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Bioactive compounds from *Rumex* plants

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ABSTRACT

Two new naphthalene acylglucosides, rumexneposides A (1) and B (2), together with 12 known compounds (3-14), were isolated from the roots of *Rumex nepalensis*. Their structures were established by chemical and spectroscopic methods. The biological activities of compounds 1-14 as well as an additional 11 compounds previously isolated from *R. nepalensis* and *Rumex hastatus* (15–25) were evaluated against *Mycobacterium tuberculosis*, *para*-aminobenzoic acid (*p*Aba) pathway, and a panel of human cancer cell lines. The results showed that compound 15 was the most active against *M. tuberculosis* with an MIC value of 2.85 μM similar to that of isoniazid. Compound 5 could inhibit *p*Aba synthetic pathway with an MIC value of 12.6 μM, comparable to that of positive control abyssomicin C, representing a new example of the rare *p*Aba pathway inhibitors.

1. Introduction

Rumex species are widely distributed in China. Among these, the roots of *Rumex nepalensis* Spreng. are traditionally used for the treatment of pain, inflammation, bleeding, tinea, tumor, and constipation in Chinese folk medicine (Li, 1998; Zhang et al., 2008), and the roots of *Rumex hastatus* are of medicinal significance for cough, headache, and fever (Editorial Commission of China, 1998). Previous studies revealed the presence of sterols in *R. nepalensis* and anthraquinones in *R. hastatus* (Khetwal et al., 1987; Tiwari and Sinha, 1980). During our search for bioactive metabolites from *Rumex* plants, we have reported several anthraquinones, naphthalenes, flavonoids and other phenolic compounds from *R. nepalensis* and *R. hastatus* (Mei et al., 2009; Zhang et al., 2009). Further study on the roots of *R. nepalensis* has resulted in the isolation of two new naphthalene acylglucosides, designated rumexneposides A (1) and B (2), together with 12 known compounds (3–14) (Fig. 1). Compounds 1–14 together with previously isolated compounds from *R. nepalensis* and *R. hastus* (15–25) (Mei et al., 2009; Zhang et al., 2009) were screened for a broad spectrum of activities, including the inhibition of *Mycobacterium tuberculosis* and *para*-aminobenzoic acid (*p*Aba) pathway, and against a panel of human cancer cell lines. In this paper, we describe the clarification of the structures from *R. nepalensis* and biological activities of compounds 1–25.

2. Results and discussion

The EtOH extracts of *R. nepalensis* roots were successively partitioned with petroleum ether, EtOAc, and H_2O. From the EtOAC-soluble fraction, two new (1 and 2) and 12 known compounds were isolated. The known compounds were identified as physcion (3) (Zhang et al., 2006), chrysophanol-8-*O*-β-D-glucopyranoside (4) (Yuan et al., 2000), torachrysone (5) (Demirezer et al., 2001), emodin-8-*O*-β-D-glucopyranoside (6) (Zhang et al., 2005), emodin-8-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (7) (Qi et al., 2005), chrysophanol (8) (Yan et al., 2001), emodin (9) (Zhang et al., 2005), citreorosein (10) (Xiang et al., 2001), resveratrol (11) (Sethi et al., 1980), nepodin-8-*O*-β-D-glucopyranoside (12) (Demirezer et al., 2001), torachrysone-8-*O*-β-D-glucopyranoside (13) (Lemli et al., 1981), and chrysophanol-8-*O*-β-D-(6′-*O*-acetyl)glucopyranoside (14) (Shi et al., 2001) based on comparisons with data in the literature.

