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Heterocycle-fused Lupane Triterpenoids Inhibit Leishmania donovani Amastigotes

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The synthesis of heterocyclic betulin derivatives and their activity against Leishmania donovani is reported. Betulonic acid was used as a versatile intermediate. Several different fused heterocycles were introduced at the 2,3-position of the lupane skeleton including isoxazole, pyrazine, pyridine, indole and pyrazole rings. Also 28-position was modified. Three compounds, 5, 8 and 25, showed low micromolar activity with IC$_{50}$ values of 13.2, 4.3 and 7.2 µM, respectively. Compound 8 showed the best activity and selectivity, and its activity was tested on infected macrophages using a concentration, 5 µM, where no macrophage toxicity was exhibited. Interestingly, the activity of compound 8 on axenic amastigotes and Leishmania-infected macrophages was similar.

Introduction

Leishmaniasis is a spectrum of diseases caused by over 20 species of protozoan parasites belonging to the genus Leishmania. These diseases affect people in more than 88 countries. There are an estimated 1 - 2 million new cases every year, 12 million people currently infected, and 350 million people living in endemic areas at risk. During past ten years leishmaniasis has spread considerably. It is transmitted by bite of infected female Phlebotomine or Lutzomyia sandflies in the Old World and the New World, respectively.

There are three major forms of leishmaniasis: cutaneous, mucocutaneous, and visceral disease. Cutaneous leishmaniasis is the mildest form of this disease and is characterized by skin ulcers on exposed areas at the site of the sand fly bite. The ulcers generally self-heal leaving scars after a few months to years. In the mucocutaneous form, which is difficult to treat, disfiguring lesions destroy the mucous membranes of the nose, mouth and throat cavity. Finally, visceral leishmaniasis (VL), the most severe form of the disease, is fatal if untreated. VL causes fever, weight loss, anaemia, and enlargement of the spleen and liver. Several treatments exist for leishmaniasis, but most of them have adverse effects. Pentavalent antimonials, the first-line treatment for leishmaniasis, have lost their efficacy in some regions endemic for VL, and liposomal amphotericin B is highly expensive. These treatments are administrated by injection and require clinical supervision or hospitalization. Miltefosine, the first effective orally administrated drug for leishmaniasis, is contraindicated in women of child-bearing age due to teratogenic effects. Hence, there is an urgent need to develop new, safe and effective treatments for these diseases.

Betulin is a plentiful naturally occurring lupane-type pentacyclic triterpene. Betulinic acid and other betulin derivatives show antiviral, anti-HIV, anti-inflammatory, anti-malarial, and anti-tumoral effects. Previously our group has shown that heterocyclic betulin derivatives have an effect against L. donovani amastigotes, which cause VL. In this study we describe a new set of heterocyclic betulin derivatives and their biological activity against Leishmania donovani amastigotes, as well as the structure-activity relationships of the compounds.
Results and discussion

Chemistry

First, betulin 1 was subjected to Jones oxidation, and the resulting betulonic acid 2 was used as a key intermediate for the synthesis of several heterocyclic adducts (Scheme 1). The indole derivatives 3-4 were prepared by the Fischer indole synthesis in 21-42% yields. Letting betulonic acid react with ethylenediamine in the presence of sulfur and morpholine gave lupa-2,20(29)-dieno[2,3-b]pyrazin-28-oic acid 5 in 68% yield. This was treated with oxalyl chloride in dichloromethane (DCM) and the resulting acyl chloride was converted to the primary amide functionality (Scheme 2) in 77% yield. The subsequent Claisen condensation with ethyl formate produced 2-hydroxymethylene adduct 6 in 56% yield. Finally, the treatment of the Claisen product with oxalyl chloride followed by the condensation/cyclization of reactions as described above for lupaj2,20(29)jdieno[2,3j isoxazol-28-oic acid 7 in 33% yield in the Beckmann rearrangement reaction by treating it with trifluoroacetic anhydride (TFAA) in DCM. Lupa-2,20(29)-dieno[2,3-b]pyridin-28-oic acid 8 was obtained from the reaction of betulonic acid and propargylamine in the presence of Cu(I)Cl in ethanol in 11% yield.

