

Article

Five New Phenolic Compounds with Antioxidant Activities from the Medicinal Insect *Blaps rynchopetera*

Huai Xiao^{1,2}, Tian-Peng Yin^{1,3}, Jian-Wei Dong¹, Xiu-Mei Wu², Qing Luo², Jian-Rong Luo², Le Cai¹ and Zhong-Tao Ding^{1,*}

¹ Functional Molecules Analysis and Biotransformation Key Laboratory of Universities in Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming 650091, China; Xiaohuai10@263.net (H.X.); tpyin89@163.com (T.-P.Y.); jwdongyn@outlook.com (J.-W.D.); caile@ynu.edu.cn (L.C.)

² Yunnan Provincial Key Laboratory of Entomological Biopharmaceutical R&D, Dali University, Dali 671000, China; wxm6865@163.com (X.-M.W.); lq690275304@163.com (Q.L.); ljrrong@vip.sina.com (J.-R.L.)

³ Zhuhai Key Laboratory of Fundamental and Applied Research in Traditional Chinese Medicine, Zunyi Medical University Zhuhai Campus, Zhuhai 519041, China

* Correspondence: ztding@ynu.edu.cn; Tel.: +86-0871-6503-2667

Received: 9 July 2017; Accepted: 31 July 2017; Published: 4 August 2017

Abstract: Five new phenolic compounds rynchopeterines A–E (1–5), in addition to thirteen known phenolics, were isolated from *Blaps rynchopetera* Fairmaire, a kind of medicinal insect utilized by the Yi Nationality in Yunnan Province of China. Their structures were established on the basis of extensive spectroscopic analyses (1D and 2D NMR, HR-MS, IR) along with calculated electronic circular dichroism method. Rynchopeterines A–E (1–4) exhibited significant antioxidant activities with IC₅₀ values of 7.67–12.3 µg/mL measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Besides, rynchopeterines B (2) and C (3) showed mild cytotoxicity against tumor cell Caco-2 and A549.

Keywords: *Blaps rynchopetera*; chemical constituents; phenolic; rynchopeterines A–E; antioxidant; anti-tumor

1. Introduction

Insects have long been utilized as medicines in the world, especially in China. Insects make up the largest and most diverse group of organisms on earth, and could serve as a vast potential medicinal resource. However, in contrast to efforts exploring the peptide and protein components present in insects, much less attention has been given to the insect derived small natural products and their biological properties. The potential roles that insect derived substances might play as lead compounds in drug discovery efforts has been largely ignored [1,2]. Fortunately, certain medicinal insects have drawn the scientists' attention because of their beneficial health effects on humans. It was reported that a huge variety of chemical substances with potent biological activities which might have use as lead compounds in drug discovery have been isolated from insects in recent years [3].

Blaps rynchopetera Fairmaire belongs to the family Tenebrionidae (Coleoptera), and is a kind of insect that has long been utilized as a folk medicine by the Yi Nationality in Yunnan Province of China. This insect is effective in the treatment of cough, gastritis, and various cancers [4]. Our previous studies have revealed that dipeptides and phenolics—which exhibited various pharmacological activities—are the main active ingredients of this drug [5]. As part of our continuous work on the systematic study of this insect, the chemical constituents of *B. rynchopetera* was investigated to afford five new phenolic compounds rynchopeterines A–E (1–5) (Figure 1), along with thirteen known

phenolics, procatechuic acid (6) [6], 3,4-dihydroxyphenylacetic acid (7) [7], 3,4-dihydroxybenzaldehyde (8) [8], 3,4-dihydroxyphenylacetaldehyde (9) [9], methyl 3,4-dihydroxybenzoate (10) [10], 3,4-dihydroxyphenylacetic acid methyl ester (11) [11], 3,4-dihydroxyphenylacetic acid ethyl ester (12) [7], 2-(3,4-dihydroxyphenyl)ethanol (13) [12], 3,4-dihydroxyphenethylacetate (14) [13], blapsins A (15) and B (16) [14], 1-(3,4-dimethoxyphenyl)-4-hydroxypentan-1-one (17) [15], and 3,3',4,4'-tetrahydroxybiphenyl (18) [16] (see Supplementary). Herein, the isolation, structural elucidation, and bioactivity of the new compounds are described.

