Phytochemistry Letters 25 (2018) 132–135

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Bioactive aromatic metabolites from the sea urchin-derived actinomycete *Streptomyces spectabilis* strain HDa1

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ARTICLE INFO

Keywords:
Sea urchin
Actinomycete
Natural product
Acetylcholinesterase inhibitor

ABSTRACT

An actinomycete strain HDa1, capable of producing new acetylcholinesterase (AchE) inhibitors, was isolated from the gut of sea urchin *Anthocidaris crassispina* and identified as *Streptomyces spectabilis* by morphology and 16S rRNA gene sequence. Chemical investigation of the fermentation broth of *Streptomyces spectabilis* HDa1 by bioactivity-guided fractionation led to the isolation of 3,4-dimethoxy-1-naphthamide (**1**), *p-O*-(3,3-dimethylallyl)-benzamide (**2**), cyclo-(L-Val-L-Pro) (**3**), cyclo-(L-Ile-L-Pro) (**4**) and cyclo-(L-Leu-L-Pro) (**5**). Their structures were determined by analysis of the high-resolution electrospray ionization mass spectrum (HRESIMS), one-dimension (^{1}H-, ^{13}C NMR) and two-dimension NMR experiments (HSQC, HMBC and ^{1}H-^{1}H COSY), as well as by comparison with those data of known compounds. Compound **1** was identified as a new compound and compound **2** was discovered as a microbial natural product for the first time. All of these isolated compounds were evaluated for acetylcholinesterase inhibitory activity, with compounds **1** and **2** displaying *in vitro* inhibitory activity against AChE with the inhibition percentage of 54.8% and 43.5%, respectively, at the concentration of 100 μM. These two natural AChE inhibitors may provide a new chance for drug development for the treatment of neural degeneration disease.

1. Introduction

Neurodegenerative diseases (NDs) like Alzheimer's disease (AD) and Parkinson's disease (PD) are dreadful neurological illnesses, because of the low efficacy of current therapies (Querfurth and LaFerla, 2010). Currently, the primary drugs approved for AD treatment are mainly acetylcholinesterase (AChE) inhibitors, such as galantamine, rivastigmine and donepezil (Anand and Singh, 2013). AChE existing in both central and peripheral nervous system and muscular motor plaques is responsible for enzymatic hydrolyzation of neurotransmitter acetylcholine (ACh), and reduction of the ACh level may lead to NDs. Thus, inhibition of this enzyme has been largely used as a rational approach to moderate memory deficits by increasing ACh level. Despite the huge efforts in drug development, most clinical trials of potential drug candidates for AD failed (Misra and Medhi, 2013; Berk and Sabbagh, 2013). Natural products have played a very important role in drug discovery for the treatment of human diseases as exemplified by the cholesterol lowering drug lovastatin, the antifungal agent nystatin, the immunosuppressant rapamycin, and the antibiotics erythromycin and vancomycin, most of them originating from the actinomycetes. The plant-derived huperzine A has been successfully developed as a drug for the treatment of AD (Raves et al., 1997). Thus, AChE inhibitors from natural sources could be used as alternative efficient drugs (John et al., 2013). *Streptomyces* is a fruitful resource for new drug leads, accounting for nearly 80% of the actinomycete natural products (Kinashi, 2011; Jensen et al., 2005). Recently, more attention has been paid to the marine natural product, especially the marine-sourced bacteria natural products (Blunt et al., 2017). In our continuing effort to find novel bioactive natural products from plant endophytes (Guo et al., 2014, 2012a,b,c), insect-associated actinomycetes (Guo et al., 2012a,b,c), and marine-derived microbes (Guo et al., 2017; Wang et al., 2015; Wang et al., 2012), we isolated an actinomycete strain *Streptomyces spectabilis* HDa1 from the gut of sea urchin *Anthocidaris crassispina*, collected from Hainan Island, China. Many strains of *Streptomyces spectabilis* have been reported to produce diverse novel antibiotic natural products (Zuo et al., 2016; Selvakumar et al., 2015; Takahashi et al., 2001; Staley and Rinehart, 1994). In our primary screening we found the ethyl acetate extract of the fermentation broth of *Streptomyces spectabilis* HDa1