Rumexneposide A (1) was obtained as a yellow powder and its molecular formula was deduced to be $C_{23}H_{26}O_9$ on the basis of its negative high-resolution fast atom bombardment mass spectros-

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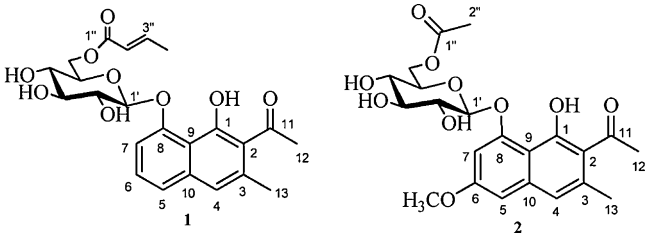

Fig. 1. Chemical structures of compounds 1–25.

copy (HRFABMS) at *m*/*z* 445.1506 ([M−H]$^-$, calcd for 445.1498). The infrared (IR) spectrum showed absorption bands representative of hydroxyl (3414 cm^{-1}) and carbonyl groups (1721 and 1698 cm^{-1}). The ^{1}H nuclear magnetic resonance (NMR) data (Table 1) exhibited three methyls (δ_H 2.48, s; 2.25, s; 1.94, d, J = 6.6 Hz), four aromatic protons (δ_H 6.97, s; 7.21, brd, J = 8.1 Hz; 7.03, dd, J = 8.1, 7.6 Hz; 6.70, brd, J = 7.6 Hz), and a sugar moiety (δ_H 2.71–4.46; 4.19, d, J = 7.2 Hz, H-1′). The ^{13}C NMR data (Table 1) were very similar to those of nepodin-8-*O*-β-D-glucopyrancoside (Demirezer et al., 2001). However, two additional olefinic carbons (δ_C 122.4, 145.4), one carbonyl (δ_C 166.2), and one methyl group (δ_C 18.1) were observed in the ^{13}C NMR spectrum of 1, which indicate the presence of a crotonoyl group in the molecule. The heteronuclear multiple bond correlations (HMBCs) of H-2″ and H-3″ with C-1″, and H-4″ with C-2″ and C-3″ confirmed the presence of such a moiety. The *trans* configurations of H-2″ and H-3″ were concluded from the *J* value (15.5 Hz) of H-2″. The HMBC of H-6′ with C-1″ indicated attachment of the *trans*-crotonoyl group to C-6′. Acid hydrolysis of 1 with 2% HCl–dioxane (1:1, v/v) produced D-glucose as the sugar residue, determined by gas chromatography (GC) analysis. Therefore, the structure of 1 was assigned as nepodin-8-*O*-β-D-(6′-*O*-*trans*-crotonoyl)glucopyranoside.

Rumexneposide B (2) was obtained as a yellow powder and its molecular formula was deduced to be $C_{22}H_{26}O_{10}$ by negative HRFABMS at *m*/*z* 449.1441 ([M−H]$^-$, calcd for 449.1447). The IR spectrum showed absorption bands attributable to hydroxyls (3422 cm^{-1}) and carbonyls (1734 and 1715 cm^{-1}). The ^{1}H and ^{3}C NMR data (Table 1) were very similar to those of 5, except that one additional acetyl signal (δ_C 171.5, 20.5) was observed in the ^{13}C NMR spectrum of 2. This was confirmed by the observed HMBC interaction of H-2″ with C-1″. The further HMBC of H-6′ with C-1″ indicates that the acetyl group is attached to C-6′. In the same manner as used for 1, acid hydrolysis of 2 with 2% HCl–dioxane (1:1, v/v) produced D-glucose as the sugar residue, determined by GC analysis. Therefore, the structure of 2 was assigned as torachrysone-8-*O*-β-D-(6′-*O*-acetyl)glucopyranoside.

Table 1
NMR data for compounds 1 (500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$) and 2 (400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$).

