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ORIGINAL ARTICLE

Ingenine F: A New Cytotoxic Tetrahydro Carboline Alkaloid from the Indonesian Marine Sponge *Acanthostrongylophora ingens*

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ABSTRACT

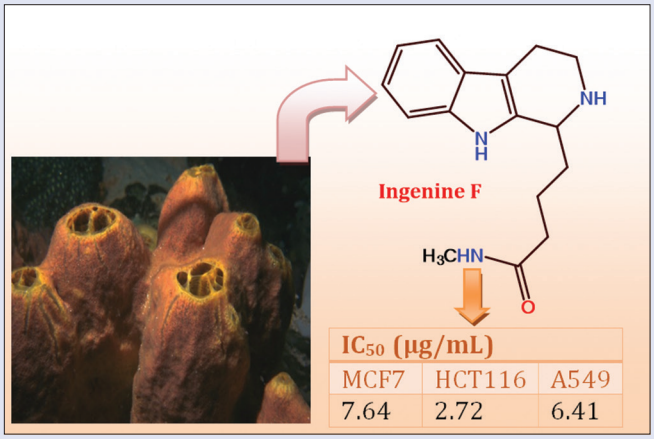
Background: Marine organisms are established to be a wealthy source of bioactive compounds with diverse chemical structures and bioactivities. *Acanthostrongylophora ingens* is known to be rich with pyrimidine b-carboline and manzamine-type alkaloids. The goal of the present work is to isolate and identify new alkaloids from A. *ingens* as well as to assess the cytotoxic potential of these metabolites towards various cancer cell lines. **Methods:** The crude MeOH extract of the sponge was separated by vacuum liquid chromatography (VLC), using *n-hexane*, EtOAc, and MeOH. The EtOAc fraction was chromatographed on VLC, SiO2, sephadex LH-20, and RP_{18} columns, affording four metabolites. Their structures were identified using infrared, ultraviolet, high-resolution mass spectrometry, and nuclear magnetic resonance spectroscopic techniques, as well as comparison with the published data. **Results:** A new 1,2,3,4-tetrahydro-β-carboline (THβCs) alkaloid, ingenine F (4) and three known compounds: Annomontine (1), acanthomine A (2), and 1-oxo-1,2,3,4-THβCs (3) were isolated and identified. Ingenine F (4) exhibited cytotoxic activity toward hormone-dependent breast carcinoma (MCF7), colon carcinoma (HCT116), and lung carcinoma (A549) cell lines with IC50 values of 2.82, 1.00, and 2.37 μM, respectively, compared to doxorubicin (IC50 0.012, 0.036, and 0.102 μM, respectively). **Conclusion:** It is the first report for the isolation of THβCs alkaloids from A. *ingens*. The THβCs alkaloid with *N*-methylbutyramide unit as found in ingenine F is very rarely encountered in nature. Ingenine F may provide new promising candidates for potential cytotoxic agent.

Key words: *Acanthostrongylophora ingens,* alkaloid, cytotoxic activity, Ingenine F, Tetrahydro-β-carboline

SUMMARY

- Ingenine F, a new 1,2,3,4-THβCs derivative (4) and three known alkaloids (1-3) were isolated from A. *ingens*. Their structures were verified by various spectroscopic analyses. Compound 4 had potent cytotoxic effect toward MCF7, HCT116, and A549 cancer cell lines.

Abbreviations used: 1D: One-dimensional; 2D: Two-dimensional; CC: Column chromatography; COSY: Correlations spectroscopy; DMSO: Dimethyl sulfoxide; HMBC: Heteronuclear multiple bond correlation experiment; HRESIMS: High resolution electrospray ionization mass spectrometry; HSQC: Heteronuclear single quantum correlation; IR: Infrared; LCQ: Liquid chromatography quadrupole; LTQ: Linear trap quadrupole; NMR: Nuclear magnetic resonance; RP: Reversed phase; SiO2: Silica gel; TLC: Thin-layer chromatography; UV: Ultraviolet; VLC: Vacuum liquid chromatography.