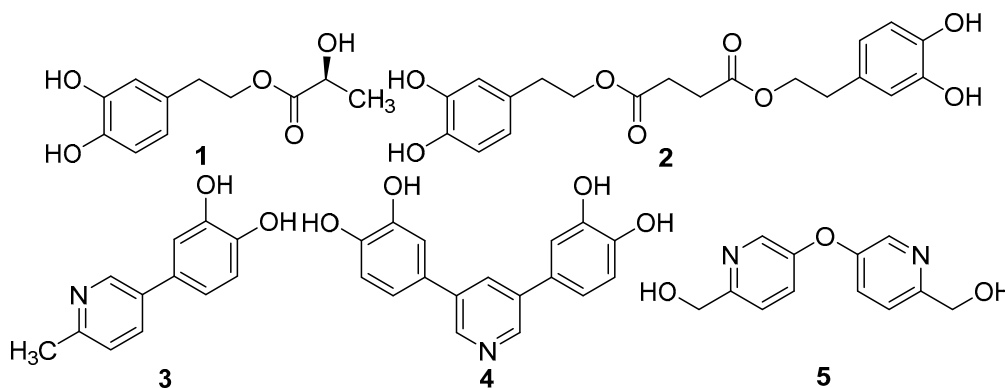


Figure 1. Structures of compounds 1–5.

2. Results

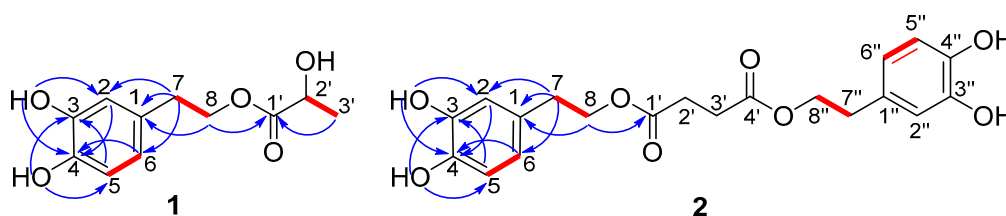
2.1. Structural Elucidation of Compounds 1–5

Compound **1** was isolated as a light yellow liquid, and its molecular formula was deduced to be $C_{11}H_{14}O_5$ with an unsaturation degree of five by HR-ESIMS at m/z : 249.0738 $[M + Na]^+$. The NMR spectra of **1** showed the presence of a 1,3,4-trisubstituted aromatic ring [δ_H 6.76 d (2.0), 6.76 d (8.0), 6.59 dd (8.0, 2.0); δ_C 129.5 s, 116.0 d, 145.0 s, 143.7 s, 115.2 d, 120.2 d], two phenolic hydroxyl groups (δ_H 7.77 brs, 7.80 brs), and two methines [δ_H 2.80 t (7.0), 4.21, 4.30 dt (7.0, 11.6); δ_C 34.2 t, 65.3 t] (Table 1), which were assigned to be a 3-hydroxytyrosol group [17]. The substituent position of the aromatic ring was further confirmed by HMBC correlations from OH-3 to C-3, OH-4 to C-4, and H-8, H-7 to C-1 (Figure 2). Besides, a lactic acid group [δ_H 4.23 q (7.0), 1.31 d (7.0); δ_C 19.9 q, 66.6 d, 174.7 s) was identified in its NMR spectra [18], which was connected to C-8 in 3-hydroxytyrosol group according to HMBC correlation from H-8 to C-1'. The absolute configuration of C-2' was assigned to be *S* by the comparison of the theoretical electronic circular dichroism (ECD) data with the experimental spectrum (Figure 2). Two geometries were previously optimized by density functional theory (DFT) method at the B3LYP/6-31G(d) level [19]. Excitation energies and rotational strengths were calculated using time-dependent density functional theory (TDDFT) at the B3LYP/6-31G(d,p) level in acetonitrile with PCM model [20]. The ECD spectrum was simulated from electronic excitation energies and velocity rotational strengths. The results showed that the theoretical ECD data for 2'*S*-isomer was in good agreement with the experimental spectrum. Therefore, the structure of compound **1** was determined as rynchopeterine A, with its assigned NMR data listed in Table 1.