The corresponding isoxazole 10 and pyrazole 11 derivatives were synthesized via the 2-hydroxymethylene adduct 12 of betulonic acid 2 followed by the condensation/cyclization reaction with NH₂OH•HCl or H₂NNH₂•H₂O in 68% and 80% yields, respectively (Scheme 2). The carboxyl group of lupa-2,20(29)-dieno[2,3-d]isoxazol-28-oic acid 10 was converted to the primary amide functionality 13 as described above in case of compound 6.

20(29)-Dihydrolup-2-en[2,3-d]isoxazol-28-oic acid 14 was obtained from benzyl betulonate in three steps (Scheme 3). First, the carbon-carbon double bond of benzyl betulonate was reduced under hydrogen atmosphere in the presence of palladium on carbon in ethyl acetate to give the corresponding dihydrobetulonic acid in 77% yield. The subsequent Claisen condensation with ethyl formate produced 2-(hydroxymethylene)-3-oxo-20(29)-dihydrolup-28-oic acid in 56% yield. Finally, the treatment of the Claisen product with NH₂OH•HCl in acetic acid gave the target 20(29)-dihydrolup-2-en[2,3-d]isoxazol-28-oic acid 14 in 90% yield.

28-Hydroxylupa-2,20(29)-dieno[2,3-d]isoxazole 15 was synthesized from betulin 1 in five steps (Scheme 4). First, the betulin C-28 hydroxy group was protected as a tetrahydropropenyl ether 16 in 80% yield, and the resulting THP ether was oxidized to the THP-protected betulonic alcohol with PCC in DCM in 46% yield. Subsequently, the same cascade of reactions as described above for lupa-2,20(29)-dieno[2,3-
d-jisoxazol-28-oic acid 10 was used to produce the isoxazole-fused 28-O-acetyl triterpene 17 in 26% yield over two steps (Scheme 4). In acidic conditions of the cyclization reaction THP protecting group was cleaved and replaced with the acetoxy group. The acetoxy group was removed with p-TsOH in methanol in quantitative yield. Finally, 28-hydroxylupa-2,20(29)-dieno[2,3-d]isoxazole 15 was treated with 2-iodobenzoic acid in THF and DMSO to give 28-oxolupa-2,20(29)-dieno[2,3-d]isoxazole 18 in 51% yield.

Allobetulin 19 was obtained in 25% yield by refluxing betulin 1 in formic acid followed by refluxing the resulting intermediate formate ester in ethanolic solution of KOH in benzene (Scheme 5). The indole derivatives of allobetulin 20-21 were obtained with the same methodology as described above for betulonic acid (cf. synthesis of compounds 3 and 4) in 57-63% yields.

Betulonic aldehyde 22 was obtained from betulin 1 by PCC oxidation in DCM in 27% yield (Scheme 6). 28-Oxolupa-2,20(29)-dieno[2,3-b]pyrazine 23 was synthesized in 17% yield using the same methods as in the preparation of lupa-2,20(29)-dieno[2,3-b]pyrazin-28-oic acid 5. It was further reacted with NH₂OH·HCl to give 28-oximinolupa-2,20(29)-dieno[2,3-b]pyrazine 24 in 77% yield.

3β-(3-Carboxy-3-methylbutanoyloxy)lup-20(29)-en-28-oic acid (bevirimat) 25 was synthesized from betulonic acid 26 by refluxing it in the presence of 2,2-dimethylsuccinic anhydride and DIPEA in DMF for 2 days in 5% yield (Scheme 7).

**Biology and structure-activity relationships**

Previously we found a set of heterocyclic betulin derivatives to have promising activity against axenic amastigotes of *L. donovani*, and based on those results we synthesized a new set of fused heterocyclic adducts of betulin, betulinic acid and betulonic acid; and varied substituents at the position C-28 to explore effects of that position as well. Leishmanicidal activity of the modified compounds was assayed using the alamarBlue (AbD Serotec, Oxford, UK) viability assay on axenic amastigotes of *L. donovani* (Table 1).

