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Evaluating the Antischistosomal Activity of Dehydrodieugenol B and Its Methyl Ether Isolated from *Nectandra leucantha*—A Preclinical Study against *Schistosoma mansoni* Infection

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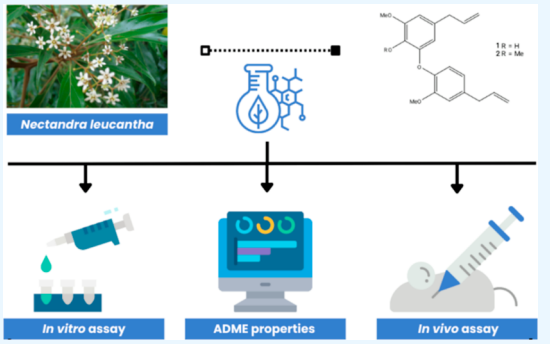
ABSTRACT: Schistosomiasis, a parasitic disease affecting nearly 250 million individuals globally, poses a significant health challenge. With praziquantel being the sole available treatment and its limited efficacy in early stage infections, the identification of novel bioactive compounds becomes imperative. This study examines the potential of dehydrodieugenol B (**1**) and its methyl ether (**2**), derived from the leaves of the Brazilian *Nectandra leucantha* plant (Lauraceae), in combatting *Schistosoma mansoni* infections through a preclinical approach. Initially, compound **1** displayed noteworthy in vitro antiparasitic activity with an EC_{50} of 31.9 μM, showcasing low toxicity in mammalian cells and an in vivo animal model (*Caenorhabditis elegans*). Conversely, compound **2** exhibited no activity. In silico predictions pointed to favorable oral bioavailability and the absence of PAINS similarities. Subsequently, a single oral dose of 400 mg/kg of compound **1** or praziquantel was administered to mice infected with adult (patent infection) or immature parasites (prepatent infection). Remarkably, in prepatent infections, **1** resulted in a significant reduction (approximately 50%) in both worm and egg burden, while praziquantel reduced worm and egg numbers by 30%. The superior efficacy of dehydrodieugenol B (**1**) compared to praziquantel in premature infections holds the potential to advance the development of new molecular prototypes for schistosomiasis treatment.



1. INTRODUCTION

Schistosomiasis is a parasitic disease that remains one of the most important neglected diseases worldwide. With an estimated 250 million people infected globally and 800 million people at risk of infection, schistosomiasis leads to significant morbidity and even mortality in several regions, particularly affecting impoverished communities lacking access to safe drinking water and proper sanitation.[1] The disease is caused by a flatworm parasite of the genus *Schistosoma*, which resides in the blood vasculature and produces eggs responsible for a variety of pathologies.[2]

For decades, schistosomiasis control in individuals, communities, and country-wide mass drug administration programs has been primarily reliant on treatment with praziquantel—PZQ.[3] However, concerns about decreased efficacy or the emergence of resistance have risen, especially as its usage continues to expand.[4] Although effective against adult worms (patent infection), juvenile worms (prepatent infection) do not respond to PZQ treatment, thereby going on to (re)establish infection with the consequent morbidity. Moreover, due to the neglect associated with this disease, there is limited pharmaceutical investment in exploring novel antischistosomal agents.[5,6] As a result, schistosomiasis has been targeted for elimination by 2030 in the World Health Organization's (WHO) neglected tropical diseases (NTDs) roadmap.[7] Consequently, there is an urgent call for the development of innovative anthelmintic agents to address this pressing global health issue.[6,8]

Natural products have long been recognized as valuable sources of substances with pharmacological properties, and among them, various metabolites with antischistosomal properties have been identified. Notably, neolignans are one such example.[9] Previous studies on *Nectandra leucantha* (Lauraceae), a plant species found in the Atlantic Forest biome of Brazil, have reported the antiparasitic potential of different 3-*O*-4′-neolignans, especially against *Leishmania donovani*,[10] *L. infantum*,[11] and *Trypanosoma cruzi*,[12] responsible for visceral leishmaniasis and Chagas disease, respectively. Notably, dehydrodieugenol B (**1**) and its methyl ether (**2**) were isolated as the main metabolites from the leaves and barks

