

First total synthesis of the (±)-2-methoxy-6-heptadecynoic acid and related 2-methoxylated analogs as effective inhibitors of the *Leishmania* topoisomerase IB enzyme*

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Abstract: The fatty acids (±)-2-methoxy-6*Z*-heptadecenoic acid, (±)-2-methoxy-6-heptadecynoic acid, and (±)-2-methoxyheptadecanoic acid were synthesized and their inhibitory activity against the *Leishmania* DNA topoisomerase IB enzyme (*LdTopIB*) determined. Both 2-OMe-17:1 fatty acids were synthesized from 4-bromo-1-pentanol, the olefinic fatty acid in 10 steps and in 7 % overall yield, while the acetylenic fatty acid in 7 steps and in 14 % overall yield. The 2-OMe-17:0 acid was prepared in 6 steps and in 42 % yield from 1-hexadecanol. The 2-OMe-17:1 acids inhibited *LdTopIB*, with the acetylenic acid displaying an $EC_{50} = 16.6 \pm 1.1 \mu\text{M}$, but the 2-OMe-17:0 acid did not inhibit *LdTopIB*. The (±)-2-methoxy-6*Z*-heptadecenoic acid preferentially inhibited *LdTopIB* over the human TopIB enzyme. Unsaturation seems to be a prerequisite for effective inhibition, rationalized in terms of weak intermolecular interactions between the active site of *LdTopIB* and either the double or triple bonds of the fatty acids. Toxicity toward *Leishmania donovani* promastigotes was also investigated, resulting in the order acetylenic > olefinic > saturated with the (±)-2-methoxy-6-heptadecynoic acid displaying an $EC_{50} = 74.0 \pm 17.1 \mu\text{M}$. Our results indicate that α -methoxylation decreases the toxicity of $C_{17:1}$ fatty acids toward *L. donovani* promastigotes, but improves their selectivity index.

Keywords: alkynes; *Leishmania donovani*; lipids; methoxylated fatty acids; synthesis; topoisomerase IB.

INTRODUCTION

Visceral leishmaniasis continues to be a serious tropical disease with around 500 000 cases reported each year, mainly in third-world countries such as India, Bangladesh, Indonesia, and Sudan [1]. The causative agent is *Leishmania donovani*, injected by a sand fly into a human in the form of promastigotes, which eventually invade the spleen and liver as amastigotes, resulting in anaemia and fever and ultimately in death if left untreated [2]. Several therapeutic venues are presently available, including

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pentavalent antimonials such as meglumine antimoniate or oral chemotherapeutic drugs such as miltefosine. A main problem with many of these drugs is emerging resistance by the parasite, and some can display considerable toxicity [3,4].

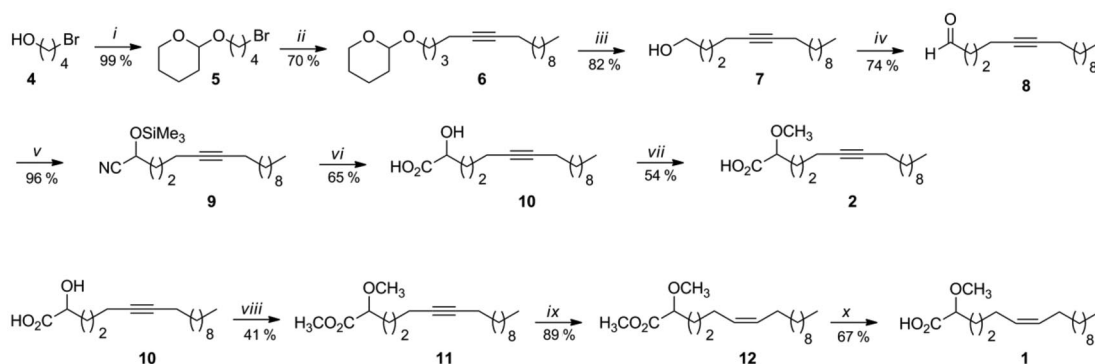
Some fatty acids have been reported to display antileishmanial activity, but earlier reports are contradictory. For example, among a series of C₄–C₁₈ fatty acids, it was reported that the C₁₈ fatty acids were the most toxic to *L. donovani* promastigotes, with the unsaturated fatty acids being more toxic than their saturated analogs [5]. In particular, oleic acid displayed a minimum inhibitory concentration (MIC) of 0.09 μmol/mL against *L. donovani*, while *n*-octadecanoic acid only showed an MIC of 1.39 μmol/mL. On the other hand, in an earlier work, *n*-decanoic acid, *n*-dodecanoic acid, and *n*-hexadecanoic acid, at 100 μg/mL, inhibited the motility of *L. donovani* promastigotes, but oleic acid was reported to have no effect [6].