Primary screen was performed at 50 µM concentration and compounds showing >70% inhibition were assayed at 15 µM concentration and finally most potent derivatives at 5 µM concentration (Table 1). In the series of A-ring fused isoxazoles, the betulin-derived compound 15 had 57% inhibition of the growth at 50 µM concentration. The betulonic acid-derived isoxazole 10 inhibits 96% at 50 µM and 16% at 15 µM. Interestingly, the dihydrobetulonic acid-derived isoxazole 14 had only 3% inhibition at 50 µM. In our earlier studies we found a similar effect, but not this strong, between betulonic acid and betulonic acid. The primary amide derivative of the betulonic acid-derived isoxazole 13 inhibits 84% of the growth at 50 µM concentration, but only 17% at 15 µM concentration. On the other hand, the betulinic aldehyde-derived isoxazole 18 has lower activity (34%) at 50 µM but slightly better activity (21%) at 15 µM concentration compared to 13. This may be due to solubility, as the aldehyde might not be completely soluble at high concentration. The least active isoxazole derivative, 28-O-acetylbetulin-derived isoxazole 17 inhibited only 6% of the growth at 50 µM concentration. It has been suggested that carboxyl group in triterpenoid skeleton enhances the observed antiprotozoal effects. However, among these A-ring fused isoxazole derivatives of betulin, the compounds 18 and 13 were more active than the betulinic acid-derived isoxazole 10. All isoxazole derivatives were less active than betulonic acid 2 (99% at 50 µM, 46% at 15 µM).

The A-ring fused pyrazine derivative of betulonic acid 5 showed 93% at 50 µM, 80% at 15 µM, and 20% inhibition at 5 µM concentration, whereas for the corresponding primary amide 6, we observed inhibition of 95% at 50 µM, and 35% at 15 µM concentration, and for its 28-oximino derivative 24 only 3% at 50 µM. Interestingly, the A-ring fused pyridine derivative of betulonic acid 9 inhibited 88% at 50 µM, but only 26% at 15 µM concentration. Here, with pyridine derivatives, we could see the importance of carboxyl group for antileishmanial activity.
The A-ring fused 5'-methoxyindole derivative of betulinic acid 4 was the most active indole derivative. At 5 µM, the lowest concentration tested, inhibition was 14%, whereas the corresponding unsubstituted indole derivative 3 inhibited only 5%. Also with the related indole derivatives the importance of carboxyl group can be seen as the A-ring fused indole derivative of allobetulin 20 and the corresponding 5'-fluorindole derivative 21 did not have activity at all. One factor affecting this might be the reduced solubility; allobetulin derivatives are not that soluble under the assay conditions. In addition, the A-ring fused pyrazole derivative of betulinic acid 11 and betulinic acid 26 precipitated at 50 µM in these assays.

4-Aza-3-oxohomo betulinic acid 8 displayed very good activity (98.2%) at 50 µM concentration and even at 5 µM concentration (inhibition 52.0%), whereas the A-ring fused pyrazole derivative of betulinic acid 11 displayed moderate 60.2% inhibition at 50 µM concentration. In addition, potent anti-HIV betulinic acid derived compound 25, bevirimat, displayed very good inhibition: 100% inhibition at 50 µM concentration and 95.7% at 50 µM concentration. The best compounds after primary screen were compounds 5, 8 and 25 that significantly inhibited parasite growth when tested at lower concentrations. The IC₅₀ values for 5, 8 and 25 were 13.2, 4.3 and 7.2 µM, respectively, with the compound 8 showing the best activity. Cytotoxicity IC₅₀ values of 8 and 25 against THP-1 cell line were 55.5 and 54.0 µM, respectively. The compound 25 (bevirimat) showed the highest activity among the compounds tested in this study. It showed 100% inhibition at 50 µM, 69% at 15 µM, and 59% at 5 µM. Interestingly, bevirimat 25 also showed good activity against HIV-infected patients in a recent phase II study. Only 4-aza-3-oxohomo betulinic acid 8 showed a similar level of activity with 98% inhibition at 50 µM, 75% at 15 µM, and 52% at 5 µM. The third most active compound was the A-ring fused pyrazin derivative of betulinic acid 5. Cytotoxicity (Table 2) using the human macrophage cell line THP-1 was determined for 8 and 25, and found to be similar (IC₅₀ ca. 50 µM) for both compounds. Compound 8 had the best selectivity index (IC₅₀ THP-1 / IC₅₀ axenic amastigotes; SI =12.9), and its activity was tested on L. donovani infected macrophages at low 5 µM concentration, where no macrophage toxicity was observed. Interestingly, activity of the compound 8 against Leishmania infected macrophages (54.0 ± 4.8% inhibition) was similar to that seen for axenic amastigotes (52.0 ± 1.2% inhibition).

