Organic LETTERS

Letter

pubs.acs.org/OrgLett

(+)- and (−)-Spiroreticulatine, A Pair of Unusual Spiro Bisheterocyclic Quinoline-imidazole Alkaloids from the South China Sea Sponge *Fascaplysinopsis reticulata*

Qi Wang,[†,∥] Xuli Tang,[‡,∥] Xiangchao Luo,[†] Nicole J. de Voogd,[§] Pinglin Li,*[,†] and Guoqiang Li*[,†]

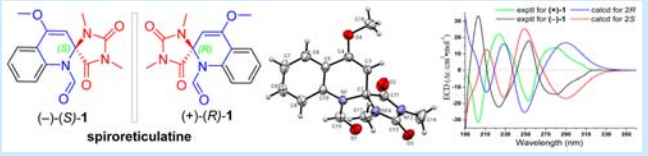
[†]Key Laboratory of Marine Drugs, Chinese Ministry of Education, School of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, People's Republic of China

[‡]College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, People's Republic of China

[§]National Museum of Natural History, 2300 RA Leiden, The Netherlands

Supporting Information

ABSTRACT: A pair of novel bisheterocyclic quinoline-imidazole alkaloids, (+)- and (−)-spiroreticulatine (**1**), were isolated from the South China Sea sponge *Fascaplysinopsis reticulata*. The structures and absolute configurations were elucidated by comprehensive spectroscopic analysis, single-crystal X-ray diffraction, and quantum chemical calculation methods. Spiroreticulatine is the first example of a sponge-derived natural spiro quinoline-imidazole alkaloid that may derive from tryptophan and 1,3-dimethylurea. Compound **1** showed inhibitory activity on IL-2 production but inactive against normal tumor cell lines.



Spirocyclic alkaloids are a class of naturally occurring alkaloids with unique structural cores and biogenetic origination.[1] So far, almost all these second metabolites were exclusively isolated from fungi and terrestrial plants,[2] and most of them have been found to have biogenetic relationships with indole alkaloids.[3] Chemically, the representative members of this family, brevianamides,[4] paraherquamides,[5] and notoamides[6] derived from the fungal genera of *Penicillium* and *Aspergillus*, featured a characteristic bicycio [2.2.2] diazaoctane ring system,[2a] while the plant-originated spirocyclic alkaloids, mostly from the genera of *Ervatamia*, *Gelsemium*, *Alstonia*, and *Uncaria*, usually architect the spirocyclic structures through an aza-unit with isoquinuclidine,[7] tropane,[8] indolizidine,[9] or pyrrolo[1,2-*b*]isoquinoline[10] core. Because of the broad bioactivities and synthetic challenges of the structures, some total synthesis works have been achieved in the past decades.[11]

Many marine organisms including sponges, tunicates, red alga, acorn worm, and symbiotic bacteria have been proven to generate indole alkaloids.[12] It was noticed that the sponge of the genus *Fascaplysinopsis* with only one species, *Fascaplysinopsis reticulata*, contains characteristic indole-imidazolidinone and pentacyclic fused bisindole alkaloids such as aplysinopsin and fascaplysin.[13] In our continuing search for new bioactive natural products from Xisha island (Paracel islands) invertebrates,[14] a pair of novel spiro bisheterocyclic quinoline-imidazole alkaloids, (+)- and (−)-spiroreticulatine (**1**), constructed by a unique *N*-carbaldehyde-1,2-dihydrogenquinoline and a 1,3-dimethyl-imidazolidin-2,4-dione unit via a chiral spiro carbon, were isolated from Xisha sponge *F. reticulata*. The initial isolation resulted in the racemate of (±)-**1**, and further chiral resolution for (±)-**1** was achieved on chiral HPLC to afford the enantiomers (+)- and (−)-**1**, respectively. The structure and absolute configuration of **1** was unambiguously elucidated by 1D and 2D NMR spectra, X-ray diffraction analysis, and calculated ECD method. Herein, we report the isolation, structural elucidation, and plausible biogenetic pathway of **1**.

