Chemico-Biological Interactions 244 (2016) 129–139

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Structural parameters, molecular properties, and biological evaluation of some terpenes targeting *Schistosoma mansoni* parasite

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ARTICLE INFO

Article history:
Received 7 September 2015
Received in revised form
21 November 2015
Accepted 10 December 2015
Available online 15 December 2015

Keywords:
Terpene
Schistosoma
Schistosomiasis
3,7-Dimethyl-1-octanol
Dihydrocitronellol
Antischistosomal compound

ABSTRACT

The use of natural products has a long tradition in medicine, and they have proven to be an important source of lead compounds in the development of new drugs. Among the natural compounds, terpenoids present broad-spectrum activity against infective agents such as viruses, bacteria, fungi, protozoan and helminth parasites. In this study, we report a biological screening of 38 chemically characterized terpenes from different classes, which have a hydroxyl group connected by hydrophobic chain or an acceptor site, against the blood fluke *Schistosoma mansoni*, the parasite responsible for schistosomiasis mansoni. *In vitro* bioassays revealed that 3,7-dimethyl-1-octanol (dihydrocitronellol) (**10**) was the most active terpene (IC_{50} values of 13–52 μM) and, thus, we investigated its antischistosomal activity in greater detail. Confocal laser scanning microscopy revealed that compound **10** induced severe tegumental damage in adult schistosomes and a correlation between viability and tegumental changes was observed. Furthermore, we compared all the inactive compounds with dihydrocitronellol structurally by using shape and charge modeling. Lipophilicity (miLogP) and other molecular properties (e.g. molecular polar surface area, molecular electrostatic potential) were also calculated. From the 38 terpenes studied, compound **10** is the one with the greatest flexibility, with a sufficient apolar region by which it may interact in a hydrophobic active site. In conclusion, the integration of biological and chemical analysis indicates the potential of the terpene dihydrocitronellol as an antiparasitic agent.

1. Introduction

Schistosomiasis is a widespread parasitic disease that affects mostly poor and marginal populations in Africa, Asia, and Latin America, and that has been neglected by the pharmaceutical industry and governments [1]. Based on the latest estimates, approximately 200 million people are currently infected worldwide, with about 800 million people at risk of infection [2]. This disease is also known as bilharzia, and it is caused by trematode flukes of the genus *Schistosoma*. Three main species of schistosomes infect humans: *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*. The transmission cycle requires the contamination of surface water by excreta, specific freshwater snails as intermediate hosts, and human contact with the water [1,3].

The control of schistosomiasis is based on improved sanitation and health education, snail control and, above all else, drug

Abbreviations: DMSO, dimethyl sulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IC_{50}, inhibitory concentrations 50%; LD_{50}, lethal dose 50%; MEP, molecular electrostatic potential; miLogP, method for LogP prediction developed at Molinspiration software; MW, molecular weight; PAIN, Pan Assay Interference compound; PSA, molecular polar surface area; PZQ, praziquantel; RPMI, Roswell Park Memorial Institute medium.

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http://dx.doi.org/10.1016/j.cbi.2015.12.003
0009-2797/

treatment [1,3]. Vaccines are not yet on release, and the available treatment for schistosomiasis is limited to the isoquinoline-pyrazino derivative, praziquantel (PZQ), which was released in the 1970s. However, schistosomes have developed resistance to treatment with this drug [4,5]. The development of new, safer, and more effective antischistosomal compounds remains a challenge because these drugs are not given high priority by the pharmaceutical industry.

The use of natural products has a long tradition in medicine, and they have proven to be an important source of lead compounds in the development of new drugs [6]. Among the natural compounds, terpenoids present broad-spectrum activity against infective agents such as viruses, bacteria, fungi, and protozoan and helminth parasites [7,8]. As reviewed elsewhere, some terpenes have been reported to have schistosomicidal activity [9].

Different computational chemistry tools have been developed to understand and predict the biological activities of new compounds, especially with regard to a specific biological target [10]. However, despite several years of experiments aimed at defining PZQ's mode of action, the precise identity and location of the molecular targets of the drug have remained undefined [11,12].

In this study, 38 terpenes were selected for biological testing, and the viability of *S. mansoni* adult worm pairs (male and female) was determined following incubation with different concentrations of compounds. *In vitro* bioassays revealed that dihydrocitronellol (compound **10**) was the most active terpene and, thus, we investigated its antischistosomal activity in greater detail. Finally, to better understand the activity of these compounds, structural and molecular analysis were performed to compare the molecular structure of compound **10** to that of inactive terpenes and to seek some common features.

