*-2-hydroxy-*N,N,N*-trimethylcyclopentanamino or the *trans*-2-hydroxy-*N,N,N*-trimethylcyclohexanamino group, the 3-(2-hydroxy-ethylidene)-8,8-dimethyl-8-azonia-bicyclo[3.2.1]octane group and (4-hydroxy-but-2-ynyl)-trimethylammonium group.

Our results indicated that introduction of cycloalkane rings in alkylphosphocholines provides compounds with enhanced activity, against *L. infantum*, and reduced cytotoxicity on the human monocytic cell line THP-1.[16] Among the ring-substituted ether phospholipid derivatives that we synthesized, those containing an adamantylidene moiety (Fig. 1) exhibited high activity against *L. infantum* amastigotes and low cytotoxicity against THP-1 macrophages (Table 1).[16,17] Furthermore, we recently reported on the trypanocidal activity of new 1-alkyl-2-aminoadamantanes and the corresponding guanyl hydrazones and congeneric thiosemicarbazones.[18]

The promising results of the new ω-adamantylidene-substituted ether phospholipid derivatives, urged us to explore further the substitution pattern of the adamantane moiety for enhanced antileishmanial activity and reduced cytotoxicity. Herein, we describe the synthesis and biological evaluation of 2-[3-(2-alkyloxyethyl)-adamantan-1-yl]-ethoxy substituted ether phospholipids. Our aim was to introduce an ethylene spacer between the adamantane tricyclic system and the phosphate group, while, at position 3 an alkoxyethylene moiety would be attached. In this manner the adamantane system would be inserted within the lipid portion thus, resulting in more constrained derivatives. In addition, the distance between the adamantane moiety and the head group would be a short ethylene spacer, instead of an oligomethylene spacer of 11 or 5 carbon atoms,[14,16] thus, giving us the opportunity to investigate the optimum spacer length. In our design we have maintained choline as head group on the basis of our previous findings[16] which indicated that this group confers to the corresponding ether phospholipids reduced cytotoxicity while, activity remained high. Our aim was also to investigate the effect of the alkoxyethylene group on activity and toxicity and to this end we varied the length or the nature of the corresponding alkyl group (butyl, hexyl, octyl, decyl, dodecyl and benzyl).

Table 1
In vitro antileishmanial activity[a] against the intracellular amastigote form of *L. infantum*

Compound	Intracellular amastigote form *L. infantum* MON 235 IC_{50} (μM)	Cytotoxicity IC_{50} (μM)	Selectivity index
I	0.031 ± 0.01	59.50 ± 2.11	1919.3
II	5.69 ± 0.93	168.7	29.6
III	1.34 ± 0.56	37.45 ± 0.69	27.9
IV	5.2 ± 1.0	75.04	14.5
V	0.30 ± 0.2	74.95	249.8
VI	0.8 ± 0.1	39.7 ± 0.3	49.6
VII	9.1 ± 0.5	147.3	16.2
Miltefosine (control)	6.7 ± 1	28.6 ± 2.5	8.7

[a] Results are expressed as mean ± SEM of three independent experiments.

The synthetic strategy followed for the preparation of the new ether phospholipids **5a–f** is depicted in Scheme 2 and involves phosphorylation of the appropriate alcohols **4a–f**, followed by hydrolysis and formation of the corresponding pyridinium salts, which were in turn coupled to choline *p*-toluenesufonate.[19] The synthesis of the required alcohols **4a–f** was effected by esterification of 1,3-bis(carboxymethyl)adamantane (**1**)[20] followed by reduction of 1,3-adamantanediacetate (**2**) by $LiAlH_4$ to the corresponding symmetric diol **3**, which was in turn monoalkylated to afford the desired alcohols **4a–f**, as shown in Scheme 1.

The antiparasitic activity of derivatives **5a–f** was assessed using the intracellular amastigote form of *L. infantum* field strain MK-1 in infected human monocytic THP-1 cells (Table 2, Supplementary data). THP-1 cells infected with the appropriate *Leishmania* species are used for the evaluation of the leishmanicidal activity of compounds against the intracellular amastigote stages of the parasite, and therefore we assessed the cytotoxicity of all new analogues

