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Isolation and total synthesis of dysidone A: a new piperidone alkaloid from the marine sponge *Dysidea* sp.[†]

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A new piperidone alkaloid, dysidone A (**1**), was isolated from the marine sponge *Dysidea* sp. The structure of **1** was elucidated by the method of spectroscopic analysis. Compound **1** represented the first example of piperidone alkaloid isolated from the sponge of the genus *Dysidea* with the exocyclic double bond. Furthermore, the total synthesis of **1** was also carried out, which was started with piperidine proceeding a PIDA/I₂-mediated α and β -C (sp³)–H bond dual oxygenation to achieve a 5-steps synthesis in a total yield of 10.6%. In addition, the anti-inflammatory activities of **1** and its derivative dysidone B (**1d**) were evaluated, which suggested that **1** showed weak anti-inflammatory activity.

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Introduction

Piperidine alkaloids have incomparable skeleton diversity and novelty, which often exhibit a wide range of biological activities including anticancer, antibacterial, anti-inflammatory, antiviral, antimalarial and antipsychotic effects, *etc.*^{1,2} Dozens of piperidine-containing drug molecules have been approved for marketing for the treatment of various diseases,^{3,4} such as Piperine and Febrifugine, which were isolated from Traditional Chinese herb *Piper nigrum* L. and *Dichroa febrifuga* Lour. for the treatment of convulsion and malaria,^{5–7} respectively. Biosynthetically, piperidines are often formed following lysine cyclization into 2-pipecolic acid.⁸ In addition, piperidines can be used to synthesize antineoplastic, antidiabetic, anti-inflammatory, antiviral (including HIV), cardiovascular and anti-influenza drugs, *etc.*^{9,10} which makes piperidine a favoured dominant scaffold in drug synthesis. However, the synthesis strategies for this kind of compounds are very limited, most of them are traditional chemical methods,^{11,12} which have problems such as narrow application range, harsh reaction conditions, complicated steps, and low yield.¹³ Therefore, there is an urgent need to find new methods for efficient synthesis.

The sponge of genus *Dysidea*, belonging to the family of Dysideidae, order Dictyoceratida, was widely distributed in the South China Sea. Abundant secondary metabolites had been isolated from this genus such as terpenes, alkaloids and sterols, which exhibited extensive biological activities such as cytotoxic,

antioxidant, antibacterial and anti-HIV activities.^{14–17} It was worth noting that there was no piperidone alkaloids had been discovered from this genus before. During the course of chemical investigation on marine sponges,^{18–23} a new piperidone alkaloid, dysidone A (**1**), featuring an exocyclic double bond moiety, was isolated from *Dysidea* sp. (Fig. 1). Herein, we describe the isolation, structure elucidation, total synthesis, and biological evaluation of **1**.

Results and discussion

Dysidone A (**1**) was obtained as light-yellow oil. Its molecular formula was determined as C₇H₁₁NO₂ by HRESIMS at *m/z* 164.0684 [M + Na]⁺ (calcd for C₇H₁₁NO₂Na: 164.0687), indicating three degrees of unsaturation. The UV absorption maximum was at 224 nm. The IR bands showed the presence of amino (3350 cm^{−1}), hydroxyl (3263 cm^{−1}) and carbonyl (1639 cm^{−1}) groups. Analysis of NMR spectra revealed that **1** had one carbonyl [δ_C 167.3 (C-2)], a trisubstituted double bonds [δ_H 6.21 (m, H-7); δ_C 141.5 (C-7) and 130.9 (C-3)], four methylenes [δ_H 4.32 (d, *J* = 5.9 Hz, H-8), 3.37 (m, H-6), 2.52 (m, H-4) and 1.87 (m, H-5); δ_C 59.4 (C-8), 42.7 (C-6), 31.9 (C-4) and 22.9 (C-5)] as well as two active hydrogen signals [δ_H 6.23 (br s, –NH) and 5.03 (s, 8-OH)]. The above data (Table 1) were similar to

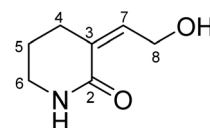


Fig. 1 Chemical structure of **1**.

