

Article

A Novel Sesterterpenoid, Petrosaspongins and γ -Lactone Sesterterpenoids with Leishmanicidal Activity from Okinawan Marine Invertebrates

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Abstract: Leishmaniasis is a major public health problem, especially affecting vulnerable populations in tropical and subtropical regions. The disease is endemic in 90 countries, and with millions of people at risk, it is seen as one of the ten most neglected tropical diseases. Current treatments face challenges such as high toxicity, side effects, cost, and growing drug resistance. There is an urgent need for safer, affordable treatments, especially for cutaneous leishmaniasis (CL), the most common form. Marine invertebrates have long been resources for discovering bioactive compounds such as sesterterpenoids. Using bioassay-guided fractionations against cutaneous-type leishmaniasis promastigotes, we identified a novel furanosesterterpenoid, petrosaspongins from Okinawan marine sponges and a nudibranch, along with eight known sesterterpenoids, hippospongins and manoalides. The elucidated structure of petrosaspongins features a β -substituted furane ring, a tetronic acid, and a conjugated triene. The sesterterpenoids with a γ -butenolide group exhibited leishmanicidal activity against *Leishmania major* promastigotes, with IC₅₀ values ranging from 0.69 to 53 μ M. The structure–activity relationship and molecular docking simulation suggest that γ -lactone is a key functional group for leishmanicidal activity. These findings contribute to the ongoing search for more effective treatments against CL.

Keywords: *Leishmania major*; furanosesterterpenoid; tetronic acid; γ -butenolide; marine sponge



Academic Editor: Junsei Taira

Received: 11 December 2024

Revised: 25 December 2024

Accepted: 27 December 2024

Published: 30 December 2024

Citation: Jomori, T.; Higa, N.; Hokama, S.; Tyas, T.A.; Matsuura, N.; Ueda, Y.; Kimura, R.; Arizono, S.; de Voogd, N.J.; Hayashi, Y.; et al. A Novel Sesterterpenoid, Petrosaspongins and γ -Lactone Sesterterpenoids with Leishmanicidal Activity from Okinawan Marine Invertebrates. *Mar. Drugs* **2025**, *23*, 16. <https://doi.org/10.3390/md23010016>

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1. Introduction

Leishmaniasis is a major public health problem affecting the world's most vulnerable and poorest populations. It is caused by parasitic protozoa and is endemic in 90 tropical and subtropical countries. Worldwide, leishmaniasis is considered one of the top ten neglected tropical diseases, with 0.9 to 1.6 million new cases and 20,000 to 30,000 deaths each year [1]. Approximately 12 million people are currently infected and another 350 million are at risk. There are three forms of the disease: cutaneous, mucosal and visceral. Cutaneous leishmaniasis (CL) is the most common form, causing skin sores and ulcers that can lead to severe scarring and social stigma.

Current treatments, such as antimonial compounds and liposomal amphotericin B, have problems including high toxicity, serious side effects, high cost, and increasing drug resistance [2,3]. The oldest chemotherapies used for leishmaniasis are antimonial compounds, which are administered intravenously or intramuscularly. These compounds contain heavy metals that cause side effects such as nausea, vomiting, headache, and, occasionally, cardiotoxicity. In addition, some parasites have developed resistance to these drugs [4]. Liposomal amphotericin B, a currently widely used intravenous formulation, reduces nephrotoxicity through liposome encapsulation and is designed to remain at the site of the lesion for prolonged periods. Miltefosine, an oral drug also used for amoebic infections, is used for all three forms of leishmaniasis, but its efficacy is variable and it is contraindicated in pregnant women [4]. These drugs are sometimes used in combination. However, these treatments are all expensive and not easily accessible to patients in endemic regions, leading most people to abandon treatment. Treatment of CL is also challenging due to the long duration required and the lack of effective topical therapies. The lack of a vaccine and better diagnostic tools makes the disease even more difficult to manage and highlights the need for safer and affordable treatments.

Marine sesterterpenoids have diverse structures that are biosynthesized by terpene cyclases and cytochrome P450s from the substrate geranyl farnesyl diphosphate (C₂₅) [5–7]. Structures comprise a significant diversity of skeletons that are organized as linear, mono-, bi-, tri-, and tetra-carbocyclic sesterterpenes. They display a broad range of bioactivity including cytotoxic, antibacterial, and antiviral activities, selective growth inhibition of hypoxia-adapted cancer cells, and anti-inflammatory activities [8–14]. There are sesterterpenoids that can be considered as drug leads in the search for new structures to fight pathologies for which effective practice is yet unknown. Although this group is relatively rarer than other terpenoids, many sesterterpenoids have been isolated from marine invertebrates, particularly from marine sponges and their predator nudibranchs [8–18].

This study focuses on marine sponges, a key component of the diverse flora and fauna of the Ryukyu archipelago in southwestern Japan. The Okinawa island group boasts a subtropical climate and unparalleled biodiversity, making it an exceptional site for discovering novel bioactive compounds. In our screening of drug candidates against leishmaniasis in the FT2023–2024 Okinawa Innovation Ecosystem Joint Research Promotion Project, ethyl acetate (EtOAc) extracts of the sponges *Petrosaspongia* sp., *Ircinia* sp., *Luffariella* sp., and the nudibranch *Chromodoris willani* demonstrated >80% growth inhibition of the promastigote *Leishmania major* at 10 µg/mL. By means of bioassay-guided fractionations using open-column chromatography or HPLC, sesterterpenoids 1–3, 4, 5–7, and 8, 9, were purified from *Petrosaspongia* sp., *Ircinia* sp., *Luffariella* sp., and *C. willani*, respectively. By comparing the NMR spectra and the HR-ESI-MS data with those in the literature (Figure 1), three of the four sesterterpenoids (2–4) were identified as hippospongin (2) [17], hipposulfate A (3) [18], and a hippospongin analogue with conjugated γ-tetronic acid 4 [19]. Compound 1, named petrosaspongin, was assumed to be a new analogue of 2 based on NMR spectra. The three sesterterpenoids from *Luffariella* sp. were identified as manoalide (5) [13], secmanoalide (6) [20], and manoalide-25-acetate (7) [21]. The analogues of 5 and 6 from *C. willani* were deoxymanoalide (8) and deoxysecmanoalide (9), thought to be bio-converted by the nudibranch from 5 and 6 found in the prey sponge *Luffariella* sp. [22]. We describe here the structure elucidation of a novel furanosesterterpenoid, petrosaspongin, along with the leishmanicidal activities of eight sesterterpenoids from three marine sponges and a nudibranch collected in Okinawa prefecture, Japan.