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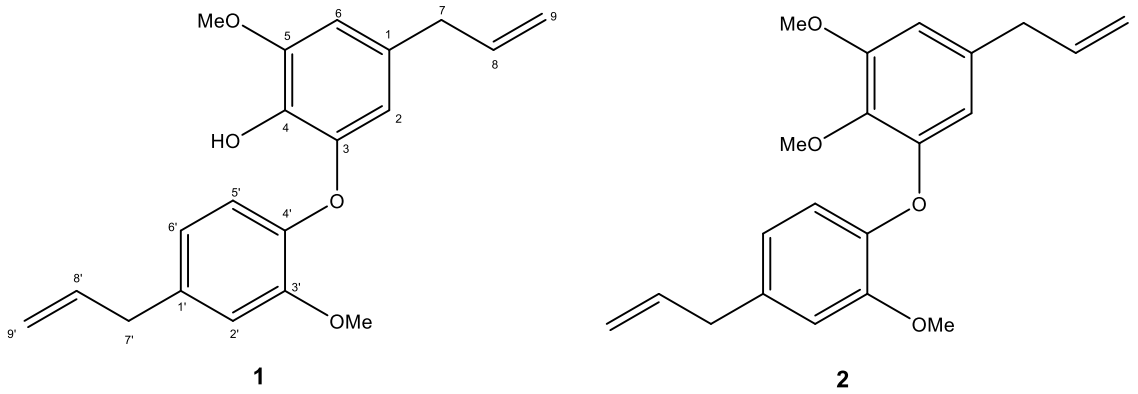

Figure 1. Chemical structures of compounds **1** and **2**.

of the studied plant species and were used as starting material for the preparation of different derivatives to perform SAR studies against *T. cruzi*.[13] More recently, the effectiveness of semisynthetic derivatives formed by cross-metathesis was investigated, and compounds containing a methoxycarbonyl group at C-9/C-9′ displayed excellent activity against *T. cruzi*.[14]

Considering the significant amounts of compounds **1** and **2** produced by *N. leucantha* and the proven effectiveness of these natural products against different parasites, the present study reports the evaluation of the antischistosomal activity of compounds **1** and **2**, isolated from *N. leucantha*, using an in vitro, in silico, and in vivo preclinical approach.

2. RESULTS AND DISCUSSION

2.1. Chemical Characterization of Compounds 1 and 2 Isolated from *N. leucantha*. ESI-HRMS analysis of compounds **1** and **2** indicated $[M + Na]^+$ ion peaks at m/z 349.1423, and 363.1585 corresponding, respectively, to molecular formulas $C_{20}H_{22}O_4$ and $C_{21}H_{24}O_4$. ^{1}H NMR spectra of both compounds indicated structural similarity since these spectra displayed similar profile, including aromatic hydrogens at δ 6.89/6.81 (d, J = 8.1 Hz, H-5′), 6.80/6.79 (d, J = 2.0 Hz, H-2′), 6.70/6.69 (dd, J = 8.1 and 2.0 Hz, H-6′), 6.49/6.48 (d, J = 1.8 Hz, H-2), and 6.40/6.27 (d, J = 1.8 Hz, H-6), allylic hydrogens at δ 3.37/3.36 (d, J = 6.6 Hz, H-7′), 3.25/3.24 (d, J = 6.6 Hz, H-7), and at δ 5.06−5.93 (H-8/H-8′ and H-9/H-9′). ^{13}C NMR spectra of compounds **1** and **2** showed 16 signals of sp^2 carbons at δ 105−153, attributed to the 12 carbons of two aromatic rings and two double bonds (C-8/C-9 and C-8′/C-9′) which, in association to the presence of two methylene carbon atoms at δ 40.0/40.1 (C-7) and 39.9/40.0 (C-7′), confirm the presence of two allyl side chains. However, the main difference between the NMR spectra of isolated compounds consists of the presence of two methoxy signals at δ 56.2/56.1 (5-OMe) and 55.9/56.0 (3′-OMe) to both compounds and an additional peak at δ 61.0 (4-OMe) to compound **2**. These data confirmed the structures of these two chemically related compounds as dehydrodieugenol B (**1**) and methyl dehydrodieugenol B (**2**), respectively (Figure 1), previously isolated from *N. leucantha*.[10,12]