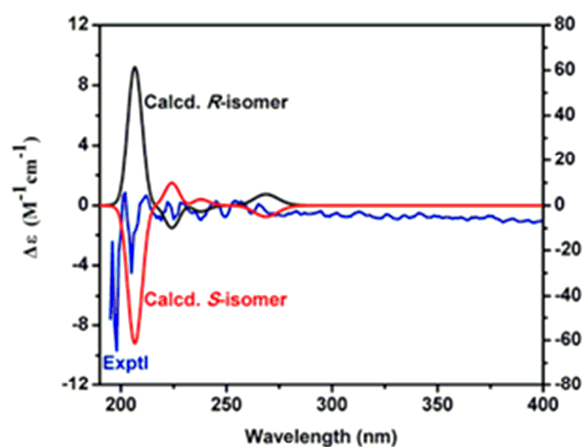
Compound **2** was isolated as light brown liquid, whose molecular formula was deduced to be $C_{20}H_{22}O_8$ with an unsaturation degree of ten by HR-ESIMS at m/z : 413.1192 $[M + Na]^+$. In combination with the MS data, two 3-hydroxytyrosol groups and an amber acid group (δ_H 2.59, s, 4H; δ_C 28.5 t, 171.8 s) were identified in its NMR spectra (Table 1) [21]. The connection of those three fragments was accomplished by the HMBC correlations from H-8 to C-1', and from H-8'' to C-5' (Figure 1). Hence, the structure of compound **2** was determined, and its assigned NMR data are listed in Table 1.

Table 1. NMR spectral data of compounds **1** (CD₃COCD₃) and **2** (CD₃COCD₃:DMSO-*d*₆ = 5:1).

No.	1		No.	2	
	δ_H (J in Hz)	δ_C		δ_H (J in Hz)	δ_C
1		129.5 s	1 (1'')		129.6 s
2	6.76 (d, 2.0)	116.0 d	2 (2'')	6.76 (2H, d, 1.6)	116.0 d
3		145.0 s	3 (3'')		145.0 s
4		143.7 s	4 (4'')		143.7 s
5	6.76 (d, 8.0)	115.2 d	5 (5'')	6.76 (2H, d, 8.0)	115.3 d
6	6.59 (dd, 8.0, 2.0)	120.2 d	6 (6'')	6.59 (2H, dd, 8.0, 1.6)	120.2 d
7	2.80 (2H, t, 7.0)	34.2 t	7 (7'')	2.78 (4H, t, 7.0)	34.2 t
8	4.21 (dt, 7.0, 11.6)	65.3 t	8 (8'')	4.20 (4H, t, 7.0)	65.2 t
	4.30 (dt, 7.0, 11.6)		1' (4')		171.8 s
1'		174.7 s	2' (3')	2.59 (4H, s)	28.5 t
2'	4.23 (q, 7.0)	66.6 d			
3'	1.31 (3H, d, 7.0)	19.9 q			
OH-3	7.80 (brs)				
OH-4	7.77 (brs)				

**Figure 2.** Key ¹H-¹H COSY (—) and HMBC (—) correlations of compounds **1** and **2**.

Compound **3** was isolated as a white powder, whose molecular formula was deduced to be C₁₂H₁₁NO₂ with an unsaturation degree of six by HR-ESIMS at *m/z*: 202.0859 [M + H]⁺. The NMR spectra of **3** displayed signals of a pyrocatechol group [22], a methyl group (δ_H 2.49 s; δ_C 23.2 t) (Table 2), and a 1,4-disubstituted pyridine ring. The pyrocatechol group was connected to C-5 in the pyridine ring according to HMBC correlation from H-4, H-6 to C-1', and H-2', H-6' to C-5. The methyl group was placed at C-2 on the basis of HMBC correlation from H-7 to C-2 (Figure 3). Therefore, the structure of compound **3** was determined as rynchopeterine C, whose assigned NMR data were listed in Table 2.

**Figure 3.** Calculated and experimental electronic circular dichroism (ECD) spectra of compound **1**.

Compound **4** was isolated as a light yellow powder, and its molecular formula was deduced to be C₁₇H₁₃NO₄ with an unsaturation degree of twelve by HR-ESIMS at *m/z*: 296.0920 [M + H]⁺. Full

analysis of the MS and NMR spectra of **4** demonstrated that it is composed of two pyrocatechol groups and a 3,5-disubstituted pyridine ring. The connection of those three fragments was established by the HMBC correlations from H-2' and H-6' to C-5, and H-4 and H-6 to C-1' (Figure 4). Hence, the structure of compound **4** was determined, with its assigned NMR data listed in Table 2.