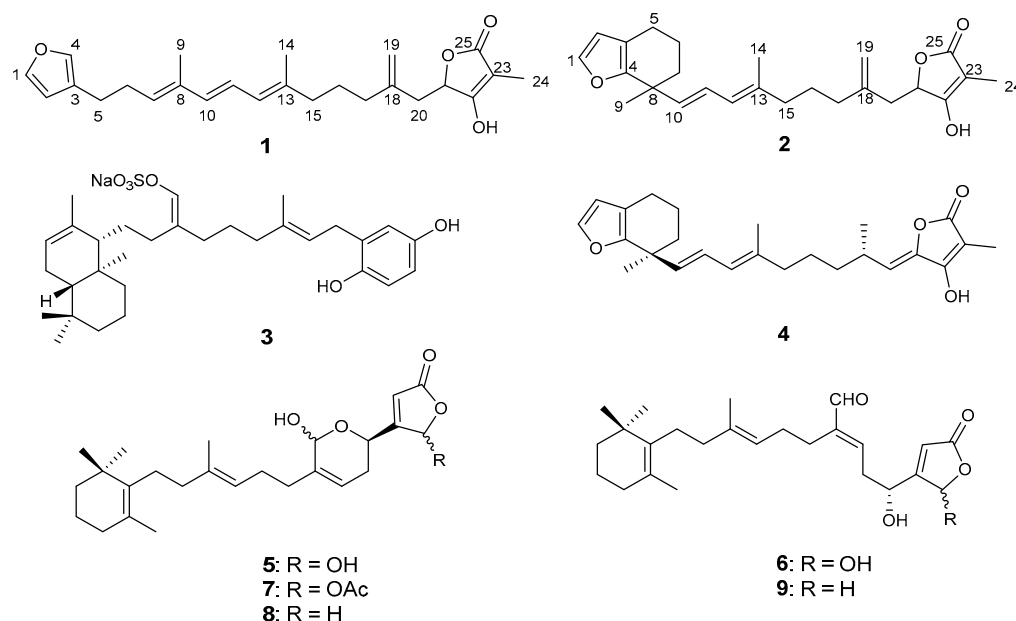


Figure 1. The structures of compounds (1–9).

2. Results and Discussion

2.1. Structure Elucidation

Petrosaspongins (1) was obtained as a colorless oil with a molecular formula of $C_{25}H_{32}O_4$, established by HR-ESI-MS, which showed an $[M + Na]^+$ ion peak at m/z 419.2195 (calculated for $C_{25}H_{32}O_4Na$, 419.2193, $\Delta -0.48$ ppm), possessing ten degrees of unsaturation. The NMR data suggest the presence of a β -substituted furan at δ_H 7.33 (brs, H-1), δ_H 6.27 (brs, H-2), δ_H 7.20 (brs, H-4), along with a terminal double bond at δ_H 4.91 (s), δ_H 4.93 (s), δ_C 113.2 (CH₂-19), δ_C 144.0 (C-18). A tetronic acid moiety was deduced by the signals at δ_H 1.71 (s, CH₃-24), δ_C 77.3, 176.6, 97.5, 5.9, and 174.8 for C-21 to C-25, also supported by IR spectrum (1749 and 1653 cm^{-1}) [17,19,23,24]. The presence of a conjugated triene was proposed at δ_H 5.48 (t, $J = 7.0$ Hz, H-7), δ_H 6.15 (d, $J = 15.3$ Hz, H-10), δ_H 6.33 (dd, $J = 15.3, 10.8$ Hz, H-11), δ_H 5.86 (d, $J = 10.8$ Hz, H-12) with two vinylic methyl groups at δ_H 1.76, δ_C 12.5, (CH₃-9), δ_H 1.77, δ_C 16.7, (CH₃-14). The HMBC correlations from CH₃-9 to C-7/C-8/C-10 and CH₃-14 to C-12/C-13/C-15 also supported the presence of two vinylic methyl groups of CH₃-9 and CH₃-14. These were revealed by the HMBC correlations from H-10 to C-7/C-8/C-9/C-11/C-12, from H-11 to C-8/C-10/C-12/C-13, and from CH₃-9 to C-7/C-8/C-10, consistent with the UV absorption of 1 at 278 nm. The NMR spectra were almost superimposable with those of 2 (Table 1). Hippospongins (2) has a disubstituted furan ring fused with a cyclohexene ring, bearing a methyl group and a long chain. The ^1H and ^{13}C NMR spectra of 1 lack the cyclohexene signals of 2 at δ_H 1.70 (H-6/H-7), δ_C 38.4 (C-7), δ_C 38.6 (C-8), and δ_C 25.8 (C-9), and compound 1 has additional signals of a β -substituted furan ring at δ_H 7.33 (H-4), a trisubstituted olefin at δ_H 5.48 (H-7), δ_C 131.2 (C-7), δ_C 134.8 (C-8) of the triene, and a vinyl methyl group at δ_H 1.76 (s, H-9), instead. Detailed analysis of the COSY data revealed five spin systems, as shown in Figure 2a. The spin system of H-1 to H-2 indicated the presence of a β -substituted furan at C-1 to C-4. The HMBC correlations from H-1 and H-2 to C-3 and C-4 supported the presence of a β -substituted furan. Moreover, the lack of cyclohexene signals of 2 fused at C-3 and C-4 of the furane ring suggested that 1 may be a ring-opened isomer of 2. Without these signals, 1 would harbor the trisubstituted olefin at δ_H 5.48 (H-7), δ_C 131.2 (C-7), δ_C 134.8 (C-8) with the vinyl methyl group at δ_H 1.76 (s), δ_C 12.5 (CH₃-9). This was later supported by the spin system of H-5 to H-7 concomitant with the HMBC correlations from H-6 (δ_H 2.40) to C-7/C-8/C-3

and from H-5 (δ_{H} 2.50) to the furan signals C-2/C-3/C-4 (δ_{C} 111.0/124.7/138.9), as shown in Figure 2a. The connectivities between the conjugated triene and the remaining spin systems (H-15 to H-17, H-20 to H-21) were established by HMBC correlations from the vinyl methyl group CH₃-14 to C-12/C-13/C-15, from the terminal vinyl protons CH₂-19 to C-17/C-20, and from H-20 (δ_{H} 2.27, 2.66) to C-18/C-19/C-25 (δ_{C} 174.8) of the terminal tetronic acid (C-21–C-25), consistent with those of **2** (Table 1) [17].