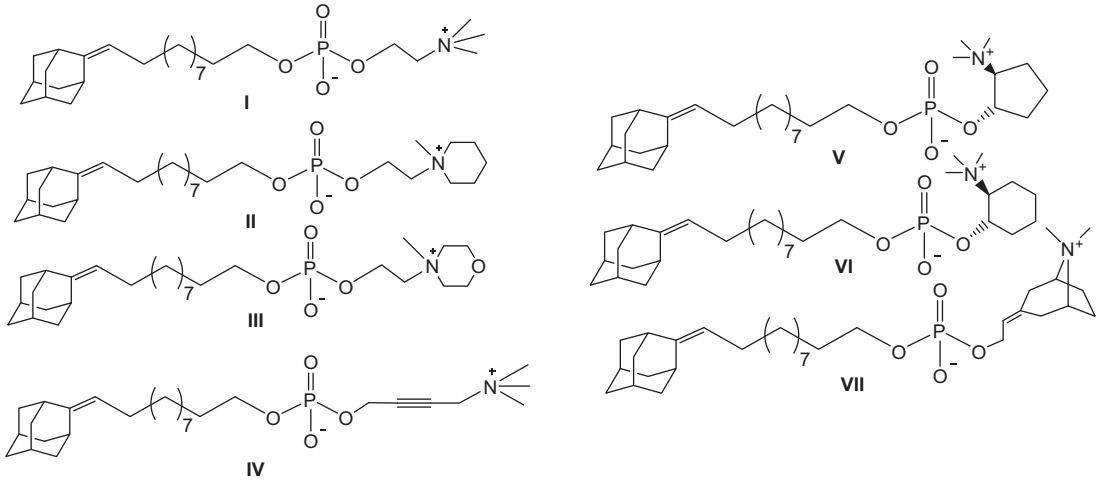
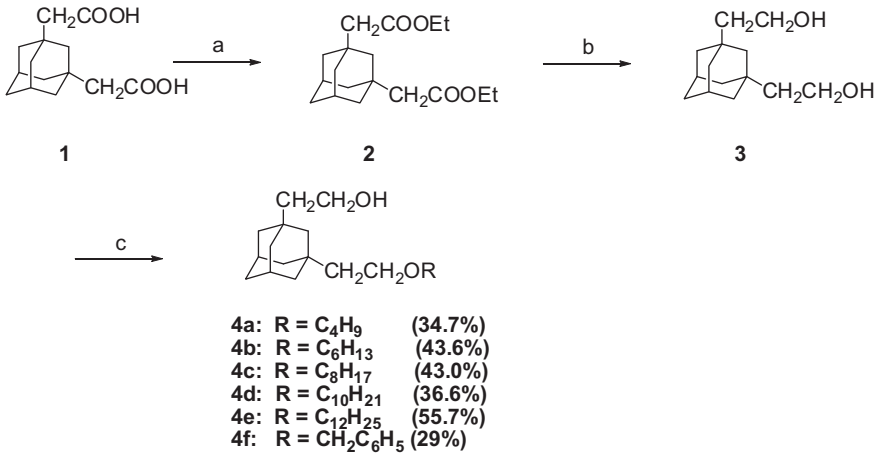
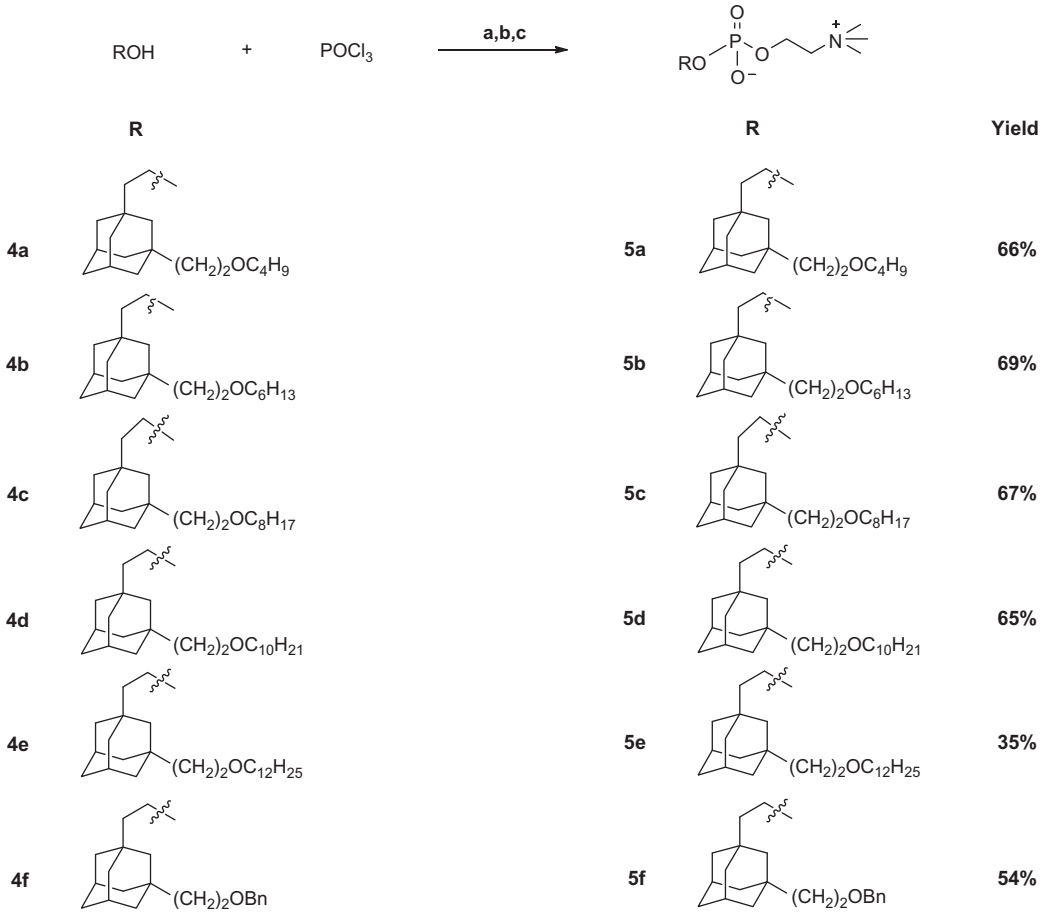