	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1		150.4 (s)		153.5 (s)
2		125.3 (s)		121.9 (s)
3		132.9 (s)		134.4 (s)
4	6.97 (s)	119.8 (d)	6.92 (s)	119.3 (d)
5	7.21 (brd, 8.1)	122.8 (d)	6.63 (d, 2.2)	100.8 (d)
6	7.03 (dd, 8.1, 7.6)	127.8 (d)		158.8 (s)
7	6.70 (brd, 7.6)	111.4 (d)	6.82 (d, 2.2)	103.7 (d)
8		154.8 (s)		155.8 (s)
9		113.5 (s)		109.2 (s)
10		136.1 (s)		137.6 (s)
OH-1	9.70 (s)			
COMe-2		208.9 (s)		206.6 (s)
COMe-2	2.48 (s)	32.5 (q)	2.55 (s)	32.2 (q)
Me-3	2.25 (s)	19.4 (q)	2.30 (s)	20.5 (q)
OMe-6			3.80 (s)	55.2 (d)
1′	4.19 (d, 7.2)	103.3 (d)	4.90 (d, 7.9)	102.6 (d)
2′	3.70 (m)	73.6 (d)	3.62 (m)	73.0 (d)
3′	3.45 (m)	76.0 (d)	3.45 (m)	76.4 (d)
4′	3.53 (m)	69.9 (d)	3.37 (m)	69.8 (d)
5′	2.71 (m)	74.6 (d)	3.65 (m)	74.3 (d)
6′	4.46 (brd, 11.0)	62.8 (t)	4.43 (dd, 12.0, 2.0)	63.4 (t)
	4.27 (dd, 11.0, 6.5)		4.25 (dd, 12.0, 7.2)	
1″		166.2 (s)		171.5 (s)
2″	5.93 (brd, 15.5)	122.4 (d)	2.09 (s)	20.5 (q)
3″	7.05 (overlap)	145.4 (d)		
4″	1.94 (d, 6.6)	18.1 (q)		

Notably, compounds 1, 2, 7, and 14 all have an acyl group on the sugar moiety, which may be characteristic of the compounds from *R. nepalensis*. Compounds 7, 10, 11, 13, and 14 were also isolated from this plant for the first time.

Considering the traditional medical uses of *Rumex* plants, compounds 1–25 were examined for their inhibitory effects on infection- and tumor-associated targets, such as *M. tuberculosis*, *p*Aba biosynthetic pathway, and a panel of human cancer cell lines.

In an anti-tuberculosis assay, the results showed that compound 1, 5, and 12–16 exhibited potent inhibitory activity towards *M. tuberculosis*, with minimum inhibitory concentration (MIC) values of 20.7, 6.1, 26.6, 8.9, 4.1, 2.85, and 10.2 μM, respectively, when isoniazid was used as the positive control (MIC: 2.04 μM), whereas the other compounds showed no activity (MIC > 40 μM) (data not shown).

The *p*Aba pathway exists in plants, algae, bacteria, fungi, and parasites, but not in mammals. Therefore, this pathway is considered to be an attractive target for the development of new chemotherapeutic agents (McConkey, 1999; Roberts et al., 1998). However, until now, only abyssomicin C, a marine natural product, has been reported to inhibit *p*Aba biosynthesis (Riedlinger et al., 2004). Because the roots of *R. nepalensis* are renowned for the treatment of tinea, the metabolites isolated from this material together with previously isolated compounds (Mei et al., 2009) were screened for their effects on *p*Aba biosynthesis. Our results show that 5 has significant inhibitory activity on the *p*Aba pathway, with an MIC value of 12.6 μM (3.1 μg/mL), similar to that of abyssomicin C (the MIC value of abyssomicin C in MP medium is 8.3 μM, used as a positive control in this study). Other compounds, including analogues of 5, showed no inhibitory effects at concentrations of up to 30 μg/mL (data not shown). Like abyssomicin C, this molecule represents another *p*Aba biosynthesis pathway inhibitor.

A cytotoxic assay of all the compounds was performed against five cancer cell lines (A549, H522, MCF-7, MCF-10A, and SKBR3) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, with cisplatin as the positive control. Compounds 1, 11, 14, and 17 showed different cytotoxicities towards the five cancer cell lines (Table 2), but the other compounds showed no cytotoxic effects at concentrations of up to 40 μM. Compound 1, in particular, showed activity against a broad spectrum of human cancer cells, including lung and breast cancer cells. The cytotoxicity of 14 was cell-type-dependent, and of the five cell lines tested, only MCF-10A cancer cells responded to 14.

In this study, we isolated and characterized 2 new naphthalene acylglucosides and 12 known compounds from *R. nepalensis* roots. We evaluated the biological activities of 25 compounds, including 11 previously reported compounds from *R. nepalensis* and *R. hastatus*, against *M. tuberculosis*, *p*Aba pathway, and 5 human cancer cell lines. Several compounds showed good activity against *M. tuberculosis*, of which compound 15 was the most active, with an MIC similar to that of isoniazid. Compound 5 was a potent *p*Aba synthetic pathway inhibitor, representing another example of the rare *p*Aba pathway inhibitors. Although most compounds showed

Table 2
Cytotoxic activities of the compounds (IC_{50} μM)[a].