Table 1. NMR data of petrosaspongins (**1**) and hippospongins (**2**).

Position	1 ^a		2 [17]	
	δ_{C} , Type	δ_{H} (J in Hz)	δ_{C} , Type	δ_{H} (J in Hz)
1	142.7, CH	7.33, brs	140.6, CH	7.24, d (1.8)
2	111.0, CH	6.27, brs	110.1, CH	6.16, d (1.8)
3	124.7, C	-	116.7, C	-
4	138.9, CH	7.20, brs	154.4, C	-
5	24.8, CH ₂	2.50, m	22.5, CH ₂	2.40, m
6	28.9, CH ₂	2.40, m	20.0, CH ₂	1.70, m
7	131.2, CH	5.48, t (7.0)	38.4, CH ₂	1.70, m
8	134.8, C	-	38.6, C	-
9	12.5, CH ₃	1.76, s	25.8, CH ₃	1.34, s
10	135.5, CH	6.15, d (15.3)	138.8, CH	5.60, d (15.2)
11	123.0, CH	6.33, dd (15.3, 10.9)	124.9, CH	5.95, dd (15.2, 10.6)
12	125.6, CH	5.86, d (10.9)	124.8, CH	5.77, d (10.6)
13	137.8, C	-	137.0, C	-
14	16.7, CH ₃	1.77, s	16.5, CH ₃	1.63, s
15	39.5, CH ₂	2.06, m	39.3, CH ₂	2.00, m
16	25.8, CH ₂	1.58, m	25.8, CH ₂	1.50, m
17	35.9, CH ₂	2.06, m	35.9, CH ₂	2.00, m
18	144.0, C	-	143.8, C	-
19	113.2, CH ₂	4.91, s 4.93, s	113.0, CH ₂	4.89, s 4.91, s
20	38.3, CH ₂	2.27, dd (15.1, 8.5) 2.66, dd (15.1, 3.7)	38.2, CH ₂	2.26, dd (15.0, 8.2) 2.62, dd (15.0, 3.9)
21	77.3, CH	4.83, dd (8.5, 3.7)	77.8, CH	4.79, dd (8.2, 3.9)
22	176.6, C	-	177.5, C	-
23	97.5, C	-	97.0, C	-
24	5.9, CH ₃	1.71, s	5.9, CH ₃	1.69, s
25	174.8, C	-	175.6, C	-

^a ¹H (500 MHz) and ¹³C (125 MHz) NMR data in CDCl₃.

The *E* geometry of $\Delta^{10,11}$ double bond was assigned based on the coupling constants ($^3J_{10,11}$ = 15.3 Hz). In ¹³C NMR spectroscopy, the chemical shifts of vinyl methyl groups can provide insight into the geometry of the double bond. Generally, when the ¹³C chemical shift of the methyl group is shifted downfield more than 20 ppm, it suggests the double bond is in a *Z* configuration. Conversely, when the chemical shift of the vinyl methyl group high field-shifted more than 20 ppm, it typically indicates that the double bond is assigned as *E* geometry [25]. The $\Delta^{7,8}$ and $\Delta^{12,13}$ double bonds were also deduced to have *E* geometries from the ¹³C NMR chemical shifts at δ_{C} 12.5 (CH₃-9) and 16.7 (CH₃-14). This assignment was further supported by the NOE correlations of CH₃-9/H-11, CH₃-14/H-11, H-10/H-12 and H-7, H-12/H-15 in Figure 2b. Consequently, the planar structure of **1** was elucidated as shown in Figure 1. The stereochemistry at C-21 of **1** was assessed using the circular dichroism exciton chirality method; however, no significant cotton effects were observed. Chemical derivatizations were also attempted for **1** by opening the tetronic acid moiety followed by modified Mosher methods. Nonetheless, **1** was easily decomposed under light,

oxygen, or heat due to the presence of a furan ring and a triene. The stereochemistry at C-21 remains to be assigned.

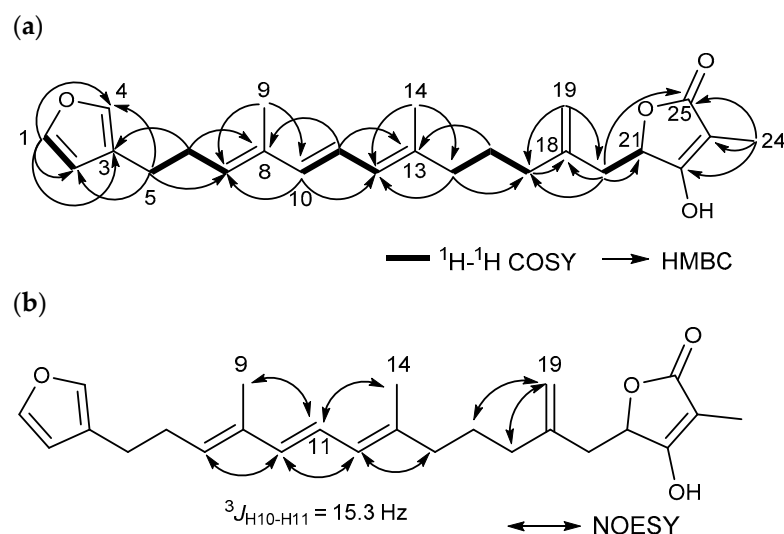


Figure 2. Key 2D NMR correlations for petrosaspongins (1). (a) ^1H - ^1H COSY and key HMBC correlations. (b) Key NOESY correlations.

