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Potent *in Vitro* Anticancer Activity of Metacycloprodigiosin and Undecylprodigiosin from a Sponge-Derived Actinomycete *Saccharopolyspora* sp. nov.

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Bioassay-guided fractionation of $CHCl_3$ extract from the fermentation broth of a sponge *Mycale plumose*-derived actinomycete *Saccharopolyspora* sp. nov., led to the isolation of two known prodigiosin analogs - metacycloprodigiosin (**1**) and undecylprodigiosin (**2**). These compounds exhibited significant cytotoxic activities against five cancer cell lines: P388, HL60, A-549, BEL-7402, and SPCA4. This is the first report on the significant cytotoxicity of metacycloprodigiosin (**1**) against human cancer cell lines.

Key words: *Mycale plumose*, Sponge-derived actinomycete, *Saccharopolyspora* sp. nov, Metacycloprodigiosin, Undecylprodigiosin, Tripyrrole pigments, Cytotoxicity

INTRODUCTION

We have been screening microbial extracts for *in vitro* anticancer activity as part of an ongoing natural product research program. Among them, a $CHCl_3$ extract from an actinomycete strain *Saccharopolyspora* sp. nov., associated with the sponge *Mycale plumose*, showed significant cytotoxic activity against tsFT210 (a mouse temperature-sensitive $p34^{cdc2}$ mutant cell line) at 1 μg/mL. Consequently, mass fermentation and activity-guided isolation of anticancer compounds from this strain have been undertaken. This led to the isolation of two prodigiosins analogs-metacycloprodigiosin (**1**) and undecylprodigiosin (**2**). Both of them exhibited potent *in vitro* activity against five cancer cell lines: P388, HL60, A-549, BEL-7402, and SPCA4.

Prodigiosins are a family of naturally occurring polypyrrole red pigments produced by a restricted group of microorganisms, including *Streptomyces* and *Serratia* strains, characterized by a common pyrrolyldipyrrolylmethene skeleton (Ricardo *et al.*, 2003). Members of this family include 2-methyl-3-phentyl-6-methoxyprodigiosene (PG), cycloprodigiosin (cPrG), nonylprodigiosin, metacycloprodigiosin, and undecylprodigiosin (prodigiosin 25-C, UP). Prodigiosins are known to exhibit a wide range of biological activities. Recent investigations on their immunosuppressive activities (Furstner *et al.*, 2001; Nakamura *et al.*, 1986; Spongia *et al.*, 1997) and proton inhibition (Matsuya *et al.*, 2000; Sato *et al.*, 1998), have sparked renewed interest in these tripyrrole pigments. In this paper, we report the isolation and cytotoxicity of two prodigiosin analogs, metacycloprodigiosin (**1**) and undecylprodigiosin (**2**), from a marine-derived actinomycete *Saccharopolyspora* sp. nov.

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MATERIALS AND METHODS

General experimental procedures

Melting points were measured using a Yanaco MP-500D micro-melting point apparatus and were uncorrected. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU® 640 spectrophotometer. IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer in KBr discs. ^1H-, ^{13}C-NMR, DEPT spectra, and 2D-NMR were recorded on a JEOL Eclips-600 spectrometer using TMS as an internal standard. Chemical shifts were recorded as δ

values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer.

Bacterial material

The strain *Saccharopolyspora* sp. nov. was isolated from a sponge collected along the coast of Qingdao, China, and the strain was identified at the China Center for Type Culture Collection. The Institute of Oceanography at the Chinese Academy of Sciences identified the sponge as *Mycale plumose*.

Fermentation and extraction

The strain was grown on a rotary shaker under 120 rpm at 28°C for 5 days in two hundreds 500-mL conical flasks containing a liquid medium (150 mL/flask) composed of soybean powder 0.5%, starch 1.5%, $(NH_4)_2SO_4$ 0.2%, NaCl 0.5%, yeast extract 0.2 %, corn steep liquor 0.5%, and seawater after adjusting its pH to 7.0. The fermented whole broth (30 L) was filtrated through cheesecloth to separate into supernatant and mycelia. The former was concentrated under reduced pressure to approximately a quarter of the original volume, then extracted three times with $CHCl_3$, while the latter was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with $CHCl_3$ to give another $CHCl_3$ solution. Both $CHCl_3$ solutions were combined and concentrated under reduced pressure to give a $CHCl_3$ extract (2.0 g).

Purification

The $CHCl_3$ extract (2.0 g) was firstly subjected to column chromatography on silica gel and gradationally eluted with petroleum ether: acetone (from 100:0 to 0:100) to yield 20 fractions. Among them, fraction 7 (petroleum ether: acetone 85:15) was further purified by RP-18 column and gradationally eluted with methanol: water to yield compound **1** (30 mg, methanol: water 80:20) as orange crystals, and compound **2** (12 mg, methanol: water 85:15) as magenta powder.

