



Discovery of andrastones from the deep-sea-derived *Penicillium allii-sativi* MCCC 3A00580 by OSMAC strategy

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ABSTRACT

Andrastones are unusual 6,6,6,5-tetracyclic meroterpenoids that are rarely found in nature. Previously, three andrastones were obtained from the rice static fermentation extract of the deep-sea-derived fungus *Penicillium allii-sativi* MCCC 3A00580. Inspired by one strain many compounds (OSMAC) approach, the oat static fermentation on *P. allii-sativi* was conducted. As a result, 14 andrastones were isolated by UV-guided isolation. The chemical structures of the nine new compounds (1–9) was established by comprehensive analysis of the NMR, MS, ECD, and X-ray crystallography and the five known ones (10–14) were assigned by comparing their NMR, MS, and OR data with those reported in literature. Compound 1 bears a novel hemiketal moiety while 2 is the first example to possess a novel tetrahydrofuran moiety via C-7 and C-15. All isolates were tested for anti-allergic bioactivity. Compound 10, 3-deacetylцитреохидронол, significantly decreased degranulation with the IC₅₀ value of 14.8 μM, compared to that of 92.5 μM for the positive control, loratadine. Mechanism study indicated 10 could decrease the generation of histamine and TNF-α by reducing the accumulation of Ca²⁺ in RBL-2H3 cells. These findings indicate andrastones could be potential to discover new anti-allergic candidate drugs.

1. Introduction

Andrastones are 6,6,6,5-tetracyclic meroterpenoids biosynthesized from a sesquiterpene and a tetraketide. They were firstly discovered from *Penicillium citreo-viride* B. IFO 6200 and 4692 in 1919 [1]. Compounds possessing such scaffold are rarely found from the nature. Up to now, around 40 derivatives were reported, including citreohybridones A and B [1], andrastins A–D [2,3], atlantinones A and B [4], citreohybridones A–C [5], citreohybridones A–G and J–L [6], citreohybridonol [7], 3-deacetylцитреохидронол [8], 15-deacetylated citreohybridone E [9], isocitreohybridones A–C and H–J [5,6], isopenicins A–C [10], asperterpenes A–C [11], and terrenoid [11]. They showed a broad spectrum of bioactivities, including anti-Alzheimer's disease (AD) [11], anti-cancer [12], and anti-neuroinflammation [13]. Their unusual structures and diverse bioactivities have made them the subject of intense synthetic interest, organically [14,15] and biologically [16–19]. Previously, chemical investigations on the rice static fermentation extract of the deep-sea-derived *Penicillium allii-sativi* MCCC3A00580

afforded three novel andrastones [20].

Intrigued by the OSMAC (one strain many compounds) strategy that was commonly used to activate many silent biosynthetic genes in microorganisms to make miscellaneous secondary metabolites by manipulating different cultivation conditions, such as media composition, temperature, pH, etc [21,22]. Fermentation of the fungus *Penicillium allii-sativi* on solid oat medium caused a significant change of the fungal metabolites compared to those grown on rice (Figure S1). On the basis of the UV absorption characteristic spectrum of the andrastones at 208, 230, and 280 nm, the chemical investigation was carried out, which afforded nine new and five known andrastones (Fig. 1). Herein, we report the isolation, structure, and bioactivities of these compounds.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded on Bruker 400 MHz spectrometer using

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TMS as an internal standard. The HRESIMS spectra were measured on a Waters Xevo G2 Q-TOF mass spectrometer. Optical rotations were measured with an Anton Paar MCP100 polarimeter. ECD spectra were measured on a Chirascan spectrometer. The semi-preparative HPLC was conducted on an Agilent technologies 1260 infinity instrument equipped with DAD detector. Column chromatography (CC) was performed on silica gel, Sephadex LH-20, and ODS.

2.2. Fermentation and isolation

Penicillium allii-sativi was isolated from western Pacific (−4302 m) as described previously [20]. Large-scale fermentation was carried out in 100 Erlenmeyer flasks (1 L) each containing oats (80 g) and tape water (120 mL, 3% marine salt). After incubation for 30 days, the fermented broth was extracted with EtOAc for three times. The filtrate was concentrated under reduced pressure to give a crude extract (200 g), which was defatted by CC on silica gel using petroleum ether (PE) to provide a CH₂Cl₂-soluble extract (63 g).

The CH₂Cl₂-soluble extract was subjected to CC on silica gel eluting with gradient PE–EtOAc to get eight fractions (Fr.1–Fr.8). Fraction Fr.3 (6.3 g) was further separated by CC over ODS to give nine subfractions (Fr.3.1–Fr.3.9). Fraction Fr.3.4 (2.8 g) was CC on Sephadex LH-20 (MeOH), followed by recrystallization in MeOH–H₂O to provide **11** (434.3 mg). Fraction Fr.4 (2.1 g) was separated subsequently by CC on Sephadex LH-20 (MeOH) and ODS (MeOH–H₂O). Final purification by semi-prep. HPLC (ACN–H₂O) afforded **2** (2.5 mg, *t_R* = 30.5 min), **3** (7.9 mg, *t_R* = 31.8 min), **6** (10.0 mg, *t_R* = 34.7 min), **9** (3.4 mg, *t_R* = 26.6 min), and **12** (39.0 mg, *t_R* = 28.0 min). Fraction Fr.5 (3.5 g) was isolated by CC over ODS (MeOH–H₂O, 60 → 100%) to get eleven sub-fractions (Fr.5.1–Fr.5.11). Subfractions Fr.5.5 (90.6 mg) was subjected to CC on Sephadex LH-20 (MeOH), followed by purification using semi-prep. HPLC (ACN–H₂O) to get **4** (6.8 mg, *t_R* = 19.8 min), **5** (0.5 mg, *t_R* = 18.5 min), and **7** (27.1 mg, *t_R* = 21.0 min). Similarly, **8** (29.0 mg, *t_R* = 18.2 min), **10** (9.1 mg, *t_R* = 25.4 min), and **13** (9.6 mg, *t_R* = 19.0 min) were obtained from fraction Fr.5.6 (310.6 mg), while **1** (5.3 mg, *t_R* = 7.8

min) and **14** (14.7 mg, *t_R* = 20.2 min) were isolated from fractions Fr.5.7 (83.5 mg) and Fr.5.8 (174.8 mg), respectively.