### Table 1 Activity of the compounds against axenic amastigotes of Leishmania donovani.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% inhibition ± s.e</th>
<th>IC₅₀ µM ± s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>50 µM</td>
<td>15 µM</td>
</tr>
<tr>
<td>2</td>
<td>98.6±0.1</td>
<td>45.9±0.7</td>
</tr>
<tr>
<td>3</td>
<td>23.4±2.2</td>
<td>27.4±2.4</td>
</tr>
<tr>
<td>4</td>
<td>29.3±4.1</td>
<td>17.5±2.7</td>
</tr>
<tr>
<td>5</td>
<td>92.7±0.1</td>
<td>79.7±0.4</td>
</tr>
<tr>
<td>6</td>
<td>94.9±0.2</td>
<td>35.2±1.4</td>
</tr>
<tr>
<td>7</td>
<td>98.2±0.1</td>
<td>75.2±1.1</td>
</tr>
<tr>
<td>8</td>
<td>87.9±0.5</td>
<td>25.6±1.5</td>
</tr>
<tr>
<td>9</td>
<td>95.7±0.3</td>
<td>15.6±1.0</td>
</tr>
<tr>
<td>10</td>
<td>60.2±0.7</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>84.3±0.8</td>
<td>16.7±2.0</td>
</tr>
<tr>
<td>12</td>
<td>2.4±3.4</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>56.9±0.4</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>6.1±3.7</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>34.2±2.0</td>
<td>21.1±1.1</td>
</tr>
<tr>
<td>16</td>
<td>19.8±2.4</td>
<td>18.5±0.8</td>
</tr>
<tr>
<td>17</td>
<td>6.8±0.7</td>
<td>10.2±4.1</td>
</tr>
<tr>
<td>18</td>
<td>2.8±4.5</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>100.4±0.1</td>
<td>69.1±3.1</td>
</tr>
<tr>
<td>20</td>
<td>61.0±0.6</td>
<td>-</td>
</tr>
</tbody>
</table>

Average two experiments; *Selectivity Index = IC₅₀/IC₅₀. *Precipitates at 50 µM, see crystals; Average three experiments; ** Average two experiments; Amphoteracin B is a positive control and was tested on infected macrophages at 1 µM.