Our research team has been actively studying fatty acids as agents to treat parasitic diseases. In an effort to further clarify the structural characteristics needed for a fatty acid to be effective against *L. donovani* promastigotes, our team synthesized and tested the natural occurring (from the plant *Sommera sabiceoides*) fatty acids 6-heptadecynoic acid and 6-icosynoic acid as well as its olefinic analogs [7]. We found that these Δ⁶ acetylenic fatty acids displayed good antiprotozoal activity toward *L. donovani* promastigotes (EC_{50s} = 1–6 μg/mL) and that the corresponding saturated fatty acids *n*-heptadecanoic acid and *n*-eicosanoic acid were not effective at all [7]. In addition, we also established that these unsaturated fatty acids were reasonably good inhibitors (EC_{50s} = 36–49 μM) of the *Leishmania* DNA topoisomerase IB enzyme (*LdTopIB*), a likely intracellular target for these fatty acids [7].

LdTopIB is a potential drug target for visceral leishmaniasis since it is different from other TopIB in the sense that it is phylogenetically unique and has an anomalous dimeric structure [8]. When the sequence of the human topoisomerase IB enzyme (hTopIB) and both subunits of the *LdTopIB* enzyme are compared, we find that the degree of homogeneity of the large subunit of *LdTopIB* with the hTopIB is low (45 %) and even less (29 %) when the corresponding regions of the hTopIB are compared with the small subunit of *LdTopIB* [8]. This comparison is important since it is exactly in these unconserved regions that we find the areas more sensitive to drugs. This difference makes the *LdTopIB* enzyme an attractive target for drug intervention, in particular if a compound can preferentially interact with *LdTopIB* over hTopIB. Therefore, our aim in this study was to synthesize and test against *LdTopIB* the 2-methoxylated analogs of the 6-heptadecynoic and 6*Z*-heptadecenoic acids previously studied [7]. The additional 2-methoxylated functionality was chosen inspired by the possibility for the α-methoxy group to favorably interact with the enzyme via hydrogen bonding as well as by the naturally occurring (±)-2-methoxy-6*Z*-heptadecenoic acid (**1**), an acid that we identified in the sponge *Calyx podatypa* and with the potential to display some interesting biological properties [9]. Based on our previous findings with the 6-heptadecynoic and 6*Z*-heptadecenoic acids, we expected the corresponding α-methoxylated fatty acids to be good inhibitors of *LdTopIB* as well as to display toxicity toward *L. donovani* promastigotes.

RESULTS AND DISCUSSION

The synthesis of the (±)-2-methoxy-6-heptadecynoic acid (**2**) started with commercially available 4-bromo-1-butanol (**4**), which was protected with 2,3-dihydro-2*H*-pyran (DHP) using catalytic amounts of PTSA in chloroform affording the 2-(4-bromobutyl)-tetrahydro-2*H*-pyran (**5**) in a 99 % yield (Scheme 1). In the subsequent step, commercially available 1-dodecyne was successfully coupled with **5** in the presence of *n*-BuLi in THF-hexamethylphosphoramide (HMPA) at –70 °C, which resulted in the tetrahydropyranyl protected alkyne **6** in a 70 % yield. The deprotection of **6** was achieved with PTSA in methanol at 60 °C, which yielded 5-hexadecynol (**7**) in an 82 % yield. Alcohol **7** was oxidized using pyridinium dichromate (PCC) in CH₂Cl₂ affording 5-hexadecynal (**8**) in a 74 % yield. The aldehyde was then reacted with trimethylsilyl cyanide (TMSCN) in dichloromethane and catalytic amounts