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Table 1 ^1H and ^{13}C data of **1** in CDCl_3 (δ , J, Hz)

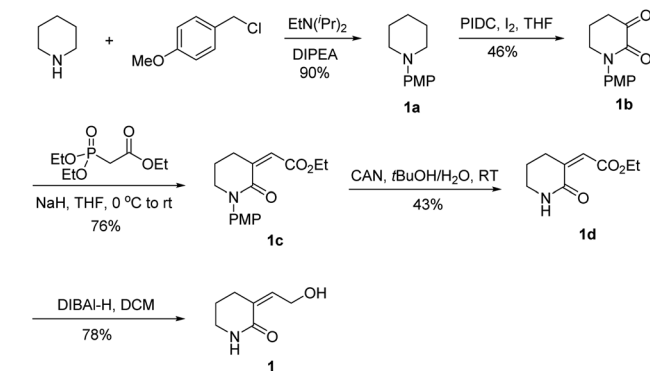
| No. | δ_{H} | δ_{C} |
|------|---------------------|---------------------|
| NH | 6.23 br s | — |
| 2 | — | 167.3 |
| 3 | — | 130.9 |
| 4 | 2.52 m | 31.9 |
| 5 | 1.87 m | 22.9 |
| 6 | 3.37 m | 42.7 |
| 7 | 6.21 m | 141.5 |
| 8 | 4.32 d (5.9) | 59.4 |
| 8-OH | 5.03 s | — |

those of valerolactam,²⁴ suggesting that **1** might be a piperidone alkaloid.

The ^1H - ^1H COSY spectrum of **1** revealed the presence of two spin coupling systems in bold as shown in Fig. 2. The HMBC correlations from H-4/H-6/H-7 to C-2, from H-4/H-5/H-8 to C-3, as well as from H-4 to C-7 indicated the basic skeleton of **1**. In addition, according to the molecular formula information and the obvious downshift at C-8 revealed that the remaining hydroxyl group should be attached at C-8. Therefore, the planer structure of **1** was determined. In the NOESY spectrum, the NOE correlation between H-7 and H-4a suggested the *Z* configuration of the double bond.

A total synthesis of **1** was also carried out to confirm its structure. Firstly, DIPEA was used to release $\text{EtN}(\text{iPr})_2$ to give compound **1a** in 90% yield, and the PMP group could be readily removed under oxidative condition. By mixing compound **1a** with iodobenzene diacetate (PIDC) and I_2 in THF, α - and β -C (sp^3)-H bond dual oxygenation of piperidine derivative **1b** was achieved in 46% yield, which made fast installation of **1** possible.²⁵ Subsequently, a HWE reaction was smoothly conducted between compound **1b** and organophosphine reagent to deliver compound **1c**, which was undertaken selective deprotection reaction with oxidant CAN to give compound **1d** in 43% yield. DIBAL-H reagent was employed to selectively reduce ester to alcohol under mild condition for delivering **1**, which was then fully characterized. The ^1H and ^{13}C NMR spectra of the synthesized **1** were nearly the same as those of the natural one (Fig. S10 and S11†). Eventually, the structure of **1** was unambiguously determined as shown in Scheme 1.

In fact, NO is an important pro-inflammatory mediator with an effect on the immune system. It can activate inflammatory pathways and induce the expression of iNOS by stimulating tumor necrosis factor (TNF- α), lipopolysaccharide (LPS), and interleukin 21 (IL-21), thereby causing an increase in NO synthesis. The previous study reported that many piperidines showed the anti-

Scheme 1 Synthetic route of **1**.

inflammatory activities, like (+)-homocrepidine A and homocrepidine B, which inhibited the production of NO with IC_{50} values of 3.6 and 27.6 μM , respectively.²⁶ Therefore, we evaluated the NO inhibitory activity of **1** and **1d**. The result indicated that compound **1** showed inhibitory activity ($\text{IC}_{50} = 378.27 \mu\text{M}$) comparable to that of SMT ($\text{IC}_{50} = 117.59 \mu\text{M}$), while compound **1d** did not show anti-inflammatory activity in this assay.