2.2.1. Citrehybridonol B (**1**)

Colorless orthorhombic crystals; [α]_D²⁰ + 17.4 (c 0.35, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (4.08), 234 (3.74), 279 (3.69) nm; ECD (MeOH) $\Delta\epsilon_{203}$ − 22.73, $\Delta\epsilon_{248}$ + 12.88, $\Delta\epsilon_{303}$ − 1.77 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS *m/z* 515.2733 [M − H][−] (calcd for C₂₉H₃₉O₈, 515.2739).

2.2.2. Andrastin G (**2**)

White amorphous solid; [α]_D²⁰ + 43.1 (c 0.13, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 206 (3.72), 232 (3.37), 256 (3.60), 345 (2.47) nm; ECD (MeOH) $\Delta\epsilon_{202}$ − 12.52, $\Delta\epsilon_{251}$ + 5.06 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS *m/z* 523.2308 [M + Na]⁺ (calcd for C₂₈H₃₆O₈Na, 523.2308).

2.2.3. Andrastone B (**3**)

White amorphous solid; [α]_D²⁰ − 49.1 (c 0.44, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (3.63), 246 (3.32), 253 (3.33), 264 (3.26), 294 (3.51) nm; ECD (MeOH) $\Delta\epsilon_{200}$ − 7.61, $\Delta\epsilon_{216}$ + 0.21, $\Delta\epsilon_{228}$ − 0.95, $\Delta\epsilon_{260}$ + 4.85 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS *m/z* and 469.2566 [M + Na]⁺ (calcd for C₂₆H₃₈O₆Na, 469.2566).

2.2.4. Andrastone C (**4**)

White amorphous solid; [α]_D²⁰ − 38.0 (c 0.30, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 205 (3.59), 301 (2.80); ECD (MeOH) $\Delta\epsilon_{200}$ − 3.26, $\Delta\epsilon_{218}$ + 2.02, $\Delta\epsilon_{234}$ + 0.82, $\Delta\epsilon_{264}$ + 3.59, $\Delta\epsilon_{316}$ − 5.56 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS *m/z* 543.2585 [M + Na]⁺ (calcd for C₂₈H₄₀O₉Na, 543.2570).

2.2.5. Andrastone D (**5**)

White amorphous solid; [α]_D²⁰ − 125.5 (c 0.20, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 204 (3.63), 286 (2.51), 309 (2.66) nm; ECD (MeOH) $\Delta\epsilon_{217}$ + 4.47, $\Delta\epsilon_{229}$ + 3.11, $\Delta\epsilon_{258}$ + 5.98, $\Delta\epsilon_{310}$ − 14.37 nm; ¹H and ¹³C NMR

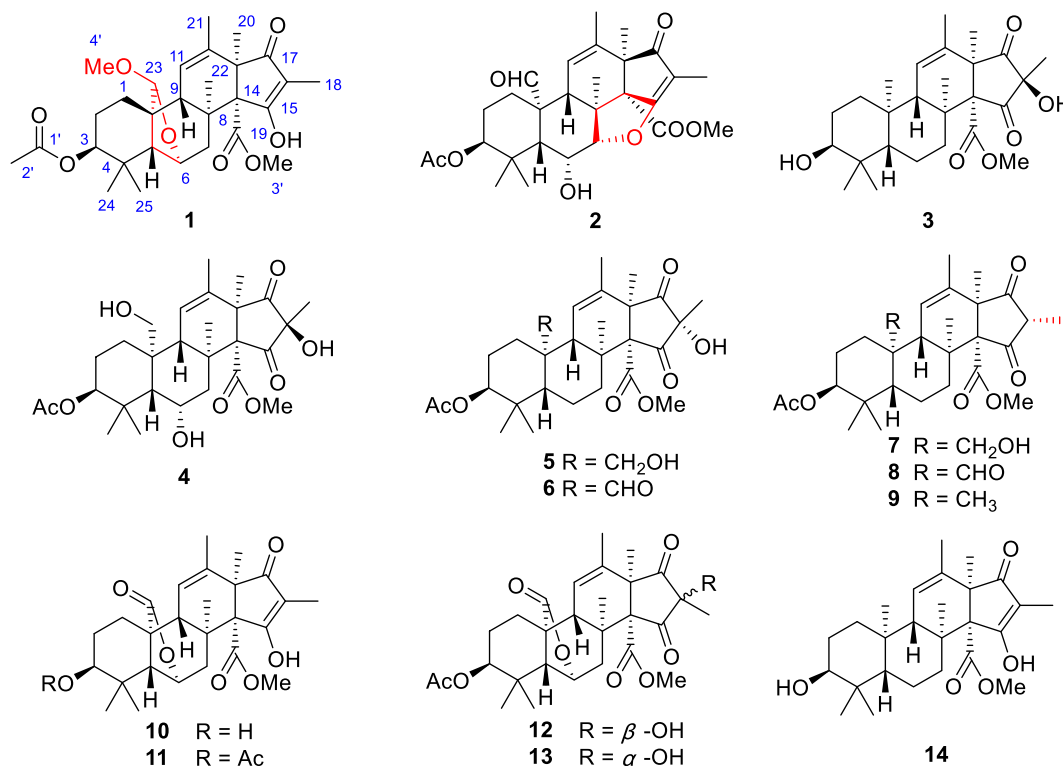


Fig. 1. Chemical structures of compounds 1–14.

Table 1
¹H NMR (400 MHz) Spectroscopic Data of Compounds 1–9.