2.2. Leishmanicidal Activity and Biological Activity

Leishmanicidal activities of isolated sesterterpenoids against *Leishmania major* promastigotes are shown in Table 2. Hippospongins (2) exhibited growth inhibition with an IC_{50} 2.8 μM . A new hippospongins analogue, petrosaspongins (1) lacking a cyclohexene moiety showed 2.8 times less activity than that of 2, with hipposulfate A (3) having the weakest activity (IC_{50} 88 μM). In addition, compound 4, the hippospongins analogue with conjugated terminal γ -tetronic acid showed 5.7 times less activity than that of 2. Since both hipposulfate A without a terminal γ -tetronic acid and compound 4 with the conjugated γ -tetronic acid showed lower activities than 1 and 2, the key functional group may be a γ -tetronic acid.

Table 2. IC_{50} of isolated compounds against promastigotes *L. major*.

Compounds	IC_{50} (μM)
petrosaspongins (1)	7.8
hippospongins (2)	2.8
hipposulfate A (3)	88
compound 4	16
manoalide (5)	24
secomanoalide (6)	11
manoalide-25-acetate (7)	0.69
deoxymanoalide (8)	53
deoxysecomanoalide (9)	3.0
amphotericin B	0.10

Manoalide-25-acetate (7) remarkably inhibited the proliferation of *L. major* with an IC_{50} 0.69 μM . Manoalide (5) and secomanoalide (6) with the presence of a terminal γ -butenolide displayed moderate activities (IC_{50} : 11 and 24 μM), while 25-deoxymanoalide (8) showed 77 times less activity than that of 7. These structure–activity relationships suggest that containing γ -butenolide or tetronic moieties may be crucial for sesterterpenoids to exhibit leishmanicidal activity.

Marine sponges are well-known sources for drug discovery, and several publications have suggested their antileishmanial potential [26–28]. The majority of anti-leishmanial compounds from marine sponges are related to alkaloids [28]. Alkaloid pseudocera-tidines demonstrated moderate activity against *Leishmania amazonensis* and *L. infantum* promastigotes with IC_{50} 19–76 μ M [29]. Other alkaloids, monalidines and batzelladines, have relatively potent activity at IC_{50} 2–4 μ M against *L. infantum* promastigotes [30]. A few papers were recently published on leishmanicidal active sesterterpenoids. Furanoses-terterpenoid, ircinin-1, showed leishmanicidal activities against *L. donovani* (IC_{50} range: 28–130 μ M) [31], and Majer et al. reported ircinianin-type sesterterpenoids exhibiting moderate leishmanicidal activity against *L. donovani* (IC_{50} 16.6 μ M) [32]. Although we cannot simply compare those published IC_{50} values with our results, it is noteworthy that the IC_{50} value 0.69 μ M of manoalide-25-acetate is the most potent leishmanicidal activity among the marine-derived sesterterpenoids.

The structure–activity relationships of isolated sesterterpenoids suggest that the γ -lactone would be a key functionality. Although we have no experimental evidence for the molecular target for these sesterterpenoids, the clerodane diterpenoid possessing a γ -butenolide ring, 16 α -hydroxycleroda-3,13 (14) Z-diene15,16-olide (clerodane) was reported as an inhibitor of DNA topoisomerase type I encoded in *L. donovani* (Ld-topoI). The clerodane inhibited catalytic activity of the recombinant Ld-topoI, which is essential for parasite growth, and ultimately, induced apoptosis. Molecular docking experiments with the clerodane showed five strong hydrogen-bonding interactions and hydrophobic interactions with Ld-topoI. Notably, the γ -butenolide ring of clerodane forms strong hydrogen bonds with the NH group of amino acid residue Arg314, oxygen of Asn452 and oxygen of Cys452 at the active site of Ld-topoI [33]. To gain more insight into the interactions between the sesterterpenoid γ -lactones with the active site of DNA topoisomerase I in *Leishmania* sp., we conducted a molecular docking simulation using the free docking Web service SwissDock [34]. Since the γ -butenolide ring of clerodane was reported as a crucial functional group for the inhibition of Ld-topoI, we used the X-ray crystal data of Ld-topoI (PDB code: 2B9S) as the target molecule [35]. Seven compounds containing γ -lactone were used as a ligand. AutoDock Vina 1.2.0, facilitating the design and execution of a simple docking simulation, was used for the calculation algorithm to generate the docking results of ligand and Ld-topoI (Figure S10) [34]. As a result, the oxygens of the γ -lactone ring have at least one or two hydrogen bonding interactions with several amino acid residues at active sites of Ld-topoI. Although this molecular docking simulation cannot conclude the specific molecular target, the hydrogen bonding interactions at the γ -lactone group may contribute to showing the leishmanicidal activities of those sesterterpenoids. The mechanism of action of sesterterpenoids possessing γ -lactones should be assessed by further investigations on the interaction of γ -lactone sesterterpenoids with DNA topoisomerase of *L. major*.

The nonprofit organization, Drugs for Neglected Diseases Initiative (DNDi), suggests that a selectivity index with cytotoxicity against a human hepatoma cell line HepG2 should be at least >5 as a first criterion for in vitro screening of drug candidates for cutaneous leishmaniasis [36]. To know the selectivity of manoalide-25-acetate (7), the cytotoxicity of 7 against HepG2 was demonstrated and did not show any cytotoxicity (the highest concentration was >20 μ M for 48 h treatment, not tested with higher concentrations). The selectivity index of 7 is 28 times higher than that recommended by the DNDi. However, manoalide-25-acetate (7) exhibits potent cytotoxicity against cancer cell lines with IC_{50} = 0.26, 0.63, 0.76, 1.68 μ M for MCF-7 (a human breast adenocarcinoma), KB (human oral epidermoid carcinoma), HT-29 (colorectal carcinoma), and HeLa (human cervical carcinoma), respectively [37]. It would therefore be inappropriate as a drug for visceral leishmaniasis with oral or intra-abdominal administration, though it would be possible to

develop it as a topical treatment for cutaneous leishmaniasis. Manoalide-25-monoacetate exhibited the most potent activity against *L. major* promastigotes but not as high potency as other sesterterpenoids with hydroxy or dehydrated γ -butenolides or tetrionic acids. This result suggested that acetylation on γ -butenolide could become a driving force for binding its target molecules or the permeability of lipophilic compounds. Manoalides are well-studied for structure–activity relationships against their molecular target, phospholipase A₂ (PLA₂) in mammalian cells. Faulkner et al. reported that γ -hydroxy butenolide ring is involved in the initial interaction of manoalide with PLA₂ and irreversibly inhibits PLA₂ [13,14]. Further studies on the mechanism of toxicity in *Leishmania* genus are ongoing.