Spiroreticulatine (**1**, 5 mg)[15] was obtained as a colorless crystal and had a molecular formula of $C_{15}H_{15}N_3O_4$ provided by the positive HRESIMS (*m*/*z* 302.1131 [M + H]$^+$, calcd. 302.1135). The IR spectrum suggested the presence of carbonyl (1702 cm^{-1}) group and aromatic ring (1651, 1600, 1501 cm^{-1}). The ^{1}H NMR spectrum of compound **1** (Table 1) displayed an aldehyde proton (δ_H 9.25), four aromatic protons characterized by an ABCD coupling system (δ_H 7.26, 7.42, 7.65, and 7.69),[16] an olefinic proton (δ_H 5.00), and three methyl protons assigned to one methoxyl group (δ_H 3.75) and two additional methyl groups (δ_H 2.54, 2.96) mostly attached to nitrogen atoms. Its ^{13}C NMR and DEPT spectra (Table 1) exhibited a total of 15 carbon resonances divided into three methyls, six methine groups, and six quaternary carbons. The HMQC spectrum clearly indicated the presence of a carbaldehyde group, and the relatively upfield carbon resonance (δ_C 162.0) suggested the connection to the remaining nitrogen in the molecule. A consecutive ^{1}H−^{1}H COSY correlation from H-6 to H-9, together with the HMBC correlations from aromatic H-7 (δ_H 7.26, dd, *J* = 7.60, 7.55 Hz) and H-9 (δ_H 7.69, d, *J* = 8.00 Hz) to C-5 (δ_C 117.9), from H-8 (δ_H 7.42, dd, *J* = 8.20, 7.50 Hz) and H-6 (δ_H 7.65, d, *J* = 7.76 Hz) to C-10 (δ_C 134.1), from olefinic H-3 (δ_H 5.00, s) to C-4 (δ_C 152.0), C-5, and C-2 (δ_C 74.3), and from aldehyde H-15 to C-2

Received: May 22, 2015
Published: June 30, 2015

DOI: 10.1021/acs.orglett.5b01503
Org. Lett. 2015, 17, 3458−3461

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for 1 in DMSO-d_6

no.	type	δ_H (J in Hz)	δ_C
2	C		74.3
3	CH	5.00 (s)	90.6
4	C		152.0
5	C		117.9
6	CH	7.65 (d, 7.76)	122.5
7	CH	7.26 (dd, 7.60, 7.55)	124.4
8	CH	7.42 (dd, 8.20, 7.50)	130.4
9	CH	7.69 (d, 8.00)	115.5
10	C		134.1
11	C		170.7
13	C		153.8
15	CH	9.25 (s)	162.0
12-NMe	CH_3	2.96 (s)	24.8
14-NMe	CH_3	2.54 (s)	24.4
4-OMe	CH_3	3.75 (s)	55.7

could establish the *N*-carbaldehyde-1,2-dihydrogenquinoline moiety (Figure 1). The HMBC correlations of the N-Me (δ_H

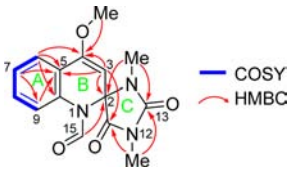


Figure 1. Key 2D NMR correlations of **1**.

2.54, s) with C-2 and C-13 (δ_C 153.8), of the other N-Me (δ_H 2.96, s) with C-13 and C-11 (δ_C 170.7), and of H-3 with C-2 and C-11 further constructed the additional moiety, 1,3-dimethylimidazolidin-2,4-dione, and allowed a connection of the two established moieties aforementioned to form the molecular skeleton via a spiro carbon (C-2). The residual methoxy group assigned at C-4 was evident from the HMBC correlation of H-OMe with C-4. Thus, the planar structure of **1** was established (Figure 1).