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https://doi.org/10.1016/j.phytol.2018.04.014
Received 31 January 2018; Received in revised form 30 March 2018; Accepted 3 April 2018

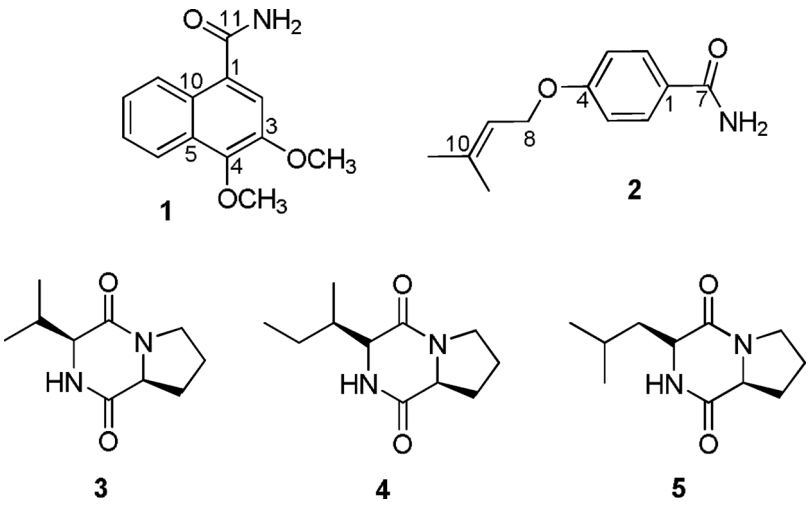

Fig. 1. Chemical structures of compounds **1**-**5**.

exhibited AChE inhibitory activity, and further chemical investigation of the crude extract resulted in the isolation of one new compound 3,4-dimethoxy-1-naphthamide (**1**), *p*-*O*-(3,3-dimethylallyl)-benzamide (**2**) firstly isolated as a microbial natural product, together with three known compounds cyclo-(L-Val-L-Pro) (**3**), cyclo-(L-Ile-L-Pro) (**4**) and cyclo-(L-Leu-L-Pro) (**5**) (Fig. 1). Herein we report the isolation, structure elucidation, and AChE inhibitory activity of the metabolites from this strain.

2. Results and discussion

The actinomycete strain HDa1 was identified as *Streptomyces spectabilis* by morphological observation and partial 16S rDNA sequence analysis, whose 16S rDNA GenBank accession number was MG745333. The ethyl acetate extract of the fermentation broth of *Streptomyces spectabilis* HDa1 showed AChE inhibitory activity with inhibition percentage of 38.2%, and further chemical investigation of the crude extract resulted in the isolation of compounds **1**-**5**.

Compound **1** was isolated as a light yellow powder, with the molecular formula $C_{13}H_{13}NO_3$ (eight degrees of unsaturation) as derived from its HRESIMS in combination with the ^{13}C NMR data (Table 1). The ^{13}C and DEPT135 NMR spectra showed signals of one aromatic amide carbon (δ_C 165.8, C-11), two oxygenated methyl carbons (δ_C 61.9, 3-OCH_3; 61.5, 4-OCH_3), and ten olefinic/aromatic carbons, including five methine carbons (δ_C 127.6, C-2; 122.2, C-6; 128.5, C-7; 126.7, C-8; 129.8, C-9). The ^1H NMR spectrum showed the presence of two doublets at δ_H 8.12 (d, J = 8.7 Hz, H-6) and 7.99 (d, J = 8.0 Hz, H-9), two triplets at δ_H 7.61 (t, J = 8.0 Hz, H-7) and 7.52 (t, J = 8.0 Hz, H-8), and one singlet at δ_H 8.33 (s, H-2). Detailed analysis of the splitting patterns of the coupled aromatic protons in the ^1H and ^1H-^1H COSY spectra of **1** revealed the presence of a 1,3,4-trisubstituted naphthalene ring. The above deduction was confirmed by the observation of the ^1H-^1H COSY correlations from H-7 to H-6 and H-8, from H-8 to H-9, and HMBC correlations from H-7 to C-5 (δ_C 131.4), from H-6 to C-4 (δ_C 148.5), C-5 and C-10 (δ_C 131.3), from H-8 to C-10, from H-9 to C-1 (δ_C 128.5), C-5 and C-10, from H-2 and 4-OCH_3 to C-4, from H-2 and 3-OCH_3 to C-3, and from H-2 to C-1, C-10 and C-11 (δ_C 165.8). Finally, the complete analysis of the ^1H, ^{13}C, DEPT135, HSQC, ^1H-^1H COSY, and HMBC NMR data (Fig. 2) allowed the determination of the structure for **1**, which was identified as 3,4-dimethoxy-1-naphthamide.

