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# Peganine hydrochloride dihydrate an orally active antileishmanial agent ☆

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ABSTRACT

Protozoic infections caused by genus *Leishmania* pose an enormous public health threat in developing countries, compounded by the toxicity and resistance to current therapies. Under the aegis of our ongoing program on drug discovery and development on antileishmanial agents from plants, we carried out bioassay guided fractionation on *Peganum harmala* seeds which resulted in the isolation of **1** as an antileishmanial agent. 2D-NMR spectral data and single crystal X-ray crystallography data indicated **1** as peganine hydrochloride in dihydrated form. The compound **1** exhibited in-vitro activity against both extracellular promastigotes as well as intracellular amastigotes residing within murine macrophages in *Leishmania donovani*. Furthermore, **1** also exhibited in-vivo activity, 79.6 (±8.07)% against established VL in hamsters at a dose of 100 mg/kg b.wt.



Visceral leishmaniasis (VL or Kala-azar) is the most devastating form of leishmaniasis among complex leishmaniasis and is caused by the invasion of the reticuloendothelial system (spleen, liver and bone marrow) by the haemoflagellate protozoan parasite *Leishmania donovani*. More than 12 millions individuals were infected by leishmaniasis around the world with 40000 new cases every year. The disease is generally restricted to areas, heavily infested by the sandfly (*Phlebotomus* spp.), the vector of this disease, which is widely distributed in the Indian subcontinent and south-west Asia.[1,2] In India, high incidence has been reported from the states of Bihar, Assam, West Bengal and Eastern Uttar Pradesh where resistance and relapse are on the increase. Since the 1940s, pentavalent antimonial compounds have been constituted the first-line treatment for all forms of leishmaniasis. In case of therapeutic resistance to these compounds, amphotericin B desoxycholate, liposomal amphotericin B and miltefosine may also be used.[3] However, most of these drugs are expensive, toxic and have side effects and complicated by the fact that they are given parenterally.[4] Moreover, cases of drug resistance are on the rise.[5]

The lack of an effective antileishmanial drug has caused a renewed interest in the study of medicinal plants as source of new chemotherapeutic compounds with better activities and fewer side effects. *Peganum harmala* Linn, commonly known as 'harmal' belonging to the family Zygophyllaceae, is one of the most important medicinal plants of India.[6] Its different parts are used in traditional systems of medicine for the treatment of variety of human ailments.[7–16] We carried out bioassay guided fractionation of *P. harmala* seeds which resulted in the isolation of **1** as an antileishmanial agent. 2D-NMR spectral data and single crystal X-ray crystallography data indicated **1** as peganine hydrochloride in dihydrated form (Figs. 2 and 3).[17,18] Recently we reported the in-vitro antileishmanial activity of **1** and its programmed cell death (apoptosis) and topoisomerase inhibiting activity in *L. donovani*.[19] Compound **1** induces apoptosis-like cell death in *L. donovani*. Strong binding interactions between **1** and DNA topoisomerase I in molecular modeling (docking) studies and our experimental studies against DNA topoisomerase of *L. donovani* was similar to some other natural as well as synthetic quinazoline alkaloids **2**-**6** (Fig. 1). The apoptosis like cell death appears to be consistent to *L. donovani*'s topoisomerase I inhibition by **1**. To further validate the **1**'s antileishmanial activity in animal studies we carried out in-vivo experiments with **1**. Here, we report its in vitro and in vivo antileishmanial efficacy in hamster model.

The concentration of **1** at which nearly 50% death of *L. donovani* promastigotes would occur, was calculated using log phase transgenic Green Fluorescent Protein (GFP) expression of promastigotes by flow cytometry. The% cell death was measured by decrease in Mean Fluorescence Intensity (MFI) values on treatment with drug. Very rapid and dose-dependent cell death occurred with **1** at concentrations between 25 and 100 μg/ml, reaching approximately 90% at around 75 μg/ml ($IC_{90}$). $IC_{50}$ was calculated to be 38 (±1.23) μg/ml. The reference drug, miltefosine, exhibited nearly

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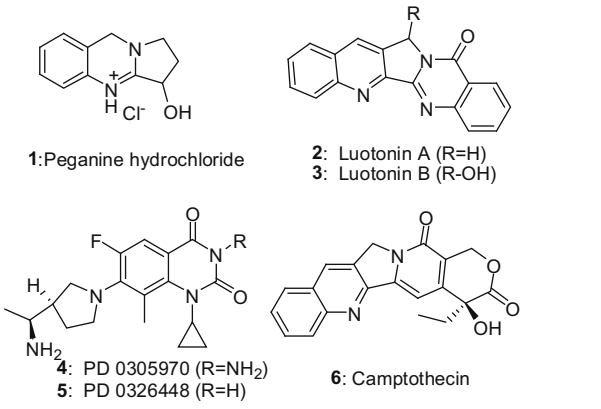


**Figure 1.** Peganine hydrochloride **1** from *Peganum harmala* and DNA topoisomerase inhibitors (Luotonin A **2** and B **3** from *P. nigellastrum*; Syntheic quinazoline alkaloids **4** and **5** Camptothecin **6** from *Camptotheca accuminata*).