Cell line	A549[b]	H522[b]	MCF-7[c]	MCF-10A[c]	SKBR3[c]
1	31.0	15.7	21.8	22.8	20.7
2	>40	>40	>40	>40	>40
3	>40	>40	>40	>40	>40
4	>40	>40	>40	>40	>40
5	>40	>40	>40	>40	>40
6	>40	>40	>40	>40	>40
7	>40	>40	>40	>40	>40
8	>40	>40	>40	>40	>40
9	>40	>40	>40	>40	>40
10	>40	>40	>40	>40	>40
11	27.8	>40	29.4	12.3	>40
12	>40	>40	>40	>40	>40
13	>40	>40	>40	>40	>40
14	>40	>40	>40	9.6	>40
15	>40	>40	>40	>40	>40
16	>40	>40	>40	>40	>40
17	29.0	38.7	>40	7.6	19.9
18	>40	>40	>40	>40	>40
19	>40	>40	>40	>40	>40
20	>40	>40	>40	>40	>40
21	>40	>40	>40	>40	>40
22	>40	>40	>40	>40	>40
23	>40	>40	>40	>40	>40
24	>40	>40	>40	>40	>40
25	>40	>40	>40	>40	>40
Cisplatin[d]	17.5	20.4	85.1	77	67

[a] Previously isolates: aloesin (**15**), (−)-epicatechin-3-*O*-gallate (**16**), orientaloside (**17**), hastatuside A (**18**), orcinol glucoside (**19**), lyoniresinol 3α-*O*-β-D-glucopyranoside (**20**), (3,5-dimethoxy-4-hydroxyphenol)-1-*O*-β-D-(6-*O*-galloyl)-glucose (**21**), (−)-epicatechin (**22**), nepalensides A (**23**) and B (**24**), and hastatuside B (**25**).
[b] Lung cancer cells.
[c] Breast cancer cells.
[d] Positive control.

no cytotoxicity against several human cancer cells, compound 1 exhibited broad activity against lung and breast cancer cells. MCF-10A was sensitive to compounds 1, 11, 14, and 17.

3. Experimental

3.1. General experimental procedures

Melting points were determined with an XRC-1 micromelting apparatus. Optical rotations were determined with a JASCO-20C digital polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained with a Bruker Tensor 27 FT-IR spectrophotometer with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or a DRX-500 spectrometer. Electron impact mass spectrometry (EIMS) (70 eV) and FABMS were recorded on a VG Auto Spec-3000 spectrometer. The silica gel (200–300 mesh and 1–40 μm) for column chromatography (CC) and GF_{254} for thin-layer chromatography (TLC) were obtained from the Qingdao Marine Chemical Factory, Qingdao, China. Sephadex LH-20 was obtained from Amersham Pharmacia Biotech, Sweden. The fractions were monitored by TLC and the spots were visualized after spraying with 10% NaOH in EtOH or anisaldehyde reagents, followed by heating.

