Supporting Information to:

**Withanolide Z, a New Chlorinated Withanolide from *Withania somnifera***

Swapan Pramanick¹, Amit Roy², Shekher Ghosh¹, Hemanta K. Majumder², Sibabrata Mukhopadhyay¹

**Affiliation**

¹ Medicinal Chemistry Division, Indian Institute of Chemical Biology, Jadavpur, Kolkata, India
² Department of Molecular Parasitology, Indian Institute of Chemical Biology, Jadavpur, Kolkata, India

**Correspondence**

*Dr. Sibabrata Mukhopadhyay*

Medicinal Chemistry Division
Indian Institute of Chemical Biology
4 Raja S.C.Mullick Road
Jadavpur
Kolkata-700 032
India
Tel.: +91/2473/3491/0492
Fax: +91/3324/735/197
sbmukh@yahoo.co.uk
Figure 1S $^1$H-$^1$H COSY correlations for compound 1.

Figure 2S $^1$H-NMR spectra for compound 1.
**Figure 3S** $^{13}$C-NMR spectra for compound 1.

**Figure 4S** HR-ESI mass spectrum for compound 1.
Comparison of the $^{13}$C-NMR chemical shifts (δ) of withanolides.

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>C6-OH</th>
<th>Name of the Compound</th>
<th>C6-Cl/C7-Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withaperuvin</td>
<td>74.5</td>
<td>4-Deoxyphysalolactone</td>
<td>66.5/--</td>
</tr>
<tr>
<td>Withanolide C</td>
<td>74.7</td>
<td>Physalolactone</td>
<td>65.5/--</td>
</tr>
<tr>
<td>Jaborosalactone</td>
<td>74.1</td>
<td>6α-Chloro-5β-hydroxywithaferin A</td>
<td>66.0/--</td>
</tr>
<tr>
<td>Withanolide Z (1)</td>
<td>75.9</td>
<td>Withanolide Z (1)</td>
<td>--/69.3</td>
</tr>
</tbody>
</table>

Mass fragmentation pattern of compound 1.

![Mass fragmentation pattern of compound 1](image)

**In vitro L. donovani bi-subunit topoisomerase I inhibition assay**

The type I DNA topoisomerase was assayed by decreased mobility of the relaxed isomers of supercoiled pBluescript (SK⁺) DNA in an agarose gel. The relaxation assay was carried out as described previously [22] with LdTOP1LS in the relaxation buffer containing 25 mM Tris-HCl, pH 7.5, 5% glycerol, 0.5 mM DTT, 10 mM MgCl₂, 2.5 mM EDTA, 150 μg/mL BSA, supercoiled pBluescript (SK⁺) DNA (85 to 95 % were negatively supercoiled with the remainder being nicked circles) and 50 mM KCl. The reaction was carried out for 30 min at 37 °C and was then stopped by addition of SDS to a final concentration of 0.5% (w/v) and electrophoresed in 1% agarose gels. Positions of supercoiled monomer (SM) and relaxed and nicked monomer (RL/NM) are indicated. The amount of supercoiled monomer DNA band florescence after ethidium bromide (EtBr; 0.5μg/mL) staining was quantified by integration using Gel Doc 2000 under UV illumination (Bio Rad -Quality one software).