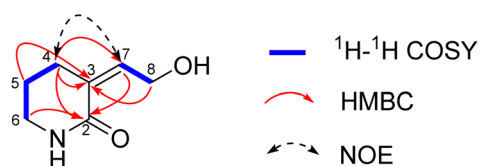
Conclusions

In conclusion, a new piperidone alkaloid, dysidone A (**1**), was isolated from the marine sponge *Dysidea* sp. Compound **1** represented the first example of piperidone alkaloid isolated from the sponge of genus *Dysidea* with the moiety of exocyclic double bond. Meanwhile, compound **1** was also fast installed from commercially available piperidine *via* a PIDA/ I_2 -mediated dual oxygenation reaction in five steps. Furthermore, the bioactivity evaluation results showed that **1** exhibited weak anti-inflammatory inhibitory activity. In the future, we will explore more bioactivities of **1**, such as antimalarial, analgesic, anti-cancer and antibacterial activities, *etc.*

Experimental section

General experimental procedures

Optical rotations were determined with a P-2000 Digital Polarimeter (JASCO International Co. Ltd, Hachioji, Japan). The UV spectra were recorded in MeOH on Shimadzu UV-2401PC spectrometer (Shimadzu, Kyoto, Japan) and the IR spectrums were measured on Thermo Nicolet iS50 FT-IR (Thermo Fisher Scientific, Waltham, MA USA). HR-ESI-MS was acquired from Agilent 6210 LC-ESI-Q/TOF mass spectrometer (Agilent Technologies Inc., California, America). The NMR spectra were obtained from Bruker Av 600/300 NMR (Bruker, Fällanden, Switzerland). HPLC was performed using YMC-Pack ODS-A [$250 \times 4.6 \text{ mm}$, D , 5 μm , 12 nm] was accomplished by an Agilent 1260 series apparatus (Agilent, Palo Alto, America). Column chromatography was performed with silica gel (300–400 mesh, Qingdao Marine Chemical Co., Qingdao, China) and GE Sephadex LH-20. The Thin-Layer Chromatography silica gel plate ($0.2 \pm 0.03 \text{ mm}$, HSGH 254) were purchased from the Qingdao Marine Chemical Factory, Qingdao, China.

Fig. 2 Key ^1H - ^1H COSY, HMBC and NOE correlations of **1**.

Materials

The sponge was collected from Xuwen County, Zhanjiang City, Guangdong Province, China, in May 2017, which was identified by the professor De-Xiang Wang as *Dysidea* sp. The specimen (2017-05) had been deposited in the Department of Chemistry, College of Chemistry and Materials Science, Jinan University.

Extraction and isolation

The sponge *Dysidea* sp. (20.0 kg, wet weight) was powdered and exhaustively extracted with 95% EtOAc to give a crude extract (1.8 kg) at room temperature, which was suspended in 2 L H₂O and partitioned by petroleum ether, EtOAc and *n*-butanol for five times, respectively. The EtOAc extract (40.0 g) was applied to silica gel column chromatography with gradient elution using CH₂Cl₂/CH₃OH (100 : 0–0 : 100) to yield 11 fractions (Frs 1–11). Fr. 8 (3.8 g) was subjected to silica gel column eluted with CH₂Cl₂/CH₃OH to gain 9 fractions (Frs 8–1–8–9). Fr. 8–7 (1.6 g) were applied to Sephadex LH-20 column with CH₂Cl₂/CH₃OH (1 : 1) to yield 7 fractions (Frs 8–7–1–8–7–7). Frs 8–7–6 (289.5 mg) was purified by preparative reversed-phase HPLC (8 mL min^{−1}, MeOH/H₂O = 5 : 95) to afford **1** (*t*_R = 64.01 min, 5.4 mg).

Natural dysidone A (1). Light-yellow oil; m. p. 170–171 °C; UV (CH₃OH) λ_{max}: 224 nm; IR (KBr) ν_{max}: 3350, 3263, 2951, 2845, 1639, 1403, 1017, 591 cm^{−1}; HR-ESI-MS: *m/z* 164.0684 [M + Na]⁺ (calcd for C₇H₁₁NO₂Na: 164.0687); ¹H NMR and ¹³C NMR data, see Table 1.

NO production assay

The anti-inflammatory activity of each sample was tested by nitric oxide (NO) production assay. In brief, RAW264.7 cells were seeded at a density of 33 × 10⁴ cells per mL in DMEM and incubated for 24 h. The seeded cells were treated with the compounds for 1 h, then treated with LPS and incubated for 24 h. After incubation for 24 h, 10 μL cells supernatant were incubated with an equal volume of Griess reagent at room temperature for 10 min, the optical density was then measured by detection of the absorbance at 540 nm on a microplate reader.

Conflicts of interest

There are no conflicts to declare.

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