### Experimental section

**Chemistry**


Betulonic acid (0.10 g, 0.22 mmol) and the corresponding phenylhydrazine hydrochloride (0.35 g, 0.24 mmol) were dissolved in acetic acid (10 mL) and refluxed (130 °C) for 3 h. Water was added and the resulting mixture was extracted with Et₂O. The organic phase was washed with water and brine, dried over anhydrous Na₂SO₄, and the solvents were evaporated. The crude product was purified with SiO₂ column chromatography (25-50% EtOAc/Hexane) to give a yellowish solid (49 mg, 42%). 1H NMR (300 MHz, CDCl₃) δ 7.71 (s, 1H), 7.37 (m, 1H), 7.29 (m, 1H), 7.08 (m, 2H), 4.79 (s, 1H), 4.65 (s, 1H), 3.08 (m, 1H), 2.83 (d, J = 15.0 Hz, 1H), 2.38–2.09 (m, 4H), 1.73 (s, 3H), 1.68–1.31 (m, 12H), 1.28 (s, 3H), 1.17 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 0.88 (s, 3H); 13C NMR (75 MHz, CDCl₃) δ 181.7, 150.4, 140.9, 136.2, 128.4, 121.0, 119.0,
Lupa-2,20(29)-dieno[2,3-b]pyrazin-28-oic acid. (5) A mixture of betulonic acid (0.20 g, 0.44 mmol), 1,2-diaminoethane (130 mg, 2.0 mmol), sulfur (130 mg, 4.1 mmol) and morpholine (4 mL) was refluxed for 21 h. Water was added and the resulting mixture was extracted with CHCl3. The organic phase was washed with 1 M hydrochloric acid, water, a saturated aqueous solution of NaHCO3 and evaporated. The crude product was purified with SiO2 column chromatography (20-50% EtOAc/n-hexane) to give a white crystalline solid (147 mg, 68%).13 1H NMR (300 MHz, CDCl3) δ 8.42 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 2.4 Hz, 1H), 4.76 (s, 1H), 4.64 (s, 1H), 3.05 (m, 2H), 2.46 (d, J = 16.5 Hz, 1H), 2.29 (m, 2H), 1.30 (s, 3H), 1.72 (s, 3H), 1.27 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H), 0.81 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 181.15, 160.0, 151.0, 150.5, 142.6, 141.5, 110.0, 56.6, 53.2, 49.4, 47.0, 45.9, 38.7, 38.3, 37.1, 34.1, 33.6, 32.2, 31.6, 29.9, 25.7, 23.1, 22.6, 21.5, 19.4, 19.2, 16.3, 15.9, 14.8; FTIR (v, cm−1): 738, 885, 1459, 2873, 2843; HRMS: m/z calcd for C18H20NO2 325.1368, found 325.1370 [M+H]+. 1H NMR spectral data is consistent with those previously reported.23

Lupa-2,20(29)-dieno[2,3-d]pyrazin-28-amide. (6) A mixture of lupa-2,20(29)-dieno[2,3-d]pyrazin-28-oic acid (141 mg, 0.28 mmol), oxalyl chloride (44 mg, 0.34 mmol), and a drop of DMF in dry THF (10 mL) was stirred at room temperature for 2 h. The solvent was evaporated, and the residue was dissolved in EtOAc. The organic phase was washed with a saturated aqueous solution of NaHCO3, water and brine, dried over anhydrous Na2SO4 and evaporated. The resulting crude lupa-2,20(29)-dieno[2,3-d]pyrazin-28-yl chloride was dissolved in CHCl3 (5 mL), and a water solution of 25% ammonia (2 mL) was added to the mixture. The resulting solution was stirred at room temperature for 30 min and evaporated to dryness to give a white crystalline solid (137 mg, quant).14 1H NMR (300 MHz, CDCl3) δ 8.85 (s, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 16.5 Hz, 1H), 2.67–2.40 (m, 2H), 2.08–1.84 (m, 2H), 1.71 (s, 3H), 1.67–1.35 (m, 13H), 1.31 (s, 3H), 1.30 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H), 0.82 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 182.4, 161.4, 152.3, 152.0, 143.8, 142.3, 110.0, 57.1, 54.3, 51.2, 50.1, 48.1, 43.7, 41.9, 40.62, 39.4, 39.1, 37.9, 34.6, 34.3, 31.9, 31.8, 30.7, 27.0, 24.4, 22.8, 21.2, 19.6, 16.6, 16.3, 15.0; FTIR (v, cm−1): 886, 1107, 1184, 1406, 1265, 2869, 2948, 3440, 3219; HRMS: m/z calcd for C20H18N2O2 429.3797, found 429.3796 [M+H]+.