Compound **5** was isolated as a white powder, whose molecular formula was deduced to be $C_{12}H_{12}N_2O_3$ with an unsaturation degree of eight by HR-ESIMS at m/z : 233.0920 $[M + H]^+$. The NMR spectra of **5** displayed signals of a 2,5-disubstituted pyridine ring, an oxygenated methylene. The oxygenated methylene was placed at C-2 on the basis of HMBC correlation from H-7 to C-2 (Figure 4). In combination with the comparison of the NMR data of **5** with those of 5-hydroxy-2-pyridinemethanol, the MS data indicated that **5** is composed of two identical fragments. Thus, the structure of compound **5** was determined, with its assigned NMR data listed in Table 2.

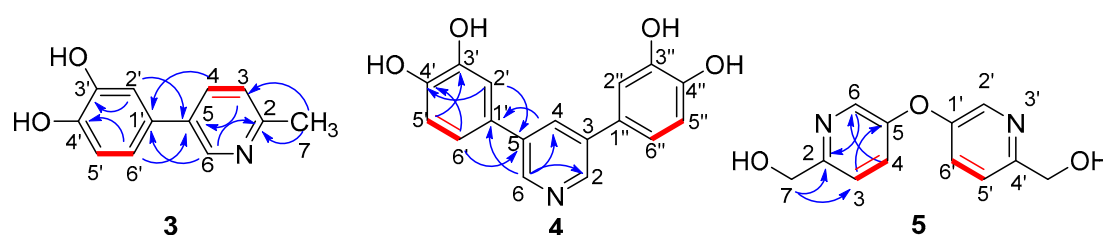


Figure 4. Key 1H - 1H COSY (—) and HMBC (—) correlations of compounds 3–5.

Table 2. NMR spectral data of compounds 3–5 (CD_3OD).

No.	3		No.	4		No.	5	
	δ_H (f in Hz)	δ_C		δ_H (f in Hz)	δ_C		δ_H (f in Hz)	δ_C
1		156.0 s	1		144.2 d	1 (3')		152.1 s
2		123.2 d	2, 6	8.59 (brs)	137.3 s	2 (4')		120.9 d
3	7.24 (d, 8.0)	123.2 d	3, 5		131.9 d	3 (5')	7.34 (d, 8.4)	122.9 d
4	7.80 (dd, 8.0, 2.2)	133.7 d	4	8.06 (t, 2.0)	128.9 s	4 (6')	7.25 (dd, 8.4, 2.8)	152.5 s
5		133.6 s	1' (1'')		113.6 d	5 (1')		136.3 d
6	8.64 (d, 2.2)	146.7 d	2' (2'')	7.14 (d, 2.2)	145.7 s	6 (2')	8.15 (d, 2.8)	64.1 t
7	2.49 (3H, s)	23.2 q	3' (3'')		145.9 s	7 (7')	4.61 (s)	
1'		129.3 s	4' (4'')		115.7 d			
2'	7.11 (d, 2.2)	113.9 d	5' (5'')	6.92 (d, 8.2)	118.4 d			
3'		146.1 s	6' (6'')	7.06 (dd, 8.2, 2.2)				
4'		145.8 s						
5'	6.90 (d, 8.1)	116.1 d						
6'	6.97 (dd, 8.1, 2.2)	117.9 d						

2.2. Antioxidant and Anti-Tumor Activities

Our previous studies have found that the ethyl acetate fraction of *B. rynchopetera* exhibited strong 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, with an IC_{50} value of 23.35 $\mu g/mL$, while the IC_{50} values of petroleum ether (PE) and n-butyl alcohol fractions were 673.0 and 620.3 $\mu g/mL$, respectively. The results indicated that the ethyl acetate fraction is abundant in antioxidant ingredients, which inspired us to measure the anti-radical activity of the isolated compounds. As shown in Table 3, some of the isolated compounds showed impressive anti-radical activity. Especially, compound **7** exhibited significant anti-radical activity with an IC_{50} value of 1.02 $\mu g/mL$. The results revealed that new compounds **1–4** were nearly equally effective as the positive controls vitamin C and rutin regarding DPPH radical-scavenging activity. Their effective anti-radical activities could be attributed to the hydroxyl group in the molecules [23].

The cytotoxicity of compounds **1–5** was tested in vitro against ten common human tumor cell lines A549, AGS, Caco-2, HeLa, HepG2, K562, MDA-MB-231, TE-1, U251, and Bel-7402 using the modified 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) method. The results showed that compound **2** exhibited anti-tumor activity against Caco-2 cell and A549 cells with IC_{50} values of 119.7 $\mu g/mL$ and 108.5 $\mu g/mL$, respectively. Compound **3** exhibited mild anti-tumor activity against

Caco-2 cells with an IC₅₀ value of 158.7 µg/mL, while the positive control cisplatin generated IC₅₀ values of 6.2 µg/mL against Caco-2 cells.

