Tetrahedron Letters 49 (2008) 6282–6285

Contents lists available at ScienceDirect

Tetrahedron Letters

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

A new antimalarial polyether from a marine *Streptomyces* sp. H668

MinKyun Na [a,b], Damaris A. F. Meujo [a], Dion Kevin [a], Mark T. Hamann [a,*], Matthew Anderson [c], Russell T. Hill [c,*]

[a] Department of Pharmacognosy, and National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, MS 38677, USA
[b] College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, South Korea
[c] Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202, USA

ARTICLE INFO

Article history:
Received 30 April 2008
Revised 1 August 2008
Accepted 7 August 2008
Available online 19 August 2008

ABSTRACT

The antimalarial guided fractionation of the culture of marine *Streptomyces* sp. strain H668 led to the isolation of a new polyether metabolite. The structure was determined by comprehensive NMR and MS assignments. This new metabolite showed in vitro antimalarial activity against both the chloroquine-susceptible (D6) and-resistant (W2) clones of *Plasmodium falciparum*, without cytotoxicity to normal cells (Vero) making it a promising first lead from this marine bacterium.

Malaria is a tropical infection caused by four different species of protozoa from the genus *Plasmodium* transmitted to humans by the *Anopheles* mosquitoes. During past decades, chloroquine and other aminoquinolines have been utilized as frontline antimalarial agents. However, an increase in drug resistance in *Plasmodium falciparum* has made it essential to develop new chemotherapeutic agents in addition to combination therapies utilizing available antimalarial drugs with different modes of action.[1] Recently, some specific polyether antibiotics have been reported to have potent antimalarial activity.[2–5] K-41, a carboxylic acid containing polyether antibiotic produced from *Streptomyces hygroscopicus* has been reported to exhibit nanomolar *in vitro* antimalarial activity against *P. falciparum* strains K1 (drug resistant) and FCR3 (drug sensitive).[3] Furthermore, it showed high selectivity *in vivo* against both *P. berghei* strain N and *P. yoelii* strain NS-infected mice, when administered orally.[3] Several polyethers from this class such as lonomycin A, nigericin, and monensin have been identified as potential antimalarial agents due to their highly potent and selective activity.[4,5] These compounds are classified as ionophores owing to their potential activity to interact with ions (cations). A unique structural feature[4,5,11] is that they possess a polycyclic alkyl backbone which confers lipophilic character to these compounds, and a terminal carboxyl group which plays an important role in the formation of an oxygen rich internal cavity which is capable of binding metal ions.[11] Though rich in oxygen atoms, these molecules are lipophilic in nature. Since the parasite infected cell membrane is vulnerable to binding with lipophilic compounds, the putative mechanism of action for these polyethers is via transfer of ions through the membrane.[4,5] This transport is potentially done after complexation with a cation (mobile carrier: true ionophores) or by formation of trans-membrane channels (quasi-ionophores). Bacteria, especially *Streptomyces* sp. (isolated from soil samples) are reported as the primary producers of such molecules (with at least 21 producer strains reported thus far).[15] A literature evaluation has yielded very little about the isolation of such compounds from marine *Streptomyces* sp.: aplasmomycin,[12] arenaric acid, and oxolonomycin.[13,14]

An antimalarial screening effort of marine microorganisms from Hawaiian sediments yielded a *Streptomyces* sp. designated strain H668 with highly potent *in vitro* activity against *P. falciparum* without significant cytotoxicity to Vero cells. The strain H668 was identified as a *Streptomyces* sp. on the basis of >700 bp of sequence of the 16S rRNA gene. Bioassay-guided fractionation of the EtOAc-soluble fraction of the H668 culture led to the isolation of a new polyether metabolite. In this report, we describe the isolation and structure elucidation of this metabolite, and evaluate the antimalarial activity.