3. Materials and Methods

3.1. General Experimental Procedures

¹H, ¹³C and 2D NMR spectra were recorded on Bruker Avance III 500 spectrometer (500 MHz for ¹H NMR, 125 MHz for ¹³C NMR). Chemical shifts are denoted in δ (ppm) relative to the residual solvent peaks as internal standards (CDCl₃, δ_{H} 7.26, δ_{C} 77.0). Data for NMR spectra were reported as follows: chemical shifts (δ) in ppm; s, singlet; d, doublet; t, triplet; m, multiplet; br, broad signal; *J*, coupling constants in Hz. Data were analyzed using Topspin 4.1.4. ESI-MS spectra were recorded on a JEOL JMS-T1000GCV spectrometer. Optical rotations were recorded on a JASCO P-1010 polarimeter. All reagents were used as supplied, unless otherwise stated. Column chromatography was performed using silica gel 60 (Merck); thin layer chromatography (TLC), silica gel 60 F254 plates (Merck). High-performance liquid chromatography (HPLC), Hitachi L-6000 pump fitted with a Hitachi L-4000 UV monitor and a Shodex RI-101 monitor to detect compounds. A 5C₁₈-AR-II column (Cosmosil) or a 5SL-II column (Cosmosil) was used for reversed-phase or normal-phase HPLC, respectively. The details of the conditions for HPLC are described below.

3.2. Preparation of Extracts from Marine Organisms

An extract library of marine organisms established in another project from 2009 to 2014 and in 2023 was used for the screening in the FT2023-2024 Okinawa Innovation Ecosystem Joint Research Promotion Project. The organisms included sponges, soft corals, and algae, hand-collected during SCUBA or rebreather diving from coral reefs in the Ryukyu archipelago near Okinawa, Kerama, Kume, Miyako, Iriomote, and Yonaguni islands of Okinawa prefecture. The specimens were brought back to the lab after collection in fresh or frozen condition and were kept frozen until extraction. The general extraction procedure involved steeping a thawed specimen in acetone three times and the combined acetone solutions were then filtered and concentrated in vacuo. The residual material was partitioned between EtOAc and water. The organic layer was concentrated to give an EtOAc extract, while the aqueous layer was concentrated to a dry material which was washed with a small amount of methanol (MeOH). The MeOH solution was then concentrated to give a MeOH extract. Both EtOAc and MeOH extracts were kept at $-30\text{ }^{\circ}\text{C}$ for screening. The sponges were identified by one of us (N.J.dV.).

3.3. Isolation of Sesterterpenoids

The extracts that exhibited >80% growth inhibition at 10 $\mu\text{g/mL}$ against *Leishmania major* promastigotes were examined further. One EtOAc bioactive extract was prepared from the sponge *Petrosaspongia* sp. (464 g, wet, registration specimen code at Naturalis: RMNH. POR.12476), collected off Kume Island, Okinawa prefecture in September 2009 (26°20'05'' N 126°44'08'' E, depth: 45 m). A portion of the EtOAc extract (241.2 mg of 7.2 g) was subjected directly to reversed-phase HPLC (Cosmosil 5C₁₈-AR-II column; ϕ 10 $\times$ 250 mm) eluted in isocratic mode with solvent, acetonitrile (CH₃CN), in water at

90% with 0.1% formic acid (HCOOH), to obtain as major compounds petrosaspongin (1) [46.2 mg; retention time (Rt) = 10.0 min], hippospongin (2) (48.0 mg; Rt = 10.7 min), and hipposulfate A (3) (22.1 mg; Rt = 7.5 min).

Another EtOAc extract was prepared from the marine sponge *Ircina* sp. (206 g, wet, specimen code; RMNH. POR.12475) collected by hand off Iriomote island, Okinawa prefecture, Japan in July 2012 (24°20'20" N 123°41'37" E, depth: 60 m). A portion of the EtOAc extract (52.0 mg of 3.8 g) was purified by reversed-phase HPLC (Cosmosil 5C₁₈-AR-II column; ϕ 10 × 250 mm) eluted in isocratic mode with solvent CH₃CN in water at 90% with 0.1% HCOOH, to obtain compound 4 (4.2 mg; Rt = 11.8 min).

An EtOAc extract (642 mg) of the fresh sponge *Luffariella* sp. (51.5 g, wet, specimen code; RMNH. POR.12477) collected at Manza, Okinawa island in July 2023 (26°30'17"N 127°50'38"E, depth: 28 m), was subjected to open column chromatography on silica gel eluted with a stepwise gradient of solvents (*n*-hexane, 90%, 75% and 33% of dichloromethane in EtOAc, and 100% EtOAc), resulting in seven fractions (1–7). Fraction 3 (4.3 mg of 226.5 mg) was further purified by normal-phase HPLC on a Cosmosil 5SL-II column (ϕ 4.6 × 250 mm), eluted with 78% *n*-hexane in EtOAc, to yield manoalide (5) (2.8 mg; Rt = 13.0 min). Fraction 5 (4.0 mg from 75.6 mg) was purified on normal-phase HPLC (Cosmosil 5SL-II column; ϕ 4.6 × 250 mm) in isocratic mode with 75% *n*-hexane in EtOAc, yielding secomanolide (6) (1.2 mg; Rt = 12.0 min). Manoalide-25-acetate (7) (22.1 mg; Rt = 5.0 min) was purified from fraction 1 (120.0 mg) with normal-phase HPLC on a 5SL-II column (ϕ 4.6 × 250 mm, eluted with 80% *n*-hexane in EtOAc).