Phenolics have been reported to exhibit various physiological effects in humans, including inhibiting platelet aggregation, reducing the risk of coronary heart disease and cancer, and preventing oxidative damage to lipids and low-density lipoproteins; these effects are generally associated with their antioxidant abilities [24]. The antioxidant phenolics in *B. rynchopetera* might be responsible for its therapeutic effect. *B. rynchopetera* are utilized to treat various cancer by the natives; the isolated compounds **2** and **3** showed mild anti-tumor activities, and could be regarded as the effective pharmaceutical constituents in *B. rynchopetera*.

Table 3. DPPH radical-scavenging activity of the compounds from *B. rynchopetera* (IC₅₀, µg/mL).

Compound	IC ₅₀	Compound	IC ₅₀
1	9.02	15	5.85
2	12.31	16	9.09
3	7.67	18	11.81
4	11.86	vitamin C	6.92
7	1.02	rutin	8.28

3. Experimental Section

3.1. General Experimental Procedures

NMR spectra were acquired with a Bruker AV-400 spectrometer (Bruker, Karlsruhe, Germany) using TMS as the internal reference. MS analyses were recorded with an Agilent G3250AA LC/MSD TOF System (Agilent, Santa Clara, CA, USA). Silica gel (300–400 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (GE Healthcare, Fairfield, NJ, USA), and ODS (Merck China, Beijing, China) were used for column chromatography (CC). GF254 plates (Qingdao Marine, Qingdao, China) were used for thin layer chromatography, and spots were visualized under UV light or by spraying with 5% H₂SO₄ in ethanol followed by heating.

3.2. Insect Material

B. rynchopetera was collected from Dali Bai Autonomous Prefecture in Yunnan Province of China in June 2008, and identified by Professor Guodong Ren from Hebei University. A voucher specimen (No. 2008071001) is deposited in the special medicinal insect development national engineering research center of medicinal insect specimens of Dali University.

3.3. Extraction and Isolation

Air-dried and powdered *B. rynchopetera* (5.0 kg) were extracted by 95% ethanol for three times under the room temperature. Removal of the solvent under reduced pressure afforded the crude extract, which was partitioned successively with PE, ethyl acetate and n-butyl alcohol to yield three soluble fractions.

The ethyl acetate fraction was subjected to silica gel CC eluted with PE–acetone gradient system (10:1 to 0:10) to give eleven fractions (FrA–FrK). Further silica gel CC purification of FrC was accomplished by elution with CHCl₃–CH₃OH (100:1 to 3:1) to afford fractions FrC1–FrC7. FrC2 was further subjected to silica gel, ODS, and Sephadex LH-20 CC to provide compounds **10** (30 mg), **11** (18 mg), **12** (10 mg), and **14** (8 mg). FrC3 was further subjected to ODS and silica gel CC to provide compounds **1** (50 mg), **8** (5 mg), **9** (8 mg), **13** (18 mg), and **16** (25 mg). FrC4 was further subjected to ODS and silica gel CC to provide compounds **5** (9 mg), **6** (8 mg), **7** (10 mg), and **15** (7 mg). Further silica gel CC purification of FrC5 was accomplished by elution with ODS, silica gel, and Sephadex LH-20 to afford compounds **2** (5 mg), **3** (8 mg), **15** (28 mg), and **18** (12 mg). FrD was further subjected to silica gel CC eluted with CHCl₃–CH₃OH (50:1 to 3:1) to afford compound **5** (30 mg). FrE was further

subjected to ODS CC eluted with CH₃OH-H₂O gradient system (1:9 to 9:1) to provide compounds **4** (30 mg) and **17** (18 mg).

3.3.1. Rynchopeterine A (1)

Light yellow liquid; $[\alpha]_D^{20} -13.8$ (c 0.24, CH₃OH); HR-ESIMS: m/z 249.0738 [M + Na]⁺ (calcd. for C₁₁H₁₄O₅Na: 249.0739); For ¹H- and ¹³C-NMR spectral data, see Table 1; IR (KBr, cm⁻¹) ν_{\max} 3410, 1736, 1607, 1591, 1553, 1490, 1456, 1381, 1250, 1165, 1033, 818.