3.2. Plant material

R. nepalensis roots were collected in the suburb of Kunming, Yunnan Province, China, in July 2006. The voucher specimen (no. CHYX0183) authenticated by Prof. Yun-Heng Ji in our institution was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried roots of *R. nepalensis* (15 kg, powdered) were extracted with 95% EtOH (3 × 30 L) under reflux and concentrated *in vacuo* to produce a crude extract, which was suspended in water and then successively partitioned with petroleum ether (3 × 3 L) and EtOAc (3 × 3 L). The EtOAC extract (488 g) was subjected to CC over silica gel (200–300 mesh, 5 kg) and eluted with a gradient of $CHCl_3$–MeOH (100:0, 98:2, 96:4, 94:6, 90:10, 85:15, 80:20, 70:30) to produce fractions A–G. Fraction C (26 g) was subjected to silica gel CC eluted with a gradient of $CHCl_3$–Me_2CO (4:1, 3:1, 2:1, 1:1) to produce three fractions (fr. C.1–3). Fraction C.2 was subjected to Sephadex LH-20 (MeOH) chromatography and repeated vacuum liquid chromatography (VLC) over silica gel to yield 1 (31 mg) and 13 (187 mg). Fraction D (22 g) was subjected to silica gel CC with a gradient of $CHCl_3$–Me_2CO (150:1, 120:1, 100:1, 80:1, 40:1, 20:1), followed by gel filtration on Sephadex LH-20 (MeOH), MCI Gel® CHP20P chromatography (MeOH–H_2O, 9:1), and repeated VLC (silica gel) to produce 2 (32 mg), 3 (17 mg), 4 (104 mg), 8 (34 mg), and 9 (75 mg). Fraction E (29 g) was fractionated by MCI Gel® CHP20P chromatography, eluted with a gradient of MeOH–H_2O (3:7, 5:5, 7:3, 1:0), followed by Sephadex LH-20 (MeOH) filtration, to produce 7 (20 mg), 10 (35 mg), 11 (76 mg), 12 (41 mg), and 14 (76 mg). The combined chromatography of fraction F by silica gel CC ($CHCl_3$–MeOH, 100:0, 80:1, 60:1, 40:1, 20:1) and Sephadex LH-20 (MeOH) chromatography yielded 5 (136 mg). Compound 6 (21 mg) was purified from fraction G (23 g) by silica gel CC ($CHCl_3$–MeOH, 40:1, 30:1, 15:1, 10:1, 6:1, 4:1, 2:1) and gel filtration over Sephadex LH-20 (MeOH).

3.4. Nepalenside A (1)

Yellow powder, mp 67–68 °C; $[\alpha]_D^{21.6}$ −45.1 (*c* 0.96, MeOH); UV λ_{max} ($CHCl_3$) nm (log ε): 334 (3.54), 299 (3.57); IR (KBr) ν_{max} cm^{-1}: 3414, 1721, 1698, 1631, 1578, 1078; ^1H and ^{13}C NMR data, see Table 1; FABMS (negative) *m*/*z*: 445 $[M-H]^-$; HRFABMS (negative) *m*/*z*: 445.1506 $[M-H]^-$ ($C_{23}H_{25}O_9$, calcd for 445.1498).

3.5. Nepalenside B (2)

Yellow powder, mp 151–153 °C; $[\alpha]_D^{22.1}$ −75.6 (*c* 0.97, MeOH); UV λ_{max} ($CHCl_3$) nm (log ε): 323 (3.62), 262 (4.18); IR (KBr) ν_{max} cm^{-1}: 3422, 1734, 1715, 1627, 1585, 1077; ^1H and ^{13}C NMR data, see Table 1; FABMS (negative) *m*/*z*: 449 $[M-H]^-$; HRFABMS (negative) *m*/*z*: 449.1441 $[M-H]^-$ ($C_{22}H_{25}O_{10}$, calcd for 449.1447).

3.6. Acid hydrolysis of 1 or 2

Compound 1 or 2 (5 mg each) was refluxed with 2% HCl–dioxane (1:1, v/v, 4 mL) in a water bath (80 °C) for 6 h. The reaction mixture was then evaporated to dryness, and partitioned between $CHCl_3$ and H_2O. The aqueous layer was neutralized with Amberlite IRA-401 resin (OH^- form). The filtrate was evaporated to dryness. The sugar residue was diluted in 2 mL of pyridine and treated with 1.5 mL of L-cysteine methyl ester hydrochloride at 60 °C for 1 h, and then treated with 1.5 mL of trimethylsilylimidazole at 60 °C for 30 min. The supernatant was analyzed by GC under the conditions: 30QC/AC-5 (30 m × 0.32 mm i.d.); detector, FID (250 °C); column temperature, 180–280 °C, rate, 3 °C/min; t_R (s), 19.450 (standard D-glucose), 19.856 (standard L-glucose), 19.402 (D-glucose from 1), or 19.412 (D-glucose from 2).