2.2. Efficacy against *S. mansoni* Ex Vivo and Toxicity Assessment. Initially, the antischistosomal effect of compounds **1** and **2**, isolated from *N. leucantha*, was evaluated in vitro against *Schistosoma mansoni* adult worms to determine their effective concentrations at 50% (EC_{50}). The gold-standard antiparasitic compound, praziquantel (PZQ), was used as a positive control, and DMSO 0.5% (drug vehicle) served as a negative control. The viability of the schistosomes exposed to tested compounds is demonstrated in Figure 2A and in the Supporting Information. Results of the EC_{50} value are summarized in Table 1. As observed, compound **1** displayed significant antiparasitic effects with an EC_{50} value of 31.9 μM. On the other hand, compound **2** showed no activity on adult schistosomes when compared to DMSO controls at 72 h, even at the highest tested concentration (100 μM). Control worms remained viable over the entire observation period, whereas PZQ displayed EC_{50} values of about 1 μM. Based on the structural differences between compounds **1** and **2**, particularly the presence of a methoxy group at the C-4 position in compound **2** instead of a hydroxy group as observed in compound **1**, it can be concluded that the phenolic moiety is crucial for the activity against schistosomes in vitro.

The bioassay also revealed that compound **1** did not interfere with worm pairing or oviposition. Additionally, as shown in the Supporting Information, male and female parasites were equally susceptible to compound **1**. At the level of light microscopy, no morphological alterations on ex vivo adult-treated worms were observed. The mechanism by which compound **1** acts against both male and female *Schistosoma* parasites remains unknown. In trypanosomatids, it has been demonstrated that compound **1** and its derivatives induce transient depolarization of the plasma membrane potential and disrupt intracellular calcium homeostasis. Additionally, these compounds interfere with the cell cycle of protozoan parasites, triggering a programmed cell death-like mechanism and impacting DNA replication.[10−14] Further studies are needed to explore the potential mode of action of these compounds on *S. mansoni*.

The antischistosomal properties of chemically related lignoids have been recently documented in the literature, with a potency similar to that demonstrated by compound **1** in this study. For example, licarin A, a neolignan isolated from *Nectandra oppositifolia* (Lauraceae), displayed in vitro effective concentrations ranging from 22.1 to 38.7 μM.[15] Additionally, *threo*-austrobailignan-6 and verrucosin, two neolignans isolated from *Saururus cernuus* (Saururaceae), showed effective concentrations of 23.6−34.7 and 15.2−22.1 μM, respectively.[16]

The evaluation of a compound safety profile is a crucial step in the antischistosomal drug discovery process, ensuring the prioritization of chemical entities that do not display overt cytotoxicity.[17] In this study, a mammalian cell line (Vero) was

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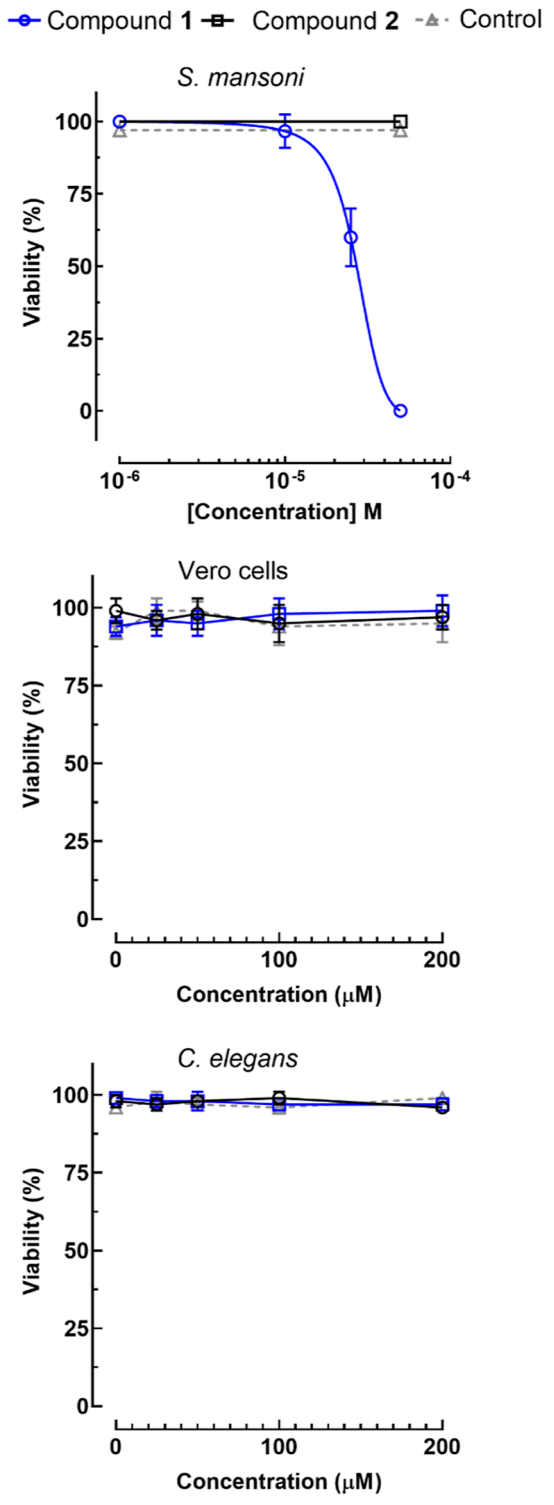