The antimalarial active fraction eluting with 70% MeOH in H_2O using reversed phase C18 VLC was further fractionated by preparative HPLC [Phenomenex C8 column (21.2 × 250 mm); flow rate 5 mL/min] using a gradient from 50% to 100% MeOH in H_2O over 80 min. The active fractions were further purified by preparative HPLC [Phenomenex C18 column (21.2 × 250 mm) at 5 mL/min] eluting with a gradient from 70% to 100% MeOH in H_2O, to afford a semipure metabolite. Purification of this semipure compound by HPLC [5 micron C18 (10 × 250 mm); flow rate 3 mL/min] using an isocratic solvent of 75% MeOH in H_2O gave 3.0 mg of the pure metabolite **1**.[6]

The ^{1}H NMR spectrum of **1** indicated the presence of 11 oxymethines (3.1–4.3 ppm), 4 methoxy groups (3.2–3.6 ppm), 8 methyls (0.9–1.3 ppm), and methylenes (1.2–2.2 ppm). This was supported by the ^{13}C and DEPT NMR spectroscopic data of **1**. In

* Corresponding authors. Tel./fax: +1 662 915 5730 (M.T.H.).
E-mail addresses: mthamann@olemiss.edu (M. T. Hamann), hillr@umbi.umd.edu (R. T. Hill).

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doi:10.1016/j.tetlet.2008.08.052

addition, two oxygenated quaternary carbon signals at δ_C 82.8 and 78.4, as well as two ketal (or hemiketal) signals at δ_C 100.9 and 106.2 were observed in the ^{13}C NMR spectrum. Four independent spin systems were identified by a COSY experiment, in which a sequential correlation from H-12 to H-23 and two methyl signals at the positions of C-26 and C-27 were observed, along with correlations for H-9 and H-10, H-3 through H-7, and H-1 and one methyl group at C-1. Several ambiguous correlations in the methylene area of the COSY data were identified based on the analysis of HSQC data. From the HSQC data, each protonated carbon for the partial structures could be assigned as shown in Table 1. The HMBC data of **1** revealed correlations from H-12 (δ_H 3.89) to C-14 (δ_C 28.2) and C-15 (δ_C 81.7) and from H-15 (δ_H 3.74) to C-12 (δ_C 81.9), suggesting a structure of a furan ring (ring C). In a similar fashion HMBC correlations assisted in identifying ring D. Ring E was deduced from the HMBC analysis, where both the methyl protons at δ_H 0.97 (3H, d, J = 6.4 Hz, H-27) and 1.01 (3H, d, J = 6.4 Hz, H-26) showed correlations toward C-22 (δ_C 74.6), and the proton H-22 (1H, t, J = 10.0 Hz) exhibited 2- and 3-bond correlation with C-20 (δ_C 75.3), C-21 (δ_C 40.9), C-23 (δ_C 47.8), and C-24 (δ_C 100.9), along with a correlation from the methyl signal at δ_H 1.30 (3H, s, H-25) to C-24. Moreover, the HMBC correlations from H-12 to C-11 (δ_C 82.8) and C-10 (δ_C 94.8), as well as from neighboring protons (H-10, H-9, H-7, and H-6) to the ketal carbon at δ_C 106.2 facilitated the construction of a spiroketal analog linked with the ring C. Above results suggest that the compound **1** is a polyether type metabolite. Comparison of the NMR spectral data of **1** with those of polyether compounds previously reported supported the NMR assignments for the rings A–D.[7–10] However, the residual part was quite different from any other polyethers reported thus far. The most significant difference is the lack of a carboxyl group in **1**. COSY data confirmed the linkage of H-3 (δ_H 4.29, 1H, d, J = 10.0 Hz) and H-4 (δ_H 4.10, 1H, br s), and of H-1 (δ_H 3.45, 1H, m) and H-32 (δ_H 1.02, 3H, d, J = 6.4 Hz). The HMBC correlations from H-3 to C-2 (δ_C 78.4), as well as to the methyl carbon at δ_C 11.6 revealed the connection from C-1 to C-3 bearing two methyl groups (C-31 and C-32). In addition, two methoxyl groups at δ_H 3.54 and 3.39 were identified and attached at the position of C-1 and C-2, respectively, by the HMBC data analysis. Taken together, the structure of **1** was determined as shown in Figure 1, and all the ^1H and ^{13}C NMR data were assigned as in Table 1. The relative configuration of **1** was determined on the basis of ROESY NMR data. NOE correlations were observed as described in Table 1, which indicated that **1** has the same relative configuration in rings A-E as those of K-41 (Fig. 2).