No.	1 ^a	2 ^b	3 ^b	4 ^b	5 ^b	6 ^a	7 ^b	8 ^b	9 ^b
1	1.84 m	2.47 (dt, 13.8, 3.0)	1.52 m	1.99 (dt, 13.7, 3.8)	2.15 (dt, 13.1, 3.2)	2.40 (dt, 12.9, 4.5)	2.14 (dt, 13.2, 3.2)	2.35 (dt, 12.8, 3.0)	1.57 m
	1.24 m	1.11 m	1.26 m	1.08 (td, 13.3, 3.4)	1.01 (dt, 13.5, 3.7)	1.03 m	1.05 (td, 13.4, 3.6)	1.01 m	1.11 (td, 13.0, 3.5)
2	2.07 (dt, 12.8, 4.2)	1.72 m	1.95 m	2.06 m	2.03 m	1.62 m	1.98 m	1.60 m	1.96 m
	1.62 m	1.64 m	1.55 m	1.68 m	1.73 m		1.66 m		1.64 m
3	4.62 (br d, 2.2)	4.61 (d, 2.4)	3.41 (t, 2.7)	4.65 (t, 2.3)	4.70 (t, 2.6)	4.66 (t, 2.8)	4.67 (t, 4.6)	4.65 (t, 2.7)	4.64 (t, 2.4)
5	1.86 m	1.91 (d, 2.7)	1.42 m	1.67 (d, 2.2)	1.55 m	1.78 m	1.48 m	1.78 m	1.35 m
6	4.47 (br d, 3.0)	4.66 (t, 2.7)	1.65 (dd, 6.1, 1.1)	4.47 (dt, 2.6, 2.0)	1.55 m	2.16 (dd, 13.9, 3.1)	1.53 m	1.77 m	1.54 m
			1.56 m			1.79 m		1.28 m	
7	2.68 (d, 14.0)	4.61 (d, 2.4)	2.67 (td, 13.2, 7.6)	3.02 (dd, 14.9, 4.2)	2.77 m	2.84 (td, 13.5, 4.2)	2.68 (td, 13.5, 6.2)	2.73 (td, 13.2, 4.2)	2.57 (td, 12.8, 5.6)
	2.36 (dd, 14.0, 4.2)		2.21 (dt, 13.2, 3.0)	2.47 (dd, 14.7, 1.6)	2.28 (d, 13.3)	2.46 (dt, 13.5, 4.1)	2.30 (dt, 13.6, 3.0)	2.34 (dt, 13.5, 3.0)	2.27 (dt, 13.3, 3.2)
9	2.22 br s	2.18 br s	1.90 br s	2.06 (t, 2.0)	1.92 (t, 2.4)	2.05 (t, 2.2)	1.95 m	2.12 (d, 3.9)	1.79 (t, 2.2)
11	5.60 br s	5.72 br s	5.70 s	6.02 s	6.03 br s	5.78 s	5.99 s	5.72 s	5.60 s
16							3.23 (q, 6.8)	3.22 (q, 6.8)	3.27 (q, 6.8)
18	1.60 s	1.70 s	1.33 s	1.39 s	1.43 s	1.41 s	1.20 (d, 6.8)	1.22 (d, 6.8)	1.21 (d, 6.8)
20	1.20 s	1.27 s	1.35 s	1.40 s	1.36 s	1.30 s	1.29 s	1.24 s	1.28 s
21	1.82 s	1.86 s	1.72 s	1.72 br s	1.66 br s	1.68 br s	1.64 br s	1.16 s	1.70 (dd, 2.4, 2.2)
22	1.53 s	1.20 s	1.24 s	1.66 s	1.43 s	1.21 s	1.41 s	1.65 s	1.24 s
23	4.74 s	10.38 s	0.88 s	4.29 (d, 12.4)	4.02 (d, 12.1)	10.20 s	3.99 (d, 12.6)	10.12 s	0.89 s
				3.94 (d, 12.4)	3.97 (d, 12.1)		3.95 (d, 12.6)		
24	1.06 s	1.06 s	0.84 s	1.25 s	0.90 s	0.93 s	0.87 s	2.10 s	2.10 s
25	0.93 s	1.18 s	0.97 s	0.98 s	0.91 s	0.99 s	0.87 s	0.93 s	0.86 s
2'	2.01 s	2.02 s		2.11 s	2.11 s	2.10 s	2.09 s	0.88 s	0.89 s
3'	3.57 s	3.74 s	3.58 s	3.62 s	3.67 s	3.62 s	3.56 s	3.58 s	3.56 s
4'	3.31 s								
6-OH		2.94 br s							

^a Measured in CD₃OD.

^b Measured in CDCl₃.

Table 2

¹³C NMR (100 MHz) Spectroscopic Data of Compounds 1–9.

NO	1 ^a	2 ^b	3 ^b	4 ^b	5 ^b	6 ^a	7 ^b	8 ^b	9 ^b
1	23.3 t	28.8 t	32.4 t	31.1 t	28.6 t	29.0 t	28.3 t	27.8 t	33.0 t
2	25.3 t	23.1 t	24.7 t	23.0 t	23.2 t	24.3 t	23.2 t	23.3 t	22.5 t
3	79.0 d	78.1 d	76.0 d	78.6 d	77.3 d	78.8 d	77.4 d	77.2 d	77.8 d
4	34.8 s	37.3 s	37.4 s	37.1 s	36.3 s	38.1 s	36.2 s	36.9 s	36.6 s
5	55.7 d	46.6 d	47.6 d	50.6 d	49.5 d	49.6 d	49.5 d	47.9 d	48.9 d
6	79.8 d	67.5 d	17.5 t	67.6 d	17.2 t	17.8 t	17.1 t	16.7 t	17.3 t
7	41.4 t	92.4 d	31.8 t	40.2 t	32.0 t	32.2 t	31.9 t	30.9 t	31.6 t
8	44.0 s	46.2 s	39.4 s	38.1 s	39.4 s	40.5 s	39.8 s	39.4 s	37.3 s
9	55.0 d	50.2 d	53.3 d	53.6 d	53.5 d	55.6 d	53.5 d	53.8 d	53.7 d
10	48.4 s	51.8 s	37.3 s	42.8 s	42.4 s	53.4 s	42.5 s	52.3 s	40.2 s
11	125.6 d	119.9 d	129.1 d	129.4 d	129.6 d	126.8 d	128.2 d	124.6 d	127.3 d
12	137.7 s	135.3 s	132.9 s	131.2 s	129.6 s	135.5 s	131.0 s	134.3 s	134.2 s
13	58.0 s*	53.7 s	60.9 s	60.9 s	58.8 s	59.6 s	60.6 s	60.7 s	60.8 s
14	70.5 s	69.3 s	71.4 s	71.8 s	73.0 s	73.0 s	73.1 s	72.3 s	73.1 s
15	197.3 s*	185.2 s	211.1 s	211.1 s	212.0 s	213.4 s	210.5 s	210.4 s	210.5 s
16	115.4 s	113.3 s	72.2 s	72.2 s	75.8 s	77.1 s	50.6 d	50.8 d	50.9 d
17	199.1 s*	202.4 s	207.7 s	207.2 s	211.6 s	212.3 s	209.6 s	208.8 s	209.6 s
18	6.3 q	5.8 q	16.8 q	19.8 q	25.3 q	26.5 q	9.5 q	9.5 q	9.3 q
19	171.9 s	168.8 s	167.7 s	167.6 s	168.7 s	169.0 s	168.2 s	168.0 s	168.3 s
20	16.8 q	20.99 q	19.7 q	16.9 q	17.2 q	17.7 q	16.1 q	15.9 q	16.3 q
21	20.0 q	20.7 q	18.9 q	19.0 q	19.2 q	19.0 q	19.0 q	18.93 q	19.0 q
22	21.0 q	15.1 q	18.4 q	18.7 q	17.8 q	19.8 q	17.3 q	18.95 q	17.8 q
23	108.4 d	208.6 d	17.0 q	62.3 t	62.7 t	206.3 d	62.8 t	204.4 d	16.9 q
24	21.7 q	26.1 q	21.7 q	24.1 q	21.7 q	21.6 q	21.6 q	21.3 q	21.47 q
25	29.0 q	23.9 q	28.0 q	27.4 q	28.0 q	27.1 q	27.9 q	26.5 q	27.5 q
1'	172.3 s	170.1 s		170.9 s	170.9 s	172.4 s	170.9 s	170.7 s	171.0 s
2'	21.0 q	20.96 q		21.5 q	21.5 q	21.1 q	21.5 q	21.4 q	21.50 q
3'	52.0 q	52.2 q	51.7 q	51.9 q	52.4 q	52.5 q	51.8 q	51.9 d	51.7 q
4'	55.6 q								