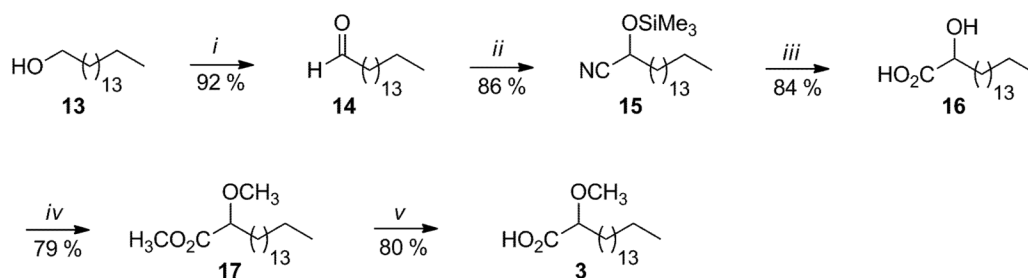


Scheme 1 Synthesis of (±)-2-methoxy-6-heptadecynoic acid (**2**) and the (±)-2-methoxy-6Z-heptadecenoic acid (**1**). (i) DHP, PTSA, CHCl_3 , rt; (ii) 1-dodecyne, *n*-BuLi, THF-HMPA, -70°C , 24 h; (iii) PTSA, MeOH, 60°C , 24 h; (iv) PCC, CH_2Cl_2 , 24 h, rt; (v) TMSCN, Et_3N , CH_2Cl_2 , 0°C , 3 h; (vi) HCl (con.), 2-MeTHF, 60°C , 4 h; (vii) NaH, THF, CH_3I , 0°C , 2 h; (viii) HCl, MeOH, reflux then NaH, THF, CH_3I , 0°C , 2 h; (ix) H_2 , Pd/C (10%), quinoline, hexane, rt; (x) KOH/EtOH (1 M), reflux, 2 h.

of triethylamine (Et_3N) at 0°C resulting in the desired 2-(trimethylsilyloxy)-6-heptadecynenitrile (**9**) in a 96% yield, which was then converted into the (±)-2-hydroxy-6-heptadecynoic acid (**10**) in a 65% yield by acid hydrolysis of nitrile **9** in 2-methyltetrahydrofuran (2-MeTHF) as solvent. In the final step, the desired acid **2** [10] was obtained in a 54% yield by methylation of **10** with sodium hydride (NaH) and methyl iodide (CH_3I) in THF as solvent (Scheme 1). This synthesis was achieved in seven steps and in 14% overall yield.

For the synthesis of the (±)-2-methoxy-6Z-heptadecenoic acid (**1**), the same synthetic route outlined in Scheme 1 was followed using the (±)-2-hydroxy-6-heptadecynoic acid (**10**) as the starting material (Scheme 1). The synthesis continued with the esterification of **10** using HCl/MeOH under reflux resulting in the desired methyl (±)-2-hydroxy-6-heptadecynoate, which was subsequently reacted with NaH and CH_3I in dry THF to obtain methyl (±)-2-methoxy-6-heptadecynoate (**11**) in a combined 41% yield. Methyl ester **11** was hydrogenated under Lindlar conditions in dry hexane, affording methyl (±)-2-methoxy-6Z-heptadecenoate (**12**) in an 89% yield. The desired acid **1** [11] was obtained in a 67% yield through the saponification of methyl ester **12** using KOH/EtOH (1 M) under reflux (Scheme 1). This represents the first synthesis for the naturally occurring (±)-2-methoxy-6Z-heptadecenoic acid (**1**), which was achieved in 10 steps and in a 7% overall yield.

The synthesis of the (±)-2-methoxyheptadecanoic acid (**3**) started with the oxidation of commercially available 1-hexadecanol (**13**) with PCC in CH_2Cl_2 , which afforded hexadecanal (**14**) in a 92% yield (Scheme 2). Hexadecanal was then reacted with TMSCN in CH_2Cl_2 using catalytic amounts of Et_3N at 0°C , affording the 2-(trimethylsilyloxy)heptadecanenitrile (**15**) in an 86% yield. The nitrile **15** was then converted into the (±)-2-hydroxyheptadecanoic acid (**16**) in an 84% yield by acid hydrolysis of **15** in 2-MeTHF as solvent. The methyl (±)-2-methoxyheptadecanoate (**17**) was obtained in a combined 79% yield by first preparing the methyl ester of acid **16** with HCl/MeOH and then methylating the hydroxy group with NaH and CH_3I in dry THF as solvent. The desired acid **3** [12] was finally obtained in an 80% yield by the saponification of **17** with KOH/EtOH (1 M) under reflux. The synthesis took six steps from 1-hexadecanol and a total overall yield of 42%.