Figure 2. Antiparasitic efficacy against *S. mansoni* ex vivo and toxicity assessment on Vero cells, and *C. elegans* at 72 h. Values are means (±SD) of three independent experiments performed in triplicate.

used to determine the cytotoxic concentrations of 50% (CC_{50}) and selectivity index (SI) of the tested compounds. Additionally, an in vivo toxicity assay was conducted using the model organism *Caenorhabditis elegans*. Encouragingly, both compounds **1** and **2** exhibited no toxicity against Vero cells (Figure 2B), with CC_{50} values exceeding 200 μM, and, therefore, compound **1** showed a favorable SI > 6.2 (Table 1).

Table 1. In Vitro Schistosomicidal and Cytotoxic Activities of Compounds 1, 2, and Praziquantel at 72 h

Compound	*S. mansoni* EC_{50} (μM)	Vero cell CC_{50} (μM)	SI[a]
1	31.9 ± 4.5	>200	>6.2
2	>100	>200	ND
PZQ	0.8 ± 0.2	>200	>250

[a]Selectivity index (SI) = CC_{50}/EC_{50}. Values are means (±SD) of three independent experiments performed in triplicate. Not determined (ND).

Furthermore, both tested compounds demonstrated no toxicity to *C. elegans* (Figure 2C). These findings are promising and contribute to the potential of these compounds as candidates for further investigation in antischistosomal drug development.

2.3. Drug-Likeness Properties. Based on the perspective on schistosomiasis drug discovery, an analysis of currently published antischistosomal compounds reveals that they tend to be lipophilic with low polar surface areas.[18] Therefore, to gain further insights into the druglike properties of compound **1**, an in silico analysis using Swiss ADME (Table 2) was

Table 2. In Silico Physicochemical Properties of Compound 1

parameters	compound **1**
MW (Da)	433.39
c log *P*	4.44
HBD	1
HBA	4
Lipinski[a]	0 violation
TPSA (Å^2)	47.92
PAINS	0 alert

[a]Lipinski's rule of five parameters: molecular weight (MW) < 500, calculated octanol/H_2O partition coefficient *c* log *P* < 5, number of hydrogen bond donor atoms (HBD) < 5 and number of hydrogen bond acceptor (HBA) < 10. TPSA: topological polar surface area (ideal: < 140 Å). PAINS: pan-assay interference compounds (ideal: 0 alerts).

performed. Initially, the compounds adherence to Lipinski's rule of five, which predicts oral bioavailability, was assessed. Remarkably, compound **1** satisfied the literature threshold, with no violations of the rule of five in each parameter. Subsequently, the topological polar surface area (TPSA), a reliable predictor of drug absorption of compound **1**, was calculated. Gratifyingly, compound **1** fell within the recommended range (47.92 Å) since values below 140 Å are ideal for good oral absorption.[19] Furthermore, no pan-assay interference compounds (PAINS) alerts, which represent promiscuous compound classes leading to false positive hits, were detected for compound **1**. These encouraging findings, in conjunction with the supportive experimental results previously reported, strengthen the rationale for evaluating the antiparasitic efficacy of compound **1** in an animal model of schistosomiasis.