* Identified in HMBC spectrum

^a Measured in CD₃OD.^b Measured in CDCl₃.

data, see Tables 1 and 2. HRESIMS m/z 527.2619 [M + Na]⁺ (calcd for C₂₈H₄₀O₈Na, 527.2621).

2.2.6. Andrastone E (6)

White amorphous solid; $[\alpha]_D^{20}$ – 61.8 (c 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 195 (2.49), 203 (3.70), 295 (2.89) nm; ECD (MeOH) $\Delta\epsilon_{214}$ + 2.80, $\Delta\epsilon_{217}$ + 3.05, $\Delta\epsilon_{252}$ + 5.36, $\Delta\epsilon_{309}$ – 13.51 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS m/z 525.2468 [M + Na]⁺ (calcd for C₂₈H₃₈O₈Na, 525.2464).

2.2.7. Andrastone F (7)

White amorphous solid; $[\alpha]_D^{20}$ – 28.4 (c 0.74, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.59), 246 (3.37) nm; ECD (MeOH) $\Delta\epsilon_{203}$ – 19.96, $\Delta\epsilon_{237}$ + 7.59, $\Delta\epsilon_{310}$ – 2.33 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS m/z and 511.2690 [M + Na]⁺ (calcd for C₂₈H₄₀O₇Na, 511.2672).

2.2.8. Andrastone G (8)

White amorphous solid; $[\alpha]_D^{20}$ – 16.5 (c 0.49, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.58), 264 (3.21), 282 (3.29) nm; ECD (MeOH) $\Delta\epsilon_{202}$ – 14.92, $\Delta\epsilon_{238}$ + 5.04, $\Delta\epsilon_{310}$ – 2.91 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS m/z and 509.2491 [M + Na]⁺ (calcd for C₂₈H₃₈O₇Na, 509.2515).

2.2.9. Andrastone H (9)

White amorphous solid; $[\alpha]_D^{20}$ – 11.8 (c 0.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.73), 245 (3.40), 262 (3.44) nm; ECD (MeOH) $\Delta\epsilon_{206}$ – 20.63, $\Delta\epsilon_{236}$ + 7.05, $\Delta\epsilon_{306}$ – 2.01 nm; ¹H and ¹³C NMR data, see Tables 1 and 2. HRESIMS m/z 495.2733 [M + Na]⁺ (calcd for C₂₈H₄₀O₆Na, 495.2723).

2.2.10. X-ray crystallography of compound 1

Compound 1 was obtained as colorless orthorhombic crystals. The crystal data were recorded with an Xcalibur Eos Gemini single-crystal

diffractometer with Cu-K α radiation (λ = 1.54184 Å). Space group $P2_12_12_1$, a = 10.8766(2) Å, b = 11.0415(2) Å, c = 23.3236(3) Å, $\alpha = \beta = \gamma = 90^\circ$, V = 2801.02 Å³, Z = 4, D_{calcd} = 1.225 g/cm³; μ = 0.723 mm^{–1}, $F(000)$ = 1112.0; The final R indices [$I > 2\sigma(I)$] R_1 = 0.0299, wR_2 = 0.0737. The absolute structure parameter was 0.00(9). Crystallographic data of 1 have been deposited in the Cambridge Crystallographic Data Center, with deposition number CCDC 1954318. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, U.K. (fax + 44(0)-1233-336033; email: deposit@ccdc.cam.ac.uk).

2.3. ECD calculation

As reported previously [23], conformational analysis was firstly performed via random searching in the Sybyl-X 2.0 using the MMFF94S force field with an energy cutoff of 7.0 kcal/mol and RMSD threshold of 0.2 Å. All conformers were consecutively optimized at PM6 and HF/6-31G(d) levels. Dominative conformers were re-optimized at B3LYP/6-31G(d) level in gas phase. The theoretical ECD spectra were calculated with the GIAO method at MPW1PW91/6-31G(d,p) level in MeOH using Gaussian 09 [24]. The ECD spectrum was simulated in SpecDis [25] by overlapping Gaussian functions for each transition.

2.4. Measurement of β -hexosaminidase

As previously reported [26], rat basophilic leukemia (RBL)-2H3 cells (ATCC, Manassas, VA, USA) were culture on the a 96-well plate for 24 h. Then the cells were washed twice with pre-warmed Tyrode's buffer (37 °C) after treating with 0.1 μ g/mL antidiinitrophenyl (DNP)-IgE (Sigma, St Louis, MO, U.S.A.). Subsequently, the tested compounds at different concentrations were added for 1 h. After stimulated with dinitrophenyl-bovine serum albumin DNP-BSA (0.1 μ g/mL) (Biosearch, Petaluma, CA) for 1 h in Tyrode's buffer, the cells were then measured the total β -hexosaminidase activity of the RBL-2H3 cells. The activity

was quantified by detecting the fluorescence intensity (360 nm excitation and 450 nm emission).