Scheme 2 Synthesis of the (±)-2-methoxyheptadecanoic acid (**3**). (i) PCC, CH₂Cl₂, rt, 24 h; (ii) TMSCN, Et₃N, CH₂Cl₂, 0 °C, 3 h; (iii) HCl (conc.), 2-MeTHF, 60 °C, 5 h; (iv) HCl, MeOH, reflux then NaH, CH₃I, THF, 0 °C, 3 h; (v) KOH/EtOH (1 M), reflux, 2 h.

Aimed at examining the effect of α -methoxylation on the inhibitory potential of these acetylenic fatty acids, the new α -methoxylated fatty acids **1–3** were tested against *LdTopIB* following our previously published procedure [7]. We were in a good position to assess the effect of a triple vs. double bond on the inhibitory activity since the number of carbon atoms remained constant in our chosen examples. As can be seen from Table 1, the (±)-2-methoxy-6-heptadecynoic acid (**2**) was the most efficient inhibitor with an $EC_{50} = 16.6 \pm 1.1 \mu\text{M}$ (Fig. 1). The effectiveness of inhibition followed the order **2** > **1** > **3**, where the (±)-2-methoxy-6Z-heptadecenoic acid (**1**) also displayed considerable inhibition of the enzyme with an $EC_{50} = 41.3 \pm 5.6 \mu\text{M}$ and the (±)-2-methoxyheptadecanoic acid (**3**) did not inhibit *LdTopIB* ($EC_{50} > 1000 \mu\text{M}$). Since the chain length (C₁₇) and unsaturation position at C-6 remained constant among our chosen examples, we can conclude that acids **1–2** were more effective inhibitors of *LdTopIB* than either 6Z-heptadecenoic acid or 6-heptadecynoic acid. Therefore, α -methoxylation seems to be a viable way of increasing the inhibitory potential of C₁₇ fatty acids toward *LdTopIB*.

Table 1 Inhibition of the relaxation activities of the *LdTopIB* enzyme by the studied fatty acids.

Fatty acid	EC_{50} (μM)
(±)-2-OMe-6-heptadecynoic (2)	16.6 ± 1.1
6-Heptadecynoic	71.7 ± 3.8
(±)-2-OMe-6Z-heptadecenoic (1)	41.3 ± 5.6
6Z-Heptadecenoic	80.4 ± 9.3
(±)-2-OMe-heptadecanoic (3)	>1000
<i>n</i> -Heptadecanoic	>1000

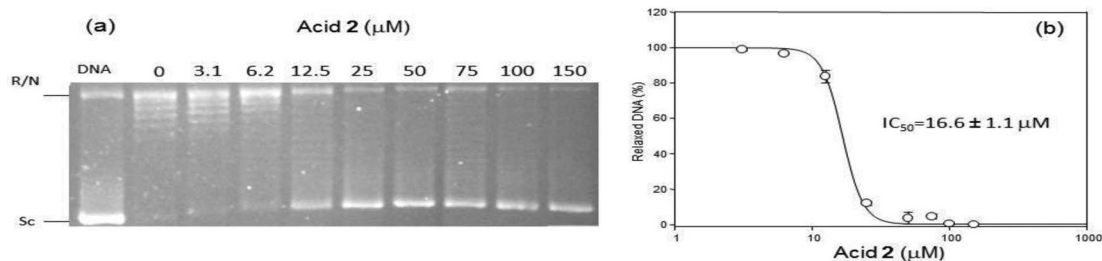


Fig. 1 Inhibition of the relaxation activity of recombinant *LdTopIB* by acid **2** (a) and the corresponding plot of the relaxation activity (%) of the *LdTopIB* enzyme versus concentration (μM) of the acetylenic fatty acid **2** (b). One unit of recombinant *LdTopI* was assayed in a plasmid DNA relaxation assay for 30 min at 37 °C in the presence of 3.1–150 μM acid **2**. Reaction products were resolved in agarose gel and subsequently visualized by ethidium bromide staining. The relative position of the negatively supercoiled DNA substrate is indicated by Sc, N is the nicked DNA, whereas the ladder of relaxed DNA topoisomer bands is in between. Reactions were stopped with a mixture of 1 % SDS and 6.1 μg of proteinase K. Lane 1 contains 0.2 μg of pHOT plasmid DNA.