The EtOAc extract (4.0 mg) of the nudibranch *C. willani* (1.1 g, wet, specimen code; RU114N) collected with the aforementioned prey sponge *Luffariella* sp., was directly subjected to HPLC on a Cosmosil 5SL-II column (ϕ 4.6 × 250 mm), eluted with 75% *n*-hexane in EtOAc, to yield deoxymanoalide (8) (0.9 mg; Rt = 8.0 min). The fraction washed with 100% EtOAc was further separated with the same column eluted with 60% of *n*-hexane in EtOAc to yield deoxysecomanolide (9) (0.1 mg; Rt = 11.0 min).

Petrosaspongin (1): Colorless oil; $[\alpha]_D^{25} +18.3^\circ$ (*c* 0.08, CHCl₃); HR-ESI-MS: *m/z* 419.2195 [M+Na]⁺ (calcd. for C₂₅H₃₂O₄Na, 419.21928, Δ -0.48 ppm), IR (film): $\nu_{\max}$ 1749, 1653 cm⁻¹; UV (CH₃CN) $\lambda_{\max}$ (log ϵ): 278 (3.9), 228 nm (4.1). The ¹H and ¹³C NMR (CDCl₃) spectra are shown in Table 1 and SI Figures S1 and S2.

3.4. In Vitro Leishmanicidal Assay

The leishmania growth medium for cutaneous-type promastigotes of *Leishmania major* (MHOM/SU/73/5ASKH) consisted of Medium-199 supplemented with 10% fetal calf serum, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Promastigotes were cultured at 27 °C and 5% CO₂ in an incubator.

The leishmanicidal effects of the extracts and isolated compounds were assessed using the improved 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl- tetrasodium bromide (MTT) method as follows: cultured promastigotes were seeded at 5 × 10⁴/50 µL of medium per well in 96-well microplates. Then 50 µL of varying concentrations of test compounds dissolved in a mixture of dimethyl sulfoxide (DMSO) and the medium were added to each well. Each concentration was tested in triplicate. The microplate was then incubated at 27 °C in 5% CO₂ for 48 h, following which 10 µL of the Cell Counting Kit-8 (Dojindo, Japan) was added to each well and the plates further incubated at 27 °C for 6 h. Optical density values were measured using an ARVO MX microplate reader (PerkinElmer Japan Co., Ltd., Kanagawa, Japan), with a test wavelength of 450 nm and a reference wavelength of 595 nm.

The IC₅₀ values were calculated by fitting the data to a non-linear regression using a dose-response inhibitory model in Microsoft Excel, with experiments conducted in triplicate (*n* = 3). Amphotericin B served as a positive control (IC₅₀ 0.10 µM).

3.5. Molecular Docking Simulation

The target X-ray crystal data of the type I DNA topoisomerase of *Leishmania donovani* (Ld-topI) was downloaded from the Protein Data Bank (PDB code: 2B9S) [35]. The nucleic acids were removed from 2B9S data and saved with PyMOL software (PyMOL Molecular Graphics System V4.6.0, Schrödinger, LLC., New York, USA) for further experiments. The 2D chemical structures of selected sesterterpenoids, petrosaspongins (1), hippospongins (2), compound 4, manoalide (5), secomanolide (6), and manoalide-25-acetate (7) were sketched with ChemDraw 20.1.1 and submitted as a ligand in SwissDock to generate a SMILES file. The calculation program, “Docking with AutoDock Vina” was chosen; <https://www.swissdock.ch/> [34]. To submit the crystal data to the web server, the double strand DNA was deleted from original 2B9S crystal data and uploaded as a target molecule. To run docking simulation in SwissDock, we had to define the calculation area. The search box center and size were defined as [Box center: 33-50-8 Å, Box size: 25-25-25 Å], which can cover the active site pocket of DNA topoisomerase type I. The number of samplings was set to “10” and generated a docking result for seven ligands. All results are shown in Supplementary Materials (Figure S10).

3.6. Cytotoxicity of Manoalide-25-Acetate

HepG2 cells were seeded in 96-well plates at a density of 2.5×10^4 cells/well. The following day, the cells were cultured with manoalide-25-acetate (7) for 48 h, and cytotoxicity was determined using the WST-8 assay with the Cell Counting Kit-8 (Dojindo).

4. Conclusions

Nine sesterterpenoids were isolated through bioassay-guided fractionations from three different Okinawan marine sponges and a nudibranch. The planar structure of the previously undescribed sesterterpenoid petrosaspongins (1) was determined by HR-ESI-MS and detailed NMR analysis. The geometries at double bonds were elucidated by chemical shifts of vinyl methyl groups, coupling constants, and NOESY correlations. Isolated sesterterpenoids that harbored γ -tetronic acid or γ -butenolide groups exhibited leishmanicidal activities, with IC_{50} values from 0.69 to 53 μ M. The sesterterpenoids exhibited noteworthy bioactivity, with manoalide-25-acetate (7) showing the highest potency. Further research will be required to elucidate the mode of actions of sesterterpenoids with γ -lactones. The findings underscore the importance of exploring marine biodiversity for novel therapeutic agents, suggesting that further investigations into the mechanisms of action and structural optimization of these compounds could lead to effective and safer treatments for leishmaniasis. This research adds to the understanding of the bioactive potential of marine organisms, and also addresses a critical need in public health for accessible and effective therapies against neglected tropical diseases.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/md23010016/s1>, Figures S1–S6: ^1H , ^{13}C , HSQC, HMBC, ^1H - ^1H COSY, and NOESY NMR spectra in $CDCl_3$ of 1. Figure S7. HR-ESI-MS spectrum of 1. Figure S8. FTIR spectrum of 1. Figure S9. UV spectrum of 1. Figure S10. Docking simulation results of sesterterpenoids.

Author Contributions: T.J., N.H., S.H. and T.A.T. performed the isolation and structure identification of compounds; N.M., Y.U., R.K., S.A., K.M.-Y. and Y.H. carried out the bioactivity assay; N.J.d.V. identified the sponge species; T.J. and K.M.-Y. wrote the manuscript; T.J., Y.H., M.Y.-H., J.T. and K.M.-Y. contributed funding acquisitions, supervised this research and corrected this manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by a grant from FT2023-2024 Okinawa Innovation Ecosystem Joint Research Promotion Project to T.J., Y.H., K.M.Y., M.Y.H. and J.T., and a Grant-in-Aid

for Research Activity Start-up (Grant No. 22K20717) from the Japan Society for the Promotion of Science (JSPS) to T.J.