2.5. Histamine release assay

Histamine was measured using the model of the IgE-mediated mast cell allergic reaction. Same as measurement of β -hexosaminidase except that the IgE-sensitized RBL-2H3 cells were pre-incubated with tested compounds for 1 h and then incubated with DNP-BSA (500 ng/mL) for 15 min. Cell culture supernatant was collected for measurement of histamine (IBL, Hamburg, Germany).

2.6. Cytokine immunoassays

Briefly, RBL-2H3 cells were sensitized by anti-DNP-IgE (0.1 μ g/mL) overnight. Then the IgE-sensitized cells were treated with test compounds for 1 h and stimulated with DNP-BSA for 6 h. According to the manufacturer's instructions, the levels of tumor necrosis factor (TNF)- α was determined by sandwich-ELISA kit (Perprotech, Princeton, NJ, USA) [27].

2.7. Measurement of intracellular Ca^{2+} concentration

Intracellular Ca^{2+} signal was measured as described previously [28]. RBL-2H3 cells were seeded to a black 96-well culture plate and treated with anti-DNP IgE as described above. After washing with HBSS twice, the IgE-sensitized cells were incubated with 100 μ L Fluo 3-AM for 1 h. After abandoning the working solution, 100 μ L HBSS containing different concentrations of samples were added to the sample group for 30 min, 100 μ L HBSS containing equal volume of PBS was added to A23187 group, 100 μ L HBSS containing 5 μ L 2% Triton X-100 was added to Fmax group, and 5 μ L 100 mmol/L EGTA was added to Fmin group. After A23187 was added, fluorescence value (F) of each pore solution was immediately monitored with an excitation wavelength of 490 nm and an emission wavelength of 530 nm using a microplate reader.

3. Results and discussion

3.1. Structure elucidation

From the extract of *Penicillium allii-sativii* MCCC 3A00580, nine new (1–9) and five known (10–14) andrastones were obtained by using repeated column chromatography (CC) over silica gel, Sephadex LH-20, ODS, and semi-preparative HPLC. By comparison of the NMR, MS, OR data with those reported in references, the known compounds were identified as 3-deacetylcitreohybridonol (10) [8], citreohybridonol (11) [16], citreohybridione A (12) [5], 16-*epi*-citreohybridione A (13) [20], and andrastin F (14) [29].

Compound 1 was isolated as colorless orthorhombic crystals. The molecular formula was established as $\text{C}_{29}\text{H}_{40}\text{O}_8$ on the basis of its negative HRESIMS spectrum at m/z 515.2733 $[\text{M} - \text{H}]^-$, requiring ten degrees of unsaturation. The ^1H NMR spectrum exhibited seven methyl singlets, two methoxys, one olefinic and three oxygenated methines. The ^{13}C and DEPT spectra, with the aid of the HMBC spectrum, revealed 29 carbons, including seven methyls, two methoxys, three methylenes, six methines (one olefinic at δ_{C} 125.6, one hemiketal at δ_{C} 108.4, two oxygenated at δ_{C} 79.8 and 79.0), and 11 nonprotonated carbons (four carbonyls at δ_{C} 199.1, 197.3, 172.3, 171.9, and two olefinics at δ_{C} 137.7 and 115.4), which were very similar to those of citreohybridonol [16]. In the COSY spectrum, H-11 (δ_{H} 5.60) was correlated to H-9 (δ_{H} 2.22)/H₃-21 (δ_{H} 1.82), H₂-2 (δ_{H} 2.07, 1.62) to H₂-1 (δ_{H} 1.84, 1.24)/H-3 (δ_{H} 4.62), and H-6 (δ_{H} 4.47) to H₂-7 (δ_{H} 2.68, 2.36)/H-5 (δ_{H} 1.86), constructing three segments (Fig. 1). In the HMBC spectrum, correlations of H₃-18,20,22,24,25 as well as H₂-1 and H-23 connected four rings of A, B, C, and E. While the HMBC cross peaks of H₃-2',3',4' and H-3 linked the acetoxyl and two methoxys to C-3, C-19, and C-23 positions,

respectively. However, the poor HMBC correlations made it impossible to construct the ring D. Fortunately, orthorhombic crystals of 1 were obtained in a mixture of MeOH-H₂O. By the single crystal X-ray diffraction using Cu-K α radiation (Fig. 2), the absolute configuration of 1 was then established unambiguously as 23-deoxy-23 α -methoxycitreo-hybridonol, and named citreohybridonol B.

Compound 2 exhibited a sodium adduct ion peak at m/z 523.2308 $[\text{M} + \text{Na}]^+$ in its HRESIMS spectrum, corresponding to the molecular formula of $\text{C}_{28}\text{H}_{36}\text{O}_8$ with eleven degrees of unsaturation. The ^1H and ^{13}C NMR spectra show 28 carbons, including seven methyl singlets, one methoxys, two methylenes, seven methines [one aldehyde (δ_{H} 10.38; δ_{C} 208.6), one olefinic (δ_{H} 5.72; δ_{C} 119.9), three oxygenated (δ_{H} 4.66, 4.61 (2 $\times$ H); δ_{C} 67.5, 78.1, 92.4), and 11 nonprotonated carbons (six sp^2 at δ_{C} 202.4, 185.2, 170.1, 168.8, 135.3, 113.3). These signals were very similar to those of andrastin A [2] except for two additional hydroxyls at C-6 and C-7 positions. The assumption was evidenced by the COSY correlations of H-6 (δ_{H} 4.66, t, J = 2.7 Hz) to H-5 (δ_{H} 1.91, d, J = 2.7 Hz)/H-7 (δ_{H} 4.61, d, J = 2.4 Hz)/6-OH (δ_{H} 2.94, br s). Taking the molecular formula into consideration, 2 was deduced to bear a furan ring via the oxygen bridge between C-7 and C-15. On the basis of their small coupling constants, H-6 and H-7 were supposed to be equatorially β -oriented [30]. This was further confirmed by the NOESY correlations of H-5 to H-6/H₃-24 while H-23 to OH-6/H₃-22/H₃-25 (Fig. 3).