Metacycloprodigiosin (1)

Orange crystal; m.p. 195-197°C; $[\alpha]_D^{25}$ -2170° (*c* 0.001, $CHCl_3$); UV ($CHCl_3$) λ_{max} nm (log ε) 504 (4.55), 536 (5.03); IR (KBr) ν_{max} 3104, 2925, 1601, 1554, 1383, 1239, 958, 658 cm^{-1}; HRESI-MS *m/z* 392.2708 $[M + H]^+$; ^1H- and ^{13}C-NMR (see Table I).

Undecylprodigiosin (2)

Magenta powder; UV ($CHCl_3$) λ_{max} nm (log ε) 370 (3.60), 500 (4.38), 530 (4.80); IR (KBr) ν_{max} 2924, 2852, 1610, 1546, 1379, 1040, 957, 658 cm^{-1}; HRESI-MS *m/z* 394.2853 $[M + H]^+$; ^1H- and ^{13}C-NMR (see Table I).

In vitro cytotoxic assays

Active fractions were assayed using the mouse temperature-sensitive $p34^{cdc2}$ mutant cell line tsFT210. The cytotoxic activity of compounds was evaluated by the SRB method (Skehan *et al.*, 1990) on five cancer cell lines: P388 (mouse lymphoma cell), HL60 (human peripheral blood promyeloblast cell), A-549 (human lung carcinoma cell), BEL-7402 (human hepatic carcinoma cell), and SPCA4 (human lung carcinoma cell). The cell lines were grown in RPMI-1640, supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37°C (tsFT210 cell line at 32°C). 200 μL of those cell suspensions at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 2 h under the above conditions. Then 2 μL of the test compound solutions (in DMSO) at different concentrations was added to each well and further incubated for 48 h in the same conditions. 50 μL of 80% trichloroacetic acid (TCA) were added to each well to fix the cells for 1.5 h in 4°C. 200 μL of an old medium containing TCA was washed repeatedly by water and stained with 100 μL 0.4% SRB dissolved in 1% acetic acid. The unbound dye was removed and the protein-bound dye was dissolved with 150 μL unbuffered Tris base for determination of optical density. Absorbance was then determined on a SPECTRA MAX PLUS plate reader at 540 nm.

RESULTS AND DISCUSSION

The two known compounds, metacycloprodigiosin (**1**) and undecylprodigiosin (**2**), were isolated from the $CHCl_3$ extract of *Saccharopolyspora* sp. nov. by bioassay-guided fractionation using the cytotoxicity against tsFT210 cells. The structures of **1** and **2** were identified by their NMR data analysis, as well as by comparing their physical and spectral data with those of literature values (Wasserman *et al.*, 1969a, 1976b). To the best of our knowledge, compounds **1** and **2** are firstly reported from the *Saccharopolyspora* sp. nov. strain.

Compound **1** was obtained as orange crystals. Its molecular formula was established as $C_{25}H_{33}N_3O$ by HRESI-MS (*m/z* 392.2708 $[M + H]^+$, calcd 392.2702), in combination with ^1H- and ^{13}C-NMR data. The IR (KBr) spectrum of **1** measured absorption bands at 3100 and 1600 cm^{-1}, and its UV spectrum in $CHCl_3$ gave a characteristic absorption of polypyrrole at 504 and 536 nm. The ^1H-NMR data of **1** summarized in Table I showed six aromatic or olefinic protons [δ_H 6.27 (1H, d, *J*=1.9), 7.05 (1H, s), 6.10 (1H, d, *J*=2.2), 7.22 (1H, m), 6.35 (1H, m), 6.91 (1H, m)], one methoxyl group at δ 4.03 (3H, s), one methyl proton, one methine proton, nine methylene protons, and two exchangeable protons (δ_H 12.77 and δ_H 12.64), assigned to NH protons. The ^{13}C-NMR spectrum showed 25 carbons

Table I. ^{1}H- and ^{13}C-NMR (600 and 150 MHz) data for compounds **1** and **2** in $CDCl_3$