The antimalarial activity of the new metabolite (**1**) was evaluated against both the chloroquine-susceptible (D6) and -resistant (W2) clones of *P. falciparum*, and their toxicity was tested against Vero cells. Compound **1** showed antiprotozoal activity against both the D6 and W2 clones, with IC_{50} values ranging from 100 to 200 ng/

Table 1
NMR assignment of compound **1** in CD_3OD

	δ_C[a]	δ_H mult. (J in Hz)[b]	HMBC	ROESY
1	81.8[c]	3.45, m[c]	C-2, 1-OMe	H-3
2	78.4	—		
3	66.6	4.29, d (10.0)	C-2, C-31	H-1, 2-OMe
4	61.4	4.10, br s		
5	32.2	1.40, m[c]	C-7	
6	78.6	3.34, m	C-4, C-8	H-7
7	36.7	1.71, m	C-5, C-8, C-30	H-6, H-29, H-30
8	106.2	—		
9	45.6	2.06, m	C-8, C-10, C-29	H-10, H-29, H-30
10	94.8	3.43, d (9.6)[c]	C-9, C-12, C-28, C-29, 10-OMe	H-9, H-28, H-29
11	82.8	—		
12	81.9[c]	3.89, t (7.2)	C-10, C-11, C-14, C-15, C-28	H-13a, H-15, 10-OMe
13	26.0	1.99, m	C-11, C-12, C-15	H-13a, H-14b, H-28
		1.83, m[c]		H-12, H-13b
14	28.2	1.71, m[c]	C-12, C-15, C-16	H-13b, H-14a, H-16
		1.87, m[c]		H-14b, H-15
15	81.7	3.74, q (6.4)	C-13, C-12 (or C-16), C-17	H-12, H-14a, H-16, H-17a
16	81.9[c]	3.99, q (6.4)	C-14, C-19	H-14b, H-15, H-17b
17	28.5	2.06, m	C-15, C-19	H-16, H-17a
		1.83, m[c]		H-15, H-17b, H-18a
18	24.8	2.00, m	C-16, C-19, C-20	H-18a, H-21
		1.91, m[c]		H-17a, H-18b, H-20
19	79.9	4.20, dt (6.4, 2.8)	C-20	H-20, H-27
20	75.3	3.40, m[c]		H-18a, H-19, H-22, H-27
21	40.9	1.25, m	C-19, C-20, C-22, C-27	H-18b, H-23, H-27
22	74.6	3.08, t (10.0)	C-20, C-21, C-23, C-24, C-26, C-27	H-20, H-26, H-27
23	47.8	1.40, m	C-22, C-24, C-25, C-26	H-21, H-25, H-26
24	100.9	—		
25	21.2	1.30, s	C-23, C-24	H-23, H-26
26	11.5	1.01, d (6.8)[c]	C-22, C-23, C-24	H-22, H-23, H-25
27	12.3	0.97, d (6.4)	C-20, C-21, C-22	H-19, H-21, H-22
28	24.7	1.37, s	C-10, C-11	H-10, H-13b
29	10.5	0.97, d (6.4)	C-9, C-10	H-7, H-9, H-10
30	12.0	0.95, d (6.8)	C-6, C-7	H-7, H-9
31	11.6	1.10, s	C-2	H-32
32	11.2	1.02, d (6.8)[c]	C-1, C-2	H-31
1-OMe	60.0	3.54, s	C-1	
2-OMe	49.1	3.39, s[c]	C-2	H-3
6-OMe	56.9	3.28, s	C-6	
10-OMe	59.6	3.41, s	C-10	H-12

[a] Assignments based on DEPT, HMQC, and HMBC NMR data (100 MHz).
[b] Assignments based on COSY and HMBC NMR data (400 MHz).
[c] Signals partially overlapped.

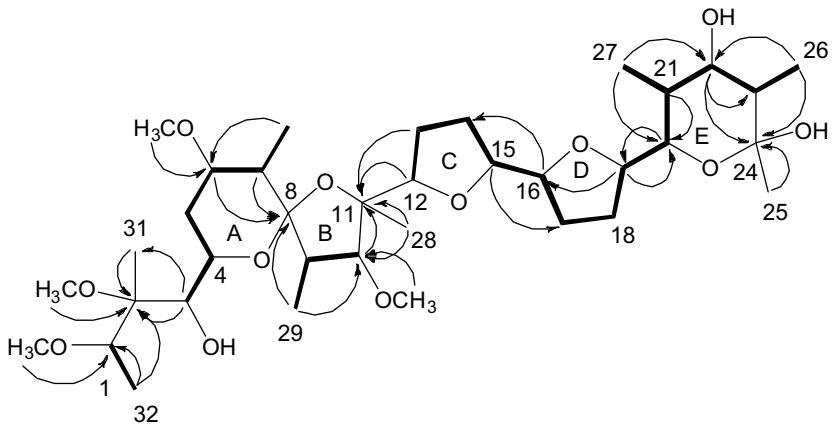

Figure 1. Key HMBC correlations (→) and 1H–1H COSY data (–) for compound **1**.