Compound **2**, isolated as a colorless lamellar crystal, gave a $[M + Na]^+$ ion peak at *m/z* 228.1010 in its HRESIMS spectrum, revealing a molecular formula of $C_{12}H_{15}NO_2$. The ^{13}C and DEPT135 NMR spectra of **2** showed signals of twelve carbons, including two methyl carbons (δ_C 25.8, C-11; 18.2, C-12), one oxygenated methylene carbon (δ_C 65.6, C-8), one aromatic amide carbon (δ_C 168.9, C-7), and eight olefinic/aromatic carbons. The ^1H NMR spectrum exhibited a pair of doublets at δ_H 7.89 (d, J = 8.7 Hz, H-2, H-6) and 6.97 (d, J = 8.7 Hz, H-3, H-5), indicating the presence of a 1,4-disubstituted benzene ring. Also the typical signals for a 3,3-dimethylallyloxy group at δ_H 5.47 (t, J = 6.5 Hz, H-9), 4.62 (d, J = 6.5 Hz, H_2-8), 1.78 (s, H_3-11) and 1.76 (s, Me, H_3-12) were observed. These deductions were confirmed by detailed analysis of the HSQC, ^1H-^1H COSY and HMBC data. Key HMBC correlations from H_2-8 to C-4 (δ_C 162.4) confirmed the linkage of 3,3-dimethylallyloxy group with the 1,4-disubstituted benzene ring through an oxygen bridge. Also the HMBC correlation from H-2 to C-7 was observed (Fig. 2). Finally the structure for **2** was elucidated as shown, which was identical to *p*-*O*-(3,3-dimethylallyl)benzamide previously isolated from the bark of *Amyris bernesii* (Hasbum and Castro, 1988). Herein this compound was discovered as a microbial natural product for the first time.

The structures of compounds **3**-**5** were identified and confirmed by comparison of its MS and NMR data with those published data (Xu et al., 2015; Young et al., 1976).

All of the isolates were evaluated for AChE inhibitory activity. As a result, compounds **1** and **2** exhibited inhibitory activity against AChE with the inhibition percentage of 54.8% and 43.5%, respectively, which were comparable to that of the positive control Tacrine with the inhibition percentage of 77.2%, whereas compounds **3**-**5** were almost

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **1** and **2** in acetone-d_6.

Position	**1**		**2**	
	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)	δ_C
1		128.5 (s)		127.6 (s)
2	8.33 (s)	127.6 (d)	7.89 (d, J = 8.7)	130.2 (d)
3		148.0 (s)	6.97 (d, J = 8.7)	114.9 (d)
3-OCH_3	4.07 (s)	61.9 (q)		
4		148.5 (s)		162.4 (s)
4-OCH_3	4.04 (s)	61.5 (q)		
5		131.4[a] (s)	6.97 (d, J = 8.7)	114.9 (d)
6	8.12 (d, J = 8.0)	122.2 (d)	7.89 (d, J = 8.7)	130.2 (d)
7	7.61 (t, J = 8.0)	128.5 (d)		168.9 (s)
8	7.52 (t, J = 8.0)	126.7 (d)	4.62 (d, J = 6.5)	65.6 (t)
9	7.99 (t, J = 8.0)	129.8 (d)	5.47 (t, J = 6.5)	120.7 (d)
10		131.3[a] (s)		138.2 (s)
11		165.8 (s)	1.78 (s)	25.8 (q)
12			1.76 (s)	18.2 (q)

[a] interchangeable signals.