No.	1			2		
	δ_C	δ_H (*J* in Hz)	HMBC	δ_C	δ_H (*J* in Hz)	HMBC
1	154.2 s			152.0 s		
2	112.3 d	6.27 (d, 1.9)	C-1, C-3, C-4	112.1 d	6.18 (d, 3.7)	C-1, C-4
3	150.3 s			128.6 d	6.81 (d, 3.7)	C-1, C-4
4	125.9 s			126.6 s		
5	–	12.77 (s)		–		
6	113.3 d	7.05 (s)	C-3, C-8	116.2 d	6.98 (s)	C-8
7	147.4 s			151.0 s		
8	165.6 s			166.4 s		
9	92.7 d	6.10 (d, 2.2)	C-7, C-10	93.3 d	6.08 (s)	C-7, C-10
10	120.5 s			122.4 s		
11	–			–		
12	122.3 s			122.2 s		
13	126.8 d	7.22 (m)	C-12, C-14, C-15	128.6 d	7.32 (brs)	
14	111.6 d	6.35 (m)	C-12, C-13	111.8 d	6.37 (dd, 3.7, 2.5)	C-12, C-13, C-15
15	116.7 d	6.91 (m)	C-12, C-13	118.4 d	6.97 (dd, 3.7, 1.1)	C-12, C-13
16	–	12.64 (s)		–		
8-OMe	58.6 t	4.03 (s)	C-8	58.7 t	4.01 (s)	C-8
1'	28.9 t	2.76 (ddd, 12.4, 8.0, 4.5) 3.20 (ddd, 12.4, 8.5, 4.0)		29.3 t	2.79 (m)	
2'	26.7 t	1.77 (m), 1.81 (m)		22.6-31.8 t 2'C-10'C	1.25-1.37 (18H, m) 2'H-10'H	
3'-7'	22.5-27.3 t	0.87-1.74 (10H, m)				
8'	34.3 t	1.41 (m), 1.74 (m)				
9'	39.6 d	2.55 (m)				
10'	29.9 t	1.60 (m), 1.68 (m)	C-3, C-9', C-11'			
11'	12.6 q	0.88 (t, 7.3)	C-9', C-10'	14.1 q	0.87 (t, 6.6)	

including 13 aromatic carbons, one methoxyl carbon, one methyl carbon, one methine carbon, and nine methylene carbons. A comparison of the ^{13}C-NMR data with the known prodigiosins (Wasserman *et al.*, 1969) revealed that compound **1** possessed the same three-pyrrole-ring skeleton, which was verified by the HMBC experiments. In addition, C-8 methoxyl and the pyrrole ring A which is disubstituted by a macrocyclic ring at the C-1 and C-3 positions was also confirmed by the HMBC correlations shown in Table I. On the basis of above data and by comparison with the literature (Wasserman *et al.*, 1969a, 1976b), compound **1** was identified as metacycloprodigiosin.

Compound **2** was obtained as magenta powder. Its molecular formula was established as $C_{25}H_{35}N_3O$ by HRESI-MS (*m/z* 394.2853 $[M + H]^+$, calcd 394.2858) in combination with ^{1}H- and ^{13}C-NMR data. The spectroscopic data showed **2** to be a member of prodigiosins. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with **1** showed the presence of the same three-pyrrole-ring skeleton, except for the C-1 and C-3 disubstituted macrocyclic ring in **1** being replaced by a C-1 monosubstituted fatty chain in **2** that was confirmed by further analysis of 2D NMR. On the basis of this interpretation, as well as by comparison with the literature (Wasserman *et al.*, 1969a), compound **2** was identified as undecylprodigiosin.

Cytotoxic effects of compounds **1** and **2** were first evaluated on P388, HL60, A-549, BEL-7402, and SPCA4 cell lines by the SRB method. As shown in Table II, both **1** and **2** displayed significant cytotoxic activities against

Table II. Inhibitory effect of **1** and **2** on the proliferation of five cancer cell lines

Compound	Cytotoxicity (IC_{50}, μM) for:				
	P388	HL60	A-549	BEL-7402	SPCA4
Metacycloprodigiosin (**1**)	0.007	0.022	0.11	0.028	7.52
Undecylprodigiosin (**2**)	0.013	0.033	0.11	0.026	0.010

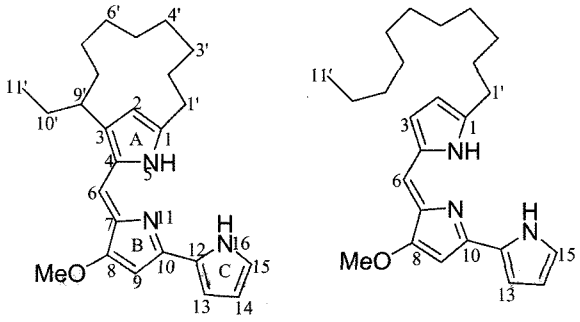

Fig. 1. Structures of metacycloprodigiosin (**1**) and undecylprodigiosin (**2**)

P388, HL60, A-549, and BEL-7402, while **2** was almost three orders of magnitude more cytotoxic than **1** against SPCA4. These results implied that the three-pyrrole-ring skeleton might be the essential part of the pharmacophore of prodigiosins, and the side chain could partially influence the cytotoxicity.

It has been recently found that the class of prodigiosins has antimicrobial, antimalarial, immunosuppressive, and cytotoxity activity *in vitro* (Ricardo *et al.*, 2003), but little has been done concerning their development. This situation may be attributed to a lack of a large amounts of compounds. To the best of our knowledge, this is the first isolation from *Saccharopolyspora* sp. nov. and the cytotoxic activity of metacycloprodigiosin (**1**) against human cancer cell lines. Prompted by the high anticancer activity and structure novelty, metacycloprodigiosin are currently being investigated for the evaluation of its anticancer activity and the biomolecular mechanism.

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