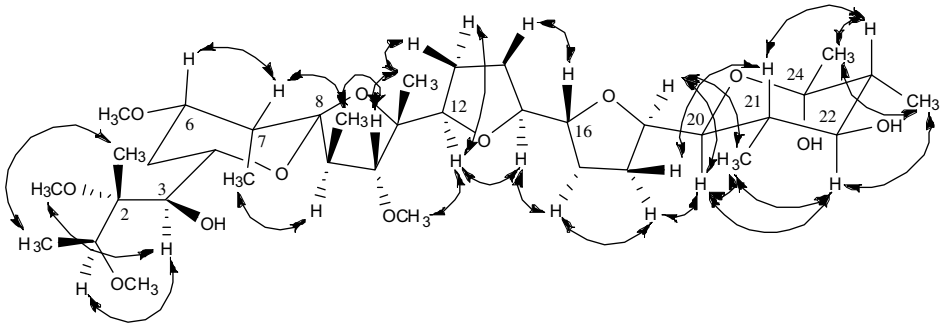

Figure 2. Key NOE correlations (↔) for compound **1**.

mL. Although the *in vitro* antimalarial activity of **1** was significantly less than the original extract, no cytotoxicity was observed at 4.75 μg/mL, the highest concentration tested. The high index of selectivity (SI = $IC_{50\ Vero}/IC_{50\ P.\ falciparum}$) for **1** indicates that the polyether metabolite **1** is highly specific to the parasite. As discussed earlier the mechanism of action for ionophores (quasi-ionophored or mobile carrier) is via alteration of normal membrane permeability to cationic species.[4,5,15] Ionophores (quasi-ionophores as mobile carriers) have a unique structural feature, which is crucial to their ability to interact with metal species such as Na^+, K^+, and, Ca^{2+}.[4,5,11] Some quasi-ionophores (alamethicin, gramicidin S, and, gramicidin D) are peptides or very large molecules, and other mobile carriers are polycyclic polyether compounds with an alkyl backbone, an internal oxygen rich pocket, and a terminal carboxyl group. These last two features play an important role in their ability to bind metal ions.[11] There are a number of examples of mobile carriers including: monensins A, B, C, nigericin, laidlomycin, grisorixin, mutalomycin, lonomycins A, B, X-206, alborixin, lenoremycin, dianemycin, carriomycin, septamycin, etheromycin, A-204A, K-41A, K-41B, and, A-601612 all have a terminal carboxyl moiety.[15]

The new polyether **1** isolated from marine *Streptomyces* sp. H668 is not a typical ionophore or a member of either of these classes, and structurally it is clearly unrelated to the quasi-ionophores that we came across in the literature. Though, it is closely related to several members of the mobile carrier group, the fact that it lacks the carboxyl functionality, suggest the mode of action against the parasite warrants additional investigation. An important issue in antimalarial chemotherapy is resistance of *P. falciparum* to current drugs making it necessary to identify new chemotherapeutic agents with unique structures and modes of action. Further investigations regarding mechanism of action studies and in vivo activity will be published in due course.

Acknowledgments

The authors are grateful to the National Institute of Health (NIAID) 1RO1AI36596, National Center for Research Resources C06 RR-14503-01, and the Medicines for Malaria Venture for funding a predoctoral fellowship for Damaris A.F. Meujo. Na. M., was supported in part by the Korean Research Foundation Grant funded by the Korean Government (MOEHRRD, Basic Research Promotion Fund, KRF-2008-331- E00451).

Supplementary data

Supplementary data (HRESIMS, ^1H, ^{13}C, and 2D NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.08.052.

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6. Compound **1**: Colorless solid, $[\alpha]_D^{24}$ +48.2 (*c* 0.17, MeOH); HRESIMS *m*/*z* 711.4301 $[M+Na]^+$ (calcd for $C_{36}H_{64}O_{12}Na$, 711.4295).
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