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Author

Liberio, Michelle S, Sooraj, Dhanya, Williams, Elizabeth D, Feng, Yunjiang, Davis, Rohan A

Published

2011

Journal Title

Tetrahedron Letters

Version

Accepted Manuscript (AM)

DOI

10.1016/j.tetlet.2011.09.151

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Graphical Abstract

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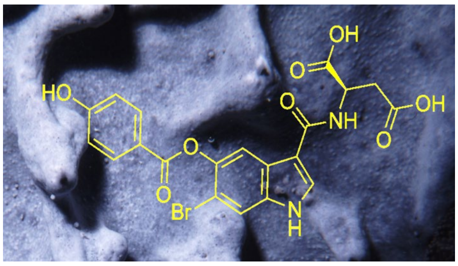

Kingamide A, a new indole alkaloid from the ascidian *Leptoclinides kingi*

Michelle S. Liberio[a], Dhanya Sooraj[b], Elizabeth D. Williams[b], Yunjiang Feng[a], Rohan A. Davis[a*]

[a] *Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia.*

[b] *Centre for Cancer Research, Monash Institute of Medical Research, Monash University, VIC 3168, Australia*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Kingamide A
Natural product
Alkaloid
Ascidian
Leptoclinides kingi

ABSTRACT

Chemical investigation of the CH_2Cl_2/MeOH extract from the Australian ascidian *Leptoclinides kingi* led to the isolation of a new brominated indole alkaloid, kingamide A (**1**). The planar structure of kingamide A was elucidated following the interpretation of 1D/2D NMR and MS data, and the absolute configuration was determined using Marfey's method. This is the first report of a natural product from *L. kingi*.

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Several thousand halogenated natural products have been identified as biosynthetic products from living organisms.[1] While various terrestrial organisms such as fungi, plants, bacteria, lichens and insects have been identified as producers of organohalogen metabolites, the marine environment is the single largest source of naturally occurring halogenated compounds.[2] The presence of halogen substituents in many natural products has been hypothesised as providing evolutionary advantage to the producing organism or symbiont, due to optimised bioactivity.[3] Ascidians belonging to the genus *Leptoclinides* have proven to be a source of new nitrogenous metabolites with 11 compounds reported to date, of which eight are indole alkaloids.[4] The first *Leptoclinides*-derived indole metabolites to be identified were *N*-(1*H*-indolyl-3-carbonyl)-D-arginine (**2**), *N*-(6-bromo-1*H*-indolyl-3-carbonyl)-L-arginine (**3**), *N*-(6-bromo-1*H*-indolyl-3-carbonyl)-L-histidine (**4**), and *N*-(6-bromo-1*H*-indolyl-3-carbonyl)-L-enduracididine (**5**)[5] (Figure 1). Other examples include leptoclinidamines A–C (**6**–**8**) from *L. durus*[6] and C^2-α-D-mannosylpyranosyl-L-tryptophan (**9**) from *L. dubius.*[7]

As part of our continuing research program aimed at the discovery of new chemistry from Australian ascidians,[8,9] 143 crude organic extracts derived from ascidians belonging to the family Didemnidae[10] were investigated using analytical HPLC and MS. Small-scale extraction of each of the 143 freeze-dried and ground Didemnid samples (300 mg) was undertaken using CH_2Cl_2 and MeOH. Both extracts were combined to afford a crude extract that was fractionated by analytical C_{18} HPLC (MeOH/H_2O/0.1% TFA) to yield 11 fractions that were subsequently analysed by off-line (±)-LRESIMS. Literature searching[4] in combination with MS data analysis identified one fraction from *Leptoclinides kingi,* which contained ions in the (-)-LRESIMS at *m/z* 489/491 (1:1) that were predicted to correspond to a new monobrominated alkaloid. Subsequent mass-directed isolation of the large-scale CH_2Cl_2/MeOH extract of *L. kingi* yielded the new indole alkaloid, kingamide A (**1**). In this Letter we report the isolation and structure elucidation of compound **1**.