In a separate experiment, we also compared the inhibition of **1** toward *LdTopIB* vs. hTopIB, and the results are shown in Fig. 2. Acid **1** was able to inhibit *LdTopIB* at the listed concentration, but it was not effective against hTopIB at the same concentrations. These results indicate that it will be possible to preferentially interfere with *LdTopIB* without inhibiting the human enzyme, a finding that could have therapeutic value. The latter seems to be a general finding for fatty acids, i.e., in all of the fatty acids that we have tested so far (unpublished data), we have found that *LdTopIB* is more sensitive to inhibition by fatty acids than hTopIB.

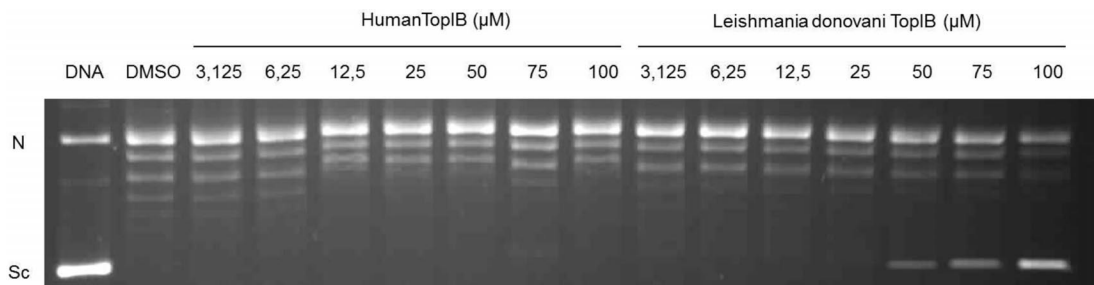


Fig. 2 Comparison of the inhibition of the relaxation activity of human TopIB (left) and recombinant *LdTopIB* by acid **1**. One unit of recombinant *LdTopIB* was assayed in a plasmid DNA relaxation assay for 30 min at 37 °C in the presence of 3.125–100 μM acid **1**. Reaction products were resolved in agarose gel and subsequently visualized by ethidium bromide staining. The relative position of the negatively supercoiled DNA substrate is indicated by Sc, N is the nicked DNA, whereas the ladder of relaxed DNA topoisomer bands is shown in between. Reactions were stopped with a mixture of 1 % SDS and 6.1 μg of proteinase K. Lane 1 contains 0.2 μg of pHOT plasmid DNA and lane 2 DMSO.

Based on the fact that the inhibition of *LdTopIB* followed the order **2** > **1** >> **3**, one can argue that the inhibition decreases in the order: triple bond \geq double bond >> single bond. Since we do not expect a big difference between the geometries of acids **2** and **3**, and we consider the fact that a fatty acid with a *cis*-cyclopropane group does not inhibit *LdTopIB* [13], other factors, such as intermolecular cation– π interactions, might be significant near the binding sites of the fatty acids with *LdTopIB*. One could hypothesize that the ammonium groups (R-NH_3^+ or $\text{R}_2\text{C}=\text{NH}_2^+$) in amino acids such as lysine (K) and/or arginine (R), known to be present in the active sites of these TopIB enzymes [14], might inter-

act with the double or triple bonds in these fatty acids. These forces are quite significant, in the proximity of 10 kcal/mol, as previous computational studies have shown [15,16].