To assign the absolute configuration of 2, a theoretical calculation of the electronic circular dichroism (ECD) was performed. Briefly, the conformational analyses were carried out for (3S,5S,6S,7S,8S,9R,10R,13R,14R,16R)-2 (2a) and its enantiomer 2b via random searching in the Sybyl-X 2.0. Three lowest energy conformers were re-optimized at the B3LYP/6-31G(d) level. The theoretical ECD spectra were calculated using the GIAO method. As a result, 2a showed the same Cotton effects as the experimental one (Fig. 4). On the basis of above evidences, 2 was elucidated as 6 α -hydroxy-7 α ,15-epoxyandrastin A, and named andrastin G.

The molecular formula of compound 3 was assigned to be $\text{C}_{26}\text{H}_{38}\text{O}_6$ according to its sodium adduct at m/z 469.2566 $[\text{M} + \text{Na}]^+$, suggesting eight degrees of unsaturation. The ^1H and ^{13}C NMR spectra of 3 were almost the same as those of andrastone A except for the absence of an acetyl unit. By detailed analysis of the 1D and 2D NMR spectroscopic data, 3 was then determined as 3-O-deacetyl andrastone A, and named andrastone B.

Compound 4 had a molecular formula $\text{C}_{28}\text{H}_{40}\text{O}_9$ as established by its positive HRESIMS at m/z 543.2585 $[\text{M} + \text{Na}]^+$. It showed similar ^1H and ^{13}C NMR spectroscopic data to those of andrastone A except for two additional hydroxyls. This was evidenced by the replacement of one methylene and one methyl in andrastone A to an oxygenated methine (δ_{C} 67.6 d) and an oxygenated methylene (δ_{C} 62.3 t) in 4. In contrast to andrastone A, C-7 and C-10 in 4 was downshifted by 8.9 and 6.2 ppm. This indicated the attachment of the hydroxy group to C-6 and C-23, respectively. Further confirmation were found by the COSY correlations of H-6 (δ_{H} 4.47, dt, J = 2.6, 2.0 Hz) to H-5 (δ_{H} 1.67, d, J = 2.2 Hz)/H-7 (δ_{H} 3.02, dd, J = 14.9, 4.2 Hz; 2.47, dd, J = 14.7, 1.6 Hz) and the HMBC cross peaks of H₂-23 (δ_{H} 4.29, d, J = 12.4 Hz; 3.94, d, J = 12.4 Hz) to C-1 (δ_{C} 31.1 t)/C-5 (δ_{C} 50.6 d)/C-9 (δ_{C} 53.6 d)/C-10 (δ_{C} 42.8 s). The α -configuration of 6-OH was judged from the small coupling constant of H-5 and H-6 ($J_{\text{H-5,H-6}}$ = 2.4 Hz). Further by comparison of the calculated and experimental ECD spectra (Figure S2), 4 was then established to be 6 α ,23-dihydroxyandrastone A, and named andrastone C.

Compound 5 possessed a molecular formula $\text{C}_{28}\text{H}_{40}\text{O}_8$ from its HRESIMS at m/z 527.2619 $[\text{M} + \text{Na}]^+$. The ^1H and ^{13}C NMR spectra were similar to those of 4 except that the oxygenated methine of C-6 position in 4 was changed to a methylene in 5, indicating the absence of 6-OH in 5. Moreover, the significant downshift of C-16 and C-18 to 3.6 and 5.5 ppm indicated the configuration of 16-OH should be α in 5 instead of β in 4. Interestingly, such phenomena could also be found for other meroterpenoid enantiomers such as citreohybridione A and 16-*epi*-citreohybridione A. In the NOESY spectrum, cross peaks were

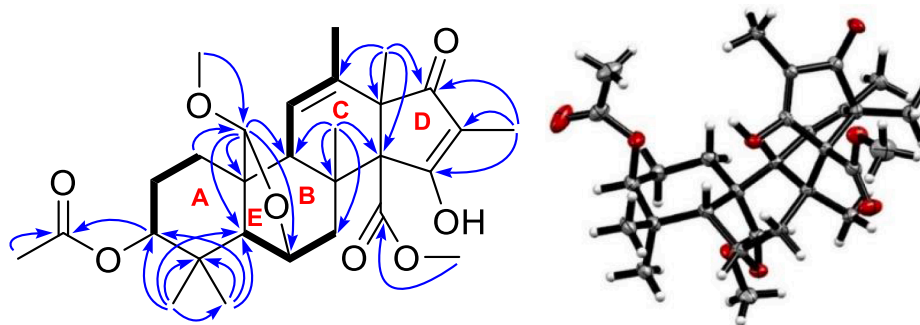


Fig. 2. Key COSY (bold) and HMBC (arrow) correlations of 1 (left) and single X-ray crystallography of 1 (right).

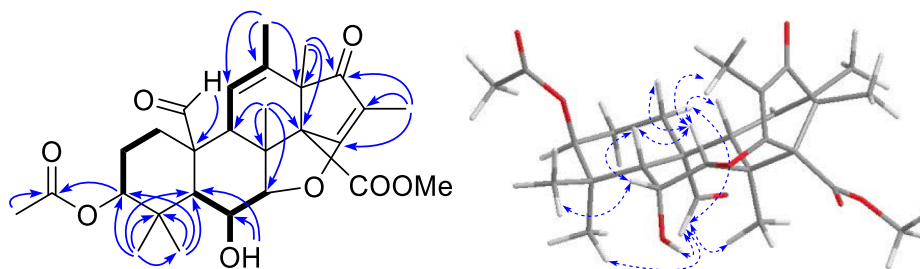


Fig. 3. The Key COSY (bold), HMBC (thin), and NOESY (dashed) correlations of 2.