A freeze-dried and ground sample (10 g) of *L. kingi*[11] was extracted sequentially with *n*-hexane, CH_2Cl_2/MeOH (4:1), and MeOH. The CH_2Cl_2/MeOH and MeOH extracts were combined and chromatographed using reversed-phase semi-preparative C_{18} HPLC (MeOH/H_2O/0.1% TFA) to yield 60 fractions that were analysed by (-)-LRESIMS. Fraction 37 contained the ions of interest (*m/z* 489/491) and was further purified by semi-preparative C_{18} HPLC (MeOH/H_2O/0.1% TFA) to yield the new

* To whom correspondence should be addressed. Tel: +61-7-3735-6043. Fax: +61-7-3735-6001; E-mail: r.davis@griffith.edu.au

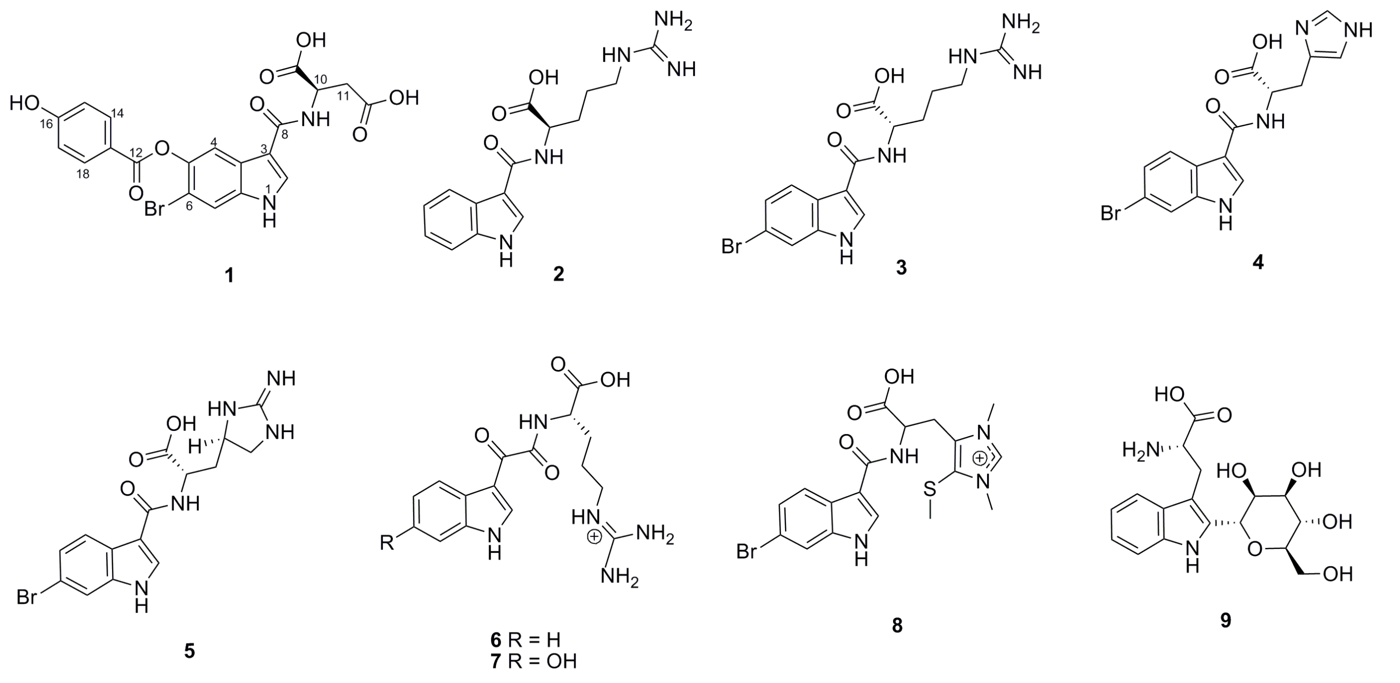

Figure 1. Chemical structures of natural products **1**–**9**.

indole alkaloid, kingamide A (**1**, 5.23 mg, 0.052% dry wt).