Data Availability Statement: Data are contained within this article and Supplementary Materials.

Acknowledgments: We thank Masayoshi Kondo for assisting in the collection of the sponge and Aya Yoshimura (Faculty of Pharmacy, Hokkaido University) for compound searches using SciFinder. We also acknowledge Takenori Kusumi (Faculty of Pharmaceutical Sciences, Tokushima University), Makoto Itoh (Faculty of Agriculture, Kyushu University), Toshiyuki Wakimoto (Faculty of Pharmacy, Hokkaido University), Hiroyuki Nakamura (Okinawa Prefectural Sea Farming Center), and Takayuki Ogi (Okinawa Industrial Technology Center) for fruitful discussions. The authors wish to acknowledge Yoshio Kusakabe (Faculty of Pharmacy, Teikyo University) for his help in interpreting the significance of the results of the molecular docking simulation.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- World Health Organization/PAHO. Neglected Tropical Diseases Factsheet. Pan American Health Organization. Available online: <https://www.paho.org/en/topics/leishmaniasis> (accessed on 30 November 2024).
- Choi, H.L.; Jain, S.; Ruiz Postigo, J.A.; Borisch, B.; Dagne, D.A. The global procurement landscape of leishmaniasis medicines. *PLoS Negl. Trop. Dis.* **2021**, *15*, e0009181. [\[CrossRef\]](#)
- Oliveira, L.F.; Schubach, A.O.; Martinsb, M.M.; Passosa, S.L.; Oliveira, R.V.; Marzochia, M.C.; Andradea, C.A. Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. *Acta Trop.* **2011**, *118*, 87–96. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sheikh, S.Y.; Shukla, F.H.D.; Faruqi, S.B.T.; Akhter, Y.; Khan, A.R.; Nasibullah, M. A review on potential therapeutic targets for the treatment of leishmaniasis. *Parasitol. Int.* **2024**, *100*, 102863. [\[CrossRef\]](#)
- Wilson, K.; de Rond, T.; Burkhardt, I.; Steele, T.S.; Schäfer, R.J.B.; Podell, S.; Allen, E.E.; Moore, B.S. Terpene biosynthesis in marine sponge animals. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2220934120. [\[CrossRef\]](#)
- Avila, C.; Angulo-Preckler, C. Bioactive compounds from marine Heterobranchs. *Mar. Drugs* **2022**, *18*, 657. [\[CrossRef\]](#)
- Liu, Y.; Zhang, S.; Abreu, P.J. Heterocyclic terpenes: Linear furano- and pyrroloterpenoids. *Nat. Prod. Rep.* **2006**, *23*, 630–651. [\[CrossRef\]](#) [\[PubMed\]](#)
- Tsukamoto, S.; Miura, S.; van Soest, R.W.; Ohta, T. Three new cytotoxic sesterterpenes from a marine sponge *Spongia* sp. *J. Nat. Prod.* **2003**, *66*, 438–440. [\[CrossRef\]](#)
- Ryu, G.; Matsunaga, S.; Fusetani, N. Three new cytotoxic sesterterpenes from the marine sponge *Hyrtios* cf. *erectus*. *J. Nat. Prod.* **1996**, *59*, 515–517. [\[CrossRef\]](#) [\[PubMed\]](#)
- Fattorusso, E.; Magno, S.; Santacroce, C.; Sica, D. Sclarin, a new pentacyclic C-25 terpenoid from the sponge *Cacospongia scalaris*. *Tetrahedron* **1972**, *28*, 5993–5997. [\[CrossRef\]](#)
- Arai, M.; Kawachi, T.; Setiawan, A.; Kobayashi, M. Hypoxia-selective growth inhibition of cancer cells by furospinosulin-1, a furanosesterterpene isolated from an Indonesian marine sponge. *ChemMedChem* **2010**, *5*, 1919–1926. [\[CrossRef\]](#)
- Arai, M.; Kawachi, T.; Kotoku, N.; Nakata, C.; Kamada, H.; Tsunoda, S.; Tsutsumi, Y.; Endo, H.; Inoue, M.; Sato, H.; et al. Furospinosulin-1, marine spongean furanosesterterpene, suppresses the growth of hypoxia-adapted cancer cells by binding to transcriptional regulators p54(nrb) and LEDGF/p75. *ChemBioChem* **2016**, *17*, 181–189. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Silva, E.D.; Scheuer, P.J. Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (polejaff). *Tetrahedron* **1980**, *21*, 1611–1614. [\[CrossRef\]](#)
- Lombardo, D.; Dennis, E.A. Cobra venom phospholipase A2 inhibition by manoalide. *J. Biol. Chem.* **1985**, *260*, 7234–7240. [\[CrossRef\]](#)
- Ebada, S.S.; Lin, W.; Proksch, P. Bioactive sesterterpenes and triterpenes from marine sponges: Occurrence and pharmacological significance. *Mar. Drugs* **2010**, *8*, 313–346. [\[CrossRef\]](#)
- Faulkner, D.J. Marine natural products. *Nat. Prod. Rep.* **2000**, *17*, 7–55. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. Hippospongion, a novel furanosesterterpene possessing antispasmodic activity from the Okinawan marine sponge *Hippospongia* sp. *Tetrahedron* **1986**, *27*, 2113–2116. [\[CrossRef\]](#)
- Musman, M.; Ohtani, I.I.; Nagaoka, D.; Tanaka, J.; Higa, T. Hipposulfates A and B, new sesterterpene sulfates from an Okinawan sponge, *Hippospongia* cf. *metachromia*. *J. Nat. Prod.* **2001**, *64*, 350–352. [\[CrossRef\]](#)
- Issa, H.H.; Tanaka, J.; Higa, T. New cytotoxic furanosesterterpenes from an Okinawan marine sponge, *Ircinia* sp. *J. Nat. Prod.* **2003**, *66*, 251–254. [\[CrossRef\]](#) [\[PubMed\]](#)
- De Silva, E.D.; Scheuer, P.J. Three new sesterterpenoid antibiotics from the marine sponge *Luffariella variabilis* (polejaff). *Tetrahedron Lett.* **1981**, *22*, 3147–3150. [\[CrossRef\]](#)