2.4. Efficacy of Compound 1 in *S. mansoni*-Infected Mice. To assess the in vivo efficacy of compound **1**, a murine model of schistosomiasis was utilized with both prepatent and patent infections, involving immature and adult-stage schistosomes, respectively. At 21 days (prepatent infections) and 42 days (patent infections) postinfection, mice were orally administered a single dose of 400 mg/kg of compound **1**. On the 56th day postinfection, the worm burden and egg production in the compound **1**-treated group were measured,

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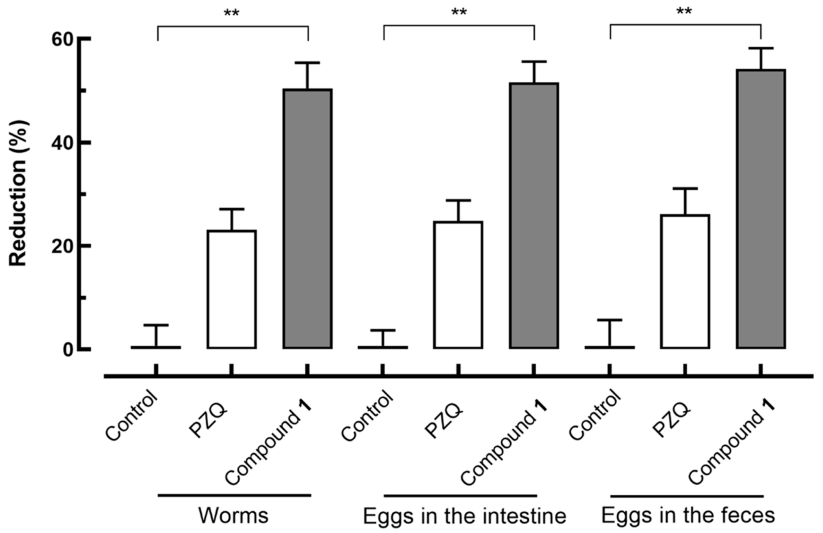

Figure 3. Efficacy of compound **1** (dehydrodieugenol B) and praziquantel (PZQ) in mice harboring prepatent *Schistosoma mansoni* infection. Drugs were administered orally using a single dose of 400 mg/kg 21 days postinfection to mice harboring juvenile schistosomes. Groups of *S. mansoni*-infected controls were given a corresponding amount of vehicle on the same timetable. Worm and egg burden were determined on day 56 postinfection. The therapeutic efficacy of egg burden was evaluated using qualitative and quantitative organs in the intestine, expressed as immature eggs, as well as the total number of eggs in the feces. Values are means (±SD) of five animals per group. $^{**}P < 0.01$ compared with the infected untreated control.

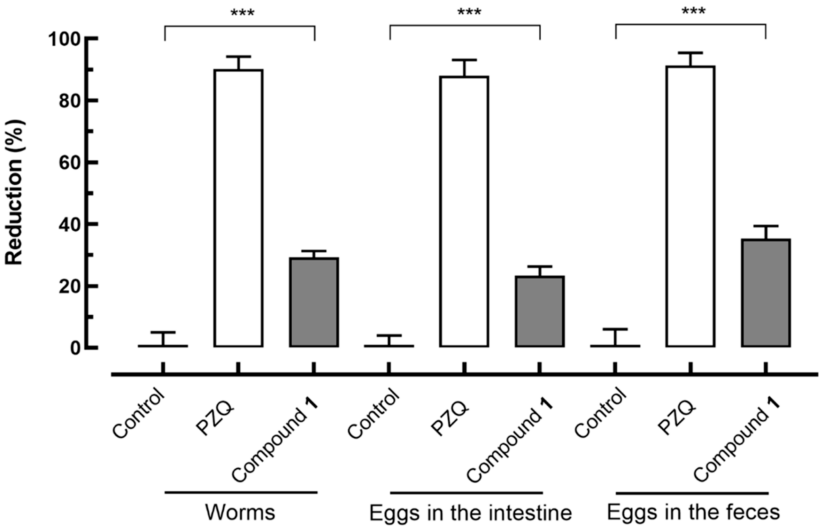

Figure 4. Efficacy of compound **1** (dehydrodieugenol B) and praziquantel (PZQ) in mice harboring patent *Schistosoma mansoni* infection. Drugs were administered orally using a single dose of 400 mg/kg 42 days postinfection to mice harboring adult schistosomes. Groups of *S. mansoni*-infected controls were given a corresponding amount of vehicle on the same timetable. Worm and egg burden were determined on day 56 postinfection. The therapeutic efficacy of egg burden was evaluated using qualitative and quantitative organs in the intestine, expressed as immature eggs, as well as the total number of eggs in the feces. Values are means (±SD) of five animals per group. $^{***}P < 0.001$ compared with the infected untreated control.

and the results were compared to those of the infected and treated animals with vehicle or PZQ controls.