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INTRODUCTION

The ability to use nature as an inspiring framework for devolving novel products is an interesting area of scientific research. In fact, large numbers of current pharmacopoeias derive directly or indirectly from natural products. Marine organisms are established to be fruitful origin of bioactive novel compounds with diverse chemical structures that are of great usefulness as new drug leads for treating various ailments. *Acanthostrongylophora ingens* (order Haplosclerida; family Petrosiidae) is considered to be a rich source of pyrimidine β-carboline[1-3] and manzamine-type alkaloids.[4-8] These compounds exhibit a diverse range of bioactivities, including antimalarial, antileishmanial, antituberculosis,[4-6] cytotoxic,[1,2,9-11] and anti-HIV.[4-6] In our further search for structurally interesting and bioactive compounds from the sponge *A. ingens*, a new 1,2,3,4-tetrahydro-β-carboline (THβCs) alkaloid, ingenine

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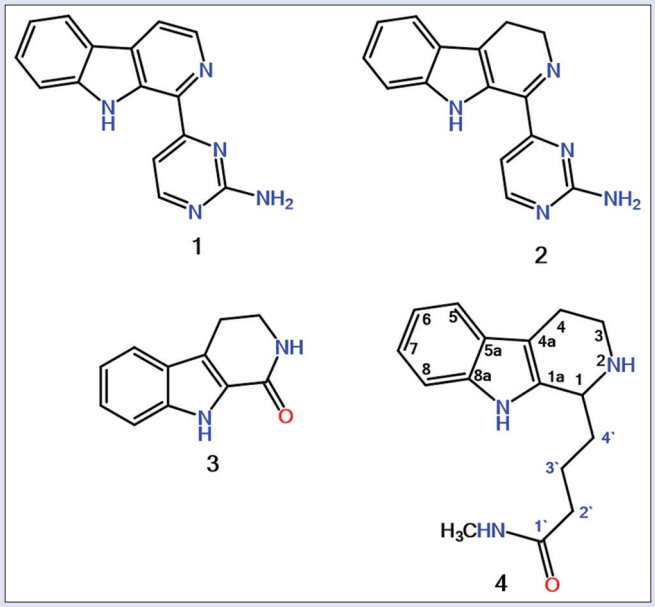

Figure 1: Structures of isolated compounds 1-4

F (4), and three known compounds were isolated [Figure 1]. The structural identities of the isolated metabolites were proven by interpreting the spectroscopic data. Herein, the isolation, structural characterization, and cytotoxic potential of 4 were described.

MATERIALS AND METHODS

General experimental procedures

A JASCO polarimeter was utilized for measuring optical rotation (Jasco Co., Tokyo, Japan). An electrothermal-9100 digital melting point instrument was used to get the melting points (Perkin-Elmer, Waltham, MA, USA). Ultraviolet (UV) data were recorded by Hitachi-300 spectrophotometer (Kyoto, Japan). The infrared (IR) absorbance was obtained by a Shimadzu Infrared 400 spectrophotometer (Shimadzu, Kyoto, Japan). A liquid chromatography quadrupole (LCQ) DECA mass spectrometer (ThermoFinnigan, Bremen, Germany) was utilized to obtain the electrospray ionization mass spectroscopy (ESIMS) spectrum. high resolution electrospray ionization mass spectrometry (HRESIMS) was assessed by linear trap quadropole (LTQ)-Orbitrap MS spectrometer. A Bruker Avance DRX 500 MHz spectrometer was used for measuring nuclear magnetic resonance (NMR) data (Bruker BioSpin, Billerica, MA, USA). High-performance liquid chromatography (HPLC) was carried out on a C-18 column Eurospher 100 (300 mm × 8 mm, Knauer, Berlin, Germany) at 5.0 ml/min flow rate. Sephadex LH-20 (0.25–0.1 mm), silica gel (0.063–0.200 mm), and RP_{18} (0.04–0.063 mm) (Merck, Darmstadt, Germany) were utilized for column chromatography. For TLC, precoated plates silica gel (60 F_{254}, 0.2 mm, Merck, Darmstadt, Germany) were used. RP_{18} six mL standard extraction tube (40–63 μm) (Merck, Darmstadt, Germany) was used for the purification of compounds.

Animal material

The sponge material was obtained in 2010 from Sulawesi Island by scuba diving at 12 m depth. The collected material was immediately freeze-dried after collection. A specimen (ZMAPOR. 17795-A) was preserved in the Zoological Museum (Amsterdam University).