Figure 1. Structures of 11-adamantylideneundecyl-substituted ether phospholipids (**I**–**VII**).



Scheme 1. Reagents and conditions: (a) (1) $SOCl_2$/reflux overnight; (2) EtOH, reflux 2 h, 100%; (b) LAH, THF, reflux 2 h, 87%; (c) (1) NaH, DMF, rt, 2 h; (2) RX, DMF, rt, six days.



Scheme 2. Reagents and conditions: (a) (1) $P(O)Cl_3$, Et_3N, THF; (2) H_2O, 2-propanol; (b) pyridine, 40 °C; (c) pyridine, TIPS-Cl, choline *p*-toluenesulfonate.

against the human monocytic THP-1 host cell line (Table 2 and Fig. 2, Supplementary data).

We examined the antileishmanial activity only against the intracellular amastigote stage of the parasite for two reasons. Firstly, this is the disease relevant form and secondly because we and others have observed that alkylphosphocholines appear to be more active against the intracellular amastigotes than the promastigote stage, an effect which can be partially explained by their ability to activate macrophages.[21]

The most active analogues are the 3-hexyloxyethyl-adamantyl-substituted analogue **5b** and the 3-octyloxyethyl-adamantyl-substituted analogue **5c** with IC_{50} values of 17.1 ± 1.8 μM and 16.2 ± 2.1 μM, respectively. Moreover, these compounds do not exhibit cytotoxicity (IC_{50}>50 μM). Decreasing the length of the alkyl chain of the alkyloxyethyl substituent at position C3 of the adamantane moiety to butyl, compound **5a**, results in dramatic decrease of activity (IC_{50}>50 μM) while cytotoxicity remains unaffected (IC_{50}>50 μM). Conversely, increasing the alkyl chain to decyl or

Table 2
In vitro antileishmanial activity[a] against the intracellular amastigote form of *L. infantum*

Compound	Intracellular amastigote form *L. infantum* field strain MK-1 IC_{50} (μM)	Cytotoxicity IC_{50} (μM)	Selectivity index
5a	>50	>50	NA
5b	17.1 ± 1.8	>50	NA
5c	16.2 ± 2.1	>50	NA
5d	>18.4	18.4 ± 2.5	NA
5e	>21.4	21.4 ± 2.8	NA
5f	41.8 ± 2.5	>50	NA
Miltefosine (control)	2.56 ± 1.2	28.6 ± 2.5	11.2

NA: not applicable.
[a] Results are expressed as mean ± SEM of three independent experiments.