In order to study these cation- π interactions further with modern computational techniques, the $\sigma\cdots\pi$ interactions between NH_4^+ and $\text{C}_2\text{H}_2/\text{C}_2\text{H}_4/\text{C}_2\text{H}_6$ were investigated by means of ab initio methods using the Gaussian 09 program [17]. All the geometries were fully optimized at the level of MP2/aug-cc-pVTZ [18], and CCSD(T)/aug-cc-pVTZ [19–21] single-point energy computations were carried out to refine the energy data. The zero-point energy (ZPE) and basis set superposition error (BSSE) corrections were also considered at the MP2/aug-cc-pVTZ level of theory. Natural bond orbital (NBO) analysis [22] was used to evaluate the charge transfer between NH_4^+ and C_2H_x . Both the $\text{NH}_4^+\cdots\text{C}_2\text{H}_2$ and $\text{NH}_4^+\cdots\text{C}_2\text{H}_4$ complexes are of C_s symmetry, while $\text{NH}_4^+\cdots\text{C}_2\text{H}_6$ has C_1 symmetry (Fig. 3). The distances between the H pointing to C_2H_x ($x = 2, 4, 6$) in NH_4^+ and the center of C–C bond ($D_{\text{H-O}}$) in the $\text{NH}_4^+\cdots\text{C}_2\text{H}_2$, $\text{NH}_4^+\cdots\text{C}_2\text{H}_4$, and $\text{NH}_4^+\cdots\text{C}_2\text{H}_6$ complexes are 2.078, 2.099, and 2.274 Å, respectively. The N–H bond ($D_{\text{N-H}}$) of NH_4^+ pointing to C_2H_x ($x = 2, 4, 6$) is elongated from 1.023 Å in the free NH_4^+ structure to 1.043, 1.046, and 1.031 Å in the corresponding complexes (Fig. 3, Table 2). Among these three complexes, the interaction between NH_4^+ and C_2H_6 is the weakest (–5.27 kcal/mol), and those between NH_4^+ and C_2H_x ($x = 2, 4$) are comparable (–9.95 and –9.99 kcal/mol, respectively), with $\text{NH}_4^+\cdots\text{C}_2\text{H}_4$ having a slightly stronger binding energy. The electrostatic interactions likely dictate the stabilities of these complexes, the charge transfers (Δq) (0.043, 0.055, and 0.015 e, respectively) correlate well with the binding energies for $\text{NH}_4^+\cdots\text{C}_2\text{H}_x$ ($x = 2, 4, 6$). These findings could help explain why acids **1** and **2** inhibit *LdTopIB*, while acid **3**, as well as all saturated fatty acids tested so far, do not inhibit the enzyme.

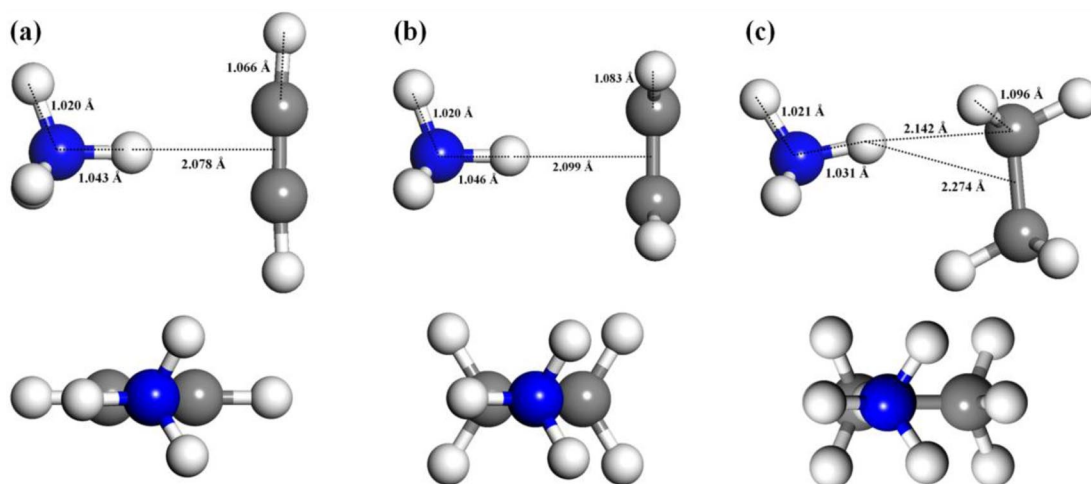


Fig. 3 The lowest-energy structures of $\text{NH}_4^+\cdots\text{C}_2\text{H}_x$ [$x = 2$ (a), 4(b), 6(c)] at MP2/aug-cc-pVTZ level of theory.