2-(Hydroxymethylene)-3-oxolup-20(29)-en-28-oic acid. (12) A mixture of betulonic acid (0.200 g, 0.440 mmol), NaH (60% dispersion in mineral oil, 0.490 g, 12.8 mmol) and dry THF (5 mL) was refluxed to the ice-water bath temperature. To this solution ethyl formate (0.749 g, 10.1 mmol) was added, the resulting mixture was warmed to room temperature and stirred overnight. A saturated aqueous solution of NH4Cl was added, and the resulting mixture was extracted with EtOAc, washed with water and brine, dried over anhydrous Na2SO4, and evaporated. The crude product was purified with SiO2 column chromatography (10-20% EtOAc/n-hexane) to yield a white crystalline solid (12 mg, 11%).15 1H NMR (300 MHz, CDCl3) δ 8.47 (m, 1H), 7.27 (m, 1H), 7.02 (dd, J = 7.6, 4.8 Hz, 1H), 4.75 (s, 1H), 4.62 (s, 1H), 3.10 (m, 1H), 2.74 (d, J = 15.9 Hz, 1H), 2.32 (m, 2H), 2.03 (m, 2H), 1.70 (s, 3H), 1.67–1.36 (m, 13H), 1.32 (s, 3H), 1.27 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.78 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 180.3, 163.6, 150.8, 146.8, 138.4, 130.3, 121.1, 109.7, 56.6, 53.8, 49.5, 49.0, 47.12 46.1, 42.7, 40.8, 39.6, 38.7, 37.2, 36.4, 33.7, 32.5, 31.6, 30.9, 30.0, 25.8, 24.2, 21.7, 20.4, 19.6, 16.0, 15.9, 14.8; FTIR (v, cm−1): 1012, 1045, 1110, 1132, 1184, 1457, 2856, 2927, 2959; HRMS: m/z calcd for C20H18N2O3 409.3685, found 409.3683 [M+H]+.
acid (0.091 g, 0.18 mmol), hydroxylamine hydrochloride (0.036 g, 0.52 mmol) and acetic acid (10 mL) was refluxed for 3 h. Water was added, and the resulting mixture was extracted with EtOAc, washed with a saturated aqueous solution of NaHCO₃, water, and brine, dried over anhydrous Na₂SO₄, and evaporated to give a white solid (59 mg, 68%).¹² ¹H NMR (300 MHz, CDCl₃) δ 10.92 (br s, 1H), 7.97 (s, 1H), 4.75 (s, 1H), 4.63 (s, 1H), 3.09–2.92 (m, 1H), 2.47 (d, J = 15.1 Hz, 1H), 2.36–2.19 (m, 3H), 2.06–1.88 (m, 3H), 1.70 (s, 3H), 1.60–1.33 (m, 15H), 1.28 (s, 3H), 1.19 (s, 3H), 1.01 (s, 3H), 0.99 (s, 3H), 0.81 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 181.3, 173.0, 150.3, 150.3, 109.8, 108.9, 56.4, 53.6, 49.2, 49.1, 46.9, 42.5, 40.8, 39.0, 38.5, 37.0, 35.9, 34.8, 33.4, 32.1, 30.6, 29.8, 28.7, 25.5, 21.4, 21.2, 19.4, 18.8, 16.1, 15.8, 14.7; FTIR (ν, cm⁻¹): 3733, 881, 1181, 1375, 1452, 1643, 1695, 2869, 2943; HRMS: m/z [M+H]⁺: calculated 479.3638; found 479.3638.

Intracellular amastigote growth in infected THP-1 cells (1.0×10⁴ cells/well) was carried out using transgenic Ld:pSSU-int/LUC promastigotes that express luciferase essentially as previously described.²⁶ Amphotericin B (1 µM) was included as a positive control on each plate. Complete medium both with and without DMSO was used as negative controls (0% inhibition of amastigote growth). Calculation of the IC₅₀’s and statistical analysis were carried out using GraphPad Prism Version 6.0b (GraphPad Software, Inc. San Diego, CA).

Conclusions

A set of betulin, betulinic acid and dihydrobetulinic acid derivatives, including eight new A-ring fused heterocycles, was synthesized and tested against L. donovani. Two heterocyclic compounds 5, 8, and potent anti-HIV drug candidate 25, had significant inhibition on parasite growth even at 5 µM concentration. Compound 8 had the best selectivity index, and showed similar good activity on Leishmania-infected macrophages and axenic amastigotes. Further improvement and optimization are needed to get more potent betulin derivatives against L. donovani.

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Notes and references

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Electronic Supplementary Information (ESI) available: Experimental procedures, characterization data, $^1$H and $^1$C NMR spectra. See DOI: 10.1039/b000000x/

‡CLJ holds the Michael and Penny Feiwel Chair of Dermatology.