Kingamide A[12] was isolated as a stable, optically active yellow gum. The (-)-LRESIMS of **1** showed two equally intense ions at *m/z* 489 and 491, which indicated that the molecule contained one bromine atom. The molecular formula, $C_{20}H_{15}BrN_2O_8$ was determined by (-)-HRESIMS in conjunction with the 1D NMR data. The ^{1}H NMR spectrum (Table 1) of **1** contained three exchangeable protons [δ_H 11.81 (br d, J = 2.4 Hz), 10.56 (br s), 8.30 (d, J = 7.8 Hz)], three aromatic methine protons [δ_H 8.19 (d, J = 2.4 Hz), 7.98 (s) and 7.80 (s)], two mutually coupled aromatic methine doublets [δ_H 8.03 (d, J = 8.4 Hz), 6.95 (d, J = 8.4 Hz)], one mid-field methine proton [δ_H 4.74 (ddd, J = 7.8, 7.8, 6.0 Hz)] and two methylene signals [δ_H 2.83 (dd, J = 16.8, 6.0 Hz) and 2.67 (dd, J = 16.8, 7.8 Hz)]. Analysis of the COSY spectrum of **1** in combination with ^{1}H–^{1}H coupling constants readily identified three spin systems that included –CH=CH–, –NH–CH–CH$_2$– and –NH–CH= fragments. Eighteen unique signals were observed in the ^{13}C NMR spectrum (Table 1) of which 16 resonated between δ_C 105 and 175. HSQC data analysis allowed all 10 non-exchangeable protons to be assigned to the carbons that they were directly attached. A 3,4,5-trisubstituted pyrrole moiety was established on the basis of HMBC correlations (Figure 2) from the proton at δ_H 11.81 to carbons at δ_C 110.2 (C-3), 126.0 (C-3a), and 134.6 (C-7a), in conjunction with the COSY data that had elucidated the –NH–HC= system (δ_H 11.81/ δ_H 8.09).

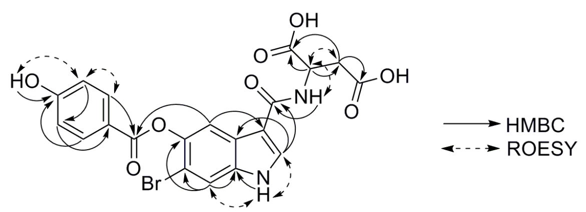

Figure 2. Key HMBC and ROESY correlations of kingamide A (**1**).

Furthermore, HMBC correlations from the aromatic methine singlet at δ_H 7.98 to carbons at δ_C 109.6 (C-6), 110.2 (C-3), 126.0 (C-3a), 141.8 (C-5), 134.6 (C-7a), and from the proton at δ_H 7.80 to carbons at δ_C 126.0 (C-3a), 141.8 (C-5), 109.6 (C-6), 134.6 (C-7a) allowed a 1,2,4,5-tetrasubstituted benzene ring to be fused to the pyrrole system, thus forming an indole skeleton. Strong ROESY correlations from the indole exchangeable proton at δ_H 11.81 to both protons at δ_H 7.80 (H-7) and δ_H 8.19 (H-2) further confirmed the presence of a 3,5,6-trisubstituted indole system in **1**. A *para*-substituted phenyl system was indicated by the two mutually coupled aromatic doublets at δ_H 8.03 (2H, J = 8.4 Hz) and 6.95 (2H, J = 8.4 Hz), and the two aromatic methine carbon signals to which they were directly attached at δ_C 132.3 (2C) and 115.7 (2C), respectively. Strong HMBC correlations from the methine proton at δ_H 8.03 to downfield carbons at δ_C 164.3 and 162.8 suggested a *para*-hydroxy benzoyl moiety. The hydroxy substitution was confirmed by HMBC correlations from the exchangeable proton at δ_H 10.56 to the carbon at δ_C 162.8 and a ROESY correlation between protons at δ_H 10.56 and 6.95. The benzoyl system was attached via an oxygen atom to C-5 of the indole skeleton through a $^4J_{CH}$ correlation from the proton at δ_H 7.98 (H-4) to the carbon at δ_C 164.3 (C-12). The ester linkage was confirmed by IR spectrum analysis, which showed a strong absorption at 1709 cm^{-1}. The bromine atom present in **1** was attached to C-6 of the indole system on the basis of the diagnostic carbon chemical shift at δ_C 109.6, which is similar to the NMR data reported for the previously isolated compound, 6-bromo-5-hydroxyindole.[13] The remaining unassigned carbons at δ_C 163.8, 171.9 and 172.9 were shown to be part of an aspartic acid moiety following HMBC data analysis of the protons associated with the –NH–CH–CH$_2$– spin system [δ_H 8.30 (H-9)/ 4.74 (H-10)/ 2.83/ 2.67 (H-11a/H-11b)]. All non-exchangeable protons showed HMBC correlations to the carbon resonating at δ_C 172.9, while both the protons at δ_H 2.83 and 2.67 showed $^2J_{CH}$ correlations to the carboxylic carbon at δ_C 171.9. HMBC data was instrumental in linking the aspartic acid unit to the indole skeleton at C-3. Strong HMBC cross-peaks were observed from both the H-2