2.4.1. Efficacy of Compound 1 in Prepatent Infections. Considering the limited efficacy of PZQ against juvenile worms,[20] the effect of compound **1** on worm burden in prepatent infection (Figure 3) was initially evaluated. As expected, PZQ achieved an insignificant reduction in the number of parasites (23.1%). In contrast, oral treatment with compound **1** resulted in a significant worm burden reduction of 50.4% ($P < 0.01$) compared to the control *S. mansoni*-infected mice.

Next, the effect of compound **1** on the egg burden in the intestine and feces was assessed. As shown in Figure 3, compound **1** caused a significant reduction in the number of immature eggs in the intestine (51.6%, $P < 0.01$) and the total number of eggs in the feces. (54.2%, $P < 0.01$) compared to control-infected animals. In comparison, PZQ caused an insignificant reduction in the number of eggs in the intestine (24.8%) and in the feces (26.1%). These results confirm that compound **1** is more active than PZQ during the prepatent phase of schistosomiasis.

2.4.2. Efficacy of Compound 1 in Patent Infections. Ideally, it is crucial that any new bioactive compound exhibits activity against both juvenile and adult stages of *S. mansoni*. Therefore, the efficacy of compound **1** in animals harboring adult worms was evaluated. As a result, the treatment with

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compound **1** caused a reduction of 29.3% in the number of worms compared to the control *S. mansoni*-infected mice. In contrast, as expected, PZQ achieved a high worm burden reduction of 90.2% (Figure 4).

Regarding egg burden, similar results were observed following the oral administration of compound **1** or praziquantel in adult worms in mouse models of the disease. As shown in Figure 4, compound **1** caused a modest reduction in the number of immature eggs in the intestine (25.3%) and the total number of eggs in the feces (35.4%) compared to control infected animals, confirming that adult worms in a mouse model of the disease do not respond to compound **1** treatment. In comparison, PZQ significantly reduced the number of eggs by 88.1% in the intestine and 91.4% in the feces.

The mechanism by which compound **1** exhibited greater efficacy against juvenile worms than against adult worms in murine schistosomiasis remains unknown. In comparison to other lignoids, a modest efficacy in experimental studies using rodents with patent *S. mansoni* infection has been reported for the neolignan licarin A.[15] These observed differences in efficacy could be attributed to various factors, including pharmacokinetic aspects (such as oral bioavailability, plasma half-life, and maximum plasma concentration) or pharmacodynamic aspects, i.e., the mechanism of action in the different stages of the parasite. Further investigations are warranted to elucidate the specific underlying reasons for the differential activity against distinct developmental stages of *S. mansoni* by compound **1**.

3. CONCLUSIONS

In conclusion, compound **1** (dehydrodieugenol B), isolated from *N. leucantha* leaves, displayed significant antiparasitic activity against adult *S. mansoni* ex vivo, without showing any toxic effects on mammalian cells or *C. elegans*. In silico studies of compound **1** revealed favorable drug-likeness properties, adhering to Lipinski's rules of five and showing no alerts for PAINS. In vivo, a single oral dose of 400 mg/kg of compound **1** resulted in higher efficacy than the reference drug PZQ in a murine model with prepatent *S. mansoni* infection, although compound **1** showed limited effectiveness in the patent phase of the disease. These findings open up possibilities for further studies using other lignans and exploring structure−activity relationships. Moreover, investigating the combination of compound **1** with PZQ not only broadens the spectrum of activity against different stages of the parasite but also holds potential for improved activity through synergism. This combination approach may also help in reducing the development of drug resistance by schistosomes, which is a critical concern in the treatment of parasitic diseases.

4. MATERIAL AND METHODS

4.1. General Procedures. NMR spectra were recorded on a Varian INOVA 500 spectrometer (Palo Alto, CA, USA), operating at 500 and 125 MHz for ^{1}H and ^{13}C nuclei, respectively, using $CDCl_3$ (Aldrich) as the solvent and TMS (Aldrich) as the internal standard. Chemical shifts are reported in d units (ppm) and coupling constants (J) in Hz. ESI-HRMS spectra were recorded on a positive ionization mode on a Bruker Daltonics MicroTOF QII spectrometer. Chromatographic procedures were performed using lipophilic Sephadex LH-20 (Aldrich) and silica gel 60 (Merck, 63-210 mesh) for column chromatography, whereas silica gel 60 PF_{254} (Merck) was used for analytical thin-layer chromatography - TLC (0.25 mm).