3.3.2. Rynchopeterine B (2)

Light brown liquid; HR-ESIMS: m/z 413.1192 [M + Na]⁺, (calcd. for C₂₀H₂₂O₈Na: 413.1212); For ¹H- and ¹³C-NMR spectral data, see Table 1; IR (KBr, cm⁻¹) ν_{\max} : 3342, 2959, 1740, 1603, 1519, 1430, 1290, 1195, 1110, 944, 805.

3.3.3. Rynchopeterine C (3)

White powder; HR-ESIMS: m/z 202.0859 [M + H]⁺ (calcd. for C₁₂H₁₂NO₂: 202.0868); For ¹H- and ¹³C-NMR spectral data, see Table 2; IR (KBr, cm⁻¹) ν_{\max} : 3380, 3029, 1603, 1595, 1519, 1479, 1380, 1287, 1184, 1065, 802.

3.3.4. Rynchopeterine D (4)

Light yellow powder; HR-ESIMS: m/z 296.0920 [M + H]⁺ (calcd. for C₁₇H₁₄NO₄: 296.0923); For ¹H- and ¹³C-NMR spectral data, see Table 2; IR (KBr, cm⁻¹) ν_{\max} : 3382, 2901, 1603, 1594, 1519, 1455, 1286, 866.

3.3.5. Rynchopeterine E (5)

White powder; HR-ESIMS: m/z 233.0920 [M + H]⁺ (calcd. for C₁₂H₁₃N₂O₃: 233.0926); For ¹H- and ¹³C-NMR spectral data, see Table 2; IR (KBr, cm⁻¹) ν_{\max} 3410, 3025, 1605, 1570, 1520, 1483, 1285, 1069, 1010, 812.

3.4. DPPH Assay

The DPPH radical-scavenging activity was assessed using a previously published method [25]. A total of 3.9 mL DPPH (0.075 mM) was mixed with 0.1 mL sample at various concentrations. The mixture was shaken and incubated at 25 °C in the dark, and the absorbance of the reaction mixture was measured at 515 nm after 30 min. The DPPH radical-scavenging activity was calculated by the following equation: inhibition (%) = (1 - A_s/A_o) × 100%; where A_o is the absorbance of DPPH without any sample, and A_s is the absorbance of a sample with DPPH in it. Inhibition was the scavenging effect, and the 50% absorbance reduction (IC₅₀) was measured from a curve relating concentration to the absorbance of a sample. Vitamin C and rutin were used as positive controls.

3.5. Anti-Tumor Assay

The cytotoxicity of compounds **1–5** against ten human cell lines, including lung cancer cell A549, gastric cancer cell AGS, colon cancer cell Caco-2, cervical cancer cell Hela, hepatoma cell HepG2 and Bel-7402, leukemia cell K562, breast cancer cell MDA-MB-231, esophageal carcinoma cell TE-1, and glioma cell U251 were determined in vitro by the MTT method [26]. Briefly, cells were seeded in 96-well plates at a density of 5 × 10³ to 1 × 10⁴ cells/well. Cells were treated with different concentrations of compounds and positive control for 48 h, following incubation with MTT solution for 4 h. The absorbance was measured using a microplate reader at 490 nm. Cisplatin was used as a positive control.

Supplementary Materials: Supplementary Materials are available online.

Acknowledgments: This work was financially supported by a grant from the National Natural Science Foundation of China (No. 81260475), and a grant from the Key Project of Applied Basic Research Program of Yunnan Province (No. 2014FA007).