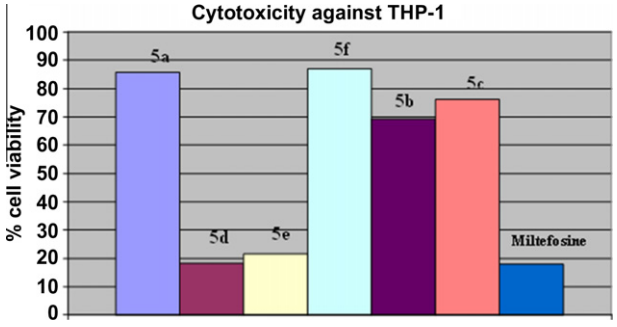


Figure 2. Cell viability (%) of THP-1 macrophages at 50 μM compound concentration.

dodecyl, compounds **5d** and **5e**, respectively, increases cytotoxicity (IC_{50} = 18.4 ± 2.5 μM and 21.4 ± 2.8 μM, respectively). However, antileishmanial activity of compounds **5d** and **5e** is higher than the cytotoxicity (IC_{50}>18.4 μM and >21.4 μM, respectively) and as a result, we cannot distinguish between specific antiparasitic activity and general cytotoxicity for these compounds. Finally the benzyl-substituted derivative **5f** is not cytotoxic (IC_{50}>50 μM), and possesses moderate antileishmanial activity IC_{50} = 41.8 ± 2.5 μM.

In general, four of the new derivatives are less cytotoxic than miltefosine (IC_{50} = 28.6 ± 2.5 μM), which is in accordance with our previous findings that the presence or cycloalkane rings in the lipid portion of alkylphosphocholines reduces cytotoxicity. Our results underscore certain structural features modulating the leishmanicidal activity of the adamantane-containing alkylphosphocholines. Firstly, the two carbon spacer between the adamantane moiety and the phosphate head group is not optimal since all the new compounds are less active than the ω-adamantylideneundecyl derivatives **I–VII** (Fig. 1), previously synthesized by us.[14,16] Secondly, the alkyl substituent of the alkyloxyethyl group at C3 modulates the relation between activity and cytotoxicity against THP-1 macrophages. Short alkyl chains (butyl to octyl) are preferred for non-toxicity versus longer (decyl to dodecyl), maybe due to detergent effects to cellular membranes.

In conclusion, we have synthesized a series of new 2-[3-(2-alkyloxy-ethyl)-adamantan-1-yl]-ethoxy substituted ether phospholipids. The majority of the new analogues were significantly less cytotoxic than miltefosine while, antiparasitic activity against *L. infantum* amastigotes depended on the length of the 2-alkyloxy substituent. The most potent compounds were {2-[[[3-(2-hexyloxy-ethyl)-adamant-1-yl]-ethoxy]hydroxyphosphinyloxy]ethyl}-*N*,*N*,*N*-trimethyl-ammonium inner salt (**5b**) and {2-[[[3-(2-octyloxy-ethyl)-adamant-1-yl]-ethoxy]hydroxyphosphinyloxy]ethyl}-*N*,*N*,*N*-trimethyl-ammonium inner salt (**5c**) which were not cytotoxic.

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Supplementary data

Experimental procedures, spectroscopic and analytical data for compounds **2**, **3**, **4a–f** and **5a–f**. Evaluation of in vitro antiparasitic activity and cytotoxicity of compounds **5a–f**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.078.

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19. General procedure for the preparation of ether phospholipids (**5a–f**) . To a solution of phosphorus oxychloride (0.12 mL, 1.3 mmol) in dry THF (5 mL) was added at −10 to −5 °C, a mixture of the corresponding alcohol **4a–f** (1 mmol) and dry triethylamine (0.25 mL, 1.8 mmol) in dry THF (7 mL). The reaction mixture was stirred at this temperature for 15 min and for additional 30 min at rt. Then H_2O (5 mL) was added and stirring was continued for 30 min. The aqueous layer was extracted with EtOAc and then with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was evaporated in vacuo to afford the corresponding phosphoric acid derivative, which was converted to the pyridinium salt by addition of 5 mL of anhydrous pyridine, stirring for 2 h at 40 °C and evaporation of the solvent in vacuo. To a solution of the above salt (1 mmol) in pyridine (7 mL) were sequentially added choline *p*-toluenesulfonate (0.413 g, 1.5 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-Cl) (0.455 g, 1.5 mmol) and the mixture was stirred at ambient temperature for 72 h. Subsequently, the mixture was hydrolyzed at °C by addition of 2-propanol/H_2O (7:2) 9 mL, and stirring for 0.5 h, the solvent was evaporated in vacuo and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 60:50/50:60/30:70) to afford the desired ether phospholipid derivatives **5a–f**.
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