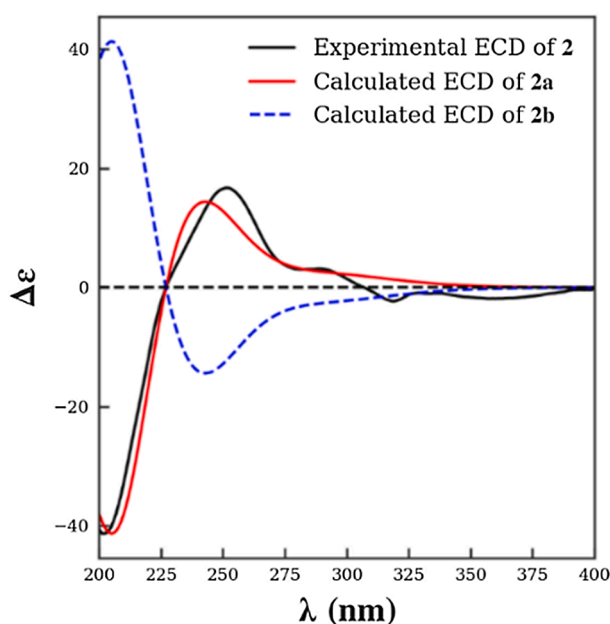


Fig. 4. Calculated and experimental ECD spectra of 2 in MeOH.

observed of H₃-22 to H₃-20/H₂-23 while H-9 to H₃-18, which further confirmed α -configuration of 16-OH. By comparison of its theoretical and experimental ECD spectra (Figure S2), 5 was thus established as 16-*epi*-23-hydroxyandrastone A, and named andrastone D.

Compound 6 presented the molecular formula of C₂₈H₃₈O₈ as evidenced by its positive HRESIMS spectrum at m/z 525.2468 [M + Na]⁺ (calcd for C₂₈H₃₈O₈Na, 525.2464). The ¹H and ¹³C NMR spectroscopic data were very similar to those of 5 except that an aldehyde group (δ_C 206.3 d) instead of a hydroxymethyl moiety (δ_C 62.7 t) was located at the C-23 position. This was confirmed by the HMBC correlation of H-23

(δ_H 10.20 s) to C-1 (δ_C 29.0 t) and C-10 (δ_C 53.4 s). Therefore, 6 was assigned as 16-*epi*-23-oxoandrastone A, and named andrastone E.

Compound 7 showed a sodium adduct molecule peak at m/z 511.2690 [M + Na]⁺ in its positive HRESIMS spectrum, corresponding to the molecular formula C₂₈H₄₀O₇. The ¹H and ¹³C NMR spectra were very close to those of 5 except that the oxygenated nonprotonated carbon (δ_C 75.8 s) at the C-16 position in 5 was displaced by a sp^3 methine (δ_C 50.6 d) in 7. Meanwhile, 18-Me was upshifted significantly by 15.8 ppm. This suggested the absence of 16-OH in 7. The assumption was supported by the significant upshift of 18-Me from δ_C 25.3 to δ_C 9.5. Further confirmation was obtained by the COSY correlation of H-16 (δ_H 3.23, quart, J = 6.8 Hz) to H₃-18 (δ_H 1.20, d, J = 6.8 Hz) and HMBC cross peaks of 18-Me to C-15 (δ_C 210.5 s)/C-16 (δ_C 50.6 d)/C-17 (δ_C 209.6 s). The α -configuration of the 18-Me was established by the NOESY correlations of H-16 to H-9 (δ_H 1.95 m), and H₃-3' (δ_H 3.56 s) to H₃-18/H₃-20 (δ_H 1.29 s). Further by comparison of its theoretical and experimental ECD spectra (Figure S2), the structure of 7 was then elucidated as 16-dehydroxy-23-hydroxyandrastone A, and named andrastone F.

Compounds 8 and 9 presented the molecular formula of C₂₈H₃₈O₇ and C₂₈H₄₀O₆ on the basis of their HRESIMS spectrum. They showed very similar ¹H and ¹³C NMR spectra with those of 7 except that the hydromethyl unit at C-23 position in 7 was changed to be an aldehyde group (δ_C 204.4 d; δ_H 10.12 s) in 8 and a methyl moiety (δ_C 16.9 q; δ_H 0.89 s) in 9. This was confirmed by the HMBC correlations of H-23 in 8 to C-1/C-10 and H₃-23 in 9 to C-1/C-5/C-9/C-10. By detailed analysis of their HSQC, COSY, HMBC, NOESY, and ECD spectra, 8 and 9 were then established as 16-dehydroxy-23-oxoandrastone A and 16-dehydroxyandrastone A, and were named andrastones G and H, respectively.

Noteworthy, compound 1 was the first natural andrastone with a hemiketal unit at C-23. Previously, a similar compound, dihydroxycitreohybridonol, was biosynthesized when a cytochrome P450 monooxygenase encoded by the cluster (CtrP450) was introduced to the production system of andrastin A [16]. Compound 2 possesses a novel tetrahydrofuran moiety via C-7 and C-15. While compounds 7–9 were the first three examples of andrastones with a doublet methyl (C-18) at the C-16 position.

3.2. Effects of compounds on degranulation and histamine release in IgE-sensitized RBL-2H3 cells

In recent years, the increasing incidence of food allergies made it a worldwide potentially life-threatening condition which, in some cases, could cause an anaphylactic shock to death [31]. Unfortunately, there are no effective approach in clinic, except in phase I clinical trial, the Chinese herbal formulation Food Allergy Herbal Formula-2 (FAHF-2) was shown to be safe and well-tolerated in food allergic individuals [32]. Currently, the only way to prevent food-allergic reactions is to strictly avoid the allergen. Therefore, it is very urgent to search for anti-allergic compounds.

As a proper *in vitro* model, the degranulation assay in IgE-mediated RBL-2H3 cells was commonly used for anti-allergic investigation [33,34]. As a result, andrastone B (**3**) and 3-deacetylцитеоhybridonol (**10**) showed potent degranulation-inhibitory activity with the IC₅₀ value of 40.4 μM and 14.8 μM, respectively (Table 3).

Therefore, **3** and **10** were further tested the histamine levels. As a result, compound **10** showed an apparent effect of inhibiting antigen-induced histamine release with the inhibition rates of 12.09%, 28.76% and 44.54% (Fig. 5).

3.3. Effects of compounds on TNF-α release in IgE-sensitized RBL-2H3 cells

Allergies are often accompanied by the production of large amounts of cytokines of TNF-α. Mast cells play an important role in initiating and perpetuating the inflammatory response in allergic reactions. Interestingly, compounds **3** and **10** could decrease the production of TNF-α in a dose-concentration dependence (Fig. 6).