Table 1. NMR data for kingamide A (**1**)[a]

Position	^{13}C	^{1}H (mult., *J* in Hz)	COSY	HMBC	ROESY
1		11.81 (br d, 2.4)	2	3, 3a, 7a, 8	2, 7
2	130.4	8.19 (d, 2.4)	1	3, 3a, 4, 7a, 8,	1
3	110.2				
3a	126.0				
4	115.2	7.98 (s)		3, 3a, 5, 6, 7a, 12	
5	141.8				
6	109.6				
7	115.7	7.80 (s)		3a, 5, 6, 7a	1
7a	134.6				
8	163.8				
9		8.30 (d, 7.8)	10	8, 10, 11, 10-COOH	10, 11b
10	48.6	4.74 (ddd, 7.8, 7.8 6.0)	9, 11a, 11b	8, 10-COOH, 11, 11-COOH	9, 11a, 11b
10-COOH	172.9	[b]			
11	36.2	2.83 (dd, 16.8, 6.0)	10, 11b	10, 10-COOH, 11-COOH	10, 11b
		2.67 (dd, 16.8, 7.8)	10, 11a	10, 10-COOH, 11-COOH	9, 10, 11a
11-COOH	171.9	[b]			
12	164.3				
13	119.2				
14	132.3	8.03 (d, 8.4)	15	12, 16, 18	15
15	115.7	6.95 (d, 8.4)	14	13, 14, 16, 17	14, 16-OH
16	162.8				
16-OH		10.56 (br s)		15, 16, 17	15, 17
17	115.7	6.95 (d, 8.4)	18	13, 15, 16, 18	16-OH, 18
18	132.3	8.03 (d, 8.4)	17	12, 14, 16	17

[a] Spectra were recorded at 600 MHz for ^{1}H and 125 MHz for ^{13}C in DMSO-d_6 at 30 °C.

[b] Signal not observed.

indole methine (δ_H 8.19) and the aspartic acid NH (δ_H 8.30) to the carbonyl signal resonating at δ_C 163.8 (C-8). These data confirmed that an amide functional group was attached to C-3 of the indole, and the NMR data was consistent with the related natural product leptoclinidamine C (**8**).[6] With this amide linkage established, the planar structure for kingamide A was assigned.

The absolute configuration of the amino acid in **1** was determined using Marfey's method.[14] This involved acid hydrolysis (6 N HCl, 105 ºC, 15 hours) of kingamide A followed by derivatisation of the hydrolysate with Marfey's reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA).[14] The FDAA derivatives of L- and D-aspartic acid were also synthesised, and used as standards. Analysis of all FDAA derivatives by LC-MS, using retention times and co-injections with standards, revealed the presence of only D-aspartic acid in **1**. With the absolute configuration determined, the structure of kingamide A was assigned to **1**.

Kingamide A has recently been added to the open-access small-molecule repository at the Queensland Compound Library (QCL),[15] where it is available for drug discovery and chemical biology research. To date, compound **1** has been screened against the breast cancer cell line MDA-MB-231, and a panel of seven prostate cancer cell lines (DU-145, PC3 ATCC, PC3-DsRed,[16] LNCaP and derivatives C4, C4-2 and C4-2B) and showed no cytotoxicity at 10 μM after 24 hours. Kingamide A has also been tested in an antimalarial *in vitro* assay using the chloroquine sensitive *Plasmodium falciparum* 3D7 line; no parasitic growth inhibition was detected at 10 μM after 24 hours.