Extraction and isolation

The dried sponge (1.3 kg) was extracted with MeOH (4 × 2 L) at room temperature (25°C). The combined extract was concentrated to get a brown residue (39.0 g). The latter was submitted to vacuum liquid chromatography (VLC), utilizing *n*-hexane, EtOAc, and MeOH (2 × 1.0 L, each) to get *n*-hexane (5.9 g), EtOAc (7.8 g), and MeOH (20.8 g) fractions. The EtOAc (7.8 g) fraction was chromatographed, using $CHCl_3$:MeOH gradient on VLC to get 13 sufractions: EA-1 to EA-13. Fraction EA-3 (380 mg) was separated using MeOH: $CHCl_3$ (90:10) on a sephadex LH-20 CC (50 g × 50 cm × 3 cm) to give 3 subfractions: EA-3A to EA-3C. Subfraction EA-3B (71 mg) was submitted to SiO_2 CC (40 g × 50 × 3 cm), utilizing $CHCl_3$:MeOH (95:5–80:20) to get impure 1. Purification of 1 was performed using a semi-preparative HPLC to yield 1 (10.2 mg, yellow needles). Fraction EA-4 (148 mg) was separated on a SiO_2 CC (40 g × 50 cm × 2 cm), utilizing a $CHCl_3$:MeOH gradient to give 2 subfractions: EA-4A and EA-4B. Subfraction EA-4A (69 mg) was subjected to SiO_2 CC (40 g × 50 cm × 2 cm) with $CHCl_3$:MeOH gradient to afford impure 2 and 3. Each compound was purified separately on RP_{18} extraction tube, using H_2O: acetonitrile gradient elution to yield 2 (9.5 mg, red needles) and 3 (7.1 mg, white amorphous powder). Fractions EA-5 (65 mg) was subjected to RP_{18} CC (20 g × 50 cm × 2 cm) eluting with H_2O: MeOH gradient to get 2 subfractions: EA-5A and EA-5B. Subfraction EA-5A (29 mg) was separated on HPLC to yield 4 (2.7 mg, pale yellow powder).

Spectral data

Ingenine F (4): pale yellow powder; $[\alpha]_D^{20}$ + 13.9 (*c* 0.3, CH_3OH); UV (MeOH) λ_{max} (log ε) 226 (4.37), 300 (3.98) nm; IR (KBr) ν_{max} 3210, 2924, 1649, 1564, 1058 cm^{-1}; NMR data [Table 1]; HRESIMS m/z 272.1764 (M + H) + (calcd for $C_{16}H_{22}N_3O$, 272.1763).

Cytotoxicity assay

The *in vitro* cytotoxic activity of 4 was assessed toward colon carcinoma (HCT116), hormone-dependent breast carcinoma (MCF7), human cervix carcinoma (Hela), lung carcinoma (A549), and brain tumor (PC12) cell lines (ATCC, Rockville, MD) as previously stated.[12-15] DMSO and doxorubicin were utilized as negative and positive standards, respectively.

RESULTS AND DISCUSSION

Compound 4 was separated as pale yellow powder and afforded positive tests for alkaloids.[16] Its molecular formula $C_{16}H_{21}N_3O$ was estimated by the pseudomolecular ion peak at m/z 272.1764 (M + H) + (calcd for $C_{16}H_{22}N_3O$, 272.1763) in HRESIMS spectrum, requiring eight DBE. Its UV absorbances at 226, and 300 nm specified the existence of a THβC chromophore.[17,18] Its IR showed bands at 3210 (NH), 2924 (C-H), and 1649 (C = O) cm^{-1}. The chemical shifts together with NMR experiments suggested 4 to have a C-1 substituted 1,2,3,4-tetrahydro β-carboline skeleton.[18] ^{13}C NMR and heteronuclear single quantum correlation (HSQC) spectra of 4 exhibited 16 carbons, including 8 aromatic carbons, an amide carbonyl (δ_C 156.9), 5 methylenes, NH-bonded methine, and N-methyl signal (δ_C 48.6). The ^1H NMR spectrum showed four-coupled aromatic protons at δ_H 7.45 (H-5), 7.01 (H-6), 7.11 (H-7), and 7.37 (H-8), suggesting the presence of an ABCD spin system characteristic for a 1,2-disubstituted benzene. They correlated to the carbons, resonating at δ_C 118.1, 119.1, 121.9, and 111.4, respectively, in the HSQC spectrum. In addition, signals for a NH-bonded methine at δ_H 4.71 (H-1)/δ_C 51.9 (C-1), two coupled methylenes at δ_H 3.58 (m, H-3)/δ_C 40.8 (C-3) and δ_H 2.91 (m, H-4)/δ_C 18.2 (C-4), and N*H* group at δ_H 8.21 (brs, 2-NH) were attributable to 1,2,3,4-tetrahydro-pyridine ring of the β-carboline moiety. The HMBC correlations of H-1 to C-1a, C-4a, and C-3, H-3 to C-1 and C-4a, H-4 to C-1a, C-4a, and C-3, H-5 to C-7 and C-8a, H-6 to C-5a and C-8, H-7 to C-5 and C-8a, and H-8 to C-5a

Table 1: NMR data of compound 4 (DMSO-d_6, 500 and 125 MHz)