4.2. Plant Material. Leaves of *N. leucantha* were collected in July/2022 on *Parque Estadual do Pereque* (23°51′06.0″S 46°25′02.3″W) on an Atlantic Forest biome region located in Cubatão city, São Paulo State, Brazil. The plant material was identified by Dr. Roberto Baptista Almeida and received a registration code at SISGEN A4123E4. A voucher specimen was compared with that deposited in the Herbarium of Institute of Biosciences, University of São Paulo, SP, Brazil, under code EM357.

4.3. Isolation of Compounds 1 and 2 from *N. leucantha*. Dried and powdered leaves of *N. leucantha* (600 g) were exhaustively extracted by maceration using hexanes (8× 1 L) at room temperature. Obtained organic phases were pooled together to afford 36 g of hexane extract after the elimination of the solvent under reduced pressure. Part of this material (10 g) was subjected to column chromatography over silica gel eluted with hexane containing increasing amounts of EtOAc. This procedure afforded 70 fractions (100 mL each), which were pooled together in five groups (A−E) after TLC analysis. Group C (2.2 g) was purified by Sephadex LH-20 column chromatography eluted with hexane/CH_2Cl_2 1:4 and CH_2Cl_2/acetone 3:2 to afford methyl dehydrodieugenol B (**2**, 783 mg). Group D (4.1 g) was purified by column chromatography over silica gel eluted with mixtures of hexane/EtOAc (8:2, 7:3, and 1:1) to afford 1773 mg of dehydrodieugenol B (**1**).

4.4. Drugs and Reagents Used in Biological Assays. Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, heat-inactivated fetal calf serum, and penicillin G-streptomycin solutions (10,000 U/mL penicillin G sodium salt, 10 mg/mL streptomycin sulfate) were obtained from VITROCELL (Campinas, SP, Brazil). Thiazolyl blue tetrazolium bromide (MTT), reagents for nematode growth medium (NGM), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer, dimethyl sulfoxide (DMSO), and cholesterol were obtained from Sigma (St. Louis, MO, USA). PZQ was supplied by *Ecovet Indústria Veterinaria Ltd.a* (Sao Paulo, Brazil). In all in vitro experiments, compounds were solubilized in DMSO and used at a maximum concentration of 0.5% (v/v). For in vivo studies, compounds were dissolved in EtOH at a final concentration of 2% (v/v).

4.5. Animals, Parasites, and Cells. The life-cycle of *S. mansoni* (BH strain) was maintained by passage through *Biomphalaria glabrata* snails and Swiss mice at the Research Center on Neglected Diseases (Guarulhos University, SP, Brazil). Both snails and mice were kept under environmentally controlled conditions (25 °C; humidity of 50%), with free access to water and food. Vero cells, obtained from the American Type Culture Collection (ATCC), were maintained in 25 cm^2 tissue culture flasks (Corning, New York, NY, USA) with DMEM supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine. *C. elegans* (strain N2) were maintained at 22 °C on nematode growth medium (NGM) agar seeded with the *Escherichia coli* strain OP50, according to standard protocols (Stiernagle, 2006).[21]

4.6. In Vitro Antischistosomal Assay. The in vitro anthelmintic assay was performed as previously described.[22] Briefly, adult schistosomes, obtained from infected mice, were incubated in the presence of 0.78−50.0 μM of the test

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compounds and RPMI 1640 culture medium supplemented with 5% heat-inactivated fetal calf serum and containing 100 U/mL penicillin and 100 μg/mL streptomycin in a 24-well culture plate (Corning) containing one pair of parasites per well (de Moraes et al., 2015). Compounds **1** and **2**, and PZQ, were dissolved in DMSO, and each concentration was tested in triplicate, with experiments repeated three times. Parasites were monitored daily at 24, 48, and 72 h using a BEL Engineering microscope (INV 100, Monza [MB], Italy) based on their motility, as shown in the Supporting Information.[23] Worms were considered dead when they remained motionless in any part of the body for at least 1 min, even when touched with tweezers. Parasite viability was averaged, and 50% effective concentrations (EC_{50}) were calculated using GraphPad Prism software.[24]