In order to determine the absolute configuration, a monocrystal of **1** was cultivated and obtained in a mixture solvent of CH_2Cl_2:MeOH (7:1) in a brown bottle. However, single crystal X-ray diffraction experiment using Cu Kα radiation (Figure 2, deposition number CCDC 1400075) was carried out and provided a structure of racemic mixture of **1** deriving from

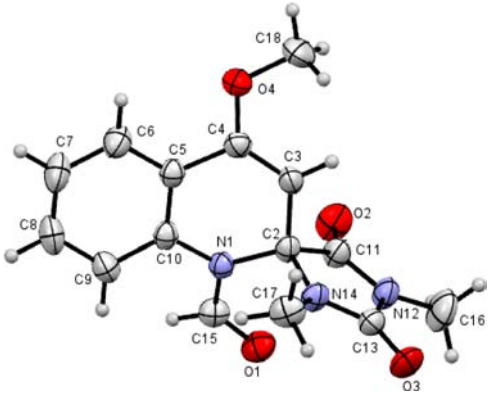


Figure 2. X-ray crystal structure of (±)-**1**.

the centrosymmetric space group $P12_1/n1$ (Supporting Information). Following resolution for **1** was achieved by a chiral HPLC method and afforded two enantiomers, (+)-**1** and (−)-**1**, in a ratio of almost 1:1 (Figure 3). The observation of opposite

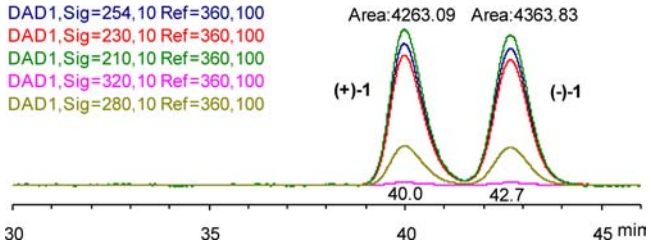


Figure 3. Chiral HPLC separation chromatogram of **1** on chiral Daicel Chiralpack IC column (250 × 4.6 mm, 5 μm).

optical rotation values and mirror ECD spectra for (+)-**1** and (−)-**1** confirmed their enantiomeric relationship (Figure 4).