Author Contributions: The list authors contributed to this work as follows: L.C. and Z.-T.D. conceived and designed the experiments, H.X., T.-P.Y., J.-W.D., X.-M.W., Q.L. and J.-R.L. performed the experiments and analyzed the data, T.-P.Y. wrote the paper, and H.X. polished the paper. H.X. and Z.-T.D. acquired funding for the research. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yan, Y.M.; Ai, J.; Shi, Y.N.; Zuo, Z.L.; Hou, B.; Luo, J.; Cheng, Y.X. (+/−)-Aspongamide A, an N-acetyldopamine trimer isolated from the insect *Aspongopus chinensis*, is an inhibitor of p-Smad3. *Org. Lett.* **2014**, *16*, 532–535. [[CrossRef](#)] [[PubMed](#)]
2. Di, L.; Shi, Y.N.; Yan, Y.M.; Jiang, L.P.; Hou, B.; Wang, X.L.; Zuo, Z.L.; Chen, Y.B.; Yang, C.P.; Cheng, Y.X. Nonpeptide small molecules from the insect *Aspongopus chinensis* and their neural stem cell proliferation stimulating properties. *RSC Adv.* **2015**, *5*, 70985–70991. [[CrossRef](#)]
3. Dossey, A.T. Insects and their chemical weaponry: New potential for drug discovery. *Nat. Prod. Rep.* **2010**, *27*, 1737–1757. [[CrossRef](#)] [[PubMed](#)]
4. Zhang, L.S.; Xia, C.L.; Yang, Y.S.; Liu, G.M. Progress on Studies of *Blapsyrin chopetera* Fairmaire of the Yi Nationality. *Lishizhen Med. Mater. Med. Res.* **2009**, *20*, 3113–3114.
5. Luo, Q.; Cai, L.; Wu, X.M.; Guo, N.N.; Luo, J.R.; Xiao, H. Cyclodipeptides from medicinal insect *Blaps rynchopetera*. *Chin. Tradit. Herb. Drugs* **2015**, *46*, 2381–2384.
6. Lee, S.J.; Jang, H.J.; Kim, Y.; Oh, H.M.; Lee, S.; Jung, K.; Kim, Y.H.; Lee, W.S.; Lee, S.W.; Rho, M.C. Inhibitory effects of IL-6-induced STAT3 activation of bio-active compounds derived from *Salvia plebeia* R.Br. *Process Biochem.* **2016**, *51*, 2222–2229. [[CrossRef](#)]
7. Bozzini, T.; Botta, G.; Delfino, M.; Onofri, S.; Saladino, R.; Amatore, D.; Sgarbanti, R.; Nencioni, L.; Palamara, A.T. Tyrosinase and Layer-by-Layer supported tyrosinases in the synthesis of lipophilic catechols with antiinfluenza activity. *Bioorg. Med. Chem.* **2013**, *21*, 7699–7708. [[CrossRef](#)] [[PubMed](#)]
8. Li, H.Z.; Zhang, Y.Q.; Liu, Q.; Sun, C.L.; Li, J.; Yang, P.; Wang, X. Preparative separation of phenolic compounds from *Chimonanthus praecox* flowers by high-speed counter-current chromatography using a stepwise elution mode. *Molecules* **2016**, *21*, 1016. [[CrossRef](#)] [[PubMed](#)]
9. Mula, S.; Banerjee, D.; Patro, B.S.; Bhattacharya, S.; Barik, A.; Bandyopadhyay, S.K.; Chattopadhyay, S. Inhibitory property of the Piper betel phenolics against photosensitization-induced biological damages. *Bioorg. Med. Chem.* **2008**, *16*, 2932–2938. [[CrossRef](#)] [[PubMed](#)]
10. Jayan, N.; Yoshio, H.; Junfa, F.; Kenneth, L.K. Convenient syntheses of biogenic aldehydes, 3,4-dihydroxyphenylacetaldehyde and 3,4-dihydroxyphenylglycolaldehyde. *Bioorg. Chem.* **2003**, *31*, 191–197.
11. Zhang, Z.; Xiao, B.; Chen, Q.; Lian, X.Y. Synthesis and biological evaluation of caffeic acid 3,4-dihydroxyphenethyl ester. *J. Nat. Prod.* **2010**, *73*, 252–254. [[CrossRef](#)] [[PubMed](#)]
12. Guazzaroni, M.; Crestini, C.; Saladino, R. Layer-by-layer coated tyrosinase: An efficient and selective synthesis of catechols. *Bioorg. Med. Chem.* **2012**, *20*, 157–166. [[CrossRef](#)] [[PubMed](#)]
13. Botta, G.; Bizzarri, B.M.; Garozzo, A.; Timpanaro, R.; Bisignano, B.; Amatore, D.; Saladino, R. Carbon nanotubes supported tyrosinase in the synthesis of lipophilic hydroxytyrosol and dihydrocaffeoyl catechols with antiviral activity against DNA and RNA viruses. *Bioorg. Med. Chem.* **2015**, *21*, 5345–5351. [[CrossRef](#)] [[PubMed](#)]
14. Yan, Y.M.; Dai, H.Q.; Du, Y.; Schneider, B.; Guo, H.; Li, D.P.; Cheng, Y.X. Identification of blapsins A and B as potent small-molecule 14–3–3 inhibitors from the insect *Blaps japonensis*. *Bioorgan. Med. Chem. Lett.* **2012**, *22*, 4179–4181. [[CrossRef](#)] [[PubMed](#)]
15. Posner, G.H.; Maxwell, J.P.; Kahraman, M.M. Fast and stereoselective epoxide opening by ketone enolate anions. application to synthesis of the norlignan curculigine. *J. Org. Chem.* **2003**, *68*, 3049–3054. [[CrossRef](#)] [[PubMed](#)]
16. Ruzza, P.; Serra, P.A.; Fabbri, D.; Dettori, M.A.; Rocchitta, G.; Delogu, G. Hydroxylated biphenyls as tyrosinase inhibitor: A spectrophotometric and electrochemical study. *Eur. J. Med. Chem.* **2017**, *126*, 1034–1038. [[CrossRef](#)] [[PubMed](#)]