In conclusion, this Letter reports the isolation and structure elucidation of the new indole alkaloid, kingamide A (**1**). This is the first report of a natural product from the ascidian species *L. kingi*. Kingamide A contains a benzoate group substituted at C-5 of an indole skeleton, which is rare among secondary metabolites.[4]

Acknowledgements

We thank H. T. Vu (Griffith University) for acquiring the HRESIMS measurements and V. Choomuenwai (Griffith University) for performing the antimalarial assay. We would also like to thank J. N. A. Hooper and P. Mather (Queensland Museum) for ascidian collection and taxonomic identification, respectively. M. S. L. is grateful for a PhD scholarship provided by the Eskitis Institute and Griffith University. E. D. W. is supported by an Australian NHMRC Career Development Award (#519539). This project was supported in part by funding from the Queensland and Victorian Government's Operational Infrastructure Support Programs.

Supplementary material

Supplementary data (1D/2D NMR spectra for kingamide A, general experimental details, extraction and isolation procedure, stereochemical determination details, and biological assays) associated with this article can be found, in the online version, at doi:

References and notes

1. Gribble, G. W. In *Prog. Heterocycl. Chem.*; Gordon, W. G.; John, A. J., Ed.; Elsevier: 2003; pp 58.
2. Gribble, G. W. *Chemosphere* **2003**, *52*, 289.
3. Neumann, C. S.; Fujimori, D. G.; Walsh, C. T. *Chem. Biol.* **2008**, *15*, 99.
4. *Dictionary of Natural Products* (DVD). 18.2 ed.; Taylor & Francis Group/CRC Press: London, UK, 2010.
5. García, A.; Vázquez, M. J.; Quiñoá, E.; Riguera, R.; Debitus, C. *J. Nat. Prod.* **1996**, *59*, 782.
6. Carroll, A. R.; Avery, V. M. *J. Nat. Prod.* **2009**, *72*, 696.
7. Garcia, A.; Lenis, L. A.; Jiménez, C.; Debitus, C.; Quiñoá, E.; Riguera, R. *Org. Lett.* **2000**, *2*, 2765.
8. Yin, S.; Boyle, G. M.; Carroll, A. R.; Kotiw, M.; Dearnaley, J.; Quinn, R. J.; Davis, R. A. *J. Nat. Prod.* **2010**, *73*, 1586.
9. Yin, S.; Cullinane, C.; Carroll, A. R.; Quinn, R. J.; Davis, R. A. *Tetrahedron Lett.* **2010**, *51*, 3403.
10. The 143 ascidian samples represented seven different genera including *Atriolum* (1 sample), *Didemnum* (60 samples), *Diplosoma* (5 samples), *Leptoclinides* (35 samples), *Lissoclinum* (7 samples), *Polysyncraton* (12 samples) and *Trididemnum* (23 samples).
11. The ascidian *Leptoclinides kingi* Michaelsen 1930 (Didemnidae) was collected by SCUBA diving at a depth of 26.7 m between Hook and Hardy Reefs in Queensland, Australia, during June 1999. A voucher sample (G315000) has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.
12. Kingamide A (**1**): stable yellow gum; $[\alpha]_D$ -18 (*c* 0.174, MeOH); UV (MeOH) λ_{max} (log ε), 256 (4.33), 274 sh (4.18), 296 sh (3.79) nm; IR ν_{max} (film) 3600-3100 (br), 2360, 2342, 1709, 1591, 1548, 1453, 1267, 1165 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR, see Table 1; (-)-LRESIMS *m/z* (35 eV) (rel. int.) 489 (100), 491 (100); (-)-HRESIMS *m/z* 488.9933 $[M-H]^-$ (calcd for $C_{20}H_{14}{}^{79}BrN_2O_8$, 488.9939).
13. Ochi, M.; Kataoka, K.; Ariki, S.; Iwatsuki, C.; Kodama, M.; Fukuyama, Y. *J. Nat. Prod.* **1998**, *61*, 1043.
14. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.
15. www.griffith.edu.au/science/queensland-compound-library
16. Zeng, Y.; Opeskin, K.; Goad, J.; Williams, E. D. *Cancer Res.* **2006**, *66*, 9566.