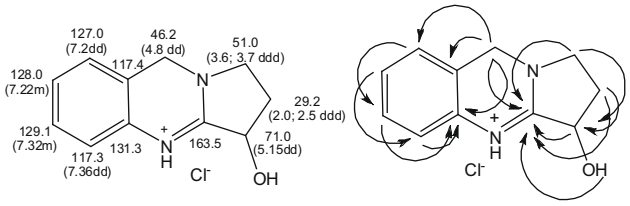

**Figure 2.** $^{1}H$ (parenthesis) and $^{13}C$ NMR spectral data and selected $^{1}H \rightarrow {}^{13}C$ HMBC correlation of peganine hydrochloride dihydrate (**1**).

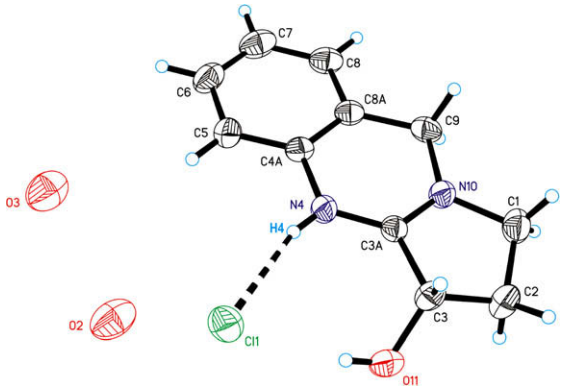

**Figure 3.** Single crystal X-ray structure of peganine hydrochloride dihydrate (**1**).

50% inhibition of parasite multiplication at a concentration of 10 μg/ml.

The leishmanicidal effect of **1** was assessed on intracellular transgenic amastigotes form of *L. donovani* expressing GFP by flow cytometry. A negligible decrease in MFI values in case of amastigotes was observed up to 25 μg/ml. Thereafter, a significant decrease was observed at the concentrations in between 30 and 100 μg/ml. Approximately 90% death was observed at 85 μg/ml ($IC_{90}$). $IC_{50}$ was measured to be 41 (±1.53) μg/ml. Miltefosine which is used as reference drug has $IC_{50}$ of 5 μg/ml.

Treatment of J774A.1 macrophages with **1** was carried out at various concentrations to assess the safety of this pure compound for mammalian cells. After 48 h, the viability of macrophages was checked by MTT assay. The compound was found to be devoid of any cytotoxic effect to macrophages even at the concentration of 200 μg/ml, which was many folds higher than $IC_{50}$ of the compound.

The in-vivo efficacy of **1** was assessed by oral route at three dose schedules (50, 100 and 200 mg/kg body weight for 5 days) against established *L. donovani* infection in hamsters. 87.5 (±9.10)% of inhibition of parasite was observed at a dose of 200 mg/kg. At half a dose of 100 mg/kg it exhibited 79.6 (±8.07)% inhibition of parasites whereas at a lower dose of 50 mg/kg compound **1** was inactive. The reference drug miltefosine resulted in 95.5 (±1.22)% inhibition of *Leishmania* parasite at a dose of 40 mg/kg × 5 days.

In conclusion peganine hydrochloride dihydrate (**1**) was identified as an orally active antileishmanial lead molecule from the seeds of *P. harmala* through activity guided fraction and isolation work for the first time. The compound **1** has simple structural features, easily synthesizable, non toxic to hosts and induces apoptosis-like cell death in *L. donovani*. The binding interactions between **1** and DNA topoisomerase I in molecular modeling studies (docking) and experimental studies suggested that the apoptosis like cell death appears to be due to *L. donovani*'s topoisomerase I inhibition by **1**. Further work is under consideration to study the pharmacokinetic, pharmacodynamic and toxicological studies of **1** and also to synthesise the analogues of **1** to develop more potent antileishmanial agent than natural lead.

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## Supplementary data

Isolation procedure, spectral data (1D and 2D) and X-ray data of **1** and experimental procedure of biological activity is available free of charge. It can be downloaded from the internet at www.sciencedirect.com. X-ray crystallography data was deposited with Cambridge Crystallographic Data Centre (Deposition number CCDC No. 718840). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.039.

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