Table 2 The main structural parameters, the charge transfer between NH_4^+ and C_2H_x , and binding energies of optimized $\text{NH}_4^+\cdots\text{C}_2\text{H}_x$ ($x = 2, 4, 6$) complexes.^a

	$\text{NH}_4^+\cdots\text{C}_2\text{H}_2$	$\text{NH}_4^+\cdots\text{C}_2\text{H}_4$	$\text{NH}_4^+\cdots\text{C}_2\text{H}_6$
$D_{\text{N-H}}$ (Å)	1.043	1.046	1.031
$D_{\text{H-O}}$ (Å)	2.078	2.099	2.274 (2.142)
Δq (e)	0.043	0.055	0.015
E_b (kcal/mol)	-9.95	-9.99	-5.27

^a $D_{\text{H-O}}$ is the distance between H of NH_4^+ pointing to C_2H_x ($x = 2, 4, 6$) and the center of C-C, the data in parentheses denotes the distance between H of NH_4^+ and its closer C atom. E_b was obtained at CCSD(T)/aug-cc-pVTZ//MP2/aug-cc-pVTZ level of theory, including the MP2-estimated ZPE and BSSE corrections.

The mode of inhibition of **1–2** against *LdTopIB* could very well be similar to the one reported for conjugated eicosapentaenoic acid (cEPA) with hTopIB [14]. In this study, it was concluded that cEPA essentially binds to a region close to the active site of the enzyme and inhibits DNA cleavage by blocking the area, but it does not prevent DNA from binding. In fact, in this computational model it was shown that cEPA binds in close proximity to K443, K587, and N722, and prevents the catalytic Y723 from performing a nucleophilic substitution on the DNA phosphate [14].

Based on the enzymatic studies, we further explored the antiprotozoal activity of the 2-methoxylated fatty acids toward *L. donovani* promastigotes following our already published procedure [7] and the results are shown in Table 3. Among the studied 2-methoxylated fatty acids, the (\pm)-2-methoxy-6-heptadecyonic acid (**2**) was the most toxic fatty acid toward *L. donovani* promastigotes with an $\text{EC}_{50} = 74.0 \pm 17.1 \mu\text{M}$. When compared to the 6-heptadecyonic acid ($\text{EC}_{50} = 25.1 \pm 1.4 \mu\text{M}$), it became evident that 2-methoxylation somewhat decreased the toxicity of 6-heptadecyonic acid toward *L. donovani*. However, the 6-heptadecyonic acid displays considerable toxicity toward murine macrophages, resulting in a low therapeutic index of 0.85 (Table 3). On the other hand, acid **2** did not display toxicity toward murine macrophages resulting in an excellent therapeutic index (>700). These results indicate that α -methoxylation decreases the toxicity of C_{17} fatty acids toward *L. donovani* promastigotes, but improves their selectivity index when compared to murine macrophages, thus making acid **2** the most viable drug candidate among the studied fatty acids.

Table 3 Antileishmanial activity of the studied fatty acids (μM).

Fatty acid	<i>L. donovani</i> promastigotes (EC_{50})	Murine macrophages (IC_{50})	Therapeutic index ($\text{IC}_{50}/\text{EC}_{50}$)
(\pm)-2-OMe-6-heptadecyonic (2)	74.0 ± 17.1	>700	>32
6-Heptadecyonic	25.1 ± 1.4	21.3 ± 0.9	0.85
(\pm)-2-OMe-6Z-heptadecenoic (1)	404.1 ± 11.9	483.9 ± 15.8	1.2
6Z-Heptadecenoic	>100	44.4 ± 1.6	n/a
(\pm)-2-OMe-heptadecanoic (3)	>1000	>1000	n/a
<i>n</i> -Heptadecanoic	>100	52.6 ± 7.5	n/a
Miltefosine	0.8 ± 0.1	1.9 ± 0.4	2.5

Interesting were the results with either the (\pm)-2-methoxy-6Z-heptadecenoic acid (**1**) or the 6Z-heptadecenoic acid, which were not effective against *L. donovani* promastigotes despite the fact that they were inhibitors of *LdTopIB*. Acid **1** presented some antileishmanial activity toward *L. donovani* promastigotes with an $\text{EC}_{50} = 404.1 \pm 11.9 \mu\text{M}$ (Table 3). These results indicate that a triple bond is

preferred over a double bond in these C₁₇ fatty acids in order to display antiprotozoal activity. This observation was further confirmed by the finding that both (±)-2-methoxyheptadecanoic acid (**3**) and *n*-heptadecanoic acid were not effective at all against the parasite. However, there seems to be other mechanisms of toxicity, besides *LdTopIB* inhibition, responsible for the toxicity of **1–2** toward *L. donovani* promastigotes. In any instance, the antiprotozoal studies with acid **2** correlate with the *LdTopIB* inhibitory studies, which indicate that acid **2** is the best overall candidate among the studied compounds.