4.7. Cytotoxicity Assay. The determination of cytotoxicity was conducted following a previously reported procedure.[25,26] Briefly, cells were seeded in 96-well plates (Corning) using DMEM supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine. After 24 h of cell adhesion at 37 °C and 5% CO_2, the 50% cytotoxic concentrations (CC_{50}) of the test compounds were determined using a concentration range of 0.12−200 μM. 72 h postincubation, MTT solution was added, and the plates were incubated for another 3 h. Subsequently, the plates were read using an Epoch Microplate Spectrophotometer (BioTek Instruments, Winooski, VT, USA) at 595 nm.[27] The assay was conducted in triplicate and repeated three times. The results are expressed as a percentage of the control. The selectivity indices (SI) of the tested compounds were calculated by dividing the CC_{50} values obtained on Vero cells by the CC_{50} values determined on *S. mansoni*.[28]

4.8. Toxicity Assay Using *C. elegans*. Toxicity assay using *C. elegans* was conducted following a previously described method.[29] Briefly, L4 stage worms were transferred to a 96-well plate with approximately 25 worms per well, containing 60% M9 buffer, 10 μg/mL cholesterol, and 40% BHI. Compound **1** was added to each well at concentrations ranging from 25 to 200 μM, with each concentration tested in triplicate. Nematodes were maintained at 22 °C for 24 h, and their viabilities were observed using a Motic inverted microscope (AE2000, Canada). Worm survival was assessed based on mobility and form. Organisms with a rigid, stick-shaped appearance were considered dead, while nematodes exhibiting a sinusoidal, worm-like shape were considered alive.[30] The experiments were repeated three times.

4.9. In Vivo Studies. Female mice (3 weeks old) were subcutaneously infected with 80 *S. mansoni* cercariae each. Compound **1** was administered as a single oral dose of 400 mg/kg to a group of five mice at two different time points: twenty-one days after infection (animals harboring juvenile worms, i.e., prepatent infection) and forty-two days after infection (animals harboring adult worms, i.e., patent infection).[31] Control groups of *S. mansoni*-infected rodents treated with a vehicle or PZQ at 400 mg/kg (five mice per group) were also included. All experimental groups of animals were weighed, euthanized, and dissected on the 56th day after infection. Worm burden was determined by collecting schistosomes from the hepatic portal system and mesenteric veins, followed by sexing and counting.[32] Therapeutic efficacy was evaluated using qualitative and quantitative organs in the intestine, expressed as immature eggs, as well as the Kato-Katz method for quantitative fecal examination to provide additional insights into the therapeutic.[33]

4.10. Randomization and Blinding. The animals included were randomly assigned to their respective experimental groups and treatment plans. Additionally, the animals were euthanized in a randomized manner within each group. All results obtained were analyzed by investigators who were blinded to the group conditions. Integral research procedures, including parasite viability assessment and worm and egg counts, were conducted by two different investigators.[34]

4.11. Physiochemical and Drug-Likeness Properties Analysis. Physicochemical parameters were obtained using the Web server SwissADME (http://www.swissadme.ch/). The following physicochemical properties for compound **1** were analyzed: molecular weight (MW), calculated octanol/water partition coefficient (c log *P*), number of hydrogen bond donor atoms (HBD), number of hydrogen bond acceptor atoms (HBA), topological polar surface area (TPSA), and the number of RO5 violations (the number of properties defined in Lipinski's Rule of 5 that the compound fails).

4.12. Statistical Analysis. Statistical analysis was performed by using GraphPad Prism 8.0. The determination of the CE_{50} and CC_{50} values was obtained from sigmoid dose−response curves. For in vivo studies, the Kruskal−Wallis nonparametric test was used. A *P* value of <0.05 was considered statistically significant.[35]

4.13. Ethics. Animal studies are reported in compliance with the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) ARRIVE guidelines. All experiments were conducted in conformity with protocols approved by the Committee for the Ethical Use of Animals in Experimentation at Guarulhos University (Guarulhos, SP, Brazil; protocol ID 47/20).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06111.

Viability score of adult *S. mansoni* exposed to praziquantel, compound **1**, and compound **2** (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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