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Short communication

Antileishmanial effect of new indeno-1,5-naphthyridines, selective inhibitors of *Leishmania infantum* type IB DNA topoisomerase

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ABSTRACT

Visceral leishmaniasis is a neglected disease of poor and developing countries. The current therapeutic approach is based on pentavalent antimonial (Sb^V) drugs and amphotericin B, both nephrotoxic and parenterally administered drugs. Therefore, there is a real need of new antileishmanial drugs. Eukaryotic type I DNA topoisomerases (TopIB) have been identified as druggable targets against leishmaniasis. These enzymes are involved in solving topological problems generated during replication, transcription and recombination of DNA. Leishmanial TopIB is a unique heterodimeric protein structurally different than that found in the mammalian host, thus making it an interesting target for drug discovery. Tetrahydro indeno-1,5-naphthyridines **5** and indeno[1,5]naphthyridines **6** were synthesized. The inhibition of Leishmania and human TopIB of these polycyclic heterocycles were studied and their antileishmanial activity on promastigotes and amastigote-infected splenocytes of *Leishmania infantum* were evaluated. In this regard, it is noteworthy that some of the prepared heterocycles, as compounds 6b, 6i and 5 h, showed selective inhibition of LtopIB while no inhibition of hTopIB was observed at evaluated conditions. In addition, the cytotoxic effects of newly synthesized compounds were assessed on host murine splenocytes in order to calculate the corresponding selective indexes (SI). Tetrahydro indeno-1,5-naphthyridines **5e** and **5h** showed good antileishmanial activity (IC_{50} values of 0.67 ± 0.06 and 0.54 ± 0.17 μM) with similar activity than the standard drug amphotericin B (0.32 ± 0.05 μM) and even tetrahydro indeno-1,5-naphthyridine **5h** showed higher (SI) towards *L. Infantum* amastigotes. Likewise, in the family of indeno-[1,5]-naphthyridines **6**, compound **6b** showed good antileishmanial activity (IC_{50} value 0.74 ± 0.08 μM) and higher selective index (SI) towards *L. Infantum* amastigotes than amphotericin B.

1. Introduction

Visceral leishmaniasis (VL) is a serious zoonotic disease caused by digenetic protozoan parasites of *Leishmania* Genus, which are transmitted by phlebotominae insects. From an epidemiological point of view, leishmaniasis is the second most widespread neglected tropical disease, after malaria [1]. The disease is fatal in Old World countries, especially in some Northern states of India (Bihar) where more than 90% of the estimated 40,000 deaths all over the world occur [2]. However, despite the good sanitary conditions of European countries, VL is endemic at the Northern shore of the Mediterranean Basin [3]. The treatment of this disease is based on chemotherapy [4], since potential human vaccines are in preclinical or early clinical stages of development [5].

Drugs for Neglected Diseases initiative (DNDi) recommends combinations of pentavalent antimony (Sb^V) with the polyene fungicide amphotericin B (AMB), the aminoglycoside antibiotic

Abbreviations: VL, Visceral Leishmaniasis:; TopIB, Type IB DNA Topoisomerase; IRFP, Infra Red Fluorescent Protein; FCS, Foetal Calf Serum; DMSO, Dimethyl sulfoxide; SI, Selectivity Index; CPT, camptothecin; CPTs, camptothecin derivatives; DDQ, dichloro-5,6-dicyanobenzoquinone; HDAr, hetero-Diels-Alder reaction; MCR, multicomponent reactions; TLC, thin layer chromatography.

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paromomycin and the phospholipid milefosine to face VL, in developing countries [6]. However, with the exception of miltefosine, these compounds have nephrotoxic side effects or require parenteral administration [7], which limit their use in countries where the frequently overwhelmed health services are very far away from the residences of the affected people [8]. Therefore, the search for new drugs [9] based on validated targets against VL remains urgently needed, especially when Big Pharma have waived funds and efforts in R + D to treat other more profitable pathologies of wealthy countries [10].

Eukaryotic type I DNA topoisomerase IB (TopIB) is a nuclear enzyme that resolves DNA tangles during replication, transcription and repair processes. All these processes are very active during cell division [11] For these reasons, TopIB is considered a validated druggable target for the treatment of different tumours in humans [12]. The most studied TopIB inhibitors are camptothecin (CPT, Fig. 1) derivatives [13], which are being used clinically against certain tumours. However, many others polynuclear heterocycle compounds such as indenoisoquinolines have improved ability to inhibit the enzyme from human cell cultures [14].

Leishmania TopIB (LTopIB) is a promising target for developing antileishmanial drugs due to two main reasons: i) the enzyme has an increased expression during the division cycle of rapidly growing *Leishmania* and especially ii) LTopIB is structurally different from that of the host [15]. LTopIB is an unusual heterodimeric enzyme, constituted by a large subunit that contains the four amino acids of the active site, and a small subunit that contains the catalytic Tyr222 involved in breaking one DNA strand [16]. In addition, LTopIB displays two non-conserved regions; one at the C-terminal end of the large protomer and the other at the N-terminal end of the small protomer, which are important in enzyme assembly, in conferring sensitivity to topoisomerase inhibitors [17] and in enzyme translocation towards the nucleus. All these distinctive features are also present in other trypanosomatids of medical interest i.e. *Trypanosoma brucei* [18] and *T. cruzi* [19], which makes this enzyme remarkably interesting for drug discovery of other neglected diseases.

