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Design, synthesis and anti-leishmanial activity of novel symmetrical bispyridinium cyclophanes

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ABSTRACT

Nine novel symmetrical bispyridinium cyclophanes have been synthesized. They are rigid derivatives with an upper spacer which joins the two exocyclic amino groups, and a lower spacer joining the two positively charged nitrogen atoms. At least one of the two spacers is an aliphatic linker, such as an alkane or oxyalkane fragment. The activity of these compounds has been evaluated against promastigotes and intracellular amastigotes of *Leishmania donovani* and *Leishmania major*. All the cyclophanes are more active against *L. major*, with EC_{50} in intracellular amastigotes of between 1 and 17 μM, they exhibit very low toxicity against mammalian cells THP-1 and in some cases they present a higher selectivity index than the reference anti-leishmanial drugs amphotericin B and miltefosine. Compound **9** [2,8-Diaza-1,9(4,1)-dipyridinacyclotetradecaphan-1^1,9^1-bis(ilium) dibromide] is the most active one among cyclophane derivatives against intracellular amastigotes of *L. donovani* (EC_{50} 7.6 ± 0.2 μM) while *L. major* amastigotes are 6-fold more susceptible to the compound (EC_{50} 1.26 ± 0.3 μM). Compound **9** produces depolarization of the mitochondrial membrane and a decrease in the ATP levels that leads to death of the parasites. The anti-leishmanial activity of this macrocyclic salts is independent of the *Leishmania* enzymes ethanolamine kinase and choline/ethanolamine kinase.

1. Introduction

Leishmaniasis is a major group of neglected tropical diseases caused by the protozoan parasite *Leishmania*. Currently it affects 12 million people in 98 countries and around 350 million people worldwide are at risk of infection [1]. Leishmaniasis is responsible for a variety of pathologies that have been classified in three main clinical manifestations including cutaneous (CL), mucocutaneous (MCL), and visceral (VL) leishmaniasis; ranging from self-healing cutaneous lesions to fatal visceral infection [2].

Since an effective vaccine against leishmaniasis is not available, chemotherapy is the only way to treat all forms of the disease. The recommended first-line therapies for leishmaniasis includes pentavalent antimonials, amphotericin B (AmB), miltefosine and paromomycin, all of which have different types of limitation including toxicity, price, efficacy and emerging resistance [3], which emphasizes the importance of development new drugs against leishmaniasis. Pentamidine [1,5-bis(4-amidinophenoxy) pentane] is an aromatic diamidine widely used for the treatment of sleeping sickness caused by *Trypanosoma brucei* [4]. It was used as a second line drug against VL in cases of antimony failure, but its use against leishmaniasis is now limited to treatment of some forms of CL in South America [5]. Pentamidine acts at the mitochondrial level by accumulating within the mitochondria and binding to DNA, thus interfering with the replication and transcription [6]. Novel diamidines derivatives with improved pharmacokinetic properties have been under development in recent years [7,8].

Abbreviations: CL, cutaneous leishmaniasis; VL, visceral leishmaniasis; AmB, amphotericin B; CK, choline kinase; CEK, choline-ethanolamine kinase; EK, ethanolamine kinase; SI, selectivity index; $DiBAC_4(3)$, bis(1,3-dibutylbarbituric acid) trimethine oxonol; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DAPI, 4′,6-diamidino-2-phenylindole dilactate; PMA, phorbol 12-myristate 13-acetate; FBS, fetal bovine serum.

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We have previously designed and synthesized a set of bispyridinium compounds as inhibitors of the human choline kinase (CK). This enzyme is a validated anti-tumor target and all the above-mentioned compounds have shown a significant antiproliferative activity [9]. These compounds can be considered as structural analogues of pentamidine in which the amidino moiety, which is protonated at physiological pH, has been replaced by a positively charged nitrogen atom as a pyridinium ring. In view of this structural resemblance and with the intention of identifying potential anti-leishmanial drugs, we analyzed the anti-leishmanial activity of a series of bispyridinium derivatives. All compounds assayed found to display activity against promastigotes and intracellular amastigotes of *Leishmania donovani* and *Leishmania major*, with EC_{50} values lower than 1 μM in most cases [10].

Rigidification is a commonly used strategy to increase the activity of a drug or to reduce its side effects. Here we present a new family of symmetrical bispyridinium cyclophanes designed as cyclic analogues of previously reported bispyridinium derivatives, by cyclization through the exocyclic nitrogen atoms at position 4 of the pyridinium moiety via linker 2, which leads to the target cyclophanes (Fig. 1). These compounds have been evaluated as anti-leishmanial agents against *L. major*, responsible of the CL, and *L. donovani*, that causes the potentially fatal VL.