2. Methods

2.1. Compounds and reagents

All terpenes (Table 1) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and PZQ was purchased from Merck (São Paulo, SP, Brazil). Drugs were dissolved in dimethyl sulfoxide (DMSO).

Roswell Park Memorial Institute (RPMI-1640) culture medium containing phenol red and L-glutamine, heat-inactivated fetal bovine serum, penicillin G, and streptomycin sulfate were obtained from Vitrocell (Campinas, SP, Brazil). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer and DMSO were obtained from Sigma–Aldrich.

2.2. Structural analysis and molecular properties

We used a set of 38 related terpenes from different classes (Table 1); all structures in the set were built and their 3D structures were generated using MarvinSketch 6.3.1, 2014, ChemAxon (http://www.chemaxon.com). Structural and molecular properties were calculated by the Molinspiration Property Engine (v2013.09) internet tool. Dipole moments were calculated with assigned Van der Waals radii.

2.3. Animals and parasite maintenance

S. mansoni (BH strain) were obtained from experimentally infected *Mesocricetus auratus* hamsters following the standard procedures of our laboratory. Animals (3 weeks old) were infected by subcutaneous injection with approximately 150 *S. mansoni* cercariae each, harvested from infected intermediate host snails *Biomphalaria glabrata* [13]. Animals were maintained with free access to rodent diet and water. For *in vitro* studies, schistosomes were collected from the hepatic portal and mesenteric veins of infected hamster 7 weeks post infection [14]. Freshly harvested schistosomes were placed in RPMI 1640 culture medium supplemented with 10% fetal bovine serum and containing 200 IU/mL penicillin and 200 μg/mL streptomycin at 37 °C, 5% CO_2 until usage.

2.4. Ethics statement

The present study was approved by the Ethics Committee at the Universidade Federal do Piauí, PI, Brazil (approval number 013/11). All the animals were handled in strict accordance with good animal practice as defined by the Universidade Federal do Piauí guidelines for animal husbandry, in accordance with the Brazilian legislation (Comissão de Ética de Uso de Animais, CEUA).

2.5. In vitro antischistosomal assay

Adult worm drug testing was performed as previously reported [15,16]. One pair of worms was added to the wells of a 24-well culture plate (TPP, St. Louis, MO, USA) containing the aforementioned RPMI 1640 medium at 37 °C, 5% CO_2. In the first step, all compounds were tested at a concentration of 100 μM in culture plates with a final volume of 2 mL. Subsequently, the most active compound, the monoterpene dihydrocitronellol, was used to obtain final test concentrations of 10–160 μM (10, 20, 40, 80, and 160 μM) [17]. Negative controls contained worms cultured in RPMI alone and in RMPI with 0.5% DMSO. Positive control wells contained worms cultured in PZQ at 3 μM. Cultures were incubated at 37 °C and 5% CO_2, and the parasites were kept for 120 h and monitored every 24 h using an inverted microscope. The effect of the drug was assessed, with an emphasis on changes in worm motor activity and alteration in the tegument. Worm mortality was assessed by lack of movement [15,18].

2.6. Confocal laser scanning microscopy studies

The effect of dihydrocitronellol on the tegument of adult S. mansoni was observed using a confocal laser scanning microscope. After death, the helminths were fixed in a formalin-acetic acid-alcohol solution (FAA) and analyzed under a confocal microscope (Laser Scanning Microscope, LSM 510 META, Carl Zeiss, Standorf Göttingen, Vertrieb, Germany) [15,17]. Autofluorescence was excited with a 488-nm line from an Argon laser, and emitted light was collected with 505 nm [19].

For assessment of changes in the tegument of parasites, three-dimensional images obtained from confocal laser microscopy were used for a quantitative method according to standard procedures [20,21]. During the microscopic analysis, the numbers of intact tubercles on the dorsal surface of male parasites were counted in a 20,000 μm^2 area.

2.7. Statistical analysis

Statistical tests were performed with GRAPHPAD PRISM (version 5.0) software. Significant differences were determined by applying Tukey's test for multiple comparisons. A P value of <0.05 was considered significant. The 50% inhibitory concentrations (IC_{50}) were also calculated using sigmoid dose–response curves, and the 95% confidence intervals are included in parentheses.

3. Results and discussion

3.1. Compounds

A set with 38 terpenes, which have a hydroxyl group connected