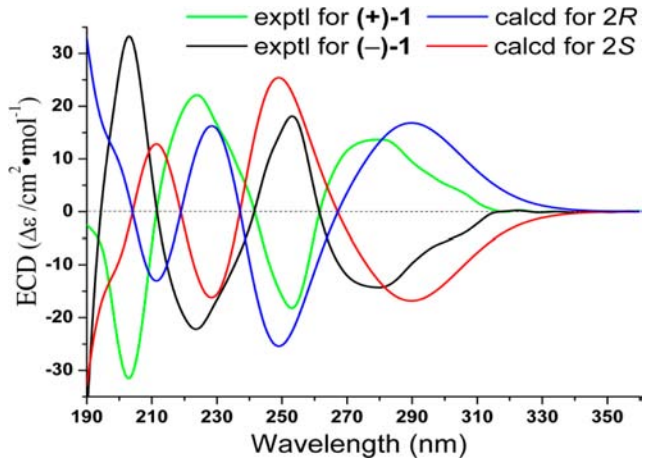


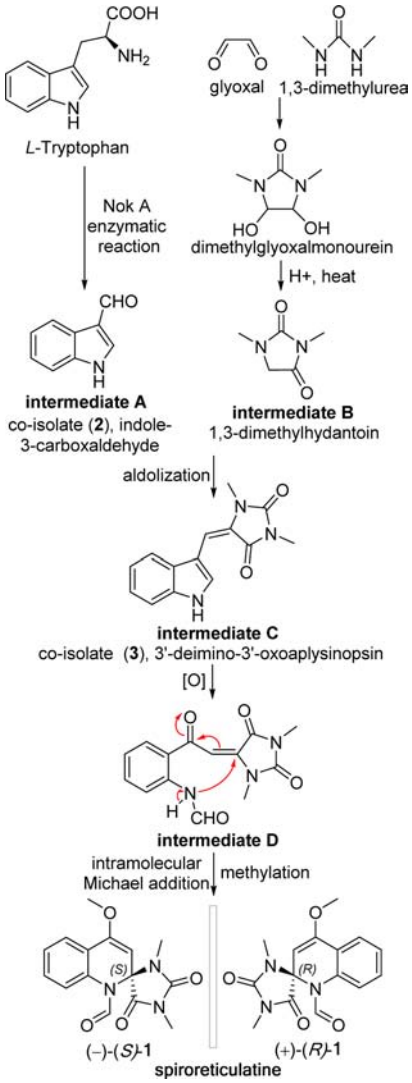
Figure 4. Experimental and theoretical ECD spectra of (+)-**1** and (−)-**1**.

Based on the consideration of the necessary chromophores surrounding chiral C-2 and conformer distribution analysis referenced to the dominant conformation from single-crystal X-ray diffraction, the ECD calculations for respective (+)- and (−)-**1** were performed by the TDDFT/ECD method at RB3LYP/DGDZVP level (Supporting Information).[17] The experimental ECD spectrum of (+)-**1** exhibited two strong positive CEs at 224.0 and 281.1 nm and two strong negative CEs at 204.5 and 253.5 nm, in good agreement with the calculated ECD spectrum for 2*R* configuration, and showed mirror-like relationship with calculated and experimental ECD spectra for 2*S* configuration (Figure 4). Therefore, 2*R* and 2*S* were finally assigned for (+)- and (−)-**1**, respectively.

The sponge *F. reticulata* is well-known for its production of the tryptophan-derived indole alkaloids.[13] A plausible biogenetic pathway for **1** could be proposed as shown in Scheme 1. The biosynthesis of (+)-**1** and (−)-**1** were assumed by way of the indispensable coisolated indole-3-carboaldehyde (intermediate A, **2**) and 3′-deimino-3′-oxoaplysinopsin (intermediate C, **3**). The conversion from L-tryptophan into indole-3-carboaldehyde could be carried out by a homologue of NokA.[18] The intermediate B, 1,3-dimethylimidazolidin-2,4-dione, could be formed from the intermolecular aldol reaction of the 1,3-dimethylurea with glyoxal and dehydration.[19] Then, the intermediates A and B could be aldolized to generate the

DOI: 10.1021/acs.orglett.5b01503
Org. Lett. 2015, 17, 3458−3461

Scheme 1. Plausible Biosynthetic Pathway of 1



intermediate C. Subsequently, the intermediate C could undergo an indole ring cleavage by oxidization to yield the intermediate D,[20] which possibly transformed into the products of (+)- and (−)-spiroreticulatine by intramolecular Michael addition through *a*-side and *b*-side attack to the olefinic bond plane and by methylation, respectively.

Cytotoxic activities for racemate (±)-**1** and enantiomers (+)-**1** and (−)-**1** were tested against four human cancer cell lines, K562, A549, and HeLa by MTT method,[21] and Jurkat by AlamarBlue method.[22] None of (±)-**1**, (+)-**1**, and (−)-**1** was active against the tested cell lines (growth inhibition percentage ≤17.5% at 50 μM). However, the determination of immune-suppressive activity[23] for (±)-**1**, (+)-**1**, and (−)-**1** showed dramatic inhibitory effects with a dose-dependent relationship on Interleukin (IL-2) secretion (Figure 5). (±)-**1** and (+)-**1** displayed almost equal inhibitory effects on IL-2 production at the concentration of 5, 25, and 50 μM, far larger than (−)-**1**, using DMSO and FK506 (Tacrolimus) as negative and positive controls, respectively (Table S4 in Supporting Information).