CONCLUSIONS

Based on the *LdTopIB* inhibitory studies and the antileishmanial studies with *L. donovani* promastigotes, the novel fatty acid (±)-2-methoxy-6-heptadecynoic acid (**2**) was identified as a viable candidate against leishmaniasis based on its high inhibition of the *LdTopIB* enzyme and its high therapeutic index. It was found that the combination of α-methoxylation and a C-6 triple bond enhances the inhibition of C₁₇ fatty acids toward the *LdTopIB* enzyme.

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10. Spectral data for the (±)-2-methoxy-6-heptadecynoic acid (**2**). IR (neat) ν_{\max} : 3365, 2926, 2855, 1734, 1457, 1380, 1119 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ (ppm): 3.83 (1H, t, *J* = 4.6 Hz), 3.44 (3H, s, -OCH₃), 2.20 (2H, t, *J* = 7.0 Hz), 2.12 (2H, t, *J* = 6.9 Hz), 1.62 (2H, q, *J* = 7.2 Hz), 1.25 (18H, brs, -CH₂-), 0.87 (3H, t, *J* = 6.9 Hz, -CH₃); ¹³C NMR (CDCl₃, 125 MHz) δ (ppm): 176.97 (C-1), 81.07 (C-7), 79.72 (C-2), 79.02 (C-6), 58.24 (C-2'), 31.88 (C-15), 31.36 (C-13), 29.57 (C-12), 29.52 (C-14), 29.30 (C-9), 29.14 (C-11), 28.88 (C-3), 24.44 (C-16), 22.66 (C-4), 18.71 (C-5), 18.45 (C-8), 14.09 (C-17). HRMS calcd for C₁₈H₃₂O₃ [M + H]⁺ 297.2424, found 297.2423.

11. Spectral data for the (\pm)-2-methoxy-6Z-heptadecenoic acid (**1**). IR (neat) ν_{\max} : 3330, 3005, 2928, 2856, 1720, 1650, 1458, 1380, 969, 778 cm^{-1} ; ^1H NMR (CDCl_3 , 500 MHz) δ (ppm): 5.35 (2H, m), 3.78 (1H, t, $J = 6.7$ Hz), 3.42 (3H, s, $-\text{OCH}_3$), 2.01 (4H, m), 1.76 (2H, m), 1.23 (18H, brs, $-\text{CH}_2-$), 0.85 (3H, t, $J = 7.0$ Hz, $-\text{CH}_3$); ^{13}C -NMR (CDCl_3 , 125 MHz) δ (ppm): 176.87 (C-1), 131.26 (C-7), 129.04 (C-6), 80.46 (C-2), 58.28 (C-2'), 31.88 (C-15), 31.81 (C-3, C-9), 29.67 (C-10), 29.61 (C-11), 29.53 (C-12), 29.41 (C-13), 29.31 (C-14), 27.23 (C-8), 26.71 (C-5), 24.83 (C-4), 22.67 (C-16), 14.13 (C-17). HRMS Calcd for $\text{C}_{18}\text{H}_{35}\text{O}_3$ $[\text{M} + \text{H}]^+$ 299.2581, found 299.2583.
12. Spectral data for the (\pm)-2-methoxyheptadecanoic acid (**3**). IR (neat) ν_{\max} : 3420, 2922, 2852, 1716, 1457, 1381, 1090 cm^{-1} ; ^1H -NMR (CDCl_3 , 500 MHz) δ (ppm): 3.79 (1H, t, $J = 5.0$ Hz), 3.46 (3H, s, $-\text{OCH}_3$), 1.52 (2H, m), 1.28 (26H, brs, $-\text{CH}_2-$), 0.87 (3H, t, $J = 6.7$ Hz, $-\text{CH}_3$); ^{13}C -NMR (CDCl_3 , 125 MHz) δ (ppm): 175.89 (C-1), 80.27 (C-2), 58.34 (C-2'), 32.22 (C-15), 31.93 (C-3), 29.70 (C-12, C-13), 29.67 (C-10, C-11), 29.63 (C-9), 29.55 (C-8), 29.42 (C-7), 29.37 (C-6), 29.32 (C-5), 29.08 (C-14), 24.82 (C-4), 22.70 (C-16), 14.13 (C-17).
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