17. Park, C.H.; Kim, K.H.; Lee, I.K.; Lee, S.Y.; Choi, S.U.; Lee, J.H.; Lee, K.R. Phenolic constituents of *Acorus gramineus*. *Arch. Pharm. Res.* **2011**, *34*, 1289. [[CrossRef](#)] [[PubMed](#)]
18. Nilsson, M.; Duarte, I.F.; Almeida, C.; Delgadillo, I.; Goodfellow, B.J.; Gil, A.M.; Morris, G.A. High-resolution NMR and diffusion-ordered spectroscopy of port wine. *J. Agric. Food Chem.* **2004**, *52*, 3736–3743. [[CrossRef](#)] [[PubMed](#)]
19. Dong, J.W.; Cai, L.; Fang, Y.S.; Xiao, H.; Li, Z.J.; Ding, Z.T. Proaporphine and aporphine alkaloids with acetylcholinesterase inhibitory activity from *Stephania epigaea*. *Fitoterapia* **2015**, *104*, 102–107. [[CrossRef](#)] [[PubMed](#)]
20. Liao, Y.; Liu, X.; Yang, J.; Lao, Y.Z.; Yang, X.W.; Li, X.N.; Zhang, J.J.; Ding, Z.J.; Xu, H.X.; Xu, G. Hypersubones A and B, new polycyclic acylphloroglucinols with intriguing adamantane type cores from *Hypericum subsessile*. *Org. Lett.* **2015**, *17*, 1172–1175. [[CrossRef](#)] [[PubMed](#)]
21. Williamson, K.L.; Hasan, M.U.; Clutter, D.R. Conformational analysis by NMR. ^{13}C nuclear magnetic resonance spectra of saturated and unsaturated carboxylic acids and their corresponding esters and anhydrides. *J. Magn. Reson.* **1978**, *30*, 367–383. [[CrossRef](#)]
22. Bernini, R.; Coratti, A.; Provenzano, G.; Fabrizi, G.; Tofani, D. Oxidation of aromatic aldehydes and ketones by $\text{H}_2\text{O}_2/\text{CH}_3\text{ReO}_3$ in ionic liquids: A catalytic efficient reaction to achieve dihydric phenols. *Tetrahedron* **2005**, *61*, 1821–1825. [[CrossRef](#)]
23. Yin, T.P.; Cai, L.; Xing, Y.; Yu, J.; Li, X.J.; Mei, R.F.; Ding, Z.T. Alkaloids with antioxidant activities from *Aconitum handelianum*. *J. Asian Nat. Prod. Res.* **2016**, *18*, 603–610. [[CrossRef](#)] [[PubMed](#)]
24. Oliveras-López, M.; Berná, G.; Jurado-Ruiz, E.; de la Serrana, H.L.-G.; Martín, F. Consumption of extra-virgin olive oil rich in phenolic compounds has beneficial antioxidant effects in healthy human adults. *J. Funct. Foods* **2014**, *10*, 475–484. [[CrossRef](#)]
25. He, J.M.; Yin, T.P.; Chen, Y.; Cai, L.; Tai, Z.G.; Li, Z.J.; Liu, C.S.; Wang, Y.R.; Ding, Z.T. Phenolic compounds and antioxidant activities of edible flowers of *Pyrus pashia*. *J. Funct. Foods* **2015**, *17*, 371–379. [[CrossRef](#)]
26. Yin, T.P.; Xing, Y.; Cai, L.; Yu, J.; Luo, P.; Ding, Z.T. A new polyketide glycoside from the rhizospheric *Clonostachys rogersoniana* associated with *Panax notoginseng*. *J. Asian Nat. Prod. Res.* **2017**. [[CrossRef](#)] [[PubMed](#)]

Sample Availability: Samples of the compounds 1–18 are available from the authors.



© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).