Spiroreticulatine (**1**) is the first example of sponge-derived spiro bisheterocyclic quinolone-imidazole alkaloid with a new skeleton. The resolution of racemate (±)-**1** was achieved by chiral HPLC to afford enantiomers (+)- and (−)-**1**. The absolute

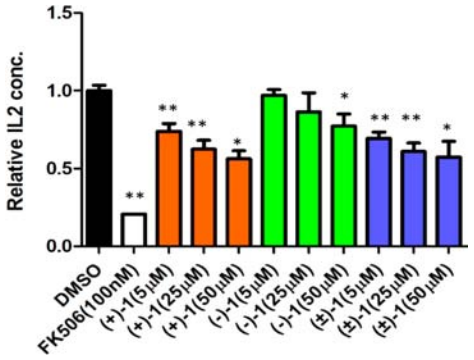


Figure 5. Immune-suppressive effects of (±)-**1**, (+)-**1**, and (−)-**1** on Interleukin 2 (IL2) secretion in Jurkat T cells. *$p < 0.1$ and **$p < 0.01$ were calculated by comparison with negative control.

configurations of the enantiomers were ambiguously determined by X-ray and calculated ECD with quantum chemistry method. Biosynthetically, (+)- and (−)-**1** were proposed to be relative with the indole alkaloids starting from the L-tryptophan. The racemate (±)-**1** and enantiomers (+)- and (−)-**1** showed significant inhibitory activity on IL-2 production but inactive against normal tumor cell lines.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, 1D and 2D NMR, MS spectra, X-ray crystal data, and computational details of compound **1**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01503.

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +86-532-82032323. Fax: +86-532-82033054. E-mail: lipinglin@ouc.edu.cn.

*E-mail: liguoqiang@ouc.edu.cn.

Author Contributions

‖Q.W. and X.T. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 41376142 and 41476107), NSFC-Shandong Joint Fund for Marine Science Research Centers (Grant No. U1406402), and Hi-tech Research and Development Program of China (Nos. 2013AA092902 and 2013AA093002). Special thanks are given to engineer L. Liu (Ocean University of China, Qingdao, China) for the cytotoxicity tests, Dr. Y. J. Dang (Fudan University, Xiamen, China) for the immunity tests, engineer Y. L. Shao (Lanzhou University, Lanzhou, China) for performing X-ray analysis, and Dr. Nicole J. de Voogd, a coauthor, for the sponge species identification.

■ REFERENCES

(1) Galliford, C. V.; Scheidt, K. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8748−8758.