21. Cambie, R.C.; Craw, P.A.; Bergquist, P.R.; Karuso, P. Chemistry of sponges III. Manoalide monoacetate and thorectolide monoacetate, two new sesterterpenoids from *Thorectandra excavatus*. *J. Nat. Prod.* **1988**, *51*, 331–334. [\[CrossRef\]](#)
22. Uddin, M.H.; Otsuka, M.; Muroi, T.; Ono, A.; Hanif, N.; Matsuda, S.; Higa, T.; Tanaka, J. Deoxymanoalides from the nudibranch *Chromodoris willani*. *Chem. Pharm. Bull.* **2009**, *57*, 885–887. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Hofheinz, W.; Schönholzer, P. Ircinianin, a novel sesterterpene from a marine sponge. *Helv. Chim. Acta* **1977**, *60*, 1367–1370. [\[CrossRef\]](#)
24. Alfano, G.; Cimino, G.; De Stefano, S. Palinurin, a new linear sesterterpene from a marine sponge. *Experientia* **1979**, *35*, 1136–1137. [\[CrossRef\]](#)
25. González, A.G.; Rodríguez, M.L.; Barrientos, A.S.M. On the stereochemistry and biogenesis of C₂₁ linear furanoterpenes in *Ircinia* sp. *J. Nat. Prod.* **1983**, *46*, 256–261. [\[CrossRef\]](#)
26. Dube, A.; Singh, N.; Saxena, A.; Lakshmi, V. Antileishmanial potential of a marine sponge, *Haliclona exigua* (Kirkpatrick) against experimental visceral leishmaniasis. *Parasitol. Res.* **2007**, *10*, 317–324. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Barreto, A.L.S.; Alonso, A.N.; Moraes, D.C.; Curvelo, J.A.R.; Miranda, K.; Portela, M.B.; Ferreira-Pereira, A.; Souto-Pradón, T.; Soares, R.M.A. Anti-*Leishmania amazonensis* activity of the marine sponge *Dercitus (Stoebe) latex* (Porifera) from São Pedro and São Paulo Archipelago, Pernambuco, Brazil. *An. Acad. Bras. Cienc.* **2022**, *94*, e20211090. [\[CrossRef\]](#)
28. Mostafa, O.; Al-Shehri, M.; Moustafa, M. Promising antiparasitic agents from marine sponges. *Saudi J. Biol. Sci.* **2022**, *29*, 217–227. [\[CrossRef\]](#)
29. Parra, L.L.L.; Bertonha, A.F.; Severo, I.R.M.; Aguiar, A.C.C.; de Souza, G.E.; Oliva, G.; Guido, R.V.C.; Grazzia, N.; Costa, T.R.; Miguel, D.C.; et al. Isolation, Derivative Synthesis, and Structure-Activity Relationships of Antiparasitic Bromopyrrole Alkaloids from the Marine Sponge *Tedania brasiliensis*. *J. Nat. Prod.* **2018**, *81*, 188–202. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Santos, M.F.; Harper, P.M.; Williams, D.E.; Mesquita, J.T.; Pinto, É.G.; da Costa-Silva, T.A.; Hajdu, E.; Ferreira, A.G.; Santos, R.A.; Murphy, P.J.; et al. Anti-parasitic Guanidine and Pyrimidine Alkaloids from the Marine Sponge *Monanchora arbuscula*. *J. Nat. Prod.* **2015**, *78*, 1101–1112. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Chianese, G.; Silber, J.; Luciano, P.; Merten, C.; Erpenbeck, D.; Topaloglu, B.; Kaiser, M.; Tasdemir, D. Antiprotozoal Linear Furanosesterterpenoids from the Marine Sponge *Ircinia oros*. *J. Nat. Prod.* **2017**, *80*, 2566–2571. [\[CrossRef\]](#)
32. Majer, T.; Bhattarai, K.; Straetener, J.; Pohlmann, J.; Cahill, P.; Zimmermann, M.O.; Hübner, M.P.; Kaiser, M.; Svenson, J.; Schindler, M.; et al. Discovery of Ircinianin Lactones B and C—Two New Cyclic Sesterterpenes from the Marine Sponge *Ircinia wistarii*. *Mar. Drugs* **2022**, *20*, 532. [\[CrossRef\]](#)
33. Misra, P.; Sashidhara, K.V.; Singh, S.P.; Kumar, A.; Gupta, R.; Chaudhary, S.S.; Gupta, S.S.; Majumder, H.K.; Saxena, A.K.; Dube, A. 16 α -Hydroxycycloproda-3,13(14)Z-dien-15,16-olide from *Polyalthia longifolia*: A safe and orally active antileishmanial agent. *Br. J. Pharmacol.* **2010**, *159*, 1143–1150. [\[CrossRef\]](#)
34. Bugnon, M.; Röhrig, U.F.; Goullieux, M.; Perez, M.A.S.; Daina, A.; Michielin, O.; Zoete, V. SwissDock 2024: Major enhancements for small-molecule docking with Attracting Cavities and AutoDock Vina. *Nucleic Acids Res.* **2024**, *52*, W324–W332. [\[CrossRef\]](#)
35. Davies, D.R.; Mushtaq, A.; Interthal, H.; Champoux, J.J.; Hol, W.G. The structure of the transition state of the heterodimeric topoisomerase I of *Leishmania donovani* as a vanadate complex with nicked DNA. *J. Mol. Biol.* **2006**, *357*, 1202–1210. [\[CrossRef\]](#)
36. Caridha, D.; Vesely, B.; van Bocxlaer, K.; Arana, B.; Mowbray, C.E.; Rafati, S.; Uliana, S.; Reguera, R.; Kreishman-Deitrick, M.; Sciotti, R.; et al. Route map for the discovery and pre-clinical development of new drugs and treatments for cutaneous leishmaniasis. *Int. J. Parasitol. Drugs Drug Resist.* **2019**, *11*, 106–117. [\[CrossRef\]](#)
37. Wonganuchitmeta, S.N.; Yuenyongsawad, S.; Keawpradub, N.; Plubrukarn, A. Antitubercular sesterterpenes from the Thai sponge *Brachiastrer* sp. *J. Nat. Prod.* **2004**, *67*, 1767–1770. [\[CrossRef\]](#)

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