3.4. Effects of compounds on intracellular Ca²⁺ concentration

The activation of mast cells is dependent on an increase in cytosolic Ca²⁺ concentration, which is associated with release from intracellular stores and influx of external Ca²⁺ through SOCs. The effect of **3** and **10** on intracellular calcium mobilization in RBL-2H3 cells were studied by monitoring intracellular Ca²⁺ fluorescence intensity. A23187 is a kind of mobile ion carrier. When A23187 is added to the cell culture medium containing calcium ion, calcium ion will enter the cytoplasm quickly. Therefore, it is widely used to increase the cytosolic free calcium concentration in cell biology research. As shown in Fig. 7, elevation of intracellular free Ca²⁺ concentration was also suppressed by compound **3** and **10** when RBL-2H3 cells were stimulated by A23187. And the effect

Table 3

Degranulation inhibitory effect of compounds 1–14 on RBL-2H3 cells (n = 3, means ± SD).

Compounds	Inhibition rate ^a (%)	IC ₅₀ (μM)
1	13.4	NT
2	23.1	NT
3	60.7	40.4 ± 13.2
4	12.5	NT
5	6.5	NT
6	7.8	NT
7	10.3	NT
8	14.2	NT
9	4.3	NT
10	77.7	14.8 ± 4.7
11	6.5	NT
12	4.8	NT
13	21.8	NT
14	35.6	NT
Loratadine	37.2	92.5 ± 3.5

NT, not tested.

Loratadine, a commercially available anti-allergic drug.

^a The work dose of all compounds was 50 μM.

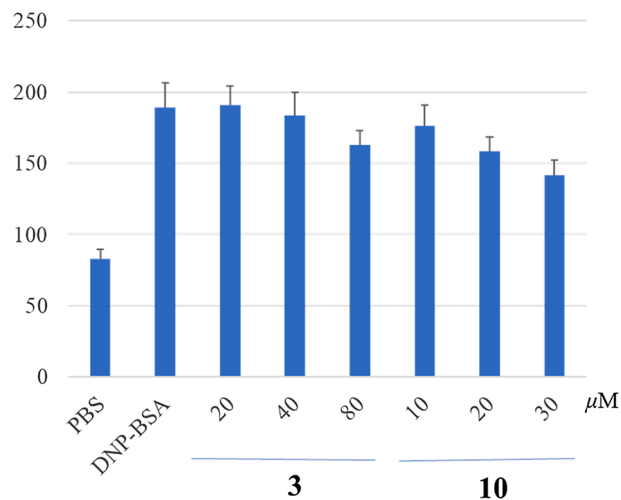


Fig. 5. Effects of compounds **3** and **10** on histamine release.

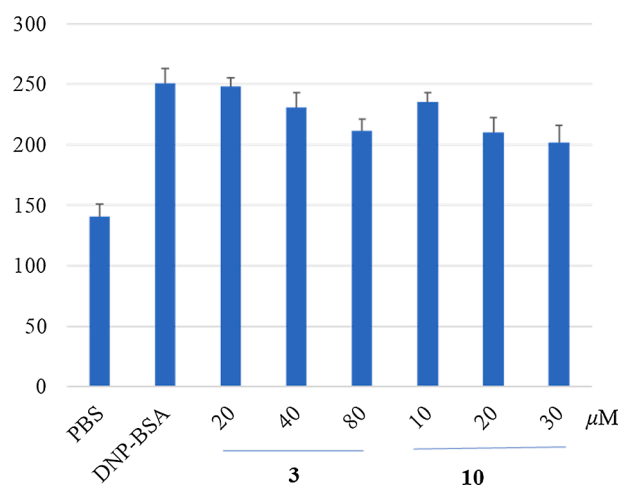


Fig. 6. Effects of compounds **3** and **10** on TNF-α release.

of **3** and **10** on the FcεRI-independent Ca²⁺ response was showed good concentration dependence, respectively.

4. Conclusions

Using the OSMAC strategy by changing the culture media from the rice to the oat, new andrastone meroterpenoids were produced. On the basis of their UV absorption characteristic spectrum, nine new (**1–9**) and five known andrastones (**10–14**) were isolated from the deep-sea-derived *Penicillium allii-sativi* MCCC 3A00580. Compound **1** has a hemiketal moiety at C-23. Compound **2** possesses a novel tetrahydrofuran moiety via C-7 and C-15. While compounds **7–9** were the first three examples of andrastones bearing a doublet methyl (C-18) at C-16. All isolates were tested for anti-allergic bioactivity. Andrastone B (**3**) and 3-deacetylцитеоhybridonol (**10**) significantly decreased degranulation with the IC₅₀ values of 40.4 μM and 14.8 μM, respectively. They could also decrease histamine release and the production of TNF-α in a dose-concentration dependence. In addition, the accumulation of Ca²⁺ in RBL-2H3 cells was found to be suppressed by **3** and **10**. Taken together, these findings indicated that andrastones may represent a novel drug lead for allergic diseases.

Fundings

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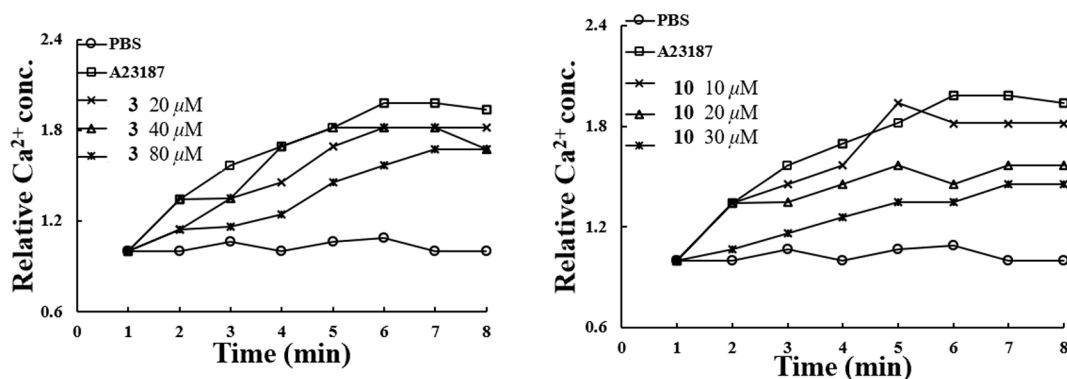


Fig. 7. Effects of compounds 3 and 10 on intracellular Ca^{2+} concentration.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2021.104671>.

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