DOI: 10.1021/acs.orglett.5b01503
Org. Lett. 2015, 17, 3458−3461

(2) (a) Finefield, J. M.; Frisvad, J. C.; Sherman, D. H.; Williams, R. M. *J. Nat. Prod.* **2012**, *75*, 812−833. (b) Herbert, R. B. *Nat. Prod. Rep.* **2003**, *20*, 494−508 and previous issues in this series .
(3) Saxton, J. E. *Nat. Prod. Rep.* **1994**, *11*, 493−531 and previous issues in this series .
(4) Birch, A. J.; Russell, R. A. *Tetrahedron* **1972**, *28*, 2999−3008.
(5) Yamazaki, M.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135−136.
(6) Tsukamoto, S.; Kawabata, T.; Kato, H.; Greshock, T. J.; Hiroshi, H.; Ohta, T.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1297−1300.
(7) Tang, B. Q.; Wang, W. J.; Huang, X. J.; Li, G. Q.; Wang, L.; Jiang, R. W.; Yang, T. T.; Shi, L.; Zhang, X. Q.; Ye, W. C. *J. Nat. Prod.* **2014**, *77*, 1839−1846.
(8) Tan, S. J.; Lim, J. L.; Low, Y. Y.; Sim, K. S.; Lim, S. H.; Kam, T. S. *J. Nat. Prod.* **2014**, *77*, 2068−2080.
(9) Yuan, D.; Ma, B.; Wu, C.; Yang, J.; Zhang, L.; Liu, S.; Wu, L.; Kano, Y. *J. Nat. Prod.* **2008**, *71*, 1271−1274.
(10) Clivio, P.; Richard, B.; Deverre, J. R.; Sevenet, T.; Zeches, M.; Men-Oliver, L. L. *Phytochemistry* **1991**, *30*, 3785−3792.
(11) (a) Edmondson, S.; Danishefsky, S. J.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*, 2147−2155. (b) Trost, B. M.; Cramer, N.; Silverman, S. M. *J. Am. Chem. Soc.* **2007**, *129*, 12396−12397.
(12) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munray, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2014**, *31*, 160−258 and previous issues in this series .
(13) (a) Kazlauskas, R.; Murphy, P. T.; Quinn, R. J.; Wells, R. J. *Tetrahedron Lett.* **1977**, *18*, 61−64. (b) Roll, D. M.; Ireland, C. M.; Lu, H. S. M.; Clardy, J. *J. Org. Chem.* **1988**, *53*, 3276−3278.
(14) (a) Liu, C. X.; Tang, X. L.; Li, P. L.; Li, G. Q. *Org. Lett.* **2012**, *14*, 1994−1997. (b) Zhang, J. R.; Tang, X. L.; Li, P. L.; Li, G. Q. *J. Nat. Prod.* **2013**, *76*, 600−606.
(15) Spiroreticulatine (**1**): colorless crystal, (+)-**1**, $[\alpha]_D^{23}$ = +79 (*c* 0.2, MeOH); (−)-**1**, $[\alpha]_D^{23}$ = −79 (*c* 0.2, MeOH); UV (DMSO) λ_{max} 256; IR (KBr) ν_{max} 1501, 1600, 1651, 1702 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 302.1131 $[M + H]^+$ ($C_{15}H_{15}N_3O_4$ calcd for 302.1135).
(16) O′Donnell, G.; Poeschl, R.; Zimhony, O.; Gunaratnam, M.; Moreira, J. B. C.; Neidle, S.; Evangelopoulos, D.; Bhakta, S.; Malkinson, J. P.; Boshoff, H. I. *J. Nat. Prod.* **2009**, *72*, 360−365.
(17) Shi, Y.; Liu, Y.; Li, Y.; Li, L.; Qu, J.; Ma, S.; Yu, S. *Org. Lett.* **2014**, *16*, 5406−5409.
(18) Chiu, H. T.; Lin, Y. C.; Lee, M. N.; Chen, Y. L.; Wang, M. S.; Lai, C. C. *Mol. BioSyst.* **2009**, *5*, 1192−1203.
(19) Martínez-López, D.; Yu, M. L.; García-Iriepa, C.; Campos, P. J.; Frutos, L. M.; Golen, J. A.; Rasapalli, S.; Sampedro, D. *J. Org. Chem.* **2015**, *80*, 3929−3939.
(20) Utsukihara, T.; Sato, M.; Itoh, K.; Sakamaki, H.; Kuniyoshi, M.; Horiuchi, C. A. *J. Mol. Catal. B: Enzym.* **2007**, *48*, 59−63.
(21) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589−601.
(22) Godugu, C.; Patel, A. R.; Desai, U.; Andey, T.; Sams, A.; Singh, M. *PLoS One* **2013**, *8*, e53708.
(23) Ren, Y. R.; Pan, F.; Parvez, S.; Fleig, A.; Chong, C. R.; Xu, J.; Dang, Y.; Zhang, J.; Jiang, H.; Penner, R.; Liu, J. O. *PLoS One* **2008**, *3*, e4009.

DOI: 10.1021/acs.orglett.5b01503
Org. Lett. 2015, 17, 3458−3461