3.7. Assay for anti-M. tuberculosis (H37Rv) in its logarithmic growth phase

M. tuberculosis (MTB) assays were carried using a constitutive green fluorescent protein (GFP) expression vector (pUV3583c–GFP) with the direct fluorescence readout as a measure of bacterial growth (Chan et al., 2002). The production of GFP by replicating cells as a marker of live cells has been shown to result in the same MIC as that measured by the direct enumeration of colony-forming units (cfu) for all front-line antituberculosis agents (Changsen et al., 2003). Briefly, MTB was grown in 50 mL of 7H9 broth (Difco) containing 0.05% Tween 80 and 25 μg/mL kanamycin. The cultures were incubated at 37 °C with rotary agitation, grown to mid-exponential phase (optical density at 595 nm [OD_{595}] of approximately 0.6–0.8), and harvested by centrifugation. The cell pellets were resuspended in a small amount of enriched 7H9 medium. The starter culture was grown to an OD_{595} of 0.3–0.5 and then diluted with 7H9 medium to an OD_{595} of approximately 0.05 (1.5×10^4 cells). The compounds were prepared at 100 × stocks in dimethyl sulfoxide (DMSO). Six serial dilutions of the compounds were prepared in the same solvent and added to the wells in a 2 μL volume. Isoniazid (purchased from Sigma) was used as the positive control for the MTB assay. Wells containing only the carrier solvent were the negative controls. Each well received 80 μL of the diluted bacterial culture. The plate was incubated at 37 °C with gentle agitation, and the OD_{600} and fluorescence were read at intervals of 24 h for three days using an EnVision 2103 multilabel reader (PerkinElmer, USA) with excitation at 485 nm and emission at 508 nm.

3.8. Assay for the inhibition of the biosynthetic pathway from chorismate to pAba

Inhibition was determined according to the previously described method (Riedlinger et al., 2004), with some modification. *Bacillus subtilis* ATCC 66333 was grown on an agar plate on minimal medium (MM) consisting of glucose 0.5%, tri-sodium citrate·$2H_2O$ 0.05%, KH_2PO_4 0.3%, K_2HPO_4 0.7%, $MgSO_4{\cdot}7H_2O$ 0.01%, and $(NH_4)_2SO_4$ 0.1% in deionized water. The *B. subtilis* strain was also grown in MP medium (MM medium supplemented with 5 mM *p*Aba). Aliquots (2 μL) of the compounds dissolved in DMSO were transferred to flat-bottomed, 96-well microtiter plates (Greiner, Germany), to which were added 80 μL of solutions diluted with MM or MP medium to a final concentration of 1×10^5 cfu/mL. The assay plates were incubated overnight in a 37 °C incubator. The MIC was determined with a broth microdilution protocol. The OD of the overnight culture was determined at 595 nm using an EnVision 2103 multilabel reader (PerkinElmer). Abyssomicin C (a kind gift from Prof. Michael Goodfellow and purified by high-performance liquid chromatography) was used as the positive control. In an assay for the inhibition of *p*Aba biosynthesis, the MIC value of abyssomicin C in MP medium was 8.3 μM. Active compounds showed selective antibacterial activity against the test organism grown on MM medium, but no activity was observed when the test organism was grown on MP medium.

3.9. Cytotoxicity assay

The cytotoxicity of the compounds for A549 (human non-small-cell lung cancer), SKBR3, MCF-10A, MCF-7, and H522 cells (non-small-cell lung carcinoma) was measured. The cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated newborn calf serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) in a humidified incubator under 5% CO_2 at 37 °C. The cells were seeded in 96-well microplates ($1–2 \times 10^4$ cells/well) and cultured for 12 h. The medium was replaced with one containing the test compounds, and the cells were further cultured at 37 °C. To evaluate the IC_{50} of all the compounds, the cells were exposed to the test compounds at various concentrations. After incubation for 48 h, 10 μL of MTT solution (Sigma) was added to each well and the cells were incubated under the same conditions for 4 h until a purple precipitate was visible. DMSO (200 μL) was added and the optical density was measured at 570 nm in a microplate reader (Bio-Tek Synergy HT). Cisplatin and DMSO were used as the positive and negative controls, respectively.

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