Position	δ_H multiplicity (*J* in Hz)	δ_C	COSY	HMBC
1	4.71 t (7.2)	51.9 CH	1'	1a, 3, 4a, 3'
1a	-	130.1 C	-	-
3	3.58 m	40.8 CH_2	4	1, 4a
4	2.91 m	18.2 CH_2	3	1a, 3, 4a
4a	-	105.9 C	-	-
5	7.45 d (7.6)	118.1 CH	6	7, 8a
5a	-	125.8 C	-	-
6	7.01 t (7.6)	119.1 CH	5, 7	5a, 8
7	7.11 t (7.6)	121.9 CH	6, 8	5, 8a
8	7.37 d (7.6)	111.4 CH	7	5a, 6
8a	-	136.2 C		-
4'	2.25 m	28.7 CH_2	2'	1, 1a, 2'
	1.95 m			
3'	1.75 m	24.2 CH_2	1', 3'	1, 1'
2'	3.21 m	40.3 CH_2	3'	1', 4'
1'	-	156.9 C	-	-
1'-NH-CH_3	3.17 s	48.6 CH_3	-	1'
NH	11.22 s	-	-	4a, 5a, 8a
2-NH	8.21 brs	-	-	-

NMR: Nuclear magnetic resonance; DMSO: Dimethyl sulfoxide

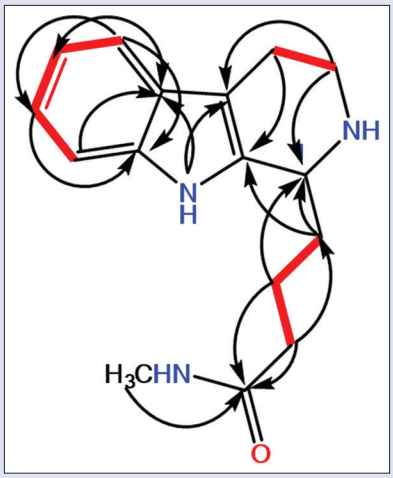

Figure 2: ^{1}H-^{1}H COSY and HMBC correlations of 4

and C-6 established the THβC moiety of 4 [Figure 2]. In the ^{1}H and ^{13}C spectra, signals for three-coupled aliphatic methylenes (δ_H 3.21 [m, H-2`]/$\delta_C$ 40.3 [C-2`], 1.75 [m, H-3`]/$\delta_C$ 24.2 [C-3`], and 2.25 and 1.95 [each m, H-4`]/$\delta_C$ 28.7 [C-4`]), and NH-bonded methyl (δ_H 3.17/δ_C 48.6), and an amide carbonyl (δ_C 156.9) characteristic for *N*-methylbutyramide moiety were observed. This moiety was assured by the cross peaks of H-2`/C-1` and C-4`, H-3`/C-1`, H-4`/C-2`, and 1`-N*H*-CH_3/C-1` in HMBC. The connectivity of the *N*-methylbutyramide moiety at C-1 of the THβC moiety was assured by the HMBC relations of H-2` and H-3`/C-1, and H-4`/C-1 and C-1a. Due to the scarcity of the compound, C-1 relative configuration was assigned to be *R*-form based on the comparison ^{1}H and ^{13}C shifts and *J* value of H-1 as well as optical rotation of 4 with those of series of analogous compounds.[19,20] On the basis of the above evidence, 4 was identified as a THβC alkaloid substituted at C-1 by N-methylbutyramide. Thus, compound 4 was finally assigned as (*R*)-N-methyl-4-(2,3,4,9-tetrahydro-1*H*-pyrido [3,4-*b*] indol-1-yl) butanamide and named ingenine F.

The NMR data of compounds 1-3 were in accordance with those reported previously for annomontine (1),[1,2] acanthomine A (2),[1] and 1-oxo-1,2,3,4-THβCs (3).[1]

Ingenine F (4) was assessed for its cytotoxic potential toward A549, MCF7, HCT116, PC12, and Hela cancer cell lines. It displayed cytotoxic activity against MCF7, HCT116, and A549 with IC_{50} values of 2.82, 1.00, and 2.37 μM, respectively, compared to doxorubicin (IC_{50} 0.012, 0.036, and 0.102 μM, respectively). However, it was inactive toward Hela and PC12 cancer cell lines.

CONCLUSION

A new 1,2,3,4-THβCs derivative (4) and three known compounds (1-3) were separated from the Indonesian marine sponge *A. ingens*. Their structures were identified by the aid of comprehensive spectroscopic analysis. Compound 4 exhibited cytotoxic effect toward MCF7, HCT116, and A549 cancer cell lines.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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