2. Results

2.1. Chemistry

A new set of 9 macrocycles were synthesized. They are symmetrical bispyridinum compounds which differ from each other in the upper and lower spacers. Four different spacers were used: two are phenyl-*p*-diylmethylene and phenyl-*m*-diylmethylene linkers, and the other two are aliphatic, such as the 1,5-pentanediyl and 3-oxa-1,5-pentanediyl moieties. At least one of the two spacers in every cyclophane is an aliphatic linker (Table 1).

The final compounds were synthesized according to Scheme 1. Dipyridines **1** and **2** were prepared from commercially available diamines and 4-bromopyridine in the presence of phenol under argon atmosphere, as previously described [11]. The novel dipyridines (**3** and **4**) were prepared from commercially available pentane-1,5-diamine and bis-2-(aminoethyl)ether, and following the same synthetic protocol previously reported [11].

Cyclophanes **B** were obtained by cyclization of dipyridines **1**–**4** and the dibromide derivatives in acetonitrile, according to our reported procedures [11]. The reaction was carried out by adding a 0.004 M solution of the dibromide drop by drop to the dipyridine in acetonitrile at the reflux temperature of the mixture for a period of 10–12 days, which favors the cyclization step and avoids the intermolecular reaction. In order to shorten the reaction time, microwave was used. Thus, dipyridine and dibromide derivatives in acetonitrile were microwave-irradiated at 140 °C for 20 min. Under these conditions, similar yields were obtained as compared to standard heating at the boiling point of the solvent (acetonitrile).

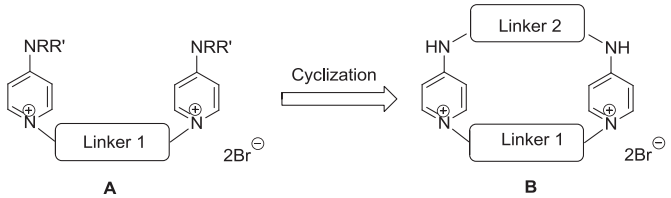
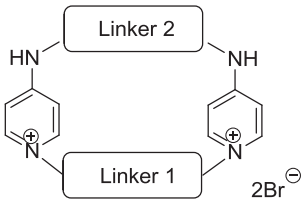
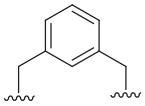
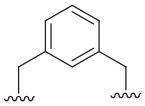
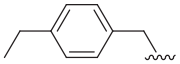
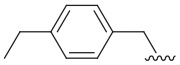
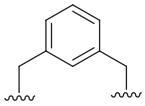


Fig. 1. Structural variations that lead to symmetrical bispyridinium cyclophanes (**B**) from symmetrical acyclic bispyridinium derivatives (**A**).

Table 1
Structures of the symmetrical bispyridinium cyclophanes.



Compound	Linker 1	Linker 2
5	—$(CH_2)_5$—	
6	—$(CH_2)_2$-O—$(CH_2)_2$—	
7	—$(CH_2)_5$—	
8	—$(CH_2)_2$-O—$(CH_2)_2$—	
9	—$(CH_2)_5$—	—$(CH_2)_5$—
10		—$(CH_2)_2$-O—$(CH_2)_2$—
11	—$(CH_2)_5$—	—$(CH_2)_2$-O—$(CH_2)_2$—
12	—$(CH_2)_2$-O—$(CH_2)_2$—	—$(CH_2)_2$-O—$(CH_2)_2$—
13	—$(CH_2)_2$-O—$(CH_2)_2$—	—$(CH_2)_5$—

2.2. Biological evaluation

2.2.1. Anti-leishmanial activity

Leishmania has two major life cycle stages: (i) the extracellular promastigote forms that proliferate inside the insect vector and (ii) the clinically relevant intracellular amastigote forms that reside inside the mammalian host. The final nine cyclophanes were tested as anti-leishmanial agents against promastigotes and intracellular amastigotes of *L. donovani* and *L. major*. The cytotoxic effect of these compounds was also investigated on the THP-1 cell line. Selectivity indexes (SI) were calculated as the ratio of the EC_{50} (the concentration of compound required to inhibit growth by 50%) for THP-1 to the EC_{50} for intracellular amastigotes. The results are shown in Table 2, where miltefosine and AmB were used as reference anti-leishmanial drugs.

All assayed compounds exhibit anti-leishmanial activity against promastigotes and intracellular amastigotes of *L. major* and *L. donovani*, being more active in *L. major*, with EC_{50} values in amastigotes of between 1 and 17 μM. Compounds **7**, **9**, **11**, **12** and **13** display EC_{50} values below 1 μM against promastigotes of *L. major*, an activity 100-fold higher than that obtained in promastigotes of *L. donovani*. However, the differences in activity decrease in the amastigote forms, because these compounds are less active in amastigotes than in promastigotes of *L. major* and more active in amastigotes than in promastigotes of *L. donovani*.

In general, from a structural point of view, compounds with two aliphatic linkers show better anti-leishmanial activity against