Some compounds showing good human TopIB inhibition, such as CPT derivatives, have been studied as LTopIB inhibitors [20]. From a chemical point of view, CPT derivatives present fused ring heterocycles and this feature may be important towards their effectiveness as TopIB inhibitors. Taking this into account, other quasi-flat condensed heterocycles, such as naphthyridine derivatives (Fig. 1), that are effective hTopIB inhibitors [21] might be also adequate candidates to act as LTopIB inhibitors and overcome some of CPTs inherent limitations.

In order to prepare such heterocyclic compounds multicomponent reactions (MCR) [22], widely applied in Medicinal Chemistry [23], represent a very appropriate strategy. MCR have some advantages over classic divergent reaction strategies, including lower costs, shorter reaction time, and energy, as well as environmentally friendlier aspects [24], diversity with atom-economy and enantiocontrol.

In addition, among the organic reactions the Hetero-Diels-Alder-reaction (HDAr) is an excellent choice for the six-membered heterocycle formation [25]. Among them, Povarov reaction [26,27] can be considered as an example of HDAr and represents an excellent method for the preparation of nitrogen-containing heterocyclic compounds, in which aldimines derived from anilines and aldehydes react with electron-rich alkenes in the presence of a Lewis acid catalyst. This process has found applications in total synthesis of natural biologically active compounds [28]. In this sense, if pyridyl amines are used in Povarov reactions this strategy can be also applied to the synthesis of nitrogen polyheterocyclic derivatives, such as 1,5-naphthyridines [29]. This structure can be found in the skeleton of a wide range of biologically active derivatives [30]. In this sense, 7*H*-indeno[2,1-c][1,5]-naphthyridine derivatives may be good candidates with interesting pharmacological activity as new enzyme inhibitors [31]. Consequently, their synthesis and biological evaluation seems to be an attractive task.

This manuscript describes for the first time the synthesis and antileishmanial effect of new synthetic indeno-1,5-naphthyridines on *L. infantum*, the aetiological agent responsible for VL in humans and dogs in Mediterranean countries. For this purpose an *ex vivo* intracellular screening on macrophages isolated from naturally infected BALB/c mice with an infrared-emitting *L. infantum* strain was used [32]. In addition, the inhibitory effect of these compounds has been studied on recombinant TopIB from both *Leishmania* and human sources, showing a selective effect over the parasite enzyme. These results point indeno-1,5-naphthyridines as promising antileishmanial drugs.

2. Chemistry

The synthesis of tetrahydroindeno-1,5-naphthyridines was developed by means of a Povarov type [4 + 2]-cycloaddition reaction by both step by step and multicomponent strategies (MCRs) and in this article only the best reaction conditions and yields are reported. Thus, the preparation of novel tetrahydroindeno-1,5-naphthyridine **5b** (R = 4-F-C_6H_4) was accomplished by reaction of *N*-(3-pyridyl)aldimine **3b**, prepared *in situ* by reaction of 3-pyridylamine **1** and aldehyde **2b** (R = 4-F-C_6H_4, Scheme 1, route A_1), and indene **4** in the presence of 2 equivalents of $BF_3 \cdot Et_2O$ in refluxing chloroform (Scheme 1, route A_2, Chart 1) with excellent yield (93%).

The structure of compound **5b** was assigned on the basis of the 1D and 2D spectroscopy., including HMQC and HMBC experiments and mass spectral data. Thus, the ^1H NMR spectrum showed one singlet at 4.68 ppm corresponding to proton of C-6, one doublet at 4.61 ppm with coupling constant of $^3J_{HH} = 6.9$ Hz corresponding to proton of C-11b, one singlet at 3.73 ppm corresponding to proton of

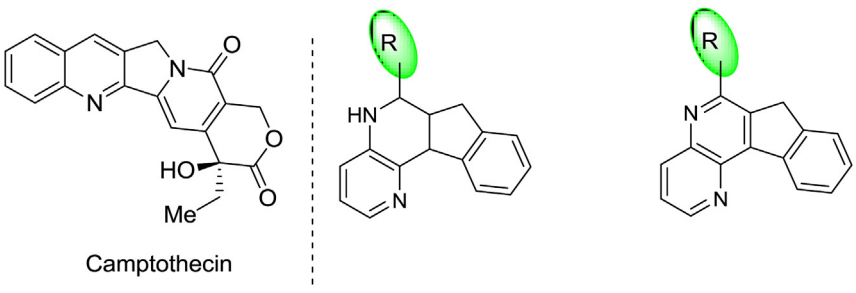

Fig. 1. Structure of camptothecin (left) and of newly synthesized 7*H*-indeno[2,1-c][1,5]-naphthyridines (right).